IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

BIO-RAD LABORATORIES, INC. and PRESIDENT AND FELLOWS OF HARVARD COLLEGE Plaintiffs, v. 10X GENOMICS, INC., Defendant.	C.A. No. 19-1699-RGA **DEMAND FOR JURY TRIAL**

FIRST AMENDED COMPLAINT

Bio-Rad Laboratories, Inc. ("Bio-Rad") and the President and Fellows of Harvard College ("Harvard University") (collectively "Plaintiffs") hereby allege for their Complaint ("Complaint") against Defendant 10X Genomics, Inc. ("10X"), on personal knowledge as to their own actions and on information and belief as to the actions of others, as follows:

NATURE OF THE ACTION

1. This is an action for patent infringement arising under the United States Patent Act 35 U.S.C. §§1 *et seq*., including 35 U.S.C. § 271.

2. Plaintiffs bring this action to halt 10X's infringement of their rights under the Patent Laws of the United States 35 U.S.C. §1, *et. seq.*, which arise under U.S. Patent No. 8,871,444 ("the '444 patent"), which is attached hereto as Exhibit 1, U.S. Patent No. 9,919,277 ("the '277 patent"), which is attached hereto as Exhibit 14.

3. Bio-Rad by itself brings this action to halt 10X's infringement of its rights under the Patent Laws of the United States 35 U.S.C. §1, *et. seq.*, and U.S. Patent No. 10,190,115 ("the '115 patent"), which is attached hereto as Exhibit 15.

THE PARTIES

4. Plaintiff Bio-Rad is a Delaware corporation having a principal place of business at 1000 Alfred Nobel Drive, Hercules, CA 94547.

5. Harvard University is a research university incorporated as a Massachusetts not-for-profit institution, with its principal place of business at 1563 Massachusetts Ave., Cambridge, Massachusetts 02138. Harvard University is a patent owner and licensor for the '444 and '277 patents.

6. 10X is a company organized and existing under the laws of Delaware, with its principal place of business at 7068 Koll Center Parkway, Suite 401, Pleasanton, CA, 94566.

JURISDICTION AND VENUE

7. This action for patent infringement arises under the patent laws of the United States, Title 35 of the United States Code.

8. This Court has subject matter jurisdiction pursuant to 28 U.S.C. §§ 1331 and 1338(a).

9. This Court has personal jurisdiction over defendant 10X. 10X has substantial contacts with the forum as a consequence of conducting business in Delaware, and has purposefully availed itself of the benefits and protections of Delaware state law by incorporating under Delaware law.

10. This Court has personal jurisdiction over nominal defendant Harvard University. Harvard University has substantial contacts with the forum as a consequence of conducting business and activities in Delaware.

11. Venue is proper in this District under 28 U.S.C. §§ 1391(b) and (c), and 1400(b) because Bio-Rad and 10X are both Delaware corporations and Delaware is a convenient forum for resolution of the parties' disputes set forth herein.

BACKGROUND

12. Bio-Rad is a leader in the field of life science research and clinical diagnostics, and today many of Bio-Rad's products and tools used in the biotechnology industry are recognized as the gold standard.

13. A centerpiece of many of Bio-Rad's products is its Droplet Digital™ technology. This technology involves partitioning biological samples by placing them in individual microdroplets that are formed based on emulsion chemistry. Using this technology, researchers can create a large numbers of partitions, each one for carrying out a reaction, with a minimum amount of sample handling and a minimum amount of sample volume. A variety of different reactions may be carried out inside the droplets, including polymerase chain reaction ("PCR"), and various reactions to prepare samples for next generation sequencing ("NGS").

14. Bio-Rad began offering its Droplet Digital™ PCR ("ddPCR™") Systems brands in 2011 following its $162 million acquisition of QuantaLife, Inc. ("QuantaLife") and its digital droplet PCR technology. The work at QuantaLife, and subsequently at Bio-Rad, led to a large number of patents being granted throughout the world concerning droplet-based emulsion systems and methods.

15. Bio-Rad's droplet digital technology was a breakthrough that greatly advanced the capabilities of PCR and NGS. Just one year after the launch of Bio-Rad's first generation product, the number of papers citing Bio-Rad's droplet digital method using PCR

nearly quintupled. Indeed, more than 250 peer-reviewed papers have been published in the fields of cancer, liquid biopsy, virology, and other diseases that cited to BioRad's technology.

16. Bio-Rad's ddSEQ™ Single-Cell Isolator uses Droplet Digital™ technology to encapsulate single cells and barcodes into subnanoliter droplets, where cell lysis and barcoding of cellular messenger RNA occur. Libraries are generated representing the messenger RNAs from single cells that can be sequenced for Single Cell Analysis.

17. Bio-Rad has spent years and hundreds of millions of dollars researching, acquiring and developing its Droplet technology and portfolio that is the foundation for many droplet-based applications such as ddPCR™ and NGS and Single Cell Analysis.

18. For instance, in addition to its $162 million acquisition of QuantaLife, Bio-Rad completed an $87 million acquisition of RainDance Technologies, Inc. ("RainDance"), and all of its intellectual property.

19. As another example, Bio-Rad is the exclusive licensee of droplet intellectual property from world-renowned institutions, such as Harvard University and Lawrence Livermore National Laboratory. Likewise, by virtue of its acquisition of RainDance, Bio-Rad acquired an exclusive licensee to foundational droplet technology developed at the University of Chicago.

20. Starting in 2012, several Bio-Rad employees, including Serge Saxanoff (the sole inventor of the '115 patent) left to found 10X Technologies, Inc., which later became Defendant 10X. This company, like Bio-Rad, focused on developing systems and methods for generating droplet-based emulsions.

21. In 2015, 10X launched a droplet-based emulsion system called GemCode that used the claimed microchips and chemistry for forming droplets that can be used in, among

other things, Next Generation Sequencing and Single Cell Analysis. Approximately one year later, 10X launched an updated version of its droplet-based emulsion system called Chromium. These platforms compete against Bio-Rad's Droplet Digital™ technology.

22. In February 2015, RainDance filed a lawsuit in this district accusing 10X's GemCode and Chromium platforms of infringing several patents developed at the University of Chicago. Following its acquisition of RainDance, Bio-Rad substituted itself as the lead Plaintiff in this litigation. In November 2018, Bio-Rad obtained a jury verdict of willful infringement against 10X Genomics, and in August 2019 Bio-Rad obtained a permanent injunction.

23. Following the jury verdict, 10X announced a new line of products, which it recently began selling under the tradename "Next GEM." The Next GEM platform consists of an instrument known as the Chromium Controller along with reagent kits for carrying out various genetic analyses, including at least 10X's Chromium Single Cell Gene Expression Solution, Chromium Single Cell Immune Profiling Solution, and Chromium Single Cell ATAC Solution. *See generally* Exs. 2-3.

24. The Next GEM platform is at the heart of a $362 million IPO that 10X will launch imminently. As 10X stated in its prospectus, "[w]e currently expect that, by the end of the third quarter of 2019, all Chromium instruments that we sell will operate exclusively with our Next GEM solutions and that our Chromium products utilizing our Next GEM microfluidic chips will constitute substantially all of our Chromium sales by the end of 2020." Ex. 4 at 7.

25. 10X, however, infringes, literally or under the doctrine of equivalents, at least the '444 patent through its activities connected to the Next GEM platform.

COUNT I

(Infringement of U.S. Patent No. 8,871,444)

26. Plaintiffs re-allege and incorporates by reference the allegations contained in paragraphs 1 through 25 above as relevant to this count.

27. On October 28, 2014, the United States Patent and Trademark Office duly and legally issued the ’444 patent, entitled “In vitro evolution in microfluidic systems.” A copy of the ’444 patent is attached as Exhibit 1.

28. Andrew David Griffiths, David A. Weitz, Darren R. Link, Keunho Link, and Jerome Bibette are the sole and true inventors of the ’444 patent. By operation of law and as a result of written assignment agreements, United Kingdom Research and Innovation (“UKRI”) and President and Fellows of Harvard College (“Harvard University”) obtained the entire right, title and interest to and in the ’444 patent.

29. Pursuant to license agreements Bio-Rad entered into with UKRI and Harvard University, Bio-Rad obtained an exclusive license to the ’444 patent in the field of microfluidic systems, kits and chips.

30. On information and belief, 10X has infringed and continues to infringe at least claims 1-2, 4, and 8 of the ’444 patent pursuant to 35 U.S.C. § 271(a), literally or under the doctrine of equivalents, by using within the United States without authority the Next GEM products. As an example, attached as Exhibit 5 is a preliminary and exemplary claim chart detailing 10X’s infringement of multiple claims of the ’444 patent. This chart is not intended to limit Plaintiff’s right to modify this chart or any other claim chart or allege that other activities of 10X infringe the identified claims or any other claims of the ’444 patent or any other patents. Exhibit 5 is hereby incorporated by reference in its entirety. Each claim element in Exhibit 5 that

is mapped to 10X's Next GEM platform shall be considered an allegation within the meaning of the Federal Rules of Civil Procedure and therefore a response to each allegation is required.

31. 10X's infringement of the '444 patent has been knowing and willful. 10X's founders, senior-most executives, and senior scientists became aware of Bio-Rad's license with Harvard University at least in connection with 10X's November 2018 trial against Bio-Rad in this district, where 10X was found to willfully infringe intellectual property from the University of Chicago. Moreover, 10X has become deeply familiar with the full scope of the Harvard University droplet patent portfolio (including the '444 patent), at least because it has licensed certain patents from Harvard University, as confirmed by Dr. Ben Hindson, 10X's co-founder and Chief Scientific Officer, during 10X's November 2018 trial. In fact, Dr. Hindson confirmed that 10X was well aware of the work of at least one named inventor of the '444 patent, including Dr. Weitz.

32. Consistent with the foregoing, going back to at least April 4, 2014, 10X has filed Information Disclosure Statements with the United States Patent Office in which it has cited U.S. Patent Application No. 2006/0078888, which is a published version of the priority application that the '444 patent is a continuation of. *See* Exs. 10-13.

33. On information and belief, in view of 10X's (1) knowledge of Bio-Rad's license with Harvard University, (2) knowledge of the Harvard University droplet patents, and (3) prior willful infringement of intellectual property controlled by Bio-Rad, 10X has carefully monitored and analyzed the Harvard University droplet patent portfolio and, through that work, become aware of the '444 patent and the fact that the Next GEM platform infringes the '444 patent. Despite being aware of these facts, 10Xs has nonetheless launched its Next GEM platform, even making it the centerpiece of a $362 million IPO. As 10X stated in in its prospectus in support of its IPO, "[w]e currently expect that, by the end of the third quarter of 2019, all Chromium

instruments that we sell will operate exclusively with our Next GEM solutions and that our Chromium products utilizing our Next GEM microfluidic chips will constitute substantially all of our Chromium sales by the end of 2020." Ex. 4 at 7.

34. In addition, 10X has had knowledge of and notice of the '444 patent and its infringement since at least, and through, the filing and service of this Complaint and despite this knowledge continues to commit the aforementioned infringing acts. For at least the reasons stated in this paragraph and in paragraphs 31-33 above, this infringement has been willful.

35. 10X actively, knowingly, and intentionally has induced, or has threatened to induce, infringement of at least claims 1-2, 4, and 8 of the '444 patent through a range of activities. First, on information and belief, 10X has induced infringement by controlling the design and manufacture of, offering for sale, and selling the Next GEM platform and/or its individual components with the knowledge and specific intent that its customers will use the Next GEM platform to infringe the '444 patent, literally or under the doctrine of equivalents, by performing the claimed method for detecting a product of an enzymatic reaction.

36. Second, on information and belief, 10X has induced infringement by its customers through the dissemination of promotional and marketing materials relating to the Next GEM platform with the knowledge and specific intent that its customers will use the Next GEM platform to infringe the '444 patent, literally or under the doctrine of equivalents, by performing the claimed method for detecting a product of an enzymatic reaction. For instance, 10X promotes the Next GEM platform on its website. As 10X states on the Technology portion of its website, its "proprietary Next GEM technology fuels our Chromium System with an innovative reagent delivery system, set of algorithms and turnkey software analysis tools that enable the discovery of previously inaccessible genetic information at massive rate and scale." Ex. 3 at 1.

37. Third, on information and belief, 10X has induced infringement by its customers through the creation of distribution channels for the Next GEM platform and/or its individual components in the United States with the knowledge and specific intent that its customers will use the Next GEM platform to infringe the ’444 patent, literally or under the doctrine of equivalents, by performing the claimed method for detecting a product of an enzymatic reaction.

38. Fourth, on information and belief, 10X has induced infringement through the distribution of other instructional materials, product manuals, and technical materials with the knowledge and the specific intent to encourage and facilitate its customer’s infringing (either literally or under the doctrine of equivalents) use of the Next GEM platform. *See, e.g.*, Exs. 2, 7-9. 10X is liable for its induced infringement of the ’444 patent pursuant to 35 U.S.C. § 271 (b).

39. 10X has engaged in the above activities with knowledge of the ’444 patent and with the specific intent to encourage and cause infringement by its customers, as shown by the allegations set forth in ¶¶ 31-38 above.

40. 10X has contributed to, or has threatened to contribute to, the infringement by its customers of the ’444 patent by, without authority, selling and offering to sell within the United States materials and apparatuses for practicing the claimed invention of the ’444 patent, including at least the Next GEM platform as a whole and/or the individual components of the Next GEM platform (including without limitation reagent kits). When, for example, the Next GEM platform is used by 10X’s customers for the various applications 10X offers, the claimed method for detecting the product of an enzymatic reaction is performed, thereby infringing, literally or under the doctrine of equivalents, at least claims 1-2, 4, and 8 of the ’444 patent. The Next GEM

platform and/or its individual components, supplied by 10X, constitute a material part of the claimed invention of the ’444 patent.

41. On information and belief, 10X knows that the Next GEM platform and/or its individual components constitute a material part of the inventions of the ’444 patent and that they are not a staple article or commodity of commerce suitable for substantial noninfringing use. As documented above and in Exhibit 5, the Next GEM platform consists of a specialized microfluidic device along with specialized reagents for conducting reactions in microfluidic droplets. *See supra* ¶ 23; Exs. 2, 7-9. As such, no part of the Next GEM platform is a staple article of commerce suitable for substantial non-infringing use. 10X knows that the Next GEM platform and its individual components are not staple articles or commodities of commerce suitable for substantial non-infringing use because the Next GEM platform and its individual components have no use apart from infringing the ’444 patent. 10X is liable for its contributory infringement of the ’444 patent pursuant to 35 U.S.C. § 271(c).

42. 10X’s infringement of the ’444 patent has injured Plaintiff in its business and property rights. 10X’s infringement of the ’444 patent has been and is deliberate and willful and constitutes egregious misconduct. Despite actual knowledge of the ’444 patent and numerous related patents and applications since at least its trial against Bio-Rad in this district in November 2018, 10X continued to develop and launch its infringing products. As set forth in Exhibit 5, when customers use 10X’s Next GEM platform, they practice every element of multiple claims of the ’444 patent. In developing and launching its product, 10X has been willfully blind to this ongoing infringement. Plaintiff is entitled to recover monetary damages for the injuries arising from 10X’s willful infringement pursuant to 35 U.S.C. § 284 in an amount to be determined at trial. 10X’s

infringement of the ’444 patent has caused irreparable harm to Plaintiff and will continue to cause such harm unless and until 10X’s infringing activities are enjoined by this Court.

COUNT II

(Infringement of U.S. Patent No. 9,919,277)

43. Plaintiffs re-allege and incorporate by reference the allegations contained in paragraphs 1 through 42 above as relevant to this count.

44. On March 20, 2018, the United States Patent and Trademark Office duly and legally issued the ’277 patent, entitled “In vitro evolution in microfluidic systems.” A copy of the ’277 patent is attached as Exhibit 14.

45. Andrew David Griffiths, David A. Weitz, Darren R. Link, Keunho Ahn, and Jerome Bibette are the sole and true inventors of the ’277 patent. By operation of law and as a result of written assignment agreements, United Kingdom Research and Innovation (“UKRI”) and President and Fellows of Harvard College (“Harvard University”) obtained the entire right, title and interest to and in the ’277 patent.

46. Pursuant to license agreements Bio-Rad entered into with UKRI and Harvard University, Bio-Rad obtained an exclusive license to the ’277 patent in the field of microfluidic systems, kits and chips.

47. On information and belief, 10X has infringed and continues to infringe at least claims 1-6, 8-9, 11, and 13-14 of the ’277 patent pursuant to 35 U.S.C. § 271(a), literally or under the doctrine of equivalents, by using within the United States without authority the Next GEM products. As an example, attached as Exhibit 16 is a preliminary and exemplary claim chart detailing 10X’s infringement of multiple claims of the ’277 patent. This chart is not intended to limit Plaintiff’s right to modify this chart or any other claim chart or allege that other activities of 10X infringe the identified claims or any other claims of the ’277 patent or any other patents.

Exhibit 16 is hereby incorporated by reference in its entirety. Each claim element in Exhibit 16 that is mapped to 10X's Next GEM platform shall be considered an allegation within the meaning of the Federal Rules of Civil Procedure and therefore a response to each allegation is required.

48. 10X's infringement of the '277 patent has been knowing and willful. 10X's founders, senior-most executives, and senior scientists became aware of Bio-Rad's license with Harvard University at least in connection with 10X's November 2018 trial against Bio-Rad in this district, where 10X was found to willfully infringe intellectual property from the University of Chicago. Moreover, 10X has become deeply familiar with the full scope of the Harvard University droplet patent portfolio (including the '277 patent), at least because it has licensed certain patents from Harvard University, as confirmed by Dr. Ben Hindson, 10X's co-founder and Chief Scientific Officer, during 10X's November 2018 trial. In fact, Dr. Hindson confirmed that 10X was well aware of the work of at least one named inventor of the '277 patent, including Dr. Weitz.

49. On information and belief, in view of 10X's (1) knowledge of Bio-Rad's license with Harvard University, (2) knowledge of the Harvard University droplet patents, and (3) prior willful infringement of intellectual property controlled by Bio-Rad, 10X has carefully monitored and analyzed the Harvard University droplet patent portfolio and, through that work, become aware of the '277 patent and the fact that the Next GEM platform infringes the '277 patent. Despite being aware of these facts, 10Xs has nonetheless launched its Next GEM platform, even making it the centerpiece of a $362 million IPO. As 10X stated in in its prospectus in support of its IPO, "[w]e currently expect that, by the end of the third quarter of 2019, all Chromium instruments that we sell will operate exclusively with our Next GEM solutions and that our Chromium products utilizing our Next GEM microfluidic chips will constitute substantially all of our Chromium sales by the end of 2020." Ex. 4 at 7.

50. In addition, 10X has had knowledge of and notice of the ’277 patent and its infringement since at least, and through, the filing and service of this Complaint and despite this knowledge continues to commit the aforementioned infringing acts. For at least the reasons stated in this paragraph and in paragraphs 47-49 above, this infringement has been willful.

51. 10X actively, knowingly, and intentionally has induced, or has threatened to induce, infringement of at least claims 1-6, 8-9, 11, and 13-14 of the ’277 patent through a range of activities. First, on information and belief, 10X has induced infringement by controlling the design and manufacture of, offering for sale, and selling the Next GEM platform and/or its individual components with the knowledge and specific intent that its customers will use the Next GEM platform to infringe the ’277 patent, literally or under the doctrine of equivalents, by performing the claimed method for conducting an enzymatic reaction.

52. Second, on information and belief, 10X has induced infringement by its customers through the dissemination of promotional and marketing materials relating to the Next GEM platform with the knowledge and specific intent that its customers will use the Next GEM platform to infringe the ’277 patent, literally or under the doctrine of equivalents, by performing the claimed method for conducting an enzymatic reaction. For instance, 10X promotes the Next GEM platform on its website. As 10X states on the Technology portion of its website, its “proprietary Next GEM technology fuels our Chromium System with an innovative reagent delivery system, set of algorithms and turnkey software analysis tools that enable the discovery of previously inaccessible genetic information at massive rate and scale.” Ex. 3 at 1.

53. Third, on information and belief, 10X has induced infringement by its customers through the creation of distribution channels for the Next GEM platform and/or its individual components in the United States with the knowledge and specific intent that its

customers will use the Next GEM platform to infringe the ’277 patent, literally or under the doctrine of equivalents, by performing the claimed method for conducting an enzymatic reaction.

54. Fourth, on information and belief, 10X has induced infringement through the distribution of other instructional materials, product manuals, and technical materials with the knowledge and the specific intent to encourage and facilitate its customer’s infringing (either literally or under the doctrine of equivalents) use of the Next GEM platform. *See, e.g.*, Exs. 2, 7-9. 10X is liable for its induced infringement of the ’277 patent pursuant to 35 U.S.C. § 271 (b).

55. 10X has engaged in the above activities with knowledge of the ’444 patent and with the specific intent to encourage and cause infringement by its customers, as shown by the allegations set forth in ¶¶ 47-54 above.

56. 10X has contributed to, or has threatened to contribute to, the infringement by its customers of the ’277 patent by, without authority, selling and offering to sell within the United States materials and apparatuses for practicing the claimed invention of the ’277 patent, including at least the Next GEM platform as a whole and/or the individual components of the Next GEM platform (including without limitation reagent kits). When, for example, the Next GEM platform is used by 10X’s customers for the various applications 10X offers, the claimed method for conducting an enzymatic reaction is performed, thereby infringing, literally or under the doctrine of equivalents, at least claims 1-6, 8-9, 11, and 13-14 of the ’277 patent. The Next GEM platform and/or its individual components, supplied by 10X, constitute a material part of the claimed invention of the ’277 patent.

57. On information and belief, 10X knows that the Next GEM platform and/or its individual components constitute a material part of the inventions of the ’277 patent and that they are not a staple article or commodity of commerce suitable for substantial noninfringing use.

As documented above and in Exhibit 16, the Next GEM platform consists of a specialized microfluidic device along with specialized reagents for conducting reactions in microfluidic droplets. *See supra* ¶ 23; Exs. 2, 7-9. As such, no part of the Next GEM platform is a staple article of commerce suitable for substantial non-infringing use. 10X knows that the Next GEM platform and its individual components are not staple articles or commodities of commerce suitable for substantial non-infringing use because the Next GEM platform and its individual components have no use apart from infringing the '277 patent. 10X is liable for its contributory infringement of the '277 patent pursuant to 35 U.S.C. § 271(c).

58. 10X's infringement of the '277 patent has injured Plaintiff in its business and property rights. 10X's infringement of the '277 patent has been and is deliberate and willful and constitutes egregious misconduct. Despite actual knowledge of the '277 patent and numerous related patents and applications since at least its trial against Bio-Rad in this district in November 2018, 10X continued to develop and launch its infringing products. As set forth in Exhibit 16, when customers use 10X's Next GEM platform, they practice every element of multiple claims of the '277 patent. In developing and launching its product, 10X has been willfully blind to this ongoing infringement. Plaintiff is entitled to recover monetary damages for the injuries arising from 10X's willful infringement pursuant to 35 U.S.C. § 284 in an amount to be determined at trial. 10X's infringement of the '277 patent has caused irreparable harm to Plaintiff and will continue to cause such harm unless and until 10X's infringing activities are enjoined by this Court.

COUNT III

(Infringement of U.S. Patent No. 10,190,115)

59. Bio-Rad re-alleges and incorporates by reference the allegations contained in paragraphs 1 through 58 above as relevant to this count.

60. On March 20, 2018, the United States Patent and Trademark Office duly and legally issued the ’115 patent, entitled “Methods and Compositions For Nucleic Acid Analysis.” A copy of the ’115 patent is attached as Exhibit 15.

61. Serge Saxonov is the sole and true inventor of the ’115 patent. By operation of law and as a result of written assignment agreements, Bio-Rad obtained the entire right, title and interest to and in the ’115 patent.

62. On information and belief, 10X has infringed and continues to infringe at least claims 1, 4-15, and 18-26 of the ’115 patent pursuant to 35 U.S.C. § 271(a), literally or under the doctrine of equivalents, by using within the United States without authority the Next GEM products. As an example, attached as Exhibit 17 is a preliminary and exemplary claim chart detailing 10X’s infringement of multiple claims of the ’115 patent. This chart is not intended to limit Plaintiff’s right to modify this chart or any other claim chart or allege that other activities of 10X infringe the identified claims or any other claims of the ’115 patent or any other patents. Exhibit 17 is hereby incorporated by reference in its entirety. Each claim element in Exhibit 17 that is mapped to 10X’s Next GEM platform shall be considered an allegation within the meaning of the Federal Rules of Civil Procedure and therefore a response to each allegation is required.

63. 10X’s infringement of the ’115 patent has been knowing and willful. 10X’s founders, senior-most executives, and senior scientists were aware of the ’115 patent at least since the founding of 10X. Serge Saxonov, a co-founder of 10X and former employee of Bio-Rad is the sole inventor on the ’115 patent and worked on the invention encompassed by the ’115 patent while he was employed at Bio-Rad. Thus, Serge Saxonov and 10X are deeply familiar with the ’115 patent.

64. Consistent with the foregoing, going back to at least December 4, 2015, 10X has filed Information Disclosure Statements with the United States Patent Office in which it has cited U.S. Patent Publication No. 2015/0376609 which is a published version of the application that resulted in the '115 patent. *See* Ex. 18.

65. Despite being aware of these facts, 10X has nonetheless launched its Next GEM platform, even making it the centerpiece of a $362 million IPO. As 10X stated in in its prospectus in support of its IPO, "[w]e currently expect that, by the end of the third quarter of 2019, all Chromium instruments that we sell will operate exclusively with our Next GEM solutions and that our Chromium products utilizing our Next GEM microfluidic chips will constitute substantially all of our Chromium sales by the end of 2020." Ex. 4 at 7.

66. In addition, 10X has had knowledge of and notice of the '115 patent and its infringement since at least, and through, the filing and service of this Complaint and despite this knowledge continues to commit the aforementioned infringing acts. For at least the reasons stated in this paragraph and in paragraphs 63-64 above, this infringement has been willful.

67. 10X actively, knowingly, and intentionally has induced, or has threatened to induce, infringement of at least claims 1, 4-15, and 18-26 of the '115 patent through a range of activities. First, on information and belief, 10X has induced infringement by controlling the design and manufacture of, offering for sale, and selling the Next GEM platform and/or its individual components with the knowledge and specific intent that its customers will use the Next GEM platform to infringe the '115 patent, literally or under the doctrine of equivalents, by using the claimed composition and device.

68. Second, on information and belief, 10X has induced infringement by its customers through the dissemination of promotional and marketing materials relating to the Next

GEM platform with the knowledge and specific intent that its customers will use the Next GEM platform to infringe the '115 patent, literally or under the doctrine of equivalents, by using the claimed composition and device. For instance, 10X promotes the Next GEM platform on its website. As 10X states on the Technology portion of its website, its "proprietary Next GEM technology fuels our Chromium System with an innovative reagent delivery system, set of algorithms and turnkey software analysis tools that enable the discovery of previously inaccessible genetic information at massive rate and scale." Ex. 3 at 1.

69. Third, on information and belief, 10X has induced infringement by its customers through the creation of distribution channels for the Next GEM platform and/or its individual components in the United States with the knowledge and specific intent that its customers will use the Next GEM platform to infringe the '115 patent, literally or under the doctrine of equivalents, by using the claimed composition and device.

70. Fourth, on information and belief, 10X has induced infringement through the distribution of other instructional materials, product manuals, and technical materials with the knowledge and the specific intent to encourage and facilitate its customer's infringing (either literally or under the doctrine of equivalents) use of the Next GEM platform. *See, e.g.*, Exs. 2, 7-9. 10X is liable for its induced infringement of the '115 patent pursuant to 35 U.S.C. § 271 (b).

71. 10X has engaged in the above activities with knowledge of the '115 patent and with the specific intent to encourage and cause infringement by its customers, as shown by the allegations set forth in ¶¶ 62-69 above.

72. 10X has contributed to, or has threatened to contribute to, the infringement by its customers of the '115 patent by, without authority, selling and offering to sell within the United States materials and apparatuses for practicing the claimed invention of the '115 patent,

including at least the Next GEM platform as a whole and/or the individual components of the Next GEM platform (including without limitation reagent kits). When, for example, the Next GEM platform is used by 10X's customers for the various applications 10X offers, the claimed composition and device is used, thereby infringing, literally or under the doctrine of equivalents, at least claims 1, 4-15, and 18-26 of the '115 patent. The Next GEM platform and/or its individual components, supplied by 10X, constitute a material part of the claimed invention of the '115 patent.

73. On information and belief, 10X knows that the Next GEM platform and/or its individual components constitute a material part of the inventions of the '115 patent and that they are not a staple article or commodity of commerce suitable for substantial noninfringing use. As documented above and in Exhibit 17, the Next GEM platform consists of a specialized microfluidic device along with specialized reagents for conducting reactions in microfluidic droplets. *See supra* ¶ 23; Exs. 2, 7-9. As such, no part of the Next GEM platform is a staple article of commerce suitable for substantial non-infringing use. 10X knows that the Next GEM platform and its individual components are not staple articles or commodities of commerce suitable for substantial non-infringing use because the Next GEM platform and its individual components have no use apart from infringing the '115 patent. 10X is liable for its contributory infringement of the '115 patent pursuant to 35 U.S.C. § 271(c).

74. 10X's infringement of the '115 patent has injured Plaintiff in its business and property rights. 10X's infringement of the '115 patent has been and is deliberate and willful and constitutes egregious misconduct. Despite actual knowledge of the '115 patent, 10X continued to develop and launch its infringing products. As set forth in Exhibit 17, when customers use 10X's Next GEM platform, they practice every element of multiple claims of the '115 patent. In developing and launching its product, 10X has been willfully blind to this ongoing infringement.

Plaintiff is entitled to recover monetary damages for the injuries arising from 10X's willful infringement pursuant to 35 U.S.C. § 284 in an amount to be determined at trial. 10X's infringement of the '115 patent has caused irreparable harm to Plaintiff and will continue to cause such harm unless and until 10X's infringing activities are enjoined by this Court.

PRAYER FOR RELIEF

WHEREFORE, Plaintiffs pray for relief as follows:

A. Judgment that 10X has infringed one or more claims of the '444 and '277 patents;

B. An order permanently enjoining 10X from further infringement of the '444 and '277 patents or in the alternative an on-going royalty;

C. An award of damages pursuant to 35 U.S.C. § 284;

D. A determination that 10X's infringement of the '444 and '277 patents has been and is willful, and an award of enhanced damages, up to and including trebling of the damages awarded to Bio-Rad;

E. An award to Bio-Rad of its costs, pre- and post-judgment interest, and reasonable expenses to the fullest extent permitted by law;

F. A declaration that this case is exceptional pursuant to 35 U.S.C. § 285, and an award of attorneys' fees and costs; and

G. An award of such other and further relief as the Court may deem just and proper.

WHEREFORE, Bio-Rad by itself prays for relief as follows:

A. Judgment that 10X has infringed one or more claims of the '115 patent;

B. An order permanently enjoining 10X from further infringement of the'115

patent or in the alternative an on-going royalty;

C. An award of damages pursuant to 35 U.S.C. § 284;

D. A determination that 10X's infringement of the '115 patent has been and is willful, and an award of enhanced damages, up to and including trebling of the damages awarded to Bio-Rad;

E. An award to Bio-Rad of its costs, pre- and post-judgment interest, and reasonable expenses to the fullest extent permitted by law;

F. A declaration that this case is exceptional pursuant to 35 U.S.C. § 285, and an award of attorneys' fees and costs; and

An award of such other and further relief as the Court may deem just and proper.

DEMAND FOR JURY TRIAL

Pursuant to Federal Rule of Civil Procedure 38(b), Bio-Rad hereby demands a trial by jury on all issues so triable.

Dated: November 4, 2019

Respectfully submitted,

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EXHIBIT 1

US008871444B2

(12) **United States Patent**
Griffiths et al.

(10) **Patent No.: US 8,871,444 B2**
(45) **Date of Patent: *Oct. 28, 2014**

(54) **IN VITRO EVOLUTION IN MICROFLUIDIC SYSTEMS**

(71) Applicants: **Medical Research Council**, London (GB); **President and Fellows of Harvard College**, Cambridge, MA (US)

(72) Inventors: **Andrew David Griffiths**, Strasbourg (FR); **David A. Weitz**, Cambridge, MA (US); **Darren R. Link**, Lenxington, MA (US); **Keunho Ahn**, Lafayette, CA (US); **Jerome Bibette**, Paris (FR)

(73) Assignees: **Medical Research Council**, London (GB); **President and Fellows of Harvard College**, Cambridge, MA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **13/693,356**

(22) Filed: **Dec. 4, 2012**

(65) **Prior Publication Data**

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Related U.S. Application Data

(63) Continuation of application No. 11/665,030, filed as application No. PCT/GB2005/003889 on Oct. 10, 2005, which is a continuation of application No. 10/961,695, filed on Oct. 8, 2004, now Pat. No. 7,968,287.

(51) **Int. Cl.**

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G01N 33/53	(2006.01)
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C12M 1/00	(2006.01)
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B01L 3/00	(2006.01)
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B01J 19/00	(2006.01)
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B01F 3/08	(2006.01)
C12P 21/00	(2006.01)

(52) **U.S. Cl.**

CPC ***B01J 19/0046*** (2013.01); *B01J 2219/005* (2013.01); *B01J 2219/00657* (2013.01); ***B01L 3/502784*** (2013.01); ***B01F 13/0071*** (2013.01); ***B01L 3/502753*** (2013.01); ***B01F 13/0076*** (2013.01); *B01L 2200/0636* (2013.01); *B01J 2219/00466* (2013.01); *B01J 2219/00468* (2013.01); *B01J 2219/00576* (2013.01); *B01J 2219/00722* (2013.01); ***B01F 5/0646*** (2013.01); *B01L 2300/0867* (2013.01); ***B01F 5/0655*** (2013.01); *B01L 2300/0864* (2013.01); *B01J 2219/0052* (2013.01); *B01L 2400/0487* (2013.01); ***C12N 15/1075*** (2013.01); ***B01F 3/0807*** (2013.01); *B01J 2219/00545* (2013.01); ***B01F 13/0062*** (2013.01); *B01L 2300/0654* (2013.01); ***C12N 15/1058*** (2013.01); *B01L 2300/0816* (2013.01); *B01L 2200/0673* (2013.01); ***C12P 21/00*** (2013.01); ***B01L 3/502776*** (2013.01); ***B01F 5/0647*** (2013.01); *B01L 2400/0415* (2013.01); ***B01L 3/502746*** (2013.01)

USPC **435/6.1**; 435/7.1; 435/91.2; 435/283.1; 422/68.1; 536/23.1; 536/24.33

(58) **Field of Classification Search**

CPC B01L 3/502784; A61K 49/1806; C12Q 1/686

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See application file for complete search history.

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(57) **ABSTRACT**

The invention describes a method for isolating one or more genetic elements encoding a gene product having a desired activity, comprising the steps of: (a) compartmentalizing genetic elements into microcapsules; and (b) sorting the genetic elements which express the gene product having the desired activity; wherein at least one step is under microfluidic control. The invention enables the in vitro evolution of nucleic acids and proteins by repeated mutagenesis and iterative applications of the method of the invention.

9 Claims, 25 Drawing Sheets

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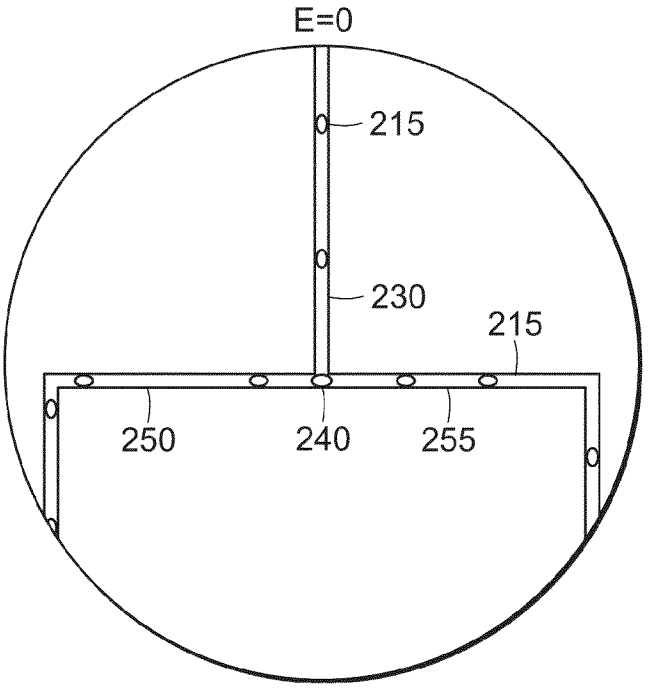

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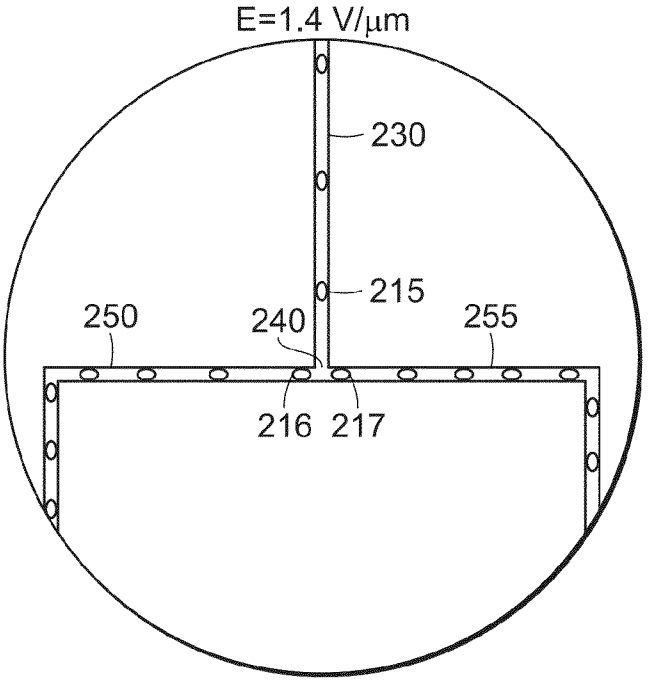

FIG. 1B

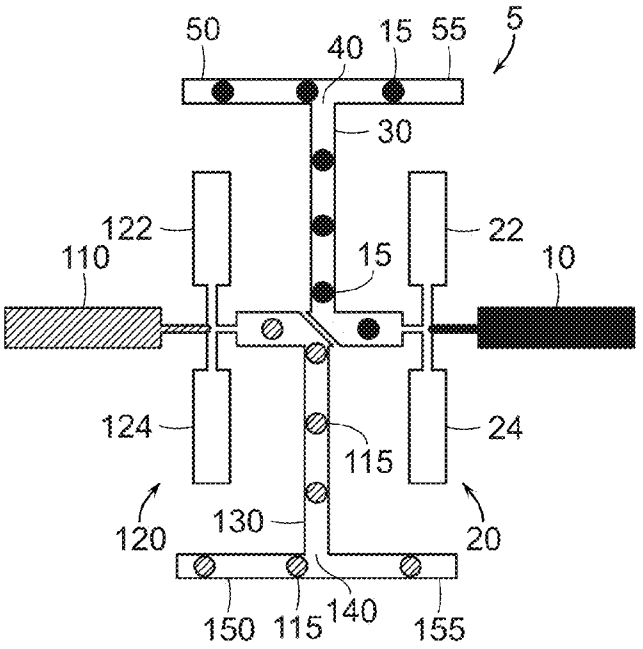
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FIG. 2A

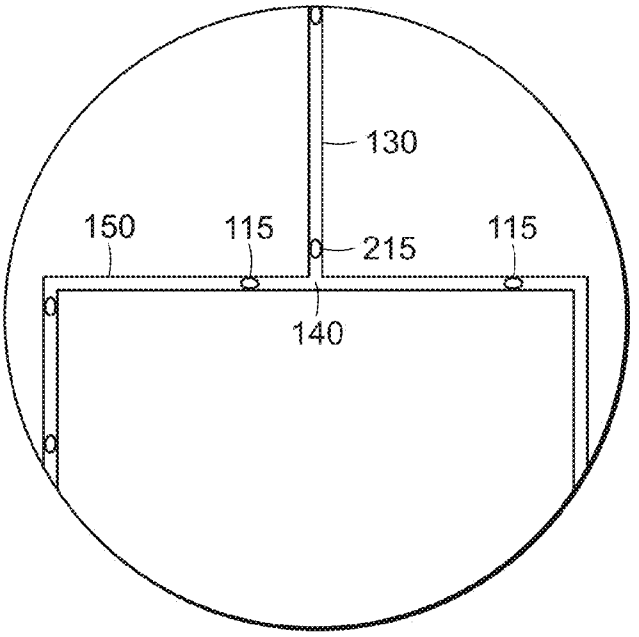
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FIG. 2B

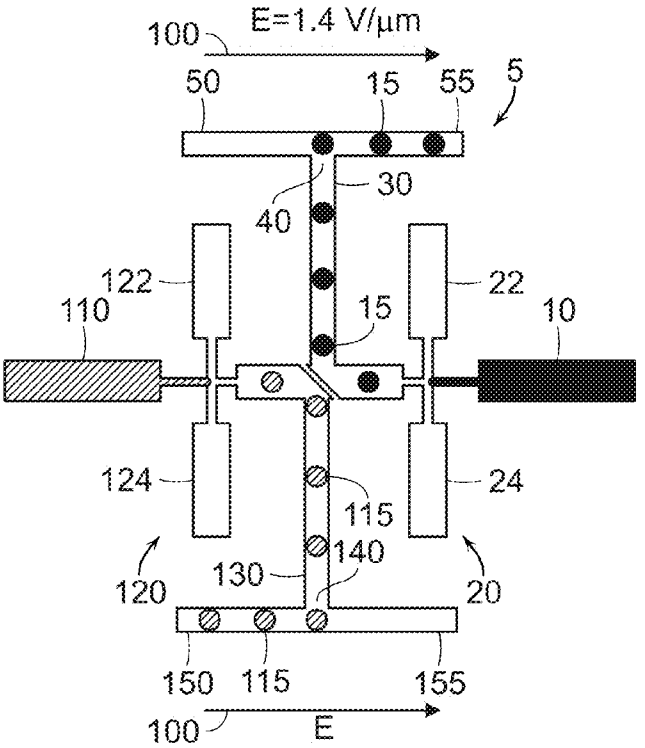
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FIG. 3A

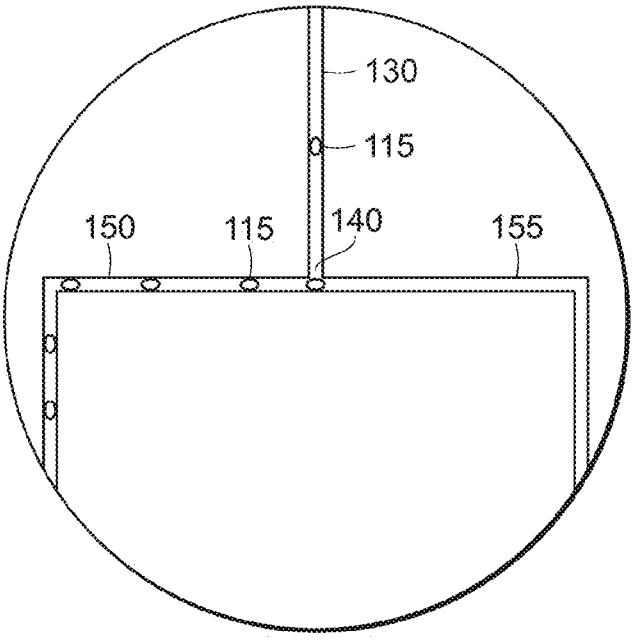
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FIG. 3B

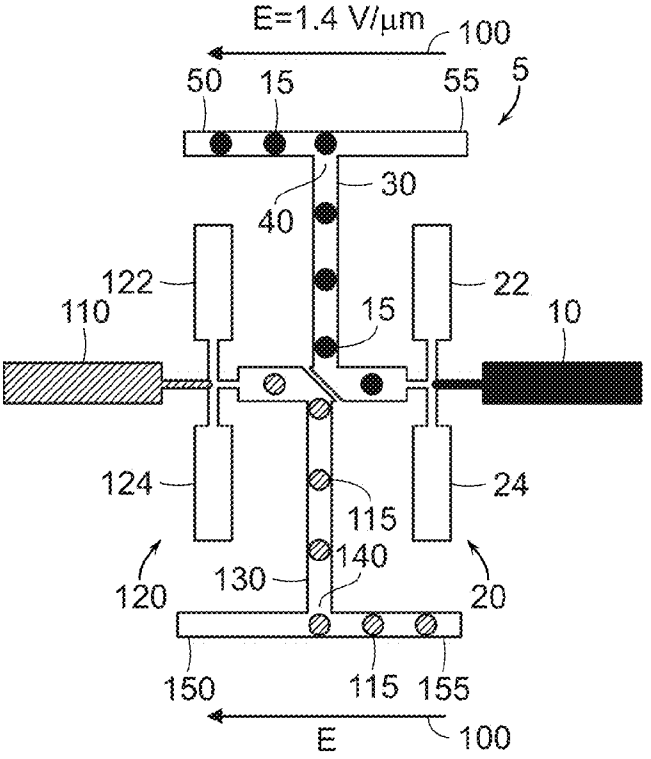
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FIG. 4A

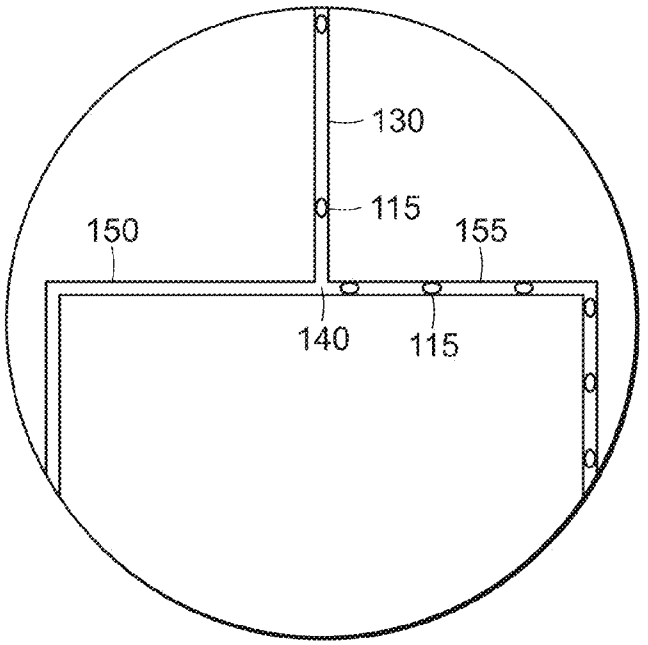
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FIG. 4B

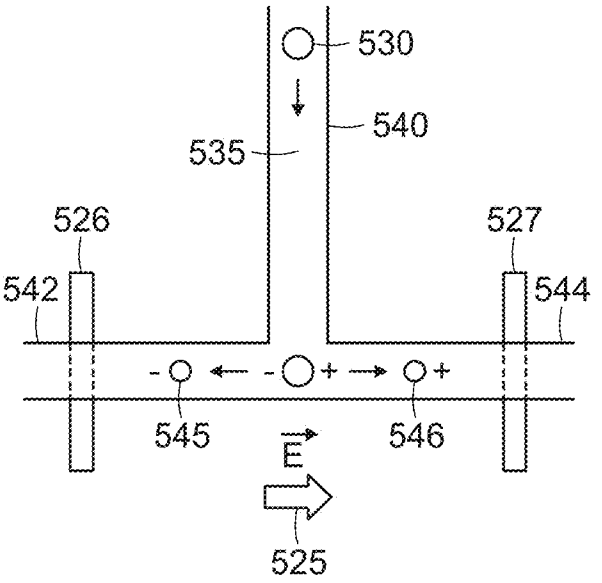

FIG. 5

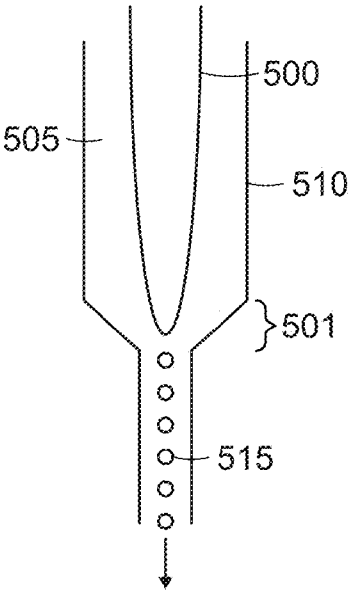

FIG. 7A

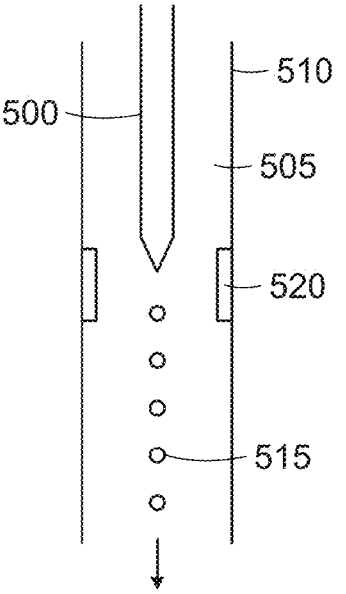

FIG. 7B

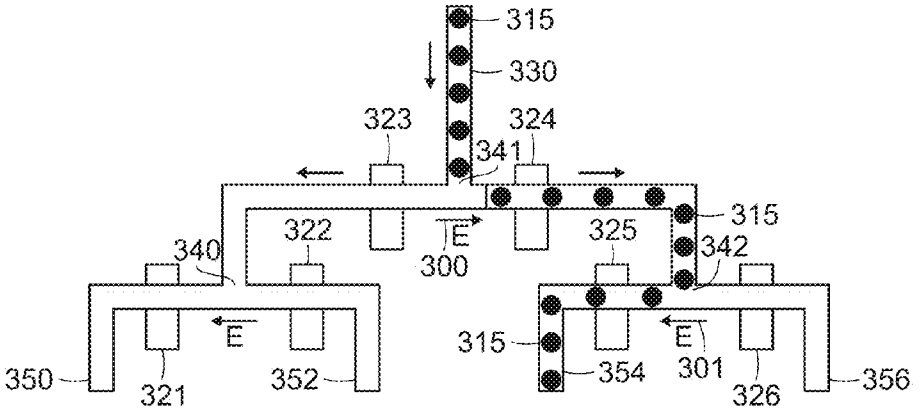
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FIG. 6A

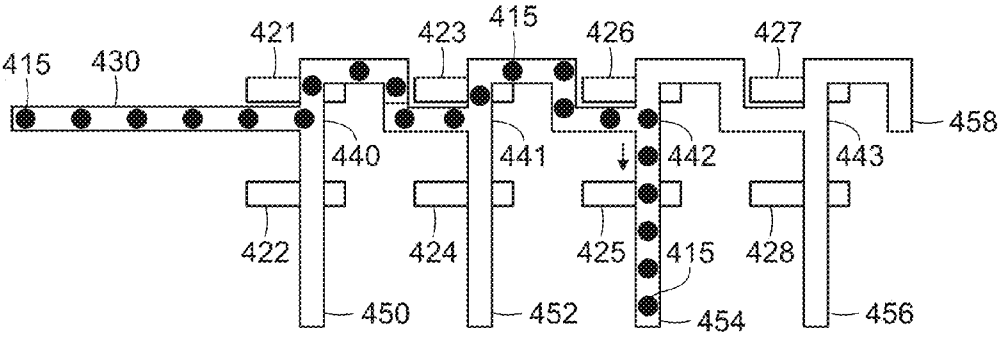
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FIG. 6B

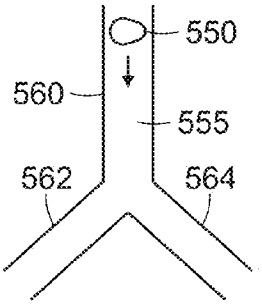
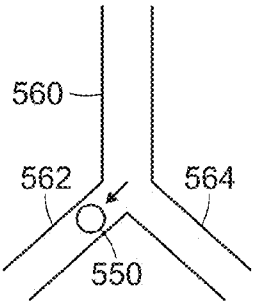


FIG. 8A



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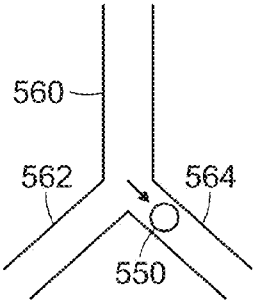


FIG. 8C

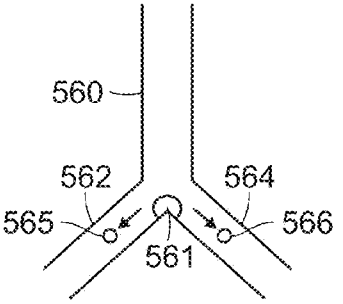


FIG. 8D

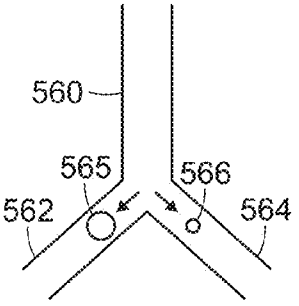


FIG. 8E

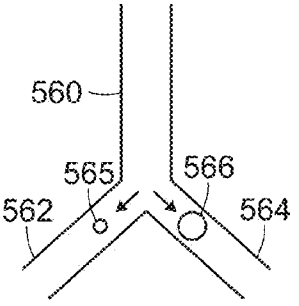


FIG. 8F

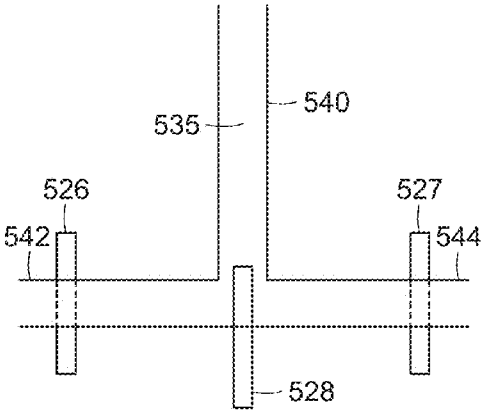


FIG. 9A

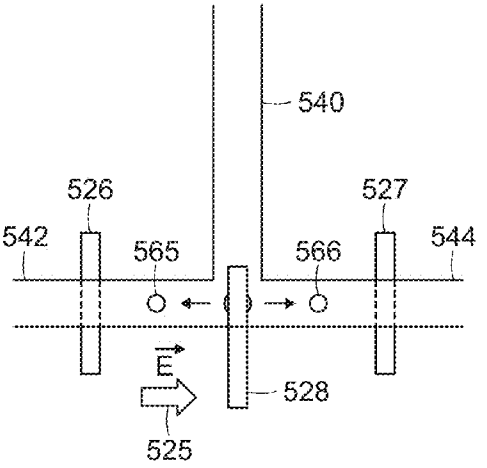


FIG. 9B

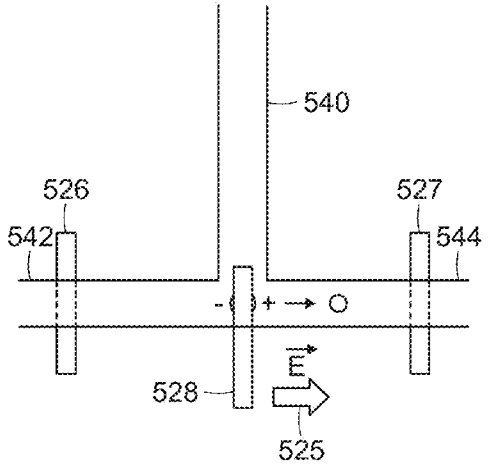


FIG. 9C

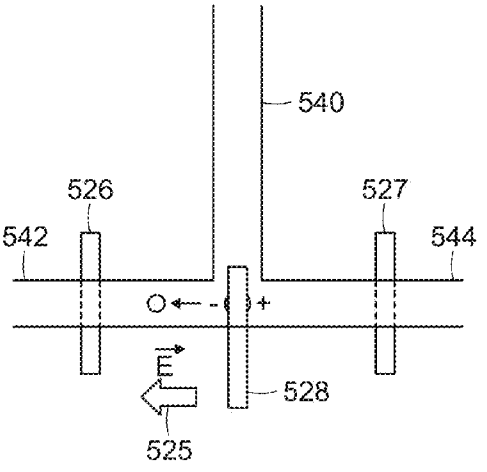


FIG. 9D

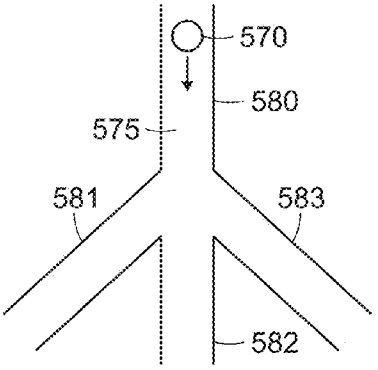
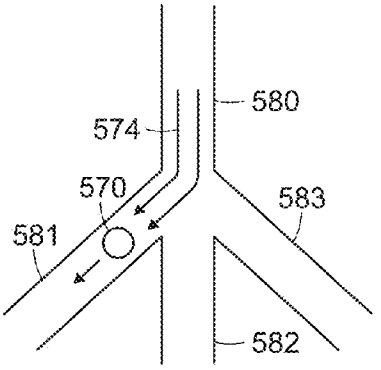


FIG. 10A



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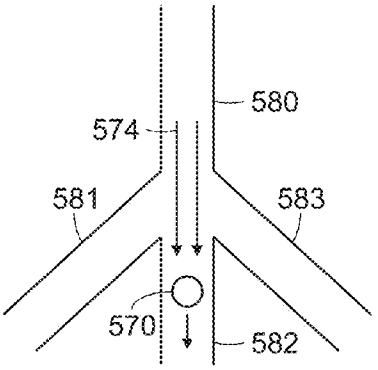

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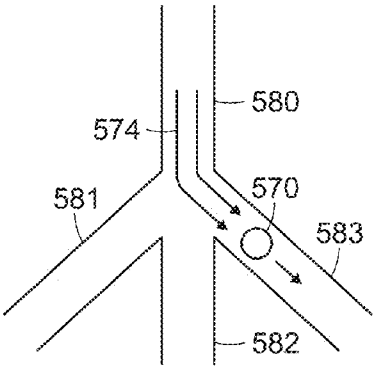


FIG. 10D

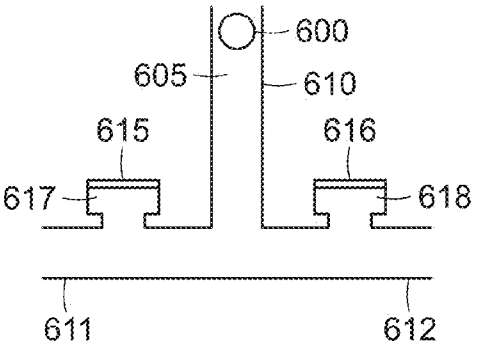
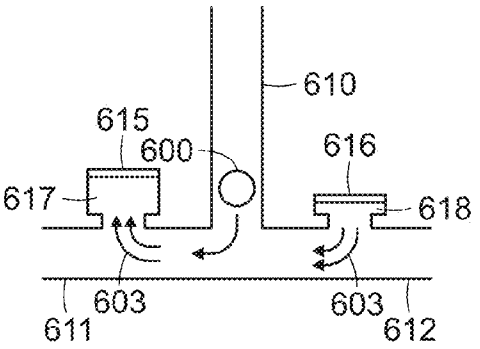


FIG. 11A



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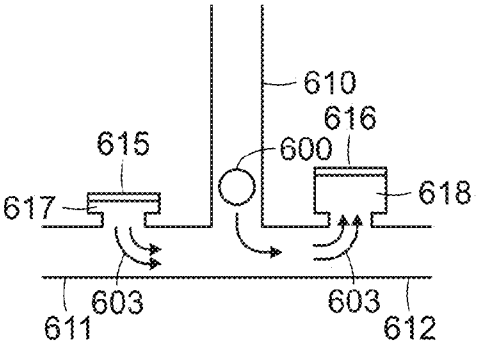


FIG. 11C

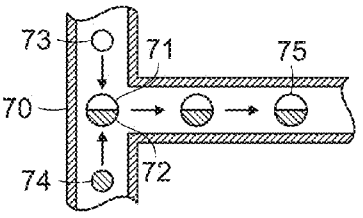
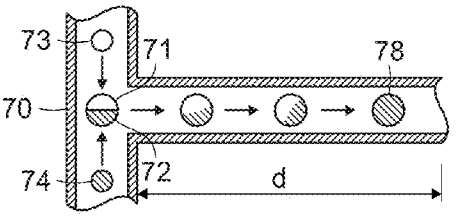


FIG. 12A



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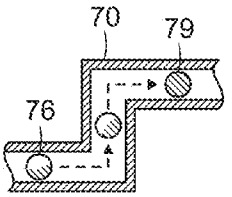

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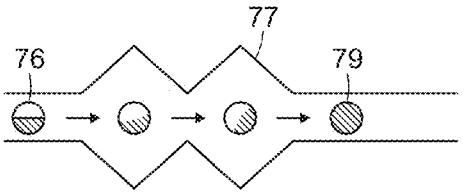

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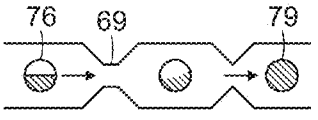

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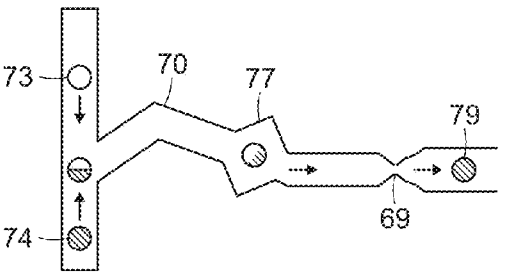

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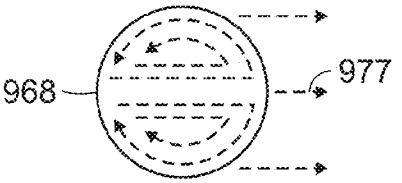


FIG. 12G

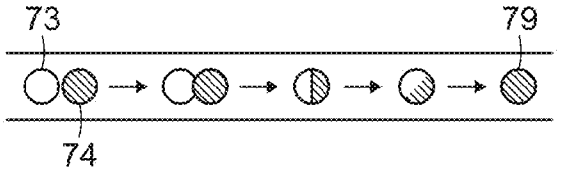


FIG. 12H

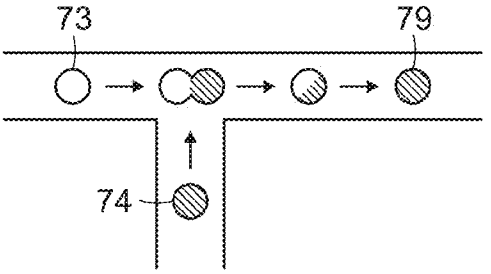


FIG. 12I

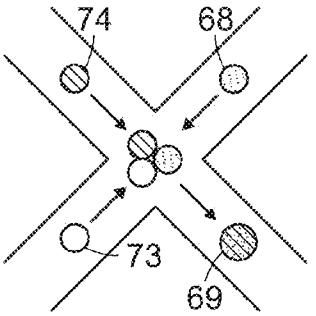


FIG. 12J

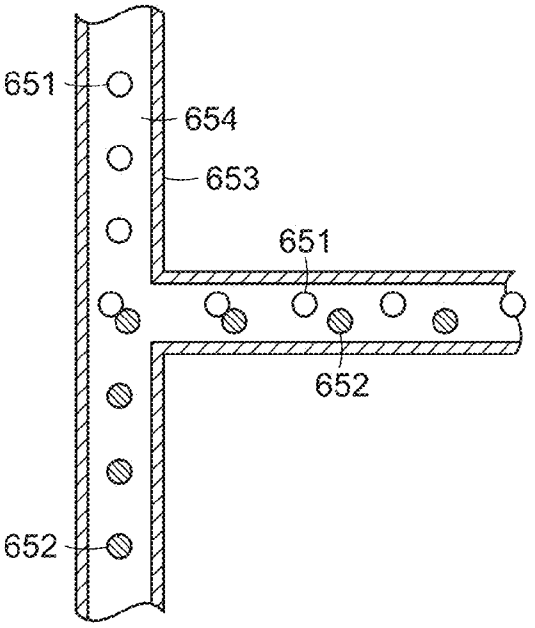


FIG. 13A

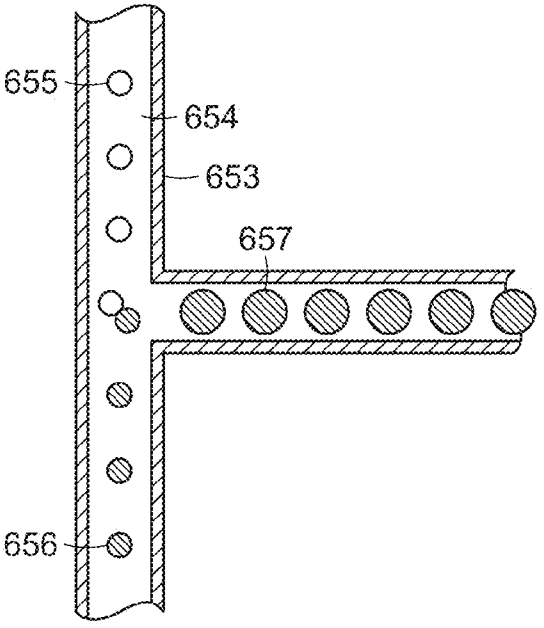


FIG. 13B

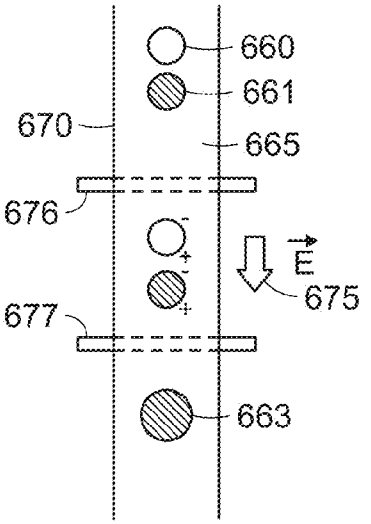


FIG. 13C

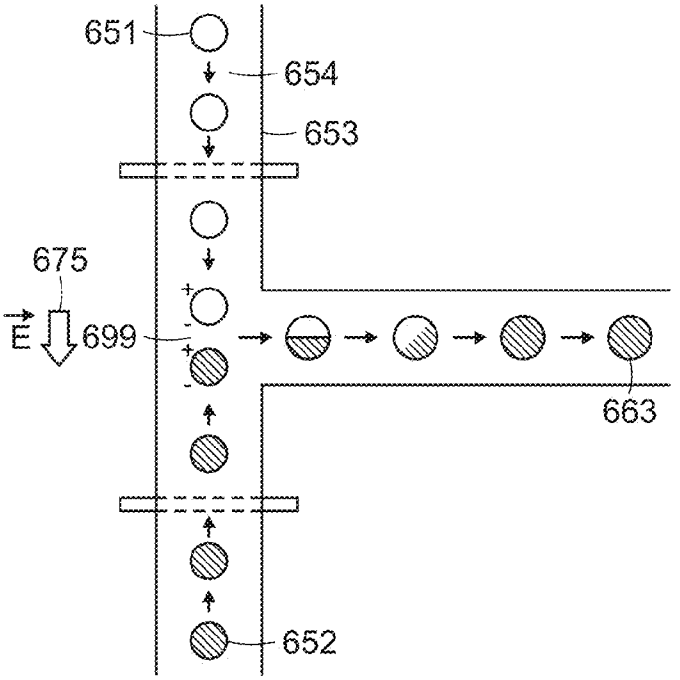


FIG. 13D

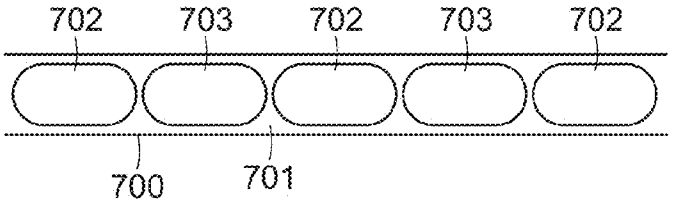


FIG. 14A

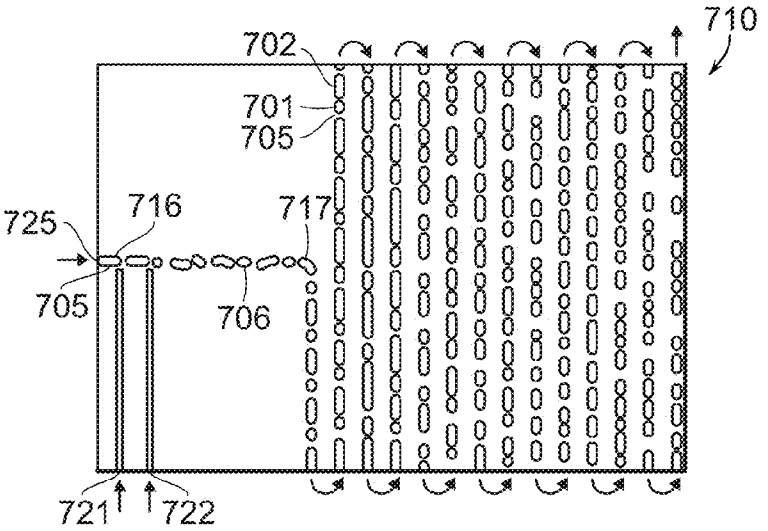


FIG. 14B

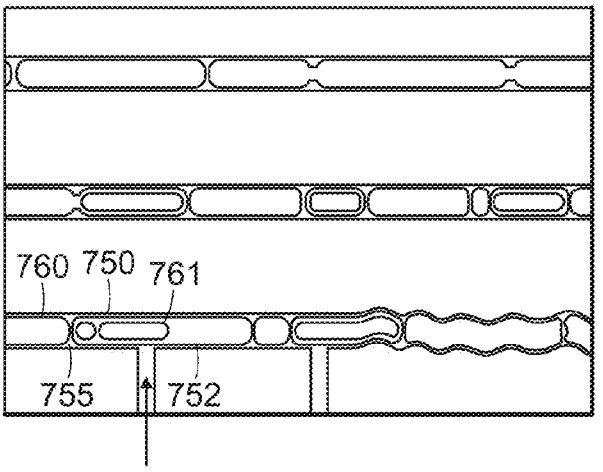


FIG. 14C

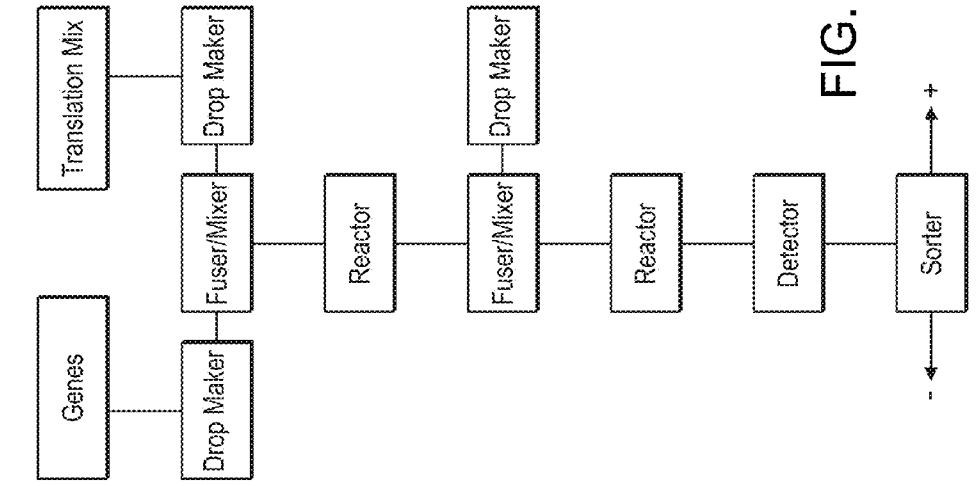


FIG. 15B

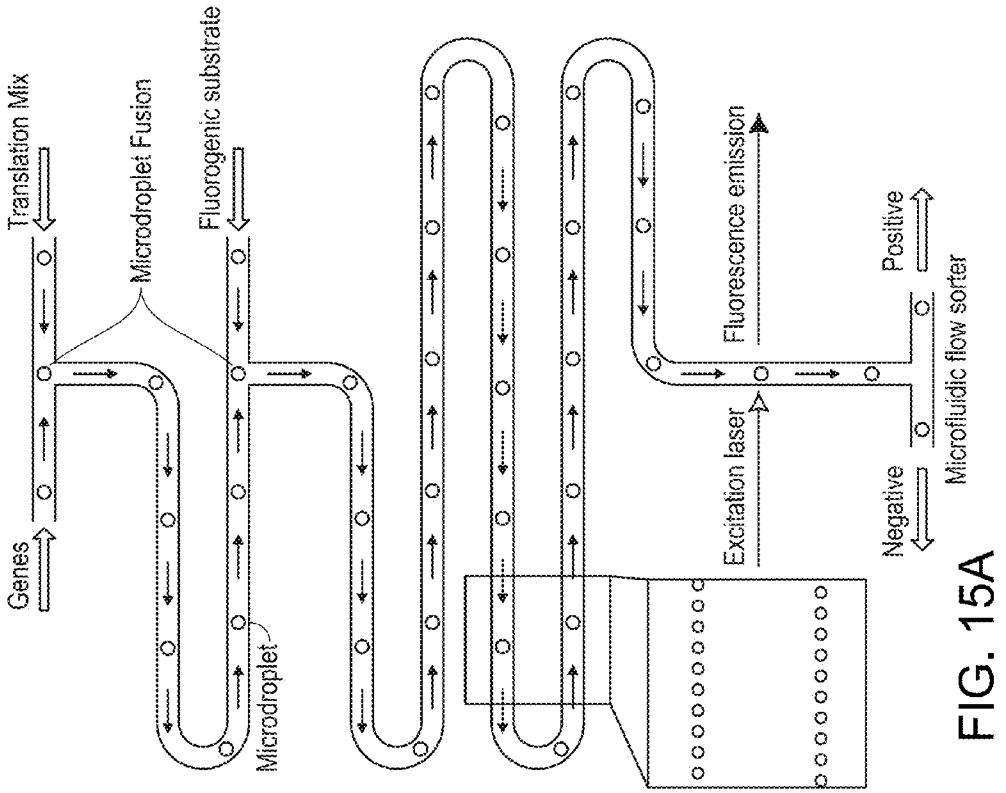


FIG. 15A

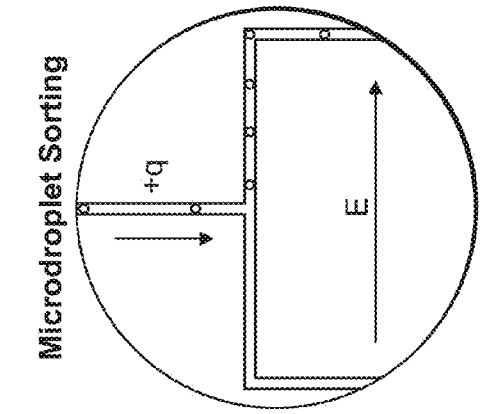


FIG. 16D

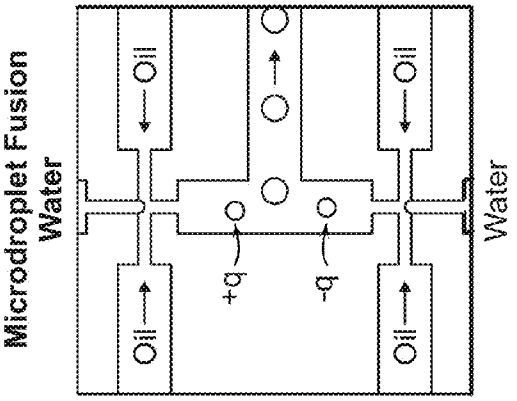


FIG. 16C

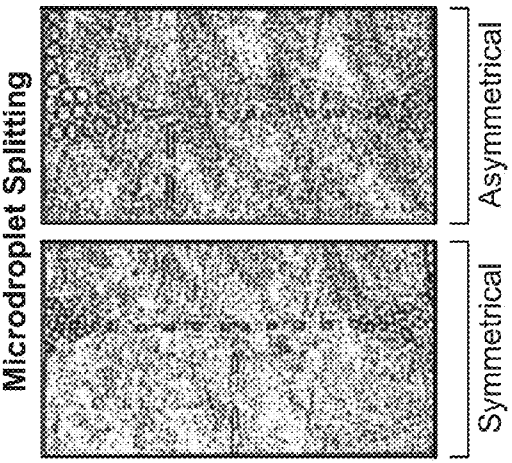


FIG. 16B

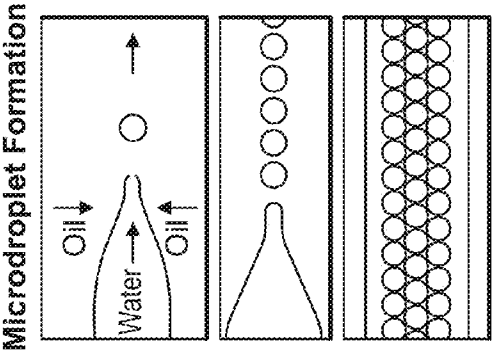


FIG. 16A

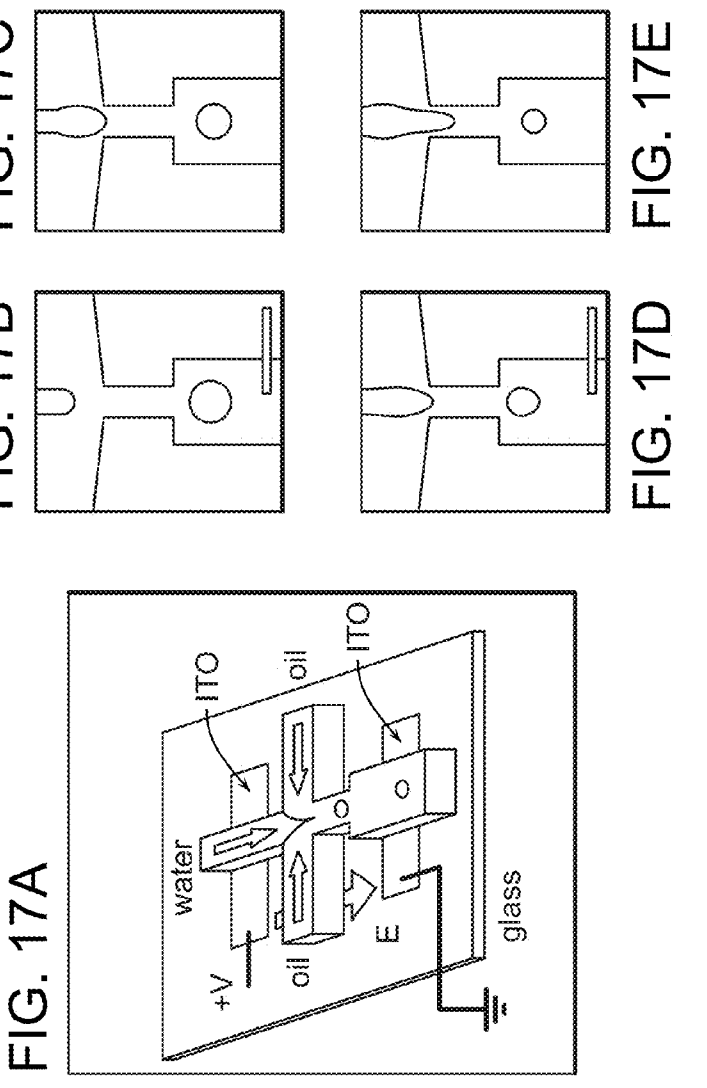


FIG. 17A

FIG. 17B

FIG. 17C

FIG. 17D

FIG. 17E

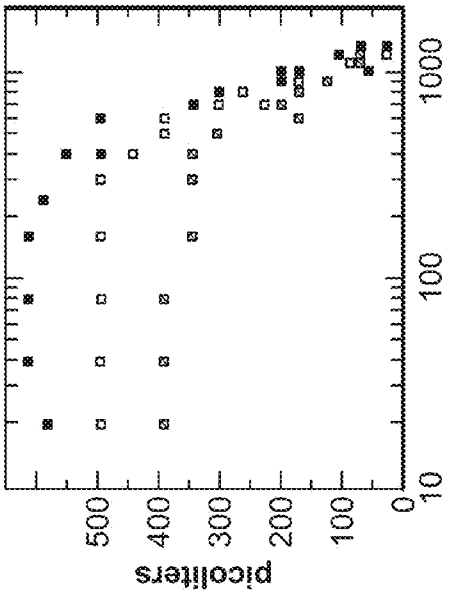


FIG. 17F

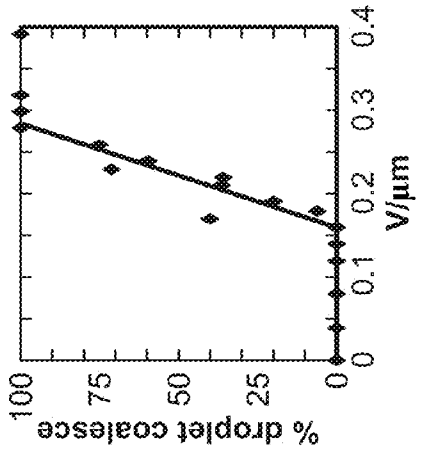


FIG. 18D

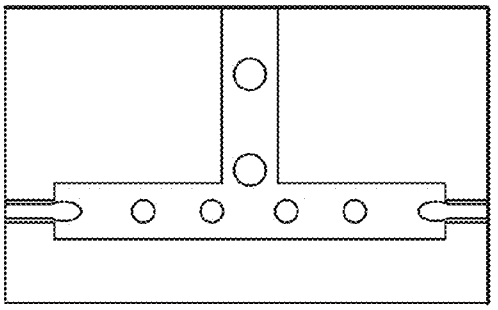


FIG. 18C

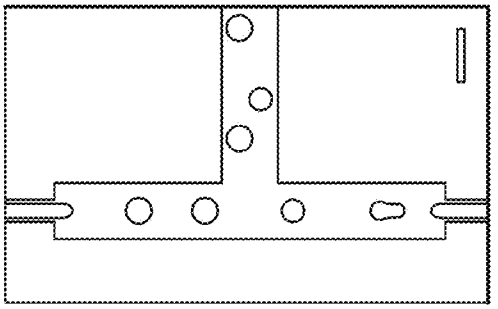


FIG. 18B

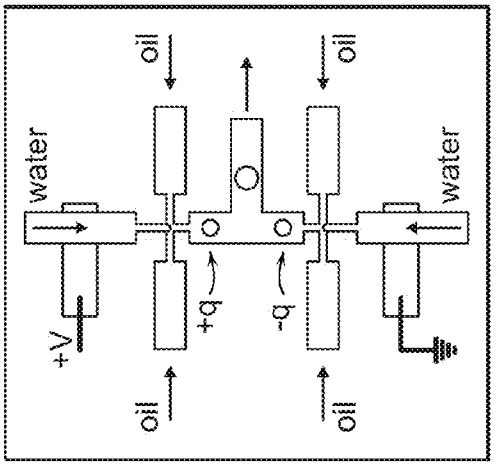


FIG. 18A

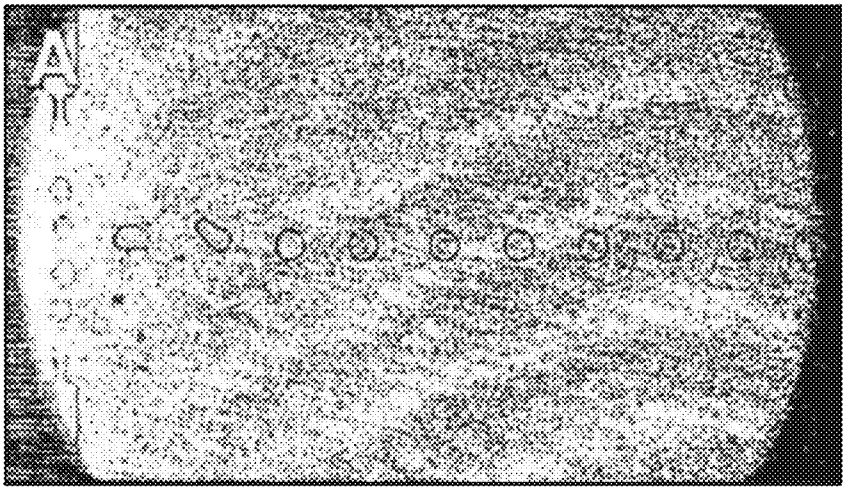


FIG. 19A

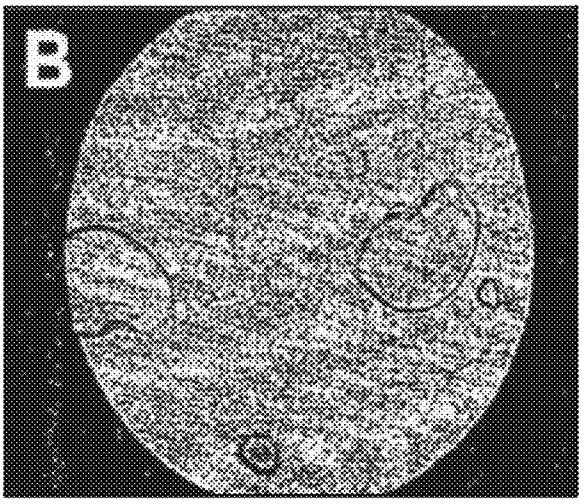


FIG. 19B

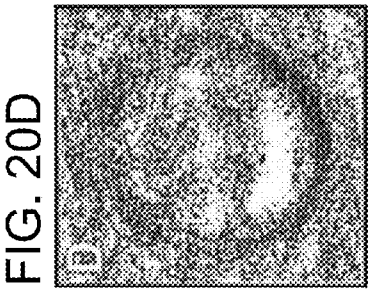


FIG. 20D

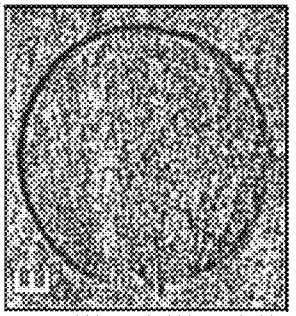


FIG. 20E

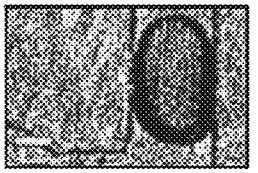


FIG. 20I

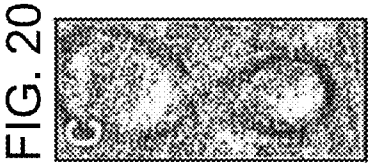


FIG. 20C

FIG. 20H

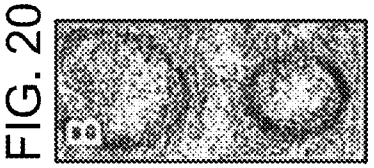


FIG. 20B

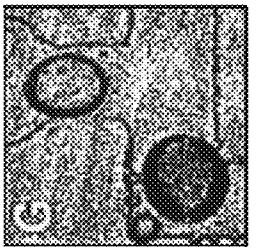


FIG. 20G

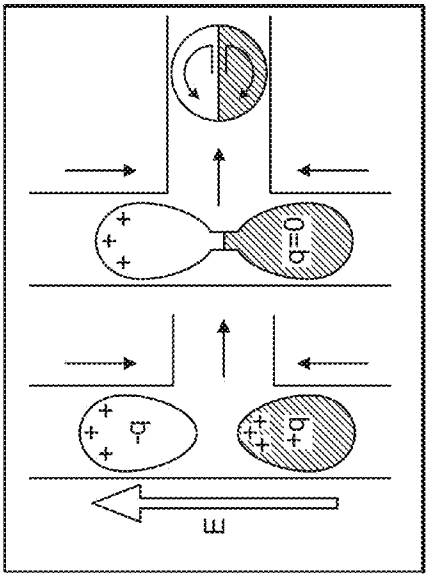


FIG. 20A

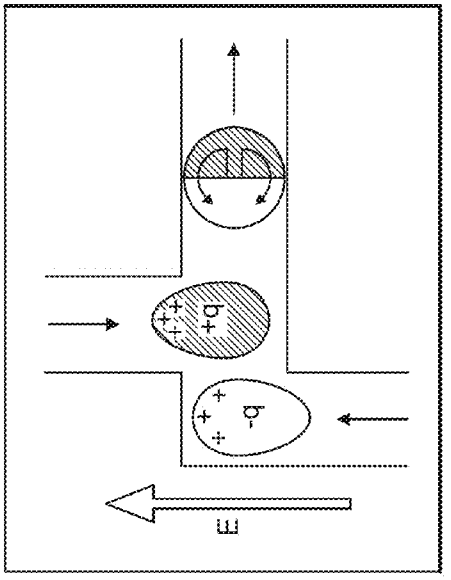


FIG. 20F

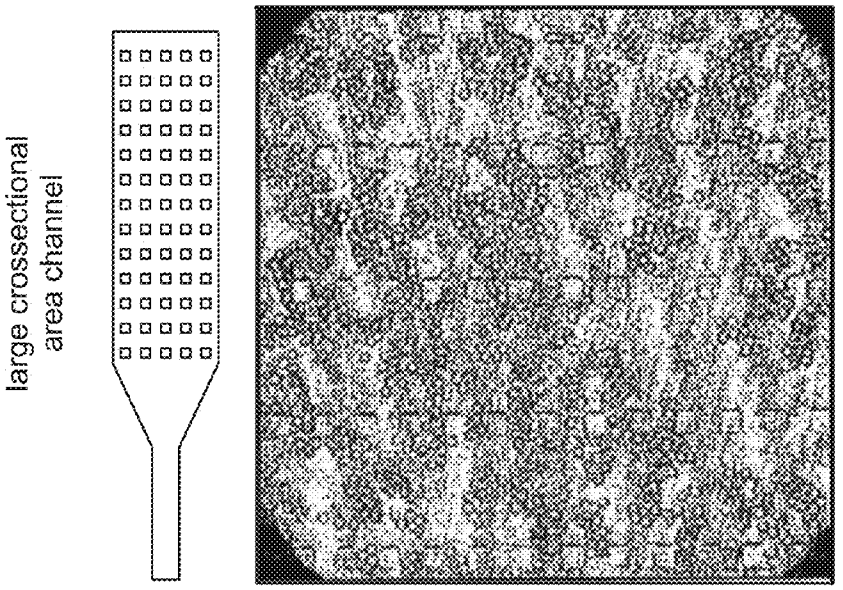


FIG. 21B

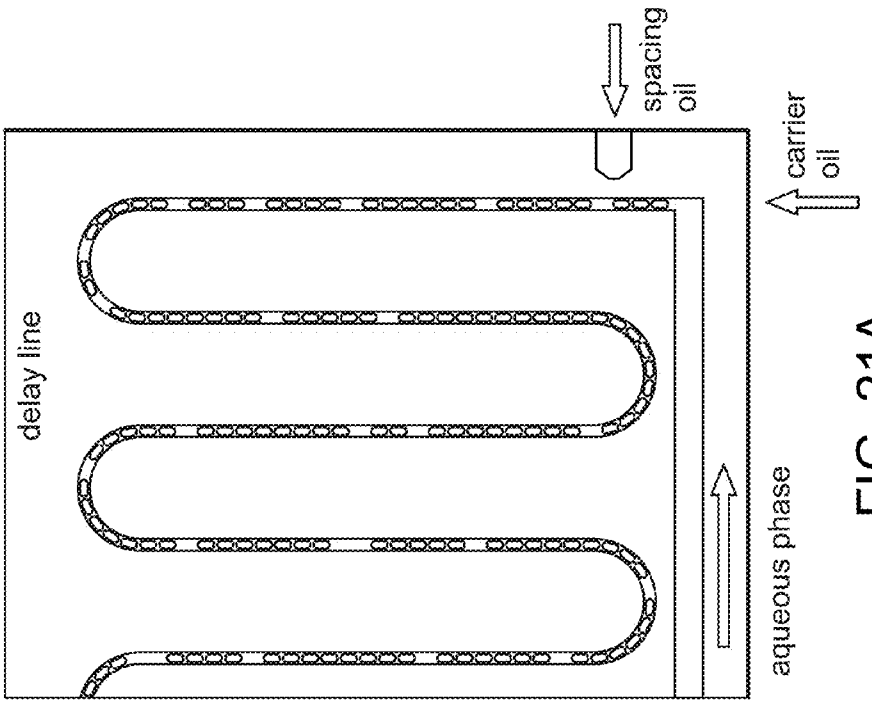


FIG. 21A

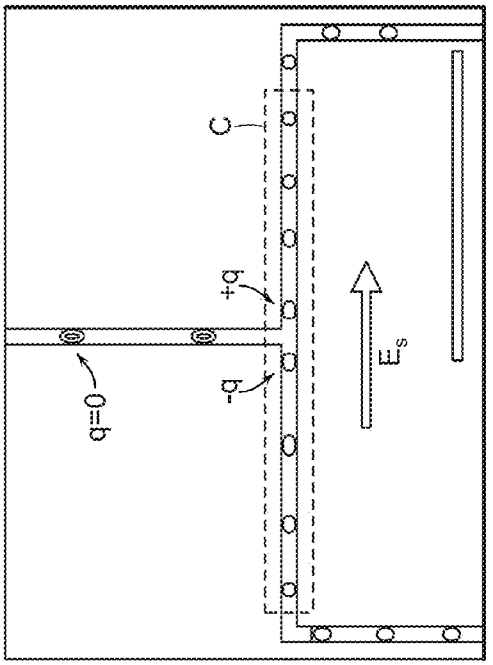


FIG. 22B

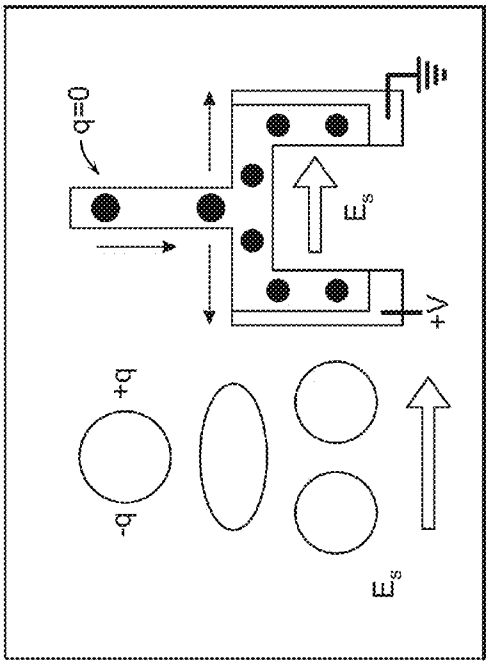


FIG. 22A

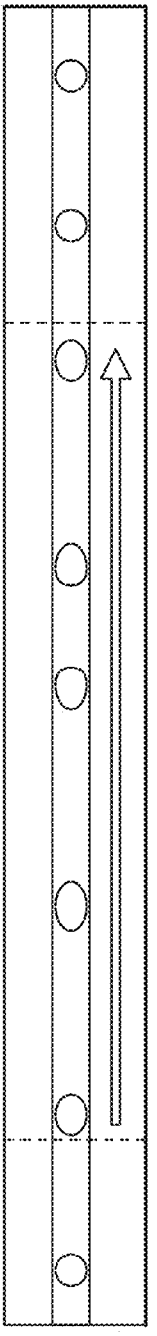


FIG. 22C

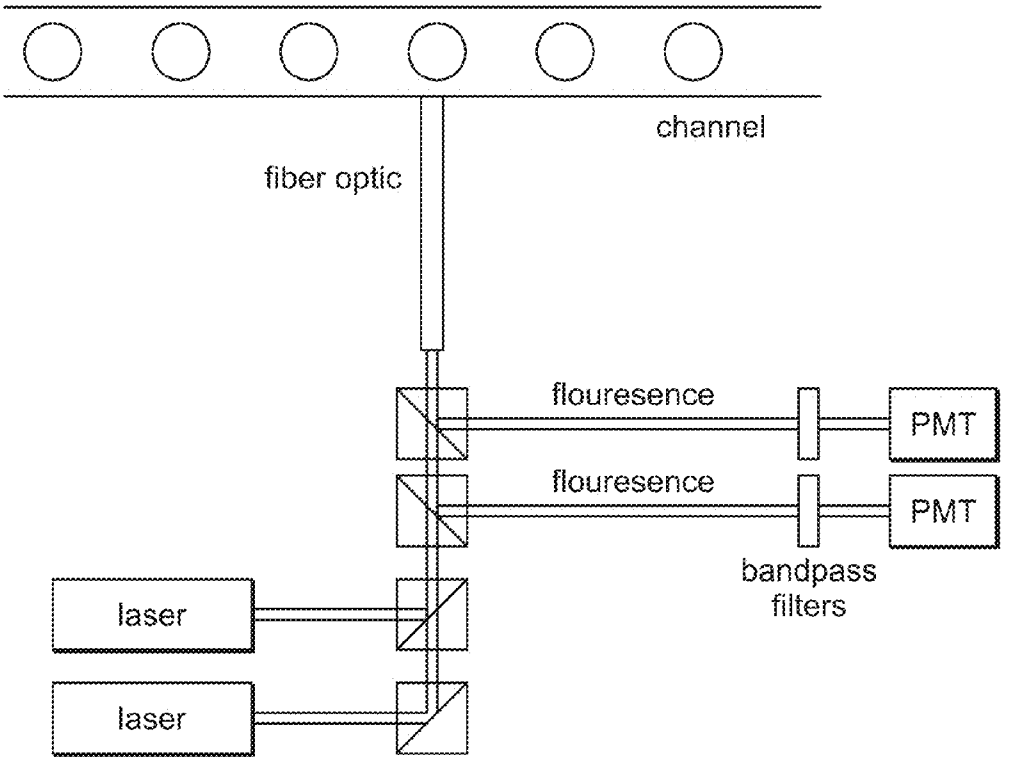

channel
fiber optic
flouresence
PMT
flouresence
PMT
bandpass
filters
laser
laser

FIG. 23

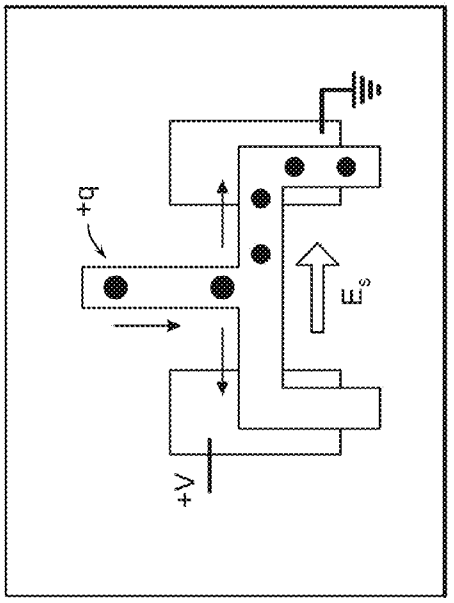


FIG. 24B

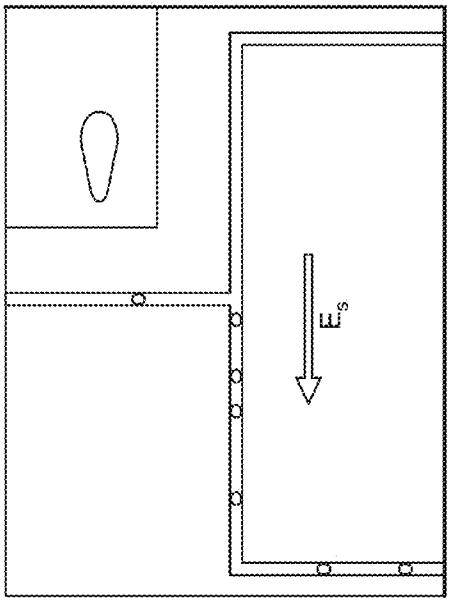


FIG. 24D

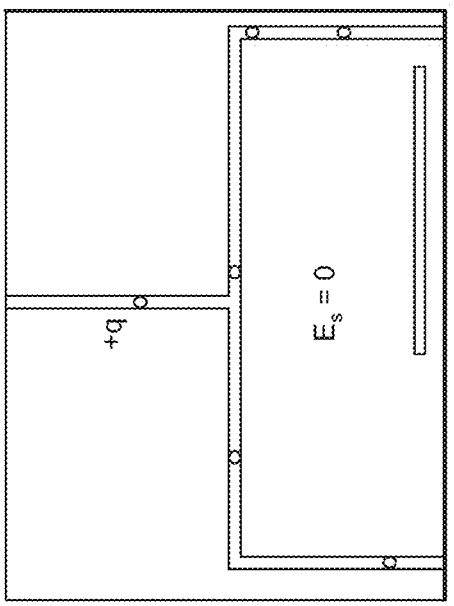


FIG. 24A

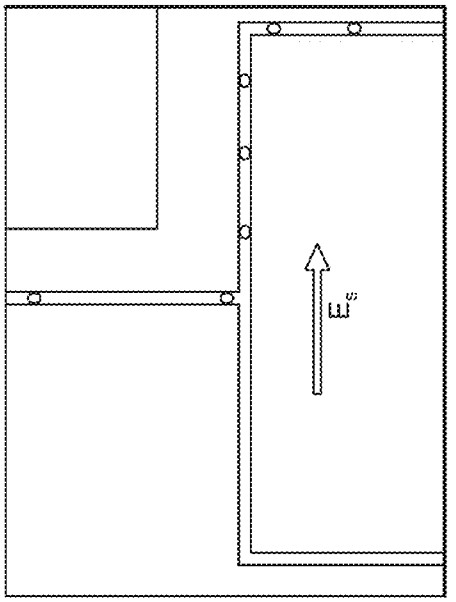


FIG. 24C

IN VITRO EVOLUTION IN MICROFLUIDIC SYSTEMS

The present application is a continuation application of U.S. Ser. No. 11/665,030, filed Apr. 14, 2009, which application is a 35 U.S.C. §371 National Phase Application of PCT/GB2005/003889, filed Oct. 10, 2005; which claims priority from U.S. Ser. No. 10/961,695 filed Oct. 8, 2004, each of which is incorporated by reference in its entirety.

The present invention relates to methods for use in in vitro evolution of molecular libraries. In particular, the present invention relates to methods of selecting nucleic acids encoding gene products in which the nucleic acid and the activity of the encoded gene product are linked by compartmentation, using microfluidic systems to create and/or handle the compartments.

Evolution requires the generation of genetic diversity (diversity in nucleic acid) followed by the selection of those nucleic acids which result in beneficial characteristics. Because the nucleic acid and the activity of the encoded gene product of an organism are physically linked (the nucleic acids being confined within the cells which they encode) multiple rounds of mutation and selection can result in the progressive survival of organisms with increasing fitness. Systems for rapid evolution of nucleic acids or proteins in vitro advantageously mimic this process at the molecular level in that the nucleic acid and the activity of the encoded gene product are linked and the activity of the gene product is selectable.

Recent advances in molecular biology have allowed some molecules to be co-selected according to their properties along with the nucleic acids that encode them. The selected nucleic acids can subsequently be cloned for further analysis or use, or subjected to additional rounds of mutation and selection.

Common to these methods is the establishment of large libraries of nucleic acids. Molecules having the desired characteristics (activity) can be isolated through selection regimes that select for the desired activity of the encoded gene product, such as a desired biochemical or biological activity, for example binding activity.

Phage display technology has been highly successful as providing a vehicle that allows for the selection of a displayed protein by providing the essential link between nucleic acid and the activity of the encoded gene product (Smith, 1985; Bass et al., 1990; McCafferty et al., 1990; for review see Clackson and Wells, 1994). Filamentous phage particles act as genetic display packages with proteins on the outside and the genetic elements which encode them on the inside. The tight linkage between nucleic acid and the activity of the encoded gene product is a result of the assembly of the phage within bacteria. As individual bacteria are rarely multiply infected, in most cases all the phage produced from an individual bacterium will carry the same genetic element and display the same protein.

However, phage display relies upon the creation of nucleic acid libraries in vivo in bacteria. Thus, the practical limitation on library size allowed by phage display technology is of the order of 10^7 to 10^{11}, even taking advantage of λ phage vectors with excisable filamentous phage replicons. The technique has mainly been applied to selection of molecules with binding activity. A small number of proteins with catalytic activity have also been isolated using this technique, however, selection was not directly for the desired catalytic activity, but either for binding to a transition-state analogue (Widersten and Mannervik, 1995) or reaction with a suicide inhibitor (Soumillion et al., 1994; Janda et al., 1997). More recently

there have been some examples of enzymes selected using phage-display by product formation (Atwell & Wells, 1999; Demartis et al., 1999; Jestin et al., 1999; Pederson, et al., 1998), but in all these cases selection was not for multiple turnover.

Specific peptide ligands have been selected for binding to receptors by affinity selection using large libraries of peptides linked to the C terminus of the lac repressor LacI (Cull et al, 1992). When expressed in *E. coli* the repressor protein physically links the ligand to the encoding plasmid by binding to a lac operator sequence on the plasmid.

An entirely in vitro polysome display system has also been reported (Mattheakis et al., 1994; Hanes and Pluckthun, 1997) in which nascent peptides are physically attached via the ribosome to the RNA which encodes them. An alternative, entirely in vitro system for linking genotype to phenotype by making RNA-peptide fusions (Roberts and Szostak, 1997; Nemoto et al., 1997) has also been described.

However, the scope of the above systems is limited to the selection of proteins and furthermore does not allow direct selection for activities other than binding, for example catalytic or regulatory activity.

In vitro RNA selection and evolution (Ellington and Szostak, 1990), sometimes referred to as SELEX (systematic evolution of ligands by exponential enrichment) (Tuerk and Gold, 1990) allows for selection for both binding and chemical activity, but only for nucleic acids. When selection is for binding, a pool of nucleic acids is incubated with immobilised substrate. Non-binders are washed away, then the binders are released, amplified and the whole process is repeated in iterative steps to enrich for better binding sequences. This method can also be adapted to allow isolation of catalytic RNA and DNA (Green and Szostak, 1992; for reviews see Chapman and Szostak, 1994; Joyce, 1994; Gold et al., 1995; Moore, 1995).

However, selection for "catalytic" or binding activity using SELEX is only possible because the same molecule performs the dual role of carrying the genetic information and being the catalyst or binding molecule (aptamer). When selection is for "auto-catalysis" the same molecule must also perform the third role of being a substrate. Since the genetic element must play the role of both the substrate and the catalyst, selection is only possible for single turnover events. Because the "catalyst" is in this process itself modified, it is by definition not a true catalyst. Additionally, proteins may not be selected using the SELEX procedure. The range of catalysts, substrates and reactions which can be selected is therefore severely limited.

Those of the above methods that allow for iterative rounds of mutation and selection are mimicking in vitro mechanisms usually ascribed to the process of evolution: iterative variation, progressive selection for a desired the activity and replication. However, none of the methods so far developed have provided molecules of comparable diversity and functional efficacy to those that are found naturally. Additionally, there are no man-made "evolution" systems which can evolve both nucleic acids and proteins to effect the full range of biochemical and biological activities (for example, binding, catalytic and regulatory activities) and that can combine several processes leading to a desired product or activity.

There is thus a great need for an in vitro system that overcomes the limitations discussed above.

In Tawfik and Griffiths (1998), and in International patent application PCT/GB98/01889, we describe a system for in vitro evolution that overcomes many of the limitations described above by using compartmentalisation in microcapsules to link genotype and phenotype at the molecular level.

In Tawfik and Griffiths (1998), and in several embodiments of International patent application WO9902671, the desired activity of a gene product results in a modification of the genetic element which encoded it (and is present in the same microcapsule). The modified genetic element can then be selected in a subsequent step.

Our subsequent international patent application WO0040712 describes a variation of this technology in which the modification of the genetic element causes a change in the optical properties of the element itself, and which has many advantages over the methods described previously.

The manipulation of fluids to form fluid streams of desired configuration, discontinuous fluid streams, droplets, particles, dispersions, etc., for purposes of fluid delivery, product manufacture, analysis, and the like, is a relatively well-studied art. For example, highly monodisperse gas bubbles, less than 100 microns in diameter, have been produced using a technique referred to as capillary flow focusing. In this technique, gas is forced out of a capillary tube into a bath of liquid, the tube is positioned above a small orifice, and the contraction flow of the external liquid through this orifice focuses the gas into a thin jet which subsequently breaks into equal-sized bubbles via a capillary instability. In a related technique, a similar arrangement was used to produce liquid droplets in air.

An article entitled "Generation of Steady Liquid Microthreads and Micron-Sized Monodisperse Sprays and Gas Streams," Phys. Rev. Lett., 80:2, Jan. 12, 1998, 285-288 (Ganan-Calvo) describes formation of a microscopic liquid thread by a laminar accelerating gas stream, giving rise to a fine spray.

An articled entitled "Dynamic Pattern Formation in a Vesicle-Generating Microfluidic Device," Phys. Rev. Lett., 86:18, Apr. 30, 2001 (Thorsen, et al.) describes formation of a discontinuous water phase in a continuous oil phase via microfluidic cross-flow, specifically, by introducing water, at a "T" junction between two microfluidic channels, into flowing oil.

U.S. Pat. No. 6,120,666, issued Sep. 19, 2000, describes a micofabricated device having a fluid focusing chamber for spatially confining first and second sample fluid streams for analysing microscopic particles in a fluid medium, for example in biological fluid analysis.

U.S. Pat. No. 6,116,516, issued Sep. 12, 2000, describes formation of a capillary microjet, and formation of a monodisperse aerosol via disassociation of the microjet.

U.S. Pat. No. 6,187,214, issued Feb. 13, 2001, describes atomised particles in a size range of from about 1 to about 5 microns, produced by the interaction of two immiscible fluids.

U.S. Pat. No. 6,248,378, issued Jun. 19, 2001, describes production of particles for introduction into food using a microjet and a monodisperse aerosol formed when the microjet dissociates.

Microfluidic systems have been described in a variety of contexts, typically in the context of miniaturised laboratory (e.g., clinical) analysis. Other uses have been described as well. For example, International Patent Publication No. WO 01/89789, published Nov. 29, 2001 by Anderson, et al., describes multi-level microfluidic systems that can be used to provide patterns of materials, such as biological materials and cells, on surfaces. Other publications describe microfluidic systems including valves, switches, and other components.

BRIEF DESCRIPTION OF THE INVENTION

According to a first aspect of the present invention, there is provided a method for isolating one or more genetic elements encoding a gene product having a desired activity, comprising the steps of:

(a) compartmentalising the genetic elements into microcapsules;

(b) sorting the genetic elements which express gene product (s) having the desired activity;

wherein at least one step is under microfluidic control.

In the method of the invention, a genetic element may be expressed to form its gene product before or after compartmentalisation; where the gene product is expressed before compartmentalisation, it is linked to the genetic element such that they are compartmentalised together.

Preferably, at least one step is performed using electronic control of fluidic species.

Advantageously at least one step involves fusion or splitting of microcapsules.

Methods for electronic control of fluidic species, as well as splitting (and fusing) of microcapsules under microfluidic control, are described herein.

Preferably, the method of the invention comprises the steps of:

(a) compartmentalising the genetic elements into microcapsules;

(c) expressing the genetic elements to produce their respective gene products within the microcapsules; and

(d) sorting the genetic elements which encode gene product (s) having the desired activity.

Alternatively, the method of the invention comprises the steps of:

(a) expressing the genetic elements to produce their respective gene products such that the gene products are linked to the genes encoding them;

(b) compartmentalising the genetic elements into microcapsules; and

(c) sorting the genetic elements which encode gene product(s) having the desired activity.

The microcapsules according to the present invention compartmentalise genetic elements and gene products such that they remain physically linked together.

As used herein, a genetic element is a molecule or molecular construct comprising a nucleic acid. The genetic elements of the present invention may comprise any nucleic acid (for example, DNA, RNA or any analogue, natural or artificial, thereof). The nucleic acid component of the genetic element may moreover be linked, covalently or non-covalently, to one or more molecules or structures, including proteins, chemical entities and groups, and solid-phase supports such as beads (including nonmagnetic, magnetic and paramagnetic beads), and the like. In the method of the invention, these structures or molecules can be designed to assist in the sorting and/or isolation of the genetic element encoding a gene product with the desired activity.

Expression, as used herein, is used in its broadest meaning, to signify that a nucleic acid contained in the genetic element is converted into its gene product. Thus, where the nucleic acid is DNA, expression refers to the transcription of the DNA into RNA; where this RNA codes for protein, expression may also refer to the translation of the RNA into protein. Where the nucleic acid is RNA, expression may refer to the replication of this RNA into further RNA copies, the reverse transcription of the RNA into DNA and optionally the transcription of this DNA into further RNA molecule(s), as well as optionally the translation of any of the RNA species produced into protein. Preferably, therefore, expression is performed by one or more processes selected from the group consisting of transcription, reverse transcription, replication and translation.

Expression of the genetic element may thus be directed into either DNA, RNA or protein, or a nucleic acid or protein containing unnatural bases or amino acids (the gene product)

within the microcapsule of the invention, so that the gene product is confined within the same microcapsule as the genetic element.

The genetic element and the gene product thereby encoded are linked by confining each genetic element and the respective gene product encoded by the genetic element within the same microcapsule. In this way the gene product in one microcapsule cannot cause a change in any other microcapsules. In addition, further linking means may be employed to link gene products to the genetic elements encoding them, as set forth below.

The term "microcapsule" is used herein in accordance with the meaning normally assigned thereto in the art and further described hereinbelow. In essence, however, a microcapsule is an artificial compartment whose delimiting borders restrict the exchange of the components of the molecular mechanisms described herein which allow the sorting of the genetic elements according to the function of the gene products which they encode.

Preferably, the microcapsules used in the method of the present invention will be capable of being produced in very large numbers, and thereby to compartmentalise a library of genetic elements which encodes a repertoire of gene products.

As used herein, a change in optical properties refers to any change in absorption or emission of electromagnetic radiation, including changes in absorbance, luminescence, phosphorescence or fluorescence. All such properties are included in the term "optical". Microcapsules and/or genetic elements can be sorted, for example, by luminescence, fluorescence or phosphorescence activated sorting. In a preferred embodiment, flow cytometry is employed to sort microcapsules and/or genetic elements, for example, light scattering (Kerker, 1983) and fluorescence polarisation (Rolland et al., 1985) can be used to trigger flow sorting. In a highly preferred embodiment genetic elements are sorted using a fluorescence activated cell sorter (FACS) sorter (Norman, 1980; Mackenzie and Pinder, 1986). Such a sorting device can be integrated directly on the microfluidic device, and can use electronic means to sort the microcapsules and/or genetic elements. Optical detection, also integrated directly on the microfluidic device, can be used to screen the microcapsules and/or genetic elements to trigger the sorting. Other means of control of the microcapsules and/or genetic elements, in addition to charge, can also be incorporated onto the microfluidic device.

Changes in optical properties may be direct or indirect. Thus, the change may result in the alteration of an optical property in the microcapsule or genetic element itself, or may lead indirectly to such a change. For example, modification of a genetic element may alter its ability to bind an optically active ligand, thus indirectly altering its optical properties.

Alternatively, imaging techniques can be used to screen thin films of genetic elements to allow enrichment for a genetic element with desirable properties, for example by physical isolation of the region where a genetic element with desirable properties is situated, or ablation of non-desired genetic elements. The genetic elements can be detected by luminescence, phosphorescence or fluorescence.

The sorting of genetic elements may be performed in one of essentially seven techniques.

(I) In a first embodiment, the microcapsules are sorted according to an activity of the gene product or derivative thereof which makes the microcapsule detectable as a whole. Accordingly, a gene product with the desired activity induces a change in the microcapsule, or a modification of one or more molecules within the microcapsule, which enables the microcapsule containing the gene product and the genetic element encoding it to be sorted. In this embodiment the microcapsules are physically sorted from each other according to the activity of the gene product(s) expressed from the genetic element(s) contained therein, which makes it possible selectively to enrich for microcapsules containing gene products of the desired activity.

(II) In a second embodiment, the genetic elements are sorted following pooling of the microcapsules into one or more common compartments. In this embodiment, a gene product having the desired activity modifies the genetic element which encoded it (and which resides in the same microcapsule) in such a way as to make it selectable in a subsequent step. The reactions are stopped and the microcapsules are then broken so that all the contents of the individual microcapsules are pooled. Selection for the modified genetic elements enables enrichment of the genetic elements encoding the gene product(s) having the desired activity. Accordingly, the gene product having the desired activity modifies the genetic element encoding it to enable the isolation of the genetic element. It is to be understood, of course, that modification may be direct, in that it is caused by the direct action of the gene product on the genetic element, or indirect, in which a series of reactions, one or more of which involve the gene product having the desired activity, leads to modification of the genetic element.

(III) In a third embodiment, the genetic elements are sorted following pooling of the microcapsules into one or more common compartments. In this embodiment, a gene with a desired activity induces a change in the microcapsule containing the gene product and the genetic element encoding it. This change, when detected, triggers the modification of the gene within the compartment. 'The reactions are stopped and the microcapsules are then broken so that all the contents of the individual microcapsules are pooled. Selection for the modified genetic elements enables enrichment of the genetic elements encoding the gene product(s) having the desired activity. Accordingly the gene product having the desired activity induces a change in the compartment which is detected and triggers the modification of the genetic element within the compartment so as to allow its isolation. It is to be understood that the detected change in the compartment may be caused by the direct action of the gene product, or indirect action, in which a series of reactions, one or more of which involve the gene product having the desired activity leads to the detected change.

(IV) In a fourth embodiment, the genetic elements may be sorted by a multi-step procedure, which involves at least two steps, for example, in order to allow the exposure of the genetic elements to conditions which permit at least two separate reactions to occur. As will be apparent to a persons skilled in the art, the first microencapsulation step of the invention must result in conditions which permit the expression of the genetic elements—be it transcription, transcription and/or translation, replication or the like. Under these conditions, it may not be possible to select for a particular gene product activity, for example because the gene product may not be active under these conditions, or because the expression system contains an interfering activity. The method therefore comprises expressing the genetic elements to produce their respective gene products within the microcapsules, linking the gene products to the genetic elements encoding them and isolating the complexes thereby formed. This allows for the genetic elements and their associated gene products to be isolated from the capsules before sorting according to gene product activity takes place. In a preferred embodiment, the complexes are subjected to a further compartmentalisation step prior to isolating the genetic elements

encoding a gene product having the desired activity. This further compartmentalisation step, which advantageously takes place in microcapsules, permits the performance of further reactions, under different conditions, in an environment where the genetic elements and their respective gene products are physically linked. Eventual sorting of genetic elements may be performed according to embodiment (I), (II) or (III) above.

Where the selection is for optical changes in the genetic elements, the selection may be performed as follows:

(V) In a fifth embodiment, the genetic elements are sorted following pooling of the microcapsules into one or more common compartments. In this embodiment, a gene product having the desired activity modifies the genetic element which encoded it (and which resides in the same microcapsule) so as to make it selectable as a result of its modified optical properties in a subsequent step. The reactions are stopped and the microcapsules are then broken so that all the contents of the individual microcapsules are pooled. The modification of the genetic element in the microcapsule may result directly in the modification of the optical properties of the genetic element. Alternatively, the modification may allow the genetic elements to be further modified outside the microcapsules so as to induce a change in their optical properties. Selection for the genetic elements with modified optical properties enables enrichment of the genetic elements encoding the gene product(s) having the desired activity. Accordingly, the gene product having the desired activity modifies the genetic element encoding it to enable the isolation of the genetic elenrient as a result in a change in the optical properties of the genetic element. It is to be understood, of course, that modification may be direct, in that it is caused by the direct action of the gene product on the genetic element, or indirect, in which a series of reactions, one or more of which involve the gene product having the desired activity, leads to modification of the genetic element.

(VI) In a sixth embodiment, the genetic elements may be sorted by a multi-step procedure, which involves at least two steps, for example, in order to allow the exposure of the genetic elements to conditions which permit at least two separate reactions to occur. As will be apparent to persons skilled in the art, the first microencapsulation step of the invention advantageously results in conditions which permit the expression of the genetic elements—be it transcription, transcription and/or translation, replication or the like. Under these conditions, it may not be possible to select for a particular gene product activity, for example because the gene product may not be active under these conditions, or because the expression system contains an interfering activity. The method therefore comprises expressing the genetic elements to produce their respective gene products within the microcapsules, linking the gene products to the genetic elements encoding them and isolating the complexes thereby formed. This allows for the genetic elements and their associated gene products to be isolated from the capsules before sorting according to gene product activity takes place. In a preferred embodiment, the complexes are subjected to a further compartmentalisation step prior to isolating the genetic elements encoding a gene product having the desired activity. This further compartmentalisation step, which advantageously takes place in microcapsules, permits the performance of further reactions, under different conditions, in an environment where the genetic elements and their respective gene products are physically linked. Eventual sorting of genetic elements may be performed according to embodiment (V) above.

The "secondary encapsulation" may also be performed with genetic elements linked to gene products by other means, such as by phage display, polysome display, RNA-peptide fusion or lac repressor peptide fusion, optionally where expression takes place prior to encapsulation; or even by the encapsulation of whole cells containing the desired genetic element.

The selected genetic element(s) may also be subjected to subsequent, possibly more stringent rounds of sorting in iteratively repeated steps, reapplying the method described above either in its entirety or in selected steps only. By tailoring the conditions appropriately, genetic elements encoding gene products having a better optimised activity may be isolated after each round of selection.

Additionally, the genetic elements isolated after a first round of sorting may be subjected to mutagenesis before repeating the sorting by iterative repetition of the steps of the method of the invention as set out above. After each round of mutagenesis, some genetic elements will have been modified in such a way that the activity of the gene products is enhanced.

Moreover, the selected genetic elements can be cloned into an expression vector to allow further characterisation of the genetic elements and their products.

(VII) In a seventh embodiment, the microcapsules may be sorted using microfluidic approaches. The microcapsules may be produced using microfluidic droplet formation techniques, such as those described herein, or by other techniques, for example conventional emulsification by forcing together two fluid phases. Sorting using microfluidics is applicable to embodiments I to VI above, and provides enhanced processing of microcapsules leading to improved sorting. Microcapsules may be split or fused according to methods described herein, or the contents thereof mixed. Moreover, the contents of the microcapsules may be analysed and the microcapsules sorted using detectors in microfluidic systems.

In a second aspect, the invention provides a product when selected according to the first aspect of the invention. As used in this context, a "product" may refer to a gene product, selectable according to the invention, or the genetic element (or genetic information comprised therein).

In a third aspect, the invention provides a method for preparing a gene product, the expression of which may result, directly or indirectly, in the modification the optical properties of a genetic element encoding it, comprising the steps of:

(a) preparing a genetic element encoding the gene product;
(b) compartmentalising genetic elements into microcapsules;
(c) expressing the genetic elements to produce their respective gene products within the microcapsules;
(d) sorting the genetic elements which produce the gene product(s) having the desired activity using the changed optical properties of the genetic elements; and
(e) expressing the gene product having the desired activity;
wherein one or both of steps (b) and (d) is performed under microfluidic control.

In accordance with the third aspect, step (a) preferably comprises preparing a repertoire of genetic elements, wherein each genetic element encodes a potentially differing gene product. Repertoires may be generated by conventional techniques, such as those employed for the generation of libraries intended for selection by methods such as phage display. Gene products having the desired activity may be selected from the repertoire, according to the present invention, according to their ability to modify the optical properties of the genetic elements in a manner which differs from that of other gene products. For example, desired gene products may

modify the optical properties to a greater extent than other gene products, or to a lesser extent, including not at all.

In a fourth aspect, the invention provides a method for screening a compound or compounds capable of modulation the activity of a gene product, the expression of which may result, directly or indirectly, in the modification of the optical properties of a genetic element encoding it, comprising the steps of:

(a) preparing a repertoire of genetic elements encoding gene product;

(b) compartmentalising genetic elements into microcapsules;

(c) expressing the genetic elements to produce their respective gene products within the microcapsules;

(d) sorting the genetic elements which produce the gene product(s) having the desired activity using the changed optical properties of the genetic elements; and

(e) contacting a gene product having the desired activity with the compound or compounds and monitoring the modulation of an activity of the gene product by the compound or compounds; wherein one or both of steps (b) and (d) is performed under microfluidic control.

Advantageously, the method further comprises the step of:

(f) identifying the compound or compounds capable of modulating the activity of the gene product and synthesising said compound or compounds.

This selection system can be configured to select for RNA, DNA or protein molecules with catalytic, regulatory or binding activity.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. **1**A and **1**B illustrate the splitting of droplets in accordance with one embodiment of the invention;

FIGS. **2**A and **2**B illustrate an apparatus in accordance with an embodiment of the invention, before the application of an electric field thereto;

FIGS. **3**A and **3**B illustrate the apparatus of FIGS. **2**A and **2**B after the application of an electric field thereto;

FIGS. **4**A and **4**B illustrate the apparatus of FIGS. **2**A and **2**B after the application of a reversed electric field thereto;

FIG. **5** is a schematic diagram of droplet splitting, in accordance with one embodiment of the invention;

FIGS. **6**A and **6**B are schematic diagrams of additional embodiments of the invention;

FIGS. **7**A and **7**B are schematic diagrams of the-formation of microfluidic droplets in accordance with the present invention;

FIGS. **8**A-F illustrate the splitting of droplets in accordance with the invention;

FIGS. **9**A-D illustrate the induction of dipoles in droplets in accordance with the invention;

FIGS. **10**A-D illustrate the sorting of microcapsules by altering the flow of carrier fluid in a microfluidic system;

FIGS. **11**A-C illustrate the use of pressure changes in the microfluidic system to control 10 the direction of flow of droplets;

FIGS. **12**A-J illustrate flow patterns for droplets in microfluidic systems in accordance with the invention;

FIGS. **13**A-D illustrate the use of oppositely charged droplets in the invention;

FIGS. **14**A-C are illustrations of the formation and maintenance of microfluidic droplets using three immiscible liquids;

FIGS. **15**A-B: Directed evolution of enzymes using microdroplets in a microfluidic system. FIG. **15**A: schematic of the core system. FIG. **15**B: process block diagram showing the modules in the core system. Libraries of mutated enzyme genes are encapsulated in aqueous microdroplets (FIG. **16**A) such that, statistically, the majority of droplets contain no more than one gene per droplet. Each of these microdroplets is fused with a second microdroplet (FIG. **16**C) containing an in vitro translation system. After allowing time for the genes to be translated into protein each microdroplet is fused with another microdroplet containing an inhibitor of translation (puromycin) and a fluorogenic enzyme substrate. The rate of the enzymatic reaction is determined by measuring the fluorescence of each microdroplet, ideally at multiple points (corresponding to different times). Microdroplets with catalytic rates over a desired threshold value (e.g. the fastest 1%) will be sorted (FIG. **16**D) and collected and the genes contained therein amplified using the polymerase chain reaction (PCR). The selected genes will then either be characterised, re-selected directly, or first re-mutated randomly, or recombined before re-selection.

FIGS. **16**A-D: Examples of microdroplet formation and manipulation using microfluidics. FIG. **16**A: microdroplets can be created at up to 10^4 sec^1 by hydrodynamic-focussing (top two panels) and show <1.5% polydispersity (bottom panel). FIG. **16**B: microdroplets can be split symmetrically or asymmetrically. FIG. **16**C: microdroplets carrying positive (+q) and negative (−q) electrical charges fuse spontaneously. FIG. **16**D: charged microdroplets can also be steered using an applied electrical field (E).

FIGS. **17**A-F: Charged droplet generation. (FIG. **17**A), Oil and water streams converge at a 30 micron orifice. A voltage V applied to indium-tin-oxide (ITO) electrodes on the glass produces an electric field E to capacitively charges the aqueous-oil interface. Drop size is independent of charge at low field strengths but decreases at higher fields, as shown in the photomicrographs, [(FIG. **17**B) V=0, (FIG. **17**C) V=400, (FIG. **17**D) V=600 and (FIG. **17**E) V=800] at higher fields. (FIG. **17**F) Droplet size as a function of voltage showing the crossover between flow-dominated and field-dominated snap-off for three different flow rates of the continuous phase oil (Q_c,=80 nL/s, 110 nL/s, and 140 nL/s). The infusion rate of the water is constant Q_d=20 nL/s.}

FIGS. **18**A-D: Coalescing drops. (FIG. **18**A) Drops having opposite sign of electrostatic charge can be generated by applying a voltage across the two aqueous streams. (FIG. **18**B) In the absence of the field the frequency and timing of drop formation at the two nozzles are independent and each nozzle produces a different size drop at a different frequency; infusion rates are the same at both nozzles. After the confluence of the two streams, drops from the upper and lower nozzles stay in their respective halves of the stream and due to surfactant there are no coalescence events even in the case of large slugs that fill the channel width. (FIG. **18**C) With an applied voltage of 200V across the 500 micron separation of the nozzles, the drops simultaneously break-off from the two nozzles and are identical; simultaneous drop formation can be achieved for unequal infusion rates of the aqueous streams even up to a factor of two difference in volumes. (FIG. **18**D) The fraction of the drops that encounter each other and coalesce increases linearly above a critical field when a surfactant, sorbiton-monooleate 3% is present.

FIGS. **19**A-B: Droplets carrying a pH sensitive dye coalesce with droplets of a different pH fluid. Chaotic advection rapidly mixes the two fluids through a combination of translation and rotation as the droplets pass around corners.

FIGS. **20**A-I: Diffusion limited and rapid mixing strategies. (FIG. **20**A) Drops meet and coalesce along the direction of E and then move off in a perpendicular direction, as sketched the counter rotating vortices after coalescence do

not mix the two fluid parts as each vortex contains a single material. (FIG. **20**B) As the drops approach each other the increasing field causes there interfaces to deform and (FIG. **20**C) a bridge to jump out connecting the drops, to create (FIG. **20**D) in the case of 20 nm silica particles and MgCl_2 a sharp interface where the particles begin to gel. (FIG. **20**E) A typical unmixed droplet with particles in one hemisphere. (FIG. **20**F) To achieve fast mixing, droplets are brought together in the direction perpendicular to the electric field and move off in the direction parallel to the direction they merged along. Counter rotating vortexes are then created where each vortex is composed of half of the contentes from each of the premerger-droplets. (FIG. **20**G) Shows a pH sensitive dye in the lower drop and a different pH fluid in the upper droplet. (FIG. **20**H) After merger the droplets are split by a sharp line. (FIG. **20**I) A uniform intensity indicating that mixing has been occurred is achieved in the droplet after it translates one diameter, typically this takes 1 to 2 ms.

FIGS. **21**A-B: Time delay reaction module. (FIG. **21**A) Droplets of perfluorodecaline alternate with aqueous droplets in a hexadecane carrier fluid. The 'single-file' ordering of the droplets provides for long delays with essentially no deviation in the precise spacing of aqueous droplets or droplet order. (FIG. **21**B) Increasing the width and height of the channel to create a 'large cross-sectional area' channel provides for extremely long time delays from minutes to hours. The exact ordering and spacing between the droplets is not maintained in this type of delay line.

FIGS. **22**A-C: Recharging neutral drops. (FIG. **22**A) Schematic to recharge neutral drops by breaking them in the presence of an electric field. Uncharged drops ($q=0$) are polarized in an electric field ($E_s \neq 0$), and provided E_s is sufficiently large, as shown in the photomicrograph of (FIG. **22**B), they break into two oppositely charged daughter .drops in the extensional flow at a bifurcation. The enlargement of the dashed rectangle, shown in (FIG. **22**C), reveals that the charged drops are stretched in the electric field E_s but return to spherical on contacting the electrodes indicated by dashed vertical lines.

FIG. **23**: Detection module. One or more lasers are coupled to an optical fibre that is used to excite the fluorescence in each droplet as it passes over the fibre. The fluorescence is collected by the same fibre and dichroic beam splitters separate off specific wavelengths of the fluorescent light and the intensity of the fluorescence is measured with a photomultiplier tube (PMT) after the light passes through a band-pass filter.

FIGS. **24**A-D: Manipulating charged drops. In (FIG. **24**A) charged drops alternately enter the right and left channels when there is no field applied ($E_s=0$). The sketch in (FIG. **24**B) shows the layout for using an electric field E_s to select the channel charged drops will enter at a bifurcation. When an electric field is applied to the right (FIG. **24**C), the drops enter the right branch at the bifurcation; they enter the left branch when the field is reversed (FIG. **24**D). After the bifurcation, the distance between drops is reduced to half what it was before indicating the oil stream is evenly divided. The inset of (FIG. **24**D) shows the deformation in the shape of a highly charged drop in an electric field.

DEFINITIONS

As used herein, "or" is understood to mean "inclusively or," i.e., the inclusion of at least one, but including more than one, of a number or list of elements. In contrast, the term "exclusively or" refers to the inclusion of exactly one element of a number or list of elements.

The indefinite articles "a" and "an," as used herein in the specification and in the claims, should be understood to mean "at least one."

The term "about," as used herein in reference to a numerical parameter (for example, a physical, chemical, electrical, or biological property), will be understood by those of ordinary skill in the art to be an approximation of a numerical value, the exact value of which may be subject to errors such as those resulting from measurement errors of the numerical parameter, uncertainties resulting from the variability and/or reproducibility of the numerical parameter (for example, in separate experiments), and the like.

The term "microcapsule" is used herein in accordance with the meaning normally assigned thereto in the art and further described hereinbelow. In essence, however, a microcapsule is an artificial compartment whose delimiting borders restrict the exchange of the components of the molecular mechanisms described herein which allow the identification of the molecule with the desired activity. The delimiting borders preferably completely enclose the contents of the microcapsule. Preferably, the microcapsules used in the method of the present invention will be capable of being produced in very large numbers, and thereby to compartmentalise a library of genetic elements. Optionally, the genetic elements can comprise genes attached to microbeads. The microcapsules used herein allow mixing and sorting to be performed thereon, in order to facilitate the high throughput potential of the methods of the invention. A microcapsule can be a droplet of one fluid in a different fluid, where the confined components are soluble in the droplet but not in the carrier fluid. In another embodiment there is a third material defining a wall, such as a membrane.

Arrays of liquid droplets on solid surfaces, multiwell plates and "plugs" in microfluidic systems, that is fluid droplets that are not completely surrounded by a second fluid as defined herein, are not microcapsules as defined herein.

The term "microbead" is used herein in accordance with the meaning normally assigned thereto in the art and further described hereinbelow. Microbeads, are also known by those skilled in the art as microspheres, latex particles, beads, or minibeads, are available in diameters from 20 nm to 1 mm and can be made from a variety of materials including silica and a variety of polymers, copolymers and terpolymers. Highly uniform derivatised and non-derivatised nonmagnetic and paramagnetic microparticles (beads) are commercially available from many sources (e.g. Sigma, Bangs Laboratories, Luminex and Molecular Probes) (Formusek and Vetvicka, 1986).

Microbeads can be "compartmentalised" in accordance with the present invention by distribution into microcapsules. For example, in a preferred aspect the microbeads can be placed in a water/oil mixture and emulsified to form a water-in-oil emulsion comprising microcapsules according to the invention. The concentration of the microbeads can be adjusted such that a single microbead, on average, appears in each microcapsule.

As used herein, the "target" is any compound, molecule, or supramolecular complex. Typical targets include targets of medical significance, including drug targets such as receptors, for example G protein coupled receptors and hormone receptors; transcription factors, protein kinases and phosphatases involved in signalling pathways; gene products specific to microorganisms, such as components of cell walls, replicases and other enzymes; industrially relevant targets, such as enzymes used in the food industry, reagents intended for research or production purposes, and the like.

A "desired activity", as referred to herein, is the modulation of any activity of a target, or an activity of a molecule which is influenced by the target, which is modulatable directly or indirectly by a genetic element or genetic elements as assayed herein. The activity of the target may be any measurable biological or chemical activity, including binding activity, an enzymatic activity, an activating or inhibitory activity on a third enzyme or other molecule, the ability to cause disease or influence metabolism or other functions, and the like. Activation and inhibition, as referred to herein, denote the increase or decrease of a desired activity 1.5 fold, 2 fold, 3 fold, 4 fold, 5 fold, 10 fold, 100 fold or more. Where the modulation is inactivation, the inactivation can be substantially complete inactivation. The desired activity may moreover be purely a binding activity, which may or may not involve the modulation of the activity of the target bound to.

A compound defined herein as "low molecular weight" or a "small molecule" is a molecule commonly referred to in the pharmaceutical arts as a "small molecule". Such compounds are smaller than polypeptides and other, large molecular complexes and can be easily administered to and assimilated by patients and other subjects. Small molecule drugs can advantageously be formulated for oral administration or intramuscular injection. For example, a small molecule may have a molecular weight of up to 2000 Dalton; preferably up to 1000 Dalton; advantageously between 250 and 750 Dalton; and more preferably less than 500 Dalton.

A "selectable change" is any change which can be measured and acted upon to identify or isolate the genetic element which causes it. The selection may take place at the level of the micro capsule, the microbead, or the genetic element itself, optionally when complexed with another reagent. A particularly advantageous embodiment is optical detection, in which the selectable change is a change in optical properties, which can be detected arid acted upon for instance in a flow sorting device to separate microcapsules or microbeads displaying the desired change.

As used herein, a change in optical properties refers to any change in absorption or emission of electromagnetic radiation, including changes in absorbance, luminescence, phosphorescence or fluorescence. All such properties are included in the term "optical". Microcapsules or microbeads can be identified and, optionally, sorted, for example, by luminescence, fluorescence or phosphorescence activated sorting. In a preferred embodiment, flow sorting is employed to identify and, optionally, sort microcapsules or microbeads. A variety of optical properties can be used for analysis and to trigger sorting, including light scattering (Kerker, 1983) and fluorescence polarisation (Rolland et al., 1985).

The genetic elements in microcapsules or on beads can be identified using a variety of techniques familiar to those skilled in the art, including mass spectroscopy, chemical tagging or optical tagging.

As used herein, "microfluidic control" refers to the use of a microfluidic system comprising microfluidic channels as defined herein to direct or otherwise control the formation and/or movement of microcapsules (or "droplets") in order to carry out the methods of the present invention. For example, "microfluidic control" of microcapsule formation refers to the creation of microcapsules using a microfluidic device to form "droplets" of fluid within a second fluid, thus creating a microcapsule. Microcapsules sorted under microfluidic control are sorted, as described herein, using a microfluidic device to perform one or more of the functions associated with the sorting procedure. "Microfluidic control of fluidic species", therefore, refers to the handling of fluids in a microfluidic system as defined in order to carry out the methods of the present invention.

As used herein, a "cell" is given its ordinary meaning as used in biology. The cell may be any cell or cell type. For example, the cell may be a bacterium or other single-cell organism, a plant cell, or an animal cell. If the cell is a single-cell organism, then the cell may be, for example, a protozoan, a trypanosome, an amoeba, a yeast cell, algae, etc. If the cell is an animal cell, the cell may be, for example, an invertebrate cell (e.g., a cell from a fruit fly), a fish cell (e.g., a zebrafish cell), an amphibian cell (e.g., a frog cell), a reptile cell, a bird cell, or a mammalian cell such as a primate cell, a bovine cell, a horse cell, a porcine cell, a goat cell, a dog cell, a cat cell, or a cell from a rodent such as a rat or a mouse. If the cell is from a multicellular organism, the cell may be from any part of the organism. For instance, if the cell is from an animal, the cell may be a cardiac cell, a fibroblast, a keratinocyte, a heptaocyte, a chondrocyte, a neural cell, a osteocyte, a muscle cell, a blood cell, an endothelial cell, an immune cell (e.g., a T-cell, a B-cell, a macrophage, a neutrophil, a basophil, a mast cell, an eosinophil), a stem cell, etc. In some cases, the cell may be a genetically engineered cell. In certain embodiments, the cell may be a Chinese hamster ovarian ("CHO") cell or a 3T3 cell.

"Microfluidic," as used herein, refers to a device, apparatus or system including at least one fluid channel having a cross-sectional dimension of less than 1 mm, and a ratio of length to largest cross-sectional dimension of at least 3:1. A "microfluidic channel," as used herein, is a channel meeting these criteria.

The "cross-sectional dimension" of the channel is measured perpendicular to the direction of fluid flow. Most fluid channels in components of the invention have maximum cross-sectional dimensions less than 2 mm, and in some cases, less than 1 mm. In one set of embodiments, all fluid channels containing embodiments of the invention are microfluidic or have a largest cross sectional dimension of no more than 2 mm or 1 mm. In another embodiment, the fluid channels may be formed in part by a single component (e.g. an etched substrate or moulded unit). Of course, larger channels, tubes, chambers, reservoirs, etc. can be used to store fluids in bulk and to deliver fluids to components of the invention. In one set of embodiments, the maximum cross-sectional dimension of the channel(s) containing embodiments of the invention are less than 500 microns, less than 200 microns, less than 100 microns, less than 50 microns, or less than 25 microns.

A "channel," as used herein, means a feature on or in an article (substrate) that at least partially directs the flow of a fluid. The channel can have any cross-sectional shape (circular, oval, triangular, irregular, square or rectangular, or the like) and can be covered or uncovered. In embodiments where it is completely covered, at least one portion of the channel can have a cross-section that is completely enclosed, or the entire channel may be completely enclosed along its entire length with the exception of its inlet(s) and outlet(s). A channel may also have an aspect ratio (length to average cross sectional dimension) of at least 2:1, more typically at least 3:1, 5:1, or 10:1 or more. An open channel generally will include characteristics that facilitate control over fluid transport, e.g., structural characteristics (an elongated indentation) and/or physical or chemical characteristics (hydrophobicity vs. hydrophilicity) or other characteristics that can exert a force (e.g., a containing force) on a fluid. The fluid within the channel may partially or completely fill the channel. In some

cases where an open channel is used, the fluid may be held within the channel, for example, using surface tension (i.e., a concave or convex meniscus).

The channel may be of any size, for example, having a largest dimension perpendicular to fluid flow of less than about 5 mm or 2 mm, or less than about 1 mm, or less than about 500 microns, less than about 200 microns, less than about 100 microns, less than about 60 microns, less than about 50 microns, less than about 40 microns, less than about 30 microns, less than about 25 microns, less than about 10 microns, less than about 3 microns, less than about 1 micron, less than about 300 nm, less than about 100 nm, less than about 30 nm, or less than about 10 nm. In some cases the dimensions of the channel may be chosen such that fluid is able to freely flow through the article or substrate. The dimensions of the channel may also be chosen, for example, to allow a certain volumetric or linear flowrate of fluid in the channel. Of course, the number of channels and the shape of the channels can be varied by any method known to those of ordinary skill in the art. In some cases, more than one channel or capillary may be used. For example, two or more channels may be used, where they are positioned inside each other, positioned adjacent to each other, positioned to intersect with each other, etc.

As used herein, "integral" means that portions of components are joined in such a way that they cannot be separated from each other without cutting or breaking the components from each other.

A "droplet," as used herein is an isolated portion of a first fluid that is completely surrounded by a second fluid. It is to be noted that a droplet is not necessarily spherical, but may assume other shapes as well, for example, depending on the external environment. In one embodiment, the droplet has a minimum cross-sectional dimension that is substantially equal to the largest dimension of the channel perpendicular to fluid flow in which the droplet is located.

The "average diameter" of a population of droplets is the arithmetic average of the diameters of the droplets. Those of ordinary skill in the art will be able to determine the average diameter of a population of droplets, for example, using laser light scattering or other known techniques. The diameter of a droplet, in a non-spherical droplet, is the mathematically-defined average diameter of the droplet, integrated across the entire surface. As non-limiting examples, the average diameter of a droplet may be less than about 1 mm, less than about 500 micrometers, less than about 200 micrometers, less than about 100 micrometers, less than about 75 micrometers, less than about 50 micrometers, less than about 25 micrometers, less than about 10 micrometers, or less than about 5 micrometers. The average diameter of the droplet may also be at least about 1 micrometer, at least about 2 micrometers, at least about 3 micrometers, at least about 5 micrometers, at least about 10 micrometers, at least about 15 micrometers, or at least about 20 micrometers in certain cases.

As used herein, a "fluid" is given its ordinary meaning, i.e., a liquid or a gas. Preferably, a fluid is a liquid. The fluid may have any suitable viscosity that permits flow. If two or more fluids are present, each fluid may be independently selected among essentially any fluids (liquids, gases, and the like) by those of ordinary skill in the art, by considering the relationship between the fluids. The fluids may each be miscible or immiscible. For example, two fluids can be selected to be immiscible within the time frame of formation of a stream of fluids, or within the time frame of reaction or interaction. Where the portions remain liquid for a significant period of time then the fluids should be significantly immiscible. Where, after contact and/or formation, the dispersed portions are quickly hardened by polymerisation or the like, the fluids need not be as immiscible. Those of ordinary skill in the art can select suitable miscible or immiscible fluids, using contact angle measurements or the like, to carry out the techniques of the invention.

As used herein, a first entity is "surrounded" by a second entity if a closed loop can be drawn around the first entity through only the second entity. A first entity is "completely surrounded" if closed loops going through only the second entity can be drawn around the first entity regardless of direction. In one aspect, the first entity may be a cell, for example, a cell suspended in media is surrounded by the media. In another aspect, the first entity is a particle. In yet another aspect of the invention, the entities can both be fluids. For example, a hydrophilic liquid may be suspended in a hydrophobic liquid, a hydrophobic liquid may be suspended in a hydrophilic liquid, a gas bubble may be suspended in a liquid, etc. Typically, a hydrophobic liquid and a hydrophilic liquid are substantially immiscible with respect to each other, where the hydrophilic liquid has a greater affinity to water than does the hydrophobic liquid. Examples of hydrophilic liquids include, but are not limited to, water and other aqueous solutions comprising water, such as cell or biological media, ethanol, salt solutions, etc. Examples of hydrophobic liquids include, but are not limited to, oils such as hydrocarbons, silicon oils, fluorocarbon oils, organic solvents etc.

The term "determining," as used herein, generally refers to the analysis or measurement of a species, for example, quantitatively or qualitatively, or the detection of the presence or absence of the species. "Determining" may also refer to the analysis or measurement of an interaction between two or more species, for example, quantitatively or qualitatively, or by detecting the presence or absence of the interaction. Example techniques include, but are not limited to, spectroscopy such as infrared, absorption, fluorescence, UV/visible, FTIR ("Fourier Transform Infrared Spectroscopy"), or Raman; gravimetric techniques; ellipsometry; piezoelectric measurements; immunoassays; electrochemical measurements; optical measurements such as optical density measurements; circular dichroism; light scattering measurements such as quasielectric light scattering; polarimetry; refractometry; or turbidity measurements.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art (e.g., in cell culture, molecular genetics, nucleic acid chemistry, hybridisation techniques and biochemistry). Standard techniques are used for molecular, genetic and biochemical methods (see generally, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. and Ausubel et al., Short Protocols in Molecular Biology (1999) 4th Ed, John Wiley & Sons, Inc. which are incorporated herein by reference) and chemical methods. In addition Harlow & Lane, A Laboratory Manual Cold Spring Harbor, N.Y. is referred to for standard Immunological Techniques.

(A) General Description

The microcapsules of the present invention require appropriate physical properties to allow the working of the invention.

First, to ensure that the genetic elements and gene products may not diffuse between microcapsules, the contents of each microcapsule are preferably isolated from the contents of the surrounding microcapsules, so that there is no or little exchange of the genetic elements and gene products between the microcapsules over the timescale of the experiment. How-

ever, the permeability of the microcapsules may be adjusted such that reagents may be allowed to diffuse into and/or out of the microcapsules if desired.

Second, the method of the present invention requires that there are only a limited number of genetic elements per microcapsule. This ensures that the gene product of an individual genetic element will be isolated from other genetic elements. Thus, coupling between genetic element and gene product will be highly specific. The enrichment factor is greatest with on average one or fewer genetic elements per microcapsule, the linkage between nucleic acid and the activity of the encoded gene product being as tight as is possible, since the gene product of an individual genetic element will be isolated from the products of all other genetic elements. However, even if the theoretically optimal situation of, on average, a single genetic element or less per microcapsule is not used, a ratio of 5, 10, 50, 100 or 1000 or more genetic elements per microcapsule may prove beneficial in sorting a large library. Subsequent rounds of sorting, including renewed encapsulation with differing genetic element distribution, will permit more stringent sorting of the genetic elements. Preferably, there is a single genetic element, or fewer, per microcapsule.

Third, the formation and the composition of the microcapsules advantageously does not abolish the function of the machinery the expression of the genetic elements and the activity of the gene products.

Consequently, any microencapsulation system used preferably fulfils these three requirements. The appropriate system(s) may vary depending on the precise nature of the requirements in each application of the invention, as will be apparent to the skilled person.

A wide variety of microencapsulation procedures are available (see Benita, 1996) and may be used to create the microcapsules used in accordance with the present invention.

Indeed, more than 200 microencapsulation methods have been identified in the literature (Finch, 1993).

Enzyme-catalysed biochemical reactions have also been demonstrated in microcapsules generated by a variety of other methods. Many enzymes are active in reverse micellar solutions (Bru & Walde, 1991; Bru & Walde, 1993; Creagh et al., 1993; Haber et al., 1993; Kumar et al., 1989; Luisi & B., 1987; Mao & Walde, 1991; Mao et al., 1992; Perez et al., 1992; Walde et al., 1994; Walde et al., 1993; Walde et al., 1988) such as the AOT-isooctane-water system (Menger & Yamada, 1979).

Microcapsules can also be generated by interfacial polymerisation and interfacial complexation (Whateley, 1996). Microcapsules of this sort can have rigid, nonpermeable membranes, or semipermeable membranes. Semipermeable microcapsules bordered by cellulose nitrate membranes, polyamide membranes and lipid-polyamide membranes can all support biochemical reactions, including multienzyme systems (Chang, 1987; Chang, 1992; Lim, 1984). Alginate/polylysine microcapsules (Lim & Sun, 1980), which can be formed under very mild conditions, have also proven to be very biocompatible, providing, for example, an effective method of encapsulating living cells and tissues (Chang, 1992; Sun et al., 1992).

Non-membranous microencapsulation systems based on phase partitioning of an aqueous environment in a colloidal system, such as an emulsion, may also be used.

Preferably, the microcapsules of the present invention are formed from emulsions; heterogeneous systems of two immiscible liquid phases with one of the phases dispersed in the other as droplets of microscopic or colloidal size (Becher, 1957; Sherman, 1968; Lissant, 1974; Lissant, 1984).

Emulsions may be produced from any suitable combination of immiscible liquids. Preferably the emulsion of the present invention has "water" (an aqueous liquid containing the biochemical components) as the phase present in the form of finely divided droplets (the disperse, internal or discontinuous phase) and a hydrophobic, immiscible liquid (an 'oil') as the matrix in which these droplets are suspended (the nondisperse, continuous or external phase). Such emulsions are termed 'water-in-oil' (W/O). This has the advantage that the entire aqueous phase containing the biochemical components is compartmentalised in discreet droplets (the internal phase). The external phase, being a hydrophobic liquid, generally contains none of the biochemical components and hence is inert.

The emulsion may be stabilised by addition of one or more surface-active agents (surfactants). These surfactants are termed emulsifying agents and act at the water/oil interface to prevent (or at least delay) separation of the phases. Many oils and many emulsifiers can be used for the generation of water-in-oil emulsions; a recent compilation listed over 16,000 surfactants, many of which are used as emulsifying agents (Ash and Ash, 1993). Suitable oils include light white mineral oil and decane. Suitable surfactants include: non-ionic surfactants (Schick, 1966) such as sorbitan monooleate (Span™ 80; ICI), sorbitan monosteatate (Span™ 60; ICI), polyoxyethylenesorbitan monooleate (Tween™ 80; ICI), and octylphenoxyethoxyethanol (Triton X-100); ionic surfactants such as sodium cholate and sodium taurocholate and sodium deoxycholate; chemically inert silicone-based surfactants such as polysiloxane-polycetyl-polyethylene glycol copolymer (Cetyl Dimethicone Copolyol) (e.g. Abi™ EM90; Goldschmidt); and cholesterol.

Emulsions with a fluorocarbon (or perfluorocarbon) continuous phase (Krafft et al., 2003; Riess, 2002) may be particularly advantageous. For example, stable water-in-perfluorooctyl bromide and water-in-perfluorooctylethane emulsions can be formed using F-alkyl dimorpholinophosphates as surfactants (Sadtler et al., 1996). Non-fluorinated compounds are essentially insoluble in fluorocarbons and perfluorocarbons (Curran, 1998; Hildebrand and Cochran, 1949; Hudlicky, 1992; Scott, 1948; Studer et al., 1997) and small drug-like molecules (typically <500 Da and Log P<5) (Lipinski et al., 2001) are compartmentalised very effectively in the aqueous microcapsules of water-in-fluorocarbon and water-in-perfluorocarbon emulsions—with little or no exchange between microcapsules.

Creation of an emulsion generally requires the application of mechanical energy to force the phases together. There are a variety of ways of doing this which utilise a variety of mechanical devices, including stirrers (such as magnetic stirbars, propeller and turbine stirrers, paddle devices and whisks), homogenisers (including rotor-stator homogenisers, high-pressure valve homogenisers and jet homogenisers), colloid mills, ultrasound and 'membrane emulsification' devices (Becher, 1957; Dickinson, 1994), and microfluidic devices (Umbanhowar et al., 2000).

Complicated biochemical processes, notably gene transcription and translation are also active in aqueous microcapsules formed in water-in-oil emulsions. This has enabled compartmentalisation in water-in-oil emulsions to be used for the selection of genes, which are transcribed and translated in emulsion microcapsules and selected by the binding or catalytic activities of the proteins they encode (Doi and Yanagawa, 1999; Griffiths and Tawfik, 2003; Lee et al., 2002; Sepp et al., 2002; Tawfik and Griffiths, 1998). This was possible because the aqueous microcapsules formed in the emulsion

were generally stable with little if any exchange of nucleic acids, proteins, or the products of enzyme catalysed reactions between microcapsules.

The technology exists to create emulsions with volumes all the way up to industrial scales of thousands of liters (Becher, 1957; Sherman, 1968; Lissant, 1974; Lissant, 1984).

The preferred microcapsule size will vary depending upon the precise requirements of any individual selection process that is to be performed according to the present invention. In all cases, there will be an optimal balance between gene library size, the required enrichment and the required concentration of components in the individual microcapsules to achieve efficient expression and reactivity of the gene products.

The processes of expression occurs within each individual microcapsule provided by the present invention. Both in vitro transcription and coupled transcription-translation become less efficient at sub-nanomolar DNA concentrations. Because of the requirement for only a limited number of DNA molecules to be present in each microcapsule, this therefore sets a practical upper limit on the possible microcapsule size. Preferably, the mean volume of the microcapsules is less that 5.2×10^{-16} m^3, (corresponding to a spherical microcapsule of diameter less than 10 μm, more preferably less than 6.5×10^{-17} m^3 (5 μm diameter), more preferably about 4.2×10^{-18} m^3 (2 μm diameter) and ideally about 9×10^{-18} m^3 (2.6 μm diameter).

The effective DNA or RNA concentration in the microcapsules may be artificially increased by various methods that will be well-known to those versed in the art. These include, for example, the addition of volume excluding chemicals such as polyethylene glycols (PEG) and a variety of gene amplification techniques, including transcription using RNA polymerases including those from bacteria such as *E. coli* (Roberts, 1969; Blattner and Dahlberg, 1972; Roberts et al., 1975; Rosenberg et al., 1975), eukaryotes e.g. (Weil et al., 1979; Manley et al, 1983) and bacteriophage such as T7, T3 and SP6 (Melton et al., 1984); the polymerase chain reaction (PCR) (Saiki et al., 1988); Qb replicase amplification (Miele et al., 1983; Cahill et al., 1991; Chetverin and Spirin, 1995; Katanaev et al., 1995); the ligase chain reaction (LCR) (Landegren et al, 1988; Barany, 1991); and self-sustained sequence replication system (Fahy et al., 1991) and strand displacement amplification (Walker et al, 1992). Gene amplification techniques requiring thermal cycling such as PCR and LCR may be used if the emulsions and the in vitro transcription or coupled transcription-translation systems are thermostable (for example, the coupled transcription-translation systems can be made from a thermostable organism such as *Thermus aquaticus*).

Increasing the effective local nucleic acid concentration enables larger microcapsules to be used effectively. This allows a preferred practical upper limit to the microcapsule volume of about 5.2×10^{-16} m^3 (corresponding to a sphere of diameter 10 μm).

The microcapsule size is preferably sufficiently large to accommodate all of the required components of the biochemical reactions that are needed to occur within the microcapsule. For example, in vitro, both transcription reactions and coupled transcription-translation reactions require a total nucleoside triphosphate concentration of about 2 mM.

For example, in order to transcribe a gene to a single short RNA molecule of 500 bases in length, this would require a minimum of 500 molecules of nucleoside triphosphate per microcapsule (8.33×10^{-22} moles). In order to constitute a 2 mM solution, this number of molecules is contained within a microcapsule of volume 4.17×10^{-19} liters (4.17×10^{-22} m^3 which if spherical would have a diameter of 93 nm.

Furthermore, particularly in the case of reactions involving translation, it is to be noted that the ribosomes necessary for the translation to occur are themselves approximately 20 nm in diameter. Hence, the preferred lower limit for microcapsules is a diameter of approximately 0.1 μm (100 nm).

Therefore, the microcapsule volume is preferably of the order of between 5.2×10^{-22} m^3 and 5.2×10^{-16} m^3 corresponding to a sphere of diameter between 0.1 μm and 10 μm, more preferably of between about 5.2×10^{-19} m^3 and 6.5×10^{-17} m^3 (1 μm and 5 μm). Sphere diameters of about 2.6 μm are most advantageous.

It is no coincidence that the preferred dimensions of the compartments (droplets of 2.6 μm mean diameter) closely resemble those of bacteria, for example, *Escherichia* are 1.1–1.5×2.0-6.0 μm rods and *Azotobacter* are 1.5-2.0 1. μm diameter ovoid cells. In its simplest form, Darwinian evolution is based on a 'one genotype one phenotype' mechanism. The concentration of a single compartmentalised gene, or genome, drops from 0.4 nM in a compartment of 2 μm diameter, to 25 pM in a compartment of 5 μm diameter. The prokaryotic transcription/translation machinery has evolved to operate in compartments of ~1-2 μm diameter, where single genes are at approximately nanomolar concentrations. A single gene, in a compartment of 2.6 μm diameter is at a concentration of 0.2 nM. This gene concentration is high enough for efficient translation. Compartmentalisation in such a volume also ensures that even if only a single molecule of the gene product is formed it is present at about 0.2 nM, which is important if the gene product is to have a modifying activity of the genetic element itself. The volume of the microcapsule is thus selected bearing in mind not only the requirements for transcription and translation of the genetic element, but also the modifying activity required of the gene product in the method of the invention.

The size of emulsion microcapsules may be varied simply by tailoring the emulsion conditions used to form the emulsion according to requirements of the selection system. The larger the microcapsule size, the larger is the volume that will be required to encapsulate a given genetic element library, since the ultimately limiting factor will be the size of the microcapsule and thus the number of microcapsules possible per unit volume.

The size of the microcapsules is selected not only having regard to the requirements.of the transcription/translation system, but also those of the selection system employed for the genetic element. Thus, the components of the selection system, such as a chemical modification system, may require reaction volumes and/or reagent concentrations which are not optimal for transcription/translation. As set forth herein, such requirements may be accommodated by a secondary re-encapsulation step; moreover, they may be accommodated by selecting the microcapsule size in order to maximise transcription/translation and selection as a whole. Empirical determination of optimal microcapsule volume and reagent concentration, for example as set forth herein, is preferred.

A "genetic element" in accordance with the present invention is as described above. Preferably, a genetic element is a molecule or construct selected from the group consisting of a DNA molecule, an RNA molecule, a partially or wholly artificial nucleic acid molecule consisting of exclusively synthetic or a mixture of naturally-occurring and synthetic bases, any one of the foregoing linked to a polypeptide, and any one of the foregoing linked to any other molecular group or construct. Advantageously, the other molecular group or construct may be selected from the group consisting of nucleic

acids, polymeric substances, particularly beads, for example polystyrene beads, and magnetic or paramagnetic substances such as magnetic or paramagnetic beads.

The nucleic acid portion of the genetic element may comprise suitable regulatory sequences, such as those required for efficient expression of the gene product, for example promoters, enhancers, translational initiation sequences, polyadenylation sequences, splice sites and the like.

As will be apparent from the following, in many cases the polypeptide or other molecular group or construct is a ligand or a substrate which directly or indirectly binds to or reacts with the gene product in order to alter the optical properties of the genetic element. This allows the sorting of the genetic element on the basis of the activity of the gene product. The ligand or substrate can be connected to the nucleic acid by a variety of means that will be apparent to those skilled in the art (see, for example, Hermanson, 1996).

One way in which the nucleic acid molecule may be linked to a ligand or substrate is through biotinylation. This can be done by PCR amplification with a 5'-biotinylation primer such that the biotin and nucleic acid are covalently linked.

The ligand or substrate can be attached to the modified nucleic acid by a variety of means that will be apparent to those of skill in the art (see, for example, Hermanson, 1996). A biotinylated nucleic acid may be coupled to a polystyrene or paramagnetic microbead (0.02 to approx. 5.0 μm in diameter) that is coated with avidin or streptavidin, that will therefore bind the nucleic acid with very high affinity. This bead can be derivatised with substrate or ligand by any suitable method such as by adding biotinylated substrate or by covalent coupling.

Alternatively, a biotinylated nucleic acid may be coupled to avidin or streptavidin complexed to a large protein molecule such as thyroglobulin (669 Kd) or ferritin (440 Kd). This complex can be derivatised with substrate or ligand, for example by covalent coupling to the E-amino group of lysines or through a non-covalent interaction such as biotin-avidin.

The substrate may be present in a form unlinked to the genetic element but containing an inactive "tag" that requires a further step to activate it such as photoactivation (e.g. of a "caged" biotin analogue, (Sundberg et al., 1995; Pirrung and Huang, 1996)). The catalyst to be selected then converts the substrate to product. The "tag" is then activated and the "tagged" substrate and/or product bound by a tag-binding molecule (e.g. avidin or streptavidin) complexed with the nucleic acid. The ratio of substrate to product attached to the nucleic acid via the "tag" will therefore reflect the ratio of the substrate and product in solution.

An alternative is to couple the nucleic acid to a product-specific antibody (or other product-specific molecule). In this scenario, the substrate (or one of the substrates) is present in each microcapsule unlinked to the genetic element, but has a molecular "tag" (for example biotin, DIG or DNP or a fluorescent group). When the catalyst to be selected converts the substrate to product, the product retains the "tag" and is then captured in the microcapsule by the product-specific antibody. In this way the genetic element only becomes associated with the "tag" when it encodes or produces an enzyme capable of converting substrate to product.

The terms "isolating", "sorting" and "selecting", as well as variations thereof; are used herein. Isolation, according to the present invention, refers to the process of separating an entity from a heterogeneous population, for example a mixture, such that it is free of at least one substance with which it was associated before the isolation process. In a preferred embodiment, isolation refers to purification of an entity essentially to homogeneity. Sorting of an entity refers to the process of preferentially isolating desired entities over undesired entities. In as far as this relates to isolation of the desired entities, the terms "isolating" and "sorting" are equivalent. The method of the present invention permits the sorting of desired genetic elements from pools (libraries or repertoires) of genetic elements which contain the desired genetic element. Selecting is used to refer to the process (including the sorting process) of isolating an entity according to a particular property thereof.

In a highly preferred application, the method of the present invention is useful for sorting libraries of genetic elements. The invention accordingly provides a method according to preceding aspects of the invention, wherein the genetic elements are isolated from a library of genetic elements encoding a repertoire of gene products. Herein, the terms "library", "repertoire" and "pool" are used according to their ordinary signification in the art, such that a library of genetic elements encodes a repertoire of gene products. In general, libraries are constructed from pools of genetic elements arid have properties which facilitate sorting.

Initial selection of a genetic element from a genetic element library using the present invention will in most cases require the screening of a large number of variant genetic elements. Libraries of genetic elements can be created in a variety of different ways, including the following.

Pools of naturally occurring genetic elements can be cloned from genomic DNA or cDNA (Sambrook et al., 1989); for example, phage antibody libraries, made by PCR amplification repertoires of antibody genes from immunised or unimmunised donors have proved very effective sources of functional antibody fragments (Winter et al., 1994; Hoogenboom, 1997). Libraries of genes can also be made by encoding all (see for example Smith, 1985; Parmley and Smith: 1988) or part of genes (see for example Lowman et al., 1991) or pools of genes (see for example Nissim et al., 1994) by a randomised or doped synthetic oligonucleotide. Libraries can also be made by introducing mutations into a genetic element or pool of genetic elements 'randomly by a variety of techniques in vivo, including; using mutator strains of bacteria such as *E. coli* mutD5 (Liao et al., 1986; Yamagishi et al., 1990; Low et at, 1996); using the antibody hypermutation system of B-lymphocytes (Yelamos et al., 1995). Random mutations can also be introduced both in vivo and in vitro by chemical mutagens, and ionising or UV irradiation (see Friedberg et al., 1995), or incorporation of mutagenic base analogues (Frees; 1959; Zaccolo et at, 1996). Random' mutations can also be introduced into genes in vitro during polymerisation for example by using error-prone polymerases (Leung et al., 1989).

Further diversification can be introduced by using homologous recombination either in vivo (see Kowalczykowski et al, 1994) or in vitro (Stemmer, 1994a; Stemmer, 1994b).

According to a further aspect of the present invention, therefore, there is provided a method of in vitro evolution comprising the steps of:

(a) selecting one or more genetic elements from a genetic element library according to the present invention;

(b) mutating the selected genetic element(s) in order to generate a further library of genetic elements encoding a repertoire to gene products; and

(c) iteratively repeating steps (a) and (b) in order to obtain a gene product with enhanced activity.

Mutations may be introduced into the genetic elements(s) as set forth above.

The genetic elements according to the invention advantageously encode enzymes, preferably of pharmacological or industrial interest, activators or inhibitors, especially of bio-

logical systems, such as cellular signal transduction mechanisms, antibodies and fragments thereof, and other binding agents (e.g. transcription factors) suitable for diagnostic and therapeutic applications. In a preferred aspect, therefore, the invention permits the identification and isolation of clinically or industrially useful products. In a further aspect of the invention, there is provided a product when isolated by the method of the invention.

The selection of suitable encapsulation conditions is desirable. Depending on the complexity and size of the library to be screened, it may be beneficial to set up the encapsulation procedure such that 1 or less than 1 genetic element is encapsulated per microcapsule. This will provide the greatest power of resolution. Where the library is larger and/or more complex, however, this may be impracticable; it may be preferable to encapsulate several genetic elements together and rely on repeated application of the method of the invention to achieve sorting of the desired activity. A combination of encapsulation procedures may be used to obtain the desired enrichment.

Theoretical studies indicate that the larger the number of genetic element variants created the more likely it is that a molecule will be created with the properties desired (see Perelson and Oster, 1979 for a description of how this applies to repertoires of antibodies). Recently it has also been confirmed practically that larger phage-antibody repertoires do indeed give rise to more antibodies with better binding affinities than smaller repertoires (Griffiths et al., 1994). To ensure that rare variants are generated and thus are capable of being selected, a large library size is desirable. Thus, the use of optimally small microcapsules is beneficial.

The largest repertoire created to date using methods that require an in vivo step (phage-display and Lad systems) has been a 1.6×10^{11} clone phage-peptide library which required the fermentation of 15 liters of bacteria (Fisch et al., 1996). SELEX experiments are often carried out on very large numbers of variants (up to 10^{15}).

Using the present invention, at a preferred microcapsule diameter of 2.6 μm, a repertoire size of at least 10^{11} can be selected using 1 ml aqueous phase in a 20 ml emulsion.

In addition to the genetic elements described above, the microcapsules according to the invention will comprise further components required for the sorting process to take place. Other components of the system will for example comprise those necessary for transcription and/or translation of the genetic element. These are selected for the requirements of a specific system from the following; a suitable buffer, an in vitro transcription/replication system and/or an in vitro translation system containing all the necessary ingredients, enzymes and cofactors, RNA polymerase, nucleotides, nucleic acids (natural or synthetic), transfer RNAs, ribosomes and amino acids, and the substrates of the reaction of interest in order to allow selection of the modified gene product.

A suitable buffer will be one in which all of the desired components of the biological system are active and will therefore depend upon the requirements of each specific reaction system. Buffers suitable for biological and/or chemical reactions are known in the art and recipes provided in various laboratory texts, such as Sambrook et al., 1989.

The in vitro translation system will usually comprise a cell extract, typically from bacteria (Zubay, 1973; Zubay, 1980; Lesley et al., 1991; Lesley, 1995), rabbit reticulocytes (Pelham and Jackson, 1976), or wheat germ (Anderson et al., 1983). Many suitable systems are commercially available (for example from Promega) including some which will allow coupled transcription/translation (all the bacterial systems and the reticulocyte and wheat germ TNT™ extract systems from Promega). The mixture of amino acids used may include synthetic amino acids if desired, to increase the possible number or variety of proteins produced in the library. This can be accomplished by charging tRNAs with artificial amino acids and using these tRNAs for the in vitro translation of the proteins to be selected (Ellman et al., 1991; Benner, 1994; Mendel et al., 1995).

After each round of selection the enrichment of the pool of genetic elements for those encoding the molecules of interest can be assayed by non-compartmentalised in vitro transcription/replication or coupled transcription-translation reactions. The selected pool is cloned into a suitable plasmid vector and RNA or recombinant protein is produced from the individual clones for further purification and assay.

In a preferred aspect, the internal environment of a microcapsule may be altered by addition of reagents to the oil phase of the emulsion. The reagents diffuse through the oil phase to the aqueous microcapsule environment. Preferably, the reagents are at least partly water-soluble; such that a proportion thereof is distributed from the oil phase to the aqueous microcapsule environment. Advantageously, the reagents are substantially insoluble in the oil phase. Reagents are preferably mixed into the oil phase by mechanical mixing, for example vortexing.

The reagents which may be added via the oil phase include substrates, buffering components, factors and the like. In particular, the internal pH of microcapsules may be altered in situ by adding acidic or basic components to the oil phase.

The invention moreover relates to a method for producing a gene product, once a genetic element encoding the gene product has been sorted by the method of the invention. Clearly, the genetic element itself may be directly expressed by conventional means to produce the gene product. However, alternative techniques may be employed, as will be apparent to those skilled in the art. For example, the genetic information incorporated in the gene product may be incorporated into a suitable expression vector, and expressed therefrom.

The invention also describes the use of conventional screening techniques to identify compounds which are capable of interacting with the gene products identified by the first aspect of the invention. In preferred embodiments, gene product encoding nucleic acid is incorporated into a vector, and introduced into suitable host cells to produce transformed cell lines that express the gene product. The resulting cell lines can then be produced for reproducible qualitative and/or quantitative analysis of the effect(s) of potential drugs affecting gene product function. Thus gene product expressing cells may be employed for the identification of compounds, particularly small molecular weight compounds, which modulate the function of gene product. Thus host cells expressing gene product are useful for drug screening and it is a further object of the present invention to provide a method for identifying compounds which modulate the activity of the gene product, said method comprising exposing cells containing heterologous DNA encoding gene product, wherein said cells produce functional gene product, to at least one compound or mixture of compounds or signal whose ability to modulate the activity of said gene product is sought to be determined, and thereafter monitoring said cells for changes caused by said modulation. Such an assay enables the identification of modulators, such as agonists, antagonists and allosteric modulators, of the gene product. As used herein, a compound or signal that modulates the activity of gene product refers to a compound that alters the activity of gene product in such a way that the activity of the gene product is

different in the presence of the compound or signal (as compared to the absence of said compound or signal).

Cell-based screening assays can be designed by constructing cell lines in which the expression of a reporter protein, i.e. an easily assayable protein, such as □-galactosidase, chloramphenicol acetyltransferase (CAT), green fluorescent protein (GFP) or luciferase, is dependent on gene product. Such an assay enables the detection of compounds that directly modulate gene product function, such as compounds that antagonise gene product, or compounds that inhibit or potentiate other cellular functions required for the activity of gene product.

The present invention also provides a method to exogenously affect gene product dependent processes occurring in cells. Recombinant gene product producing host cells, e.g. mammalian cells, can be contacted with a test compound, and the modulating effect(s) thereof can then be evaluated by comparing the gene product-mediated response in the presence and absence of test compound, or relating the gene product-mediated response of test cells, or control cells (i.e., cells that do not express gene product), to the presence of the compound.

In a further aspect, the invention relates to a method for optimising a production process which involves at least one step which is facilitated by a polypeptide. For example, the step may be a catalytic step, which is facilitated by an enzyme. Thus, the invention provides a method for preparing a compound or compounds comprising the steps of:

(a) providing a synthesis protocol wherein at least one step is facilitated by a polypeptide;

(b) preparing genetic elements encoding variants of the polypeptide which facilitates this step, the expression of which may result, directly or indirectly, in the modification of the optical properties of the genetic elements;

(c) compartmentalising genetic elements into microcapsules;

(d) expressing the genetic elements to produce their respective gene products within the microcapsules;

(e) sorting the genetic elements which produce polypeptide gene product(s) having the desired activity using the changed optical properties of the genetic elements; and

(f) preparing the compound or compounds using the polypeptide gene product identified in (g) to facilitate the relevant step of the synthesis.

By means of the invention, enzymes involved in the preparation of a compound may be optimised by selection for optimal activity. The procedure involves the preparation of variants of the polypeptide to be screened, which equate to a library of polypeptides as refereed to herein. The variants may be prepared in the same manner as the libraries discussed elsewhere herein.

The size of emulsion microcapsules may be varied simply by tailoring the emulsion conditions used to form the emulsion according to requirements of the screening system. The larger the microcapsule size, the larger is the volume that will .be required to encapsulate a given library, since the ultimately limiting factor will be the size of the microcapsule and thus the number of microcapsules possible per unit volume.

Water-in-oil emulsions can be re-emulsified to create water-in-oil-in water double emulsions with an external (continuous) aqueous phase. These double emulsions can be analysed and, optionally, sorted using a flow cytometer (Bernath et al., 2004).

Highly monodisperse microcapsules can be produced using microfluidic techniques. For example, water-in-oil emulsions with less than 1.5% polydispersity can be generated by droplet break off in a co-flowing steam of oil (Umbanhowar et al., 2000). Microfluidic systems can also be used for laminar-flow of aqueous microdroplets dispersed in a stream of oil in microfluidic channels (Thorsen et al., 2001). This allows the construction of microfluidic devices for flow analysis and, optionally, flow sorting of microdroplets (Fu et al., 2002).

Advantageously, highly monodisperse microcapsules can be formed using systems and methods for the electronic control of fluidic species. One aspect of the invention relates to systems and methods for producing droplets of fluid surrounded by a liquid. The fluid and the liquid may be essentially immiscible in many cases, i.e., immiscible on a time scale of interest (e.g., the time it takes a fluidic droplet to be transported through a particular system or device). In certain cases, the droplets may each be substantially the same shape or size, as further described below. The fluid may also contain other species, for example, certain molecular species (e.g., as further discussed below), cells, particles, etc.

In one set of embodiments, electric charge may be created on a fluid surrounded by a liquid, which may cause the fluid to separate into individual droplets within the liquid. In some embodiments, the fluid and the liquid may be present in a channel, e.g., a microfluidic channel, or other constricted space that facilitates application of an electric field to the fluid (which may be "AC" or alternating current, "DC" or direct current etc.), for example, by limiting movement of the fluid with respect to the liquid. Thus, the fluid can be present as a series of individual charged and/or electrically inducible droplets within the liquid. In one embodiment, the electric force exerted on the fluidic droplet may be large enough to cause the droplet to move within the liquid. In some cases, the electric force exerted on the fluidic droplet may be used to direct a desired motion of the droplet within the liquid, for example, to or within a channel or a microfluidic channel (e.g., as further described herein), etc. As one example, in apparatus **5** in FIG. **3**A, droplets **15** created by fluid source **10** can be electrically charged using an electric filed created by electric field generator **20**.

Electric charge may be created in the fluid within the liquid using any suitable technique, for example, by placing the fluid within an electric field (which may be AC, DC, etc.), and/or causing a reaction to occur that causes the fluid to have an electric charge, for example, a chemical reaction, an ionic reaction, a photocatalyzed reaction, etc. In one embodiment, the fluid is an electrical conductor. As used herein, a "conductor" is a material having a conductivity of at least about the conductivity of 18 megohm (MOhm or MΩ) water. The liquid surrounding the fluid may have a conductivity less than that of the fluid. For instance, the liquid may be an insulator, relative to the fluid, or at least a "leaky insulator," i.e., the liquid is able to at least partially electrically insulate the fluid for at least a short period of time. Those of ordinary skill in the art will be able to identify the conductivity of fluids. In one non-limiting embodiment, the fluid may be substantially hydrophilic, and the liquid surrounding the fluid may be substantially hydrophobic.

In some embodiments, the charge created on the fluid (for example, on a series of fluidic droplets) may be at least about 10^{-22} C/micrometer^3. In certain cases, the charge may be at least about 10^{-21} C/micrometer^3, and in other cases, the charge may be at least about 10^{-20} C/micrometer^3, at least about 10^{-19} C/micrometer^3, at least about 10^{-18} C/micrometer^3, at least about 10^{-17} C/micrometer^3, at least about 10^{-16} C/micrometer^3, at least about 10^{-15} C/micrometer^3, at least about 10^{-14} C/micrometer^3, at least about 10^{-13} C/micrometer^3, at least about 10^{-12} C/micrometer^3, at least about 10^{-11} C/micrometer^3, at least about 10^{-10} C/micrometer^3, or at least

about 10^{-9} C/micrometer^3 or more. In certain embodiments, the charge created on the fluid may be at least about 10^{-21} C/micrometer^2, and in some cases, the charge may be at least about 10^{-20} C/micrometer^2, at least about 10^{-19} C/micrometer^2, at least about 10^{-18} C/micrometer^2, at least about 10^{-17} C/micrometer^2, at least about 10^{-16} C/micrometer^2, at least about 10^{-15} C/micrometer^2, at least about 10^{-14} C/micrometer^2, or at least about 10^{-13} C/micrometer^2 or more. In other embodiments, the charge may be at least about 10^{-14} C/droplet, and, in some cases, at least about 10^{-13} C/droplet, in other cases at least about 10^{-12} C/droplet, in other cases at least about 10^{-11} C/droplet, in other cases at least about 10^{-10} C/droplet, or in still other cases at least about 10^{-9} C/droplet.

The electric field, in some embodiments, is generated from an electric field generator, i.e., a device or system able to create an electric field that can be applied to the fluid. The electric field generator may produce an AC field (i.e., one that varies periodically with respect to time, for example, sinusoidally, sawtooth, square, etc.), a DC field (i.e., one that is constant with respect to time), a pulsed field, etc. The electric field generator may be constructed and arranged to create an electric field within a fluid contained within a channel or a microfluidic channel. The electric field generator may be integral to or separate from the fluidic system containing the channel or microfluidic channel, according to some embodiments. As used herein, "integral" means that portions of the components integral to each other are joined in such a way that the components cannot be manually separated from each other without cutting or breaking at least one of the components.

Techniques for producing a suitable electric field (which may be AC, DC, etc.) are known to those of ordinary skill in the art. For example, in one embodiment, an electric field is produced by applying voltage across a pair of electrodes, which may be positioned on or embedded within the fluidic system (for example, within a substrate defining the channel or microfluidic channel), and/or positioned proximate the fluid such that at least a portion of the electric field interacts with the fluid. The electrodes can be fashioned from any suitable electrode material or materials known to those of ordinary skill in the art, including, but not limited to, silver, gold, copper, carbon, platinum, copper, tungsten, tin, cadmium, nickel, indium tin oxide ("ITO"), etc., as well as combinations thereof. In some cases, transparent or substantially transparent electrodes can be used. In certain embodiments, the electric field generator can be constructed and arranged (e.g., positioned) to create an electric field applicable to the fluid of at least about 0.01 V/micrometer, and, in some cases, at least about 0.03 V/micrometer, at least about 0.05 V/micrometer, at least about 0.08 V/micrometer, at least about 0.1 V/micrometer, at least about 0.3 V/micrometer, at least about 0.5 V/micrometer, at least about 0.7 V/micrometer, at least about 1 V/micrometer, at least about 1.2 V/micrometer, at least about 1.4 V/micrometer, at least about 1.6 V/micrometer, or at least about 2 V/micrometer. In some embodiments, even higher electric field intensities may be used, for example, at least about 2 V/micrometer, at least about 3 V/micrometer, at least about 5 V/micrometer, at least about 7 V/micrometer, or at least about 10 V/micrometer or more.

In some embodiments, an electric field may be applied to fluidic droplets to cause the droplets to experience an electric force. The electric force exerted on the fluidic droplets may be, in some cases, at least about 10^{-16} N/micrometer^3. In certain cases, the electric force exerted on the fluidic droplets may be greater, e.g., at least about 10^{-15} N/micrometer^3, at least about 10^{-14} N/micrometer^3, at least about 10^{-13} N/micrometer^3, at least about 10^{-12} N/micrometer^3, at least about 10^{-11} N/micrometer^3, at least about 10^{-10} N/micrometer^3, at least about 10^{-9} N/micrometer^3, at least about 10^{-8} N/micrometer^3, or at least about 10^{-7} N/micrometer^3 or more. In other embodiments, the electric force exerted on the fluidic droplets, relative to the surface area of the fluid, may be at least about 10^{-15} N/micrometer^2, and in some cases, at least about 10^{-14} N/micrometer^2, at least about 10^{-13} N/micrometer^2, at least about 10^{-12} N/micrometer^2, at least about 10^{-11} N/micrometer^2, at least about 10^{-10} N/micrometer^2, at least about 10^{-9} N/micrometer^2, at least about 10^{-8} N/micrometer^2, at least about 10^{-7} N/micrometer^2, or at least about 10^{-6} N/micrometer^2 or more. In yet other embodiments, the electric force exerted on the fluidic droplets may be at least about 10^{-9} N, at least about 10^{-8} N, at least about 10^{-7} N, at least about 10^{-6} N, at least about 10^{-5} N, or at least about 10^{-4} N or more in some cases.

In some embodiments of the invention, systems and methods are provided for at least partially neutralizing an electric charge present on a fluidic droplet, for example, a fluidic droplet having an electric charge, as described above. For example, to at least partially neutralize the electric charge, the fluidic droplet may be passed through an electric field and/or brought near an electrode, e.g., using techniques such as those described herein. Upon exiting of the fluidic droplet from the electric field (i.e., such that the electric field no longer has a strength able to substantially affect the fluidic droplet), and/or other elimination of the electric field, the fluidic droplet may become electrically neutralized, and/or have a reduced electric charge.

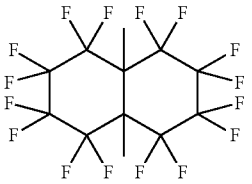
In another set of embodiments, droplets of fluid can be created from a fluid surrounded by a liquid within a channel by altering the channel dimensions in a manner that is able to induce the fluid to form individual droplets. The channel may, for example, be a channel that expands relative to the direction of flow, e.g., such that the fluid does not adhere to the channel walls and forms individual droplets instead, or a channel that narrows relative to the direction of flow, e.g., such that the fluid is forced to coalesce into individual droplets. One example is shown in FIG. **7**A, where channel **510** includes a flowing fluid **500** (flowing downwards), surrounded by liquid **505**. Channel **510** narrows at location **501**, causing fluid **500** to form a series of individual fluidic droplets **515**. In other embodiments, internal obstructions may also be used to cause droplet formation to occur. For instance, baffles, ridges, posts, or the like may be used to disrupt liquid flow in a manner that causes the fluid to coalesce into fluidic droplets.

In some cases, the channel dimensions may be altered with respect to time (for example, mechanically or electromechanically, pneumatically, etc.) in such a manner as to cause the formation of individual fluidic droplets to occur. For example, the channel may be mechanically contracted ("squeezed") to cause droplet formation, or a fluid stream may be mechanically disrupted to cause droplet formation, for example, through the use of moving baffles, rotating blades, or the like. As a non-limiting example, in FIG. **7**B, fluid **500** flows through channel **510** in a downward direction. Fluid **500** is surrounded by liquid **505**. Piezoelectric devices **520** positioned near or integral to channel **510** may then mechanically constrict or "squeeze" channel **510**, causing fluid **500** to break up into individual fluidic droplets **515**.

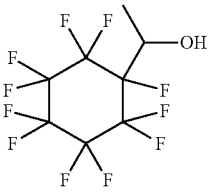
In yet another set of embodiments, individual fluidic droplets can be created and maintained in a system comprising three essentially mutually immiscible fluids (i.e., immiscible on a time scale of interest), where one fluid is a liquid carrier, and the second fluid and the third fluid alternate as individual fluidic droplets within the liquid carrier. In such a system, surfactants are not necessarily required to ensure separation

of the fluidic droplets of the second and third fluids. As an example, with reference to FIG. **14**A, within channel **700**, a first fluid **701** and a second fluid **702** are each carried within liquid carrier **705**. First fluid **701** and second fluid **702** alternate as a series of alternating, individual droplets, each carried by liquid carrier **705** within channel **700**. As the first fluid, the second fluid, and the liquid carrier are all essentially mutually immiscible, any two of the fluids (or all three fluids) can come into contact without causing droplet coalescence to occur. A photomicrograph of an example of such a system is shown in FIG. **14**B, illustrating first fluid **701** and second fluid **702**, present as individual, alternating droplets, each contained within liquid carrier **705**.

One example of a system involving three essentially mutually immiscible fluids is a silicone oil, a mineral oil, and an aqueous solution (i.e., water, or water containing one or more other species that are dissolved and/or suspended therein, for example, a salt solution, a saline solution, a suspension of water containing particles or cells, or the like). Another example of a system is a silicone oil, a fluorocarbon oil, and an aqueous solution. Yet another example of a system is a hydrocarbon oil (e.g., hexadecane), a fluorocarbon oil, and an aqueous solution. In these examples, any of these fluids may be used as the liquid carrier. Non-limiting examples of suitable fluorocarbon oils include octadecafluorodecahydro naphthalene:



or 1-(1,2,2,3,3,4,4,5,5,6,6-undecafluorocyclohexyl)ethanol:



A non-limiting example of such a system is illustrated in FIG. **14**B. In this figure, fluidic network **710** includes a channel containing liquid carrier **705**, and first fluid **701** and second fluid **702**. Liquid carrier **705** is introduced into fluidic network **710** through inlet **725**, while first fluid **701** is introduced through inlet **721**, and second fluid **702** is introduced through inlet **722**. Channel **716** within fluidic network **710** contains liquid carrier **715** introduced from inlet **725**. Initially, first fluid **701** is introduced into liquid **10** carrier **705**, forming fluidic droplets therein. Next, second fluid **702** is introduced into liquid **705**, forming fluidic droplets therein that are interspersed with the fluidic droplets containing first fluid **701**. Thus, upon reaching channel **717**, liquid carrier **705** contains a first set of fluidic droplets containing first fluid **701**, interspersed with a second set of fluidic droplets containing second fluid **702**. In the embodiment illustrated, channel **706** optionally comprises a series of bends, which may allow mixing to occur within each of the fluidic droplets, as further discussed below. However, it should be noted that in this embodiment, since first fluid **701** and second fluid **702** are essentially immiscible, significant fusion and/or mixing of the droplets containing first fluid **701** with the droplets containing second fluid **702** is not generally expected.

Other examples of the production of droplets of fluid surrounded by a liquid are described in International Patent Application Serial No. PCT/US2004/010903, filed Apr. 9, 2004 by Link, et al. and International Patent Application Serial No. PCT/US03/20542, filed Jun. 30, 2003 by Stone, et al., published as WO 2004/002627 on Jan. 8, 2004, each incorporated herein by reference.

In some embodiments, the fluidic droplets may each be substantially the same shape and/or size. The shape and/or size can be determined, for example, by measuring the average diameter or other characteristic dimension of the droplets. The term "determining," as used herein, generally refers to the analysis or measurement of a species, for example, quantitatively or qualitatively, and/or the detection of the presence or absence of the species. "Determining" may also refer to the analysis or measurement of an interaction between two or more species, for example, quantitatively or qualitatively, or by detecting the presence or absence of the interaction. Examples of suitable techniques include, but are not limited to, spectroscopy such as infrared, absorption, fluorescence, UV/visible, FTIR ("Fourier Transform Infrared Spectroscopy"), or Raman; gravimetric techniques; ellipsometry; piezoelectric measurements; immunoassays; electrochemical measurements; optical measurements such as optical density measurements; circular dichroism; light scattering measurements such as quasielectric light scattering; polarimetry; refractometry; or turbidity measurements.

The "average diameter" of a plurality or series of droplets is the arithmetic average of the average diameters of each of the droplets. Those of ordinary skill in the art will be able to determine the average diameter (or other characteristic dimension) of a plurality or series of droplets, for example, using laser light scattering, microscopic examination, or other known techniques. The diameter of a droplet, in a non-spherical droplet, is the mathematically-defined average diameter of the droplet, integrated across the entire surface. The average diameter of a droplet (and/or of a plurality or series of droplets) may be, for example, less than about 1 mm, less than about 500 micrometers, less than about 200 micrometers, less than about 100 micrometers, less than about 75 micrometers, less than about 50 micrometers, less than about 25 micrometers, less than about 10 micrometers, or less than about 5 micrometers in some cases. The average diameter may also be at least about 1 micrometer, at least about 2 micrometers, at least about 3 micrometers, at least about 5 micrometers, at least about 10 micrometers, at least about 15 micrometers, or at least about 20 micrometers in certain cases.

In certain instances, the invention provides for the production of droplets consisting essentially of a substantially uniform number of entities of a species therein (i.e., molecules, compounds, cells, genetic elements, particles, etc.). For example, about 90%, about 93%, about 95%, about 97%, about 98%, or about 99%, or more of a plurality or series of droplets may each contain the same number of entities of a particular species.

For instance, a substantial number of fluidic droplets produced, e.g., as described above, may each contain 1 entity, 2 entities, 3 entities, 4 entities, 5 entities, 7 entities, 10 entities, 15 entities, 20 entities, 25 entities, 30 entities, 40 entities, 50 entities, 60 entities, 70 entities, 80 entities, 90 entities, 100 entities, etc., where the entities are molecules or macromolecules, cells, particles, etc. In some cases, the droplets may

each independently contain a range of entities, for example, less than 20 entities, less than 15 entities, less than 10 entities, less than 7 entities, less than 5 entities, or less than 3 entities in some cases. In one set of embodiments, in a liquid containing droplets of fluid, some of which contain a species of interest and some of which do not contain the species of interest, the droplets of fluid may be screened or sorted for those droplets of fluid containing the species as further described below (e.g., using fluorescence or other techniques such as those described above), and in some cases, the droplets may be screened or sorted for those droplets of fluid containing a particular number or range of entities of the species of interest, e.g., as previously described. Thus, in some cases, a plurality or series of fluidic droplets, some of which contain the species and some of which do not, may be enriched (or depleted) in the ratio of droplets that do contain the species, for example, by a factor of at least about 2, at least about 3, at least about 5, at least about 10, at least about 15, at least about 20, at least about 50, at least about 100, at least about 125, at least about 150, at least about 200, at least about 250, at least about 500, at least about 750, at least about 1000, at least about 2000, or at least about 5000 or more in some cases. In other cases, the enrichment (or depletion) may be in a ratio of at least about 10^4, at least about 10^5, at least about 10^6, at least about 10^7, at least about 10^8, at least about 10^9, at least about 10^{10}, at least about 10^{11}, at least about 10^{12}, at least about 10^{13}, at least about 10^{14}, at least about 10^{15}, or more. For example, a fluidic droplet containing a particular species may be selected from a library of fluidic droplets containing various species, where the library may have about 10^5, about 10^6, about 10^7, about 10^8, about 10^9, about 10^{10}, about 10^{11}, about 10^{12}, about 10^{13}, about 10^{14}, about 10^{15}, or more items, for example, a DNA library, an RNA library, a protein library, a combinatorial chemistry library, a library of genetic elements, etc. In certain embodiments, the droplets carrying the species may then be fused, reacted, or otherwise used or processed, etc., as further described below, for example, to initiate or determine a reaction.

The use of microfluidic handling to create microcapsoules according to the invention has a number of advantages:

(a) They allow the formation of highly monodisperse microcapsules (<1.5% polydispersity), each of which functions as an almost identical, very small microreactor;

(b) The microcapsules can have volumes ranging from about 1 femtoliter to about 1 nanoliter;

(c) Compartmentalisation in microcapsules prevents diffusion and dispersion due to parabolic flow;

(d) By using a perfluorocarbon carrier fluid it is possible to prevent exchange of molecules between microcapsules;

(e) Reagents in microcapsules cannot react or interact with the fabric of the microchannels as they are separated by a layer of inert perfluorocarbon carrier fluid.

(f) Microcapsules can be created at up to 10,000 per second and screened using optical methods at the same rate. This is a throughput of 10^9 per day.

Microcapsules (or droplets; the terms may be used intechangeably for the purposes envisaged herein) can, advantageously, be fused or split. For example, aqueous microdroplets can be merged and split using microfluidics systems (Link et al., 2004; Song et al., 2003). Microcapsule fusion allows the mixing of reagents. Fusion, for example, of a microcapsule containing the genetic element with a microcapsule containing a transcription factor could initiate transcription of the genetic information. Microcapsule splitting allows single microcapsules to be split into two or more smaller microcapsules. For example a single microcapsule containing a ragent can be split into multiple microcapsules which can then each be fused with a different microcapsule containing a different reagent or genetic element. A single microcapsule containing a reagent can also be split into multiple microcapsules which can then each be fused with a different microcapsule containing a different genetic element, or other reagents, for example at different concentrations.

In one aspect, the invention relates to microfluidic systems and methods for splitting a fluidic droplet into two or more droplets. The fluidic droplet may be surrounded by a liquid, e.g., as previously described, and the fluid and the liquid are essentially immiscible in some cases. The two or more droplets created by splitting the original fluidic droplet may each be substantially the same shape and/or size, or the two or more droplets may have different shapes and/or sizes, depending on the conditions used to split the original fluidic droplet. In many cases, the conditions used to split the original fluidic droplet can be controlled in some fashion, for example, manually or automatically (e.g., with a processor, as discussed below). In some cases, each droplet in a plurality or series of fluidic droplets may be independently controlled. For example, some droplets may be split into equal parts or unequal parts, while other droplets are not split.

According to one set of embodiments, a fluidic droplet can be split using an applied electric field. The electric field may be an AC field, a DC field, etc. The fluidic droplet, in this embodiment, may have a greater electrical conductivity than the surrounding liquid, and, in some cases, the fluidic droplet may be neutrally charged. In some embodiments, the droplets produced from the original fluidic droplet are of approximately equal shape and/or size. In certain embodiments, in an applied electric field, electric charge may be urged to migrate from the interior of the fluidic droplet to the surface to be distributed thereon, which may thereby cancel the electric field experienced in the interior of the droplet. In some embodiments, the electric charge on the surface of the fluidic droplet may also experience a force due to the applied electric field, which causes charges having opposite polarities to migrate in opposite directions. The charge migration may, in some cases, cause the drop to be pulled apart into two separate fluidic droplets. The electric field applied to the fluidic droplets may be created, for example, using the techniques described above, such as with a reaction an electric field generator, etc.

As a non-limiting example, in FIG. **1**A, where no electric field is applied, fluidic droplets **215** contained in channel **230** are carried by a surrounding liquid, which.flows towards intersection **240**, leading to channels **250** and **255**. In this example, the surrounding liquid flows through channels **250** and **255** at equal flowrates. Thus, at intersection **240**, fluidic droplets **215** do not have a preferred orientation or direction, and move into exit channels **250** and **255** with equal probability due to the surrounding liquid flow. In contrast, in FIG. **1**B, while the surrounding liquid flows in the same fashion as FIG. **1**A, under the influence of an applied electric field of 1.4 V/micrometers, fluidic droplets **215** are split into two droplets at intersection **240**, forming new droplets **216** and **217**. Droplet **216** moves to the left in channel **250**, while droplet **217** moves to the right in channel **255**.

A schematic of this process can be seen in FIG. **5**, where a neutral fluidic droplet **530**, surrounded by a liquid **535** in channel **540**, is subjected to applied electric field **525**, created by electrodes **526** and **527**. Electrode **526** is positioned near channel **542**, while electrode **527** is positioned near channel **544**. Under the influence of electric field **525**, charge separation is induced within fluidic droplet **530**, i.e., such that a

positive charge is induced at one end of the droplet, while a negative charge is induced at the other end of the droplet. The droplet may then split into a negatively charged droplet **545** and a positively charged droplet **546**, which then may travel in channels **542** and **544**, respectively. In some cases, one or both of the electric charges on the resulting charged droplets may also be neutralized, as previously described.

Other examples of splitting a fluidic droplet into two droplets are described in International Patent Application Serial No. PCT/US2004/010903, filed Apr. 9, 2004 by Link, et al.; U.S. Provisional Patent Application Ser. No. 60/498,091, filed Aug. 27, 2003, by Link, et. al.; and International Patent Application Serial No. PCT/US03/20542, filed Jun. 30, 2003 by Stone, et al., published as WO 2004/002627 on Jan. 8, 2004, each incorporated herein by reference.

The invention, in yet another aspect, relates to systems and methods for fusing or coalescing two or more fluidic droplets into one droplet. For example, in one set of embodiments, systems and methods are provided that are able to cause two or more droplets (e.g., arising from discontinuous streams of fluid) to fuse or coalesce into one droplet in cases where the two or more droplets ordinarily are unable to fuse or coalesce, for example, due to composition, surface tension, droplet size, the presence or absence of surfactants, etc. In certain microfluidic systems, the surface tension of the droplets, relative to the size of the droplets, may also prevent fusion or coalescence of the droplets from occurring in some cases.

In one embodiment, two fluidic droplets may be given opposite electric charges (i.e., positive and negative charges, not necessarily of the same magnitude), which may increase the electrical interaction of the two droplets such that fusion or coalescence of the droplets can occur due to their opposite electric charges, e.g., using the techniques described herein. For instance, an electric field may be applied to the droplets, the droplets may be passed through a capacitor, a chemical reaction may cause the droplets to become charged, etc. As an example, as is shown schematically in FIG. **13**A, uncharged droplets **651** and **652**, carried by a liquid **654** contained within a microfluidic channel **653**, are brought into contact with each other, but the droplets are not able to fuse or coalesce, for instance, due to their size and/or surface tension. The droplets, in some cases, may not be able to fuse even if a surfactant is applied to lower the surface tension of the droplets. However, if the fluidic droplets are electrically charged with opposite charges (which can be, but are not necessarily of, the same magnitude), the droplets may be able to fuse or coalesce. For instance, in FIG. **13**B, positively charged droplets **655** and negatively charged droplets **656** are directed generally towards each other such that the electrical interaction of the oppositely charged droplets causes the droplets to fuse into fused droplets **657**.

In another embodiment, the fluidic droplets may not necessarily be given opposite electric charges (and, in some cases, may not be given any electric charge), and are fused through the use of dipoles induced in the fluidic droplets that causes the fluidic droplets to coalesce. In the example illustrated in FIG. **13**C, droplets **660** and **661** (which may each independently be electrically charged or neutral), surrounded by liquid **665** in channel **670**, move through the channel such that they are the affected by an applied electric field **675**. Electric field **675** may be an AC field, a DC field, etc., and may be created, for instance, using electrodes **676** and **677**, as shown here. The induced dipoles in each of the fluidic droplets, as shown in FIG. **13**C, may cause the fluidic droplets to become electrically attracted towards each other due to their local opposite charges, thus causing droplets **660** and **661** to fuse to produce droplet **663**. In FIG. **13**D, droplets **651** and **652** flow together to fuse to form droplet **653**, which flows in a third channel.

It should be noted that, in various embodiments, the two or more droplets allowed to coalesce are not necessarily required to meet "head-on". Any angle of contact, so long as at least some fusion of the droplets initially occurs, is sufficient. As an example, in FIG. **12**H, droplets **73** and **74** each are traveling in substantially the same direction (e.g., at different velocities), and are able to meet and fuse. As another example, in FIG. **12**I, droplets **73** and **74** meet at an angle and fuse. In FIG. **12**J, three fluidic droplets **73**, **74** and **68** meet and fuse to produce droplet **79**.

Other examples of fusing or coalescing fluidic droplets are described in International Patent Application Serial No. PCT/US2004/010903, filed Apr. 9, 2004 by Link, et al., incorporated herein by reference.

Fluidic handling of microcapsules therefore results in further advantages:

(a) Microcapsules can be split into two or more smaller microdroplets allowing the reagents contained therein to be reacted with a series of different molecules in parallel or assayed in multiplicate.

(b) Microcapsules can be fused. This allows molecules to be: (a) diluted, (b) mixed with other molecules, and (c) reactions initiated, terminated or modulated at precisely defined times.

(c) Reagents can be mixed very rapidly (in <2 ms) in microcapsules using chaotic advection, allowing fast kinetic measurements and very high throughput.

(d) Reagents can be mixed in a combinatorial manner. For example, allowing the effect of all possible pairwise combinations of compounds in a library to be tested.

Creating and manipulating microcapsules in microfluidic systems means that:

(a) Stable streams of microcapsules can be formed in microchannels and identified by their relative positions.

(b) If the reactions are accompanied by an optical signal (e.g. a change in fluorescence) a spatially-resolved optical image of the microfluidic network allows time resolved measurements of the reactions in each microcapsules.

(c) Microcapsules can be separated using a microfluidic flow sorter to allow recovery and further analysis or manipulation of the molecules they contain.

Screening/Sorting of Microcapsules

In still another aspect, the invention provides systems and methods for screening or sorting fluidic droplets in a liquid, and in some cases, at relatively high rates. For example, a characteristic of a droplet may be sensed and/or determined in some fashion (e.g., as further described below), then the droplet may be directed towards a particular region of the device, for example, for sorting or screening purposes.

In some embodiments, a characteristic of a fluidic droplet may be sensed and/or determined in some fashion, for example, as described herein (e.g., fluorescence of the fluidic droplet may be determined), and, in response, an electric field may be applied or removed from the fluidic droplet to direct the fluidic droplet to a particular rgion (e.g. a channel). In some cases, high sorting speeds may be achievable using certain systems and methods of the invention. For instance, at least about 10 droplets per second may be determined and/or sorted in some cases, and in other cases, at least about 20 droplets per second, at least about 30 droplets per second, at least about 100 droplets per second, at least about 200 droplets per second, at least about 300 droplets per second, at least about 500 droplets per second, at least about 750 droplets per

second, at least about 1000 droplets per second, at least about 1500 droplets per second, at least about 2000 droplets per second, at least about 3000 droplets per second, at least about 5000 droplets per second, at least about 7500 droplets per second, at least about 10,000 droplets per second, at least about 15,000 droplets per second, at least about 20,000 droplets per second, at least about 30,000 droplets per second, at least about 50,000 droplets per second, at least about 75,000 droplets per second, at least about 100,000 droplets per second, at least about 150,000 droplets per second, at least about 200,000 droplets per second, at least about 300,000 droplets per second, at least about 500,000 droplets per second, at least about 750,000 droplets per second, at least about 1,000,000 droplets per second, at least about 1,500,000 droplets per second, at least about 2,000,000 or more droplets per second, or at least about 3,000,000 or more droplets per second may be determined and/or sorted in such a fashion.

In one set of embodiments, a fluidic droplet may be directed by creating an electric charge (e.g., as previously described) on the droplet, and steering the droplet using an applied electric field, which may be an AC field, a DC field, etc. As an example, in reference to FIGS. **2-4**, an electric field may be selectively applied and removed (or a different electric field may be applied, e.g., a reversed electric field as shown in FIG. **4**A) as needed to direct the fluidic droplet to a particular region. The electric field may be selectively applied and removed as needed, in some embodiments, without substantially altering the flow of the liquid containing the fluidic droplet. For example, a liquid may flow on a substantially steady-state basis (i.e., the average flowrate of the liquid containing the fluidic droplet deviates by less than 20% or less than 15% of the steady-state flow or the expected value of the flow of liquid with respect to time, and in some cases, the average flowrate may deviate less than 10% or less than 5%) or other predetermined basis through a fluidic system of the invention (e.g., through a channel or a microchannel), and fluidic droplets contained within the liquid may be directed to various regions, e.g., using an electric field, without substantially altering the flow of the liquid through the fluidic system. As a particular example, in FIGS. **2**A, **3**A and **4**A, a liquid containing fluidic droplets **15** flows from fluid source **10**, through channel **30** to intersection **40**, and exits through channels **50** and **55**. In FIG. **2**A, fluidic droplets **15** are directed through both channels **50** and **55**, while in FIG. **3**A, fluidic droplets **15** are directed to only channel **55** and, in FIG. **4**A, fluidic droplets **15** are directed to only channel **50**.

In another set of embodiments, a fluidic droplet may be sorted or steered by inducing a dipole in the fluidic droplet (which may be initially charged or uncharged), and sorting or steering the droplet using an applied electric field. The electric field may be an AC field, a DC field, etc. For example, with reference to FIG. **9**A, a channel **540**, containing fluidic droplet **530** and liquid **535**, divides into channel **542** and **544**. Fluidic droplet **530** may have an electric charge, or it may be uncharged. Electrode **526** is positioned near channel **542**, while electrode **527** is positioned near channel **544**. Electrode **528** is positioned near the junction of channels **540**, **542**, and **544**. In FIGS. **9**C and **9**D, a dipole is induced in the fluidic droplet using electrodes **526**, **527**, and/or **528**. In FIG. **9**C, a dipole is induced in droplet **530** by applying an electric field **525** to the droplet using electrodes **527** and **528**. Due to the strength of the electric field, the droplet is strongly attracted to the right, into channel **544**. Similarly, in FIG. **9**D, a dipole is induced in droplet **530** by applying an electric field **525** to the droplet using electrodes **526** and **528**, causing the droplet to be attracted into channel **542**. Thus, by applying the proper electric field, droplet **530** can be directed to either channel **542** or **544** as desired.

In other embodiments, however, the fluidic droplets may be screened or sorted within a fluidic system of the invention by altering the flow of the liquid containing the droplets. For instance, in one set of embodiments, a fluidic droplet may be steered or sorted by directing the liquid surrounding the fluidic droplet into a first channel, a second channel, etc. As a non-limiting example, with reference to FIG. **10**A, fluidic droplet **570** is surrounded by a liquid **575** in channel **580**. Channel **580** divides into three channels **581**, **582**, and **583**. The flow of liquid **575** can be directed into any of channels **581**, **582**, and **583** as desired, for example, using flow-controlling devices known to those of ordinary skill in the art, for example, valves, pumps, pistons, etc. Thus, in FIG. **10**B, fluidic droplet **570** is directed into channel **581** by directing liquid **575** to flow into channel **581** (indicated by arrows **574**); in FIG. **10**C, fluidic droplet **570** is directed into channel **582** by directing liquid **575** to flow into channel **582** (indicated by arrows **574**); and in FIG. **10**D, fluidic droplet **570** is directed into channel **583** by directing liquid **575** to flow into channel **583** (indicated by arrows **574**).

However, it is preferred that control of the flow of liquids in microfluidic systems is not used to direct the flow of fluidic droplets therein, but that an alternative method is used. Advantageously, therefore, the microcapsules are not sorted by altering the direction of the flow of a carrier fluid in a microfluidic system.

In another set of embodiments, pressure within a fluidic system, for example, within different channels or within different portions of a channel, can be controlled to direct the flow of fluidic droplets. For example, a droplet can be directed toward a channel junction including multiple options for further direction of flow (e.g., directed toward a branch, or fork, in a channel defining optional downstream flow channels). Pressure within one or more of the optional downstream flow channels can be controlled to direct the droplet selectively into one of the channels, and changes in pressure can be effected on the order of the time required for successive droplets to reach the junction, such that the downstream flow path of each successive droplet can be independently controlled. In one arrangement, the expansion and/or contraction of liquid reservoirs may be used to steer or sort a fluidic droplet into a channel, e.g., by causing directed movement of the liquid containing the fluidic droplet. The liquid reservoirs may be positioned such that, when activated, the movement of liquid caused by the activated reservoirs causes the liquid to flow in a preferred direction, carrying the fluidic droplet in that preferred direction. For instance, the expansion of a liquid reservoir may cause a flow of liquid towards the reservoir, while the contraction of a liquid reservoir may cause a flow of liquid away from the reservoir. In some cases, the expansion and/or contraction of the liquid reservoir may be combined with other flow-controlling devices and methods, e.g., as described herein. Non-limiting examples of devices able to cause the expansion and/or contraction of a liquid reservoir include pistons and piezoelectric components. In some cases, piezoelectric components may be particularly useful due to their relatively rapid response times, e.g., in response to an electrical signal.

As a non-limiting example, in FIG. **11**A, fluidic droplet **600** is surrounded by a liquid **605** in channel **610**. Channel **610** divides into channels **611**, **612**. Positioned in fluidic communication with channels **611** and **612** are liquid reservoirs **617** and **618**, which may be expanded and/or contracted, for instance, by piezoelectric components **615** and **616**, by a

piston (not shown), etc. In FIG. **11**B, liquid reservoir **617** has been expanded, while liquid reservoir **618** has been contracted. The effect of the expansion/contractions of the reservoirs is to cause a net flow of liquid towards channel **611**, as indicated by arrows **603**. Thus, fluidic droplet **600**, upon reaching the junction between the channels, is directed to channel **611** by the movement of liquid **605**. The reverse situation is shown in FIG. **11**C, where liquid reservoir **617** has contracted while liquid reservoir **618** has been expanded. A net flow of liquid occurs towards channel **612** (indicated by arrows **603**), causing fluidic droplet **600** to move into channel **612**. It should be noted, however, that reservoirs **617** and **618** do not both need to be activated to direct fluidic droplet **600** into channels **611** or **612**. For example, in one embodiment, fluidic droplet **600** may be directed to channel **611** by the expansion of liquid reservoir **617** (without any alteration of reservoir **618**), while in another embodiment, fluidic droplet **600** may be directed to channel **611** by the contraction of liquid reservoir **618** (without any alteration of reservoir **617**). In some cases, more than two liquid reservoirs may be used.

In some embodiments, the fluidic droplets may be sorted into more than two channels. Non-limiting examples of embodiments of the invention having multiple regions within a fluidic system for the delivery of droplets are shown in FIGS. **6**A and **6**B. Other arrangements are shown in FIGS. **10**A-**10**D. In FIG. **6**A, charged droplets **315** in channel **330** may be directed as desired to any one of exit channels **350**, **352**, **354**, or **356**, by applying electric fields to control the movement of the droplets at intersections **340**, **341**, and **342**, using electrodes **321**/**322**, **323**/**324**, and **325**/**326**, respectively. In FIG. **6**A, droplets **315** are directed to channel **354** using applied electric fields **300** and **301**, using 5 principles similar to those discussed above. Similarly, in FIG. **6**B, charged droplets **415** in channel **430** can be directed to any one of exit channels **450**, **452**, **454**, **456**, or **458**, by applying electric fields to control the movement of the droplets at intersections **440**, **441**, **442**, and **443**, using electrodes **421**/**422**, **423**/**424**, **425**/**426**, and **427**/**428**, respectively. In this figure, droplets **415** are directed to channel **454**; of course, the charged droplets may be directed to any other exit channel as desired.

In another example, in apparatus **5**, as schematically illustrated in FIG. **2**A, fluidic droplets **15** created by fluid source **10** are positively charged due to an applied electric field created using electric field generator **20**, which comprises two electrodes **22**, **24**. Fluidic droplets **15** are directed through channel **30** by a liquid containing the droplets, and are directed towards intersection **40**. At intersection **40**, the fluidic droplets do not have a preferred orientation or direction, and move into exit channels **50** and **55** with equal probability (in this embodiment, liquid drains through both exit channels **50** and **55** at substantially equal rates). Similarly, fluidic droplets **115** created by fluid source **110** are negatively charged due to an applied electric field created using electric field generator **120**, which comprises electrodes **122** and **124**. After traveling through channel **130** towards intersection **140**, the fluidic droplets do not have a preferred orientation or direction, and move into exit channels **150** and **155** with equal probability, as the liquid exits through exit channels **150** and **155** at substantially equal rates. A representative photomicrograph of intersection **140** is shown in FIG. **2**B.

In the schematic diagram of FIG. **3**A, an electric field **100** of 1.4 V/micrometer has been applied to apparatus **5** of FIG. **2**A, in a direction towards the right of apparatus **5**. Positively-charged fluidic droplets **15** in channel **30**, upon reaching intersection **40**, are directed to the right in channel **55** due to the applied electric field **100**, while the liquid containing the droplets continues to exit through exit channels **50** and **55** at substantially equal rates. Similarly, negatively-charged fluidic droplets **115** in channel **130**, upon reaching intersection **140**, are directed to the left in channel **150** due to the applied electric field **100**, while the liquid fluid continues to exit the device through exit channels **150** and **155** at substantially equal rates. Thus, electric field **100** can be used to direct fluidic droplets into particular channels as desired. A representative photomicrograph of intersection **140** is shown in FIG. **3**B.

FIG. **4**A is a schematic diagram of apparatus **5** of FIG. **2**A, also with an applied electric field **100** of 1.4 V/micrometer, but in the opposite direction (i.e., −1.4 V/micrometer). In this figure, positively-charged fluidic droplets **15** in channel **30**, upon reaching intersection **40**, are directed to the left into channel **50** due to the applied electric field **100**, while negatively-charged fluidic droplets **115** in channel **130**, upon reaching intersection **140**, are directed to the right into channel **155** due to applied electric field **100**. The liquid containing the droplets exits through exit channels **50** and **55**, and **150** and **155**, at substantially equal rates. A representative photomicrograph of intersection **140** is shown in FIG. **4**B.

In some embodiments of the invention, a fluidic droplet may be sorted and/or split into two or more separate droplets, for example, depending on the particular application. Any of the above-described techniques may be used to spilt and/or sort droplets. As a non-limiting example, by applying (or removing) a first electric field to a device (or a portion thereof), a fluidic droplet may be directed to a first region or channel; by applying (or removing) a second electric field to the device (or a portion thereof), the droplet may be directed to a second region or channel; by applying a third electric field to the device (or a portion thereof), the droplet may be directed to a third region or channel; etc., where the electric fields may differ in some way, for example, in intensity, direction, frequency, duration, etc. In a series of droplets, each droplet may be independently sorted and/or split; for example, some droplets may be directed to one location or another, while other droplets may be split into multiple droplets directed to two or more locations.

As one particular example, in FIG. **8**A, fluidic droplet **550**, surrounding liquid **555** in channel **560** may be directed to channel **556**, channel **557**, or be split in some fashion between channels **562** and **564**. In FIG. **8**B, by directing surrounding liquid **555** towards channel **562**, fluidic droplet **550** may be directed towards the left into channel **562**; in FIG. **8**C, by directing surrounding liquid **555** towards channel **564**, fluidic droplet **550** may be directed towards the right into channel **564**, In FIG. **8**D, an electric field may be applied, in combination with control of the flow of liquid **555** surrounding fluidic droplet **550**, that causes the droplet to impact junction **561**, which may cause the droplet to split into two separate fluidic droplets **565**, **566**. Fluidic droplet **565** is directed to channel **562**, while fluidic droplet **566** is directed to channel **566**. A high degree of control of the applied electric field may be achieved to control droplet formation; thus, for example, after fluidic droplet **565** has been split into droplets **565** and **566**, droplets **565** and **566** may be of substantially equal size, or either of droplets **565** and **566** may be larger, e.g., as is shown in FIGS. **8**E and **8**F, respectively.

As another, example, in FIG. **9**A, channel **540**, carrying fluidic droplet **530** and liquid **535**, divides into channel **542** and **544**. Fluidic droplet **530** may be electrically charged, or it may uncharged. Electrode **526** is positioned near channel **542**, while electrode **527** is positioned near channel **544**. Electrode **528** is positioned near the junction of channels **540**, **542**, and **544**. When fluidic droplet **530** reaches the junction,

it may be subjected to an electric field, and/or directed to a channel or other region, for example, by directing the surrounding liquid into the channel. As shown in FIG. **9**B, fluidic droplet **530** may be split into two separate droplets **565** and **566** by applying an electric field **525** to the droplet using electrodes **526** and **527**. In FIG. **9**C, a dipole can be induced in droplet **530** by applying an electric field **525** to the droplet using electrodes **527** and **528**. Due to the strength of the applied electric field, the droplet may be strongly attracted to the right, into channel **544**. Similarly, in FIG. **9**D, a dipole may be induced in droplet **530** by applying an electric field **525** to the droplet using electrodes **526** and **528**, causing the droplet to be attracted into channel **542**. By controlling which electrodes are used to induce an electric field across droplet **530**, and/or the strength of the applied electric field, one or more fluidic droplets within channel **540** may be sorted and/or split into two droplets, and each droplet may independently be sorted and/or split.

Microcapsules can be optically tagged by, for example, incorporating fluorochromes. In a preferred configuration, the microcapsules are optically tagged by incorporating quantum dots: quantum dots of 6 colours at 10 concentrations would allow the encoding of 10^6 microcapsules (Han et al., 2001). Microcapsules flowing in an ordered sequence in a microfluidic channel can be encoded (wholly or partially) by their sequence in the stream of microcapsules (positional encoding).

By means of the invention, enzymes involved in the preparation of a compound may be optimised by selection for optimal activity. The procedure involves the preparation of variants of the polypeptide to be screened, which equate to a library of polypeptides as refereed to herein. The variants may be prepared in the same manner as the libraries discussed elsewhere herein.

(B) Selection Procedures

The system can be configured to select for RNA, DNA or protein gene product molecules with catalytic, regulatory or binding activity.

(i) Selection for Binding

In the case of selection for a gene product with affinity for a specific ligand the genetic element may be linked to the gene product in the microcapsule via the ligand. Only gene products with affinity for the ligand will therefore bind to the genetic element and only those genetic elements with gene product bound via the ligand will acquire the changed optical properties which enable them to be retained in the selection step. In this embodiment, the genetic element will thus comprise a nucleic acid encoding the gene product linked to a ligand for the gene product.

The change in optical properties of the genetic element after binding of the gene product to the ligand may be induced in a variety of ways, including:

(1) the gene product itself may have distinctive optical properties, for example, it is fluorescent (e.g. green fluorescent protein, (Lorenz et al., 1991)).

(2) the optical properties of the gene product may be modified on binding to the ligand, for example, the fluorescence of the gene product is quenched or enhanced on binding (Guixe et al., 1998; Qi and Grabowski, 1998)

(3) the optical properties of the ligand may be modified on binding of the gene product, for example, the fluorescence of the ligand is quenched or enhanced on binding (Voss, 1993; Masui and Kuramitsu, 1998).

(4) the optical properties of both ligand and gene product are modified on binding, for example, there can be a fluorescence resonance energy transfer (FRET) from ligand to gene product (or vice versa) resulting in emmission at the "acceptor" emmission wavelength when excitation is at the "donor" absoption wavelength (Heim & Tsien, 1996; Mahaj an et al., 1998; Miyawaki et al., 1997).

In this embodiment, it is not necessary for binding of the gene product to the genetic element via the ligand to directly induce a change in optical properties. All the gene products to be selected can contain a putative binding domain, which is to be selected for, and a common feature—a tag. The genetic element in each microcapsule is physically linked to the ligand. If the gene product produced from the genetic element has affinity for the ligand, it will bind to it and become physically linked to the same genetic element that encoded it, resulting in the genetic element being 'tagged'. At the end of the reaction, all of the microcapsules are combined, and all genetic elements and gene products pooled together in one environment. Genetic elements encoding gene products exhibiting the desired binding can be selected by adding reagents which specifically bind to, or react specifically with, the "tag" and thereby induce a change in the optical properties of the genetic element allowing there sorting. For example, a fluorescently-labelled anti-"tag" antibody can be used, or an anti-"tag" antibody followed by a second fluorescently labelled antibody which binds the first.

In an alternative embodiment, genetic elements may be sorted on the basis that the gene product, which binds to the ligand, merely hides the ligand from, for example, further binding partners which would otherwise modify the optical properties of the genetic element. In this case genetic elements with ummodified optical properties would be selected.

In an alternative embodiment, the invention provides a method according to the first aspect of the invention, wherein in step (b) the gene products bind to genetic elements encoding them. The gene products together with the attached genetic elements are then sorted as a result of binding of a ligand to gene products having the desired binding activity. For example, all gene products can contain an invariant region which binds covalently or non-covalently to the genetic element, and a second region which is diversified so as to generate the desired binding activity.

In an alternative embodiment, the ligand for the gene product is itself encoded by the genetic element and binds to the genetic element. Stated otherwise, the genetic element encodes two (or indeed more) gene products, at least one of which binds to the genetic element, and which can potentially bind each other. Only when the gene products interact in a microcapsule is the genetic element modified in a way that ultimately results in a change in a change in its optical properties that enables it to be sorted. This embodiment, for example, isused to search gene libraries for pairs of genes encoding pairs of proteins which bind each other.

Fluorescence may be enhanced by the use of Tyramide Signal Amplification (TSA™) amplification to make the genetic elements fluorescent. This involves peroxidase (linked to another protein) binding to the genetic elements and catalysing the conversion of fluorescein-tyramine in to a free radical form which then reacts (locally) with the genetic elements. Methods for performing TSA are known in the art, and kits are available commercially from NEN.

TSA may be configured such that it results in a direct increase in the fluorescence of the genetic element, or such that a ligand is attached to the genetic element which is bound by a second fluorescent molecule, or a sequence of molecules, one or raore of which is fluorescent.

(ii) Selection for Catalysis

When selection is for catalysis, the genetic element in each microcapsule may comprise the substrate of the reaction. If

the genetic element encodes a gene product capable of acting as a catalyst, the gene product will catalyse the conversion of the substrate into the product. Therefore, at the end of the reaction the genetic element is physically linked to the product of the catalysed reaction.

It may also be desirable, in some cases, for the substrate not to be a component of the genetic element. In this case the substrate would contain an inactive "tag" that requires a further step to activate it such as photoactivation (e.g. of a "caged" biotin analogue, (Sundberg et al., 1995; Pirrung and Huang, 1996)). The catalyst to be selected then converts the substrate to product. The "tag" is then activated and the "tagged" substrate and/or product bound by a tag-binding molecule (e.g. avidin or streptavidin) complexed with the nucleic acid. The ratio of substrate to product attached to the nucleic acid via the "tag" will therefore reflect the ratio of the substrate and product in solution.

The optical properties of genetic elements with product attached and which encode gene products with the desired catalytic activity can be modified by either:

(1) the product-genetic element complex having characteristic optical properties not found in the substrate-genetic element complex, due to, for example;
  (a) the substrate and product having different optical properties (many fluorogenic enzyme substrates are available commercially (see for example Haugland, 1996) including substrates for glycosidases, phosphatases, peptidases and proteases (Craig et al., 1995; Huang et al., 1992; Brynes et al., 1982; Jones et al., 1997; Matayoshi et al., 1990; Wang et al., 1990)), or
  (b) the substrate and product having similar optical properties, but only the product, and not the substrate binds to, or reacts with, the genetic element;
(2) adding reagents which specifically bind to, or react with, the product and which thereby induce a change in the optical properties of the genetic elements allowing their sorting (these reagents can be added before or after breaking the microcapsules and pooling the genetic elements). The reagents;
  (a) bind specifically to, or react specifically with, the product, and not the substrate, if both substrate and product are attached to the genetic element, or
  (b) optionally bind both substrate and product if only the product, and not the substrate binds to, or reacts with, the genetic element.

The pooled genetic elements encoding catalytic molecules can then be enriched by selecting for the genetic elements with modified optical properties.

An alternative is to couple the nucleic acid to a product-specific antibody (or other product-specific molecule). In this scenario, the substrate (or one of the substrates) is present in each microcapsule unlinked to the genetic element, but has a molecular "tag" (for example biotin, DIG or DNP or a fluorescent group). When the catalyst to be selected converts the substrate to product, the product retains the "tag" and is then captured in the microcapsule by the product-specific antibody. In this way the genetic element only becomes associated with the "tag" when it encodes or produces an enzyme capable of converting substrate to product. When all reactions are stopped and the microcapsules are combined, the genetic elements encoding active enzymes will be "tagged" and may already have changed optical properties, for example, if the "tag" was a fluorescent group. Alternatively, a change in optical properties of "tagged" genes can be induced by adding a fluorescently labelled ligand which binds the "tag" (for example fluorescently-labelled avidin/streptavidin, an anti-"tag" antibody which is fluorescent, or a nonfluorescent anti-"tag" antibody which can be detected by a second fluorescently-labelled antibody).

Alternatively, selection may be performed indirectly by coupling a first reaction to subsequent reactions that takes place in the same microcapsule. There are two general ways in which this may be performed. In a first embodiment, the product of the first reaction is reacted with, or bound by, a molecule which does not react with the substrate of the first reaction. A second, coupled reaction will only proceed in the presence of the product of the first reaction. A genetic element encoding a gene product with a desired activity can then be purified by using the properties of the product of the second reaction to induce a change in the optical properties of the genetic element as above.

Alternatively, the product of the reaction being selected may be the substrate or cofactor for a second enzyme-catalysed reaction. The enzyme to catalyse the second reaction can either be translated in situ in the microcapsules or incorporated in the reaction mixture prior to microencapsulation. Only when the first reaction proceeds will the coupled enzyme generate a product which can be used to induce a change in the optical properties of the genetic element as above.

This concept of coupling can be elaborated to incorporate multiple enzymes, each using as a substrate the product of the previous reaction. This allows for selection of enzymes that will not react with an immobilised substrate. It can also be designed to give increased sensitivity by signal amplification if a product of one reaction is a catalyst or a cofactor for a second reaction or series of reactions leading to a selectable product (for example, see Johannsson and Bates, 1988; Johannsson, 1991). Furthermore an enzyme cascade system can be based on the production of an activator for an enzyme or the destruction of an enzyme inhibitor (see Mize et al., 1989). Coupling also has the advantage that a common selection system can be used for a whole group of enzymes which generate the same product and allows for the selection of complicated chemical transformations that cannot be performed in a single step.

Such a method of coupling thus enables the evolution of novel "metabolic pathways" in vitro in a stepwise fashion, selecting and improving first one step and then the next. The selection strategy is based on the final product of the pathway, so that all earlier steps can be evolved independently or sequentially without setting up a new selection system for each step of the reaction.

Expressed in an alternative manner, there is provided a method of isolating one or more genetic elements encoding a gene product having a desired catalytic activity, comprising the steps of:

(1) expressing genetic elements to give their respective gene products;
(2) allowing the gene products to catalyse conversion of a substrate to a product, which may or may not be directly selectable, in accordance with the desired activity;
(3) optionally coupling the first reaction to one or more subsequent reactions, each reaction being modulated by the product of the previous reactions, and leading to the creation of a final, selectable product;
(4) linking the selectable product of catalysis to the genetic elements by either:
  a) coupling a substrate to the genetic elements in such a way that the product remains associated with the genetic elements, or

b) reacting or binding the selectable product to the genetic elements by way of a suitable molecular "tag" attached to the substrate which remains on the product,

or

c) coupling the selectable product (but not the substrate) to the genetic elements by means of a product-specific reaction or interaction with the product; and

(5) selecting the product of catalysis, together with the genetic element to which it is bound, either by means of its characteristic optical properties, or by adding reagents which specifically bind to, or react specifically with, the product and which thereby induce a change in the optical properties of the genetic elements wherein steps (1) to

(6) each genetic element and respective gene product is contained within a microcapsule.

(iii) Selecting for Enzyme Substrate Specificity/Selectivity

Genetic elements encoding enzymes with substrate specificity or selectivity can be specifically enriched by carrying out a positive selection for reaction with one substrate and a negative selection for reaction with another substrate. Such combined positive and negative selection pressure should be of great importance in isolating regio-selective and stereo-selective enzymes (for example, enzymes that can distinguish between two enantiomers of the same substrate). For example, two substrates (e.g. two different enantiomers) are each labelled with different tags (e.g. two different fluorophores) such that the tags become attached to the genetic element by the enzyme-catalysed reaction. If the two tags confer different optical properties on the genetic element the substrate specificity of the enzyme can be determined from the optical properties of the genetic element and those genetic elements encoding gene products with the wrong (or no) specificity rejected. Tags conferring no change in optical activity can also be used if tag-specific ligands with different optical properties are added (e.g. tag-specific antibodies labelled with different fluorophores).

(iv) Selection for Regulation

A similar system can be used to select for regulatory properties of enzymes.

In the case of selection for a regulator molecule which acts as an activator or inhibitor of a biochemical process, the components of the biochemical process can either be translated in situ in each microcapsule or can be incorporated in the reaction mixture prior to microencapsulation. If the genetic element being selected is to encode an activator, selection can be performed for the product of the regulated reaction, as described above in connection with catalysis. If an inhibitor is desired, selection can be for a chemical property specific to the substrate of the regulated reaction.

There is therefore provided a method of sorting one or more genetic elements coding for a gene product exhibiting a desired regulatory activity, comprising the steps of:

(1) expressing genetic elements to give their respective gene products;

(2) allowing the gene products to activate or inhibit a biochemical reaction, or sequence of coupled reactions, in accordance with the desired activity, in such a way as to allow the generation or survival of a selectable molecule;

(3) linking the selectable molecule to the genetic elements either by

a) having the selectable molecule, or the substrate from which it derives, attached to the genetic elements, or

b) reacting or binding the selectable product to the genetic elements, by way of a suitable molecular "tag" attached to the substrate which remains on the product,

or

c) coupling the product of catalysis (but not the substrate) to the genetic elements, by means of a product-specific reaction or interaction with the product;

(4) selecting the selectable product, together with the genetic element to which it is bound, either by means of its characteristic optical properties, or by adding reagents which specifically bind to, or react specifically with, the product and which thereby induce a change in the optical properties of the genetic elements wherein steps (1) to (3) each genetic element and respective gene product is contained within a microcapsule.

(v) Selection for Optical Properties of the Gene Product

It is possible to select for inherent optical properties of gene products if, in the microcapsules, the gene product binds back to the genetic element, for example through a common element of the gene product which binds to a ligand which is part of the genetic element. After pooling the genetic elements they can then be sorted using the optical properties of the bound gene products. This embodiment can be used, for example, to select variants of green fluorescent protein (GFP) (Cormack et al., 1996; Delagrave et al., 1995; Ehrig et al., 1995), with improved fluorescence and/or novel absoption and emission spectra.

(vi) Screening Using Cells

In the current drug discovery paradigm, validated recombinant targets form the basis of in vitro high-throughput screening (HTS) assays. Isolated genetic constructs or polypeptides cannot, however, be regarded as representative of complex biological systems; hence, cell-based systems can provide greater confidence in compound activity in an intact biological system. A wide range of cell-based assays for drug leads are known to those skilled in the art. Cells can be compartmentalised in microcapsules, such as the aqueous microdroplets of a water-in-oil emulsion (Ghadessy, 2001). The effect of a compound(s) on a target can be determined by compartmentalising a cell (or cells) in a microcapsule together with a genetic element(s) and using an appropriate cell-based assay to identify those compartments containing genetic elements with the desired effect on the cell(s). The use of water-in-fluorocarbon emulsions may be particularly advantageous: the high gas dissolving capacity of fluorocarbons can support the exchange of respiratory gases and has been reported to be beneficial to cell culture systems (Lowe, 2002).

(vii) Flow Analysis and Sorting

In a preferred embodiment of the invention the microcapsules will be analysed and, optionally, sorted by flow cytometry. Many formats of microcapsule can be analysed and, optionally, sorted directly using flow cytometry.

In a highly preferred embodiment, microfluidic devices for flow analysis and, optionally, flow sorting (Fu, 2002) of microcapsules will be used. Such a sorting device can be integrated directly on the microfluidic device, and can use electronic means to sort the microcapsules and/or genetic elements. Optical detection, also integrated directly on the microfluidic device, can be used to screen the microcapsules to trigger the sorting. Other means of control of the microcapsules, in addition to charge, can also be incorporated onto the microfluidic device.

A variety of optical properties can be used for analysis and to trigger sorting, including light scattering (Kerker, 1983) and fluorescence polarisation (Rolland et al., 1985). In a

highly preferred embodiment the difference in optical properties of the microcapsules or microbeads will be a difference in fluorescence and, if required, the microcapsules or microbeads will be sorted using a microfluidic or conventional fluorescence activated cell sorter (Norman, 1980; Mackenzie and Pinder, 1986), or similar device. Flow cytometry has a series of advantages:

(1) fluorescence activated cell sorting equipment from established manufacturers (e.g. Becton-Dickinson, Coulter, Cytomation) allows the analysis and sorting at up to 100,000 microcapsules or microbeads per second.

(2) the fluorescence signal from each microcapsule or microbead corresponds tightly to the number of fluorescent molecules present. As little as few hundred fluorescent molecules per microcapsules or microbeads can be quantitatively detected;

(3) the wide dynamic range of the fluorescence detectors (typically 4 log units) allows easy setting of the stringency of the sorting procedure, thus allowing the recovery of the optimal number microcapsules or microbeads from the starting pool (the gates can be set to separate microcapsules or microbeads with small differences in fluorescence or to only separate out microcapsules or microbeads with large differences in fluorescence, dependant on the selection being performed);

(4) fluorescence-activated cell sorting equipment can perform simultaneous excitation and detection at multiple wavelengths (Shapiro, 1995) allowing positive and negative selections to be performed simultaneously by monitoring the labelling of the microcapsules or microbeads with two to thirteen (or more) fluorescent markers, for example, if substrates for two alternative targets are labelled with different fluorescent tags the microcapsules or microbeads can labelled with different fluorophores dependent on the target regulated.

If the microcapsules or microbeads are optically tagged, flow cytometry can also be used to identify the genetic element or genetic elements in the microcapsule or coated on the microbeads (see below). Optical tagging can also be used to identify the concentration of reagents in the microcapsule (if more than one concentration is used in a single experiment) or the number of compound molecules coated on a microbead (if more than one coating density is used in a single experiment). Furthermore, optical tagging can be used to identify the target in a microcapsule (if more than one target is used in a single experiment). This analysis can be performed simultaneously with measuring activity, after sorting of microcapsules containing microbeads, or after sorting of the microbeads.

(viii) Microcapsule Identification and Sorting

The invention provides for the identification and, optionally, the sorting of intact microcapsules where this is enabled by the sorting techniques being employed. Microcapsules may be identified and, optionally, sorted as such when the change induced by the desired genetic element either occurs or manifests itself at the surface of the microcapsule or is detectable from outside the microcapsule. The change may be caused by the direct action of the gene product, or indirect, in which a series of reactions, one or more of which involve the gene product having the desired activity leads to the change. For example, where the microcapsule is a membranous microcapsule, the microcapsule may be so configured that a component or components of the biochemical system comprising the target are displayed at its surface and thus accessible to reagents which can detect changes in the biochemical system regulated by the gene product within the microcapsule.

In a preferred aspect of the invention, however, microcapsule identification and, optionally, sorting relies on a change in the optical properties of the microcapsule, for example absorption or emission characteristics thereof, for example alteration in the optical properties of the microcapsule resulting from a reaction leading to changes in absorbance, luminescence, phosphorescence or fluorescence associated with the microcapsule. All such preperties are included in the term "optical". In such a case, microcapsules can be identified and, optionally, sorted by luminescence, fluorescence or phosphorescence activated sorting. In a highly preferred embodiment, flow cytometry is employed to analyse and, optionally, sort microcapsules containing gene products having a desired activity which result in the production of a fluorescent molecule in the microcapsule.

The methods of the current invention allow reagents to be mixed rapidly (in <2 ms), hence a spatially-resolved optical image of microcapsules in microfluidic network allows time resolved measurements of the reactions in each microcapsule. Microcapsules can, optionally, be separated using a microfluidic flow sorter to allow recovery and further analysis or manipulation of the molecules they contain. Advantageously, the flow sorter would be an electronic flow sorting device. Such a sorting device can be integrated directly on the microfluidic device, and can use electronic means to sort the microcapsules. Optical detection, also integrated directly on the microfluidic device, can be used to screen the microcapsules to trigger the sorting. Other means of control of the microcapsules, in addition to charge, can also be incorporated onto the microfluidic device.

In an alternative embodiment, a change in microcapsule fluorescence, when identified, is used to trigger the modification of the microbead within the compartment. In a preferred aspect of the invention, microcapsule identification relies on a change in the optical properties of the microcapsule resulting from a reaction leading to luminescence, phosphorescence or fluorescence within the microcapsule. Modification of the microbead within the microcapsules would be triggered by identification of luminescence, phosphorescence or fluorescence. For example, identification of luminescence, phosphorescence or fluorescence can trigger bombardment of the compartment with photons (or other particles or waves) which leads to modification of the microbead or molecules attached to it. A similar procedure has been described previously for the rapid sorting of cells (Keij et al., 1994). Modification of the microbead may result, for example, from coupling a molecular "tag", caged by a photolabile protecting group to the microbeads: bombardment with photons of an appropriate wavelength leads to the removal of the cage. Afterwards, all microcapsules are combined and the microbeads pooled together in one environment. Genetic elements exhibiting the desired activity can be selected by affinity purification using a molecule that specifically binds to, or reacts specifically with, the "tag".

(ix) Flow Sorting of Genetic Elements

In a preferred embodiment of the invention the genetic elements will be sorted by flow cytometry. A variety of optical properties can be used to trigger sorting, including light scattering (Kerker, 1983) and fluorescence polarisation (Rolland et al., 1985). In a highly preferred embodiment the difference in optical properties of the genetic elements will be a difference in fluorescence and the genetic elements will be sorted using a fluorescence activated cell sorter (Norman, 1980; Mackenzie and Pinder, 1986), or similar device. Such a sorting device can be integrated directly on the microfluidic device, and can use electronic means to sort the genetic elements. Optical detection, also integrated directly on the

microfluidic device, can be used to screen the genetic elements to trigger the sorting. Other means of control of the genetic elements, in addition to charge, can also be incorporated onto the microfluidic device. In an especially preferred embodiment the genetic element comprises of a nonfluorescent nonmagnetic (e.g. polystyrene) or paramagnetic microbead (see Formusek and Vetvicka, 1986), optimally 0.6 to 1.0 μm diameter, to which are attached both the gene and the groups involved in generating a fluorescent signal:

(1) commercially available fluorescence activated cell sorting equipment from established manufacturers (e.g. Becton-Dickinson, Coulter) allows the sorting of up to 10^8 genetic elements (events) per hour;

(2) the fluorescence signal from each bead corresponds tightly to the number of fluorescent molecules attached to the bead. At present as little as few hundred fluorescent molecules per particle can be quantitatively detected;

(3) the wide dynamic range of the fluorescence detectors (typically 4 log units) allows easy setting of the stringeney of the sorting procedure, thus allowing the recovery of the optimal number of genetic elements from the starting pool (the gates can be set to separate beads with small differences in fluorescence or to only separate out beads with large differences in fluorescence, dependant on the selection being performed;

(4) commercially available fluorescence-activated cell sorting equipment can perform simultaneous excitation at up to two different wavelengths and detect fluoreseence at up to four different wavelengths (Shapiro, 1983) allowing positive and negative selections to be performed simultaneously by monitoring the labelling of the genetic element with two (or more) different fluorescent markers, for example, if two alternative substrates for an enzyme (e.g. two different enantiomers) are labelled with different fluorescent tags the genetic element can labelled with different fluorophores dependent on the substrate used and only genes encoding enzymes with enantioselectivity selected.

(5) highly uniform derivatised and non-derivatised nonmagnetic and paramagnetic microparticles (beads) are commercially available from many sources (e.g. Sigma, and Molecular Probes) (Formusek and Vetvicka, 1986).

(x) Multi-Step Procedure

It will be also be appreciated that according to the present invention, it is not necessary for all the processes of transcription/replication and/or translation, and selection to proceed in one single step, with all reactions taking place in one microcapsule. The selection procedure may comprise two or more steps. First, transcription/replication and/or translation of each genetic element of a genetic element library may take place in a first microcapsule. Each gene product is then linked to the genetic element which encoded it (which resides in the same microcapsule), for example via a gene product-specific ligand such as an antibody. The microcapsules are then broken, and the genetic elements attached to their respective gene products optionally purified. Alternatively, genetic elements can be attached to their respective gene products using methods which do not rely on encapsulation. For example phage display (Smith, G. P., 1985), polysome display (Mattheakkis et al., 1994), RNA-peptide fusion (Roberts and Szostak, 1997) or lac repressor peptide fusion (Cull, et al., 1992).

In the second step of the procedure, each purified genetic element attached to its gene product is put into a second microcapsule containing components of the reaction to be selected. This reaction is then initiated. After completion of the reactions, the microcapsules are again broken and the modified genetic elements are selected. In the case of complicated multistep reactions in which many individual components and reaction steps are involved, one or more intervening steps may be performed between the initial step of creation and linking of gene product to genetic element, and the final step of generating the selectable change in the genetic element.

If necessary, release of the gene product from the genetic element within a secondary microcapsule can be achieved in a variety of ways, including by specific competition by a low-molecular weight product for the binding site or cleavage of a linker region joining the binding domain of the gene product from the catalytic domain either enzymatically (using specific proteases) or autocatalytically (using an integrin domain).

(xi) Selection by Activation of Reporter Gene Expression in Situ

The system can be configured such that the desired binding, catalytic or regulatory activity encoded by a genetic element leads, directly or indirectly to the activation of expression of a "reporter gene" that is present in all microcapsules. Only gene products with the desired activity activate expression of the reporter gene. The activity resulting from reporter gene expression allows the selection of the genetic element (or of the compartment containing it) by any of the methods described herein.

For example, activation of the reporter gene may be the result of a binding activity of the gene product in a manner analogous to the "two hybrid system" (Fields and Song; 1989). Activation can also result from the product of a reaction catalysed by a desirable gene product. For example, the reaction product can be a transcriptional inducer of the reporter gene. For example arabinose may be used to induce transcription from the araBAD promoter. The activity of the desirable gene product can also result in the modification of a transcription factor, resulting in expression of the reporter gene. For example, if the desired gene product is a kinase or phosphatase the phosphorylation or dephosphorylation of a transcription factor may lead to activation of reporter gene expression.

(xii) Amplification

According to a further aspect of the present invention the method comprises the further step of amplifying the genetic elements. Selective amplification may be used as a means to enrich for genetic elements encoding the desired gene product.

In all the above configurations, genetic material comprised in the genetic elements may be amplified and the process repeated in iterative steps. Amplification may be by the polymerase chain reaction (Saiki et al., 1988) or by using one of a variety of other gene amplification techniques including; Qb replicase amplification (Cahill, Foster and Mahan, 1991; Chetverin and Spirin, 1995; Katanaev, Kurnasov and Spirin, 1995); the ligase chain reaction (LCR) (Landegren et al., 1988; Barany, 1991); the self-sustained sequence replication system (Fahy, Kwoh and Gingeras, 1991) and strand displacement amplification (Walker et al., 1992). Advantageously, the amplification procedure can be performed in a microfluidic device.

(C) Rapid Mixing of Reagents in Microcapsules

Advantageously, after fusion of microcapsules, the reagents contained in the fused microcapsule can be mixed rapidly using chaotic advection by passing the droplets through channels that disrupt the laminar flow lines of the fluid within the droplets, their contents can be rapidly mixed, fully initiating any chemical reactions.

(D) Sensing Microcapsule Characteristics

In certain aspects of the invention, sensors are provided that can sense and/or determine one or more characteristics of the fluidic droplets, and/or a characteristic of a portion of the fluidic system containing the fluidic droplet (e.g., the liquid surrounding the fluidic droplet) in such a manner as to allow the determination of one or more characteristics of the fluidic droplets. Characteristics determinable with respect to the droplet and usable in the invention can be identified by those of ordinary skill in the art. Non-limiting examples of such characteristics include fluorescence, spectroscopy (e.g., optical, infrared, ultraviolet, etc.), radioactivity, mass, volume, density, temperature, viscosity, pH, concentration of a substance, such as a biological substance (e.g., a protein, a nucleic acid, etc.), or the like.

In some cases, the sensor may be connected to a processor, which in turn, causes an operation to be performed on the fluidic droplet, for example, by sorting the droplet, adding or removing electric charge from the droplet, fusing the droplet with another droplet, splitting the droplet, causing mixing to occur within the droplet, etc., for example, as previously described. For instance, in response to a sensor measurement of a fluidic droplet, a processor may cause the fluidic droplet to be split, merged with a second fluidic droplet, sorted etc.

One or more sensors and/or processors may be positioned to be in sensing communication with the fluidic droplet. "Sensing communication," as used herein, means that the sensor may be positioned anywhere such that the fluidic droplet within the fluidic system (e.g., within a channel), and/or a portion of the fluidic system containing the fluidic droplet may be sensed and/or determined in some fashion. For example, the sensor may be in sensing communication with the fluidic droplet and/or the portion of the fluidic system containing the fluidic droplet fluidly, optically or visually, thermally, pneumatically, electronically, or the like. The sensor can be positioned proximate the fluidic system, for example, embedded within or integrally connected to a wall of a channel, or positioned separately from the fluidic system but with physical, electrical, and/or optical communication with the fluidic system so as to be able to sense and/or determine the fluidic droplet and/or a portion of the fluidic system containing the fluidic droplet (e.g., a channel or a microchannel, a liquid containing the fluidic droplet, etc.). For example, a sensor may be free of any physical connection with a channel containing a droplet, but may be positioned so as to detect electromagnetic radiation arising from the droplet or the fluidic system, such as infrared, ultraviolet, or visible light. The electromagnetic radiation may be produced by the droplet, and/or may arise from other portions of the fluidic system (or externally of the fluidic system) and interact with the fluidic droplet and/or the portion of the fluidic system containing the fluidic droplet in such as a manner as to indicate one or more characteristics of the fluidic droplet, for example, through absorption, reflection, diffraction, refraction, fluorescence, phosphorescence, changes in polarity, phase changes, changes with respect to time, etc. As an example, a laser may be directed towards the fluidic droplet and/or the liquid surrounding the fluidic droplet, and the fluorescence of the fluidic droplet and/or the surrounding liquid may be determined. "Sensing communication," as used herein may also be direct or indirect. As an example, light from the fluidic droplet may be directed to a sensor, or directed first through a fiber optic system, a waveguide, etc., before being directed to a sensor.

Non-limiting examples of sensors useful in the invention include optical or electromagnetically-based systems. For example, the sensor may be a fluorescence sensor (e.g., stimulated by a laser), a microscopy system (which may include a camera or other recording device), or the like. As another example, the sensor may be an electronic sensor, e.g., a sensor able to determine an electric field or other electrical characteristic. For example, the sensor may detect capacitance, inductance, etc., of a fluidic droplet and/or the portion of the fluidic system containing the fluidic droplet.

As used herein, a "processor" or a "microprocessor" is any component or device able to receive a signal from one or more sensors, store the signal, and/or direct one or more responses (e.g., as described above), for example, by using a mathematical formula or an electronic or computational circuit. The signal may be any suitable signal indicative of the environmental factor determined by the sensor, for example a pneumatic signal, an electronic signal, an optical signal, a mechanical signal, etc.

As a particular non-limiting example, a device of the invention may contain fluidic droplets containing one or more cells. The desired activity of one or more gene products may result in the expression (or inhibition of expression) of a 'marker' gene, for example a gene for green fluorescent protein (GFP). The cells may be exposed to a fluorescent signal marker that binds if a certain condition is present, for example, the marker may bind to a first cell type but not a second cell type, the marker may bind to an expressed protein, the marker may indicate viability of the cell (i.e., if the cell is alive or dead), the marker may be indicative of the state of development or differentiation of the cell, etc., and the cells may be directed through a fluidic system of the invention based on the presence/absence, and/or magnitude of the fluorescent signal marker. For instance, determination of the fluorescent signal marker may cause the cells to be directed to one region of the device (e.g., a collection chamber), while the absence of the fluorescent signal marker may cause the cells to be directed to another region of the device (e.g., a waste chamber). Thus, in this example, a population of cells may be screened and/or sorted on the basis of one or more determinable or targetable characteristics of the cells, for example, to select live cells, cells expressing a certain protein, a certain cell type, etc.

(E) Materials

A variety of materials and methods, according to certain aspects of the invention, can be used to form any of the above-described components of the microfluidic systems and devices of the invention. In some cases, the various materials selected lend themselves to various methods. For example, various components of the invention can be formed from solid materials, in which the channels can be formed via micromachining, film deposition processes such as spin coating and chemical vapor deposition, laser fabrication, photolithographic techniques, etching methods including wet chemical or plasma processes, and the like. See, for example, *Scientific American,* 248:44-55, 1983 (Angell, et al). In one embodiment, at least a portion of the fluidic system is formed of silicon by etching features in a silicon chip. Technologies for precise and efficient fabrication of various fluidic systems and devices of the invention from silicon are known. In another embodiment, various components of the systems and devices of the invention can be formed of a polymer, for example, an elastomeric polymer such as polydimethylsiloxane ("PDMS"), polytetrafluoroethylene ("PTFE" or Teflon®), or the like.

Different components can be fabricated of different materials. For example, a base portion including a bottom wall and side walls can be fabricated from an opaque material such as silicon or PDMS, and a top portion can be fabricated from a transparent or at least partially transparent material, such as glass or a transparent polymer, for observation and/or control of the fluidic process. Components can be coated so as to

expose a desired chemical functionality to fluids that contact interior channel walls, where the base supporting material does not have a precise, desired functionality. For example, components can be fabricated as illustrated, with interior channel walls coated with another material. Material used to fabricate various components of the systems and devices of the invention, e.g., materials used to coat interior walls of fluid channels, may desirably be selected from among those materials that will not adversely affect or be affected by fluid flowing through the fluidic system, e.g., material(s) that is chemically inert in the presence of fluids to be used within the device.

In one embodiment, various components of the invention are fabricated from polymeric and/or flexible and/or elastomeric materials, and can be conveniently formed of a hardenable fluid, facilitating fabrication via molding (e.g. replica molding, injection molding, cast molding, etc.). The hardenable fluid can be essentially any fluid that can be induced to solidify, or that spontaneously solidifies, into a solid capable of containing and/or transporting fluids contemplated for use in and with the fluidic network. In one embodiment, the hardenable fluid comprises a polymeric liquid or a liquid polymeric precursor (i.e. a "prepolymer"). Suitable polymeric liquids can include, for example, thermoplastic polymers, thermoset polymers, or mixture of such polymers heated above their melting point. As another example, a suitable polymeric liquid may include a solution of one or more polymers in a suitable solvent, which solution forms a solid polymeric material upon removal of the solvent, for example, by evaporation. Such polymeric naaterials, which can be solidified from, for example, a melt state or by solvent evaporation, are well known to those of ordinary skill in the art. A variety of polymeric materials, many of which are elastomeric, are suitable, and are also suitable for forming molds or mold masters, for embodiments where one or both of the mold masters is composed of an elastomeric material. A non-limiting list of examples of such polymers includes polymers of the general classes of silicone polymers, epoxy polymers, and acrylate polymers. Epoxy polymers are characterized by the presence of a three-membered cyclic ether group commonly referred to as an epoxy group, 1,2-epoxide, or oxirane. For example, diglycidyl ethers of bisphenol A can be used, in addition to compounds based on aromatic amine, triazine, and cycloaliphatic backbones. Another example includes the well-known Novolac polymers. Non-limiting examples of silicone elastomers suitable for use according to the invention include those formed from precursors including the chlorosilanes such as methylchlorosilanes, ethylchlorosilanes, phenylchlorosilanes, etc.

Silicone polymers are preferred in one set of embodiments, for example, the silicone elastomer polydimethylsiloxane. Non-limiting examples of PDMS polymers include those sold under the trademark Sylgard by Dow Chemical Co., Midland, Mich., and particularly Sylgard 182, Sylgard 184, and Sylgard 186. Silicone polymers including PDMS have several beneficial properties simplifying fabrication of the microfluidic structures of the invention. For instance, such materials are inexpensive, readily available, and can be solidified from a prepolymeric liquid via curing with heat. For example, PDMSs are typically curable by exposure of the prepolymeric liquid to temperatures of about, for example, about 65° C. to about 75° C. for exposure times of, for example, about an hour. Also, silicone polymers, such as PDMS, can be elastomeric and thus may be useful for forming very small features with relatively high aspect ratios, necessary in certain embodiments of the invention. Flexible (e.g., elastomeric) molds or masters can be advantageous in this regard.

One advantage of forming structures such as microfluidic structures of the invention from silicone polymers, such as PDMS, is the ability of such polymers to be oxidized, for example by exposure to an oxygen-containing plasma such as an air plasma, so that the oxidized structures contain, at their surface, chemical groups capable of cross-linking to other oxidized silicone polymer surfaces or to the oxidized surfaces of a variety of other polymeric and non-polymeric materials. Thus, components can be fabricated and then oxidized and essentially irreversibly sealed to other silicone polymer surfaces, or to the surfaces of other substrates reactive with the oxidized silicone polymer surfaces, without the need for separate adhesives or other sealing means. In most cases, sealing can be completed simply by contacting an oxidized silicone surface to another surface without the need to apply auxiliary pressure to form the seal. That is, the pre-oxidized silicone surface acts as a contact adhesive against suitable mating surfaces. Specifically, in addition to being irreversibly sealable to itself, oxidized silicone such as oxidized PDMS can also be sealed irreversibly to a range of oxidized materials other than itself including, for example, glass, silicon, silicon oxide, quartz, silicon nitride, polyethylene, polystyrene, glassy carbon, and epoxy polymers, which have been oxidized in a similar fashion to the PDMS surface (for example, via exposure to an oxygen-containing plasma). Oxidation and sealing methods useful in the context of the present invention, as well as overall molding techniques, are described in the art, for example, in an article entitled "Rapid Prototyping of Microfluidic Systems and Polydimethylsiloxane," *Anal. Chem.,* 70:474-480, 1998 (Duffy et al.), incorporated herein by reference.

Another advantage to forming microfluidic structures of the invention (or interior, fluid contacting surfaces) from oxidized silicone polymers is that these surfaces can be much more hydrophilic than the surfaces of typical elastomeric polymers (where a hydrophilic interior surface is desired). Such hydrophilic channel surfaces can thus be more easily filled and wetted with aqueous solutions than can structures comprised of typical, unoxidized elastomeric polymers or other hydrophobic materials.

In one embodiment, a bottom wall is formed of a material different from one or more side walls or a top wall, or other components. For example, the interior surface of a bottom wall can comprise the surface of a silicon wafer or microchip, or other substrate. Other components can, as described above, be sealed to such alternative substrates. Where it is desired to seal a component comprising a silicone polymer (e.g. PDMS) to a substrate (bottom wall) of different material, the substrate may be selected from the group of materials to which oxidized silicone polymer is able to irreversibly seal (e.g., glass, silicon, silicon oxide, quartz, silicon nitride, polyethylene, polystyrene, epoxy polymers, and glassy carbon surfaces which have been oxidized). Alternatively, other sealing techniques can be used, as would be apparent to those of ordinary skill in the art, including, but not limited to, the use of separate adhesives, thermal bonding, solvent bonding, ultrasonic welding, etc.

Various aspects and embodiments of the present invention are illustrated in the following examples. It will be appreciated that modification of detail may be made without departing from the scope of the invention.

All documents mentioned in the text are incorporated by reference.

EXAMPLES

Example 1

Microfluidic Device for Selection of Genes Using In Vitro Compartmentalisation

A schematic representation of the microfluidic device is shown in FIG. **15**. Microchannels are fabricated with rectangular cross-sections using rapid prototyping in poly(dimethylsiloxane) (PDMS) (McDonald and Whitesides, 2002) and rendered hydrophobic as (Song and Ismagilov, 2003). Syringe pumps were used to drive flows (Harvard Apparatus PHD 2000 Infusion pumps). For aqueous solutions, 250 μi Hamilton Gastight syringes (1700 series, TLL) with removeable needles of 27-gauge are used with 30-gauge Teflon tubing (WeiCo Wire and Cable). For the carrier fluid, 1 ml Hamilton Gastight syringes (1700 series, TLL) are used with 30-gauge Teflon needles with one hub from Hamilton (Song and Ismagilov, 2003). The carrier fluid is 9% (v/v) $C_6F_{11}C_2H_4OH$ in perfluorodecaline (PFD) (Song et al., 2003). The microfluidic device consists of a series of interconnected modules. Each module has a specific function. These include modules that will produce droplets, fuse droplets, mix droplets, react droplets, detect droplets, and 20 sort droplets (see FIG. **16**). In one example, droplets are made, consisting of different molecules or different concentrations of molecules. Droplets are made at rates of up to 10^4 sec^{-1}, and are made with a polydispersity of less than 1.5% and with sizes ranging from 1 μm to 100 μm. Each droplet is fused with a second droplet containing a second set of reactants, and is rapidly mixed to initiate the chemical reaction. This chemical reaction is allowed to proceed in each droplet by passing it through a delay channel. Each droplet is then fused with another droplet containing a second set of reactants, and is subsequently rapidly mixed to initiate the second set of chemical reactions. After the second reaction has proceeded in a delay module, the results of the reaction is determined using an optical sensor or other form of detection module. Finally, the desired droplets are sorted into two populations based on signal form the optical detection module, one population is kept for further processing and the other discarded. These and other modules can be used in this combination, or in other combinations.

Droplet Generation Module:

We use a flow-focusing geometry to form the drops. A water stream is infused from one channel through a narrow constriction; counter propagating oil streams hydrodynamically focus the water stream reducing its size as it passes through the constriction as shown in FIG. **17**A. This droplet generator can be operated in a flow regime that produces a steady stream of uniform droplets of water in oil. The size of the water droplets is controlled by the relative flow rates of the oil and the water; the viscous forces overcome surface tension to create uniform droplets. If the flow rate of the water is too high a longer jet of fluid passes through the orifice and breaks up into droplets further down stream; these droplets are less uniform in size. If the flow rate of the water is too low, the droplet breakup in the orifice becomes irregular again, producing a wider range of droplet sizes. While this emulsification technology is robust, it is limited to producing droplets of one size at any given flow rate; this droplet size is largely determined by the channel dimensions. Moreover, the timing of the droplet production cannot be controlled.

We overcome these limitations by incorporating electric fields to create an electrically addressable emulsification system. To achieve this, we apply high voltage to the aqueous stream and charge the oil water interface, as shown schematically in FIG. **17**A. The water stream behaves as a conductor while the oil is an insulator; electrochemical reactions charge the fluid interface like a capacitor. At snap-off, charge on the interface remains on the droplet. In addition, the droplet volume, V_d, and frequency, f, can be tailored over nearly three orders of magnitude without changing the infusion rate of the oil or water. Droplet size and frequency are not independent; instead their product is determined by the infusion rate of the dispersed phase $Q_d=fV_d$. The droplet size decreases with increasing field strength, as shown in FIG. **17**, B to E. The dependence of the droplet size on applied voltage for three different flow rates is summarized in FIG. **17**F. At low applied voltages the electric field has a negligible effect, and droplet formation is driven exclusively by the competition between surface tension and viscous flow. By contrast, at high electric field strengths, there is a significant additional force on the growing drop, F=qE, where q is the charge on the droplet. Since the droplet interface behaves as a capacitor, q is proportional to the applied voltage, V. This leads to a V^2 dependence of the force, which accounts for the decrease in droplet size with increasing applied field shown in FIG. **17**F. If the electric field becomes too large, the charged interface of the water stream is repelled by the highly charged drops; this destabilizes the production and increases the variation in droplet size.

The electronic control afforded by the field-induced droplet formation provides an additional valuable benefit: it allows the phase of the droplet break-off to be adjusted within the production cycle. This is accomplished by increasing the field above the critical break-off field only at the instant the droplet is required. This provides a convenient means to precisely synchronize the production and arrival of individual droplets at specific locations.

Droplet Coalesces Module:

An essential component in any droplet-based reaction confinement system is a droplet coalescing module which combines two or more reagents to initiate a chemical reaction. This is particularly difficult to achieve in a microfluidic device because surface tension, surfactant stabilization, and drainage forces all hinder droplet coalescence; moreover, the droplets must cross the stream lines that define their respective flows and must be perfectly synchronized to arrive at a precise location for coalescence.

Use of electrostatic charge overcomes these difficulties; placing charges of opposite sign on each droplet and applying an electric field forces them to coalesce. As an example we show a device consisting of two separate nozzles that generate droplets with different compositions and opposite charges, sketched in FIG. **18**A. The droplets are brought together at the confluence of the two streams. The electrodes used to charge the droplets upon formation also provide the electric field to force the droplets across the stream lines, leading to coalesce. Slight variations in the structure of the two nozzles result in slight differences in the frequency and phase of their droplet generation in the absence of a field. Thus the droplets differ in size even though the infusion rates are identical. Moreover, the droplets do not arrive at the point of confluence at exactly the same time. As a result the droplets do not coalesce as shown in FIG. **18**B. By contrast, upon application of an electric field, droplet formation becomes exactly synchronized, ensuring that pairs of identically sized droplets each reach the point of confluence simultaneously. Moreover, the droplets are oppositely charged, forcing them to traverse the stream

lines and contact each other, thereby causing them to coalesce, as shown in FIG. **18**C. The remarkable synchronization of the droplet formation results from coupling of the break-off of each of the pair of droplets as mediated by the electric field; the magnitude of the electric field varies as the separation between the leading edges of the two droplets changes and the frequency of droplet break-off is mode-locked to the electric field. A minimum charge is required to cause droplets to coalesce, presumably because of the stabilizing effects of the surfactant coating; this is clear from FIG. **18**D which shows the voltage dependence of the percentage of drops that contact each other that actually coalesce.

Droplet Mixer Module:

Rapid mixing is achieved through either successive iterations of translation and rotation, FIG. **19**, or by coalescing drops along the direction parallel to the flow direction, FIG. **20**.

Droplet Reactor/Time Delay Module:

A delay line is used to provide a fixed time for a reaction. Two non-limiting examples of how this can be achieved are 'single file' and 'large cross-section' channels. The 'single file' delay line uses length to achieve a fixed reaction time. As this often results in exceptionally long channels, it is desirable to place spacer droplets of a third fluid, immicible with both the carrier oil and the aqueous droplets inbetween aqueous droplet pairs. There is then an alternation between aqueous and non-aqueous droplets in a carrier oil. This is shown in FIG. **21**A. A second possibility for achieving a long time delay is to use wide and deap channel having a 'large cross-sectiononal area' to slow the average velocity of the droplets. An example of this is shown in FIG. **21**B.

Recharging Module:

The use of oppositely charged droplets and an electric field to combine and mix reagents is extremely robust, and 100% of the droplets coalesce with their partner from the opposite stream. However, after they coalesce the resultant drops carry no electrostatic charge. While it is convenient to charge droplets during formation, other methods must be employed in any robust droplet-based micro fluidic system to recharge the mixed droplets if necessary for further processing. This is readily accomplished through the use of extensional flow to split neutral droplets in the presence of an electric field which polarizes them, resulting in two oppositely charged daughter droplets; this is sketched in FIG. **22**A. The photomicrograph in FIG. **22**B shows neutral droplets entering a bifurcation and splitting into charged daughter droplets. The dashed region in FIG. **22**B is enlarged in FIG. **22**C to illustrate the asymmetric stretching of the charged droplets in the electric field. The vertical dashed lines indicate the edges of the electrodes where the droplets return to their symmetric spherical shape. The electric field also allows precision control of the droplet splitting providing the basis for a robust droplet division module which allows the splitting of the contents into two or more aliquots of identical reagent, facilitating multiple assays on the contents of the same microreactor.

Detection Module:

The detection module consists of an optical fiber, one or more laser, one or more dichroic beam splitter, bandpass filters, and one or more photo multiplying tube (PMT) as sketched in FIG. **23**.

Sorting Module:

The C.ontents of individual droplets must be probed, and selected droplets sorted into discreet streams. The use of electrostatic charging of droplets provides a means for sorting that can be precisely controlled, can be switched at high frequencies, and requires no moving parts. Electrostatic charge on the droplets enables drop-by-drop sorting based on the linear coupling of charge to an external electric field. As an example, a T-junction bifurcation that splits the flow of carrier fluid equally will also randomly split the droplet population equally into the two streams, as shown in FIG. **24**A. However, a small electric field applied at the bifurcation precisely dictates which channel the drops enter; a schematic of the electrode configuration is shown in FIG. **24**B. Varying the direction of the field varies the direction of the sorted droplets as shown in FIGS. **24**C and **24**D. The large forces that can be imparted on the droplets and the high switching frequency make this a fast and robust sorting engine with no moving parts; thus the processing rate is limited only by the rate of droplet generation.

Example 2

Enrichment of lacZ Genes from a Pool of Mutant lacZ Genes Based on β-Galactosidase Activity Inside Aqueous Droplets in a Microlluidic Device

An example is given how single genes encoding enzymes with a desired activity can be selected from a pool of genes by making and manipulating aqueous droplets using the microfluidic device described in Example 1. It is demonstrated that lacZ genes encoding for active β-galactosidase enzyme can be selected from a pool of mutant lacZ genes by:

(1) forming droplets containing (a) a coupled in vitro transcription/translation system and (b) genes; (2) fusing droplets (a) and (b) to initiate translation with the concentration of genes such that the majority of combined droplets (c) contain no more than one gene per droplet; (3) passing the combined droplets (c) down a microfluidic channel to allow translation; (4) fusing each droplet (c) with a droplet (d) which contains an inhibitor of translation (puromycin) and the fluorogenic substrate, fluorescein digalactoside (FDG); (5) passing the combined droplets (e) down a microfluidic channel to allow catalysis and; (6) monitoring the fluorescence of the droplets. When the gene present in the aqueous droplet encodes for an active β-galactosidase enzyme, FDG inside the compartment will be converted into the fluorescent product fluorescein (excitation 488 nm, emission 514 nm). After a single round of selection, lacZ genes can be enriched from a mixture of genes by over 100-fold.

DNA Preparation

The lacZ gene encoding for β-galactosidase is amplified from genomic DNA isolated from strain BL21 of *Escherichia coli* using primers GALBA and GALFO (GALBA: 5'-CAGACTGCACCATGGCCATGATTACGGATTCACTGGCCGTCGTTTTAC-3' (SEQ ID NO: 1); GALFO: 5'-ACGATGTCAGGATCCTTATTATTTTTGACACCAGACCAACTG GTAA TGGTAG-3' (SEQ ID NO: 2)) The PCR product is digested with restriction endonueleases NcoI and BamHI (New England Biolabs Inc., Beverly, Mass., USA). Digested DNA is gel purified and ligated into vector pIVEX2.2b (Roche Biochemicals GmbH, Mannheim, Germany) that is digested with the same enzymes. The ligation product is transformed into XL-10 gold cells (Stratagene). Minicultures are grown from 5 single colonies in 3 ml LB medium supplemented with 100 μg/ml ampicillin at 37° C. over night. From these overnight cultures, plasmid DNA (pDNA) is isolated and sequenced for the presence of the right insert. Linear DNA constructs are generated by PCR using pDNA from a sequenced clone (containing the correct lacZ sequence) as template and primers LMB2-10E (5'-GATGGCGCCCAACAGTCC-3' (SEQ ID NO: 3)) and PIVB-4 (5'-TTTGGCCGCCGCCCAGT-3' (SEQ ID NO: 4)).

Full-length mutant lacZ (lacZmut), which has an internal frameshift and hence does not encode an active β-galactosidase, is obtained by cutting pIVEX2.2b-LacZ with restriction enzyme SacI (NEB). Digested DNA is blunted by ineubation for 15 min at 12° C. with T4 DNA polymerase (2 U) and dNTPs (500 μM final concentration). The reaction is quenched by adding EDTA to a final concentration of 10 mM and. heating to 75° C. for 20 minutes. Blunted DNA is purified and self-ligated with T4 DNA ligase (1 Weiss unit) in the presence of 5% PEG 4,000 by incubating for 2 hrs at 22° C. pDNA is directly transformed into XL-10 Gold cells. Minicultures are grown from 5 single colonies in 3 ml LB medium supplemented with 100 μg/ml ampicillin at 37° C. over night and plasmid DNA is isolated. pDNA is digested with SacI and one of the clones lacking the internal SacI site is used to generate linear DNA constructs as described above.

In Vitro Transcription and Translation Inside Aqueous Droplets in a Microfluidic System

LacZ and lacZmut linear DNA constructs are mixed at a molar ratio of 1:5, 1:100 and 1:1000, respectively in nuclease-free water.

A commercial in vitro translation system (EcoProT7, Novagen/EMD biosciences Ltd, Madison, Wi, USA) is used according to the manufacturer's protocol. Using the device described in Example 1, EcoProT7 extract is compartmentalised into droplets (a) of mean μm diameter (520 fl volume). Droplets (b), of mean 7.4 μm diameter (220 fl volume) are formed containing 0.67 mM $_L$-methionine and 0.25 mM 7-hydroxycoumarin-3-carboxylic acid (Sigma Aldrich) (excitation 386 nm, emission 448 nm), and 0.75 pM DNA (mixes of LacZ and lacZmut linear DNA at the ratios described above) in nuclease-free water. The droplets are formed in a carrier fluid consisting of perfluorinated oil; the perfluorinated oil can either consist of the mixture described in example 1 or alternatively one of the 3M™ Fluorinert™ liquids. Each droplet (a) is fused with a droplet (b). The concentration of DNA is such that the majority of combined droplets (c) contain no more than one gene per droplet (the mean number of genes per droplet=0.1). According to the Poisson Distribution, $P(a)=e^{-m}$ $[m^a/a!]$, where m=0.1=the mean number of genes per droplet, and P(a)=the probability of finding a genes per droplet, 90.5% of droplets contain no genes, 9.05% contain 1 gene, and 0.45% contain 2 genes and 0.016% contain more than two genes). The combined droplets (c) are passed down the microfluidic channel held at 30° C. for 30 minutes to allow in vitro transcription and translation.

Screening and Selection for β-Galactosidase Activity

After the translation step, a series of droplets (d) of 11.2 μm diameter (740 fl volume, equal in volume to droplets (c)) and which contain 4 mM puromycin (to stop translation) and 1 mM FDG (Molecular Probes) in water. Each droplet (c) is fused with a droplet (d) to stop translation and start the catalytic reaction. The combined droplets (e) are passed down the microfluidic channel held at 30° C. for 10 minutes to allow catalysis. The fluorescence of the droplets is monitored. All droplets contain 7-hydroxycoumarin-3-carboxylic acid allowing their identification. Monitoring of the fluoroscence signal from individual droplets is achieved by coupling both excitation and fluorescent signals to the droplets through an optical fiber. The continuous wave emission from two diode lasers (363 nm and 488 nm) is used for excitation dichroic beam splitters and band pass filters (450±20 nm and 530±20 nm) are used to isolate the fluorescent emission to detect the 7-hydroxycoumarin-3-carboxylic acid fluorescence and the fluorescein fluorescence as measured with photomultiplying tubes. Droplets with the highest fluorescein fluorescence (with a sorting gate set such that less than 0.05% of the population of droplets from a negative control without DNA) are sorted. For each sort, 10,000 droplets are collected.

DNA Recovery from Sorted Droplets

DNA from the sorted droplets is precipitated by adding 100 μl 0.3 M sodium acetate pH 5.2 and 70 μl isopropanol in the presence of 20 μg glycogen as carrier (Roche 20 Biochemicals GmbH, Mannheim, Germany). DNA is pelleted by centrifugation at 20,000×g for 15 rain at 4° C. Precipitated DNA is ished twice with 100 μl 70% ethanol and the DNA pellet is dried using a Speedvac (Eppendorf). DNA is resuspended into 10 μl nuclease-free water.

PCR Amplification of- Recovered DNA

PCR reactions are set up at 50 μl total volume, using Expand Long Template PCR mix with buffer 1 according to the manufacturer's protocol (Roche). Primers LMB2-11E (5'-GCCCGATCTTCCCCATCGG-3' (SEQ ID NO: 5)) and PIVB-8 (5'-CACACCCGTCCTGTGGA-3' (SEQ ID NO: 6)) are used at a concentration of 300 μM each. Reactions are incubated for 2 min at 94° C. and subsequently subjected to 10 cycles at 94° C., 15 s; 55° C., 30 s; 68° C., 2 min, another 22 cycles with an increment in elongation time of 10 s/cycle and a final incubation step for 7 min at 68° C. PCR products are purified using a Wizard PCR prep kit (Promega).

SacI, Digestion of PCR Products

To be able to distinguish between lacZ DNA and lacZmut DNA, purified PCR products are digested with 20 U of SacI enzyme. SacI cuts the lacZ gene but not lacZmut. SacI enzyme is heat-inactivated (15 min at 65° C.) and 5 μl of digested DNA is loaded onto a 1% agarose gel in TAE. DNA is electrophoresed at 5V/cm. DNA is visualized by staining with ethidium bromide and quantified using ImageQuant TL gel analysis software (Amersham Biosciences).

Genes encoding an active β-galactosidase (lacZ genes) are significantly enriched from a pool of mutant genes (lacZmut genes) encoding an inactive β-galactosidase with all ratios of lacZ:lacZmut tested. With an initial gene concentration of 0.1% lacZ genes, the lacZ genes could be enriched over 100-fold in a single round of selection.

Example 3

Mutants with Improved β-Galactosidase Activity can be Selected from a Random Mutagenesis Library of Evolved β-Galactosidase (Ebg) Using Compartmentalisation of Genes in Aqueous Droplets in a Microfluidic Device

The gene encoding for evolved β-galactosidase (Ebg) is often used as a model to study the evolution of novel enzyme functions within an organism. The wild type ebgA gene of *Escherichia coli* encodes an enzyme—with feeble β-galactosidase activity, but ebgA has the potential to evolve sufficient activity to replace the lacZ gene for growth on the sugars lactose and lactulose. Genetic analysis of these mutants has revealed that only two amino acid replacements account for the drastic increase in β-galactosidase activity.

Here we show that similar mutants can be obtained in vitro by creating a random mutagenesis library of the ebg gene and subjecting them to selection for β-galactosidase activity by making and manipulating aqueous droplets using the microfluidic device described in Example 1.

Error Prone Mutagenesis of EbgAC Using Base Analogues

A gene segment encoding for the A domain and the C domain of evolved β-galactosidase enzyme is amplified from genomic DNA of *E. coli* strain BL21 using primers EbgACFw (5'-CAGACTGCACCGCGGGAT-

GAATCGCTGGGAAAACATTCAGC-3' (SEQ ID NO: 7)) and EbgACBw (5'-GCGAGGAGCTCTTATTGTTATG-GAAATAACCATCTTCG-3' (SEQ ID NO: 8)). The PCR product is cloned into vector pIVEX2.2b using restriction endonucleases SacIi and SacI (NEB). DNA is transfected into XLIO-gold cells and single colonies are screened for the presence of the EbgAC gene construct with the right nucleotide sequence. pDNA from a single clone with the right EbgAC gene sequence is used as template to generate a random mutagenesis library using nucleoside analogues essentially as described by Zaccolo et al. (*J Mol Biol* 255(4): 589-603, 1996). A mixture of the 5'-triphosphates of 6-(2-deoxy-b-D-ribofuranosyl)-3,4-dihydro-8H-pyrimido-[4,5-C][1,2]oxazin-7-one (dPTP) and of 8-oxo-2' deoxyguanosine (8-oxodG) is prepared in PCR grade water at 2 mM and 10 mM concentrations, respectively. This base analogue mix is diluted 167× and 333× in expand long template PCR buffer 1 (Roche); containing $MgCl_2$ (2 mM), dNTPs (500 μM), expand long template PCR polymerase enzyme mix (Roche), primer LMB2-9E (5'-GCATTTATCAGGGTTATTGTC-3 (SEQ ID NO: 9); 500 nM') and triple biotinylated primer PIVB-1 (5'-3Bi-GCGTTGATGCAATTTCT-3' (SEQ ID NO: 10); 500 nM) in a total reaction volume of 50 μl. Five nanograms of pIVEX2.2b-EbgAC DNA is added and samples are subjected to 1 cycle of 2 minutes at 94° C., followed by 3 cycles at 94° C., 1 min; at 50° C., 1 min; at 68° C., 4 min), followed by a final extension of 7 min at 68° C. Ten micrograms of molecular biology-grade glycogen is added to the DNA prior to purification using a Qiaquick PCR purification kit. After purification DNA is recovered in 50 μl PCR-grade water. Ten micrograms of Streptavidin-coated magnetic beads (Dynabeads M-280 streptavidin, Dynal Biotech, Oslo, Norway) are rinsed in 2× binding buffer provided with the beads, resuspended into 50 μl 2× binding buffer and added to the purified DNA. Beads and DNA are incubated for 2.5 hrs at room temperature in a rotating device. Beads are collected with a magnet and rinsed twice with ish buffer that is provided with the beads and twice with PCR-grade water. Finally, beads are resuspended into 25 μl water. 5 ml of bead-bound DNA is used as template in a second PCR reaction (25 cycles of 15 s at 94° C., 30 s at 55° C. and 2 min at 68° C.). PCR product is purified using a Qiaquick PCR purification kit and recovered in 50 μl of PCR-grade water.

Iterative Rounds of In Vitro Selection Using a Microfluidic System

The generated random mutagenesis library of ebgAC is subjected to 2 successive rounds of selection. Each selection round consisted of 7 separate steps: (1) forming droplets containing (a) a coupled in vitro transcription/translation system and (b) genes; (2) fusing droplets (a) and (b) to initiate translation with the concentration of genes such that the majority of combined droplets (c) contain no more than one gene per droplet; (3) passing the combined droplets (c) down a microfluidic channel to allow translation; (4) fusing each droplet (c) with a droplet (d) which contains an inhibitor of translation (puromycin) and the fluorogenic substrate, fluorescein digalactoside (FDG); (5) passing the combined droplets (e) down a microfluidic channel to allow catalysis; (6) monitoring the fluorescence of the droplets. When the gene present in the aqueous droplet encodes for an active β-galactosidase enzyme, FDG inside the compartment will be converted into the fluorescent product fluorescein (excitation 488 nm, emission 514 nm) and; (7) recovery and amplification of genes from the selected double emulsion droplets. The entire procedure is described in detail above (Example 2). Sets of nested primers are used for subsequent selection rounds (Table 1).

TABLE 1

list of primers used to amplify recovered DNA from successive rounds of selection

Selection round	Forward primer	Backward primer
0	LMB2-9E 5'-GCATTTATCAGGG TTATTGTC-3' (SEQ ID NO: 11)	PIVB-1 5'-GCGTTGATGCAA TTTCT-3' (SEQ ID NO: 12)
1	LMB2-10E 5'-GATGGCGCCCAAC AGTCC-3' (SEQ ID NO: 13)	PIVB-4 5'-TTTGGCCGCCGC CCAGT-3' (SEQ ID NO: 14)
2	LMB2-11 5'-ATGCGTCCGGCGT AGAGG-3' (SEQ ID NO: 15)	PIVB-11 5'-AGCAGCCAACTC AGCTTCC-3' (SEQ ID NO: 16)

After each selection round, the number of positive droplets within the Ebg library increased by at least 10-fold.

Characterisation of the β-Galactosidase Activity of Single Members of the Ebg Library

After the 2^{nd} selection round, DNA is recovered from the double emulsions by standard isopropanol precipitation and PCR amplified using primers LMB2-11 and PIVB-11. Amplified DNA is digested with restriction endonucleases SacI and SacII and cloned into pIVEX2.2b that is digested with the same enzymes. The ligation product is transformed into ElectroBlue electrocompetent cells (Strategene) by electroporation (at 17 kV/cm, 600Ω, 25 μF) and plated onto LB agar plates with ampicillin. Ebg gene constructs are amplified from single colonies by colony PCR using primers LMB2-10E and PIVB-4. One microliter of PCR product is added fo 14 μl of WT mix (Novagen's EcoProT7 extract, supplemented with 200 μM L-methionine) and incubated for 90 min at 30° C. Forty microliters substrate solution (250 μM FDG, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT and 100 μg/ml BSA in 10 mM Tris-HCl, pH 7.9) is added and the conversion of FDG into fluorescein is monitored every 45 s for 90 min at 37° C.

The screened clones show a broad variety of β-galactosidase activities. ~50% of colonies have β-galactosidase activities that are comparable to or lower than wild type Ebg. ~12.5% of clones show β-galactosidase activity that is comparable to the Class I and Class II mutants (single point mutations) described by Hall et al. (*FEMS Microbiol Lett* 174(1): 1-8, 1999; *Genetica* 118(2-3): 143-56, 2003). In conclusion, the system described here can be used for the selection of ebg variants with improved β-galactosidase activity from a large gene library.

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Zaug, A. J. and Cech, T. R. (1986) Science, 231, 470-5.

Zaug, A. J., Been, M. D. and Cech, T. R. (1986) Nature, 324, 429-33.

Zubay, G. (1973) Annu Rev Genet, 7, 267-87.

Zubay, G. (1980) Methods Enzyinol, 65, 856-77.

All publications mentioned in the above specification, and references cited in said publications, are herein incorporated, by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be-unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claim.

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The invention claimed is:

1. A method for detecting a product of an enzymatic reaction, comprising the steps of:

providing a droplet generator to produce, under microfluidic control, a plurality of aqueous microcapsules surrounded by an immiscible continuous phase that comprises a fluorinated oil that comprises a fluorinated polymer surfactant, each of the plurality of microcapsules comprising an enzyme, a genetic element, and reagents for the enzymatic reaction;

pooling the microcapsules into one or more common compartments such that a portion of the plurality of microcapsules contact each other but do not fuse with each other due to the presence of the surfactant;

conducting the enzymatic reaction on the genetic element of at least one of the plurality of microcapsules within the one or more common compartments; and

detecting the product of the enzymatic reaction.

2. The method of claim **1**, wherein the genetic elements are nucleic acids, proteins, or cells.

3. The method of claim 2, wherein the nucleic acids are primers for a polymerase chain reaction (PCR).

4. The method of claim 1, wherein the genetic elements are labeled.

5. The method of claim 4, wherein the genetic elements are optically labeled.

6. The method of claim 4, wherein the genetic elements are fluorescently labeled antibodies.

7. The method of claim 4, wherein the genetic elements are chemically labeled antibodies.

8. The method of claim 1, wherein the microcapsules are monodisperse with respect to each other.

9. The method of claim 1, wherein the droplet generator further comprises an aqueous fluid channel and two immiscible continuous phase channels and producing the plurality of microcapsules surrounded by the immiscible continuous phase under the microfluidic control comprises partitioning an aqueous fluid that is flowing through the aqueous fluid channel with two counter propagating streams of the immiscible continuous phase that are flowing through the immiscible continuous phase channels.

* * * * *

EXHIBIT 2

10x GENOMICS

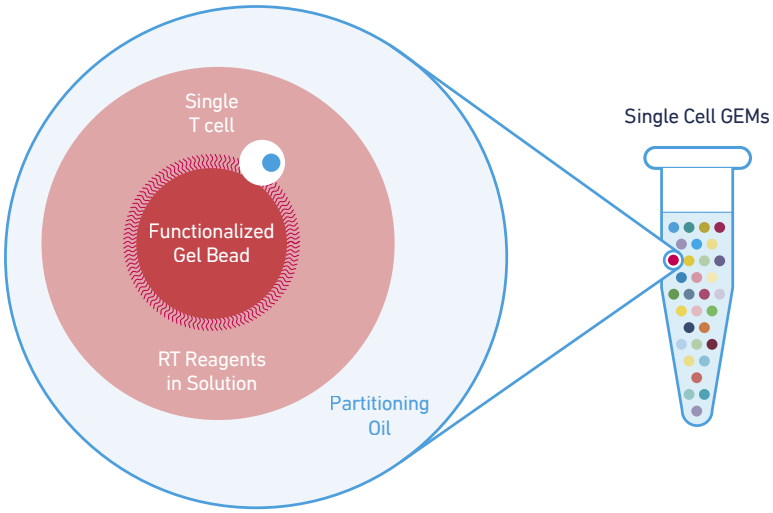
THE CHROMIUM SYSTEM

The Power of Massively Parallel Partitioning

Inside the Chromium Controller

The Power of Partitioning

The Chromium System, powered by GemCode Technology, provides a precisely engineered reagent delivery method that enables thousands of micro-reactions in parallel.



Massive Partitioning and Barcoding

Encapsulate your sample into hundreds to tens of thousands of uniquely addressable partitions in minutes, each containing an identifying barcode for downstream analysis.

Each Gel Bead, infused with millions of unique oligonucleotide sequences, is mixed with a sample, which can be high molecular weight (HMW) DNA, individual cells, cells labeled using Feature Barcoding technology, nuclei, nuclei treated with transposase, or Cell Beads (see page 6). Gel Beads and samples are then added to an oil-surfactant solution to create Gel Beads in EMulsion (GEMs), which act as individual reaction vesicles in which the Gel Beads are dissolved and the sample is barcoded (Figure 1). Barcoded products are pooled for downstream reactions to create short-read sequencer compatible libraries. After sequencing, the resulting barcoded short read sequences are fed into turnkey analysis pipelines that use the identifying barcodes to map reads back to their original HMW DNA, single cell, or single nucleus of origin.

Figure 1 A GEM is a "Gel bead in EMulsion" droplet that encapsulates each micro-reaction within the Chromium System. Here we show a Single Cell GEM with a single T cell, reagents, and barcoded gel bead all partitioned within a single droplet.

Single Cell Partitioning for Transcriptome Analysis

The Chromium System also enables single cell transcriptional profiling of up to tens of thousands of single cells. Single cell suspensions loaded onto the system are partitioned into GEMs, where transcripts are tagged with cell specific barcodes. The barcoded cDNA is then pooled for downstream processing and library preparation (Figure 3).

Our precise and efficient microfluidics allow 100-80,000+ cells to be recovered in droplets in each efficient run with a low doublet rate, facilitating the profiling of precious and rare cell populations. After sequencing, downstream bioinformatics tools use the cellular barcodes to group transcripts that originated from the same cell, revealing the transcriptome. Feature Barcoding technology uses specific oligonucleotide sequences to identify cell surface proteins or CRISPR perturbations associated with each individual cell for more robust cellular phenotyping and characterization.

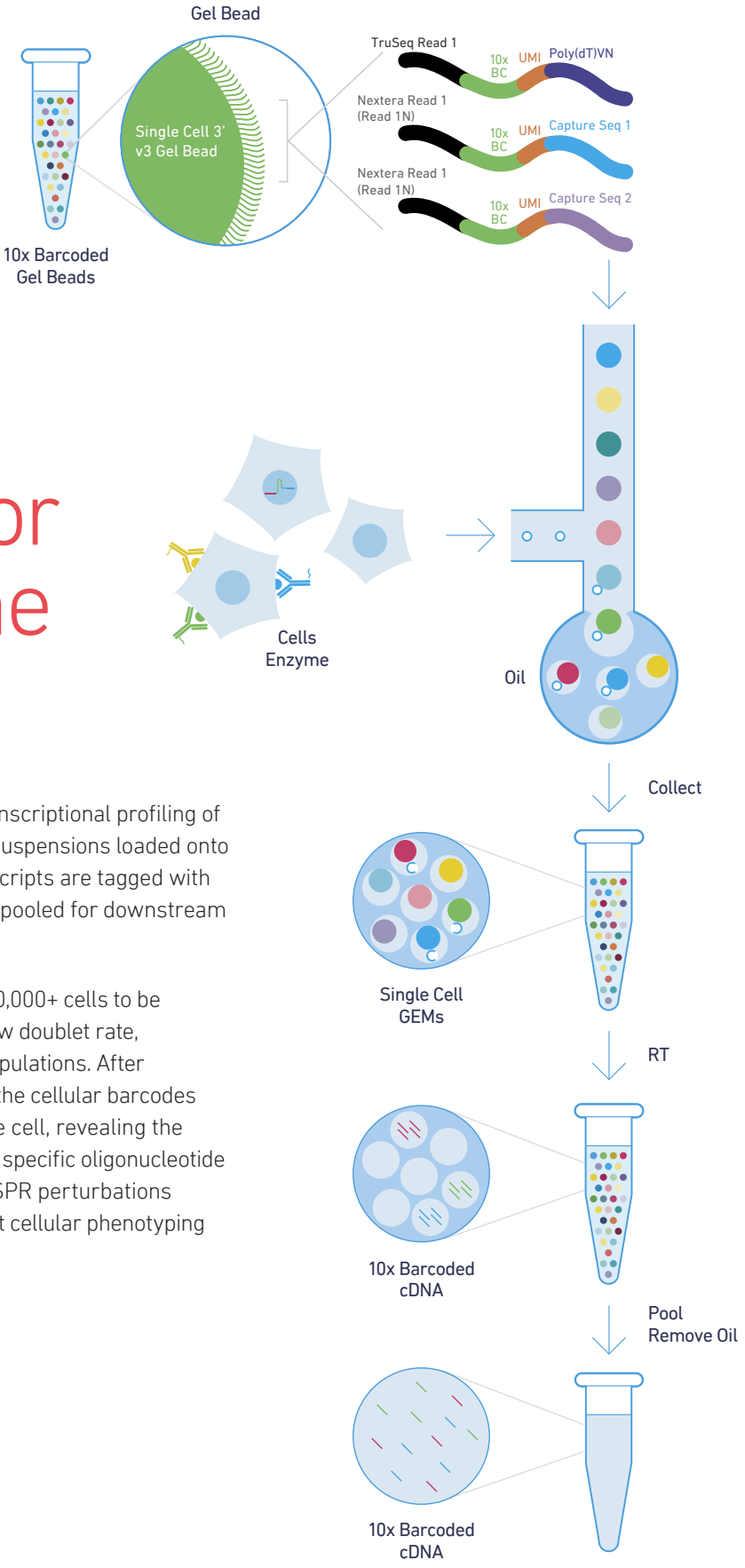


Figure 3 The Chromium partitioning workflow for single cell transcriptome analysis.

Single Cell Partitioning for Immunology Applications

Immune profiling of up to tens of thousands of T and B cells on a cell-by-cell basis can also be performed with the Chromium System. Single cell suspensions, including flow sorted cells, or cells labeled with antibodies or peptide-MHC multimers using Feature Barcoding technology, are loaded into the system and partitioned into GEMs. Transcripts are generated and tagged with cell-specific barcodes and the cDNA is then pooled for downstream processing and library preparation (Figure 4). For immune repertoire profiling, the cDNA undergoes targeted enrichment for T or B cell receptor transcripts prior to library preparation.

Our precise and efficient microfluidics allow 100-80,000+ cells to be recovered in droplets rapidly, with a low doublet rate, facilitating the profiling of precious and rare cell populations. After sequencing, downstream bioinformatics tools use the cellular barcodes to group transcripts that originated from the same cell, revealing the transcriptome and the full-length paired T or B cell receptor sequences of each individual cell. The Feature Barcode sequence is also used to identify the specific antigen or cell surface protein associated with each individual cell, allowing for a more accurate characterization of innate and adaptive immunity.

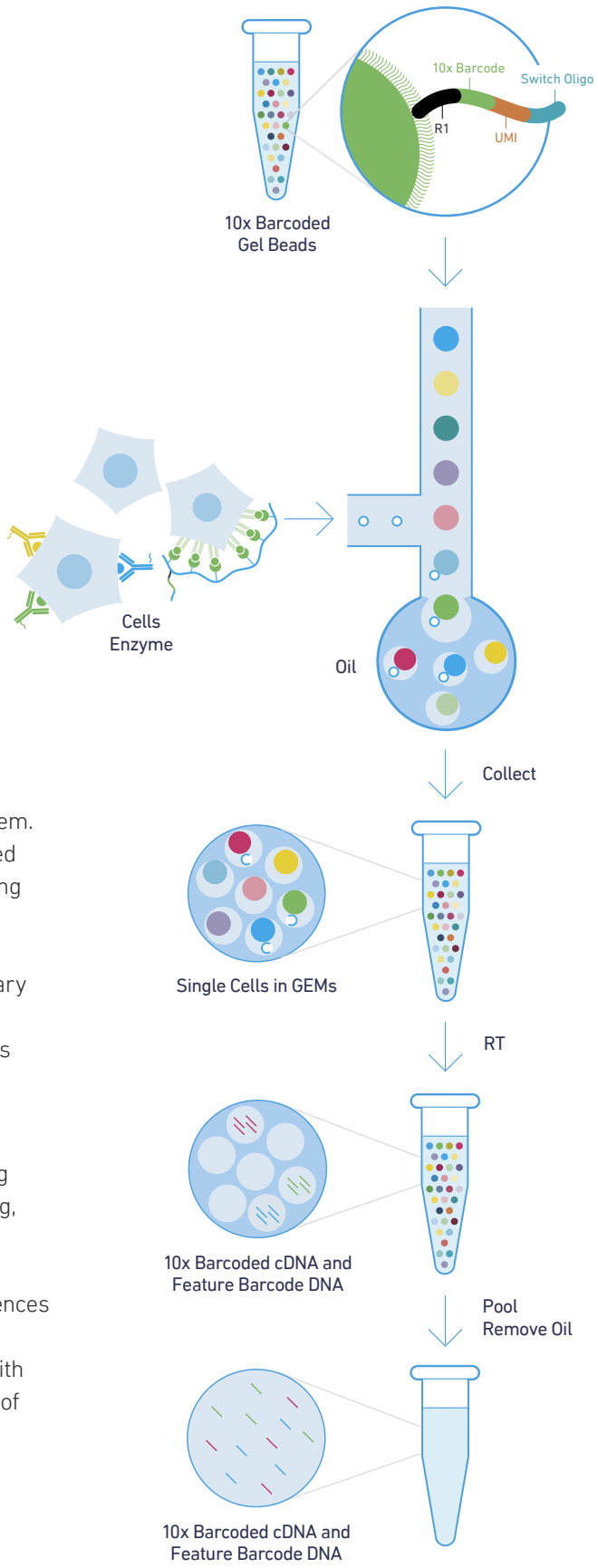


Figure 4 The Chromium partitioning workflow for single cell immunology applications.

Single Nuclei Partitioning for Epigenome Analysis

The Chromium System enables the analysis of chromatin accessibility at single cell resolution. In the Chromium Single Cell ATAC (Assay for Transposase Accessible Chromatin) workflow, single nuclei are first treated in bulk with transposase enzyme to preferentially insert sequencing adaptors into accessible DNA regions. Transposed nuclei are then partitioned into GEMs in the Chromium Controller. All accessible DNA fragments from the same nucleus share a common 10x barcode. The barcoded, accessible DNA fragments are subsequently pooled for downstream processing and library preparation (Figure 5).

The precise and efficient microfluidics allow 500-80,000+ nuclei to be recovered in droplets in each run with a low doublet rate, facilitating the open chromatin profiling of heterogeneous sample types and detecting rare cell populations. The barcoded library fragments can then be easily traced back to the open chromatin landscape for each nucleus from which they originated using intuitive bioinformatic tools.

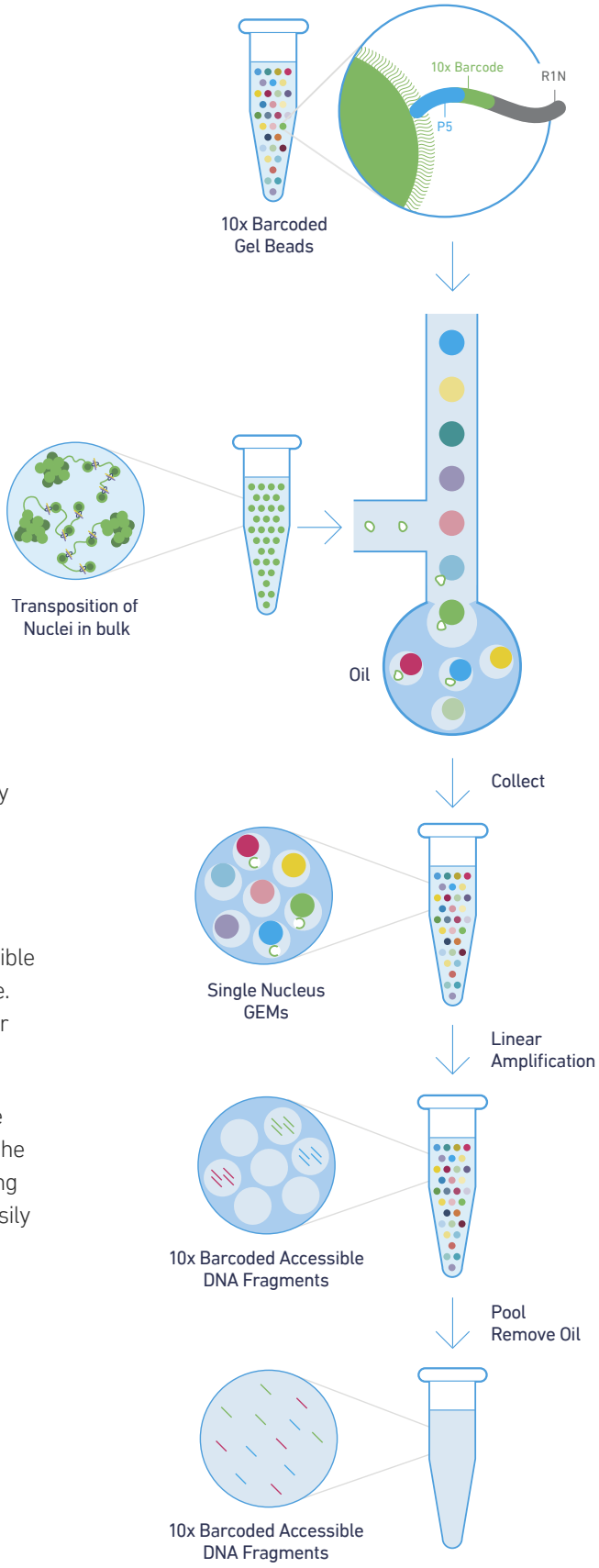


Figure 5 The Chromium partitioning workflow for single cell epigenomic profiling.

Single Cell Partitioning for Genome Analysis

Using the 10x technology, DNA from single cells is prepared in a two-step partitioning process. In the Chromium Single Cell DNA[1] workflow, single cells are first encapsulated in hydrogel Cell Beads (CBs), allowing the cells to be subjected to protein digestion and denaturation, while still retaining intact DNA in the hydrogel matrix (Figure 2). After the cells have been lysed, CBs containing DNA are partitioned with 10x barcoded Gel Beads (GBs), to generate Cell Bead Gel Bead (CBGB) GEMs (Figure 2). DNA from single cells is barcoded within the CBGB partitions and the barcoded fragments are then pooled for library production. The barcoded library fragments can be easily traced back to the cells from which they originated using downstream bioinformatics tools.

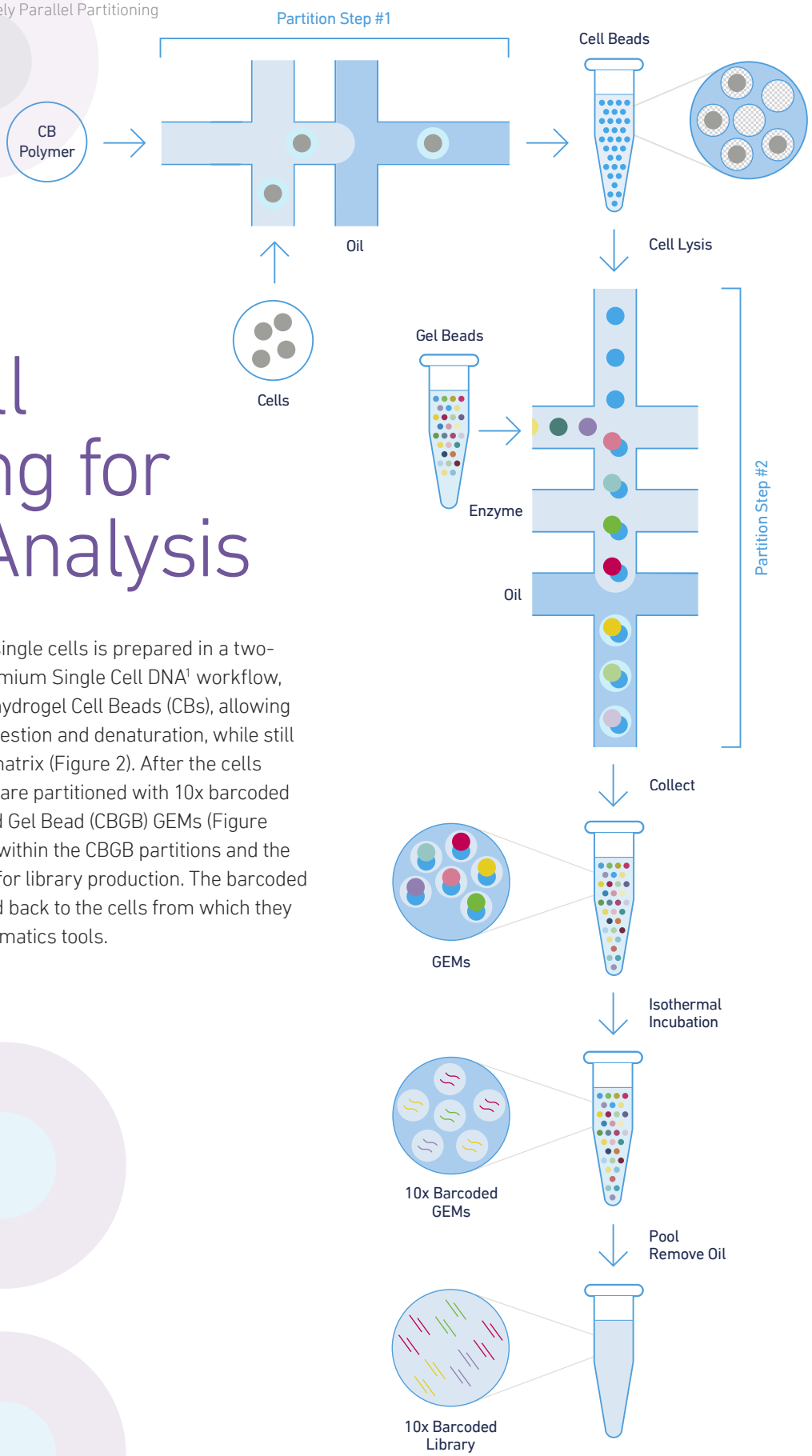


Figure 2 The Chromium partitioning workflow for single cell genome analysis.

[1] Available for use only with the indicated Chromium Controller (PN-120223;120246) or Chromium Single Cell Controller (PN-120263;120212).

High Molecular Weight DNA Partitioning

For whole genome[2] or exome[2] analyses, the Chromium Controller allows researchers to create sequencing-ready libraries with >1,000,000 unique barcodes from ~1 ng of HMW genomic DNA (Figure 6). Massive partitioning of the genome provides haplotype level dilution and enables the barcoding of long input DNA molecules, which are then sequenced in bulk to produce a unique data-type known as Linked-Reads. The long range information encoded in barcoded Linked-Reads is leveraged by innovative bioinformatics pipelines to assemble sequences over long genomic distances, including across repetitive regions. The precise assembly of Linked-Reads leverages heterozygous loci to resolve individual haplotypes, enabling diploid *de novo* assembly and phased calling of the full spectrum of human genetic variations, including SNPs, small indels, and complex structural variants.

[2] Available for use only with the indicated Chromium Controller (PN 120223 or 120246).

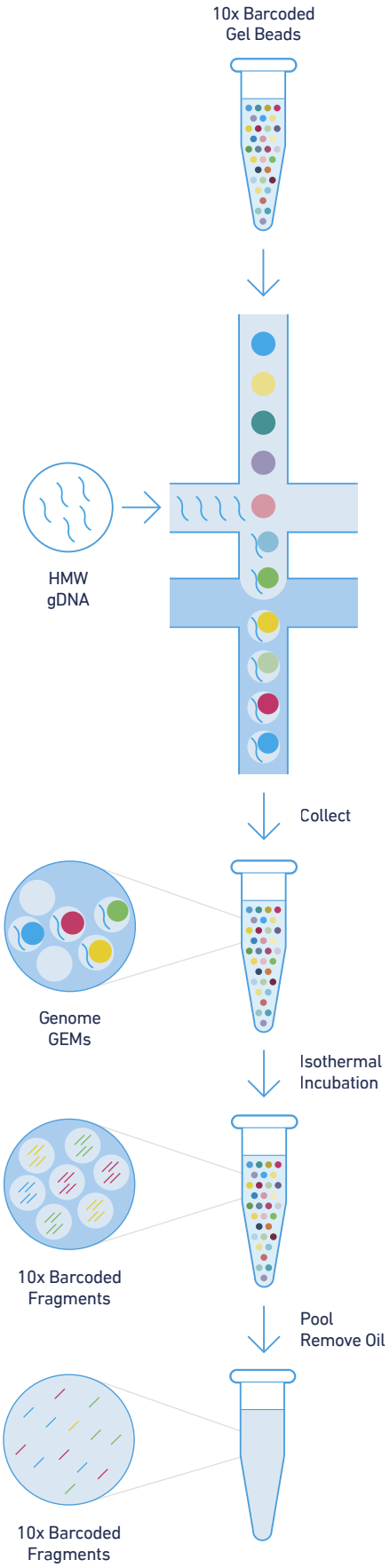


Figure 6 The Chromium DNA partitioning workflow for genome sequencing.

Linking Data, Developers and Discovery

We are dedicated to helping you get the most out of your 10x Genomics system by offering multiple helpful resources:

Solutions and Products

Along with our suite of complete solutions, we offer an ever-growing catalogue of services to help you find the answers to your research questions.

10x Compatible Products

Access our list of key partner products that have been certified compatible to work with our various solutions.

10x University

Immerse yourself in 10x University, a comprehensive step-by-step learning and training environment containing video tutorials and trainings.

Blog

Keep up to date with the 10x Genomics Blog, where you'll find everything from tips and tricks to the latest 10x news.

10x Library

Easy access to our complete library of product literature, customer publications, application notes, protocols, and many other useful resources.

Support

Visit the support site for documentation, software, and datasets that will help you get the most out of your 10x Genomics products.

Contact Us

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For more locations in US, EU and Asia visit:
10xgenomics.com/company/#locations

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Join the 10x Community. Get exclusive access to user forums, blogs, tips and tricks and more. Then, give us your feedback to help make this your community.

- Share your experience
- Read our blog
- Give feedback
- Interact with us

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LIT000025 *Rev C The Power of Massively Parallel Partitioning: Inside the Chromium Controller*

10x GENOMICS

EXHIBIT 3

THE NEXT GEM TECHNOLOGY

An integrated solution for RNA and DNA analysis

TECHNOLOGY

The Chromium System is powered by Next GEM Technology

Our proprietary Next GEM technology fuels our Chromium System with an innovative reagent delivery system, set of algorithms and turnkey software analysis tools that enable the discovery of previously inaccessible genetic information at massive rate and scale.

The Next GEM technology is built on a new chip architecture that integrates seamlessly into existing solutions. This technology will enable future solutions and product improvements. The Next GEM technology combines new chips and reagents, and is currently offered for the following solutions:

- Chromium Single Cell Gene Expression Solution – See Details (https://support.10xgenomics.com/single-cell-gene-expression/index/doc/getting-started-next-gem-technology-for-single-cell-gene-expression)
- Chromium Single Cell Immune Profiling Solution – See Details (https://support.10xgenomics.com/single-cell-vdj/index/doc/getting-started-next-gem-technology-for-single-cell-immune-profiling)
- Chromium Single Cell ATAC Solution – See Details (https://support.10xgenomics.com/single-cell-atac/index/doc/getting-started-next-gem-technology-for-single-cell-atac)

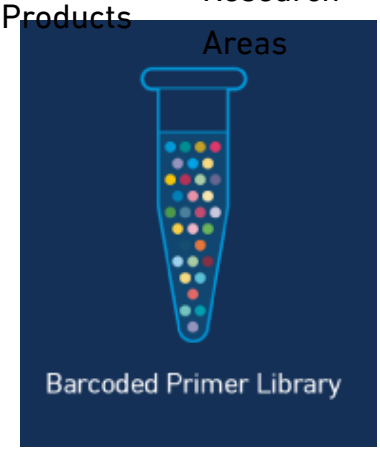
Download Chromium Technology Brochure

REAGENT DELIVERY SYSTEM

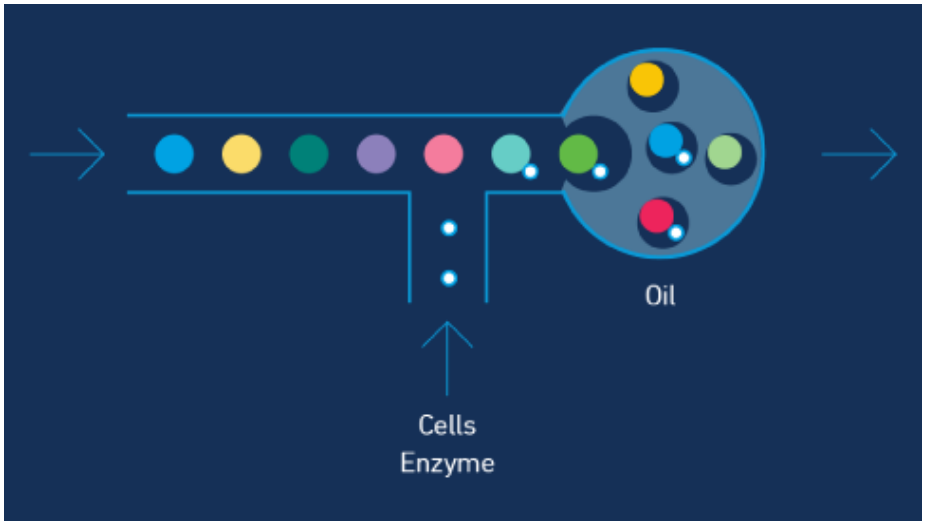
The Next GEM technology creates a unique reagent delivery system that partitions cells or nuclei and prepares sequencing libraries in parallel such that all fragments produced within a partition share a common barcode.

A simple workflow combines large partition numbers with a massively diverse barcode library to generate >100,000 barcode containing partitions in a matter of minutes.

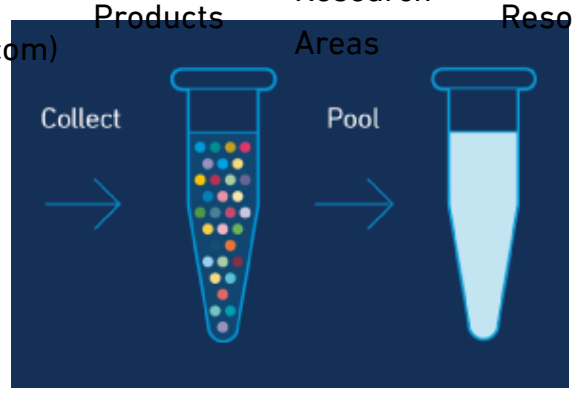
SOLID PHASE REAGENT DELIVERY



FLUID PARTITIONING



LIQUID PHASE BIOCHEMISTRY

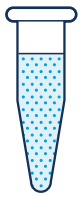


LAB INTEGRATION

Seamless Integration into Existing Lab Workflows

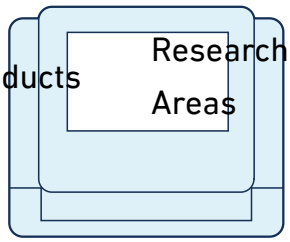
Our Next GEM technology is a transformative technology that fits easily into existing lab infrastructure.

Cell or Nuclei Suspension



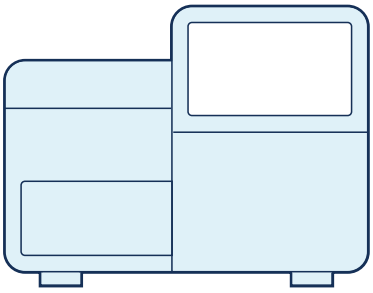
Library Construction

Barcoding & Library Construction



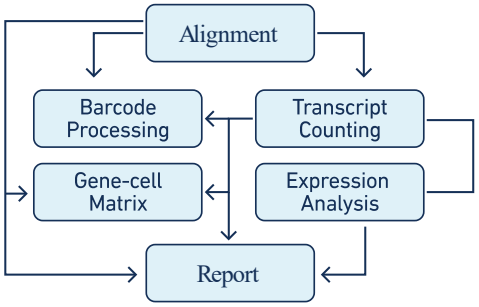
Sequencer

Sequence Libraries



Data Analysis

Cell Ranger Pipelines



Data Visualization

Report & Visualization

Order your 10x system, today.

Request Pricing

English (https://www.10xgenomics.com/technology/)
日本語 (https://www.10xgenomics.com/jp/technology/)
中文 (https://www.10xgenomics.com/cn/technology/)

EXHIBIT 4

S-1 1 d737378ds1.htm S-1

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As filed with the Securities and Exchange Commission on August 19, 2019.
Registration No. 333-

UNITED STATES
SECURITIES AND EXCHANGE COMMISSION

Washington, D.C. 20549

FORM S-1
REGISTRATION STATEMENT

UNDER
THE SECURITIES ACT OF 1933

10x Genomics, Inc.

(Exact Name of Registrant as Specified in Its Charter)

Delaware	3826	45-5614458
(State or Other Jurisdiction of Incorporation or Organization)	(Primary Standard Industrial Classification Code Number)	(I.R.S. Employer Identification Number)

6230 Stoneridge Mall Road
Pleasanton, California 94588
(925) 401-7300
(Address, Including Zip Code, and Telephone Number, Including Area Code, of Registrant's Principal Executive Offices)

Serge Saxonov
Chief Executive Officer
10x Genomics, Inc.
6230 Stoneridge Mall Road
Pleasanton, California 94588
(925) 401-7300
(Name, Address, Including Zip Code, and Telephone Number, Including Area Code, of Agent For Service)

Copies to:

Kevin P. Kennedy	Eric S. Whitaker	Charles S. Kim
Simpson Thacher & Bartlett LLP	Randy Wu	David Peinsipp
2475 Hanover Street	James Bryant	Kristin VanderPas
Palo Alto, California 94304	10x Genomics, Inc.	Cooley LLP
(650) 251-5000	6230 Stoneridge Mall Road	4401 Eastgate Mall
	Pleasanton, California 94588	San Diego, California 92121
	(925) 401-7300	(858) 550-6000

Approximate date of commencement of proposed sale to the public: As soon as practicable after the effective date of this Registration Statement.

If any of the securities being registered on this form are to be offered on a delayed or continuous basis pursuant to Rule 415 under the Securities Act of 1933, check the following box. ☐

If this form is filed to register additional securities for an offering pursuant to Rule 462(b) under the Securities Act, please check the following box and list the Securities Act registration statement number of the earlier effective registration statement for the same offering. ☐

If this form is a post-effective amendment filed pursuant to Rule 462(c) under the Securities Act, check the following box and list the Securities Act registration statement number of the earlier effective registration statement for the same offering. ☐

If this form is a post-effective amendment filed pursuant to Rule 462(d) under the Securities Act, check the following box and list the Securities Act registration statement number of the earlier effective registration statement for the same offering. ☐

Indicate by check mark whether the registrant is a large accelerated filer, an accelerated filer, a non-accelerated filer, a smaller reporting company or an emerging growth company. See the definitions of "large accelerated filer", "accelerated filer", "smaller reporting company" and "emerging growth company" in Rule 12b-2 of the Exchange Act.

Large accelerated filer	☐	Accelerated filer	☐
Non-accelerated filer	☒	Smaller reporting company	☐
		Emerging growth company	☒

If an emerging growth company, indicate by check mark if the registrant has elected not to use the extended transition period for complying with any new or revised financial accounting standards provided pursuant to Section 7(a)(2)(B) of the Securities Act. ☐

CALCULATION OF REGISTRATION FEE

Title of each Class of Securities to be Registered	Proposed Maximum Aggregate Offering Price(1)(2)	Amount of Registration Fee
Class A Common stock, par value $0.00001 per share	$100,000,000	$12,120

(1) Includes the aggregate offering price of additional shares that the underwriters have the option to purchase.
(2) Estimated solely for the purpose of computing the amount of the registration fee pursuant to Rule 457(o) under the Securities Act of 1933.

The registrant hereby amends this registration statement on such date or dates as may be necessary to delay its effective date until the registrant shall file a further amendment which specifically states that this registration statement shall thereafter become effective in accordance with Section 8(a) of the Securities Act of 1933, or until the registration statement shall become effective on such date as the Securities and Exchange Commission, acting pursuant to said Section 8(a), may determine.

Table of Contents

The information in this preliminary prospectus is not complete and may be changed. We may not sell these securities until the registration statement filed with the Securities and Exchange Commission is effective. This preliminary prospectus is not an offer to sell these securities and it is not soliciting an offer to buy these securities in any jurisdiction where the offer or sale is not permitted.

Subject to Completion, dated August 19, 2019

Preliminary prospectus

shares

10x GENOMICS

Class A common stock

This is an initial public offering of shares of Class A common stock by 10x Genomics, Inc. We are offering shares of our Class A common stock to be sold in the offering. The initial public offering price is expected to be between $ and $ per share.

We have two classes of common stock, Class A common stock and Class B common stock. The rights of the holders of Class A common stock and Class B common stock are different with respect to voting, conversion and transfer rights. Each share of Class A common stock is entitled to one vote per share. Each share of Class B common stock is entitled to ten votes per share and is convertible at any time into one share of Class A common stock. Following this offering, outstanding shares of Class B common stock will represent approximately % of the voting power of our outstanding capital stock. This means that, for the foreseeable future, investors in this offering and holders of our Class A common stock in the future will not have a meaningful voice in our corporate affairs.

Prior to this offering, there has been no public market for our Class A common stock. We have applied to list our Class A common stock on the Nasdaq Global Select Market under the symbol “TXG”.

We are an “emerging growth company” as defined under the federal securities laws and, as such, have elected to comply with certain reduced public company reporting requirements.

	Per share	Total
Initial public offering price	$	$
Underwriting discounts and commissions(1)	$	$
Proceeds to 10x Genomics, Inc., before expenses	$	$

(1) See the section titled “*Underwriting*” for a description of the compensation payable to the underwriters.

We have granted the underwriters an option for a period of 30 days to purchase up to additional shares of Class A common stock at the initial public offering price less the underwriting discounts and commissions.

Investing in our Class A common stock involves a high degree of risk. See the section titled “*Risk factors*” beginning on page 15.

Neither the Securities and Exchange Commission nor any other state securities commission has approved or disapproved of these securities or passed on the adequacy or accuracy of this prospectus. Any representation to the contrary is a criminal offense.

The underwriters expect to deliver the shares to purchasers on or about , 2019.

J.P. Morgan **Goldman Sachs & Co. LLC** **BofA Merrill Lynch**

Cowen

, 2019

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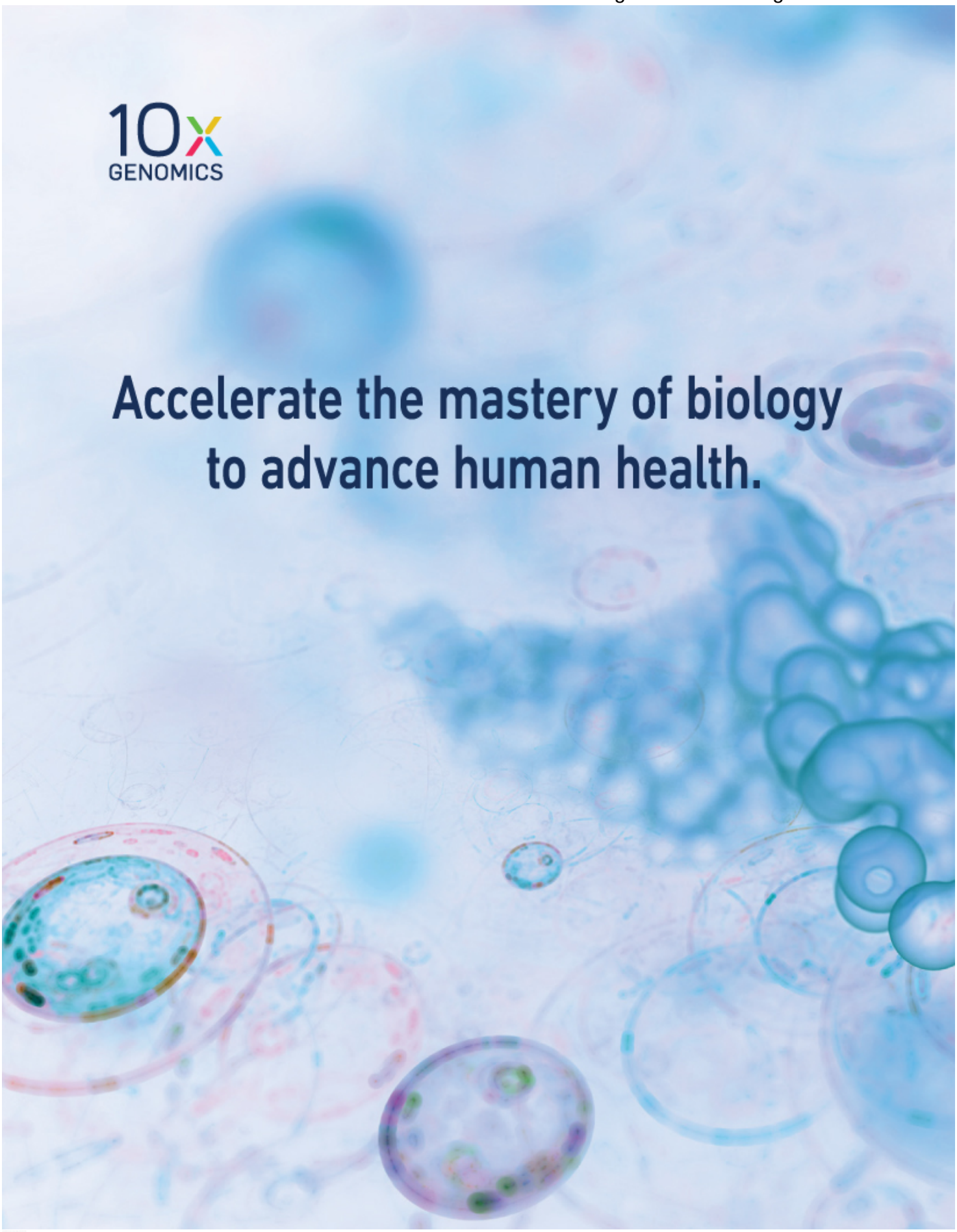
10X
GENOMICS
Accelerate the mastery of biology
to advance human health.

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In this prospectus, "10x", "10x Genomics", the "Company", "we", "us" and "our" refer to 10x Genomics, Inc. and, as appropriate, its consolidated subsidiaries, and references to our "common stock" include our Class A common stock and Class B common stock. We and the underwriters have not authorized anyone to provide any information or to make any representations other than those contained in this prospectus or in any free writing prospectuses prepared by us or on our behalf. We and the underwriters take no responsibility for, and can provide no assurance as to the reliability of, any other information that others may provide you. We are offering to sell, and seeking offers to buy, shares of Class A common stock only in jurisdictions where offers and sales are permitted. The information contained in this prospectus is accurate only as of the date of this prospectus, regardless of the time of delivery of this prospectus or of any sale of the Class A common stock. Our business, financial condition, results of operations and future growth prospects may have changed since that date.

"10X GENOMICS", "10x", "10X", the "10x" and "10X" logos, "Chromium", "Visium", "Feature Barcoding", "Chromium Connect", "GEM", "Next GEM" and other trade names, trademarks or service marks of 10x appearing in this prospectus are the property of 10x Genomics. For ease of reference, the trademarks, trade names and service marks used in this prospectus are used without the ™ and ® symbols, but that does not mean that we will not assert, to the full extent permitted by law, our full rights and the rights of our licensors over our

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trademarks. Other trademarks and trade names appearing in this prospectus are the property of their respective holders.

Through and including , 2019 (the 25th day after the date of this prospectus), all dealers that effect transactions in these securities, whether or not participating in this offering, may be required to deliver a prospectus. This is in addition to the dealers' obligation to deliver a prospectus when acting as underwriters and with respect to their unsold allotments or subscriptions.

For investors outside of the United States: We have not, and the underwriters have not, done anything that would permit this offering or possession or distribution of this prospectus in any jurisdiction where action for that purpose is required, other than the United States. Persons outside of the United States who come into possession of this prospectus must inform themselves about, and observe any restrictions relating to, the offering of the shares of Class A common stock and the distribution of this prospectus outside of the United States.

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Prospectus summary

This summary highlights information contained elsewhere in this prospectus. This summary may not contain all of the information that you should consider before deciding to invest in our Class A common stock. You should read this entire prospectus carefully, including the sections titled "Risk factors", "Management's discussion and analysis of financial condition and results of operations" and "Business" and our consolidated financial statements and related notes included elsewhere in this prospectus before making an investment decision.

10x Genomics, Inc.

Mission

Our mission is to accelerate the mastery of biology to advance human health.

Overview

We are a life science technology company building products to interrogate, understand and master biology. Our integrated solutions include instruments, consumables and software for analyzing biological systems at a resolution and scale that matches the complexity of biology. We have built deep expertise across diverse disciplines including chemistry, biology, hardware and software. Innovations in all of these areas have enabled our rapidly expanding suite of products, which allow our customers to interrogate biological systems at previously inaccessible resolution and scale. Our products have enabled researchers to make fundamental discoveries across multiple areas of biology, including oncology, immunology and neuroscience, and have helped empower the single cell revolution hailed by *Science* magazine as the 2018 'Breakthrough of the Year'. Since launching our first product in mid-2015 through June 30, 2019, we have sold 1,284 instruments to researchers around the world, including 93 of the top 100 global research institutions by publications, and 13 of the top 15 global pharmaceutical companies by 2018 revenue. We believe that this represents the very beginning of our penetration into multiple large markets. We expect that 10x will power a "Century of Biology", in which many of humanity's most pressing health challenges will be solved by precision diagnostics, targeted therapies and cures to currently intractable diseases.

The "10x" in our name refers to our focus on opportunities with the greatest potential for exponential advances and impact. We believe that the scientific and medical community currently understands only a tiny fraction of the full complexity of biology. The key to advancing human health lies in accelerating this understanding. The human body consists of over 40 trillion cells, each with a genome of 3 billion DNA base pairs and a unique epigenetic program regulating the transcription of tens of thousands of different RNAs, which are then translated into tens of thousands of different proteins. Progress in the life sciences will require the ability to measure and to experiment on biological systems at fundamental resolutions and massive scales, which are inaccessible with existing technologies. We believe that our technologies overcome these limitations, unlocking fundamental biological insights essential for advancing human health.

Resolution and scale are the imperatives underlying our technologies and products. Our Chromium and recently announced Visium product lines provide this resolution and scale along distinct but complementary dimensions of biology. Our Chromium products enable high throughput analysis of individual biological components, such as up to millions of single cells. They use our precisely engineered reagent delivery system to divide a sample into individual components in up to a million or more partitions, enabling large numbers of parallel micro-reactions. In this manner, a large population of cells can be segregated into partitions and analyzed on a cell by cell basis. Our Visium products, the first of which we expect to launch in late 2019, will enable analysis of

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biological molecules within their spatial context, providing the locations of analytes that give insight into higher order biological structure and function. Our Visium products will use high density DNA arrays with DNA sequences that encode the physical locations of biological analytes within a sample, such as a tissue section. Our products utilize our sensitive and robust molecular assays to convert biological analytes into detectable signals, enabling researchers to obtain vast amounts of information about diverse biological analytes together with their single cell and spatial context. Finally, we provide highly sophisticated and scalable software for analyzing the raw data researchers generate and presenting it in a form that is readily understood by biologists.

Our product portfolio consists of multiple integrated solutions that include instruments, consumables and software. These solutions guide customers through the workflow from sample preparation to next-generation sequencing to subsequent analysis and visualization. Our products are compatible with third-party sequencers that are commonly available in research settings. Each of our solutions are designed to interrogate a major class of biological information that is impactful to researchers:

- Our single cell solutions, all of which run on our Chromium instruments, include:
    - Single Cell Gene Expression solution for measuring gene activity on a cell-by-cell basis;
    - Single Cell Immune Profiling solution for measuring the activity of immune cells and their targets;
    - Single Cell ATAC solution for measuring epigenetics, including the physical organization of DNA; and
    - Single Cell CNV solution for measuring cellular heterogeneity through DNA changes such as copy number variation.
- Our Visium solution will measure the spatial gene expression patterns across a tissue sample.

Our Feature Barcoding technology, which is currently compatible with our Single Cell Gene Expression and Immune Profiling solutions, allows researchers to simultaneously measure multiple analytes, such as protein and RNA, within the same set of cells or tissues.

As of June 30, 2019, we employed a commercial team of over 190 employees, many of whom hold Ph.D. degrees, who help drive adoption of our products and support our vision. We prioritize creating a superior user experience from pre-sales to onboarding through the generation of novel publishable discoveries, which drive awareness and adoption of our products. We have a scalable, multi-channel commercial infrastructure including a direct sales force in North America and certain regions of Europe and distribution partners in Asia, certain regions of Europe, South America, the Middle East and Africa that drives our customer growth. This is supplemented with an extensive and highly specialized customer service infrastructure with Ph.D.-level specialists. We currently have customers in approximately 40 countries.

As of June 30, 2019, worldwide we owned or exclusively licensed over 175 issued or allowed patents and 470 pending patent applications. We also license additional patents on a non-exclusive and/or territory restricted basis. Our intellectual property portfolio includes foundational patents related to single cell analysis, epigenomics, spatial analysis and multi-omics.

Our revenue was $71.1 million and $146.3 million for 2017 and 2018, respectively, representing an annual growth rate of 106%, and $59.2 million and $109.4 million for the six months ended June 30, 2018 and 2019, respectively, representing an annual growth rate of 85%. We generated net losses of $18.8 million and $112.5 million for 2017 and 2018. Our 2018 net loss resulted substantially from charges of $62.4 million associated with intellectual property acquisitions for research and development in addition to the litigation contingency accrual of $38.0 million which was recorded in the fourth quarter of 2018. We generated net losses of $21.6 million and $14.5 million for the six months ended June 30, 2018 and 2019, respectively. The $14.5 million net loss included a $15.9 million accrual for estimated royalties related to ongoing litigation.

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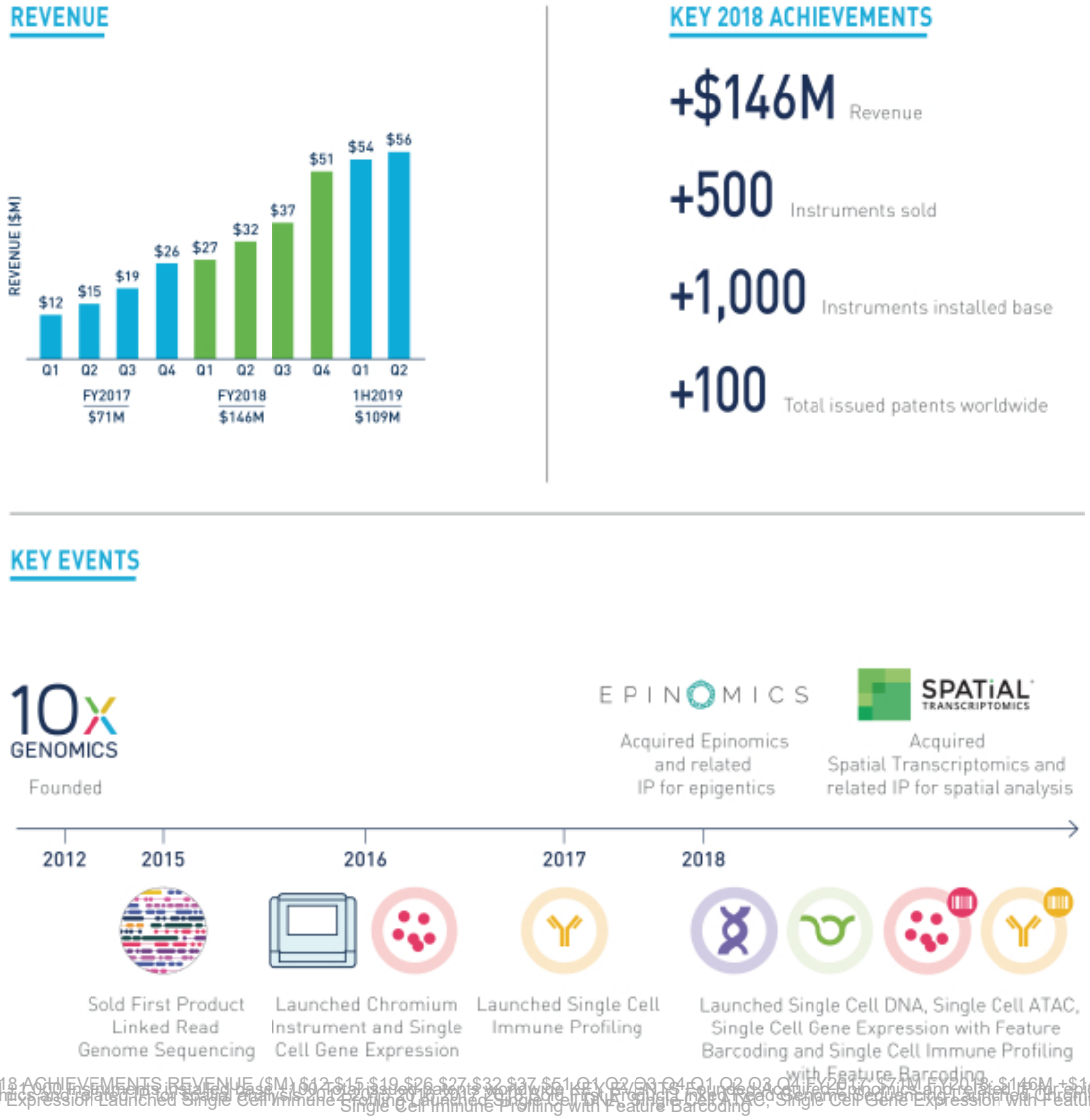

Directions in Genomics

Biology is staggeringly complex. The cell is the basic, fundamental organizational unit of all biological organisms. A human being starts from a single cell, which divides into over 40 trillion cells to create the tissues that enable all necessary functions in the human body. Within each of these trillions of cells exists a distinct genome, epigenome, transcriptome and proteome, which together collectively constitute the rich architecture of biology. Genomics is a broad, highly-interdisciplinary field that studies this architecture at a system-wide level.

The Human Genome Project, which was completed in 2003, and subsequent genomics research have been foundational in enabling new research and clinical applications. However, we believe that much of the promise

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of genomics remains unfulfilled due to the tremendous underlying complexity of biology, of which the scientific and medical community currently understands only a tiny fraction. We believe technologies that enable researchers to measure the full complexity of biology are needed to understand how cell-to-cell variations in genomes, epigenomes, transcriptomes and proteomes give rise to function or dysfunction. To accomplish this, we believe researchers need to characterize every cell type in every tissue in the human body at a full molecular and cellular level, including how cells are spatially arranged. Technologies are also needed for moving beyond the cataloguing of biological complexity and into performing experiments to understand the impact of active changes to biological systems.

This presents an enormous challenge because of the limited capabilities of existing tools for accessing biology at the molecular and cellular level. Some of these limitations are:

- Average, or “bulk”, measurements obscure underlying differences between different biological units, such as individual cells;
- Low throughput prevents requisite sampling of the underlying complexity—for example, when only a few hundred cells can be evaluated at a time;
- Limited number of biological analytes are interrogated, giving a myopic view of only a few biological processes;
- Limited ability for multi-omic interrogation;
- Inefficient use of sample to generate a signal of sufficient strength to analyze the biological molecules of interest; and
- Inadequate bioinformatics and software tools.

We believe technologies that address these limitations will serve large and unmet market needs by providing a better understanding of molecular and cellular function, the origin of disease and how to improve treatment.

Our solutions

Our solutions allow researchers to interrogate, understand and master biological systems at a resolution and scale commensurate with the complexity of biology, overcoming the limitations of existing tools.

Our Chromium platform, recently announced Visium platform, molecular assays and software constitute the building blocks of our integrated solutions. These shared building blocks allow us to rapidly build and improve our solutions:

- ***Our Chromium platform*** enables high-throughput analysis of individual biological components, such as up to millions of single cells.
- ***Our Visium platform*** is being designed to identify where biological components are located and how they are arranged with respect to each other, otherwise referred to as “spatial analysis”.
- ***Our molecular assays*** are used with our Chromium platform, and with our planned Visium platform, to provide sensitive and robust biochemistries that convert minute amounts of biological analytes into detectable signals.
- ***Our software*** transforms large amounts of raw data into usable results, giving researchers user-friendly tools to dynamically explore these results.

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To date, more than 500 peer-reviewed articles have been published based on data generated using our products. More than 90 of these articles were published in three of the most highly-regarded journals: *Cell*, *Nature* and *Science*.

Our market opportunity

We believe our solutions, which enable a comprehensive view of biology, target numerous market opportunities across the more than $50 billion global life sciences research tools market. We view much of this total market opportunity as ultimately accessible to us due to our ability to answer a broad diversity of biological questions. Based on the capabilities of our current solutions, and focusing solely on cases where our current solutions offer alternative or complementary approaches to existing tools, we believe, based on our internal estimates, we could access approximately $13 billion of the global life sciences research tools market. We believe we can further drive growth across our current and adjacent markets by improving, or enabling new uses and applications of, existing tools and technologies, as our solutions allow researchers to answer questions that may be impractical or impossible to address using other products.

Our competitive strengths

We believe our continued growth will be driven by the following competitive strengths:

- Our position as a leader in a large and growing market;
- Our proprietary technologies;
- Our rigorous product development processes and scalable infrastructure;
- Our customer experience and broad commercial reach; and
- Our experienced multidisciplinary team.

Our growth strategy

Our growth strategy includes the following key elements:

- Develop critical enabling technologies;
- Expand the installed base of our Chromium instruments;
- Strengthen use and adoption of our consumables;
- Identify the most relevant technologies, create or acquire such technologies and develop them into new products; and
- Promote our platforms as the standard for single cell and spatial analysis.

Risk factors

Investing in our Class A common stock involves risk. You should carefully consider all the information in this prospectus prior to investing in our Class A common stock. These risks are discussed more fully in the section titled "*Risk factors*" immediately following this prospectus summary.

Risks related to our business and industry

Risks and uncertainties related to our business and industry include, but are not limited to, the following:

- We have incurred significant losses since inception, we expect to incur losses in the future and we may not be able to generate sufficient revenue to achieve and maintain profitability;

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- The life sciences technology market is highly competitive. If we fail to compete effectively, our business and operating results will suffer;
- Our business depends significantly on the success of our Next GEM microfluidic chip;
- We are significantly dependent upon revenue generated from the sale of our Chromium solutions, and in particular our Single Cell Gene Expression solutions;
- Our business currently depends significantly on research and development spending by academic institutions, a reduction in which could limit demand for our products and adversely affect our business and operating results;
- Our failure to effectively manage product transitions or accurately forecast customer demand could result in excess or obsolete inventory and resulting charges;
- Our future success is dependent upon our ability to increase penetration in our existing markets;
- We may not be able to develop new products, enhance the capabilities of our existing products to keep pace with rapidly changing technology and customer requirements or successfully manage the transition to new product offerings, any of which could have a material adverse effect on our business and operating results;
- If our existing and new products fail to achieve and sustain sufficient scientific acceptance, we will not generate expected revenue and our prospects may be harmed; and
- the other risk factors set forth in the section titled "*Risk factors—Risks related to our business and industry".*

Risks related to litigation and our intellectual property

We are currently involved in litigation matters related to substantially all of our products, the loss of any of which could have a material adverse effect on our business, operations, financial results and reputation. Furthermore, parties making claims against us have obtained and may in the future be able to obtain injunctive or other relief, which effectively could block our ability to further develop, commercialize, market or sell products or services and could result in the award of substantial damages against us. In November 2018, a jury concluded that our Chromium instruments operating our Gel bead in Emulsion microfluidic chips ("GEM microfluidic chips") and associated consumables infringed certain of Bio-Rad Laboratories, Inc.'s ("Bio-Rad") patents and that the infringement was willful. The Court entered final judgment in August 2019 with damages in the amount of approximately $35 million. In the fourth quarter of 2018, we began recording an accrual for estimated royalties as cost of revenue. This accrual is based on an estimated royalty rate of 15% of worldwide sales of our Chromium instruments operating our GEM microfluidic chips and associated consumables. As of June 30, 2019, we had accrued a total of $55.3 million relating to this matter which includes the $35 million judgment and our estimated 15% royalty for sales through that date.

The Court also granted Bio-Rad a permanent injunction against our GEM microfluidic chips and associated consumables that were found to infringe the Bio-Rad patents, which have historically constituted substantially all of our product sales. However, under the injunction, we are permitted to continue to sell our GEM microfluidic chips and associated consumables for use with our historical installed base of instruments provided that we pay a royalty of 15% into escrow on our net revenue related to such sales. We have appealed the injunction to the Federal Circuit and expect that it will not take effect until the Federal Circuit rules on our request for a stay of the injunction.

We have dedicated significant resources to designing and manufacturing our new microfluidic chips (our "Next GEM microfluidic chips") which use fundamentally different physics from our GEM microfluidic chips. Neither

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the jury verdict nor the injunction relate to our Next GEM microfluidic chips and associated consumables which we launched in May 2019 for three of our single cell solutions — Single Cell Gene Expression, Single Cell Immune Profiling and Single Cell ATAC. We currently expect that, by the end of the third quarter of 2019, all Chromium instruments that we sell will operate exclusively with our Next GEM solutions and that our Chromium products utilizing our Next GEM microfluidic chips will constitute substantially all of our Chromium sales by the end of 2020.

Although our Next GEM microfluidic chips were designed to replace our GEM microfluidic chips, we cannot assure you that we will be able to make our Next GEM microfluidic chip work with all of our solutions, that our Next GEM microfluidic chip will allow our customers to maintain the level of performance or quality of our GEM microfluidic chip, that our Next GEM microfluidic chip will replace the sales of our GEM microfluidic chip or that we will be able to manufacture our Next GEM microfluidic chips in sufficient volumes in a timely fashion. Our Next GEM microfluidic chips may be subject to future claims of infringement by Bio-Rad or others and are currently the subject of the litigation described below. For additional information, see "*Risk factors—Risks related to litigation and our intellectual property*".

In addition, unless the injunction relating to our GEM microfluidic chips is stayed, we will be unable to sell our Single Cell CNV and Linked-Read solutions for use on new instruments unless and until we develop a Next GEM microfluidic chip for such solutions. Though these solutions have not significantly contributed to our revenue to date, our Single Cell CNV solution, for example, has proved crucial in understanding how cancers evolve and providing researchers with valuable insights into cancer treatments.

As we enter new markets or introduce new products, we expect that competitors will likely claim that our products infringe their intellectual property rights. Our success depends in part on our ability to defend ourselves against such claims and maintain the validity of our patents and other proprietary rights. Risks and uncertainties relating to litigation and intellectual property include, but are not limited to, the following:

- We are involved in significant litigation which has consumed significant resources and management time, and adverse resolution of these lawsuits could require us to pay significant damages and prevent us from selling our products, which would severely adversely impact our business, financial condition or results of operations;
- We are involved in lawsuits to protect, enforce or defend our patents and other intellectual property rights, which are expensive, time consuming and could ultimately be unsuccessful; and
- the other risk factors set forth in the section titled "*Risk factors—Risks related to litigation and our intellectual property*".

Risks related to this offering and ownership of our Class A common stock

- The multi-class structure of our common stock will have the effect of concentrating voting control with those stockholders who held our capital stock prior to the completion of this offering, and it may depress the trading price of our Class A common stock; and
- the other risk factors set forth in the section titled "*Risk factors—Risks related to this offering and ownership of our Class A common stock*".

Corporate information

We were incorporated in the State of Delaware on July 2, 2012 under the name Avante Biosystems, Inc. We changed our name to 10X Technologies, Inc. in September 2012 and to 10x Genomics, Inc. in November 2014.

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Our principal executive offices are located at 6230 Stoneridge Mall Road, Pleasanton, California 94588, and our telephone number is (925) 401-7300. Our website is https://www.10xgenomics.com. Neither our website nor the information contained in or accessible from our website is incorporated into this prospectus or the registration statement of which it forms a part, and investors should not rely on such information in deciding whether to invest in our Class A common stock.

Implications of being an emerging growth company

We qualify as an "emerging growth company" as defined in the Jumpstart Our Business Startups Act of 2012 (the "JOBS Act"). For so long as we remain an emerging growth company, we are permitted and currently intend to rely on the following provisions of the JOBS Act that contain exceptions from disclosure and other requirements that otherwise are applicable to companies that conduct initial public offerings and file periodic reports with the Securities and Exchange Commission (the "SEC"). These provisions include, but are not limited to:

- being permitted to present only two years of audited financial statements in this prospectus and only two years of related "*Management's discussion and analysis of financial condition and results of operations*" in our periodic reports and registration statements, including this prospectus;
- not being required to comply with the auditor attestation requirements of Section 404 of the Sarbanes-Oxley Act of 2002, as amended ("SOX");
- reduced disclosure obligations regarding executive compensation in our periodic reports, proxy statements and registration statements, including in this prospectus; and
- exemptions from the requirements of holding a nonbinding advisory vote on executive compensation and stockholder approval of any golden parachute payments not previously approved.

We will remain an emerging growth company until:

- the first to occur of the last day of the fiscal year (i) that follows the fifth anniversary of the completion of this offering, (ii) in which we have total annual gross revenue of at least $1.07 billion or (iii) in which we are deemed to be a "large accelerated filer", as defined in the Securities Exchange Act of 1934, as amended (the "Exchange Act"); or
- if it occurs before any of the foregoing dates, the date on which we have issued more than $1 billion in non-convertible debt over a three-year period.

We have elected to take advantage of certain of the reduced disclosure obligations in this prospectus and may elect to take advantage of other reduced reporting requirements in our future filings with the SEC. As a result, the information that we provide to our stockholders may be different than what you might receive from other public reporting companies in which you hold equity interests.

We have elected to avail ourselves of the provision of the JOBS Act that permits emerging growth companies to take advantage of an extended transition period to comply with new or revised accounting standards applicable to public companies. As a result, we will not be subject to new or revised accounting standards at the same time as other public companies that are not emerging growth companies.

For additional information, see the section titled "*Risk factors—Risks related to this offering and ownership of our Class A common stock—We are an "emerging growth company" and the reduced disclosure requirements applicable to emerging growth companies may make our Class A common stock less attractive to investors*".

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The offering

Class A common stock offered by us	shares.
Underwriters' option to purchase additional shares of Class A common stock from us	The underwriters have been granted an option to purchase up to additional shares of Class A common stock from us at any time within 30 days from the date of this prospectus.
Class A common stock outstanding immediately after giving effect to this offering	shares (or shares if the underwriters exercise their option to purchase additional shares in full).
Class B common stock outstanding immediately after giving effect to this offering	shares.
Total Class A common stock and Class B common stock outstanding immediately after giving effect to this offering	shares.
Use of proceeds	We estimate that the net proceeds to us from this offering, after deducting estimated underwriting discounts and commissions and estimated offering expenses payable by us, will be approximately $ million (or approximately $ million if the underwriters exercise their option to purchase additional shares in full), assuming an initial public offering price of $ per share, which is the midpoint of price range set forth on the cover page of this prospectus. Each $1.00 increase or decrease in the initial public offering price per share would increase or decrease, as applicable, our net proceeds, after deducting estimated underwriting discounts and commissions, by $ million (assuming that the number of shares offered by us remains the same and no exercise by the underwriters of their option to purchase additional shares). Similarly, each increase or decrease of 1.0 million shares in the number of shares of our Class A common stock offered by us would increase or decrease, as applicable, our net proceeds by $ million, assuming an initial public offering price of $ per share, which is the midpoint of the price range set forth on the cover page of this prospectus, and after deducting estimated underwriting discounts and commissions. We intend to use the net proceeds from this offering for general corporate purposes, including working capital,

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	operating expenses and capital expenditures. Additionally, we may use a portion of the net proceeds we receive from this offering to acquire businesses, products or technologies. However, we do not have agreements or commitments for any material acquisitions at this time. See the section titled "*Use of proceeds*".
Voting rights	Each share of our Class A common stock entitles its holder to one vote on all matters to be voted on by stockholders generally. Each share of our Class B common stock entitles its holder to ten votes on all matters to be voted on by stockholders generally. Holders of our Class A common stock and Class B common stock will generally vote together as a single class, unless otherwise required by law or our amended and restated certificate of incorporation. Additionally, our executive officers, directors and holders of 5% or more of our common stock will hold, in the aggregate, approximately % of the voting power of our outstanding capital stock following this offering. See the sections titled "*Principal stockholders*" and "*Description of capital stock*" for additional information.
Dividend policy	We do not intend to pay dividends on our Class A common stock in the foreseeable future. See the section titled "*Dividend policy*".
Risk factors	See the section titled "*Risk factors*" for a discussion of risks you should carefully consider before investing in our Class A common stock.
Proposed Nasdaq trading symbol	"TXG"

Unless we specifically state otherwise or the context otherwise requires, the number of shares of our Class A common stock and Class B common stock that will be outstanding after this offering is based on 8,095,382 shares of our Class A common stock and 75,754,278 shares of our Class B common stock (including our Convertible Preferred Stock on an as-converted basis) outstanding as of June 30, 2019 and excludes:

- 15,634,182 shares of Class A common stock issuable upon exercise of stock options outstanding as of June 30, 2019, at a weighted-average exercise price of $3.61 per share;
- 266,099 shares of Class of A common stock issuable upon exercise of warrants outstanding as of June 30, 2019, at a weighted-average exercise price of $1.17 per share;
- 842,475 shares of Class A common stock issuable upon exercise of stock options granted after June 30, 2019, at a weighted-average exercise price of $30.00 per share; and

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- 11,000,000 shares of Class A common stock to be reserved and available for future issuance under our 10x Genomics, Inc. 2019 Omnibus Incentive Plan (the "Omnibus Incentive Plan"), which will become effective in connection with this offering, as more fully described in the section titled "*Executive compensation—Equity incentive plans*", including:
  - 1,323,858 shares of Class A common stock reserved for future grants under our 10x Genomics, Inc. Amended and Restated 2012 Stock Plan (the "2012 Stock Plan"), as of June 30, 2019, which will be added to the shares reserved under our Omnibus Incentive Plan; plus
  - any shares of Class A common stock issuable upon exercise of stock options outstanding under the 2012 Stock Plan that will be added to our Omnibus Incentive Plan available reserve upon expiration or termination of such stock options; plus
  - automatic increases in the number of shares of Class A common stock reserved for future grants pursuant to our Omnibus Incentive Plan; plus
  - 2,000,000 shares of Class A common stock to be reserved and available for future issuance under our 10x Genomics, Inc. 2019 Employee Stock Purchase Plan (the "ESPP"), which will become effective in connection with this offering, as well as automatic increases in the number of shares of Class A common stock reserved for future issuance under the ESPP.

Unless we specifically state otherwise or the context otherwise requires, this prospectus reflects and assumes the following:

- no exercise of the outstanding stock options and warrants described above;
- outstanding shares include 198,250 shares of Class A common stock issued upon the early exercise of stock options and subject to repurchase as of June 30, 2019;
- no exercise by the underwriters of their option to purchase additional shares of our Class A common stock in this offering;
- the filing and effectiveness of our amended and restated certificate of incorporation, to be in effect at the closing of this offering;
- the reclassification of all outstanding shares of our Historical Class A common stock into Class B common stock and of our Historical Class B common stock (including outstanding stock options and warrants to purchase such shares) into Class A common stock prior to the closing of this offering as described under "*Description of capital stock—Reclassification of common stock and conversion of Convertible Preferred Stock*"; and
- the automatic conversion of all shares of our Convertible Preferred Stock outstanding as of June 30, 2019 into 67,704,278 shares of Class B common stock prior to the closing of this offering as described under "*Description of capital stock—Reclassification of common stock and conversion of Convertible Preferred Stock*".

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Summary consolidated financial and other data

The following tables summarize our consolidated financial and other data for the years and as of the dates indicated. We have derived the summary consolidated statements of operations data for the years ended December 31, 2017 and 2018 from our audited consolidated financial statements and related notes included elsewhere in this prospectus. We have derived the summary consolidated statements of operations data for the six months ended June 30, 2018 and 2019, and the summary consolidated balance sheet data as of June 30, 2019 from our unaudited consolidated interim financial statements and related notes included elsewhere in this prospectus. Our unaudited consolidated interim financial statements were prepared in accordance with generally accepted accounting principles in the United States ("GAAP"), on the same basis as our audited consolidated financial statements and include, in the opinion of management, all adjustments, consisting of normal recurring adjustments, that are necessary for the fair presentation of the financial information set forth in those financial statements. Our historical results are not necessarily indicative of results that may be expected in the future. You should read the following summary consolidated financial and other data together with our consolidated financial statements and related notes included elsewhere in this prospectus and the information in the sections titled "*Selected consolidated financial data*" and "*Management's discussion and analysis of financial condition and results of operations*".

	Year ended December 31,		Six months ended June 30,	
(in thousands, except share and per share data)	**2017**	**2018**	**2018**	**2019**
			(unaudited)	
Consolidated statements of operations data:				
Revenue	$ 71,085	$ 146,313	$ 59,152	$ 109,397
Cost of revenue(1)	10,560	28,661	8,520	28,971
Gross profit	60,525	117,652	50,632	80,426
Operating expenses:				
Research and development(1)	32,164	47,537	23,372	32,999
In-process research and development	—	62,363	6,206	—
Selling, general and administrative(1)	46,736	87,936	41,920	59,464
Accrued contingent liabilities	—	30,580	—	1,360
Total operating expenses	78,900	228,416	71,498	93,823
Loss from operations	(18,375)	(110,764)	(20,866)	(13,397)
Other income (expense):				
Interest income	308	1,024	461	505
Interest expense	(811)	(2,409)	(1,062)	(1,379)
Other income (expense), net	137	(249)	(120)	(141)
Total other income (expense)	(366)	(1,634)	(721)	(1,015)
Loss before provision for income taxes	(18,741)	(112,398)	(21,587)	(14,412)
Provision for income taxes	21	87	29	102
Net loss	$ (18,762)	$ (112,485)	$ (21,616)	$ (14,514)
Net loss per share attributable to common stockholders, basic and diluted(2)	$ (1.62)	$ (8.40)	$ (1.66)	$ (0.96)
Weighted-average shares used to compute net loss per share attributable to common stockholders, basic and diluted(2)	11,587,751	13,392,273	12,985,535	15,187,258
Pro forma net loss per share attributable to common stockholders, basic and diluted (unaudited)(2)		$ (1.45)		$ (0.18)
Weighted-average shares used to compute pro forma net loss per share attributable to common stockholders, basic and diluted (unaudited)(2)		77,494,992		82,891,536

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(1) Includes stock-based compensation expense as follows:

(in thousands)	Year ended December 31, 2017	Year ended December 31, 2018	Six months ended June 30, 2018	Six months ended June 30, 2019
			(unaudited)	
Cost of revenue	$ 44	$ 85	$ 36	$ 90
Research and development	801	1,030	440	1,798
Selling, general and administrative	816	1,543	530	2,496
Total stock-based compensation expense	$ 1,661	$ 2,658	$ 1,006	$ 4,384

(2) See Note 2 and Note 11 to our consolidated financial statements included elsewhere in this prospectus for further details on the calculation of net loss per share attributable to common stockholders, basic and diluted, the weighted-average shares used to compute net loss per share attributable to common stockholders, basic and diluted, and unaudited pro forma information.

(in thousands)	As of June 30, 2019 Actual	Pro forma(1)	Pro forma as adjusted(2)(3)
	(unaudited)	(unaudited)	(unaudited)
Consolidated balance sheet data:			
Cash and cash equivalents	$ 56,034	$	$
Working capital(4)	63,999		
Total assets	155,594		
Total current liabilities	43,227		
Total liabilities	140,298		
Total convertible preferred stock	243,244		
Accumulated deficit	(245,630)		
Total stockholders' equity (deficit)	(227,948)		

(1) The pro forma column in the consolidated balance sheet data table above reflects: (a) the reclassification of all outstanding shares of our Historical Class A common stock into Class B common stock and of our Historical Class B common stock into Class A common stock and (b) the automatic conversion of all shares of our Convertible Preferred Stock into 67,704,278 shares of Class B common stock, in each case, prior to the closing of this offering and as described under "*Description of capital stock—Reclassification of common stock and conversion of Convertible Preferred Stock*".

(2) The pro forma as adjusted column in the consolidated balance sheet data table above reflects (a) the pro forma adjustments set forth in footnote (1) above and (b) the issuance and sale of shares of Class A common stock by us in this offering at an assumed initial public offering price $ per share, which is the midpoint of the price range set forth on the cover page of this prospectus, after deducting estimated underwriting discounts and commissions and estimated offering expenses payable by us.

(3) Each $1.00 increase or decrease in the assumed initial public offering price of $ per share, which is the midpoint of the price range set forth on the cover page of this prospectus, would increase or decrease, as applicable, the amount of each of our pro forma as adjusted cash and cash equivalents, working capital, total assets and total stockholders' equity (deficit) by $, assuming that the number of shares offered by us, as set forth on the cover page of this prospectus, remains the same, and after deducting estimated underwriting discounts and commissions payable by us. Similarly, each increase or decrease of 1.0 million shares in the number of shares of our Class A common stock offered by us would increase or decrease, as applicable, the amount of each of our pro forma as adjusted cash and cash equivalents, working capital, total assets and total stockholders' equity (deficit) by $, assuming the assumed initial public offering price remains the same, and after deducting estimated underwriting discounts and commissions payable by us.

(4) Working capital is calculated as current assets less current liabilities. See our consolidated financial statements and related notes included elsewhere in this prospectus for further details regarding our current assets and current liabilities.

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Key business metrics

We review a number of operating and financial metrics, including the following key metrics, to evaluate our business, measure our performance, identify trends affecting our business, formulate business plans and make strategic decisions.

Instrument installed base

We define the instrument installed base as the cumulative number of Chromium instruments sold since inception.

The table below sets forth our instrument installed base as of the dates presented:

	As of December 31,		As of June 30,	
	2017	2018	2018	2019
Instrument installed base	491	1,021	701	1,284

Consumable pull-through per instrument

We define consumable pull-through per instrument as the total consumables revenue in the given quarter divided by the average instrument installed base during that quarter. We calculate the average instrument installed base for a given quarter using the instrument installed base as of the last day of the prior quarter and the instrument installed base as of the last day of the given quarter. We also calculate a year-to-date consumable pull-through per instrument figure by summing the quarterly pull-through for the quarters in a given year.

The table below sets forth the consumable pull-through per instrument for the periods presented:

	Year ended December 31,		Six months ended June 30,	
(in thousands)	2017	2018	2018	2019
Consumable pull-through per instrument	$ 140	$ 148	$ 72	$ 81

See the section titled "*Management's discussion and analysis of financial condition and results of operations—Key business metrics*" for additional information.

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Risk factors

Investing in our Class A common stock involves a high degree of risk. You should carefully consider the risks described below, as well as the other information in this prospectus, including our financial statements and the related notes and the section titled "Management's discussion and analysis of financial condition and results of operations" in this prospectus, before deciding whether to invest in our Class A common stock. The occurrence of any of the events or developments described below could harm our business, financial condition, results of operations and growth prospects. In such an event, the market price of our Class A common stock could decline and you may lose all or part of your investment. Additional risks and uncertainties not presently known to us or that we currently deem immaterial also may impair our business operations.

Risks related to our business and industry

We have incurred significant losses since inception, we expect to incur losses in the future and we may not be able to generate sufficient revenue to achieve and maintain profitability.

We have incurred significant losses since we were formed in 2012 and expect to incur losses in the future. We incurred net losses of $18.8 million and $112.5 million for the years ended December 31, 2017 and 2018, respectively. We incurred net losses of $21.6 million and $14.5 million for the six months ended June 30, 2018 and 2019, respectively. As of June 30, 2019, we had an accumulated deficit of $245.6 million. We expect that our losses will continue in the near term as we continue to invest significant additional funds toward ongoing research and development and toward the timely commercialization of both new products and improved versions of existing products. We also expect that our operating expenses will increase as a result of becoming a public company and will continue to increase as we grow our business. To date, we have financed our operations principally from the sale of convertible preferred stock, revenue from sales of our products and the incurrence of indebtedness. There can be no assurance that our revenue and gross profit will increase sufficiently such that our net losses decline, or we attain profitability, in the future. Further, our limited operating history and rapid revenue growth over the last several years make it difficult to effectively plan for and model future growth and operating expenses. Our ability to achieve or sustain profitability is based on numerous factors, many of which are beyond our control, including the impact of market acceptance of our products, future product development, our market penetration and margins and current and future litigation. We may never be able to generate sufficient revenue to achieve or sustain profitability and our recent and historical growth should not be considered indicative of our future performance. Our failure to achieve or maintain profitability could negatively impact the value of our Class A common stock.

In particular, we are subject to significant risks of losses related to current litigation matters. See "*—Risks related to litigation and our intellectual property*".

The life sciences technology market is highly competitive. If we fail to compete effectively, our business and operating results will suffer.

We face significant competition in the life sciences technology market. We currently compete with both established and early-stage life sciences technology companies that design, manufacture and market instruments, consumables and software for, among other applications, genomics, single cell analysis, spatial analysis and immunology. We believe our competitors in the life sciences technology market include Becton, Dickinson and Company, Bio-Rad and Nanostring Technologies, Inc., each of which has products that compete to varying degrees with some but not all of our product solutions, as well as a number of other emerging and established companies.

Some of our current competitors are large publicly-traded companies, or are divisions of large publicly-traded companies, and may enjoy a number of competitive advantages over us, including:

- greater name and brand recognition;

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- greater financial and human resources;
- broader product lines;
- larger sales forces and more established distributor networks;
- substantial intellectual property portfolios;
- larger and more established customer bases and relationships; and
- better established, larger scale and lower cost manufacturing capabilities.

We also face competition from researchers developing their own solutions. The area in which we compete involves rapid innovation and some of our customers have in the past, and more may in the future, elect to create their own platform or assays rather than rely on a third-party supplier such as ourselves. This is particularly true for the largest research centers and labs who are continually testing and trying new technologies, whether from a third-party vendor or developed internally. We also compete for the resources our customers allocate for purchasing a wide range of products used to analyze biological systems, some of which are additive to or complementary with our own but not directly competitive.

We cannot assure investors that our products will compete favorably or that we will be successful in the face of increasing competition from products and technologies introduced by our existing competitors, companies entering our markets or developed by our customers internally. In addition, we cannot assure investors that our competitors do not have or will not develop products or technologies that currently or in the future will enable them to produce competitive products with greater capabilities or at lower costs than ours or that are able to run comparable experiments at a lower total experiment cost. Any failure to compete effectively could materially and adversely affect our business, financial condition and operating results.

Our business depends significantly on the success of our Next GEM microfluidic chip.

Since our inception through the first half of 2019, substantially all of our Chromium instruments utilized our GEM microfluidic chips and associated consumables. In November 2018, a jury concluded that our Chromium instruments operating these chips and associated consumables infringe certain of Bio-Rad's patents. We have dedicated significant resources to designing and manufacturing our new Next GEM microfluidic chip, which uses a microfluidic architecture with fundamentally different physics from our GEM microfluidic chip. We introduced our Next GEM microfluidic chips for our Single Cell Gene Expression, Single Cell Immune Profiling and Single Cell ATAC solutions in the second quarter of 2019. We plan to gradually phase out our GEM microfluidic chips and anticipate that our Chromium products utilizing our Next GEM microfluidic chips will become an increasing percentage of our sales and will constitute substantially all of our Chromium sales by the end of 2020. In addition, we have not yet developed Next GEM microfluidic chips for our Single Cell CNV and Linked-Read solutions. Unless the injunction issued under the Bio-Rad litigation is stayed, we will be unable to sell our Single Cell CNV and Linked-Read solutions for use on new instruments unless and until we develop a Next GEM microfluidic chip for such solutions. Until we are able to completely transition to our Next GEM microfluidic chip design, our margins will be negatively impacted by any royalty obligations that result from ongoing litigation matters.

Although our Next GEM microfluidic chips were designed to replace our GEM microfluidic chips, we cannot assure you that we will be able to make our Next GEM microfluidic chip work with all of our solutions, that our Next GEM microfluidic chip will allow our customers to retain the level of performance or quality they have come to expect using our GEM microfluidic chip, that our Next GEM microfluidic chip will replace the sales of our GEM microfluidic chip or that we will be able to manufacture our Next GEM microfluidic chip in sufficient volumes and in sufficient quality in a timely fashion. While we believe that our Chromium solutions, when used with our Next GEM microfluidic chip, do not infringe the asserted Bio-Rad patents, we cannot assure you that our Next GEM microfluidic chip would not be found to infringe other patents, which could prevent us from making, selling and importing our Next GEM microfluidic chips or substantially all of our products. We currently

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expect that, by the end of the third quarter of 2019, all Chromium instruments that we sell will operate exclusively with our Next GEM solutions. We believe that these solutions are very important to our customers research but the delay caused by the injunction may slow customer adoption of our products or cause customers to investigate the availability of competing products or technologies.

We expect to incur increased research and development expenses in the near term and increased inventory and other expenses related to the introduction of, and transition to, our Next GEM microfluidic chip. Our failure to effectively manage product transitions or accurately forecast customer demand with respect to both instruments and consumables may lead to an increased risk of excess or obsolete inventory and resulting charges. We expect that as we transition to our Next GEM microfluidic chips we may need to write down the value of our GEM microfluidic chips and associated consumables we currently hold in inventory. As of June 30, 2019, we held approximately $0.3 million of GEM microfluidic chips in inventory. As we transition to our Next GEM microfluidic chips, we cannot guarantee that our customers will quickly switch to using our Next GEM microfluidic chips in their research. Customers may delay transitioning to our Next GEM microfluidic chips for a variety of reasons, including if they have experiments underway for which they do not want to introduce additional variables. More significantly, customers may decline to purchase our products altogether if they do not believe that our Next GEM microfluidic chips can produce results that are reliable, consistent and comparable to our GEM microfluidic chips.

For additional information relating to this litigation, see the section titled *"Risk factors—Risks related to litigation and our intellectual property—We are involved in significant litigation which has consumed significant resources and management time and adverse resolution of these lawsuits could require us to pay significant damages, and prevent us from selling our products, which would severely adversely impact our business, financial condition or results of operations".*

We are significantly dependent upon revenue generated from the sale of our Chromium solutions, and in particular our Single Cell Gene Expression solutions.

We currently generate substantially all of our revenue from the sale of our Chromium instruments, which we refer to as "instruments", and our proprietary microfluidic chips, slides, reagents and other consumables for both our Visium and Chromium solutions, which we refer to as "consumables". In particular, we are dependent upon revenue generated from sales of our Single Cell Gene Expression consumables, which accounted for approximately half of our revenue in each of the years ended December 31, 2017 and 2018 and for the six months ended June 30, 2019. There can be no assurance that we will be able to design future products, particularly non-Chromium product lines, that will meet the expectations of our customers or that our future products will become commercially viable. As technologies change in the future for research equipment in general and in genomics solutions specifically, we will be expected to upgrade or adapt our products in order to keep up with the latest technology. To date we have limited experience simultaneously designing, testing, manufacturing and selling non-Chromium products and there can be no assurance we will be able to do so. Our sales expectations are based in part on the assumption that our Chromium Connect instrument will increase workflows for our future customers and their associated purchases of our consumables. If sales of our Chromium Connect instruments fail to materialize so will the related consumable sales and associated revenue. Our sales expectations are also based in part on the continued success of our Single Cell Gene Expression solutions. If our recently introduced solutions, such as our Single Cell Immune Profiling and Single Cell ATAC solutions, or our upcoming Visium solution, fail to achieve sufficient market acceptance or sales of our Single Cell Gene Expression consumables decrease, our consumables revenue could be materially and adversely impacted.

Our business currently depends significantly on research and development spending by academic institutions, a reduction in which could limit demand for our products and adversely affect our business and operating results.

In each of the year end December 31, 2018 and the six months ended June 30, 2019, approximately 70% of our direct sales revenue came from sales to academic institutions. Much of their funding was, in turn, provided by

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various state, federal and international government agencies. In the near term, we expect that a large portion of our revenue will continue to be derived from sales of Chromium products, including our instruments and consumables, to academic institutions. As a result, in the near term, the demand for our products will depend upon the research and development budgets of these customers, which are impacted by factors beyond our control, such as:

- decreases in government funding of research and development;
- changes to programs that provide funding to research laboratories and institutions, including changes in the amount of funds allocated to different areas of research or changes that have the effect of increasing the length of the funding process;
- macroeconomic conditions and the political climate;
- scientists' and customers' opinions of the utility of new products or services;
- citation of new products or services in published research;
- changes in the regulatory environment;
- differences in budgetary cycles;
- competitor product offerings or pricing;
- market-driven pressures to consolidate operations and reduce costs; and
- market acceptance of relatively new technologies, such as ours.

In addition, various state, federal and international agencies that provide grants and other funding may be subject to stringent budgetary constraints that could result in spending reductions, reduced grant making, reduced allocations or budget cutbacks, which could jeopardize the ability of these customers, or the customers to whom they provide funding, to purchase our products. For example, congressional appropriations to the National Institutes of Health (the "NIH") have generally increased year-over-year for the last 18 years, and reached a new high in 2018, but the NIH also experiences occasional year-over-year decreases in appropriations, including as recently as 2013. In addition, funding for life science research has increased more slowly during the past several years compared to previous years and has actually declined in some countries. There is no guarantee that NIH appropriations will not decrease in the future, and a decrease may be more likely under the current administration, whose annual budget proposals have repeatedly decreased NIH appropriations. A decrease in the amount of, or delay in the approval of, appropriations to NIH or other similar United States or international organizations, such as the Medical Research Council in the United Kingdom, could result in fewer grants benefiting life sciences research. These reductions or delays could also result in a decrease in the aggregate amount of grants awarded for life sciences research or the redirection of existing funding to other projects or priorities, any of which in turn could cause our customers and potential customers to reduce or delay purchases of our products. Our operating results may fluctuate substantially due to any such reductions and delays. Any decrease in our customers' budgets or expenditures, or in the size, scope or frequency of their capital or operating expenditures, could materially and adversely affect our business, operating results and financial condition.

Our failure to effectively manage product transitions or accurately forecast customer demand could result in excess or obsolete inventory and resulting charges.

Because the market for our products is characterized by rapid technological advances, we frequently introduce new products with improved ease-of-use, improved performance or additional features and functionality. We

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pre-announce products and services, in some cases before such products and services have been fully developed or tested, and risk failing to meet expectations when such products and services become available. The risks associated with the introduction of new products include the difficulties of predicting customer demand and effectively managing inventory levels to ensure adequate supply of the new product and avoiding excess supply of the legacy product.

We may strategically enter into non-cancelable commitments with vendors to purchase materials for our products in advance of demand to take advantage of favorable pricing, address concerns about the availability of future supplies or build safety stock to help ensure customer shipments are not delayed should we experience higher than anticipated demand for materials with long lead times. For example, inventories increased 79% from $4.8 million as of December 31, 2017 to $8.6 million as of December 31, 2018 and inventories increased 43% from $8.6 million as of December 31, 2018 to $12.3 million as of June 30, 2019, primarily to fulfill the increased level of expected demand of our products, as well as to build inventory in anticipation of product transitions.

Our future success is dependent upon our ability to increase penetration in our existing markets.

Our customer base includes academic, government, biopharmaceutical, biotechnology and other institutions. In both the year ended December 31, 2018 and the six months ended June 30, 2019, approximately 70% of our direct sales revenue came from sales to academic institutions. Our success will depend upon our ability to increase our market penetration among these customers and to expand our market by developing and marketing new products and new applications for existing products. We recently announced our intention to introduce our Visium product line for spatial analysis and our future success will partially depend on our ability to commercialize this product line. As we continue to scale our business, we may find that certain of our products, certain customers or certain markets, including the biopharmaceutical market, may require a dedicated sales force or sales personnel with different experience than those we currently employ. Identifying, recruiting and training additional qualified personnel would require significant time, expense and attention.

We cannot assure investors that we will be able to further penetrate our existing market or that the market will be able to sustain our current and future product offerings. Any failure to increase penetration in our existing markets would adversely affect our ability to improve our operating results.

We may not be able to develop new products, enhance the capabilities of our existing products to keep pace with rapidly changing technology and customer requirements or successfully manage the transition to new product offerings, any of which could have a material adverse effect on our business and operating results.

Our success depends on our ability to develop new products and applications for our technology in existing and new markets, while improving the performance and cost-effectiveness of our existing products, in each case in ways that address current and anticipated customer requirements. Such success is dependent upon several factors, including functionality, competitive pricing and integration with existing and emerging technologies. New technologies, techniques or products could emerge that might offer better combinations of price and performance or better address customer requirements as compared to our current or future products. Existing markets for our products, including the genomics, single cell analysis, spatial analysis and other relevant markets, are characterized by rapid technological change and innovation. Competitors may be able to respond more quickly and effectively than we can to new or changing opportunities, technologies, standards or customer requirements. Due to the significant lead time involved in bringing a new product to market, we are required to make a number of assumptions and estimates regarding the commercial feasibility of a new product, including assumptions and estimates regarding the biological analytes that researchers will want to measure, the appropriate method of measuring such analytes, how researchers intend to use the resulting data and the scope and type of data that will be most useful to researchers. As a result, it is possible that we may introduce a new product that uses technologies or methods of analysis that have been displaced by the time of

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launch, addresses a market that no longer exists or is smaller than previously thought, targets biological analytes or produces data that provides less utility to researchers than previously thought or otherwise is not competitive at the time of launch. We anticipate that we will face increased competition in the future as existing companies and competitors develop new or improved products and as new companies enter the market with new technologies. Our ability to mitigate downward pressure on our selling prices will be dependent upon our ability to maintain or increase the value we offer to researchers. The expenses or losses associated with unsuccessful product development or launch activities, or a lack of market acceptance of our new products, could adversely affect our business, financial condition or results of operations.

Because our solutions are used with other products, such as sequencers, to conduct an experiment, we also expect to face competition from these complementary products, either directly or indirectly, as researchers and labs look to reduce the total cost of any given experiment. For example, if a sequencer manufacturer was successful in vertically integrating their product to provide functionality equivalent to our instruments, they would likely be able to deliver a solution that is capable of running comparable experiments with a total experiment cost that is significantly less than the cost of running such experiments using our products together with third-party sequencers. Conversely, if genome sequencing falls out of favor as a preferred approach for genomic research, whether through the development of alternative solutions or real or perceived problems with sequencing itself, the utility of our products could be significantly impacted. It is critical to our success that we anticipate changes such as these in technology and customer requirements and successfully introduce new, enhanced and competitive technologies to meet our customers' and prospective customers' needs on a timely and cost-effective basis. If we do not successfully innovate and introduce new technology into our product lines, our business and operating results will be adversely impacted.

Our ability to attract new customers and increase revenue from existing customers depends in large part on our ability to enhance and improve our existing solutions and to introduce compelling new solutions. The success of any enhancement to our solutions depends on several factors, including timely completion and delivery, competitive pricing, adequate quality testing, integration with existing technologies and overall market acceptance. Any new solution that we develop may not be introduced in a timely or cost-effective manner, may contain errors, vulnerabilities or bugs, or may not achieve the market acceptance necessary to generate significant revenue. If we are unable to successfully develop new solutions, enhance our existing solutions to meet customer requirements, or otherwise gain market acceptance, our business, results of operations and financial condition would be harmed.

Our ability to attract new customers and increase revenue from existing customers also depends on our ability to deliver any enhanced or new solutions to our customers in a format where they can be easily and consistently deployed by most or all users without significant customer service. If our customers believe that deploying our enhanced or new solutions would be overly time-consuming, confusing or technically challenging, then our ability to grow our business would be substantially harmed. We need to create and deliver a repeatable, user-friendly, prescriptive approach to deployment that allows users of all kinds to effectively and easily deploy our solutions, and if we fail to do so, our business and results of operations would be harmed.

The typical development cycle of new life sciences products can be lengthy and complicated, and may require new scientific discoveries or advancements and complex technology and engineering. Such developments may involve external suppliers and service providers, making the management of development projects complex and subject to risks and uncertainties regarding timing, timely delivery of required components or services and satisfactory technical performance of such components or assembled products. If we do not achieve the required technical specifications or successfully manage new product development processes, or if development work is not performed according to schedule, then such new technologies or products may be adversely impacted and our business and operating results may be harmed.

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If our existing and new products fail to achieve and sustain sufficient scientific acceptance, we will not generate expected revenue and our prospects may be harmed.

The life sciences scientific community is comprised of a small number of early adopters and key opinion leaders who significantly influence the rest of the community. The success of life sciences products is due, in large part, to acceptance by the scientific community and their adoption of certain products as best practice in the applicable field of research. The current system of academic and scientific research views publishing in a peer-reviewed journal as a measure of success. In such journal publications, the researchers will describe, not only their discoveries, but also the methods and typically the products used to fuel such discoveries. Mentions in peer-reviewed journal publications is a good barometer for the general acceptance of our products as best practices. Ensuring that early adopters and key opinion leaders publish research involving the use of our products is critical to ensuring our products gain widespread acceptance and market growth. Continuing to maintain good relationships with such key opinion leaders is vital to growing our market. The number of times our products were mentioned in peer-reviewed publications has increased significantly in the last two years. During this time our revenue has also increased significantly. We cannot assure investors that our products will continue to be mentioned in peer-reviewed articles with any frequency or that any new products that we introduce in the future will be mentioned in peer-reviewed articles. If too few researchers describe the use of our products, too many researchers shift to a competing product and publish research outlining their use of that product or too many researchers negatively describe the use of our products in publications, it may drive existing and potential customers away from our products, which could harm our operating results.

If we do not sustain or successfully manage our growth and anticipated growth, our business and prospects will be harmed.

We have experienced rapid growth in recent periods. This growth and our anticipated growth will place significant strains on our management, operational and manufacturing systems and processes, financial systems and internal controls and other aspects of our business. For example, we consummated two acquisitions in 2018 and intend to continue to make investments that meet management's criteria to expand or add key technologies that we believe will facilitate the commercialization of new products in the future. In addition, we launched six new products and new versions of existing products in 2018 and intend to launch additional new products and new versions of existing products in the next six to twelve months. Further development and commercialization of our current and future products are key elements of our growth strategy. Developing and launching new products and innovating and improving our existing products have required us to hire and retain additional scientific, sales and marketing, software, manufacturing, distribution and quality assurance personnel. As a result, we have experienced rapid headcount growth from 110 employees as of December 31, 2015 to 500 employees as of June 30, 2019. As we have grown, our employees have become more geographically dispersed. We currently serve thousands of researchers in approximately 40 countries and plan to continue to expand to new international jurisdictions as part of our growth strategy which will lead to increased dispersion of our employees. Moreover, we expect that we will need to hire additional accounting, finance and other personnel in connection with our becoming, and our efforts to comply with the requirements of being, a public company. Once public, our management and other personnel will need to devote a substantial amount of time towards maintaining compliance with these requirements. We may face challenges integrating, developing and motivating our rapidly growing and increasingly dispersed employee base. In addition, certain members of our management have not previously worked together for an extended period of time, do not have experience managing a public company or do not have experience managing a global business, which may affect how they manage our growth. To effectively manage our growth, we must continue to improve our operational and manufacturing systems and processes, our financial systems and internal controls and other aspects of our business and continue to effectively expand, train and manage our personnel. As our organization continues to grow, and we are required to implement more complex organizational management

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structures, we may find it increasingly difficult to maintain the benefits of our corporate culture, including our ability to quickly develop and launch new and innovative products. If we do not successfully manage our anticipated growth, our business, results of operations and growth prospects will be harmed.

Our limited operating history and rapid revenue growth make it difficult to evaluate our future prospects and the risks and challenges we may encounter.

We launched our first product in mid-2015 and have experienced significant revenue growth in recent periods, including an increase in revenue of $75.2 million, or 106%, for the year ended December 31, 2018 as compared to the year ended December 31, 2017. In addition, we operate in highly competitive markets characterized by rapid technological advances and our business has, and we expect it to continue, to evolve over time to remain competitive. Our limited operating history, evolving business and rapid growth make it difficult to evaluate our future prospects and the risks and challenges we may encounter and may increase the risk that we will not continue to grow at or near historical rates.

If we fail to address the risks and difficulties that we face, including those described elsewhere in this "*Risk factors*" section, our business, financial condition and results of operations could be adversely affected. We have encountered in the past, and will encounter in the future, risks and uncertainties frequently experienced by growing companies with limited operating histories in rapidly changing industries. If our assumptions regarding these risks and uncertainties, which we use to plan and operate our business, are incorrect or change, or if we do not address these risks successfully, our results of operations could differ materially from our expectations and our business, financial condition and results of operations could be adversely affected.

Our operating results have in the past fluctuated significantly and may continue to fluctuate significantly in the future, which makes our future operating results difficult to predict and could cause our operating results to fall below expectations or any guidance we may provide.

Our quarterly and annual operating results may fluctuate significantly, which makes it difficult for us to predict our future operating results. These fluctuations may occur due to a variety of factors, many of which are outside of our control, including, but not limited to:

- the level of demand for our products, which may vary significantly, and our ability to increase penetration in our existing markets and expand into new markets;
- customers accelerating, canceling, reducing or delaying orders as a result of developments related to our litigation or to our transition to Next GEM microfluidic chips;
- the outcomes of and related rulings in the litigation and administrative proceedings in which we are currently involved;
- our ability to successfully manufacture and transition our existing customers to our Next GEM microfluidic chips;
- the timing and cost of, and level of investment in, research and development and commercialization activities relating to our products, which may change from time to time;
- the volume and mix of our instrument and consumable sales or changes in the manufacturing or sales costs related to our instruments and consumables;
- the success of our recently announced products, such as our Chromium Connect and Visium platform, and the introduction of other new products or product enhancements by us or others in our industry;
- the timing and amount of expenditures that we may incur to acquire, develop or commercialize additional products and technologies or for other purposes, such as the expansion of our facilities;

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- changes in governmental funding of life sciences research and development or changes that impact budgets, budget cycles or seasonal spending patterns of our customers;
- future accounting pronouncements or changes in our accounting policies;
- the outcome of any future litigation or governmental investigations involving us, our industry or both;
- difficulties encountered by our commercial carriers in delivering our instruments or consumables, whether as a result of external factors such as weather or internal issues such as labor disputes;
- general market conditions and other factors, including factors unrelated to our operating performance or the operating performance of our competitors;
- higher than anticipated warranty costs; and
- the other factors described in this "*Risk factors*" section.

The cumulative effects of the factors discussed above could result in large fluctuations and unpredictability in our quarterly and annual operating results. As a result, comparing our operating results on a period-to-period basis may not be meaningful. Investors should not rely on our past results as an indication of our future performance.

This variability and unpredictability could also result in our failing to meet the expectations of industry or financial analysts or investors for any period. If our revenue or operating results fall below the expectations of analysts or investors or below any guidance we may provide, or if the guidance we provide is below the expectations of analysts or investors, the price of our Class A common stock could decline substantially. Such a stock price decline could occur even when we have met or exceeded any previously publicly stated guidance we may provide.

The sizes of the markets for our solutions may be smaller than estimated and new market opportunities may not develop as quickly as we expect, or at all, limiting our ability to successfully sell our solutions.

The market for genomics products is new and evolving, making it difficult to predict with any accuracy the sizes of the markets for our current and future solutions. Our estimates of the annual total addressable market for our current and future solutions are based on a number of internal and third-party estimates and assumptions. In particular, our estimates are based on our expectations that: (a) researchers in the market for certain life sciences research tools and technologies, such as flow cytometry, next generation sequencing, laboratory automation, microscopy and sample preparation, among others, will view our solutions as competitive alternatives to, or better options than, such existing tools and technologies; (b) researchers who already own such existing tools and technologies will recognize the ability of our solutions to complement, enhance and enable new applications of their current tools and technologies and find the value proposition offered by our solutions convincing enough to purchase our solutions in addition to the tools and technologies they already own; and (c) the trends we have seen among our customers with respect to placements of our instruments in comparison to the installed base of RT-PCR units and next generation sequencers are representative of the broader market. Underlying each of these expectations are a number of estimates and assumptions, including the assumption that government or other sources of funding will continue to be available to life sciences researchers at times and in amounts necessary to allow them to purchaser our solutions.

In addition, our growth strategy involves launching new solutions and expanding sales of existing solutions into new markets in which we have limited or no experience, such as the biopharmaceutical market. Sales of new or existing solutions into new market opportunities may take several years to develop and mature and we cannot be certain that these market opportunities will develop as we expect. For example, new life sciences technology is often not adopted by the relevant market until a sufficient amount of research conducted using such technology has been published in peer-reviewed publications. Because there can be a considerable delay

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between the launch of a new life sciences product and publication of research using such product, new life sciences products do not generally contribute a meaningful amount of revenue in the year they are introduced. In certain markets, such as the biopharmaceutical market, new life sciences technology, even if sufficiently covered in peer-reviewed publications, may not be adopted until the consistency and accuracy of such technology, method or device has been proven. As a result, the sizes of the annual total addressable market for new markets and new products are even more difficult to predict.

While we believe our assumptions and the data underlying our estimates of the total annual addressable market for our solutions are reasonable, these assumptions and estimates may not be correct and the conditions supporting our assumptions or estimates, or those underlying the third-party data we have used, may change at any time, thereby reducing the accuracy of our estimates. As a result, our estimates of the annual total addressable market for our solutions may be incorrect.

The future growth of the market for our current and future solutions depends on many factors beyond our control, including recognition and acceptance of our solutions by the scientific community as best practice and the growth, prevalence and costs of competing products and solutions. Such recognition and acceptance may not occur in the near term, or at all. If the markets for our current and future solutions are smaller than estimated or do not develop as we expect, our growth may be limited and our business, financial condition and operational results may be adversely affected.

Our management uses certain key business metrics to evaluate our business, measure our performance, identify trends affecting our business, formulate financial projections and make strategic decisions and such metrics may not accurately reflect all of the aspects of our business needed to make such evaluations and decisions, in particular as our business continues to grow.

In addition to our consolidated financial results, our management regularly reviews a number of operating and financial metrics, including our instrument installed base and consumable pull-through per instrument, to evaluate our business, measure our performance, identify trends affecting our business, formulate financial projections and make strategic decisions. We define the instrument installed base as the cumulative number of instruments sold since inception and define consumable pull-through per instrument as the total consumables revenue in the given quarter divided by the average instrument installed base during that quarter. We believe that these metrics are representative of our current business; however, these metrics may not accurately reflect all aspects of our business and we anticipate that these metrics may change or may be substituted for additional or different metrics as our business grows and as we introduce new products. For example, we expect that our expansion into new markets and adoption by new customers who may not have the same financial resources to devote to consumable purchases as our existing customer base could adversely impact our pull-through figures. These metrics also do not accurately reflect information relating to customers who purchase consumables but do not own an instrument, whom we refer to as “halo users”. Halo users and the future introduction of consumables that may not use instruments, such as our recently announced Visium solution, or instruments that are expected to use a greater amount of consumables, such as our Chromium Connect instrument, could reduce the utility of our consumable pull-through per instrument metric and make it difficult to compare such figures over time. Moreover, we expect some of our halo users to purchase instruments of their own which would decrease the consumables sold per instrument and therefore decrease our annual consumable pull-through per instrument. Though we expect the introduction of enhanced features and additional solutions on our Chromium instrument to increase consumable pull-through per instrument and to offset this decline, there are no assurances we will be successful in doing so. If our management fails to review other relevant information or change or substitute the key business metrics they review as our business grows and we introduce new products, their ability to accurately formulate financial projections and make strategic decisions may be compromised and our business, financial results and future growth prospects may be adversely impacted.

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We are dependent on single source and sole source suppliers for some of the components and materials used in our products and the loss of any of these suppliers could harm our business.

We do not have long-term contracts with our suppliers for the significant majority of the services, materials and components we use for the manufacture and delivery of our products. In certain cases, we also rely on single suppliers for all of our requirements for some of our materials or components. In most cases we do not have long term contracts with these suppliers, and even in the cases where we do the contracts include significant qualifications that would make it extremely difficult for us to force the supplier to provide us with their services, materials or components should they choose not to do so. We are therefore subject to the risk that these third-party suppliers will not be able or willing to continue to provide us with materials and components that meet our specifications, quality standards and delivery schedules. Factors that could impact our suppliers' willingness and ability to continue to provide us with the required materials and components include disruption at or affecting our suppliers' facilities, such as work stoppages or natural disasters, adverse weather or other conditions that affect their supply, the financial condition of our suppliers and deterioration in our relationships with these suppliers. In addition, we cannot be sure that we will be able to obtain these materials and components on satisfactory terms. Any increase in material and component costs could reduce our sales and harm our gross margins. In addition, any loss of a material supplier may permanently cause a change in one or more of our products that may not be accepted by our customers or cause us to eliminate that product altogether.

For example, we depend on a limited number of suppliers for enzymes and amplification mixes used in our consumables. In some cases, these manufacturers are the sole source of certain types of enzymes and reagents. We do not have long-term contracts with any of these sole source suppliers. Lead times for some of these components can be several months or more. In the event that demand increases, a manufacturing 'lot' does not meet our specifications or we fail to forecast and place purchase orders sufficiently in advance, this could result in a material shortage. Some of the components and formulations are proprietary to our vendors, thereby making second sourcing and development of a replacement difficult. Furthermore, such vendors may have intellectual property rights that could prevent us from sourcing such reagents from other vendors. If enzymes and reagents become unavailable from our current suppliers and we are unable to find acceptable substitutes for these suppliers, we may be required to produce them internally or change our product designs.

We have not qualified secondary sources for all materials or components that we source through a single supplier and we cannot assure investors that the qualification of a secondary supplier will prevent future supply issues. Disruption in the supply of materials or components would impair our ability to sell our products and meet customer demand, and also could delay the launch of new products, any of which could harm our business and results of operations. If we were to have to change suppliers, the new supplier may not be able to provide us materials or components in a timely manner and in adequate quantities that are consistent with our quality standards and on satisfactory pricing terms. In addition, alternative sources of supply may not be available for materials that are scarce or components for which there are a limited number of suppliers.

If our facilities or our third-party manufacturers' facilities become unavailable or inoperable, our research and development program could be adversely impacted and manufacturing of our instruments and consumables could be interrupted.

The manufacturing process for our Chromium Controller takes place at our third-party manufacturer's facilities in California. The majority of our consumables are manufactured at our facilities in Pleasanton, California using proprietary equipment. Certain raw materials, such as oligonucleotides and enzymes, are custom manufactured by outside partners. We periodically review the manufacturing capacity of our consumables and we expect to manufacture an increasing amount of consumables in-house. Our Pleasanton facilities also house the majority of our research and development and quality assurance teams. Our planned Chromium Connect will be

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manufactured by our partner at their facility. The facilities and the equipment we and our third-party manufacturers use to manufacture our instruments and consumables and that we use in our research and development program would be costly to replace and could require substantial lead times to repair or replace.

Our facilities in Pleasanton are vulnerable to natural disasters and catastrophic events. For example, our Pleasanton facilities are located near earthquake fault zones and are vulnerable to damage from earthquakes as well as other types of disasters, including fires, floods, power loss, communications failures and similar events. If any disaster or catastrophic event were to occur, our ability to operate our business would be seriously, or potentially completely, impaired. If our facilities or any of our third-party manufacturers' facilities become unavailable for any reason, we cannot provide assurances that we will be able to secure alternative manufacturing facilities with the necessary capabilities and equipment on acceptable terms, if at all. We may encounter particular difficulties in replacing our Pleasanton facilities given the specialized equipment housed within it. The inability to manufacture our instruments and/or consumables, combined with our limited inventory of manufactured instruments and consumables, may result in the loss of customers or harm our reputation, and we may be unable to reestablish relationships with those customers in the future. Because certain of our consumables and the raw materials we use to manufacture consumables at our Pleasanton facilities are perishable and must be kept in temperature controlled storage, the loss of power to our facilities, mechanical or other issues with our storage facilities or other events that impact our temperature controlled storage could result in the loss of some or all of such consumables and raw materials and we may not be able to replace them without disruption to our customers or at all.

In both the year ended December 31, 2018 and the six months ended June 30, 2019, approximately 70% of our direct sales revenue came from sales to academic institutions, whose research often requires long uninterrupted studies performed on a consistent basis over time; thus interruptions in our ability to supply consumables could be particularly damaging to these studies and our reputation. In addition, the budgetary planning and approval process for academic research programs can be lengthy and begin well in advance of the planned purchase of our instrument and/or consumables. If our products become unavailable during the planning process, researchers may use alternative products.

If our research and development program were disrupted by a disaster or catastrophe, the launch of new products and the timing of improvements to existing products could be significantly delayed and could adversely impact our ability to compete with other available products and solutions. If our or our third-party manufacturers' capabilities are impaired, we may not be able to manufacture and ship our products in a timely manner, which would adversely impact our business. Although we possess insurance for damage to our property and the disruption of our business, this insurance may not be sufficient to cover all of our potential losses and may not continue to be available to us on acceptable terms, or at all.

We may be unable to consistently manufacture our instruments and consumables to the necessary specifications or in quantities necessary to meet demand at an acceptable cost or at an acceptable performance level.

Our products are integrated solutions with many different components that work together. As such, a quality defect in a single component can compromise the performance of the entire solution. Certain of our consumables are manufactured at our Pleasanton, California facilities using complex processes, sophisticated equipment and strict adherence to specifications and quality systems procedures. In many cases, the consumables we manufacture are bundled with products or components that we source from third parties and assemble, package and perform quality assurance testing at our Pleasanton facilities. Our Chromium Controllers are manufactured by our third-party manufacturers at their facilities. In order to successfully generate revenue from our products, we need to supply our customers with products that meet their expectations for quality and functionality in accordance with established specifications. In order to ensure we are able to meet these expectations, our

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Pleasanton, California manufacturing facilities, as well as the facilities of our third-party manufacturers, have obtained International Organization for Standardization ("ISO") quality management certifications and employ other quality control measures. While customer complaints regarding defects in our products and consumables have historically been low, our customers have experienced quality control and manufacturing defects in the past. For example, a manufacturing defect in certain of our Chromium Controllers resulted in an unacceptable level of LCD screen failures and we launched a free replacement program in 2018 to allow customers to replace affected LCD screens as a result. As we continue to grow and introduce new products, and as our products incorporate increasingly sophisticated technology, it will be increasingly difficult to ensure our products are produced in the necessary quantities without sacrificing quality. There is no assurance that we or our third-party manufacturers will be able to continue to manufacture our products so that they consistently achieve the product specifications and quality that our customers expect. Certain of our consumables are subjected to a shelf life, after which their performance is not ensured. Shipment of consumables that effectively expire early or shipment of defective instruments or consumables to customers may result in recalls and warrantee replacements, which would increase our costs, and depending upon current inventory levels and the availability and lead time for additional inventory, could lead to availability issues. Any future design issues, unforeseen manufacturing problems, such as contamination of our or their facilities, equipment malfunctions, aging components, quality issues with components and materials sourced from third-party suppliers, or failures to strictly follow procedures or meet specifications, may have a material adverse effect on our brand, business, financial condition and operating results and could result in us or our third-party manufacturers losing ISO quality management certifications. If we or our third-party manufacturers fail to maintain ISO quality management certifications, our customers might choose not to purchase products from us. Furthermore, we or our third-party manufacturers may not be able to increase manufacturing to meet anticipated demand or may experience downtime.

In addition, as we increase manufacturing capacity, we will also need to make corresponding improvements to other operational functions, such as our customer service and billing systems, compliance programs and our internal quality assurance programs. We will also need additional equipment, manufacturing and warehouse space and trained personnel to process higher volumes of products. We cannot assure you that any increases in scale, related improvements and quality assurance will be successfully implemented or that equipment, manufacturing and warehouse space and appropriate personnel will be available. As we develop additional products, we may need to bring new equipment on-line, implement new systems, technology, controls and procedures and hire personnel with different qualifications. Our ability to increase our manufacturing capacity at our Pleasanton, California location is complicated by the use of our proprietary equipment that is not readily available from third-party manufacturers.

The risk of manufacturing defects or quality control issues is generally higher for new products, whether produced by us or a third-party manufacturer, products that are transitioned from one manufacturer to another, particularly if manufacturing is transitioned or initiated with a manufacturer we have not worked with in the past, and products that are transferred from one manufacturing facility to another. Our current product roadmap calls for the introduction of new instruments, such as our Chromium Connect, which integrates our Chromium Controller with complex robotics manufactured by our partner. We also expect to transition manufacturing of our Chromium Controller to a new third-party manufacturer with greater capacity in the near future.

As a result, both of our instruments will soon be manufactured by companies with which we have no prior manufacturing experience and the risk of manufacturing defects or quality control issues could increase as a result. Similarly, we also expect to expand our manufacturing facilities in Pleasanton, California during 2019. This expansion will result in the relocation of certain manufacturing processes and the risk of manufacturing defects or quality control issues in the consumables we manufacture there could increase as a result. We cannot assure investors that we and our third-party manufacturers will be able to launch new products on time, transition manufacturing of existing products to new manufacturers, transition our manufacturing capabilities

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to a new location or transition manufacturing of any additional consumables in-house without manufacturing defects.

An inability to manufacture products and components that consistently meet specifications, in necessary quantities and at commercially acceptable costs will have a negative impact and may have a material adverse effect on our business, financial condition and results of operations.

Undetected errors or defects in our solutions could harm our reputation and decrease market acceptance of our solutions.

Our instruments and consumables, as well as the software that accompanies them, may contain undetected errors or defects when first introduced or as new versions are released. Disruptions or other performance problems with our products or software may adversely impact our customers' research or business, harm our reputation and result in reduced revenue or increased costs associated with product repairs or replacements. If that occurs, we may also incur significant costs, the attention of our key personnel could be diverted or other significant customer relations problems may arise. We may also be subject to warranty claims or breach of contract for damages related to errors or defects in our solutions.

Certain disruptions in supply of, and changes in the competitive environment for, raw materials integral to the manufacturing of our products may adversely affect our profitability.

We use a broad range of materials and supplies, including metals, chemicals and other electronic components, in our products. A significant disruption in the supply of these materials could decrease production and shipping levels, materially increase our operating costs and materially adversely affect our profit margins. Shortages of materials or interruptions in transportation systems, labor strikes, work stoppages, war, acts of terrorism or other interruptions to or difficulties in the employment of labor or transportation in the markets in which we purchase materials, components and supplies for the production of our products, in each case may adversely affect our ability to maintain production of our products and sustain profitability. Unforeseen end-of-life for certain components, such as enzymes, could cause backorders as we modify our product specifications to accommodate replacement components. If we were to experience a significant or prolonged shortage of critical components from any of our suppliers and could not procure the components from other sources, we would be unable to manufacture our products and to ship such products to our customers in a timely fashion, which would adversely affect our sales, margins and customer relations.

We depend on certain technologies that are licensed to us. We do not control these technologies and any loss of our rights to them could prevent us from selling our products.

We rely on licenses in order to be able to use various proprietary technologies that are used in a substantial majority of our consumables. We do not own the patents that are the subject matter of these licenses. Our rights to use these patented technologies in our business are subject to the continuation of and compliance with the terms of those licenses.

We may need to license other technologies to commercialize future products. We may also need to negotiate licenses to patents and patent applications after launching new products. Our business may suffer if the technologies, patents or patent applications are unavailable for license or if we are unable to enter into necessary licenses on acceptable terms.

If we fail to offer high quality customer service, our business and reputation could suffer.

We differentiate ourselves from our competition through our commitment to an exceptional customer experience. Accordingly, high quality customer service is important for the growth of our business and any failure to maintain such standards of customer service, or a related market perception, could affect our ability

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to sell products to existing and prospective customers. Additionally, we believe our customer service team has a positive influence on recurring consumables revenue. Providing an exceptional customer experience requires significant time and resources from our customer service team. Therefore, failure to scale our customer service organization adequately may adversely impact our business results and financial condition.

Customers utilize our service teams and online content for help with a variety of topics, including how to use our products efficiently, how to integrate our products into existing workflows, how to determine which of our other products may be needed for a given experiment and how to resolve technical, analysis and operational issues if and when they arise. While we have developed significant resources for remote training, including an extensive library of online videos, we may need to rely more on these resources for future customer training, or we may experience increased expenses to enhance our online and remote solutions. If our customers do not adopt these resources, we may be required to increase the staffing of our customer service team, which would increase our costs. Also, as our business scales, we may need to engage third-party customer service providers, which could increase our costs and negatively impact the quality of the customer experience if such third parties are unable to provide service levels equivalent to ours.

The number of our customers has grown significantly and such growth, as well as any future growth, will put additional pressure on our customer service organization. We may be unable to hire qualified staff quickly enough or to the extent necessary to accommodate increases in demand.

In addition, as we continue to grow our operations and reach a global customer base, we need to be able to provide efficient customer service that meets our customers' needs globally at scale. In geographies where we sell through distributors, we rely on those distributors to provide customer service. If these third-party distributors do not provide a high quality customer experience, our business operations and reputation may suffer.

We depend on our key personnel and other highly qualified personnel, and if we are unable to recruit, train and retain our personnel, we may not achieve our goals.

Our future success depends on our ability to recruit, train, retain and motivate key personnel, including our senior management, research and development, manufacturing and sales, customer service and marketing personnel. In particular, Dr. Saxonov, our Chief Executive Officer and one of our co-founders, and Dr. Hindson, our Chief Scientific Officer, President and one of our co-founders, are critical to our vision, strategic direction, culture and products. Competition for qualified personnel is intense, particularly in the San Francisco Bay Area. As we grow, we may continue to make changes to our management team, which could make it difficult to execute on our business plans and strategies. New hires also require significant training and, in most cases, take significant time before they achieve full productivity. Our failure to successfully integrate these key personnel into our business could adversely affect our business.

Our continued growth depends, in part, on attracting, retaining and motivating highly-trained sales personnel with the necessary scientific background and ability to understand our systems at a technical level to effectively identify and sell to potential new customers. In addition, the continued development of complementary software tools, such as our analysis tools and visualization software, requires us to compete for highly trained software engineers in the San Francisco Bay Area and for highly trained customer service personnel globally. We also compete for computational biologists and qualified scientific personnel with other life science companies, academic institutions and research institutions. Many of our scientific personnel are qualified foreign nationals whose ability to live and work in the United States is contingent upon the continued availability of appropriate visas. Due to the competition for qualified personnel in the San Francisco Bay Area, we expect to continue to rely on foreign nationals to fill part of our recruiting needs. As a result, changes to United States immigration policies could restrain the flow of technical and professional talent into the United

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States and may inhibit our ability to hire qualified personnel. The current United States administration has made restricting immigration and reforming the work visa process a key focus of its initiatives and these efforts may adversely affect our ability to find qualified personnel.

We do not maintain key man life insurance or fixed term employment contracts with any of our employees. As a result, our employees could leave our company with little or no prior notice and would be free to work for a competitor. Because of the complex and technical nature of our products and the dynamic market in which we compete, any failure to attract, train, retain and motivate qualified personnel could materially harm our operating results and growth prospects.

Acquisitions could disrupt our business, cause dilution to our stockholders and otherwise harm our business.

We have and may continue to acquire other businesses and legal entities to add specialized employees, products or technologies as well as pursue technology licenses or investments in complementary businesses. In 2018, we acquired Epinomics, Inc. (“Epinomics”), an epigenetics company based in California, and Spatial Transcriptomics Holdings AB (“Spatial Transcriptomics”), a spatial analysis company based in Stockholm, Sweden. We believe we are successfully integrating the technologies acquired from those companies into our business, but the long term success of these acquisitions is not guaranteed. These transactions and any future transactions could be material to our financial condition and operating results and expose us to many risks, including:

- disruption in our relationships with customers, distributors, manufacturers or suppliers as a result of such a transaction;
- unanticipated liabilities related to acquired companies, including liabilities related to acquired intellectual property or litigation relating thereto;
- difficulties integrating acquired personnel, technologies and operations into our existing business;
- diversion of management time and focus from operating our business;
- failure to realize anticipated benefits or synergies from such a transaction;
- increases in our expenses and reductions in our cash available for operations and other uses;
- possible write-offs or impairment charges relating to acquired businesses; and
- potential higher taxes if our tax position relating to the acquisitions were challenged.

Foreign acquisitions, such as our acquisition of Spatial Transcriptomics, involve unique risks in addition to those mentioned above, including those related to integration of operations across different cultures and languages, currency risks and the particular economic, political and regulatory risks associated with specific countries.

Even if we identify a strategic transaction that we wish to pursue, we may be prohibited from consummating such transaction due to the terms of our existing or any future indebtedness. For example, our Second Amended and Restated Loan and Security Agreement, dated February 9, 2018, with Silicon Valley Bank (as amended, restated or supplemented from time to time, the “Loan and Security Agreement”) includes a covenant that limits our ability to consummate acquisitions and the exceptions to this covenant are limited. If we were to pursue an acquisition that is not permitted by the Loan and Security Agreement, we would be required to seek a waiver from the lender under the Loan and Security Agreement and we cannot assure investors that the lender would grant such a waiver.

Future acquisitions or dispositions could result in potentially dilutive issuances of our equity securities, the incurrence of debt, contingent liabilities or amortization expenses or write-offs of goodwill, any of which could

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harm our financial condition. We cannot predict the number, timing or size of future acquisitions, or the effect that any such transactions might have on our operating results.

Seasonality may cause fluctuations in our revenue and results of operations.

We operate on a December 31st year end and believe that there are significant seasonal factors which may cause sales of our products, and particularly our Chromium Controller, to vary on a quarterly or yearly basis and increase the magnitude of quarterly or annual fluctuations in our operating results. We believe that this seasonality results from a number of factors, including the procurement and budgeting cycles of many of our customers, especially government- or grant-funded customers, whose cycles often coincide with government fiscal year ends. For example, the United States government's fiscal year end occurs in our third quarter and may result in increased sales of our products during this quarter if government-funded customers have unused funds that may be forfeited, or future budgets that may be reduced, if such funds remain unspent at such fiscal year end. Furthermore, the academic budgetary cycle similarly requires grantees to 'use or lose' their grant funding, which seems to be tied disproportionately to the end of the calendar year, driving sales higher during the fourth quarter. Similarly, our biopharmaceutical customers typically have calendar year fiscal years which also result in a disproportionate amount of their purchasing activity occurring during our fourth quarter. These factors have contributed, and may contribute in the future, to substantial fluctuations in our quarterly operating results. Because of these fluctuations, it is possible that in some quarters our operating results will fall below the expectations of securities analysts or investors. If that happens, the market price of our Class A common stock would likely decrease. These fluctuations, among other factors, also mean that our operating results in any particular period may not be relied upon as an indication of future performance. Seasonal or cyclical variations in our sales have in the past, and may in the future, become more or less pronounced over time, and have in the past materially affected, and may in the future materially affect, our business, financial condition, results of operations and prospects.

Our reliance on distributors for sales of our products in certain geographies outside of the United States could limit or prevent us from selling our products and impact our revenue.

We sell our products through third-party distributors in Asia, certain regions of Europe, South America, the Middle East and Africa. We intend to continue to grow our business internationally and to do so we must attract additional distributors and retain existing distributors to maximize the commercial opportunity for our products. There is no guarantee that we will be successful in attracting or retaining desirable sales and distribution partners or that we will be able to enter into such arrangements on favorable terms. Most of our distribution relationships are non-exclusive and permit such distributors to distribute competing products. As such, our distributors may not commit the necessary resources to market our products to the level of our expectations or may choose to favor marketing the products of our competitors. If current or future distributors do not perform adequately or we are unable to enter into effective arrangements with distributors in particular geographic areas, we may not realize long-term international revenue growth.

We rely exclusively on commercial carriers to transport our products, including perishable consumables, to our customers in a timely and cost-efficient manner and if these delivery services are disrupted, our business will be harmed.

Our business depends on our ability to quickly and reliably deliver our products and in particular, our consumables, to our customers. Certain of our consumables are perishable and must be kept below certain temperatures. As such, we ship certain of our refrigerated consumables on dry ice and only ship such consumables on certain days of the week to reach customers on a timely basis. Disruptions in the delivery of our products, whether due to labor disruptions, bad weather, natural disasters, terrorist acts or threats or for other reasons could result in our customers receiving consumables that are not fit for usage, and if used, could

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result in inaccurate results or ruined experiments. While we work with customers to replace any consumables that are impacted by delivery disruptions, our reputation and our business may be adversely impacted even if we replace perished consumables free of charge. In addition, if we are unable to continue to obtain expedited delivery services on commercially reasonable terms, our operating results may be adversely affected.

In addition, should our commercial carriers encounter difficulties in delivering our instruments or consumables to customers, particularly at the end of any financial quarter, it could adversely impact our ability to recognize revenue for those products in that period and accordingly adversely affect our financial results for that period.

Ethical, legal, privacy and social concerns or governmental restrictions surrounding the use of the genomic and multi-omic information and gene editing could reduce demand for our products.

While we do not make gene sequencing or gene editing products, our products are used to better understand genomic information that could further gene editing endeavors. For example, our single cell gene expression solutions allow users to examine cells that have been genetically perturbed using clustered regularly interspaced short palindromic repeats ("CRISPR") gene editing technology. Recent advances in genome editing or gene therapy, using CRISPR systems such as CRISPR Cas9 technology have been subject to negative publicity and increased regulatory scrutiny, in part due to the underlying ethical, legal, privacy and social concerns regarding the use or potential misuse of such technology. Governmental authorities could, for safety, social or other purposes, call for limits on or regulation of technologies and products used in the genome editing or gene therapy fields. Such concerns or governmental restrictions could limit the use of our products. Because the science and technology of genome editing or gene therapy is incredibly complex, any regulations or restrictions placed on such technology or aimed at curtailing its usage could, intentionally or inadvertently, limit or restrict the usage of our products. Any such restrictions or any reduction in usage of our products as a result of concerns regarding the usage of genome editing technology could have a material adverse effect on our business, financial condition and results of operations.

We are subject to certain manufacturing restrictions related to licensed technologies that were developed with the financial assistance of United States government grants.

We are subject to certain United States government regulations because we have licensed technologies that were developed with United States government grants. Such licensed technologies are used, for example, in a substantial majority of our consumables. In accordance with these regulations, these licenses provide that products embodying the technologies are subject to domestic manufacturing requirements. If this domestic manufacturing requirement is not met, the government agency that funded the relevant grant is entitled to exercise specified rights ("march-in rights") which if exercised would allow the government agency to require the licensors or us to grant a non-exclusive, partially exclusive or exclusive license in any field of use to a third-party designated by such agency. The exercise of march-in rights or the termination of our license of the relevant technologies could materially adversely affect our business, operations and financial condition. As of June 30, 2019, all of our products embodying licensed technology subject to march-in rights were manufactured in the United States. While we do not expect to move manufacturing of these products to facilities located outside of the United States, we cannot assure investors that such products will always be manufactured in the United States or that the applicable government agency would grant a waiver of such requirement. These restrictions may limit our ability to manufacture our products in geographies where it may be more economically favorable to do so which could limit our ability to respond to competitive developments or otherwise adversely affect our results of operations.

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Our products could become subject to government regulation and the regulatory approval and maintenance process for such products may be expensive, time-consuming and uncertain both in timing and in outcome.

Our products are not subject to the clearance or approval of the U.S. Food and Drug Administration (the "FDA"), as they are not intended to be used for the diagnosis, treatment or prevention of disease. However, as we continue to expand our product line and the applications and uses of our existing products into new fields, certain of our current or future products could become subject to regulation by the FDA, or comparable international agencies, including requirements for regulatory clearance or approval of such products before they can be marketed. Such regulatory approval processes or clearances may be expensive, time-consuming and uncertain, and our failure to obtain or comply with such approvals and clearances could have an adverse effect on our business, financial condition and operating results. In addition, changes to the current regulatory framework, including the imposition of additional or new regulations, including regulation of our products, could arise at any time during the development or marketing of our products, which may negatively affect our ability to obtain or maintain FDA or comparable regulatory approval of our products, if required. Further, sales of devices for diagnostic purposes may subject us to additional healthcare regulation and enforcement by the applicable government agencies. Such laws include, without limitation, state and federal anti-kickback, fraud and abuse, false claims, privacy and security and physician sunshine laws and regulations.

Diagnostic products are regulated as medical devices by the FDA and comparable international agencies and may require either clearance from the FDA following the 510(k) pre-market notification process or pre-market approval from the FDA, in each case prior to marketing. Obtaining the requisite regulatory approvals can be expensive and may involve considerable delay. None of our products are currently regulated as medical devices, however, if our products labeled as "For Research Use Only. Not for use in diagnostic procedures" ("RUO") are used, or could be used, for the diagnosis of disease, the regulatory requirements related to marketing, selling and supporting such products could change or be uncertain, even if such use by our customers is without our consent.

If the FDA or other regulatory authorities assert that any of our products are subject to regulatory clearance or approval, our business, financial condition or results of operations could be adversely affected.

Enhanced trade tariffs, import restrictions, export restrictions, Chinese regulations or other trade barriers may materially harm our business.

We are continuing to expand our international operations as part of our growth strategy and have experienced an increasing concentration of sales in certain regions outside the United States, especially in the Asia-Pacific region. For the year ended December 31, 2018 and the six months ended June 30, 2019, sales outside of North America constituted approximately 42% and 44%, respectively, of our sales revenue and our largest markets outside of North America were China and Germany. There is currently significant uncertainty about the future relationship between the United States and various other countries, most significantly China, with respect to trade policies, treaties, government regulations and tariffs. The current United States presidential administration has called for substantial changes to United States foreign trade policy with respect to China and other countries, including the possibility of imposing greater restrictions on international trade and significant increases in tariffs on goods imported into the United States. In September 2018, the United States Trade Representative (the "USTR") enacted a tariff on the import of other Chinese products with a combined import value of approximately $200 billion. The tariff became effective on September 24, 2018, with an initial rate of 10% and increased to 25% effective on May 10, 2019.

Additionally, our business may be adversely impacted by retaliatory trade measures taken by China or other countries. Such measures could include restrictions on our ability to sell or import our instruments and/or consumables into certain countries or have the effect of increasing the prices of our instruments and/or

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consumables. For example, China has promised to impose retaliatory tariffs in response to the USTR tariffs referred to above and any such retaliatory tariffs could adversely impact our ability to sell instruments and consumables in China. While at this time neither the United States nor China has specifically imposed additional tariffs on healthcare related products, the nature of this dispute is evolving and additional products such as ours could become subject to tariffs, which could adversely affect the marketability of our products and our results of operations. Further, the continued threats of tariffs, trade restrictions and trade barriers could have a generally disruptive impact on the global economy and, therefore, negatively impact our sales. Given the relatively fluid regulatory environment in China and the United States and uncertainty how the United States or foreign governments will act with respect to tariffs, international trade agreements and policies, there could be additional tax or other regulatory changes in the future. Any such changes could directly and adversely impact our financial results and results of operations.

Additionally, in November 2018, the United States Commerce Department's Bureau of Industry and Security released an advance notice of proposed rulemaking to control the export of emerging technologies. This notice included "[b]iotechnology, including nanobiology; synthetic biology; genomic and genetic engineering; or neurotech" as possible areas of increased export controls. Therefore, it is possible that our ability to export our products may be restricted in the future.

The imposition of new, or changes in existing, tariffs, trade restrictions, trade barriers, export controls or retaliatory trade measures taken by other countries could adversely impact our business, financial condition and results of operations.

Doing business internationally creates operational and financial risks for our business.

We currently serve thousands of researchers in approximately 40 countries and plan to continue to expand to new international jurisdictions as part of our growth strategy. For the year ended December 31, 2018 and the six months ended June 30, 2019, approximately 42% and 44%, respectively, of our revenue was generated from sales to customers located outside of North America. We believe that a significant portion of our future revenue will come from international sources. We sell directly in North America and certain regions of Europe and have a significant portion of our sales and customer service personnel in the United States. We sell our products through third-party distributors in Asia, certain regions of Europe, South America, the Middle East and Africa. As a result, we or our distribution partners may be subject to additional regulations. Conducting operations on an international scale requires close coordination of activities across multiple jurisdictions and time zones. If we fail to coordinate and manage these activities effectively, our business, financial condition or results of operations could be materially and adversely affected and failure to comply with laws and regulations applicable to business operations in foreign jurisdictions may also subject us to significant liabilities and other penalties. International operations entail a variety of other risks, including, without limitation:

- challenges in staffing and managing foreign operations;
- potentially longer sales cycles and more time required to engage and educate customers on the benefits of our products outside of the United States;
- the potential need for localized software, documentation and post-sales support;
- reduced protection for intellectual property rights in some countries and practical difficulties of enforcing intellectual property and contract rights abroad;
- complexities associated with managing a third-party contract manufacturer located outside of the United States;

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- United States and foreign government trade restrictions, including those which may impose restrictions on the importation, exportation, re-exportation, sale, shipment or other transfer of programming, technology, components and/or services to foreign persons;
- changes in diplomatic and trade relationships, including new tariffs, trade protection measures, import or export licensing requirements, trade embargoes and other trade barriers;
- tariffs imposed by the United States on goods from other countries and tariffs imposed by other countries on United States goods, or increases in existing tariffs;
- deterioration of political relations between the United States and Canada, China, the United Kingdom and the European Union, which could have a material adverse effect on our sales and operations in these countries;
- changes in social, political and economic conditions or in laws, regulations and policies governing foreign trade, manufacturing, development and investment both domestically as well as in the other countries and jurisdictions into which we sell our products, including as a result of the referendum held in the United Kingdom approving the separation of the United Kingdom from the European Union;
- difficulties in obtaining export licenses or in overcoming other trade barriers and restrictions resulting in delivery delays or our inability to sell our products in certain countries;
- increased financial accounting and reporting burdens and complexities; and
- significant taxes or other burdens of complying with a variety of foreign laws, including laws relating to privacy and data protection such as the General Data Protection Regulation (the “GDPR”) which took effect in the European Union in 2018.

In conducting our international operations, we are subject to United States laws relating to our international activities, such as the Foreign Corrupt Practices Act of 1977, as well as foreign laws relating to our activities in other countries, such as the United Kingdom Bribery Act of 2010. Additionally, we are subject to laws that prohibit the conduct of business with persons that are subject to “sanctions”, including but not limited to persons listed on the United States Department of Commerce’s List of Denied Persons and the United States Department of Treasury’s Specially Designated Nationals and Blocked Persons List. Failure to comply with these laws and other applicable laws may subject us to claims or financial and/or other penalties in the United States and/or foreign countries that could materially and adversely impact our operations or financial condition. These risks have become increasingly prevalent as we have expanded our sales into countries that are generally recognized as having a higher risk of corruption.

Historically, most of our revenue has been denominated in U.S. dollars, although we have sold our products and services in local currency outside of the United States, principally the euro. For the year ended December 31, 2018 and the six months ended June 30, 2019, approximately 16% and 14%, respectively, of our sales were denominated in currencies other than the U.S. dollar. Our expenses are generally denominated in the currencies in which our operations are located, which is primarily in the United States. As our operations in countries outside of the United States grow, our results of operations and cash flows will become increasingly subject to fluctuations due to changes in foreign currency exchange rates, which could harm our business in the future. For example, if the value of the U.S. dollar increases relative to foreign currencies, in the absence of a corresponding change in local currency prices, our revenue could be adversely affected as we convert revenue from local currencies to U.S. dollars. In addition, because we conduct business in currencies other than U.S. dollars, but report our results of operations in U.S. dollars, we also face remeasurement exposure to fluctuations in currency exchange rates, which could hinder our ability to predict our future results and earnings and could materially impact our results of operations. We do not currently maintain a program to hedge exposures to non-U.S. dollar currencies.

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Violations of complex foreign and United States laws and regulations could result in fines and penalties, criminal sanctions against us, our officers or our employees, prohibitions on the conduct of our business and on our ability to offer our products and services in one or more countries, and could also materially affect our brand, our international growth efforts, our ability to attract and retain employees, our business and our operating results. Even if we implement policies or procedures designed to ensure compliance with these laws and regulations, there can be no assurance that our distribution partners, our employees, contractors or agents will not violate our policies and subject us to potential claims or penalties.

Significant U.K. or European developments stemming from the U.K.'s decision to withdraw from the European Union could have a material adverse effect on us.

In June 2016, the United Kingdom held a referendum and voted in favor of leaving the European Union, and in March 2017, the government of the United Kingdom formally initiated the withdrawal process. Negotiations for the United Kingdom's exit from the European Union ("Brexit") have created political and economic uncertainty, particularly in the United Kingdom and the European Union, and this uncertainty may last for years. Our business in the United Kingdom, the European Union and worldwide could be affected during this period of uncertainty, and perhaps longer, by the impact of the United Kingdom's referendum. There are many ways in which this business could be affected, only some of which we are able to currently identify.

The decision of the United Kingdom to withdraw from the European Union has caused and, along with events that could occur in the future as a consequence of the United Kingdom's withdrawal, may continue to cause significant volatility in global financial markets, including in global currency and debt markets. This volatility could cause a slowdown in economic activity in the United Kingdom, Europe or globally, which could adversely affect our operating results and growth prospects. In addition, our business could be negatively affected by new trade agreements or data transfer agreements between the United Kingdom and other countries, including the United States, and by the possible imposition of trade or other regulatory and immigration barriers in the United Kingdom. In addition, access to European Union research funding by research scientists based in the United Kingdom may be reduced or cut off altogether. It also is unclear whether Brexit may limit the ability or willingness of the United Kingdom's Medical Research Council to continue funding genomic or single cell research by local research centers and labs. For the year ended December 31, 2018 and the six months ended June 30, 2019, the United Kingdom comprised approximately $8.0 million and $5.6 million, respectively, of our worldwide product revenue. The impact of the United Kingdom's withdrawal from the European Union could negatively impact our revenue as a result of currency fluctuations, a slowdown in research funding or restricted budgets. In addition, the growth of sales in the United Kingdom may be slowed or those sales may even decline as a result of this withdrawal. Additionally, distribution costs for products sold in the United Kingdom may be increased due to trade agreements and incremental importation expenses. These possible negative impacts, and others resulting from the United Kingdom's actual or threatened withdrawal from the European Union, may increase our cost of doing business in Europe, disrupt our European operations and adversely affect our operating results and growth prospects.

The illegal distribution and sale by third parties of counterfeit or unfit versions of our products or stolen products could have a negative impact on our reputation and business.

Third parties might illegally distribute and sell counterfeit or unfit versions of our products, which do not meet our rigorous manufacturing, distribution and quality standards. As we expand our business internationally, we expect to encounter counterfeit versions of our products, particularly our consumables. A researcher who receives and uses counterfeit consumables could obtain erroneous results, experience failed experiments or potentially damage his or her instrument. Our reputation and business could suffer harm as a result of counterfeit products sold under our brand name. In addition, inventory that is stolen from warehouses, plants

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or while in-transit, and that is subsequently improperly stored and sold through unauthorized channels, could adversely impact our customers' experiments, our reputation and our business.

We currently plan to implement a new company-wide enterprise resource planning system in 2020 and such implementation could adversely affect our business and results of operations or the effectiveness of internal control over financial reporting.

We currently plan to implement a new company-wide enterprise resource planning ("ERP") system in 2020 to handle the business and financial processes within our operations, manufacturing and corporate functions. ERP implementations are complex and time-consuming projects that involve substantial expenditures on system software, the need to hire consultants and additional personnel for the implementation and implementation activities that can continue for several years. ERP implementations also require transformation of business and financial processes in order to reap the benefits of the ERP system. Our business and results of operations could be adversely affected if we experience operating problems and/or cost overruns during the ERP implementation process, or if the ERP system and the associated process changes do not give rise to the benefits that we expect. If we do not effectively implement and transition to the new ERP system as planned or if the system does not operate as intended, our business, results of operations and internal controls over financial reporting could be adversely affected.

Our solutions contain third-party open source software components and failure to comply with the terms of the underlying open source software licenses could restrict our ability to sell our products.

Our solutions contain software tools licensed by third parties under open source software licenses. Use and distribution of open source software may entail greater risks than use of third-party commercial software, as open source software licensors generally do not provide warranties or other contractual protections regarding infringement claims or the quality of the code. Some open source software licenses contain requirements that the licensee make its source code publicly available if the licensee creates modifications or derivative works using the open source software, depending on the type of open source software the licensee uses and how the licensee uses it. If we combine our proprietary software with open source software in a certain manner, we could, under certain open source software licenses, be required to release the source code of our proprietary software to the public for free. This would allow our competitors to create similar products with less development effort and time and ultimately could result in a loss of product sales and revenue. In addition, some companies that use third-party open source software have faced claims challenging their use of such open source software and their compliance with the terms of the applicable open source license. We may be subject to suits by third parties claiming ownership of what we believe to be open source software, or claiming non-compliance with the applicable open source licensing terms. Use of open source software may also present additional security risks because the public availability of such software may make it easier for hackers and other third parties to compromise or attempt to compromise our technology platform and systems.

Although we review our use of open source software to avoid subjecting our solutions to conditions we do not intend, the terms of many open source software licenses have not been interpreted by United States courts, and there is a risk that these licenses could be construed in a way that could impose unanticipated conditions or restrictions on our ability to commercialize our solutions. Moreover, we cannot assure investors that our processes for monitoring and controlling our use of open source software in our solutions will be effective. If we are held to have breached the terms of an open source software license, we could be required to seek licenses from third parties to continue offering our solutions on terms that are not economically feasible, to re-engineer our solutions, to discontinue the sale of our solutions if re-engineering could not be accomplished on a timely basis, or to make generally available, in source code form, our proprietary code, any of which could adversely affect our business, operating results and financial condition.

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We collect, process, store, share, disclose and use personal information and other data, which subjects us to governmental regulations and other legal obligations related to privacy and security, and our actual or perceived failure to comply with such obligations could harm our business.

We collect, process, store, transmit, disclose and use information from our employees, customers and others, including personal information and other data, some of which may be sensitive in nature. There are numerous federal, state and foreign laws and regulations regarding data protection, privacy and security. We strive to comply with applicable laws, our posted policies and legal contractual obligations relating to privacy and data protection. However, the scope of these laws is changing, is subject to differing interpretations, may be costly to comply with and may be inconsistent among countries and jurisdictions or conflict with other rules. Our business, including our ability to operate and expand internationally, could be adversely affected if legislation or regulations are adopted, interpreted or implemented in a manner that is inconsistent with our current business practices and that require changes to these practices.

The global data protection landscape is rapidly evolving and new laws and regulations are likely to be enacted and violations of existing and new laws and regulations may subject companies to significant penalties and fines, government investigations and/or enforcement actions, private litigation and other claims. For example, the European Union's recent adoption of the GDPR introduced stringent requirements for processing personal data. The GDPR is likely to increase compliance burdens on us, including by mandating potentially burdensome documentation requirements and granting certain rights to individuals to control how we collect, use, disclose, retain and leverage information about them or how we obtain consent from them. The processing of sensitive personal data, such as physical health condition, may impose heightened compliance burdens under the GDPR and is a topic of active interest among foreign regulators. In addition, the GDPR provides for breach reporting requirements, more robust regulatory enforcement and greater penalties for noncompliance than previous data protection laws, including fines of up to €20 million or 4% of a noncompliant company's global annual revenue for the preceding financial year, whichever is greater. As we continue to expand into other foreign countries and jurisdictions, we may be subject to additional laws and regulations that may affect how we conduct business.

In the United States, California recently enacted the California Consumer Privacy Act (the "CCPA"), which may limit or impose requirements on how we may collect and use personal information and is expected to come into effect in January 2020. The impact of this law on us and others in our industry is and will remain unclear until proposed bills amending the CCPA have wound their way through the legislative process and until regulations are issued by the California Attorney General. Similar privacy and data protection laws have also been proposed in other states and at the federal level.

Any failure or perceived failure by us or our vendors or partners to comply with these laws and regulations, our privacy policies, our privacy-related obligations to employees, customers or other third parties or privacy or security-related legal obligations, or any actual or perceived compromise of security that results in the unauthorized access to or disclosure, alteration, theft, loss, transfer or use of personal or other information, including personally identifiable information or other sensitive data, may result in governmental enforcement actions, fines and penalties, litigation or public statements critical of us by consumer advocacy groups or others and could cause our customers, partners or others to lose trust in us, which could have an adverse effect on our business.

If we experience a significant disruption in our information technology systems or breaches of data security, our business could be adversely affected.

We rely on information technology systems to keep financial records, facilitate our research and development initiatives, manage our manufacturing operations, maintain quality control, fulfill customer orders, maintain

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corporate records, communicate with staff and external parties and operate other critical functions. Our information technology systems are potentially vulnerable to disruption due to breakdown, malicious intrusion and computer viruses or other disruptive events including but not limited to natural disasters and catastrophes. Cyberattacks and other malicious internet-based activity continue to increase and cloud-based platform providers of services have been and are expected to continue to be targeted. In addition to traditional computer “hackers”, malicious code (such as viruses and worms), employee theft or misuse, denial-of-service attacks and sophisticated nation-state and nation-state supported actors now engage in attacks (including advanced persistent threat intrusions). Despite significant efforts to create security barriers to such threats, it is virtually impossible for us to entirely mitigate these risks. If our security measures are compromised as a result of third-party action, employee or customer error, malfeasance, stolen or fraudulently obtained log-in credentials or otherwise, our reputation could be damaged, our business may be harmed and we could incur significant liability. If we were to experience a prolonged system disruption in our information technology systems or those of certain of our vendors, it could negatively impact our ability to serve our customers, which could adversely impact our business. If operations at our facilities were disrupted, it may cause a material disruption in our business if we are not capable of restoring functionality on an acceptable timeframe. In addition, our information technology systems (and those of our vendors and partners) are potentially vulnerable to data security breaches, whether by internal bad actors (e.g., employees) or external bad actors (attacks of which are becoming increasingly sophisticated, including social engineering and phishing scams), which could lead to the exposure of personal data, sensitive data and confidential information to unauthorized persons. Such data security breaches could lead to the loss of trade secrets or other intellectual property, or could lead to the exposure of personal information (including sensitive personal information) of our employees, customers and others, any of which could have a material adverse effect on our business, reputation, financial condition and results of operations.

We have not always been able in the past and may be unable in the future to anticipate or prevent techniques used to obtain unauthorized access or to compromise our systems because the techniques used change frequently and are generally not detected until after an incident has occurred. Concerns regarding data privacy and security may cause some of our customers to stop using our solutions and fail to renew their subscriptions. This discontinuance in use or failure to renew could substantially harm our business, operating results and growth prospects.

In addition, any such access, disclosure or other loss or unauthorized use of information or data could result in legal claims or proceedings, regulatory investigations or actions, and other types of liability under laws that protect the privacy and security of personal information, including federal, state and foreign data protection and privacy regulations, violations of which could result in significant penalties and fines. In addition, although we seek to detect and investigate all data security incidents, security breaches and other incidents of unauthorized access to our information technology systems and data can be difficult to detect and any delay in identifying such breaches or incidents may lead to increased harm and legal exposure of the type described above.

The cost of investigating, mitigating and responding to potential data security breaches and complying with applicable breach notification obligations to individuals, regulators, partners and others can be significant. Our insurance policies may not be adequate to compensate us for the potential costs and other losses arising from such disruptions, failures or security breaches. In addition, such insurance may not be available to us in the future on economically reasonable terms, or at all. Further, defending a suit, regardless of its merit, could be costly, divert management attention and harm our reputation.

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We rely on on-premise, co-located and third-party data centers and platforms to host our website and other online services, as well as for research and development purposes and any interruptions of service or failures may impair and harm our business.

Our proprietary software is a crucial component of our solutions, as our software allows our end users to visualize genomic and multi-omic information provided by our instruments and reagents. All of our software is currently downloadable free of charge from our website for installation and use by end users on their computer systems. Our website is hosted with various third-party service providers located in the United States. We rely on on-premises, co-located and third-party infrastructure in the San Francisco Bay Area and other regions in the United States to perform computationally demanding analysis tasks for our research and development program and for other business purposes.

In the event of any technical problems that may arise in connection with our on-premise, co-located or third-party data centers, we could experience interruptions in our ability to provide products and services to our customers or in our internal functions, including research and development, which rely on such services. Interruptions or failures may be caused by a variety of factors, including infrastructure changes, human or software errors, viruses, security attacks, fraud, spikes in customer usage and denial of service issues. Interruptions or failures in our operations or services may reduce our revenue, result in the loss of customers, adversely affect our ability to attract new customers or harm our reputation. Significant interruptions to our research and development program could cause us to delay the introduction of new products or improvements to existing products, which could adversely impact our business, our results of operations and the competitiveness of our products.

Our current solutions are capable of generating large datasets, the analysis of which can be time consuming without access to a high-performance computing system. The visualization of such data can also be computationally intensive. As we iterate and improve our products and as the related technologies advance, our continued growth may require an ability to provide our customers with direct access to a high-performance computing system and/or alternative means of obtaining our software. As a result, we expect our reliance on internal and third-party data centers to increase in the future.

Further, as we rely on third-party and public-cloud infrastructure, we will depend in part on third-party security measures to protect against unauthorized access, cyberattacks and the mishandling of customer data. In addition, failures to meet customers' expectations with respect to security and confidentiality of their data and information could damage our reputation and affect our ability to retain customers, attract new customers and grow our business. In addition, a cybersecurity event could result in significant increases in costs, including costs for remediating the effects of such an event, lost revenue due to a decrease in customer trust and network downtime; increases in insurance coverage due to cybersecurity incidents; and damages to our reputation because of any such incident.

Our indebtedness may impair our financial and operating flexibility.

The Loan and Security Agreement provides for up to $50.0 million of term loans and a $25.0 million revolving asset-backed credit facility. As of June 30, 2019, $30.0 million of term loan borrowings were outstanding. As of June 30, 2019, revolving loan borrowings of $25.0 million were available to be drawn and $20.0 million of additional term loan borrowings were available to be drawn before January 1, 2020, subject to certain conditions. We currently intend to partially draw under our revolving line of credit, prior to the consummation of this offering, in order to provide us with additional liquidity in connection with our operations. The Loan and Security Agreement contains affirmative and negative covenants, including a covenant requiring us to maintain minimum revenue over specified periods of time and covenants that restrict, among other things, our ability to dispose of assets, change our business, management, ownership or business locations, enter into mergers or acquisitions, incur additional indebtedness or encumber any of our assets. Borrowings under the Loan and Security Agreement are secured by substantially all of our assets, excluding our intellectual property but

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including the proceeds from the sale of any of our intellectual property. These restrictions could limit our operational flexibility and the need to make principal and interest payments on our debt will reduce our ability to fund other aspects of our business, such as our research and development program. Our ability to make principal and interest payments on our indebtedness will depend on our ability to generate cash. If we default under the Loan and Security Agreement and if the default is not cured or waived, the lender could terminate its commitments to lend to us and cause any amounts outstanding to be payable immediately. Under certain circumstances, the lender could also exercise its rights with respect to the collateral securing such loans. Such a default could also result in cross-defaults under other debt instruments. Moreover, any such default would limit our ability to obtain additional financing, which may have an adverse effect on our cash flow and liquidity.

We may incur additional indebtedness in the future. The debt instruments governing such indebtedness could contain provisions that are as, or more, restrictive than our existing debt instruments. If we incur additional debt, a greater portion of our cash flows may be needed to satisfy our debt service obligations. While we do not anticipate that we will need to raise additional financing in the future to fund our operations, in the event that additional financing is required, we may not be able to raise it on terms acceptable to us or at all. As a result, we would be more vulnerable to general adverse economic, industry and capital markets conditions in addition to the risks associated with indebtedness described above.

Our ability to use net operating losses to offset future taxable income may be subject to certain limitations.

As of December 31, 2018, we had federal net operating loss carryforwards ("NOLs") of approximately $116.1 million and federal tax credit carryforwards of approximately $8.3 million. Our federal NOLs generated after January 1, 2018, which total $5.5 million, are carried forward indefinitely, while all of our other federal NOLs and tax credit carryforwards expire beginning in 2032. As of December 31, 2018, we had state NOLs of approximately $93.5 million, which expire beginning in 2032. In addition, we had state tax credit carryforwards of approximately $7.9 million, which do not expire. Our ability to utilize such carryforwards for income tax savings is subject to certain conditions and may be subject to certain limitations in the future due to ownership changes, if any, as defined by rules enacted with the Tax Cuts and Jobs Act of 2017 (the "2017 Tax Act"). As such, there can be no assurance that we will be able to utilize such carryforwards. We have experienced a history of losses and a lack of future taxable income would adversely affect our ability to utilize these NOLs and research and development credit carryforwards. We currently maintain a full valuation allowance against these tax assets.

Under Sections 382 and 383 of the Internal Revenue Code of 1986, as amended (the "Code"), if a corporation undergoes an "ownership change", the corporation's ability to use its pre-change net operating loss carryforwards and other pre-change attributes, such as research tax credits, to offset its post-change income may be limited. In general, an "ownership change" will occur if there is a cumulative change in our ownership by "5% shareholders" that exceeds 50 percentage points over a rolling three-year period. Similar rules may apply under state tax laws. We completed a study in early 2019 to determine whether an ownership change had occurred under Section 382 or 383 of the Code as of December 31, 2018 and we determined at that time that an ownership change occurred in 2013. As a result, our net operating losses generated through November 1, 2013 may be subject to limitation under Section 382 of the Code. The amount of pre-change loss carryforwards which may be subject to this limitation is $4.8 million. Our ability to use net operating loss carry forwards, research and development credit carryforwards and other tax attributes to reduce future taxable income and liabilities may be subject to limitations based on the ownership change in 2013, possible changes since the completion of the study or as a result of this offering. As a result, if we earn net taxable income, our ability to use our pre-change net operating loss carryforwards or other pre-change tax attributes to offset United States federal and state taxable income may still be subject to limitations, which could potentially result in increased future tax liability to us.

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We are subject to risks related to taxation in multiple jurisdictions.

We are subject to income taxes in both the United States and foreign jurisdictions. Significant judgments based on interpretations of existing tax laws or regulations are required in determining our provision for income taxes. Our effective income tax rate could be adversely affected by various factors, including, but not limited to, changes in the mix of earnings in tax jurisdictions with different statutory tax rates, changes in the valuation of deferred tax assets and liabilities, changes in existing tax policies, laws, regulations or rates, changes in the level of non-deductible expenses (including share-based compensation), changes in the location of our operations, changes in our future levels of research and development spending, mergers and acquisitions or the result of examinations by various tax authorities. Although we believe our tax estimates are reasonable, if the United States Internal Revenue Service or other taxing authority disagrees with the positions taken on our tax returns, we could have additional tax liability, including interest and penalties. If material, payment of such additional amounts upon final adjudication of any disputes could have a material impact on our results of operations and financial position.

Changes in tax laws or regulations that are applied adversely to us or our customers may have a material adverse effect on our business, cash flow, financial condition or results of operations.

New income, sales, use or other tax laws, statutes, rules, regulations or ordinances could be enacted at any time, which could affect the tax treatment of our domestic and foreign earnings. Any new taxes could adversely affect our domestic and international business operations and our business and financial performance. Further, existing tax laws, statutes, rules, regulations or ordinances could be interpreted, changed, modified or applied adversely to us. For example, the 2017 Tax Act significantly revised the Code. The recently enacted federal income tax law, among other things, contains significant changes to corporate taxation, including a reduction of the federal statutory rates from a top marginal rate of 35% to a flat rate of 21%, limitation of the tax deduction for interest expense to 30% of adjusted earnings (except for certain small businesses), limitation of the deduction for net operating losses to 80% of current year taxable income, elimination of net operating loss carrybacks, one time taxation of offshore earnings at reduced rates regardless of whether they are repatriated, elimination of U.S. tax on foreign earnings (subject to certain important exceptions), immediate deductions for certain new investments instead of deductions for depreciation expense over time and modifying or repealing many business deductions and credits. Notwithstanding the reduction in the corporate income tax rate, the overall impact of the new federal tax law is uncertain and our business and financial condition could be adversely affected. It is also unknown if and to what extent various states will conform to the newly enacted federal tax law. The impact of this tax reform on us and on holders of our Class A common stock is likewise uncertain and could be adverse.

If we fail to maintain an effective system of disclosure controls and internal control over financial reporting, our ability to produce timely and accurate financial statements or comply with applicable regulations could be impaired.

As a public company, we will be subject to the reporting requirements of the Exchange Act, SOX and the rules and regulations of the applicable listing standards of the Nasdaq Global Select Market (“Nasdaq”). We expect that the requirements of these rules and regulations will continue to increase our legal, accounting and financial compliance costs, make some activities more difficult, time-consuming and costly, and place significant strain on our personnel, systems and resources.

SOX requires, among other things, that we maintain effective disclosure controls and procedures and internal control over financial reporting. We are continuing to develop and refine our disclosure controls and other procedures that are designed to ensure that information required to be disclosed by us in the reports that we will file with the SEC is accurately recorded, processed, summarized and reported within the time periods

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specified in SEC rules and forms and that information required to be disclosed in reports under the Exchange Act is accumulated and communicated to our principal executive and financial officers. We are also continuing to improve our internal control over financial reporting. In order to maintain and improve the effectiveness of our disclosure controls and procedures and internal control over financial reporting, we have expended, and anticipate that we will continue to expend, significant resources including accounting-related costs and significant management oversight.

Our current controls and any new controls that we develop may become inadequate because of changes in conditions in our business. Further, weaknesses in our disclosure controls and internal control over financial reporting may be discovered in the future. Any failure to develop or maintain effective controls or any difficulties encountered in their implementation or improvement could harm our results of operations or cause us to fail to meet our reporting obligations and may result in a restatement of our financial statements for prior periods. Any failure to implement and maintain effective internal control over financial reporting also could adversely affect the results of periodic management evaluations and annual independent registered public accounting firm attestation reports regarding the effectiveness of our internal control over financial reporting that we will eventually be required to include in our periodic reports that will be filed with the SEC. Ineffective disclosure controls and procedures and internal control over financial reporting could also cause investors to lose confidence in our reported financial and other information, which would likely have a negative effect on the trading price of our Class A common stock. In addition, if we are unable to continue to meet these requirements, we may not be able to remain listed on Nasdaq. We are not currently required to comply with the SEC rules that implement Section 404 of SOX and are therefore not required to make a formal assessment of the effectiveness of our internal control over financial reporting for that purpose. As a public company, we will be required to provide an annual management report on the effectiveness of our internal control over financial reporting commencing with our second annual report on Form 10-K.

We cannot provide any assurance that significant deficiencies or material weaknesses in our internal controls over financial reporting will not be identified in the future. If we fail to remediate any significant deficiencies or material weaknesses that may be identified in the future or encounter problems or delays in the implementation of internal controls over financial reporting, we may be unable to conclude that our internal controls over financial reporting are effective. We are currently implementing an internal audit function and any failure to correctly do so could lead to significant deficiencies or material weaknesses in our financial reporting. Any failure to develop or maintain effective controls or any difficulties encountered in our implementation of our internal controls over financial reporting could result in material misstatements that are not prevented or detected on a timely basis, which could potentially subject us to sanctions or investigations by the SEC or other regulatory authorities. Ineffective internal controls could cause investors to lose confidence in us and the reliability of our financial statements and cause a decline in the price of our Class A common stock.

Our independent registered public accounting firm is not required to formally attest to the effectiveness of our internal control over financial reporting until our first annual report filed with the SEC where we are an “accelerated filer” or a “large accelerated filer”. At such time, our independent registered public accounting firm may issue a report that is adverse in the event it is not satisfied with the level at which our internal control over financial reporting is documented, designed or operating. Any failure to maintain effective disclosure controls and internal control over financial reporting could materially and adversely affect our business, results of operations and financial condition and could cause a decline in the trading price of our Class A common stock.

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If our estimates or judgments relating to our critical accounting policies are based on assumptions that change or prove to be incorrect, our operating results could fall below our publicly announced guidance or the expectations of securities analysts and investors, resulting in a decline in the market price of our Class A common stock.

The preparation of financial statements in conformity with generally accepted accounting principles in the United States ("GAAP") requires management to make estimates and assumptions that affect the amounts reported in our financial statements and accompanying notes. We base our estimates on historical experience and on various other assumptions that we believe to be reasonable under the circumstances, the results of which form the basis for making judgments about the carrying values of assets, liabilities, equity, revenue and expenses that are not readily apparent from other sources. For example, in connection with our adoption and implementation of the new revenue accounting standard, management will make judgments and assumptions based on our interpretation of the new standard. The new revenue standard is principle-based and interpretation of those principles may vary from company to company based on their unique circumstances. It is possible that interpretation, industry practice and guidance may evolve as we apply the new standard. If our assumptions underlying our estimates and judgements relating to our critical accounting policies change or if actual circumstances differ from our assumptions, estimates or judgements, our operating results may be adversely affected and could fall below our publicly announced guidance or the expectations of securities analysts and investors, resulting in a decline in the market price of our Class A common stock.

Risks related to litigation and our intellectual property

We are involved in significant litigation which has consumed significant resources and management time and adverse resolution of these lawsuits could require us to pay significant damages, and prevent us from selling our products, which would severely adversely impact our business, financial condition or results of operations.

Our success depends in part on our non-infringement of the patents or proprietary rights of third parties. Third parties have asserted and may in the future assert that our products infringe patents that they have obtained and may in the future obtain. We could incur substantial costs and divert the attention of our management and technical personnel in defending ourselves against any of these claims. Any adverse ruling or perception of an adverse ruling in defending ourselves against these claims could have an adverse impact on our business, financial condition or results of operations. Furthermore, parties making claims against us have obtained and may in the future be able to obtain injunctive or other relief, which effectively could block our ability to further develop, commercialize, market or sell products or services and have resulted and could in the future result in the award of substantial damages against us. In the event of a successful infringement claim against us, we may be required to pay damages and obtain one or more licenses from third parties or be prohibited from selling certain products or services. In addition, we may be unable to obtain these licenses at a reasonable cost, if at all. We could therefore incur substantial costs related to royalty payments for licenses obtained from third parties, which could negatively affect our gross margins and earnings per share. In addition, we could encounter delays in product introductions while we attempt to develop alternative methods or products. Defense of any lawsuit or failure to obtain any of these licenses on favorable terms could prevent us from commercializing products and the prohibition of sale of any of our products or services could adversely affect our ability to grow or achieve or maintain profitability. Regardless of merit or eventual outcome, lawsuits brought against us may result in decreased demand for our products, injury to our reputation and increased insurance costs.

In particular, we are currently involved in the following litigation matters related to substantially all of our products, the loss of any of which could have a material adverse effect on our business, operations, financial results and reputation. Beginning in 2015, Bio-Rad has filed five separate patent infringement cases against

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substantially all of our products, including instruments and consumables. These litigations are generally distinct and involve different Bio-Rad patents, however, the patents asserted by Bio-Rad in the ITC are also asserted in the district court case filed in the Northern District of California. In addition, in November 2018, Becton Dickinson filed a patent infringement suit alleging that our gel beads, which are used in substantially all of our products, infringe their patents.

The details of these litigation matters are described below:

The 2015 Delaware Action

In February 2015, Raindance Technologies, Inc. ("Raindance") and the University of Chicago filed suit against us in the U.S. District Court for the District of Delaware, accusing that substantially all of our products that use our GEM microfluidic chips are infringing seven U.S. patents owned by or exclusively licensed to Raindance (the "Delaware Action"). In May 2017, Bio-Rad was substituted as the plaintiff following its acquisition of Raindance. A jury trial was held in November 2018. The jury found that all of our accused products infringed one or more of U.S. Patent Nos. 8,304,193, 8,329,407 and 8,889,083. The jury also concluded that our infringement was willful and awarded Bio-Rad approximately $24 million in damages. Post-trial, Bio-Rad moved for a permanent injunction, treble damages for willful infringement, attorneys' fees, supplemental damages for the period from the second quarter of 2018 through the end of the trial as well as pre- and post-judgment interest.

The Court denied Bio-Rad's request for attorneys' fees and enhanced damages for willful infringement. The Court awarded supplemental damages for the period from the second quarter of 2018 through the end of trial as well as pre- and post-judgment interest. The Court entered final judgment against us in the amount of approximately $35 million in August 2019. In the fourth quarter of 2018, we began recording an accrual for estimated royalties as cost of revenue. This accrual is based on an estimated royalty rate of 15% of worldwide sales of our Chromium instruments operating our GEM microfluidic chips and associated consumables. As of June 30, 2019, we had accrued a total of $55.3 million relating to this matter which includes the $35 million judgment and our estimated 15% royalty for sales through that date.

The Court also granted Bio-Rad a permanent injunction against our GEM microfluidic chips and associated consumables that were found to infringe the Bio-Rad patents, which have historically constituted substantially all of our product sales. However, under the injunction, we are permitted to continue to sell our GEM microfluidic chips and associated consumables for use with our historical installed base of instruments provided that we pay a royalty of 15% into escrow on our net revenue related to such sales. We have appealed the injunction to the Federal Circuit and expect that it will not take effect until the Federal Circuit rules on our request for a stay of the injunction.

We have dedicated significant resources to designing and manufacturing our Next GEM new microfluidic chips which use fundamentally different physics from our GEM microfluidic chips. Neither the jury verdict nor the injunction relate to our Next GEM microfluidic chips and associated consumables which we launched in May 2019 for three of our single cell solutions – Single Cell Gene Expression, Single Cell Immune Profiling and Single Cell ATAC. We currently expect that, by the end of the third quarter of 2019, all Chromium instruments that we sell will operate exclusively with our Next GEM solutions and that our Chromium products utilizing our Next GEM microfluidic chips will constitute substantially all of our Chromium sales by the end of 2020.

Although our Next GEM microfluidic chips were designed to replace our GEM microfluidic chips, we cannot assure you that we will be able to make our Next GEM microfluidic chip work with all of our solutions, that our Next GEM microfluidic chip will allow our customers to maintain the level of performance or quality of our GEM microfluidic chip, that our Next GEM microfluidic chip will replace the sales of the GEM microfluidic chip or that we will be able to manufacture the Next GEM microfluidic chips in sufficient volumes in a timely fashion. Our Next GEM microfluidic chips may be subject to future claims of infringement by Bio-Rad or others and are

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currently the subject of the litigation described in this risk factor. While we believe that our Chromium solutions, when used with our Next GEM microfluidic chip, would not infringe the asserted Bio-Rad patents, we cannot assure you that our Next GEM microfluidic chip would not become subject to additional patent infringement litigation, which could prevent us from making, selling and importing our Next GEM microfluidic chips. In addition, it is possible that Bio-Rad could, in the future, claim that our continued sale of products violates orders issued by the court and request that the court impose sanctions or other penalties on us for such violations.

In addition, unless the injunction relating to our GEM microfluidic chips is stayed, we will be unable to sell our Single Cell CNV and Linked-Read solutions for use on new instruments unless and until we develop a Next GEM microfluidic chip for such solutions. Though our Single Cell CNV and Linked-Read solutions have not significantly contributed to our revenue to date, our Single-Cell CNV solution, for example, has proved crucial in understanding how cancers evolve and providing researchers with valuable insights into cancer treatments. Developing a Next GEM microfluidic chip for solutions may require significant uses of our resources and there may be a substantial delay before such products are available to sell to our customers.

As of June 30, 2019, we had accrued a total of $55.3 million relating to this matter. Depending upon the ultimate outcome of the litigation with Bio-Rad, we may be required to pay damages, interest and other amounts at a time specified by the court in excess of these reserves should our accruals prove insufficient to cover the actual damages awarded in the case. While we will continue to evaluate and review our estimate of amounts payable from time to time for any indications that could require us to change our assumptions relating to the amounts already recorded, we cannot assure investors that our estimates and related reserves will be sufficient.

Also in 2015, we filed multiple petitions for *inter partes* review (“IPR”) at the Patent Trial and Appeal Board (“PTAB”) of the U.S. Patent and Trademark Office (“USPTO”) against Raindance and the University of Chicago relating to the patents asserted in the Delaware Action, including U.S. Patent Nos. 7,129,091, 8,658,430, 8,304,193, 8,273,573, 8,329,407, 8,889,083 and 8,822,148. Among these proceedings, all the claims in the ‘430 patent were determined by the PTAB to be invalid, all the claims in the ‘573 patent were canceled, and our invalidity challenges to the remaining Bio-Rad patents were unsuccessful. Accordingly, we may be precluded from challenging the ‘091, ‘193, ‘407 and ‘148 patents at the PTAB in the future as a result of these decisions. Further, because all the claims in the ‘083 patent survived the IPR challenge, we will be precluded from making certain invalidity challenges to this patent at the PTAB, or in a district court or ITC litigation in the future.

The ITC 1068 Action

On July 31, 2017, Bio-Rad and Lawrence Livermore National Security, LLC filed a complaint against us in the U.S. International Trade Commission (“ITC”) pursuant to Section 337 of the Tariff Act of 1930, accusing substantially all of our products of infringing U.S. Patents Nos. 9,089,844, 9,126,160, 9,500,664, 9,636,682 and 9,649,635 (the “ITC 1068 Action”). Bio-Rad is seeking an exclusion order preventing us from importing the accused microfluidic chips, including (1) our GEM microfluidic chip, (2) our gel bead manufacturing microfluidic chip and (3) our Next GEM microfluidic chip, into the United States and a cease and desist order preventing us from selling such imported chips. An evidentiary hearing for the ITC 1068 Action was held in May of 2018 and the presiding judge issued an Initial Determination in September 2018, finding that our GEM microfluidic chips infringe the ‘664, ‘682 and ‘635 patents but not the ‘160 patent. The judge further found that our gel bead manufacturing microfluidic chip and Next GEM microfluidic chip do not infringe any claim asserted against them.

The judge recommended entry of an exclusion order against our GEM microfluidic chips, which are currently being imported into the United States. If the ITC were to adopt the judge’s recommendation regarding the

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exclusion order, we would be prevented from importing such chips, which are used in substantially all of our products, into the United States. The judge also recommended a cease and desist order that would prevent us from selling such imported chips. The ITC is not reviewing the judge's findings that our GEM microfluidic chips directly infringe the '664, '682 and '635 patents. The ITC is currently reviewing the judge's findings that (1) we indirectly infringe the '682 and '635 patents, (2) our gel bead manufacturing microfluidic chip does not infringe certain claims in the '664 patent and (3) our Next GEM microfluidic chip does not infringe certain claims in the '160 and '664 patents. A Final Determination is expected to be issued in late September 2019. The Final Determination is subject to a 60-day presidential review period before taking effect. If the Initial Determination were to be upheld, then we would be unable to import our GEM microfluidic chips and sell such imported chips, which are used in substantially all of our products. The judge recommended a bond of 100% of the entered value of accused products imported during the Presidential review period.

In order to allow our customers to continue their important research, we have dedicated significant resources to developing the capabilities to manufacture our microfluidic chips in the United States prior to the entry of an exclusion order or cease and desist order which could take effect in late November 2019. Prior to the second quarter of 2019, all of our microfluidic chips were manufactured outside of the United States. We expect our United States manufacturing facilities to achieve volume production of certain of our GEM microfluidic chips accounting for the majority of our United States consumable revenue beginning in the fourth quarter of 2019. We cannot assure investors that our U.S. manufacturing facilities can produce our microfluidic chips to the same level of functionality, quality or quantity as our current foreign manufacturer. Moreover, Bio-Rad has also filed suit against us in the U.S. District Court for the Northern District of California, which is discussed separately below. If Bio-Rad succeeds in obtaining an injunction in the district court case, we could be prohibited from selling our GEM microfluidic chips, regardless of where they are manufactured. If we are prohibited from selling our GEM microfluidic chips, our business, operations, financial results and reputation would be significantly adversely impacted.

Further, although the Next GEM microfluidic chips were designed to replace our GEM microfluidic chips, we cannot assure investors that the ITC will not reverse the finding of the Initial Determination in its Final Determination currently expected to be issued in late September 2019 that our Next GEM microfluidic chips or other products do not infringe the patents asserted against them in the ITC 1068 Action. If the ITC reverses the non-infringement finding about our Next GEM microfluidic chips and prohibits us from importing such chips or selling previously imported chips, our business, operations, financial results and reputation would be significantly adversely impacted.

In addition, if Bio-Rad obtains an exclusion order and/or cease and desist order in the ITC 1068 Action, it is possible that Bio-Rad could, in the future, file enforcement proceedings claiming that we have violated such orders and requesting that the ITC impose sanctions or other penalties on us for such violations. Our Next GEM microfluidic chips could also become subject to other patent infringement litigations. If we are prohibited from selling our Next GEM microfluidic chips, our business, operations, financial results and reputation would be significantly adversely impacted.

The Northern District of California Action

On July 31, 2017, Bio-Rad and Lawrence Livermore National Security, LLC also filed suit against us in the U.S. District Court for the Northern District of California, alleging that substantially all of our products infringe U.S. Patents Nos. 9,216,392, 9,347,059 and the five patents asserted in the ITC 1068 Action. The complaint seeks injunctive relief, unspecified monetary damages, costs and attorneys' fees. This litigation has been stayed pending resolution of the ITC 1068 Action. If we are found to infringe these patents or if we are prohibited from selling our products, our business, operations, financial results and reputation could be significantly adversely impacted.

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In 2017 and 2018, we filed multiple petitions for IPR at the PTAB against Bio-Rad regarding U.S. Patent Nos. 9,126,160, 9,216,392, 9,649,635, 9,089,844, 9,636,682 and 9,500,664, all of which were also asserted in the ITC 1068 Action or the Northern District of California Case. The PTAB denied institution of all the IPRs, which may preclude us from challenging these patents at the PTAB in the future.

The Germany Action

On February 13, 2018, Bio-Rad filed suit against us in Germany in the Munich Region Court alleging that our Chromium instruments, GEM microfluidic chips and certain accessories infringe German Utility Model No. DE 20 2011 110 979. Bio-Rad seeks unspecified damages and an injunction prohibiting sales of these products in Germany and requiring us to recall these products sold in Germany subsequent to February 11, 2018. The accused GEM microfluidic chips are currently manufactured in Germany and are currently used in substantially all of our solutions. An initial hearing was held on November 27, 2018, and a subsequent hearing was held on May 15, 2019. The Court has not yet issued a ruling on the merits. If we are prohibited from selling our products in Germany, or if our products are recalled in Germany, our business, operations, financial results and reputation could be adversely impacted.

The 2018 Delaware Action

On October 25, 2018, Bio-Rad filed suit against us in the U.S. District Court for the District of Delaware, alleging that substantially all of our products infringe U.S. Patent Nos. 9,562,837 and 9,896,722. Bio-Rad seeks injunctive relief, unspecified monetary damages, costs and attorneys' fees. Discovery is in progress. If we are found to infringe these patents or if we are prohibited from selling our products, our business, operations, financial results and reputation could be significantly adversely impacted.

The Becton Dickinson Action

On November 15, 2018, Becton, Dickinson and Company and Cellular Research, Inc. filed suit against us in the U.S. District Court for the District of Delaware, alleging that we infringe U.S. Patent Nos. 8,835,358, 9,845,502, 9,315,857, 9,816,137, 9,708,659, 9,290,808, 9,290,809, 9,567,645, 9,567,646, 9,598,736 and 9,637,799. The complaint asserted that substantially all of our products infringe these patents. Plaintiffs seek injunctive relief, unspecified monetary damages, costs and attorneys' fees. On January 18, 2019, we filed a motion to dismiss certain of the asserted claims on the grounds that they are directed to patent ineligible abstract ideas. Discovery is in progress. The Court has not yet ruled on or set a hearing date for the motion. The accused products constitute a substantial majority of our revenue, and if we are found to infringe these patents or if we are prohibited from selling our products, our business, operations, financial results and reputation would be significantly adversely impacted.

As we enter new markets or introduce new products, we expect that competitors will likely claim that our products infringe their intellectual property rights. Our success depends in part on our ability to defend ourselves against such claims and maintain the validity of our patents and other proprietary rights.

We are involved in lawsuits to protect, enforce or defend our patents and other intellectual property rights, which are expensive, time consuming and could ultimately be unsuccessful.

On January 11, 2018, we filed a complaint against Bio-Rad at the ITC pursuant to Section 337 of the Tariff Act of 1930 alleging that Bio-Rad infringes our U.S. Patent Nos. 9,644,204, 9,689,024, 9,695,468 and 9,856,530 (the "ITC 1100 Action"). Our complaint in the ITC 1100 Action seeks an exclusion order preventing Bio-Rad from importing certain microfluidic chips and other products into the United States and a cease and desist order preventing Bio-Rad from selling such importing chips and other products. An evidentiary hearing for the ITC 1100 Action was held in March of 2019.

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The judge issued an Initial Determination on July 12, 2019 finding that Bio-Rad's ddSEQ product for single cell analysis infringes the '024, '468 and '530 patents. The judge found all of our asserted patents to be valid. The judge also rejected Bio-Rad's claim of ownership in all of the asserted patents. The Target Date for the Final Determination is scheduled for November 12, 2019.

Also in January 2018, we filed a related but separate suit against Bio-Rad in the U.S. District Court for the Northern District of California, alleging that Bio-Rad infringes the '204, '024, '468 and '530 patents. The '204, '024, '468 and '530 patents generally relate to gel bead reagents that are used in our Chromium products, which constitute substantially all of our current sales. This litigation has been stayed pending resolution of the ITC 1100 Action.

In January 2019, Bio-Rad also filed petitions for IPR of the '024, '468 and '530 patents at the PTAB seeking to invalidate these patents. In July and August of 2019, the PTAB denied institution of all Bio-Rad's IPRs.

In addition to the litigation and legal proceedings discussed above, we are currently and may in the future be a party to other litigation or legal proceedings to determine the scope and validity of our intellectual property, which, if resolved adversely to us, could invalidate or render unenforceable our intellectual property or generally preclude us from restraining, enjoining or otherwise seeking to exclude competitors from commercializing products using technology developed or used by us. For example, our patents and any patents which we in-license may be challenged, narrowed, invalidated or circumvented. If patents we own or license are invalidated or otherwise limited, other companies may be better able to develop products that compete with ours, which would adversely affect our competitive position, business prospects, results of operations and financial condition.

The following are examples of litigation and other adversarial proceedings or disputes that we could become a party to involving our patents or patents licensed to us:

- we have initiated, and in the future may initiate, litigation or other proceedings against third parties to enforce our patent rights;
- third parties have initiated, and in the future may initiate, litigation or other proceedings seeking to invalidate patents owned by or licensed to us or to obtain a declaratory judgment that their product or technology does not infringe our patents or patents licensed to us or that such patents are invalid or unenforceable;
- third parties have initiated, and in the future may initiate, oppositions, IPRs, post grant reviews or reexamination proceedings challenging the validity or scope of our patent rights, requiring us and/or licensors to participate in such proceedings to defend the validity and scope of our patents;
- there are, and in the future may be, more challenges or disputes regarding inventorship or ownership of patents currently identified as being owned by or licensed to us; or
- at our initiation or at the initiation of a third-party, the USPTO may initiate an interference between patents or patent applications owned by or licensed to us and those of our competitors, requiring us and/or licensors to participate in an interference proceeding to determine the priority of invention, which could jeopardize our patent rights.

Furthermore, many of our employees were previously employed at universities or other life sciences companies, including our competitors or potential competitors. We or our employees may be subject to claims that these employees or we have inadvertently or otherwise used or disclosed trade secrets or other proprietary information of their former employers. Although no such claims are currently pending, litigation may be necessary to defend against such claims if they arise in the future. If we fail to successfully defend such

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claims, in addition to paying monetary damages, we may be subject to injunctive relief and lose valuable intellectual property rights. A loss of key research personnel work product could hamper or prevent our ability to commercialize certain potential products, which could severely harm our business. Even if we are successful in defending against these claims, litigation could result in substantial costs and be a distraction to management.

If we are unable to protect our intellectual property effectively, our business would be harmed.

We rely on patent protection as well as trademark, copyright, trade secret and other intellectual property rights protection and contractual restrictions to protect our proprietary technologies, all of which provide limited protection and may not adequately protect our rights or permit us to gain or keep any competitive advantage. As of June 30, 2019, worldwide we owned or exclusively licensed over 175 issued or allowed patents and 470 pending patent applications. We also license additional patents on a non-exclusive and/or territory restricted basis. We continue to file new patent applications to attempt to obtain further legal protection of the full range of our technologies. If we fail to protect our intellectual property, third parties may be able to compete more effectively against us and we may incur substantial litigation costs in our attempts to recover or restrict the use of our intellectual property.

Our success depends in part on obtaining patent protection for our products and processes, preserving trade secrets, patents, copyrights and trademarks, operating without infringing the proprietary rights of third parties and acquiring licenses for technology or products. We may exercise our business judgment and choose to relinquish rights in trade secrets by filing applications that disclose and describe our inventions and certain trade secrets when we seek patent protection for certain of our products and technology. We cannot assure investors that any of our currently pending or future patent applications will result in issued patents and we cannot predict how long it will take for such patents to be issued. Further, in some cases, we have only filed provisional patent applications on certain aspects of our products and technologies and each of these provisional patent applications is not eligible to become an issued patent until, among other things, we file a non-provisional patent application within 12 months of the filing date of the applicable provisional patent application. Such provisional patents may not become issued patents for a variety of reasons, including our failure to file a non-provisional patent application within the permitted timeframe or a decision that doing so no longer makes business or financial sense. Publications of discoveries in scientific literature often lag behind the actual discoveries and patent applications in the United States and other jurisdictions are typically not published until 18 months after filing or in some cases not at all. Therefore, we cannot know with certainty whether we were the first to make the inventions claimed in our owned or licensed patents or pending patent applications, or that we were the first to file for patent protection of such inventions. As a result, the issuance, scope, validity, enforceability and commercial value of our patent rights are highly uncertain, despite the importance of seeking patent protection in our industry.

Further, we cannot assure investors that other parties will not challenge any patents issued to us or that courts or regulatory agencies will hold our patents to be valid or enforceable. We cannot guarantee investors that we will be successful in defending challenges made against our patents and patent applications, even if we spend significant resources defending such challenges. Any successful third-party challenge to our patents could result in the unenforceability or invalidity of such patents and could deprive us of the ability to prevent others from using the technologies claimed in such issued patents.

Changes in either the patent laws or in interpretations of patent laws in the United States or other countries may diminish the value of our intellectual property. We cannot predict the breadth of claims that may be allowed or enforced in our patents or in third-party patents.

In addition to pursuing patents on our technology, we take steps to protect our intellectual property and proprietary technology by entering into confidentiality agreements and intellectual property assignment

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agreements with our employees, consultants, corporate partners and, when needed, our advisors. Such agreements may not be enforceable or may not provide meaningful protection for our trade secrets or other proprietary information in the event of unauthorized use or disclosure or other breaches of the agreements and we may not be able to prevent such unauthorized disclosure. Monitoring unauthorized disclosure is difficult and we do not know whether the steps we have taken to prevent such disclosure are, or will be, adequate. If we were to enforce a claim that a third-party had illegally obtained and was using our trade secrets, it would be expensive and time consuming and the outcome would be unpredictable.

We also seek trademark registration to protect key trademarks such as our 10X and CHROMIUM marks, however, we have not yet registered all of our trademarks in all of our current and potential markets. If we apply to register these trademarks, our applications may not be allowed for registration and our registered trademarks may not be maintained or enforced. In addition, opposition or cancellation proceedings may be filed against our trademark applications and registrations and our trademarks may not survive such proceedings. If we do not secure registrations for our trademarks, we may encounter more difficulty in enforcing them against third parties than we otherwise would.

With respect to all categories of intellectual property protection, our competitors could purchase our products and attempt to replicate some or all of the competitive advantages we derive from our development efforts, willfully infringe our intellectual property rights, design around our protected technology or develop their own competitive technologies that fall outside of our intellectual property rights. In addition, competitors may develop their own versions of our products in countries where we did not apply for patents, where our patents have not issued or where our intellectual property rights are not recognized, and compete with us in those countries and markets.

The laws of some countries do not protect intellectual property rights to the same extent as the laws of the United States and many companies have encountered significant problems in protecting and defending such rights in foreign jurisdictions. The legal systems of certain countries, particularly certain developing countries, do not favor the enforcement of patents and other intellectual property protection, particularly those relating to biotechnology, which could make it difficult for us to stop the infringement of our patents. Proceedings to enforce our patent rights in foreign jurisdictions could result in substantial costs and divert our efforts and attention from other aspects of our business.

The U.S. law relating to the patentability of certain inventions in the life sciences is uncertain and rapidly changing, which may adversely impact our existing patents or our ability to obtain patents in the future.

Various courts, including the U.S. Supreme Court, have rendered decisions that impact the scope of patentability of certain inventions or discoveries relating to the life sciences. Specifically, these decisions stand for the proposition that patent claims that recite laws of nature (for example, the relationships between gene expression levels and the likelihood of risk of recurrence of cancer) are not themselves patentable unless those patent claims have sufficient additional features that provide practical assurance that the processes are genuine inventive applications of those laws rather than patent drafting efforts designed to monopolize the law of nature itself. What constitutes a “sufficient” additional feature is uncertain. Furthermore, in view of these decisions, in December 2014 the USPTO, published revised guidelines for patent examiners to apply when examining process claims for patent eligibility. This guidance was updated by the USPTO in July 2015 and additional illustrative examples provided in May 2016. The USPTO provided additional guidance on examination procedures pertaining to subject matter eligibility in April 2018 and June 2018. The guidance indicates that claims directed to a law of nature, a natural phenomenon or an abstract idea that do not meet the eligibility requirements should be rejected as non-statutory, patent ineligible subject matter; however, method of treatment claims that practically apply natural relationships should be considered patent eligible. We cannot assure you that our patent portfolio will not be negatively impacted by the current uncertain state of the law,

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new court rulings or changes in guidance or procedures issued by the USPTO. From time to time, the U.S. Supreme Court, other federal courts, the U.S. Congress or the USPTO may change the standards of patentability and validity of patents within the life sciences and any such changes could have a negative impact on our business.

Risks related to this offering and ownership of our Class A common stock

The market price of our Class A common stock may be volatile, which could result in substantial losses for investors purchasing shares in this offering.

The initial public offering price for our Class A common stock will be determined through negotiations with the underwriters. This initial public offering price may differ from the market price of our Class A common stock after the offering. As a result, you may not be able to sell your Class A common stock at or above the initial public offering price. Some of the factors that may cause the market price of our Class A common stock to fluctuate include:

- the timing of our launch of future products and degree to which the launch and commercialization thereof meets the expectations of securities analysts and investors;
- the outcomes of and related rulings in the litigation and administrative proceedings in which we are currently involved;
- the failure or discontinuation of any of our product development and research programs;
- changes in the structure or funding of research at academic and research laboratories and institutions, including changes that would affect their ability to purchase our instruments or consumables;
- the success of existing or new competitive businesses or technologies;
- announcements about new research programs or products of our competitors;
- developments or disputes concerning patent applications, issued patents or other proprietary rights;
- the recruitment or departure of key personnel;
- litigation and governmental investigations involving us, our industry or both;
- regulatory or legal developments in the United States and other countries;
- volatility and variations in market conditions in the life sciences sector generally, or the genomics sector specifically;
- investor perceptions of us or our industry;
- the level of expenses related to any of our research and development programs or products;
- actual or anticipated changes in our estimates as to our financial results or development timelines, variations in our financial results or those of companies that are perceived to be similar to us or changes in estimates or recommendations by securities analysts, if any, that cover our Class A stock or companies that are perceived to be similar to us;
- whether our financial results meet the expectations of securities analysts or investors;
- the announcement or expectation of additional financing efforts;

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- stock-based compensation expense under applicable accounting standards;
- sales of our Class A common stock or Class B common stock by us, our insiders or other stockholders;
- the expiration of market standoff or lock-up agreements;
- general economic, industry and market conditions;
- natural disasters or major catastrophic events; and
- the other factors described in this "*Risk factors*" section.

In recent years, stock markets in general, and the market for life science technology companies in particular (including companies in the genomics, biotechnology, diagnostics and related sectors), have experienced significant price and volume fluctuations that have often been unrelated or disproportionate to changes in the operating performance of the companies whose stock is experiencing those price and volume fluctuations. Broad market and industry factors may seriously affect the market price of our Class A common stock, regardless of our actual operating performance. These fluctuations may be even more pronounced in the trading market for our stock shortly following this offering. Following periods of such volatility in the market price of a company's securities, securities class action litigation has often been brought against that company. Because of the potential volatility of our stock price, we may become the target of securities litigation in the future. Securities litigation could result in substantial costs and divert management's attention and resources from our business.

If securities analysts do not publish research or reports about our business or if they publish negative evaluations of our Class A common stock, the price of our Class A common stock could decline.

The trading market for our Class A common stock will rely in part on the research and reports that industry or securities analysts publish about us or our business. We do not currently have and may never obtain research coverage by industry or securities analysts. If no or few analysts commence coverage of us, the trading price of our Class A common stock could decrease. Even if we do obtain analyst coverage, if one or more of the analysts covering our business downgrade their evaluations of our Class A common stock, the price of our Class A common stock could decline. If one or more of these analysts cease to cover our Class A common stock, we could lose visibility in the market for our Class A common stock, which in turn could cause the price of our Class A common stock to decline.

Prior to this offering, there has been no public market for shares of our Class A common stock and an active trading market for our Class A common stock may never develop or be sustained.

Prior to this offering, there has been no public market for shares of our Class A common stock. We have applied to list our Class A common stock on Nasdaq under the symbol "TXG". We cannot assure you that an active trading market for our Class A common stock will develop on that exchange or elsewhere. If an active trading market does not develop, or develops but is not maintained, you may have difficulty selling any of our Class A common stock that you purchase due to the limited public float. Accordingly, we cannot assure you of your ability to sell your shares of Class A common stock when desired or the prices that you may obtain for your shares.

Sales of a substantial number of shares of our Class A common stock by our existing stockholders following this offering could cause the price of our Class A common stock to decline.

Sales of a substantial number of shares of our Class A common stock in the public market could occur at any time following the expiration of the market standoff and lock-up agreements or the early release of these agreements or the perception in the market that the holders of a large number of shares of Class A common

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stock intend to sell shares and could reduce the market price of our Class A common stock. After giving effect to (i) the filing and effectiveness of our amended and restated certificate of incorporation, (ii) the reclassification of all outstanding shares of our Historical Class A common stock into Class B common stock and of our Historical Class B common stock into Class A common stock, (iii) the automatic conversion of all shares of our Convertible Preferred Stock outstanding as of June 30, 2019 into 67,704,278 shares of Class B common stock and (iv) the issuance and sale of shares of Class A common stock by us in this offering, we will have shares of Class A common stock outstanding and 75,754,278 shares of Class B common stock outstanding. Of these shares, the shares of Class A common stock we are selling in this offering may be resold in the public market immediately, unless purchased by our affiliates. The remaining shares of Class A common stock, or % of our outstanding shares of Class A common stock after this offering and all shares of our Class B common stock (and any share of Class A common stock into which they are converted) are currently prohibited or otherwise restricted under securities laws, market standoff agreements entered into by our stockholders with us, or lock-up agreements entered into by our stockholders with the underwriters; however, subject to applicable securities law restrictions and excluding shares of Class A common stock issued pursuant to the early exercise of unvested stock options that will remain unvested, the shares of our Class A common stock outstanding after this offering will be able to be sold in the public market beginning on , 2020. The representatives may, in their sole discretion, release all or some portion of the shares subject to lock-up agreements at any time and for any reason. Shares issued upon the exercise of stock options outstanding under our equity incentive plans or pursuant to future awards granted under those plans will become available for sale in the public market to the extent permitted by the provisions of applicable vesting schedules, any applicable market standoff and lock-up agreements, and Rule 144 and Rule 701 under the Securities Act of 1933, as amended (the "Securities Act").

Moreover, after this offering, holders of an aggregate of shares of our Class B common stock will have rights, subject to conditions, to require us to file registration statements with the SEC covering their shares or to include their shares in registration statements that we may file for ourselves or other stockholders as described under "*Description of capital stock—Registration rights*". We also plan to register all shares of Class A common stock that we may issue under our equity compensation and employee stock purchase plans. Once we register these shares, they can be freely sold in the public market upon issuance and, if applicable, vesting, subject to volume limitations applicable to affiliates and the lock-up agreements described in the section titled "*Underwriting*" in this prospectus. Sales of Class A common stock in the public market as restrictions end or pursuant to registration rights may make it more difficult for us to sell equity securities in the future at a time and at a price that we deem appropriate. These sales also could cause the trading price of our Class A common stock to fall and make it more difficult for you to sell shares of our Class A common stock. See the section titled "*Shares eligible for future sale*" for more information regarding shares of Class A common stock that may be sold in the public market after this offering.

If you purchase our Class A common stock in this offering, you will incur immediate and substantial dilution as a result of this offering.

If you purchase our Class A common stock in this offering, you will incur immediate and substantial dilution of $ per share, representing the difference between the assumed initial public offering price of $ per share, which is the midpoint of the price range set forth on the cover page of this prospectus, after deducting the estimated underwriting discounts and commissions and estimated offering expenses payable by us, and our pro forma net tangible book value per share after giving effect to (i) the filing and effectiveness of our amended and restated certificate of incorporation, (ii) the reclassification of all outstanding shares of our Historical Class A common stock into Class B common stock and of our Historical Class B common stock into Class A common stock, (iii) the automatic conversion of all shares of our Convertible Preferred Stock outstanding as of June 30, 2019 into 67,704,278 shares of Class B common stock and (iv) the issuance and sale

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of shares of Class A common stock by us in this offering. As of June 30, 2019, there were 15,634,182 shares of our Class A common stock subject to outstanding stock options with a weighted-average exercise price of $3.61 per share and 266,099 shares of Class A common stock issuable upon exercise of warrants outstanding with a weighted-average exercise price of $1.17 per share. To the extent that these outstanding stock options and warrants are ultimately exercised or the underwriters exercise their option to purchase additional shares of our Class A common stock, you will incur further dilution. See the section titled "*Dilution*" for more information.

Raising additional capital may cause dilution to our existing stockholders or restrict our operations.

We anticipate that we will seek additional capital through a combination of public and private equity offerings, debt financings, strategic partnerships and alliances and licensing arrangements in the future to fund our operations. We, and indirectly, our stockholders, will bear the cost of issuing and servicing such securities. Because our decision to issue debt or equity securities in any future offering will depend on market conditions and other factors beyond our control, we cannot predict or estimate the amount, timing or nature of any future offerings. Our decision to issue debt or equity securities will also depend on contractual, legal and other restrictions that may limit our ability to raise additional capital. For example, the terms of our Loan and Security Agreement prohibit, subject to certain exceptions, our ability to incur additional indebtedness. Further, our election to borrow up to an additional $20.0 million of term loans under the Loan and Security Agreement will obligate us to issue warrants to purchase 133,000 shares of our Class A common stock at an exercise price of $1.62 per share to the lender thereof, which will result in further dilution of your ownership interest. To the extent that we raise additional capital through the sale of equity or debt securities, your ownership interest will be diluted and the terms may include liquidation or other preferences that adversely affect your rights as a stockholder. The incurrence of indebtedness would result in increased fixed payment obligations and could involve restrictive covenants, such as limitations on our ability to incur additional debt, limitations on our ability to acquire, sell or license intellectual property rights and other operating restrictions that could adversely impact our ability to conduct our business. Certain of the foregoing transactions may require us to obtain stockholder approval, which we may not be able to obtain.

The multi-class structure of our common stock will have the effect of concentrating voting control with those stockholders who held our capital stock prior to the completion of this offering and it may depress the trading price of our Class A common stock.

Our Class A common stock, which is the stock we are offering in this offering, has one vote per share, and our Class B common stock has ten votes per share, except as otherwise required by law. Following this offering, our directors, executive officers and holders of more than 5% of our common stock, and their respective affiliates, will hold in the aggregate % of the voting power of our capital stock. Because of the ten-to-one voting ratio between our Class B common stock and Class A common stock, the holders of our Class B common stock collectively will continue to control a majority of the combined voting power of our common stock and therefore be able to control all matters submitted to our stockholders for approval. This concentrated control will limit or preclude your ability to influence corporate matters for the foreseeable future, including the election of directors, amendments of our organizational documents and any merger, consolidation, sale of all or substantially all of our assets or other major corporate transaction requiring stockholder approval. In addition, this may prevent or discourage unsolicited acquisition proposals or offers for our capital stock that you may feel are in your best interest as one of our stockholders.

Future transfers by holders of Class B common stock will generally result in those shares converting to Class A common stock, subject to limited exceptions, such as certain transfers effected for estate planning purposes where sole dispositive power and exclusive voting control with respect to the shares of Class B common stock is retained by the transferring holder and transfers between our co-founders. In addition, each outstanding share

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of Class B common stock held by a stockholder who is a natural person, or held by the permitted entities of such stockholder (as described in our amended and restated certificate of incorporation), will convert automatically into one share of Class A common stock upon the death of such natural person. In the event of the death or permanent and total disability of a co-founder, shares of Class B common stock held by such co-founder or his permitted entities will convert to Class A common stock, provided that the conversion will be deferred for nine months, or up to 18 months if approved by a majority of our independent directors, following his death or permanent and total disability. Transfers between our co-founders are permitted transfers and will not result in conversion of the shares of Class B common stock that are transferred. See the section titled "Description of capital stock—Common stock—Conversion of Class B common stock" for additional information about conversions. The conversion of Class B common stock to Class A common stock will have the effect, over time, of increasing the relative voting power of those individual holders of Class B common stock who retain their shares in the long term.

In addition, in July 2017, FTSE Russell and Standard & Poor's announced that they would cease to allow most newly public companies utilizing dual or multi-class capital structures to be included in their indices. Affected indices include the Russell 2000 and the S&P 500, S&P MidCap 400, and S&P SmallCap 600, which together make up the S&P Composite 1500. Under the announced policies, our multi-class capital structure would make us ineligible for inclusion in any of these indices, and as a result, mutual funds, exchange-traded funds and other investment vehicles that attempt to passively track these indices will not be investing in our stock. These policies are new and it is as of yet unclear what effect, if any, they have had and will have on the valuations of publicly traded companies excluded from the indices, but it is possible that they may depress these valuations compared to those of other similar companies that are included.

We are an "emerging growth company" and the reduced disclosure requirements applicable to emerging growth companies may make our Class A common stock less attractive to investors.

We are an "emerging growth company", as defined in the JOBS Act. For so long as we remain an emerging growth company, we are permitted by SEC rules and plan to rely on exemptions from certain disclosure requirements that are applicable to other SEC-registered public companies that are not emerging growth companies. These exemptions include not being required to comply with the auditor attestation requirements of Section 404 of the SOX, not being required to comply with any requirement that may be adopted by the Public Company Accounting Oversight Board regarding mandatory audit firm rotation or a supplement to the auditor's report providing additional information about the audit and the financial statements, reduced disclosure obligations regarding executive compensation and exemptions from the requirements of holding a nonbinding advisory vote on executive compensation and stockholder approval of any golden parachute payments not previously approved. As a result, the information we provide stockholders will be different than the information that is available with respect to other public companies. In this prospectus, we have not included all of the executive compensation related information that would be required if we were not an emerging growth company. We cannot predict whether investors will find our Class A common stock less attractive if we rely on these exemptions. If some investors find our Class A common stock less attractive as a result, there may be a less active trading market for our Class A common stock and our stock price may be more volatile.

In addition, the JOBS Act provides that an emerging growth company can take advantage of an extended transition period for complying with new or revised accounting standards. This allows an emerging growth company to delay the adoption of certain accounting standards until those standards would otherwise apply to private companies. We have elected to avail ourselves of this exemption from new or revised accounting standards and, therefore, we will not be subject to the same new or revised accounting standards as other public companies that are not emerging growth companies. As a result, our financial statements may not be

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comparable to companies that comply with new or revised accounting pronouncements as of public company effective dates.

We will incur increased costs as a result of operating as a public company and be subject to additional potential liability. Our management will be required to devote substantial time to new compliance initiatives and corporate governance practices.

As a public company, and particularly after we are no longer an emerging growth company, we will incur significant legal, accounting and other expenses that we did not incur as a private company. The Dodd-Frank Wall Street Reform and Consumer Protection Act, SOX, the listing requirements of Nasdaq and other applicable federal and Delaware rules and regulations impose various requirements on public companies, including establishment and maintenance of effective disclosure and financial controls and corporate governance practices. We expect that we will need to hire additional accounting, finance and other personnel in connection with our becoming, and our efforts to comply with the requirements of being, a public company, and our management and other personnel will need to devote a substantial amount of time towards maintaining compliance with these requirements. These requirements will increase our legal and financial compliance costs and will make some activities more time-consuming and costly.

For example, we expect that the rules and regulations applicable to us as a public company and recent trends in the insurance market make it more expensive for us to obtain director and officer liability insurance. While we will continue to evaluate options for director and officer liability insurance, we currently intend to only obtain director and officer liability coverage (commonly referred to as “Side A” coverage). This means that while our directors and officers direct insurance coverage for acts which the company is not legally required or permitted to indemnify them, the company itself will not have coverage for amounts incurred in defending, among other things, stockholder derivative or securities class action lawsuits or in the event of certain investigative actions, for amounts it must pay as a result of such suits or amounts it must pay to indemnify our directors or officers. We will in essence be self-insuring for these costs. Any costs incurred in connection with such litigation could have a material adverse effect on our business, financial condition and results of operations.

In September 2018, California enacted a law that requires publicly held companies headquartered in California to have at least one female director by the end of 2019 and at least three by the end of 2021, depending on the size of the board. The law would impose financial penalties for failure to comply. We are currently in compliance with the requirements of the law but we may incur costs associated with complying with the law in future years, including costs associated with expanding our board of directors or identifying qualified candidates for appointment to our board of directors, or financial penalties or harm to our brand and reputation if we fail to comply. We cannot predict or estimate the amount of additional costs we may incur or the timing of such costs.

Pursuant to SOX Section 404, we will be required to furnish a report by our management on our internal control over financial reporting beginning with our second filing of an Annual Report on Form 10-K with the SEC after we become a public company. However, while we remain an emerging growth company, we will not be required to include an attestation report on internal control over financial reporting issued by our independent registered public accounting firm. To achieve compliance with SOX Section 404 within the prescribed period, we will be engaged in a process to document and evaluate our internal control over financial reporting, which is both costly and challenging. In this regard, we will need to continue to dedicate internal resources, potentially engage outside consultants, adopt a detailed work plan to assess and document the adequacy of internal control over financial reporting, continue steps to improve control processes as appropriate, validate through testing that controls are functioning as documented and implement a continuous reporting and improvement process for internal control over financial reporting. Despite our efforts, there is a risk that we will not be able to conclude, within the prescribed timeframe or at all, that our internal control over financial reporting is effective as required by SOX

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Section 404. If we identify one or more material weaknesses, it could result in an adverse reaction in the financial markets due to a loss of confidence in the reliability of our financial statements.

Additionally, we have historically operated our business as a private company. After this offering, we will be required to file with the SEC annual and quarterly information and other reports that are specified in Section 13 of the Exchange Act. We will also be required to ensure that we have the ability to prepare financial statements that are fully compliant with all SEC reporting requirements on a timely basis. We will also become subject to other reporting and corporate governance requirements, including the requirements of Nasdaq and certain provisions of SOX and the regulations promulgated thereunder, which will impose significant compliance obligations upon us. As a public company, we will, among other things:

- prepare and distribute periodic public reports and other stockholder communications in compliance with our obligations under the federal securities laws and applicable Nasdaq rules;
- create or expand the roles and duties of our board of directors and committees of the board;
- institute more comprehensive financial reporting and disclosure compliance functions;
- supplement our internal accounting, auditing and reporting function, including hiring additional staff with expertise in accounting and financial reporting for a public company;
- enhance and formalize closing procedures at the end of our accounting periods;
- enhance our internal audit and tax functions;
- enhance our investor relations function;
- establish new internal policies, including those relating to disclosure controls and procedures; and
- involve and retain to a greater degree outside counsel and accountants in the activities listed above.

We may not be successful in implementing these requirements and the significant commitment of resources required for implementing them could adversely affect our business, financial condition and results of operations. In addition, if we fail to implement the requirements with respect to our internal accounting and audit functions, our ability to report our results of operations on a timely and accurate basis could be impaired and we could suffer adverse regulatory consequences or violate the Nasdaq rules. There could also be a negative reaction in the financial markets due to a loss of investor confidence in us and the reliability of our financial statements.

The changes necessitated by becoming a public company require a significant commitment of resources and management oversight that has increased and may continue to increase our costs and might place a strain on our systems and resources. As a result, our management's attention might be diverted from other business concerns. If we fail to maintain an effective internal control environment or to comply with the numerous legal and regulatory requirements imposed on public companies, we could make material errors in, and be required to restate, our financial statements. Any such restatement could result in a loss of public confidence in the reliability of our financial statements and sanctions imposed on us by the SEC. In addition, the rules and regulations imposed on public companies are often subject to varying interpretations and, as a result, their application in practice may evolve over time as new guidance is provided by regulatory and governing bodies. This could result in continuing uncertainty regarding compliance matters and higher costs necessitated by ongoing revisions to disclosure and governance practices.

We have broad discretion in the use of the net proceeds from this offering and may not use them effectively.

We cannot specify with certainty the particular uses of the net proceeds we will receive from this offering. Our management will have broad discretion in the application of the net proceeds, including for any of the purposes

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described in the section titled "*Use of proceeds*" in this prospectus. Our management may spend a portion or all of the net proceeds from this offering in ways that our stockholders may not desire or that may not yield a favorable return. The failure by our management to apply these funds effectively could harm our business, financial condition, results of operations and prospects. Pending their use, we may invest the net proceeds from this offering in a manner that does not produce income or that loses value.

We do not expect to pay any dividends for the foreseeable future. Investors in this offering may never obtain a return on their investment.

You should not rely on an investment in our Class A common stock to provide dividend income. We do not anticipate that we will pay any dividends to holders of our Class A common stock in the foreseeable future. Instead, we plan to retain any earnings to maintain and expand our existing operations and fund our research and development programs. In addition, our Loan and Security Agreement contains, and any future credit facility or financing we obtain may contain, terms prohibiting or limiting the amount of dividends that may be declared or paid on our Class A common stock. Accordingly, investors must rely on sales of their Class A common stock after price appreciation, which may never occur, as the only way to realize any return on their investment. As a result, investors seeking cash dividends should not purchase our Class A common stock.

Delaware law and provisions in our amended and restated certificate of incorporation and amended and restated bylaws that will be in effect prior to the closing of this offering might discourage, delay or prevent a change in control of our company or changes in our management and, therefore, depress the trading price of our Class A common stock.

Our status as a Delaware corporation and the anti-takeover provisions of the Delaware General Corporation Law may discourage, delay or prevent a change in control by prohibiting us from engaging in a business combination with an interested stockholder for a period of three years after the person becomes an interested stockholder, even if a change of control would be beneficial to our existing stockholders. In addition, our restated certificate of incorporation and restated bylaws contain provisions that may make the acquisition of our company more difficult, including the following:

- any transaction that would result in a change in control of our company requires the approval of a majority of our outstanding Class B common stock voting as a separate class;
- our multi-class common stock structure provides our holders of Class B common stock with the ability to significantly influence the outcome of matters requiring stockholder approval, even if they own significantly less than a majority of the shares of our outstanding Class A common stock and Class B common stock;
- our board of directors will be classified into three classes of directors with staggered three-year terms and directors will only be able to be removed from office for cause by the affirmative vote of holders of at least two-thirds of the voting power of our then outstanding capital stock;
- certain amendments to our amended and restated certificate of incorporation will require the approval of stockholders holding two-thirds of the voting power of our then outstanding capital stock;
- certain amendments to our amended and restated bylaws will require the approval of stockholders holding two-thirds of the voting power of our then outstanding capital stock;
- our stockholders will only be able to take action at a meeting of stockholders and will not be able to take action by written consent for any matter;
- our stockholders will be able to act by written consent only if the action is first recommended or approved by the board of directors;

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- vacancies on our board of directors will be able to be filled only by our board of directors and not by stockholders;
- only our chairman of the board of directors, chief executive officer or a majority of the board of directors are authorized to call a special meeting of stockholders;
- certain litigation against us can only be brought in Delaware;
- our restated certificate of incorporation authorizes undesignated preferred stock, the terms of which may be established and shares of which may be issued, without the approval of the holders of our capital stock; and
- advance notice procedures apply for stockholders to nominate candidates for election as directors or to bring matters before an annual meeting of stockholders.

These anti-takeover defenses could discourage, delay, or prevent a transaction involving a change in control of our company. These provisions could also discourage proxy contests and make it more difficult for stockholders to elect directors of their choosing and to cause us to take other corporate actions they desire, any of which, under certain circumstances, could limit the opportunity for our stockholders to receive a premium for their shares of our capital stock and could also affect the price that some investors are willing to pay for our Class A common stock.

Our amended and restated bylaws will designate a state or federal court located within the State of Delaware as the exclusive forum for substantially all disputes between us and our stockholders, which could limit our stockholders' ability to choose the judicial forum for disputes with us or our directors, officers or employees.

Our amended and restated bylaws, which will become effective immediately prior to the completion of this offering, will provide that, unless we consent in writing to the selection of an alternative forum, the sole and exclusive forum for the following types of actions or proceedings under Delaware statutory or common law (i) any derivative action or proceeding brought on our behalf, (ii) any action asserting a claim of breach of a fiduciary duty owed by any of our directors, officers or other employees to us or our stockholders, (iii) any action arising pursuant to any provision of the Delaware General Corporation Law, our certificate of incorporation or our amended and restated bylaws or (iv) any other action asserting a claim that is governed by the internal affairs doctrine shall be a state or federal court located within the State of Delaware, in all cases subject to the court having jurisdiction over indispensable parties named as defendants. Nothing in our amended and restated bylaws will preclude stockholders that assert claims under the Securities Act or the Exchange Act from bringing such claims in state or federal court, subject to applicable law.

Any person or entity purchasing or otherwise acquiring any interest in any of our securities shall be deemed to have notice of and consented to the foregoing forum selection provisions. These exclusive-forum provisions may limit a stockholder's ability to bring a claim in a judicial forum of its choosing for disputes with us or our directors, officers, or other employees, which may discourage lawsuits against us and our directors, officers and other employees. If a court were to find either exclusive-forum provision in our amended and restated bylaws to be inapplicable or unenforceable in an action, we may incur additional costs associated with resolving the dispute in other jurisdictions, which could harm our results of operations.

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Special note regarding forward-looking statements

This prospectus contains forward-looking statements, particularly in the sections titled "*Prospectus summary*", "*Risk factors*", "*Management's discussion and analysis of financial condition and results of operations*" and "*Business*". In some cases, you can identify these statements by forward-looking words such as "anticipate", "believe", "continue", "could", "estimate", "expect", "intend", "may", "might", "plan", "potential", "predict", "should", "would" or "will", the negative of these terms and other comparable terminology. These forward-looking statements, which are subject to risks, uncertainties and assumptions about us, may include projections of our future financial performance, our future products, our technology, our potential market opportunity, our anticipated growth strategies and anticipated trends in our business.

These statements are only predictions based on our current expectations and projections about future events and trends. There are important factors that could cause our actual results, level of activity, performance or achievements to differ materially and adversely from the results, level of activity, performance or achievements expressed or implied by the forward-looking statements, including those factors discussed in the section titled "*Risk factors*". You should specifically consider the numerous risks described in the section titled "*Risk factors*". Moreover, we operate in a competitive and rapidly changing environment. New risks emerge from time to time. It is not possible for our management to predict all risks, nor can we assess the impact of all factors on our business or the extent to which any factor, or combination of factors, may cause actual results to differ materially and adversely from those contained in any forward-looking statements we may make.

Although we believe the expectations reflected in the forward-looking statements are reasonable, we cannot guarantee future results, level of activity, performance or achievements. These statements are inherently uncertain. Except to the extent required by law, we undertake no obligation to update any of these forward-looking statements after the date of this prospectus to conform our prior statements to actual results or revised expectations or to reflect new information or the occurrence of unanticipated events. Given these risks, uncertainties and assumptions, you are cautioned not to place undue reliance on such forward-looking statements as predictions of future performance or otherwise.

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Industry and market data

Unless otherwise indicated, information contained in this prospectus concerning our industry and the markets in which we operate, including our general expectations and market position, market opportunity and market size, is based on information from various sources on assumptions that we have made that are based on such information and other similar sources and on our knowledge of, and expectations about, the markets for our products. This information involves a number of assumptions and limitations and you are cautioned not to give undue weight to such estimates. While we believe the market position, market opportunity and market size information included in this prospectus is generally reliable, such information is inherently imprecise. In addition, projections, assumptions and estimates of our future performance and the future performance of the industry in which we operate is necessarily subject to a high degree of uncertainty and risk due to a variety of factors, including those described in the section titled "*Risk factors*" and elsewhere in this prospectus. These and other factors could cause results to differ materially from those expressed in the estimates made by independent third parties and by us.

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Use of proceeds

We estimate that the net proceeds to us from the issuance and the sale of shares of our Class A common stock in this offering will be approximately $, based on the assumed initial public offering price of $ per share, which is the midpoint of the price range set forth on the cover page of this prospectus, and after deducting estimated underwriting discounts and commissions and estimated offering expenses payable by us. If the underwriters exercise their option to purchase additional shares in full, we estimate that the net proceeds to us would be approximately $, after deducting estimated underwriting discounts and commissions and estimated offering expenses payable by us.

Each $1.00 increase or decrease in the assumed initial public offering price of $ per share, which is the midpoint of the price range set forth on the cover page of this prospectus, would increase or decrease, as applicable, the net proceeds that we receive from this offering by approximately $, assuming that the number of shares offered by us, as set forth on the cover page of this prospectus, remains the same, and after deducting estimated underwriting discounts and commissions payable by us. Similarly, each increase or decrease of 1.0 million in the number of shares of our Class A common stock offered by us would increase or decrease, as applicable, the net proceeds that we receive from this offering by approximately $, assuming the assumed initial public offering price remains the same, and after deducting estimated underwriting discounts and commissions payable by us.

The principal purposes of this offering are to increase our capitalization and financial flexibility, create a public market for our Class A common stock and enable access to the public equity markets for us and our stockholders.

We intend to use the net proceeds from this offering for general corporate purposes, including working capital, operating expenses and capital expenditures. Additionally, we may use a portion of the net proceeds we receive from this offering to acquire businesses, products or technologies. However, we do not have agreements or commitments for any material acquisitions at this time.

The expected use of net proceeds from this offering represents our intentions based upon our present plans and business conditions. We cannot predict with certainty all of the particular uses for the proceeds of this offering or the amounts that we will actually spend on the uses set forth above. Accordingly, our management will have significant flexibility in applying the net proceeds of this offering. The timing and amount of our actual expenditures will be based on many factors, including cash flows from operations and the anticipated growth of our business. Pending the uses described above, we intend to invest the net proceeds from this offering in short-term, interest-bearing obligations, investment-grade instruments, certificates of deposit or direct or guaranteed obligations of the United States government.

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Dividend policy

We have never declared or paid any cash dividends on our capital stock. We currently intend to retain any future earnings and do not expect to pay any dividends in the foreseeable future. Any future determination to declare cash dividends will be made at the discretion of our board of directors, subject to applicable laws, and will depend on a number of factors, including our financial condition, results of operations, capital requirements, contractual restrictions, general business conditions and other factors that our board of directors may deem relevant. In addition, the terms of our Loan and Security Agreement place certain limitations on the amount of cash dividends we can pay, even if no amounts are currently outstanding.

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Capitalization

The following table sets forth our cash and cash equivalents, and capitalization as of June 30, 2019:

- on an actual basis;
- on a pro forma basis to reflect: (i) the filing and effectiveness of our amended and restated certificate of incorporation, (ii) the reclassification of all outstanding shares of our Historical Class A common stock into Class B common stock and of our Historical Class B common stock into Class A common stock and (iii) the automatic conversion of all shares of our Convertible Preferred Stock outstanding as of June 30, 2019 into 67,704,278 shares of Class B common stock, in each case, prior to the closing of this offering and as described under "*Description of capital stock—Reclassification of common stock and conversion of Convertible Preferred Stock*"; and
- on a pro forma as adjusted basis to reflect: (i) the pro forma adjustments set forth above and (ii) the issuance and sale of shares of Class A common stock by us in this offering at an assumed initial public offering price $ per share, which is the midpoint of the price range set forth on the cover page of this prospectus, after deducting estimated underwriting discounts and commissions and estimated offering expenses payable by us.

The pro forma as adjusted information set forth in the table below is illustrative only and will be adjusted based on the actual initial public offering price and other terms of this offering determined at pricing. This table should be read in conjunction with the sections titled "*Selected consolidated financial data*" and "*Management's discussion and analysis of financial condition and results of operations*" and our consolidated financial statements and related notes included elsewhere in this prospectus.

	As of June 30, 2019		
(in thousands, except share and per share data)	Actual	Pro forma	Pro forma as adjusted(1)
	(unaudited)	(unaudited)	(unaudited)
Cash and cash equivalents	$ 56,034	$	$
Total debt, less current portion	$ 24,777	$	$
Convertible preferred stock, $0.00001 par value per share, 67,904,871 shares authorized, 67,704,278 shares issued and outstanding, actual; no shares authorized, issued or outstanding, pro forma and pro forma as adjusted	243,244	—	—
Stockholders' equity (deficit):			
Historical Class A common stock, $0.00001 par value per share, 75,955,000 shares authorized, 8,050,000 shares issued and outstanding, actual; no shares authorized, issued or outstanding, pro forma and pro forma as adjusted(2)	1	—	—
Historical Class B common stock, $0.00001 par value per share, 115,000,000 shares authorized, 8,095,382 shares issued and outstanding, actual; no shares authorized, issued or outstanding, pro forma and pro forma as adjusted(3)	—	—	—
Preferred stock, $0.00001 par value per share, no shares authorized, issued or outstanding, actual; 100,000,000 shares authorized, and no shares issued or outstanding, pro forma and pro forma as adjusted	—	—	—

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(in thousands, except share and per share data)	As of June 30, 2019		
	Actual	Pro forma	Pro forma as adjusted(1)
	(unaudited)	(unaudited)	(unaudited)
Class A common stock, $0.00001 par value per share, no shares authorized, issued or outstanding, actual; 1,000,000,000 shares authorized, and shares issued and outstanding pro forma and shares issued and outstanding pro forma as adjusted(2)	—		
Class B common stock, $0.00001 par value per share, no shares authorized, issued or outstanding, actual; 100,000,000 shares authorized, and 75,754,278 shares issued and outstanding, pro forma and pro forma as adjusted(3)	—		
Additional paid-in capital	17,715		
Accumulated other comprehensive loss	(34)		
Accumulated deficit	(245,630)		
Total stockholders' equity (deficit)	(227,948)		
Total capitalization	$ 40,073	$	$

(1) Each $1.00 increase or decrease in the assumed initial public offering price of $ per share, which is the midpoint of the price range set forth on the cover page of this prospectus, would increase or decrease, as applicable, the amount of each of our pro forma as adjusted cash and cash equivalents, additional paid-in capital, total stockholders' equity (deficit) and total capitalization by approximately $, assuming that the number of shares offered by us, as set forth on the cover page of this prospectus, remains the same and after deducting estimated underwriting discounts and commissions payable by us. Similarly, each increase or decrease of 1.0 million shares in the number of shares of our Class A common stock offered by us would increase or decrease, as applicable, the amount of each of our pro forma as adjusted cash and cash equivalents, additional paid-in capital, total stockholders' equity (deficit) and total capitalization by approximately $, assuming the assumed initial public offering price remains the same and after deducting estimated underwriting discounts and commissions payable by us.

(2) In connection with the reclassification of all outstanding shares of common stock, our Historical Class A common stock was reclassified into Class B common stock. See the section titled "*Description of capital stock—Reclassification of common stock and conversion of Convertible Preferred Stock*".

(3) In connection with the reclassification of all outstanding shares of common stock, our Historical Class B common stock was reclassified into Class A common stock. See the section titled "*Description of capital stock—Reclassification of common stock and conversion of Convertible Preferred Stock*".

If the underwriters exercise their option to purchase additional shares in full, each of our pro forma as adjusted cash and cash equivalents, additional paid-in capital, total stockholders' equity (deficit), total capitalization and pro forma as adjusted shares of Class A common stock outstanding as of June 30, 2019 would be $ million, $ million, $ million, $ million and shares, respectively.

The number of shares of our Class A common stock and Class B common stock issued and outstanding, pro forma and pro forma as adjusted in the table above is based on 8,095,382 shares of our Class A common stock and 75,754,278 shares of our Class B common stock (including our Convertible Preferred Stock on an as-converted basis) outstanding as of June 30, 2019 and excludes:

- 15,634,182 shares of Class A common stock issuable upon exercise of stock options outstanding as of June 30, 2019, at a weighted-average exercise price of $3.61 per share;
- 266,099 shares of Class of A common stock issuable upon exercise of warrants outstanding as of June 30, 2019, at a weighted-average exercise price of $1.17 per share;
- 842,475 shares of Class A common stock issuable upon exercise of stock options granted after June 30, 2019, at a weighted-average exercise price of $30.00 per share; and

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- 11,000,000 shares of Class A common stock to be reserved and available for future issuance under the Omnibus Incentive Plan, which will become effective in connection with this offering, as more fully described in the section titled "*Executive compensation—Equity incentive plans*", including:
  - 1,323,858 shares of Class A common stock reserved for future grants under the 2012 Stock Plan, as of June 30, 2019, which will be added to the shares reserved under our Omnibus Incentive Plan, plus
  - any shares of Class A common stock issuable upon exercise of stock options outstanding under the 2012 Stock Plan that will be added to our Omnibus Incentive Plan available reserve upon expiration or termination of such stock options, plus
  - automatic increases in the number of shares of Class A common stock reserved for future grants pursuant to our Omnibus Incentive Plan; plus
  - 2,000,000 shares of Class A common stock to be reserved and available for future issuance under the ESPP, which will become effective in connection with this offering, as well as automatic increases in the number of shares of Class A common stock reserved for future issuance under the ESPP.

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Dilution

If you purchase shares of our Class A common stock in this offering, your ownership interest will be diluted to the extent of the difference between the initial public offering price per share of our Class A common stock in this offering and the pro forma as adjusted net tangible book value per share of our common stock immediately after this offering.

Our historical net tangible book value as of June 30, 2019 was $(228.5) million or $(14.16) per share of common stock. Our historical net tangible book value per share represents our tangible assets, less liabilities and Convertible Preferred Stock, divided by the aggregate number of shares of common stock outstanding as of June 30, 2019.

Our pro forma net tangible book value as of June 30, 2019 was $ million or $ per share of common stock. Our pro forma net tangible book value per share represents our tangible assets, less liabilities and Convertible Preferred Stock, divided by the aggregate number of shares of common stock outstanding as of June 30, 2019, after giving effect to: (i) the filing and effectiveness of our amended and restated certificate of incorporation, (ii) the reclassification of all outstanding shares of our Historical Class A common stock into Class B common stock and of our Historical Class B common stock into Class A common stock and (iii) the automatic conversion of all shares of our Convertible Preferred Stock outstanding into 67,704,278 shares of Class B common stock, in each case, prior to the closing of the offering and as described under "*Description of capital stock—Reclassification of common stock and conversion of Convertible Preferred Stock*".

After giving effect to the issuance and sale of shares of Class A common stock by us in this offering at an assumed initial public offering price of $ per share, which is the midpoint of the price range set forth on the cover page of this prospectus, and after deducting estimated underwriting discounts and commissions and estimated offering expenses payable by us, our pro forma as adjusted net tangible book value as of June 30, 2019 would have been $ million or $ per share. This represents an immediate increase in pro forma net tangible book value to existing stockholders of $ per share and an immediate dilution in pro forma net tangible book value to new investors of $ per share. Dilution per share represents the difference between the price per share to be paid by new investors for the shares of Class A common stock sold in this offering and the pro forma net tangible book value per share immediately after this offering. The following table illustrates this per share dilution:

Assumed initial public offering price per share		$
Historical net tangible book value per share as of June 30, 2019	$(14.16)	
Pro forma increase in net tangible book value per share as of June 30, 2019		
Pro forma net tangible book value per share as of June 30, 2019		
Increase in pro forma net tangible book value per share attributable to new investors purchasing shares of Class A common stock in this offering		
Pro forma as adjusted net tangible book value per share		
Dilution per share to new investors participating in this offering		$

Each $1.00 increase or decrease in the assumed initial public offering price of $ per share, which is the midpoint of the price range set forth on the cover page of this prospectus, would increase or decrease, as applicable, our pro forma as adjusted net tangible book value per share after this offering by $ per share and the dilution in pro forma net tangible book value per share to investors participating in this offering by $ per share, assuming that the number of shares of Class A common stock offered by us, as set forth on the cover page of this prospectus, remains the same, and after deducting estimated underwriting discounts and commissions and estimated offering expenses payable by us. Similarly, each increase or decrease of 1.0 million

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shares in the number of shares of our Class A common stock offered by us would increase or decrease, as applicable, our pro forma as adjusted net tangible book value per share after this offering by $ per share and the dilution in pro forma as adjusted net tangible book value per share to investors participating in this offering by $ per share, assuming the assumed initial public offering price remains the same, and after deducting estimated underwriting discounts and commissions and estimated offering expenses payable by us.

If the underwriters exercise their option to purchase additional shares in full, the pro forma as adjusted net tangible book value per share of our Class A common stock after this offering would be $ per share and the dilution in pro forma net tangible book value per share to investors in this offering would be $ per share of Class A common stock.

The following table sets forth, on a pro forma as adjusted basis, as of June 30, 2019, the number of shares of Class A common stock purchased from us, the total consideration paid, or to be paid, and the average price per share paid, or to be paid, by existing stockholders and by the new investors, at an assumed initial public offering price of $ per share, which is the midpoint of the price range set forth on the cover page of this prospectus, before deducting estimated underwriting discounts and commissions and estimated offering expenses payable by us:

	Shares purchased		Total consideration		Weighted-average price per share
	Number	Percent	Amount	Percent	
Existing stockholders		%	$	%	$
New investors					$
Total		100%	$	100%	

Each $1.00 increase or decrease in the assumed initial public offering price per share would increase or decrease, as applicable, the total consideration paid by new investors, total consideration paid by all stockholders and the average price per share paid by all stockholder by approximately $ million, $ million and $, respectively, assuming that the number of shares of Class A common stock offered by us, as set forth on the cover page of this prospectus, remains the same, after deducting estimated underwriting discounts and commissions and estimated offering expenses payable by us. Similarly, each increase or decrease of 1.0 million shares in the number of shares of our Class A common stock offered by us would increase or decrease, as applicable, the total consideration paid by new investors, total consideration paid by all stockholders and the average price per share paid by all stockholders by approximately $ million, $ million and $, respectively, assuming an initial public offering price of $ per share, which is the midpoint of the price range set forth on the cover page of this prospectus, after deducting estimated underwriting discounts and commissions and estimated offering expenses payable by us.

The foregoing tables assume no exercise by the underwriters of their option to purchase additional shares and no exercise of outstanding stock options or warrants after June 30, 2019. If the underwriters exercise their option to purchase additional shares in full, the number of shares of Class A common stock held by our existing stockholders will represent approximately % of the total number of shares of our Class A common stock outstanding after this offering; and the number of shares held by new investors will represent approximately % of the total number of shares of our Class A common stock outstanding after this offering. In addition, to the extent any outstanding stock options or warrants are exercised, investors participating in this offering will experience further dilution.

The foregoing tables and calculations (other than the historical net tangible book value calculation) are based on 8,095,382 shares of our Class A common stock and 75,754,278 shares of our Class B common stock

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(including our Convertible Preferred Stock on an as-converted basis) outstanding as of June 30, 2019 and excludes:

- 15,634,182 shares of Class A common stock issuable upon exercise of stock options outstanding as of June 30, 2019, at a weighted-average exercise price of $3.61 per share;
- 266,099 shares of Class of A common stock issuable upon exercise of warrants outstanding as of June 30, 2019, at a weighted-average exercise price of $1.17 per share;
- 842,475 shares of Class A common stock issuable upon exercise of stock options granted after June 30, 2019, at a weighted-average exercise price of $30.00 per share; and
- 11,000,000 shares of Class A common stock to be reserved and available for future issuance under the Omnibus Incentive Plan, which will become effective in connection with this offering, as more fully described in the section titled "*Executive compensation—Equity incentive plans*", including:
    - 1,323,858 shares of Class A common stock reserved for future grants under the 2012 Stock Plan, as of June 30, 2019, which will be added to the shares reserved under our Omnibus Incentive Plan, plus
    - any shares of Class A common stock issuable upon exercise of stock options outstanding under the 2012 Stock Plan that will be added to our Omnibus Incentive Plan available reserve upon expiration or termination of such stock options, plus
    - automatic increases in the number of shares of Class A common stock reserved for future grants pursuant to our Omnibus Incentive Plan; plus
    - 2,000,000 shares of Class A common stock to be reserved and available for future issuance under the ESPP, which will become effective in connection with this offering, as well as automatic increases in the number of shares of Class A common stock reserved for future issuance under the ESPP.

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Selected consolidated financial data

The following tables present our selected consolidated financial data for the years and as of the dates indicated. We have derived the selected consolidated statements of operations data for the years ended December 31, 2017 and 2018, and the selected consolidated balance sheet data as of December 31, 2017 and 2018, from our audited consolidated financial statements and related notes included elsewhere in this prospectus. We have derived the selected consolidated statements of operations data for the six months ended June 30, 2018 and 2019, and the selected consolidated balance sheet data as of June 30, 2019 from our unaudited consolidated interim financial statements and related notes included elsewhere in this prospectus. Our unaudited consolidated interim financial statements were prepared in accordance with generally accepted accounting principles in the United States ("GAAP"), on the same basis as our audited consolidated financial statements and include, in the opinion of management, all adjustments, consisting of normal recurring adjustments, that are necessary for the fair presentation of the financial information set forth in those financial statements. Our historical results are not necessarily indicative of results that may be expected in the future. You should read the following selected consolidated financial data together with our consolidated financial statements and related notes included elsewhere in this prospectus and the information in the section titled "*Management's discussion and analysis of financial condition and results of operations*".

	Year ended December 31,		Six months ended June 30,	
(in thousands, except share and per share data)	**2017**	**2018**	**2018**	**2019**
			(unaudited)	
Consolidated statements of operations data:				
Revenue	$ 71,085	$ 146,313	$ 59,152	$ 109,397
Cost of revenue(1)	10,560	28,661	8,520	28,971
Gross profit	60,525	117,652	50,632	80,426
Operating expenses:				
Research and development(1)	32,164	47,537	23,372	32,999
In-process research and development	—	62,363	6,206	—
Selling, general and administrative(1)	46,736	87,936	41,920	59,464
Accrued contingent liabilities	—	30,580	—	1,360
Total operating expenses	78,900	228,416	71,498	93,823
Loss from operations	(18,375)	(110,764)	(20,866)	(13,397)
Other income (expense):				
Interest income	308	1,024	461	505
Interest expense	(811)	(2,409)	(1,062)	(1,379)
Other income (expense), net	137	(249)	(120)	(141)
Total other income (expense)	(366)	(1,634)	(721)	(1,015)
Loss before provision for income taxes	$ (18,741)	$ (112,398)	$ (21,587)	$ (14,412)
Provision for income taxes	21	87	29	102
Net loss	$ (18,762)	$ (112,485)	$ (21,616)	$ (14,514)
Net loss per share attributable to common stockholders, basic and diluted(2)	$ (1.62)	$ (8.40)	$ (1.66)	$ (0.96)
Weighted-average shares used to compute net loss per share attributable to common stockholders, basic and diluted(2)	11,587,751	13,392,273	12,985,535	15,187,258
Pro forma net loss per share attributable to common stockholders, basic and diluted (unaudited)(2)		$ (1.45)		$ (0.18)
Weighted-average shares used to compute pro forma net loss per share attributable to common stockholders, basic and diluted (unaudited)(2)		77,494,992		82,891,536

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(1) Includes stock-based compensation expense as follows:

(in thousands)	Year ended December 31, 2017	Year ended December 31, 2018(1)	Six months ended June 30, 2018	Six months ended June 30, 2019
			(unaudited)	
Cost of revenue	$ 44	$ 85	$ 36	$ 90
Research and development	801	1,030	440	1,798
Selling, general and administrative	816	1,543	530	2,496
Total stock-based compensation expense	$ 1,661	$ 2,658	$ 1,006	$ 4,384

(2) See Note 2 and Note 11 to our consolidated financial statements included elsewhere in this prospectus for further details on the calculation of net loss per share attributable to common stockholders, basic and diluted, the weighted-average shares used to compute net loss per share attributable to common stockholders, basic and diluted, and unaudited pro forma information.

(in thousands)	As of December 31, 2017	As of December 31, 2018	As of June 30, 2019
			(unaudited)
Consolidated balance sheet data:			
Cash and cash equivalents	$ 47,857	$ 65,080	$ 56,034
Working capital(1)	45,966	73,874	63,999
Total assets	75,609	124,310	155,594
Total current liabilities	22,141	32,362	43,227
Total liabilities	29,704	101,053	140,298
Total convertible preferred stock	158,414	243,244	243,244
Accumulated deficit	(118,631)	(231,116)	(245,630)
Total stockholders' equity (deficit)	(112,509)	(219,987)	(227,948)

(1) Working capital is calculated as current assets less current liabilities. See our consolidated financial statements and related notes included elsewhere in this prospectus for further details regarding our current assets and current liabilities.

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Management's discussion and analysis of financial condition and results of operations

The following discussion and analysis of our financial condition and results of operations should be read in conjunction with the section titled "Selected consolidated financial data" and the consolidated financial statements and related notes thereto included elsewhere in this prospectus. This discussion contains forward-looking statements that involve risks and uncertainties. Factors that could cause or contribute to such differences include those identified below and those in the section titled "Risk factors" and other parts of this prospectus. Our historical results are not necessarily indicative of the results that may be expected for any future period.

Overview

We are a life sciences technology company focused on building innovative products and solutions to interrogate, understand and master biological systems at resolution and scale that matches the complexity of biology. Our expanding suite of offerings leverages our cross-functional expertise across biology, chemistry, software and hardware to provide a comprehensive, dynamic and high-resolution view of complex biological systems. We have launched multiple products that enable researchers to understand and interrogate biological analytes in their full biological context. Our commercial product portfolio leverages our Chromium instruments, which we refer to as "instruments", and our proprietary microfluidic chips, slides, reagents and other consumables for both our Visium and Chromium solutions, which we refer to as "consumables". We bundle our software with these products to guide customers through the workflow, from sample preparation through analysis and visualization. Since launching our first product in mid-2015, and as of June 30, 2019, we have sold 1,284 instruments to customers around the world, including 93 of the top 100 global research institutions by publications, and 13 of the top 15 global pharmaceutical companies by 2018 revenue.

Our products cover a wide variety of applications and allow researchers to analyze biological systems at fundamental resolutions and on massive scales, such as at the single cell level for millions of cells. Our Chromium instruments and Chromium consumables are designed to work together exclusively. After buying a Chromium instrument, customers purchase consumables from us for use in their experiments. Accordingly, as the installed base of our instruments grows, we expect recurring revenue from consumable sales to become an increasingly important driver of our operating results. As such, our revenue growth is expected to outpace growth in our instrument placements as our business develops. In addition to instrument and consumable sales, we derive revenue from post-warranty service contracts for our Chromium instruments. For the year ended December 31, 2018, sales of our Chromium instruments accounted for 25% of our revenue, sales of our consumables accounted for 74% of our revenue and sales of services accounted for 1% of our revenue. For the six months ended June 30, 2019, sales of our Chromium instruments accounted for 14% of our revenue, sales of our consumables accounted for 84% of our revenue and sales of services accounted for 2% of our revenue.

We currently serve thousands of researchers in approximately 40 countries. Our customers include a range of academic, government, biopharmaceutical, biotechnology and other leading institutions around the globe. In both the year ended December 31, 2018 and the six months ended June 30, 2019, approximately 70% of our direct sales revenue came from sales to academic institutions.

As of June 30, 2019, we employed a commercial team of over 190 employees, including more than 75 commissioned sales representatives, many with Ph.D. degrees and many with significant industry experience. We follow a direct sales model in North America and certain regions of Europe, representing the majority of our revenue. We sell our products through third-party distributors in Asia, certain regions of Europe, South America, the Middle East and Africa. We currently sell our products for research use only. For the year ended December 31, 2018 and six months ended June 30, 2019, sales within North America accounted for approximately 58% and 56% of our revenue, respectively.

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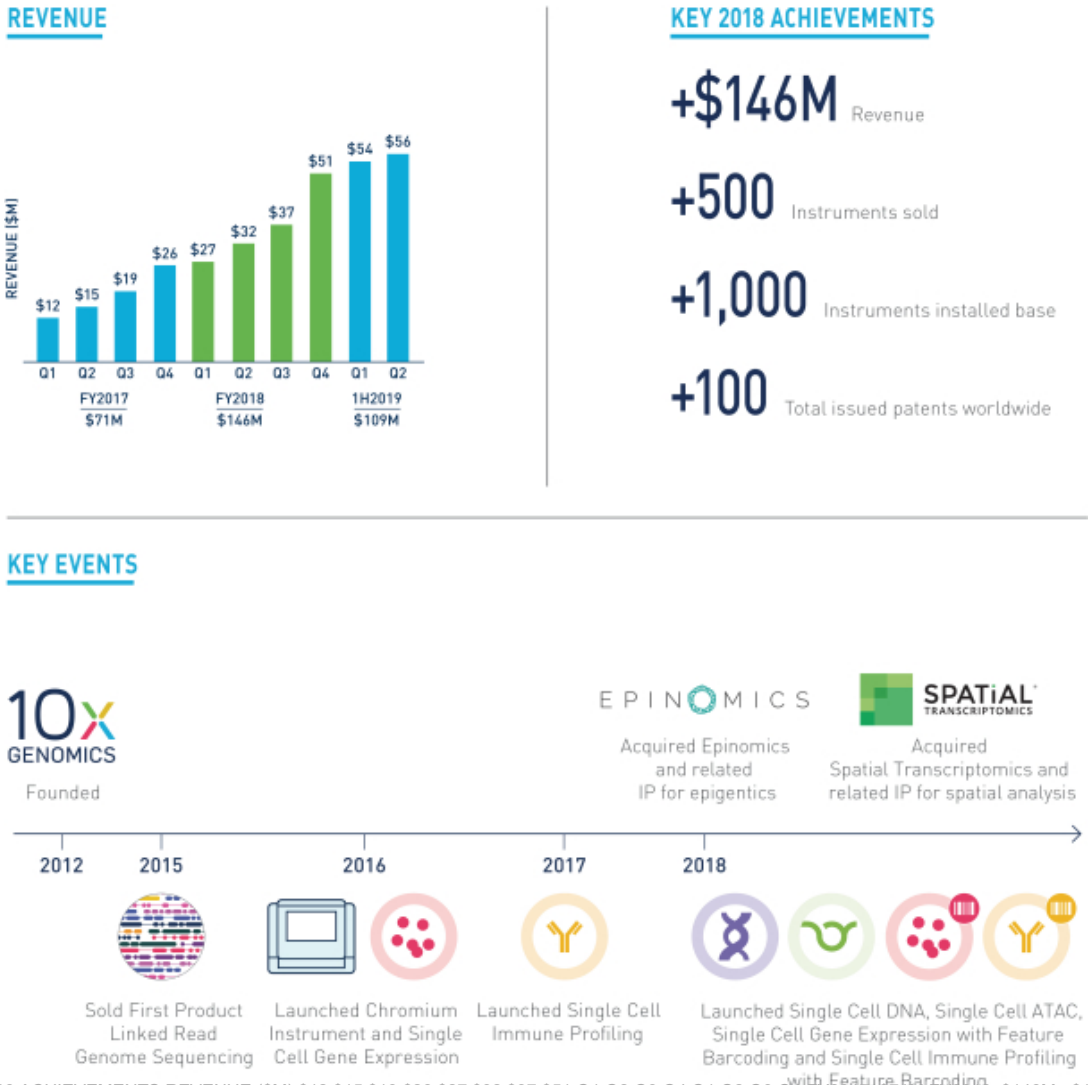
The following is our revenue for the last ten quarters, key 2018 achievements and a chronology of key events since our inception



Revenue increased 106% to $146.3 million in the year ended December 31, 2018 as compared to $71.1 million in the prior year, and increased 85% to $109.4 million for the six months ended June 30, 2019 as compared to $59.2 million for the six months ended June 30, 2018, primarily due to the adoption of our platform by customers as reflected by the doubling in size of our installed base to more than 1,000 instruments as of December 31, 2018 and more than 1,280 instruments as of June 30, 2019, and the associated consumables pull-through on those instruments.

We focus a substantial portion of our resources on developing new products and solutions. Our research and development efforts are centered around: improving the performance of our existing assays and software, developing new Chromium solutions such as multi-omics solutions, developing our Visium platform, improving

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and developing new capabilities for our Chromium platform, developing combined software and workflows across multiple solutions and investigating new technologies. We incurred research and development expenses of $32.2 and $47.5 million for the years ended December 31, 2017 and 2018, respectively, and $23.4 million and $33.0 million for the six months ended June 30, 2018 and 2019, respectively. We intend to continue to make significant investments in this area for the foreseeable future. In addition, in 2018, we made acquisitions for an aggregate purchase price of $62.4 million. See the section titled "*—Recent acquisitions*".

Our instrument manufacturing is contracted out to third-party contract manufacturers and we manufacture the majority of our consumable products in-house, with a small amount of our components outsourced to key suppliers. We have designed our operating model to be capital efficient and to scale efficiently as our product volumes grow.

To date, we have financed our operations primarily from the sale of our instruments and consumable products, the issuance and sale of our convertible preferred stock and common stock and with issuances of debt. Since our inception in 2012, we have incurred net losses in each year. Our net losses were $18.8 million and $112.5 million for the years ended December 31, 2017 and 2018, respectively, and $21.6 million and $14.5 million for the six months ended June 30, 2018 and 2019, respectively. The $14.5 million net loss included a $15.9 million accrual related to estimated royalties for ongoing litigation. The increase in our net loss for 2018 resulted substantially from charges of $62.4 million associated with intellectual property acquisitions for research and development in addition to the litigation contingency accrual of $38.0 million. The decrease in our net loss for the six months ended June 30, 2019 resulted primarily from increased revenue. As of June 30, 2019, we had an accumulated deficit of $245.6 million and cash and cash equivalents totaling $56.0 million. We expect to continue to incur significant expenses for the foreseeable future and to incur operating losses in the near term. We expect our expenses will increase substantially in connection with our ongoing activities, as we:

- attract, hire and retain qualified personnel;
- scale our technology platforms and introduce new products and services;
- protect and defend our intellectual property;
- acquire businesses or technologies; and
- invest in processes, tools and infrastructure to support the growth of our business.

Recent acquisitions

Epinomics

In March 2018, we completed the acquisition of Epinomics Inc. ("Epinomics"), a privately-held company based in California, for an all cash purchase price of $22.2 million. Epinomics' patent portfolio includes foundational intellectual property and a worldwide exclusive license relating to ATAC-seq, which supplements our existing patent portfolio and enables us to provide ATAC-seq solutions for single cell and other epigenetic applications. All of our obligations under the Epinomics acquisition agreement have been fully performed.

Spatial Transcriptomics

In November 2018, we completed the acquisition of Spatial Transcriptomics Holding AB ("Spatial Transcriptomics"), a privately-held company based in Stockholm, Sweden, for an all cash purchase price of $38.6 million. With the acquisition of Spatial Transcriptomics, we obtained intellectual property relating to the spatial interrogation of biological analytes, which we believe will open up the possibilities for discoveries in oncology, neuroscience and immunology, as well as in the broader area of biology. Pursuant to the Spatial Transcriptomics acquisition agreement, we are obligated to make contingent payments to the sellers through December 31, 2022. Aside from this obligation, all of our obligations under the Spatial Transcriptomics acquisition agreement have been fully performed. See "Business–Intellectual property" for more information regarding our contingent payment obligations.

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Prognosys

In November 2018, we completed the acquisition of a worldwide exclusive license to foundational intellectual property relating to spatial analysis technologies from Prognosys Biosciences, Inc. ("Prognosys"), for a combination of cash and common stock. All of our obligations under the Prognosys license agreement have been fully performed.

Litigation developments and product transitions

Bio-Rad 2015 litigation

In November 2018, a jury found that we willfully infringed three patents exclusively licensed to Bio-Rad Laboratories, Inc. ("Bio-Rad") and awarded Bio-Rad approximately $24.0 million in damages. In response to this award, we established an accrual of $30.6 million in November 2018 which we recorded as an operating expense for the year ended December 31, 2018 and accrued an additional $1.4 million in the first half of 2019 related to pre- and post-judgment interest, which we also recorded as an operating expense. In the fourth quarter of 2018, we began recording an accrual for estimated royalties as cost of revenue. For the year ended December 31, 2018 and the six months ended June 30, 2019 (unaudited), we accrued royalties of $7.4 million and $15.9 million, respectively. As of December 31, 2018 and June 30, 2019 (unaudited), we had accrued total amounts of $38 million and $55.3 million, respectively, related to this matter. This accrual is based on an estimated royalty rate of 15% of worldwide sales of our Chromium instruments operating our GEM microfluidic chips and associated consumables. Prior to the end of the first quarter of 2019, substantially all of our Chromium instruments and consumable sales utilized our GEM microfluidic chips. In August 2019, the Court granted Bio-Rad a permanent injunction against our GEM microfluidic chips and associated consumables that were found to infringe the Bio-Rad patents, which have historically constituted substantially all of our product sales. However, under the injunction, we are permitted to continue to sell our GEM microfluidic chips and associated consumables for use with our historical installed base of instruments provided that we pay a royalty of 15% into escrow on our net revenue related to such sales. We have appealed the injunction to the Federal Circuit and expect that it will not take effect until the Federal Circuit rules on our request for a stay of the injunction.

Neither the jury verdict nor the injunction relate to our Next GEM microfluidic chips based on our new proprietary design and associated consumables which we launched in May 2019 for three of our single cell solutions — Single Cell Gene Expression, Single Cell Immune Profiling and Single Cell ATAC. Unless the injunction relating to our GEM microfluidic chips is stayed, we will be unable to sell our Single Cell CNV and Linked-Read solutions for use on our instruments unless and until we develop a Next GEM microfluidic chip for such solutions. We believe that our Chromium solutions, when used with our Next GEM microfluidic chip, would not infringe the asserted Bio-Rad patents. We plan to gradually phase out our GEM microfluidic chips and anticipate that our Chromium products utilizing our Next GEM microfluidic chips will become an increasing percentage of our sales and will constitute substantially all of our Chromium sales by the end of 2020. We currently expect that, by the end of the third quarter of 2019, all Chromium instruments that we sell will operate exclusively with our Next GEM solutions. Until we are able to completely transition to our Next GEM microfluidic chip, our margins will be negatively impacted by any royalty obligations that result from this litigation. Furthermore, we expect to incur increased research and development expenses in the near term and increased inventory and other expenses related to the introduction of, and transition to, our Next GEM microfluidic chip. Depending upon the ultimate outcome of our appeal, our accruals may prove insufficient to cover the actual damages awarded in the case. Conversely, should we ultimately obtain a more favorable outcome in this litigation we may be able to reverse all or a portion of our litigation reserve and the related accruals.

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International Trade Commission action

In a related but separate action, in September 2018, a judge of the U.S. International Trade Commission ("ITC") found that our GEM microfluidic chips infringed three Bio-Rad patents and recommended entry of an exclusion order against our GEM microfluidic chips which would prevent importation of such chips into the United States and a cease and desist order that would prevent us from selling such imported chips in the United States which have historically constituted substantially all of our product sales. The judge further found that our gel bead manufacturing microfluidic chip does not infringe any asserted claims and that our Next GEM microfluidic chip does not infringe any asserted claims. The judge's recommendations are currently under review by the ITC, which is expected to issue a Final Determination in late September 2019. The ITC's Final Determination is subject to a 60-day presidential review period. In order to allow our customers to continue their important research, we have dedicated significant resources to developing the capabilities to manufacture our microfluidic chips in the United States. Prior to the second quarter of 2019, all of our microfluidic chips were manufactured outside of the United States. We expect our United States manufacturing facilities to achieve volume production of certain of our GEM microfluidic chips accounting for the majority of our United States consumable revenue beginning in the fourth quarter of 2019. We do not expect the transition to manufacturing our microfluidic chips in the United States to have a material impact on our margins.

The timing of the incurrence of legal expenses relating to pending litigation is difficult to predict and the outcome of litigation is inherently uncertain. If one or more legal matters were resolved against us in a reporting period for amounts in excess of management's expectations, our financial condition and operating results for that reporting period could be materially adversely affected. In addition, changes to the scheduling of significant events, such as trial dates, for pending litigation can result in the incurrence of significant legal expenses during periods in which such expenses were not expected, which could materially and adversely impact our results of operations for such reporting period. Finally, the achievement of certain litigation milestones, outcomes or events could trigger the payment of contingent payments which could significantly impact our financial results in any given period. Such events are inherently difficult to predict.

See the sections titled "*Risk factors—Risks related to litigation and our intellectual property*" and "*Business—Legal proceedings*" for more information regarding these matters.

Key business metrics

We regularly review a number of operating and financial metrics, including the following key business metrics, to evaluate our business, measure our performance, identify trends affecting our business, formulate financial projections and make strategic decisions. We believe that the following metrics are representative of our current business; however, we anticipate these may change or may be substituted for additional or different metrics as our business grows and as we introduce new products.

Instrument installed base

	As of December 31,		As of June 30,	
	2017	**2018**	**2018**	**2019**
Instrument installed base	491	1,021	701	1,284

Our products are sold to academic, government, biopharmaceutical, biotechnology and other leading institutions around the globe. Our Chromium Controller instrument is user installable and does not require in-person training. We expect our Chromium Connect instrument to require installation and we expect to offer in-person training in its use. We believe the instrument installed base is one of the indicators of our ability to drive customer adoption of our products.

We define the instrument installed base as the cumulative number of Chromium instruments sold since inception.

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Consumable pull-through per instrument

(in thousands)	Year ended December 31, 2017	Year ended December 31, 2018	Six months ended June 30, 2018	Six months ended June 30, 2019
Consumable pull-through per instrument	$ 140	$ 148	$ 72	$ 81

Our consumables portfolio includes proprietary microfluidic chips, slides, reagents and other consumables for both our Visium and Chromium solutions. Our Chromium instruments and Chromium consumables are designed to work together exclusively. This Chromium closed-system model generates recurring revenue from each instrument we sell. We believe that quarterly consumable pull-through per instrument is an indicator of our ability to generate future consumable revenue and the rate of customer adoption of our new applications.

We define consumable pull-through per instrument as the total consumables revenue in the given quarter divided by the average instrument installed base during that quarter. We calculate the average instrument installed base for a given quarter using the instrument installed base as of the last day of the prior quarter and the instrument installed base as of the last day of the given quarter. We also calculate a year-to-date consumable pull-through per instrument figure by summing the quarterly pull-through for the quarters in a given year. The figures in the table above represent the year-to-date consumable pull-through per instrument for the years ended December 31, 2017 and 2018 and the six months ended June 30, 2018 and 2019.

Key factors affecting our performance

We believe that our financial performance has been and in the foreseeable future will continue to be primarily driven by the following factors. While each of these factors presents significant opportunities for our business, they also pose important challenges that we must successfully address in order to sustain our growth and improve our results of operations. Our ability to successfully address the factors below is subject to various risks and uncertainties, including those described under the heading "*Risk factors*".

Instrument sales

Our financial performance has largely been driven by, and in the future will continue to be impacted by, the rate of sales of our Chromium instruments. Management focuses on instrument sales as an indicator of current business success and a leading indicator of likely future sales of consumables. We expect our instrument sales to continue to grow as we increase penetration in our existing markets and expand into, or offer new features and solutions that appeal to, new markets.

We plan to grow our instrument sales in the coming years through multiple strategies including expanding our sales efforts globally and continuing to enhance the underlying technology and applications for life sciences research. As part of this strategy and in an effort to increase the rate of sales of our instruments, we increased our sales force by 144% from January 1, 2018 through June 30, 2019, with 78 commissionable sales representatives as of June 30, 2019. We regularly solicit feedback from our customers and focus our research and development efforts on enhancing the Chromium Controller instrument and enabling its ability to use additional applications that address their needs, which we believe in turn helps to drive additional sales of our instruments and consumables. We are developing our Chromium Connect instrument, which is an automated version of our current Chromium Controller instrument, with a targeted release in 2020. We believe the automated features of the Chromium Connect will increase our addressable market by increasing utilization by biopharmaceutical customers.

Our sales process varies considerably depending upon the type of customer to whom we are selling. Our sales process with small laboratories and individual researchers is often short, and in some cases, we receive

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purchase orders from these customers in under a month. Our sales process with other institutions can be longer with most customers submitting purchase orders within six months. Given the variability of our sales cycle, we have in the past experienced, and likely will in the future experience, fluctuations in our instrument sales on a period-to-period basis.

Recurring consumable revenue

We regularly assess trends relating to recurring consumable revenue based on our product offerings, our customer base and our understanding of how our customers use our products. There may be quarterly variability in our consumable revenue and in the relative revenue contribution of our product offerings. For example, while revenue generated from sales of our Single Cell Gene Expression consumables accounted for approximately half of our revenue for each of the years ended December 31, 2017 and 2018 and for the six months ended June 30, 2019, we expect the revenue contribution from these and other consumable products to vary on a quarterly basis due to, among other factors, our introduction of enhanced features and additional solutions. Funding cycles of our customers vary leading to seasonality in their consumables order patterns. For example, a significant portion of our current customers are reliant on government funding and research grants. Our current customer base, a portion of which have budget cycles that typically expire at year end, exhibits seasonality resulting in a higher consumable pull-through per instrument in the fourth quarter relative to the first three quarters of the year. As our instrument installed base expands, consumables revenue on an absolute basis is expected to increase and over time should be an increasingly important contributor to our revenue.

Our current customer base includes customers who purchase consumables for use on a shared or centralized instrument. We refer to customers who purchase consumables but do not own an instrument as “halo users”. For each of the years ended December 31, 2017 and 2018 and for the six months ended June 30, 2019, halo users represented approximately half of our revenue from sales of consumables. Halo users, as well as the future introduction of consumables that may not use instruments, such as our recently announced Visium solution, or Chromium instruments that are expected to use a greater amount of consumables, such as our Chromium Connect instrument, could reduce the utility of this metric and make it difficult to compare consumable pull-through per instrument metrics over time.

We expect our annual consumable pull-through per instrument to be relatively stable as the instrument installed base increases. Our expansion into new markets with less experienced users could adversely impact average pull-through, but we expect the introduction of our Visium products as well as the release of new products and applications for our Chromium instruments to increase consumable pull-through per instrument and offset these declines. We will initially report our Visium product revenue as part of consumable revenue and include it in the average pull-through per instrument calculation. Even though Visium is not processed through a Chromium instrument, we will sell the product primarily to Chromium instrument users and view it as pull-through from a business perspective.

Revenue mix and gross margin

Our revenue is derived from sales of our instruments, consumables and service. There will be fluctuations in mix between instruments and consumables from period to period. Over time, as our instrument installed base grows and our Visium products are introduced, we expect consumables revenue to constitute a larger percentage of revenue. In addition, our margins are higher for those instruments and consumables that we sell directly to customers as compared to those that we sell through distributors. While we expect the mix of direct sales as compared to sales through distributors to remain relatively constant in the near term, we are currently evaluating increasing our direct sales capabilities in certain geographies.

From the fourth quarter of 2016 to the first quarter of 2019, we offered two versions of the Chromium Controller, one at a $125,000 list price with firmware that enabled the use of all our Chromium consumables and another at a

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$75,000 list price with firmware that enabled the use of only our Single Cell Chromium consumables. Beginning in the first quarter of 2019, we standardized our instrument offering on the fully-enabled Chromium Controller with a list price of $75,000 and as a result our Chromium Controller average selling price decreased in the first half of 2019 from those realized in 2017 and 2018. The list prices of our consumables vary by solution. Future instrument and consumable selling prices and gross margins may fluctuate due to a variety of factors, including the introduction by others of competing products and solutions or the attempted integration by third-parties of capabilities similar to ours into their existing products, such as sequencers. We aim to mitigate downward pressure on our average selling prices by increasing the value proposition offered by our instruments and consumables, primarily by, for example, expanding the applications for our instruments and increasing the quantity and quality of data that can be obtained using our consumables.

In the near term, we expect our expansion of manufacturing, warehousing and product distribution facilities, and the litigation described above under "*—Litigation developments and product transitions*", to have the greatest impact on our margins. In addition to the impact of competing products entering the market, the future margin profiles of our instruments and consumables will depend upon the outcome of such litigation, any royalties we are required to pay and the royalty rates and products to which such royalties apply.

Continued investment in growth

Our significant revenue growth has been driven by rapid innovation towards novel solutions that command price premiums and quick adoption of our solutions by our customer base. In 2018 alone, we introduced six new products or updates to existing products. We intend to continue to make focused investments to increase revenue and scale operations to support the growth of our business and therefore expect expenses in this area to increase. We have invested, and will continue to invest, significantly in our manufacturing capabilities and commercial infrastructure. The transition to our new Pleasanton global headquarters and research and development center in 2019 will help us achieve these goals in the near term by providing additional manufacturing, research and development and general office space. We plan to further invest in research and development as we hire employees with the necessary scientific and technical backgrounds to enhance our existing products and help us bring new products to market, and expect to incur additional research and development expenses and higher stock-based compensation expenses as a result. We also plan to invest in sales and marketing activities, expect to incur additional general and administrative expenses and to have higher stock-based compensation expenses as we support our growth and our transition to becoming a publicly traded company. As cost of revenue, operating expenses and capital expenditures fluctuate over time, we may experience short-term, negative impacts to our results of operations and cash flows, but we are undertaking such investments in the belief that they will contribute to long-term growth.

Acquisitions of key technologies

We have made, and intend to continue to make, investments that meet management's criteria to expand or add key technologies that we believe will facilitate the commercialization of new products in the future. Such investments could take the form of an asset acquisition, the acquisition of a business or the exclusive or non-exclusive license of patented technology. Any such acquisitions we make may affect our future financial results. For example, our acquisitions of Spatial Transcriptomics and Epinomics were largely comprised of purchases of intellectual property which were expensed as in-process research and development in the quarter during which such acquisitions occurred. While we have not previously entered into material joint-development, partnership or joint-venture agreements, we may in the future decide to do so and any such arrangements may limit our rights and the commercial opportunities of any jointly-developed technology.

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Components of results of operations

Revenue

We generate virtually all of our revenue through the sale of our instruments and consumables to customers. We also generate a small portion of our revenue from instrument service agreements which relate to extended warranties. Our revenue is subject to fluctuation based on the foreign currency in which our products are sold, principally for sales denominated in the euro.

Revenue from consumables is largely driven by the size of our instrument installed base and the volume of consumables sold per instrument. Our instruments and consumables are generally sold without the right of return. Revenue is recognized as instruments and consumables are shipped. Revenue is recognized net of any sales incentive, distributor rebates and commissions and any taxes collected from customers. Some of our recently announced products, such as our Chromium Connect instrument, may result in our recognizing revenue with respect to such products upon installation rather than upon shipment. Instrument service agreements are typically entered into for a one-year term, with the coverage period beginning after the expiration of the standard one-year warranty period. Revenue from the sale of instrument service agreements are recognized ratably over the coverage period.

Cost of revenue, gross profit and gross margin

Cost of revenue. Cost of revenue primarily consists of manufacturing costs incurred in the production process including personnel and related costs, costs of component materials, labor and overhead, packaging and delivery costs and allocated costs including facilities and information technology. We plan to hire additional employees as well as expand our manufacturing, warehousing and product distribution facilities, including increasing manufacturing automation to support our growth. In addition, cost of revenue includes royalty costs for licensed technologies included in our products, warranty costs, provisions for slow-moving and obsolete inventory and personnel and related costs and component costs incurred in connection with our obligations under our instrument service agreements. Beginning with the three months ended December 31, 2018, we began recording royalty accruals relating to sales of our GEM microfluidic chips and associated consumables, which are the subject of the Bio-Rad litigation, as cost of revenue. We expect cost of revenue to increase in absolute dollars in future periods.

Gross profit/gross margin. Gross profit is calculated as revenue less cost of revenue. Gross margin is gross profit expressed as a percentage of revenue. Our gross profit in future periods will depend on a variety of factors, including: market conditions that may impact our pricing; sales mix changes among consumables, instruments and services; product mix changes between established products and new products; excess and obsolete inventories; royalties; our cost structure for manufacturing operations relative to volume; and product warranty obligations. We expect an increase in absolute dollars of both revenue and cost of revenue; however, we expect gross margins to remain relatively constant in the near term as result of the royalty accrual related to litigation. As we transition customers to our Next GEM microfluidic chips, we expect our gross margins to increase from these levels, as the percentage of our revenue attributable to our Next GEM microfluidic chips increases. Further developments in our litigation with Bio-Rad could have a material impact on our gross margins in the near term and potentially beyond. See the section titled "—*Litigation developments and product transitions*".

Operating expenses

Research and development. Research and development expense primarily consists of personnel and related costs, independent contractor costs, laboratory supplies, equipment maintenance prototype and materials expenses, amortization of developed technology and intangibles and allocated costs including facilities and information technology.

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We plan to continue to invest in our research and development efforts, including hiring additional employees, to enhance existing products and develop new products. We expect allocated facilities costs to increase in the periods following the transition to our global headquarters and research and development center in Pleasanton, California in July 2019 and the expected implementation of a new enterprise resource planning system in 2020. We expect research and development expense will increase in absolute dollars in future periods and vary from period to period as a percentage of revenue.

In-process research and development. In-process research and development consists of costs incurred to acquire intellectual property for research and development. We expect these costs to be recognized only in periods during which we complete an acquisition of assets comprised in whole or part of intellectual property for research and development. While we periodically evaluate acquisitions of this nature from time to time, we have no definitive agreements currently in place to acquire additional intellectual property for research and development.

Selling, general and administrative. Selling, general and administrative expense primarily consists of costs related to the selling and marketing of our products, including sales incentives and advertising expenses and costs associated with our finance, accounting, legal (excluding accrued contingent liabilities), human resources and administrative personnel. Related costs associated with these functions, such as attorney and accounting fees, recruiting services, administrative services, insurance, public relations and communication activities, marketing programs and trade show appearances, travel, customer service costs and allocated costs including facilities and information technology, are also included in selling, general and administrative expenses.

We expect to incur additional selling, general and administrative expenses due to continued investment in our sales, marketing and customer service efforts to support the anticipated growth of our business. We also expect increased infrastructure costs, as well as increased costs for accounting, human resources, legal, insurance, investor relations and other costs associated with becoming a public company. We expect to continue our hiring, in the United States as well as internationally, in all these areas in line with the continued growth of our business. We also expect allocated facilities costs to increase in the periods following the transition to our global headquarters and research and development center in Pleasanton, California in July 2019 and allocated information technology costs to increase following the expected implementation of a new enterprise resource planning system in 2020. We expect selling, general and administrative expenses to vary from period to period as a percentage of revenue, increase in absolute dollars in future periods and decrease as a percentage of revenue.

We expect our stock-based compensation expense allocated to cost of revenue, research and development expenses and selling, general and administrative expenses to increase in absolute dollars.

Accrued contingent liabilities

Accrued contingent liabilities is comprised of changes in our litigation reserve, primarily relating to our litigation with Bio-Rad. The litigation reserve currently consists of accruals we make for our estimated losses in these pending legal proceedings. We record a liability when it is probable that a loss has been incurred and the amount is reasonably estimable, the determination of which requires significant judgment. Changes in the reserve are made as we change our estimates or make payments in damages or settlement. In 2018, we took a $30.6 million charge to reflect our best estimate of loss in resolving our ongoing disputes. In the six months ended June 30, 2019, we recorded an additional $1.4 million charge related to additional pre- and post- judgment interest. Beginning with the three months ended December 31, 2018, we began recording an accrual for estimated royalties as cost of revenue. For the year ended December 31, 2018 and the six months ended June 30, 2019 (unaudited), we accrued royalties of $7.4 million and $15.9 million, respectively. As of December 31, 2018 and June 30, 2019 (unaudited), we had accrued total amounts of $38 million and

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$55.3 million, respectively, related to this matter. Should we ultimately obtain a more favorable outcome in this litigation any reversal of the accrual related to the litigation would be reflected as a change to this item in the period in which it occurs. Any reversal for amounts recorded as estimated royalty accruals would be credited to our cost of revenue in such period. See the section titled "*—Litigation developments and product transactions*".

Interest income

Interest income consists of interest earned on our cash and cash equivalents which are invested in bank deposit and in money market funds.

Interest expense

Interest expense consists of interest on our outstanding debt. See the section titled "*—Contractual obligations and commitments*".

Other income (expense), net

Other income (expense), net primarily consists of realized and unrealized gains and losses related to foreign exchange rate remeasurements recorded from consolidating our foreign subsidiaries each period-end.

Provision for income taxes

Our provision for income taxes consists primarily of foreign taxes and state minimum taxes in the United States. As we expand the scale and scope of our international business activities, any changes in the United States and foreign taxation of such activities may increase our overall provision for income taxes in the future.

As of December 31, 2018, we had federal net operating loss carryforwards ("NOLs") of approximately $116.1 million and federal tax credit carryforwards of approximately $8.3 million. Our federal NOLs generated after January 1, 2018, which total $5.5 million, are carried forward indefinitely, while all of our other federal NOLs expire beginning in 2032. As of December 31, 2018, we had state NOLs of approximately $93.5 million, which expire beginning in 2032. In addition, we had state tax credit carryforwards of approximately $7.9 million, which do not expire. Our ability to utilize such carryforwards for income tax savings is subject to certain conditions and may be subject to certain limitations in the future due to ownership changes, if any, as defined by rules enacted with the Tax Cuts and Jobs Act of 2017. As such, there can be no assurance that we will be able to utilize such carryforwards. We have experienced a history of losses and a lack of future taxable income would adversely affect our ability to utilize these NOLs and research and development credit carryforwards. We currently maintain a full valuation allowance against these tax assets.

Under Sections 382 and 383 of the Internal Revenue Code of 1986, as amended (the "Code"), if a corporation undergoes an "ownership change", the corporation's ability to use its pre-change net operating loss carryforwards and other pre-change attributes, such as research tax credits, to offset its post-change income may be limited. In general, an "ownership change" will occur if there is a cumulative change in our ownership by "5% shareholders" that exceeds 50 percentage points over a rolling three-year period. Similar rules may apply under state tax laws. We completed a study in early 2019 to determine whether an ownership change had occurred under Section 382 or 383 of the Code as of December 31, 2018, and we determined at that time that an ownership change occurred in 2013. As a result, our net operating losses generated through November 1, 2013 may be subject to limitation under Section 382 of the Code. The amount of pre-change loss carryforwards which may be subject to this limitation is $4.8 million. Our ability to use net operating loss carry forwards, research and development credit carryforwards and other tax attributes to reduce future taxable income and liabilities may be subject to limitations based on the ownership change in 2013, possible changes since the completion of the study or as a result of this offering. As a result, if we earn net taxable income, our ability to use our pre-change net operating loss carryforwards or other pre-change tax attributes to offset United States federal

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and state taxable income may still be subject to limitations, which could potentially result in increased future tax liability to us.

Results of operations

The results of operations presented below should be reviewed in conjunction with the consolidated financial statements and related notes included elsewhere in this prospectus. The following table sets forth our consolidated results of operations data for the periods presented:

(in thousands)	Year ended December 31, 2017	Year ended December 31, 2018	Six months ended June 30, 2018	Six months ended June 30, 2019
			(unaudited)	
Revenue	$ 71,085	$ 146,313	$ 59,152	$ 109,397
Cost of revenue(1)	10,560	28,661	8,520	28,971
Gross profit	60,525	117,652	50,632	80,426
Operating expenses:				
Research and development(1)	32,164	47,537	23,372	32,999
In-process research and development	—	62,363	6,206	—
Selling, general and administrative(1)	46,736	87,936	41,920	59,464
Accrued contingent liabilities	—	30,580	—	1,360
Total operating expenses	78,900	228,416	71,498	93,823
Loss from operations	(18,375)	(110,764)	(20,866)	(13,397)
Other income (expense):				
Interest income	308	1,024	461	505
Interest expense	(811)	(2,409)	(1,062)	(1,379)
Other income (expense), net	137	(249)	(120)	(141)
Total other income (expense)	(366)	(1,634)	(721)	(1,015)
Loss before provision for income taxes	$ (18,741)	$ (112,398)	$ (21,587)	$ (14,412)
Provision for income taxes	21	87	29	102
Net loss	$ (18,762)	$ (112,485)	$ (21,616)	$ (14,514)

(1) Includes stock-based compensation expense as follows:

(in thousands)	Year ended December 31, 2017	Year ended December 31, 2018	Six months ended June 30, 2018	Six months ended June 30, 2019
			(unaudited)	
Cost of revenue	$ 44	$ 85	$ 36	$ 90
Research and development	801	1,030	440	1,798
Selling, general and administrative	816	1,543	530	2,496
Total stock-based compensation expense	$ 1,661	$ 2,658	$ 1,006	$ 4,384

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The following table sets forth our consolidated results of operations data as a percentage of revenue for the periods presented:

	Year ended December 31,		Six months ended June 30,	
	2017	2018	2018	2019
			(unaudited)	
Revenue	100.0%	100.0%	100.0%	100.0%
Cost of revenue(1)	14.9%	19.6%	14.4%	26.5%
Gross profit	85.1%	80.4%	85.6%	73.5%
Operating expenses:				
Research and development(1)	45.3%	32.5%	39.5%	30.2%
In-process research and development	—	42.6%	10.5%	—
Selling, general and administrative(1)	65.7%	60.1%	70.9%	54.4%
Accrued contingent liabilities	—	20.9%	—	1.2%
Total operating expenses	111.0%	156.1%	120.9%	85.8%
Loss from operations	(25.9)%	(75.7)%	(35.3)%	(12.3)%
Other income (expense):				
Interest income	0.4%	0.7%	0.8%	0.5%
Interest expense	(1.1)%	(1.6)%	(1.8)%	(1.3)%
Other income (expense), net	0.2%	(0.2)%	(0.2)%	(0.1)%
Total other income (expense)	(0.5)%	(1.1)%	(1.2)%	(0.9)%
Loss before provision for income taxes	(26.4)%	(76.8)%	(36.5)%	(13.2)%
Provision for income taxes	0%	0.1%	0%	0.1%
Net loss	(26.4)%	(76.9)%	(36.5)%	(13.3)%

(1) Includes stock-based compensation expense as follows:

	Year ended December 31,		Six months ended June 30,	
	2017	2018	2018	2019
			(unaudited)	
Cost of revenue	0.1%	0.1%	0.1%	0.1%
Research and development	1.1%	0.7%	0.7%	1.6%
Selling, general and administrative	1.1%	1.0%	0.9%	2.3%
Total stock-based compensation expense	2.3%	1.8%	1.7%	4.0%

Comparison of six months ended June 30, 2018 and 2019

Revenue

	Six months ended June 30,		Change	
(dollars in thousands)	2018	2019	$	%
	(unaudited)			
Revenue	$ 59,152	$ 109,397	$50,245	85%

Revenue increased $50.2 million, or 85%, for the six months ended June 30, 2019 as compared to the six months ended June 30, 2018. The increase was driven primarily by an increase in consumables revenue. Consumables revenue increased $49.9 million, or 117%, to $92.4 million for the six months ended June 30, 2019 as compared to the six months ended June 30, 2018. $39.5 million of the increase in consumables revenue was due to growth in the instrument installed base and $10.4 million of the increase was due to increased pull-through per instrument driven by new product introductions and updates to existing products.

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Instrument revenue decreased $0.7 million, or 5%, for the six months ended June 30, 2019 as compared to the six months ended June 30, 2018 due to lower average selling prices, partially offset by higher volumes of instruments sold. The number of instruments sold during the six months ended June 30, 2019 was 263 units, an increase of 25% as compared to the prior year, resulting in an ending installed base of 1,284 instruments. The Chromium Controller average selling price decreased by 24% from the six months ended June 30, 2018, contributing to the $0.7 million decrease in instruments revenue. The first quarter list price reduction for our fully-enabled Chromium Controller and various discount incentives to drive product adoption contributed to a $4.7 million decrease in revenue which was largely offset by $4.0 million of incremental unit sales.

Cost of revenue, gross profit and gross margin

	Six months ended June 30,		Change	
(dollars in thousands)	**2018**	**2019**	**$**	**%**
	(unaudited)			
Cost of revenue	$ 8,520	$ 28,971	$20,451	240%
Gross profit	$ 50,632	$ 80,426	$29,794	59%
Gross margin	86%	74%		

Cost of revenue increased $20.5 million, or 240%, in the six months ended June 30, 2019 as compared to the six months ended June 30, 2018. In addition to higher cost of sales in line with revenue growth, the increase was primarily due to additional accrued royalties of $15.9 million related to the judgment in the Bio-Rad litigation.

Gross profit increased $29.8 million, or 59%, for the six months ended June 30, 2019 as compared to the six months ended June 30, 2018, primarily due to increased revenue partially offset by additional accrued royalties. Gross margin percentage decreased by 12 points for the six months ended June 30, 2019 as compared to the six months ended June 30, 2018, driven almost exclusively by higher accrued royalties in the six months ended June 30, 2019.

Operating expenses

	Six months ended June 30,		Change	
(dollars in thousands)	**2018**	**2019**	**$**	**%**
	(unaudited)			
Research and development	$ 23,372	$ 32,999	$ 9,627	41%
In-process research and development	6,206	—	(6,206)	N/M
Selling, general and administrative	41,920	59,464	17,544	42%
Accrued contingent liabilities	—	1,360	1,360	N/M
Total operating expenses	$ 71,498	$ 93,823	$22,325	31%

N/M: result not meaningful.

Research and development expense increased $9.6 million, or 41%, for the six months ended June 30, 2019 as compared to the six months ended June 30, 2018. The increase was primarily driven by an increase in personnel expenses of $5.8 million and laboratory materials and supplies expenses of $2.1 million, which were attributable to an increase in headcount and expenses supporting our continued research and development efforts to enhance our existing products and develop new products.

In-process research and development expense for the six months ended June 30, 2018 relates to intellectual property we purchased in connection with our acquisition of Epinomics. There were no similar purchases in the six months ended June 30, 2019. See the section titled "*—Recent acquisitions*".

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Selling, general and administrative expenses increased $17.5 million, or 42%, for the six months ended June 30, 2019 as compared to the six months ended June 30, 2018. The increase in expenses was primarily driven by an increase in personnel expenses of $12.8 million to support our future sales growth and the overall expansion of our operations and increased allocated costs of $4.0 million for facilities with the transition to our global headquarters.

Accrued contingent liabilities consisted of $1.4 million of expenses for pre- and post-judgment interest relating to the litigation with Bio-Rad, for which we established an accrual in November 2018. There was no similar accrual in the six months ended June 30, 2018.

Other income (expense), net

	Six months ended June 30,		Change	
(dollars in thousands)	**2018**	**2019**	**$**	**%**
	(unaudited)			
Interest income	$ 461	$ 505	$ 44	10%
Interest expense	(1,062)	(1,379)	(317)	30%
Other income (expense), net	(120)	(141)	(21)	18%
Total other income (expense), net	$ (721)	$ (1,015)	$ (294)	41%

Interest income increased $44,000, or 10%, for the six months ended June 30, 2019 as compared to the six months ended June 30, 2018. The increase was driven primarily by higher cash and cash-equivalent balances in interest bearing accounts along with increased yields on such balances.

Interest expense increased $0.3 million, or 30%, for the six months ended June 30, 2019 as compared to the six months ended June 30, 2018. The increase was driven primarily by higher outstanding term loan borrowings in 2018 following the refinancing of our previous loan and security agreement in February 2018 and increased interest rates.

The change in other income (expense), net during the six months ended June 30, 2019 was driven by realized and unrealized losses from foreign currency rate measurement fluctuations. Foreign currency losses increased compared to the six months ended June 30, 2018 as a result of the overall strengthening of the U.S. dollar when compared to the foreign currencies in which we operate.

Comparison of years ended December 31, 2017 and 2018

Revenue

	Year ended December 31,		Change	
(dollars in thousands)	**2017**	**2018**	**$**	**%**
Revenue	$ 71,085	$ 146,313	$ 75,228	106%

Revenue increased $75.2 million, or 106%, for the year ended December 31, 2018 as compared to the prior year. The increase was driven primarily by an increase in consumables revenue. Consumables revenue increased $61.3 million, or 133%, to $107.5 million for the year ended December 31, 2018 as compared to the prior year. $54.9 million of the increase in consumables revenue was due to growth in the instrument installed base and $6.4 million of the increase was due to increased pull-through per instrument driven by new product introductions and updates to existing products.

Instrument revenue increased $12.1 million, or 49%, for the year ended December 31, 2018 as compared to the prior year due to higher volumes of instruments sold, partially offset by lower average selling prices. The number

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of instruments sold during the year ended December 31, 2018 was 530 units, an increase of 74% as compared to the prior year, resulting in an ending installed base of 1,021 instruments. The Chromium Controller average selling price decreased by 14% from the prior year, contributing to the $6.0 million decrease in instruments revenue. The incremental discounts offered to drive product adoption resulted in $4.4 million of this decrease in instrument revenue and the shift towards the version of the Chromium Controller with firmware that enabled the use of only our Single Cell Chromium Consumables, which was offered at a lower price than the fully-enabled version, resulted in $1.6 million of this decrease in instrument revenue.

Cost of revenue, gross profit and gross margin

	Year ended December 31,		Change	
(dollars in thousands)	**2017**	**2018**	**$**	**%**
Cost of revenue	$ 10,560	$ 28,661	$18,101	171%
Gross profit	$ 60,525	$ 117,652	$57,127	94%
Gross margin	85%	80%		

Cost of revenue increased $18.1 million, or 171%, for the year ended December 31, 2018 as compared to the prior year. In addition to higher cost of sales in line with revenue growth, the increase was primarily due to additional royalties of $7.4 million related to the Bio-Rad litigation which we began accruing in the fourth quarter of 2018, higher inventory reserves of $1.2 million as we transitioned to newer versions of our products and higher warranty-related expenses of $1.2 million.

Gross profit increased $57.1 million, or 94%, for the year ended December 31, 2018 as compared to the prior year, primarily due to increased revenue partially offset by additional accrued royalties. Gross margin percentage decreased by 5 points for the year ended December 31, 2018 as compared to the prior year, driven primarily by accrued royalties in the fourth quarter of 2018.

Operating expenses

	Year ended December 31,		Change	
(dollars in thousands)	**2017**	**2018**	**$**	**%**
Research and development	$ 32,164	$ 47,537	$ 15,373	48%
In-process research and development	—	62,363	62,363	—
Selling, general and administrative	46,736	87,936	41,200	88%
Accrued contingent liabilities	—	30,580	30,580	—
Total operating expenses	$ 78,900	$ 228,416	$149,516	190%

Research and development expense increased $15.4 million, or 48%, for the year ended December 31, 2018 as compared to the prior year. The increase was primarily driven by an increase in personnel expenses of $7.8 million and laboratory materials and supplies expenses of $4.4 million, which were attributable to an increase in headcount and expenses supporting our continued research and development efforts to enhance our existing products and develop new products.

In-process research and development expense relates to intellectual property we purchased in 2018 in connection with our acquisitions of Spatial Transcriptomics and Epinomics and our acquisition of an exclusive license to certain intellectual property from Prognosys, in each case to be used as part of our research and development efforts to enhance our existing products and develop new products. There were no similar purchases in 2017. See the section titled "—*Recent acquisitions*".

Selling, general and administrative expenses increased $41.2 million, or 88%, for the year ended December 31, 2018 as compared to the prior year. The increase in expenses was primarily driven by an increase in personnel

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expenses of $15.0 million to support our sales growth and the overall expansion of our operations and increased outside legal fees of $16.5 million.

Accrued contingent liabilities consisted of $30.6 million of expenses relating to the litigation with Bio-Rad, for which we established an accrual in November 2018. There was no similar accrual in 2017.

Other income (expense), net

	Year ended December 31,		Change	
(dollars in thousands)	**2017**	**2018**	**$**	**%**
Interest income	$ 308	$ 1,024	$ 716	N/M
Interest expense	(811)	(2,409)	(1,598)	N/M
Other income (expense), net	137	(249)	(386)	N/M
Total other income (expense), net	$ (366)	$ (1,634)	$(1,268)	N/M

N/M: result not meaningful.

Interest income increased $0.7 million for the year ended December 31, 2018 as compared to the prior year. The increase was driven primarily by higher cash and cash-equivalent balances in interest bearing accounts along with increased yields on such balances.

Interest expense increased $1.6 million for the year ended December 31, 2018 as compared to the prior year. The increase was driven primarily by higher outstanding term loan borrowings in 2018 following the refinancing of our previous loan and security agreement in February 2018 and increased interest rates.

The change in other income (expense), net during the year ended December 31, 2018 was driven by realized and unrealized losses from foreign currency rate measurement fluctuations. Foreign currency losses increased compared to the prior year as a result of the overall strengthening of the U.S. dollar when compared to the foreign currencies in which we operate.

Quarterly results of operations

The following tables set forth our selected unaudited quarterly statements of operations data for each of the ten quarters in the period ended June 30, 2019. The information for each of these quarters has been prepared in accordance with GAAP, on the same basis as our audited consolidated financial statements and include, in the opinion of management, all adjustments, consisting of normal recurring adjustments, that are necessary for the fair presentation of the results of operations for these periods. This data should be read in conjunction with the consolidated financial statements and related notes included elsewhere in this prospectus. These quarterly results of operations are not necessarily indicative of the results we may achieve in any future period.

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The following table sets forth our selected unaudited quarterly consolidated statements of operations data for the periods presented:

	Three months ended									
(in thousands)	Mar. 31, 2017	June 30, 2017	Sept. 30, 2017	Dec. 31, 2017	Mar. 31, 2018	June 30, 2018	Sept. 30, 2018	Dec. 31, 2018	Mar. 31, 2019	June 30, 2019
	(unaudited)									
Revenue	$ 12,020	$ 14,901	$ 18,541	$ 25,623	$ 27,408	$ 31,744	$ 36,607	$ 50,554	$ 53,578	$ 55,819
Cost of revenue(1)	1,997	2,112	2,601	3,850	3,970	4,550	5,241	14,900	13,965	15,006
Gross profit	10,023	12,789	15,940	21,773	23,438	27,194	31,366	35,654	39,613	40,813
Operating expenses:										
Research and development(1)	7,218	7,338	8,077	9,531	11,928	11,444	11,085	13,080	14,965	18,034
In-process research and development	—	—	—	—	6,206	—	16,104	40,053	—	—
Selling, general and administrative(1)	8,920	9,174	12,294	16,348	20,720	21,200	19,110	26,906	26,893	32,571
Accrued contingent liabilities	—	—	—	—	—	—	—	30,580	790	570
Total operating expenses	16,138	16,512	20,371	25,879	38,854	32,644	46,299	110,619	42,648	51,175
Loss from operations	(6,115)	(3,723)	(4,431)	(4,106)	(15,416)	(5,450)	(14,933)	(74,965)	(3,035)	(10,362)
Other income (expense):										
Interest income	30	74	96	108	122	339	294	269	263	242
Interest expense	(193)	(199)	(209)	(210)	(428)	(634)	(659)	(688)	(684)	(695)
Other income (expense), net	(7)	95	10	39	42	(162)	(31)	(98)	(146)	5
Total other income (expense)	(170)	(30)	(103)	(63)	(264)	(457)	(396)	(517)	(567)	(448)
Loss before provision for income taxes	$ (6,285)	$ (3,753)	$ (4,534)	$ (4,169)	$ (15,680)	$ (5,907)	$ (15,329)	$ (75,482)	$ (3,602)	$ (10,810)
Provision for income taxes	—	—	7	14	13	16	16	42	34	68
Net loss	$ (6,285)	$ (3,753)	$ (4,541)	$ (4,183)	$ (15,693)	$ (5,923)	$ (15,345)	$ (75,524)	$ (3,636)	$ (10,878)

(1) Includes stock-based compensation expense as follows:

	Three months ended									
(in thousands)	Mar. 31, 2017	June 30, 2017	Sept. 30, 2017	Dec. 31, 2017	Mar. 31, 2018	June 30, 2018	Sept. 30, 2018	Dec. 31, 2018	Mar. 31, 2019	June 30, 2019
	(unaudited)									
Cost of revenue	$ 11	$ 11	$ 11	$ 11	$ 16	$ 20	$ 27	$ 22	$ 32	$ 58
Research and development	204	184	184	229	214	226	230	360	507	1,291
Selling, general and administrative	174	196	197	249	258	272	332	681	820	1,676
Total stock-based compensation expense	$ 389	$ 391	$ 392	$ 489	$ 488	$ 518	$ 589	$ 1,063	$ 1,359	$ 3,025

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The following table sets forth our consolidated results of operations data as a percentage of revenue for the periods presented:

									Three months ended	
(in thousands)	Mar. 31, 2017	June 30, 2017	Sept. 30, 2017	Dec. 31, 2017	Mar. 31, 2018	June 30, 2018	Sept. 30, 2018	Dec. 31, 2018	Mar. 31, 2019	June 30, 2019
					(unaudited)					
Revenue	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Cost of revenue	16.6%	14.2%	14.0%	15.0%	14.5%	14.3%	14.3%	29.5%	26.1%	26.9%
Gross profit	83.4%	85.8%	86.0%	85.0%	85.5%	85.7%	85.7%	70.5%	73.9%	73.1%
Operating expenses:										
Research and development	60.0%	49.2%	43.6%	37.2%	43.5%	36.1%	30.3%	25.9%	27.9%	32.3%
In-process research and development	—	—	—	—	22.6%	—	44.0%	79.2%	—	—
Selling, general and administrative	74.3%	61.6%	66.3%	63.8%	75.6%	66.8%	52.2%	53.2%	50.2%	58.4%
Accrued contingent liabilities	—	—	—	—	—	—	—	60.5%	1.5%	1.0%
Total operating expenses	134.3%	110.8%	109.9%	101.0%	141.7%	102.9%	126.5%	218.8%	79.6%	91.7%
Loss from operations	(50.9)%	(25.0)%	(23.9)%	(16.0)%	(56.2)%	(17.2)%	(40.8)%	(148.3)%	(5.7)%	(18.6)%
Other income (expense):										
Interest income	0.3%	0.5%	0.5%	0.4%	0.4%	1.1%	0.8%	0.5%	0.5%	0.4%
Interest expense	(1.6)%	(1.3)%	(1.1)%	(0.8)%	(1.6)%	(2.0)%	(1.8)%	(1.4)%	(1.3)%	(1.2)%
Other income (expense), net	(0.1)%	0.6%	0.1%	0.2%	0.2%	(0.5)%	(0.1)%	(0.1)%	(0.3)%	—
Total other income (expense)	(1.4)%	(0.2)%	(0.5)%	(0.2)%	(1.0)%	(1.4)%	(1.1)%	(1.0)%	(1.1)%	(0.8)%
Loss before provision for income taxes	(52.3)%	(25.2)%	(24.4)%	(16.2)%	(57.2)%	(18.6)%	(41.9)%	(149.3)%	(6.8)%	(19.4)%
Provision for income taxes	—	—	0.1%	0.1%	—	0.1%	—	0.1%	0.1%	0.1%
Net loss	(52.3)%	(25.2)%	(24.5)%	(16.3)%	(57.2)%	(18.7)%	(41.9)%	(149.4)%	(6.9)%	(19.5)%

Quarterly trends

Revenue

Our quarterly revenue increased for all periods presented primarily due to an increase in consumables revenue resulting from growth of the instrument installed base and higher consumable pull-through per instrument. The revenue for the increase in instrument unit volumes was partially offset by a decrease in the average instrument selling price.

Cost of revenue

Our quarterly cost of revenue increased for all periods presented, except for the first quarter of 2019, primarily due to an increase in volume of sales. The first quarter of 2019 had lower excess and obsolete inventory and lower warranty reserves. Commencing in the fourth quarter of 2018 and continuing in the first and second quarters of 2019, cost of revenue as a percentage of revenue was higher than in prior periods as a result of additional royalties related to the judgment in the Bio-Rad litigation.

Operating expenses

Our quarterly research and development expenses increased for all periods presented, except for the second and third quarters of 2018, primarily due to increases in personnel expenses and laboratory materials and supplies which were attributable to an increase in headcount and expenses supporting our continued research and development efforts to enhance our existing products and develop new products.

Our in-process research and development expense consisted of expenses incurred in the first, third and fourth quarters of 2018 related to intellectual property we purchased in connection with our acquisitions of Spatial Transcriptomics and Epinomics and our acquisition of an exclusive license to certain intellectual property from Prognosys, respectively. There were no acquisitions of in-process research and development in other quarters in 2017 or 2018.

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Our selling, general and administrative expenses increased for all periods presented, except for the third quarter of 2018, primarily due to increases in outside legal fees and increases in personnel expenses driven by increases in headcount. The decrease in selling general and administrative expenses from the second quarter of 2018 to the third quarter of 2018 was primarily driven by lower outside legal fees during the third quarter due to the timing of litigation matters. Beginning in the fourth quarter of 2018, we also incurred higher consulting and professional expenses.

Our accrued contingent liabilities consisted of $30.6 million of expenses relating to the litigation with Bio-Rad, for which we established an accrual in the fourth quarter of 2018. There was no similar expense accrual in 2017 or in prior quarters in 2018. An additional $0.8 million and $0.6 million was recorded in the first and second quarter of 2019, respectively, related to pre- and post- judgment interest.

Liquidity and capital resources

As of June 30, 2019, we had approximately $56.0 million in cash and cash equivalents which were primarily held in U.S. bank deposit accounts and money market funds, $26.8 million in accounts receivable and an accumulated deficit of $245.6 million. Approximately $5.0 million of cash, which serves as collateral for an outstanding letter of credit, was classified as noncurrent restricted cash as of June 30, 2019. Since our inception, we have generated negative cash flows from operations.

We have asked the U.S. District Court for the District of Delaware to allow us to post a bond for approximately $35 million in connection with our litigation with Bio-Rad. We expect that prior to posting the bond, we will be required to deposit cash as collateral in a segregated cash account in an amount between $30 and $35 million. The collateral will be held until conclusion of the appeal.

We currently anticipate placing cash in escrow each quarter of an amount equal to 15% of net sales of our GEM microfluidic chips and associated consumables subsequent to the effective date of the injunction, which is anticipated to be August 28, 2019. The amounts will be held until conclusion of the appeal.

We currently anticipate making aggregate capital expenditures of between approximately $45.0 million and $55.0 million during the next 18 months, which includes the construction costs of our global expansion and for equipment to be used for manufacturing and research and development. Our future capital requirements will depend on many factors including our revenue growth rate, research and development efforts, the timing and extent of additional capital expenditures to invest in existing and new facilities, the expansion of sales and marketing and international activities, the timing of capital expenditures relating to our planned implementation of a new enterprise resource planning system and the introduction of new products. We have and may in the future enter into arrangements to acquire or invest in businesses, services and technologies, including intellectual property rights, and any such acquisitions or investments could significantly increase our capital needs.

We believe that our existing cash and cash equivalents, cash generated from sales of our products and either, or a combination of, the deferral of anticipated capital expenditures or partially borrowing under our existing credit agreements will be sufficient to meet our anticipated cash needs for the next 12 months. However, our liquidity assumptions may prove to be incorrect, and we could exhaust our available financial resources sooner than we currently expect. We intend to partially borrow under our existing revolving line of credit for our operations. Other than such borrowing, we do not anticipate that we will need to raise additional financing in the future to fund our operations. In the event that additional financing is required, we may not be able to raise it on terms acceptable to us or at all. If we raise additional funds through the issuance of additional debt or equity securities, it could result in dilution to our existing stockholders, increased fixed payment obligations, and the existence of securities with rights that may be senior to those of our common stock. If we incur

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indebtedness, we could become subject to covenants that would restrict our operations and impair our competitiveness. Further, if we elect to borrow up to an additional $20.0 million of term loans under the Loan and Security Agreement, we will be obligated to issue warrants to purchase 133,000 shares of our Class A common stock at an exercise price of $1.62 per share to the lender thereof. If we are unable to raise additional capital when desired, our business, results of operations and financial condition would be adversely affected. We are subject to all the risks typically related to the development of new products and we may encounter unforeseen expenses, difficulties, complications, delays and other unknown factors that may adversely affect our business.

Sources of liquidity

Since our inception, we have generated negative cash flows from operations and have financed our operations and capital expenditures primarily through non-registered sales of convertible preferred stock and common stock and issuances of debt. Through June 30, 2019, we have raised a total of $243.2 million from the sale of convertible preferred stock, net of costs associated with such financings.

Silicon Valley Bank Loan and Security Agreement

We are party to a Second Amended and Restated Loan and Security Agreement, dated February 9, 2018, with Silicon Valley Bank (as amended, restated or supplemented from time to time, the "Loan and Security Agreement"), under which (i) $30.0 million of term loan borrowings were outstanding, (ii) no borrowings were outstanding under the $25.0 million revolving line of credit and (iii) up to $20.0 million of additional term loan borrowings, which, subject to certain conditions, are available to be drawn before December 31, 2019, in each case as of June 30, 2019. We are obligated to issue warrants to purchase 133,000 shares of our Class A common stock, at an exercise price of $1.62 per share to the lender if we elect to borrow the additional term loan referred to in the preceding sentence. We currently intend to partially draw under our revolving line of credit, prior to the consummation of this offering, in order to provide us with additional liquidity in connection with our operations.

Borrowings under the term loan mature on December 1, 2022 and accrue interest at a floating rate equal to the greater of *The Wall Street Journal* prime rate plus 2.0% or 6.25% per annum. Monthly payments of interest are due on the term loan through December 31, 2019, after which equal monthly installments of principal and interest are due. The revolving line of credit terminates on December 1, 2022 and the amount available under the revolving line of credit is based on 80% of eligible receivables and is subject to a borrowing base calculation. As of June 30, 2019, our revolving line of credit was $25.0 million. Borrowings under the revolving line of credit accrue interest at a floating rate equal to the greater of *The Wall Street Journal* prime rate plus 0.25% or 4.5% per annum. Borrowings under the revolving line of credit are repayable monthly. As of June 30, 2019, the borrowings under the term loan accrued interest at a rate of 7.50% per annum and the interest rate applicable to borrowings under the revolving line of credit would have been 5.75% per annum.

The Loan and Security Agreement contains affirmative and negative covenants, including a covenant requiring us to maintain minimum revenue equal to at least 70% of projected revenue for the applicable periods through and including December 31, 2020 and covenants that restrict, among other things, our ability to dispose of assets, change our business, management, ownership or business locations, enter into mergers or acquisitions, incur additional indebtedness or encumber any of our assets. Because the minimum revenue requirements referred to above are based on the revenue forecasts we provide to the lender, our inability to accurately forecast our revenue for future periods could result in a failure to comply with this covenant, which would be an event of default under the Loan and Security Agreement. We were in compliance with all covenants under the Loan and Security Agreement as of June 30, 2019 and remain in compliance with such covenants as of the date of the registration statement of which this prospectus forms a part.

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Cash flow summary

The following table sets forth our cash flows for the periods presented:

(in thousands)	Year ended December 31, 2017	Year ended December 31, 2018	Six months ended June 30, 2018	Six months ended June 30, 2019
			(unaudited)	
Net cash provided by (used in):				
Operating activities	$ (10,699)	$ (76,409)	$ (20,226)	$ 13,401
Investing activities	(3,756)	(6,709)	(3,261)	(22,508)
Financing activities	20,583	105,367	69,078	59
Effect of exchange rates on cash, cash equivalents and restricted cash	(14)	(18)	11	2
Net increase in cash, cash equivalents and restricted cash	$ 6,114	$ 22,231	$ 45,602	$ (9,046)

Operating activities

The net cash provided by operating activities of $13.4 million in the six months ended June 30, 2019 was due primarily to a net loss of $14.5 million with adjustments for stock-based compensation expense of $4.4 million and depreciation and amortization of $2.2 million. The inflow from operating assets and liabilities was primarily due to an increase in accrued contingent liabilities of $17.3 million, an increase in noncurrent deferred rent of $11.7 million and an increase in accrued expenses and other current liabilities of $3.0 million partially offset by an increase in tenant allowances receivable of $6.5 million, an increase in inventory of $3.8 million and an increase in prepaid expenses and other assets of $1.2 million.

The net cash used in operating activities of $20.2 million in the six months ended June 30, 2018 was due primarily to a net loss of $21.6 million with adjustments for depreciation and amortization of $2.2 million and stock-based compensation expense of $1.0 million. The outflow from operating assets and liabilities was primarily due to an increase in accounts receivable of $4.0 million, an increase in inventory of $1.6 million, a decrease in accrued compensation and other related benefits of $1.3 million, an increase in prepaid expenses and other current assets and other assets of $0.4 million, and a decrease in accrued expenses and other current liabilities of $0.4 million, partially offset by an increase in accounts payable of $5.1 million and an increase in deferred revenue of $0.7 million.

The net cash used in operating activities of $76.4 million in the year ended December 31, 2018 was due primarily to a net loss of $112.5 million with adjustments for depreciation and amortization of $3.9 million and stock-based compensation expense of $2.7 million. The inflow from operating assets and liabilities was primarily due to the establishment of an accrual for contingent liabilities of $38.0 million, an increase in noncurrent deferred rent of $3.3 million, an increase in accounts payable of $2.6 million, an increase in accrued compensation and other related benefits of $2.6 million, an increase in accrued expenses and other current liabilities of $1.7 million and an increase in deferred revenue of $1.7 million, partially offset by an increase in accounts receivable of $14.7 million, an increase in inventory of $3.7 million, and increase in tenant allowances receivable of $1.5 million and an increase in prepaid expenses and other current assets of $1.0 million.

The net cash used in operating activities of $10.7 million in the year ended December 31, 2017 was due primarily to a net loss of $18.8 million with adjustments for depreciation and amortization of $4.3 million and stock-based compensation expense of $1.7 million. The inflow from operating assets and liabilities was primarily due to an increase in accrued compensation and other related benefits of $3.5 million, an increase in accounts payable of $3.0 million, an increase in accrued expenses and other current liabilities of $1.8 million, and an

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increase in deferred revenue of $1.3 million, partially offset by an increase in accounts receivable of $5.1 million, an increase in inventory of $2.0 million and an increase in prepaid expenses and other current assets of $0.7 million.

The increase in cash used for operating activities in the year ended December 31, 2018 as compared to the prior year is primarily due to $60.8 million in cash paid for the acquisition of intellectual property to be used in research and development efforts to enhance existing products and develop new products.

Investing activities

The net cash used in investing activities of $22.5 million in the six months ended June 30, 2019 was due to purchases of property and equipment of $22.5 million.

The net cash used in investing activities of $3.3 million in the six months ended June 30, 2018 was due to purchases of property and equipment of $3.3 million.

The net cash used in investing activities of $6.7 million in the year ended December 31, 2018 was due to purchases of property and equipment of $6.3 million and the purchase of intangible assets of $0.4 million.

The net cash used in investing activities of $3.8 million in the year ended December 31, 2017 was due to purchases of property and equipment of $3.8 million.

Financing activities

The net cash provided by financing activities of $0.1 million in the six months ended June 30, 2019 was primarily from proceeds of $2.0 million from the issuance of common stock from the exercise of stock options, primarily offset by payments of deferred financing costs of $1.9 million.

The net cash provided by financing activities of $69.1 million in the six months ended June 30, 2018 was primarily from proceeds from the issuance of convertible preferred stock, net of issuance costs, of $49.9 million, proceeds from additional borrowings of $19.5 million and proceeds of $0.5 million from the issuance of common stock from the exercise of stock options, partially offset by payments of debt obligations of $0.7 million.

The net cash provided by financing activities of $105.4 million in the year ended December 31, 2018 was primarily from proceeds from the issuance of convertible preferred stock, net of issuance costs, of $84.8 million, net proceeds from additional borrowings of $19.5 million, and proceeds of $1.8 million from the issuance of common stock from the exercise of stock options, partially offset by payments on debt obligations of $0.7 million.

The net cash provided by financing activities of $20.6 million in the year ended December 31, 2017 was primarily from proceeds from the issuance of convertible preferred stock, net of issuance costs, of $20.0 million and proceeds of $1.1 million from the issuance of common stock from the exercise of stock options, partially offset by payments of capital lease obligation of $0.4 million.

Concentrations of credit risk

As of December 31, 2017 and 2018 and June 30, 2019, no single customer, including distributors, represented 10% or more of our accounts receivable balance. There was no single customer, including distributors, that individually exceeded 10% of our revenue during each of the years ended December 31, 2017 or 2018 or for the six months ended June 30, 2019.

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Contractual obligations and commitments

The following table summarizes our commitments to settle contractual obligations as of December 31, 2018:

		Payments due by period			
(in thousands)	Total	Less than 1 year	1 – 3 years	3 – 5 years	More than 5 years
Debt obligations, including interest(1)	$ 37,028	$ 6,495	$ 19,809	$ 10,724	$ —
Lease commitments(2)	66,059	2,847	12,595	11,905	38,712
Other obligations(3)	8,083	1,754	1,929	1,100	3,300
Total	$ 111,170	$ 11,096	$ 34,333	$ 23,729	$ 42,012

(1) As of June 30, 2019, the outstanding principal balance of our term loan under our Loan and Security Agreement was $ 29.7 million. Monthly payments of interest are due under the term loan through December 31, 2019, with equal monthly installments of principal and interest due for thirty-six months thereafter. We have an option to borrow an additional $20.0 million of term loans before January 1, 2020. An end of term payment of $1.8 million, which is due to the lender upon maturity in 2022, prepayment or acceleration of the term loan, is reflected as additional interest expense over the term of the loan. Borrowings under the term loan may be prepaid, subject to a prepayment penalty. See Note 5 to our consolidated financial statements included elsewhere in this prospectus for more information regarding the terms of the Loan and Security Agreement. In June 2019, our loan and security agreement was amended to extend our option to borrow an additional $20.0 million as a term loan through December 31, 2019. Monthly payments of interest are due through December 31, 2019, with monthly installments of principal and interest due for 36 months thereafter. As a result, annual payments due on the term loan decreased by approximately $4.2 million in 2019 and increased by $1.7 million, $1.6 million and $1.5 million in 2020, 2021 and 2022, respectively. This amendment is not reflected in the table above.

(2) We have entered into various non-cancelable leases for certain offices with contractual lease periods expiring between 2019 and 2029. As of December 31, 2018, we had an unused letter of credit in the amount of $5.0 million outstanding associated with the lease of our new Pleasanton global headquarters and research and development center.

(3) Other obligations include purchase obligations, prepaid services and royalties. Purchase obligations relate to our contract manufacturer which manufacturers our instruments and makes advance purchases of components based on our sales forecasts and the placement of purchase orders by us. To the extent components are purchased by the contract manufacturer on our behalf and cannot be used by the contract manufacturer's other customers, we are obligated to purchase such components. In addition, certain supplier agreements require us to make minimum annual purchases under the agreements. To date, we have met the minimum purchase commitments. Prepaid services includes subscription software services for which we have entered into non-cancelable arrangements. Royalties include minimum commitments for license arrangements. In 2019, we entered into additional purchase commitment for professional services for $0.7 million which is not reflected in the table above.

Off-balance sheet arrangements

We did not have during the periods presented, and we do not currently have, any off-balance sheet financing arrangements or any relationships with unconsolidated entities or financial partnerships, including entities sometimes referred to as structured finance or special purpose entities, that were established for the purpose of facilitating off-balance sheet arrangements or other contractually narrow or limited purposes.

Qualitative and quantitative disclosures about market risk

We are exposed to market risks in the ordinary course of our business. Market risk represents the risk of loss that may impact our financial position due to adverse changes in financial market prices and rates. Our market risk exposure is primarily the result of fluctuations in foreign currency exchange rates.

Interest rate risk

As of December 31, 2018, we had cash and cash equivalents of $65.1 million, which consisted primarily of bank deposits and money market funds. Our historical interest income has not fluctuated significantly. A hypothetical 10% change in interest rates would have not had a material impact on our financial statements included in this prospectus. We do not enter into investments for trading or speculative purposes and have not used any derivative financial instruments to manage our interest rate risk exposure.

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Foreign currency exchange risk

Our reporting currency is the U.S. dollar and the functional currency of each of our subsidiaries is either its local currency or the U.S. dollar depending on the circumstances. Historically, most of our revenue has been denominated in U.S. dollars, although we have sold our products and services in local currency outside of the United States, principally the euro. For the year ended December 31, 2018 and for the six months ended June 30, 2019, approximately 16% and 14%, respectively, of our sales were denominated in currencies other than U.S. dollars. Our expenses are generally denominated in the currencies in which our operations are located, which is primarily in the United States. As our operations in countries outside of the United States grow, our results of operations and cash flows will be subject to fluctuations due to changes in foreign currency exchange rates, which could harm our business in the future. For example, if the value of the U.S. dollar increases relative to foreign currencies, in the absence of a corresponding change in local currency prices, our revenue could be adversely affected as we convert revenue from local currencies to U.S. dollars. In addition, because we conduct business in currencies other than U.S. dollars, but report our results of operations in U.S. dollars, we also face remeasurement exposure to fluctuations in currency exchange rates, which could hinder our ability to predict our future results and earnings and could materially impact our results of operations. We do not currently maintain a program to hedge exposures to non-U.S. dollar currencies. We do not believe that an immediate 10% increase or decrease in the relative value of the U.S. dollar to other currencies would have a material effect on our operating results.

Critical accounting policies and estimates

Our consolidated financial statements and the related notes thereto included elsewhere in this prospectus are prepared in accordance with GAAP. The preparation of consolidated financial statements also requires us to make estimates and assumptions that affect the reported amounts of assets, liabilities, revenue, costs and expenses and related disclosures. We base our estimates on historical experience and on various other assumptions that we believe to be reasonable under the circumstances. Actual results could differ significantly from our estimates. To the extent that there are differences between our estimates and actual results, our future financial statement presentation, financial condition, results of operations and cash flows will be affected.

We believe that the accounting policies described below involve a significant degree of judgment and complexity. Accordingly, we believe these are the most critical to aid in fully understanding and evaluating our consolidated financial condition and results of operations. For further information, see Note 2 of the notes to our consolidated financial statements included elsewhere in this prospectus.

Revenue recognition

We generate revenue from sales of our products and services. Our products consist of instruments and consumables, including proprietary microfluidic chips, slides, reagents and other consumables for both our Visium and Chromium solutions. We also generate a small portion of our revenue from instrument service agreements which relate to extended warranties.

Effective January 1, 2019, we adopted Accounting Standards Codification (“ASC”) Topic 606, *Revenue from Contracts with Customers*, using the modified retrospective transition method. The cumulative effect of initially adopting ASC Topic 606 was immaterial.

The revenue recognition accounting policy described below relates to revenue transactions from January 1, 2019 and onward, which are accounted for in accordance with ASC Topic 606—Revenue from Contracts with Customers.

We recognize revenue when control of the products and services is transferred to our customers in an amount that reflects the consideration we expect to receive from our customers in exchange for those products and services. This process involves identifying the contract with a customer, determining the performance

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obligations in the contract, determining the contract price, allocating the contract price to the distinct performance obligations in the contract, and recognizing revenue when the performance obligations have been satisfied. A performance obligation is considered distinct from other obligations in a contract when it provides a benefit to the customer either on its own or together with other resources that are readily available to the customer and is separately identified in the contract. We consider a performance obligation satisfied once we have transferred control of a good or service to the customer, meaning the customer has the ability to use and obtain the benefit of the good or service.

Revenue from product sales is recognized when control of the product is transferred, which is generally upon shipment to the customer. In instances where right of payment or transfer of title is contingent upon the customer's acceptance of the product, revenue is deferred until all acceptance criteria have been met. Instrument service agreements, which relate to extended warranties, are typically entered into for one-year terms, following the expiration of the standard one-year warranty period. Revenue for extended warranties is recognized ratably over the term of the extended warranty period as a stand ready performance obligation. Revenue is recorded net of discounts, distributor commissions and sales taxes collected on behalf of governmental authorities. Customers are invoiced generally upon shipment, or upon order for services, and payment is typically due within 45 days. Cash received from customers in advance of product shipment or providing services is recorded as a contract liability. Our contracts with our customer generally do not include rights of return or a significant financing component.

We regularly enter into contracts that include various combinations of products and services which are generally distinct and accounted for as separate performance obligations. The transaction price is allocated to each performance obligation in proportion to its standalone selling price. We determine standalone selling price using average selling prices with consideration of current market conditions. If the product or service has no history of sales or if the sales volume is not sufficient, we rely upon prices set by management, adjusted for applicable discounts.

The revenue recognition accounting policy described below relates to revenue transactions prior to January 1, 2019, which are accounted for in accordance with ASC Topic 605—Revenue Recognition.

We recognize revenue when persuasive evidence of an arrangement exists, delivery has occurred or services have been rendered, the price to the customer is fixed or determinable and collectability is reasonably assured. We assess collectability based on factors such as the customer's creditworthiness and past collection history, if applicable. If collection is not reasonably assured, revenue recognition is deferred until receipt of payment. We also assess whether a price is fixed or determinable by, among other things, reviewing contractual terms and conditions related to payment. Delivery occurs when there is a transfer of title and risk of loss passes to the customer.

Certain of our sales arrangements involve the delivery of multiple products and services within contractually binding arrangements. Multiple-deliverable sales transactions typically consist of the sale and delivery of one or more instruments and consumables together and may include an instrument service agreement.

For sales arrangements that include multiple deliverables, we use the stated contractual price for the instrument service agreements, if and when sold, and allocate the remaining contract consideration at the inception of the contract to the other units of accounting based upon their relative selling price. We may use our best estimate of selling price for individual deliverables when vendor specific objective evidence or third-party evidence is unavailable. A delivered item is considered to be a separate unit of accounting when it has value to the customer on a stand-alone basis.

Our products, other than instrument service agreements, are typically delivered together or within a short time frame, generally within one to three months of the contract date. Instrument service agreements are typically

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entered into for a one-year term, following the expiration of the standard one-year warranty period. Our products are generally sold without the right of return. Amounts received before revenue recognition criteria are met are classified in the balance sheets as deferred revenue.

Contract costs

Beginning January 1, 2019, sales commissions earned by our sales force are considered incremental and recoverable costs of obtaining a contract with a customer. Sale commissions related to the sale of extended warranties are deferred and amortized on a straight-line basis over the service term, which is typically greater than one year from the contract date. Amortization of deferred commissions is included in sales and marketing expenses.

Inventory

Inventory is recorded at the lower of cost, determined on a first-in, first-out basis, or net realizable value. We use judgment to analyze and determine if the composition of its inventory is obsolete, slow-moving or unsalable and frequently reviews such determinations. We write down specifically identified unusable, obsolete, slow-moving or known unsalable inventory in the period that it is first recognized by using a number of factors including product expiration dates, open and unfulfilled orders and sales forecasts. Any write-down of its inventory to net realizable value establishes a new cost basis and will be maintained even if certain circumstances suggest that the inventory is recoverable in subsequent periods. Costs associated with the write-down of inventory are recorded to cost of revenue on our consolidated statements of operations. We make assumptions about future demand, market conditions and the release of new products that may supersede old ones. However, if actual market conditions are less favorable than anticipated, additional inventory write-downs could be required.

Stock-based compensation

We estimate the fair value of share-based payment awards granted to employees and directors on the grant date using the Black-Scholes option-pricing model. The fair value of share-based payment awards is recognized as compensation expense on a straight-line basis over the requisite service period in which the awards are expected to vest, which is generally four years, and forfeitures are recognized as they occur. Share-based payment awards that include both a service condition and a performance condition are considered expected to vest when the performance condition is probable of being met.

The Black-Scholes model considers several variables and assumptions in estimating the fair value of stock-based awards. These variables include the per share fair value of the underlying common stock, exercise price, expected term, risk-free interest rate, expected annual dividend yield and expected stock price volatility over the expected term. For all stock options granted, we calculated the expected term using the simplified method for “plain vanilla” stock option awards. We have no publicly available stock information. Therefore, we have used the historical volatility of the stock price of similar publicly traded peer companies. The risk-free interest rate is based on the yield available on U.S. Treasury zero-coupon issues similar in duration to the expected term of the equity-settled award.

Equity instruments granted to nonemployees are valued using the Black-Scholes option pricing model and are subject to periodic revaluation over their vesting terms. Nonemployee stock-based compensation is recognized over the related performance period, which is generally the vesting term of the awards.

Common stock valuation

There has been no public market for our common stock to date. As such, the estimated fair value of our common stock and underlying stock options has been determined at each grant date by our board of directors,

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with input from management, based on the information known to us on the grant date and upon a review of any recent events and their potential impact on the estimated per share fair value of our common stock. As part of these fair value determinations, our board of directors obtained and considered valuation reports prepared by a third-party valuation firm in accordance with the guidance outlined in the American Institute of Certified Public Accountants Technical Practice Aid, *Valuation of Privately-Held-Company Equity Securities Issued as Compensation*.

Beginning December 31, 2018, in contemplation of an initial public offering, in determining the fair value of our common stock, we estimated the enterprise value of our business using the hybrid approach. The hybrid method is a probability-weighted expected return method ("PWERM"), which utilizes the probability of discrete exit scenarios and the probability of the remaining private scenario. The common stock value is based on the probability-weighted present value of expected future investment returns considering each of the possible outcomes available, as well as the rights of each class of stock. The future value of the common stock under each outcome is discounted back to the valuation date at an appropriate risk-adjusted discount rate and probability weighted to arrive at an indication of value for the common stock. We estimated the enterprise value of our business for the exit scenario using the market approach. Under the market approach, a group of guideline publicly-traded companies with financial and operating characteristics similar to our company are selected and valuation multiples based on the guideline public companies' financial information and market data are calculated. Based on the observed valuation multiples from our guideline public company universe, an appropriate multiple was selected to apply to our historical and forecasted revenue results. We estimated the enterprise value of the business under the remaining private scenario by reference to the closest round of equity financing preceding the date of the valuation using the option pricing method ("OPM"). The OPM treats common stock and preferred stock as call options on the total equity value of a company, with exercise prices based on the value thresholds at which the allocation among the various holders of a company's securities changes. Under this method, the common stock has value only if the funds available for distribution to stockholders exceeded the value of the preferred stock liquidation preferences at the time of the liquidity event. A discount for lack of marketability ("DLOM") of the common stock is then applied to arrive at an indication of value for the common stock. A DLOM is meant to account for the lack of marketability of a stock that is not traded on public exchanges.

Based on our early stage of development and other relevant factors, we determined that a hybrid approach of the OPM and the PWERM methods was the most appropriate method for allocating our enterprise. Previously, we estimated the enterprise value of our business either by reference to the closest round of equity financing preceding the date of the valuation using the OPM (by "backsolving" the implied enterprise value based on the price paid for each new preferred security sold), by the market approach, or by the income approach.

In addition to considering the results of these third-party valuation reports, our board of directors used assumptions based on various objective and subjective factors, combined with management judgement, to determine the fair value of our common stock as of each grant date, including:

- the prices at which we sold shares of preferred stock and the superior rights and preferences of the preferred stock relative to our common stock at the time of each grant;
- external market conditions affecting the life sciences research and development industry and trends within the industry;
- our stage of development and business strategy;
- our financial condition and operating results, including our levels of available capital resources and forecasted results;

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- developments in our business;
- the progress of our research and development efforts;
- equity market conditions affecting comparable public companies;
- general United States market conditions and the lack of marketability of our common stock; and
- for purposes of determining our stock-based compensation expense for option grants in 2019, we re-evaluated the grant date fair value of our common stock solely for accounting purposes based on external market factors and progress in and input related to this offering through August 19, 2019.

Application of these approaches involves the use of estimates, judgement and assumptions that are highly complex and subjective, such as those regarding our expected future revenue, expenses and future cash flows, discount rates, market multiples, the selection of comparable companies and the probability of possible future events. Changes in any or all of these estimates and assumptions or the relationships between those assumptions impact our valuations as of each valuation date and may have a material impact on the valuation of our common stock. For valuations after the completion of this initial public offering, our board of directors will determine the fair value of each share of underlying common stock-based on the closing price of our Class A common stock as reported on the date of grant.

As of June 30, 2019, based on the assumed initial public offering price per share of $, which is the midpoint of the price range set forth on the cover page of this prospectus, the aggregate intrinsic value of our outstanding stock options, was $ million, with $ million related to vested stock options. As of June 30, 2019, we had $39.2 million of unrecognized stock-based compensation which is expected to be recognized over a weighted-average period of approximately 3.4 years. In addition, subsequent to June 30, 2019, we granted options to purchase 845,475 shares of our common stock that vest over four years.

Accrued contingent liabilities

We have been and are currently involved in various legal proceedings, the outcomes of which are not within our complete control or may not be known for prolonged periods of time. Management is required to assess the probability of loss and amount of such loss, if any, in preparing our consolidated financial statements. We evaluate the likelihood of a potential loss from legal proceedings to which we are a party. We record a liability for such claims when a loss is deemed probable and the amount can be reasonably estimated. Significant judgment may be required in the determination of both probability and whether an exposure is reasonably estimable. Our judgments are subjective based on the status of the legal proceedings, the merits of our defenses and consultation with in-house and outside legal counsel. As additional information becomes available, we reassess the potential liability related to pending claims and may revise our estimates. Due to the inherent uncertainties of the legal processes in the multiple jurisdictions in which we operate, our judgments may be materially different than the actual outcomes, which could have material adverse effects on our business, financial conditions and results of operations.

Acquisitions of intellectual property

We evaluate acquisitions of assets and other similar transactions to assess whether or not the transaction should be accounted for as a business combination or asset acquisition by first applying a screen to determine if substantially all of the fair value of the gross assets acquired is concentrated in a single identifiable asset or group of similar identifiable assets. If the screen is met, the transaction is accounted for as an asset acquisition. If the screen is not met, further determination is required as to whether or not we have acquired inputs and processes that have the ability to create outputs, which would meet the requirements of a business.

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We account for an asset acquisition under Accounting Standards Codification, *Business Combinations Topic 805, Subtopic 50*, which requires the acquiring entity in an asset acquisition to recognize net assets based on the cost to the acquiring entity on a relative fair value basis, which includes transaction costs in addition to consideration given. Goodwill is not recognized in an asset acquisition and any excess consideration transferred over the fair value of the net assets acquired is allocated to the non-monetary identifiable assets based on relative fair values. In-process research and development expenses are expensed as incurred provided there is no alternative future use.

Contingent consideration payments in asset acquisitions are recognized when the contingency is resolved and the consideration is paid or becomes payable (unless the contingent consideration meets the definition of a derivative, in which case the amount becomes part of the basis in the asset acquired). Upon recognition of the contingent consideration payment, the amount is included in the cost of the acquired asset or group of assets.

JOBS Act accounting election

We are an emerging growth company, as defined in the JOBS Act. Under the JOBS Act, emerging growth companies can delay adopting new or revised accounting standards issued subsequent to the enactment of the JOBS Act until such time as those standards apply to private companies. We have elected to use this extended transition period for complying with new or revised accounting standards that have different effective dates for public and private companies until the earlier of the date we (i) are no longer an emerging growth company or (ii) affirmatively and irrevocably opt out of the extended transition period provided in the JOBS Act. As a result, our financial statements may not be comparable to companies that comply with new or revised accounting pronouncements as of public company effective dates.

Recent accounting pronouncements and recently adopted accounting standards

See Note 2 to our consolidated financial statements included elsewhere in this prospectus for more information.

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Business

Mission

Our mission is to accelerate the mastery of biology to advance human health.

Overview

We are a life science technology company building products to interrogate, understand and master biology. Our integrated solutions include instruments, consumables and software for analyzing biological systems at a resolution and scale that matches the complexity of biology. We have built deep expertise across diverse disciplines including chemistry, biology, hardware and software. Innovations in all of these areas have enabled our rapidly expanding suite of products, which allow our customers to interrogate biological systems at previously inaccessible resolution and scale. Our products have enabled researchers to make fundamental discoveries across multiple areas of biology, including oncology, immunology and neuroscience, and have helped empower the single cell revolution hailed by *Science* magazine as the 2018 'Breakthrough of the Year'. Since launching our first product in mid-2015 through June 30, 2019, we have sold 1,284 instruments to researchers around the world, including 93 of the top 100 global research institutions as ranked by *Nature* in 2018 based on publications, and 13 of the top 15 global pharmaceutical companies by 2018 revenue. We believe that this represents the very beginning of our penetration into multiple large markets. We expect that 10x will power a "Century of Biology", in which many of humanity's most pressing health challenges will be solved by precision diagnostics, targeted therapies and cures to currently intractable diseases.

The "10x" in our name refers to our focus on opportunities with the greatest potential for exponential advances and impact. We believe that the scientific and medical community currently understands only a tiny fraction of the full complexity of biology. The key to advancing human health lies in accelerating this understanding. The human body consists of over 40 trillion cells, each with a genome of 3 billion DNA base pairs and a unique epigenetic program regulating the transcription of tens of thousands of different RNAs, which are then translated into tens of thousands of different proteins. Progress in the life sciences will require the ability to measure biological systems in a much more comprehensive fashion and to experiment on biological systems at fundamental resolutions and massive scales, which are inaccessible with existing technologies. We believe that our technologies overcome these limitations, unlocking fundamental biological insights essential for advancing human health.

Resolution and scale are the imperatives underlying our technologies and products. Our Chromium and recently announced Visium product lines provide this resolution and scale along distinct but complementary dimensions of biology. Our Chromium products enable high throughput analysis of individual biological components, such as up to millions of single cells. They use our precisely engineered reagent delivery system to divide a sample into individual components in up to a million or more partitions, enabling large numbers of parallel micro-reactions. In this manner, a large population of cells can be segregated into partitions and analyzed on a cell by cell basis. Our Visium products, the first of which we expect to launch in late 2019, will enable analysis of biological molecules within their spatial context, providing the locations of analytes that give insight into higher order biological structure and function. Our Visium platform will use high density DNA arrays with DNA sequences that encode the physical locations of biological analytes within a sample, such as a tissue section. Our products utilize our sensitive and robust molecular assays to convert biological analytes into detectable signals, enabling researchers to obtain vast amounts of information about diverse biological analytes together with their single cell and spatial context. Finally, we provide highly sophisticated and scalable software for analyzing the raw data researchers generate and presenting it in a form that is readily understood by biologists.

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Our product portfolio consists of multiple integrated solutions that include instruments, consumables and software. These solutions guide customers through the workflow from sample preparation to sequencing on third-party sequencers that are commonly available in research settings to subsequent analysis and visualization.

Each of our solutions is designed to interrogate a major class of biological information that is impactful to researchers:

- Our single cell solutions, all of which run on our Chromium instruments, include:
    - Single Cell Gene Expression solution for measuring gene activity on a cell-by-cell basis;
    - Single Cell Immune Profiling solution for measuring the activity of immune cells and their targets;
    - Single Cell ATAC solution for measuring epigenetics, including the physical organization of DNA; and
    - Single Cell CNV solution for measuring cellular heterogeneity through DNA changes such as copy number variation.
- Our upcoming Visium solution will measure the spatial gene expression patterns across a tissue sample.

Our Feature Barcoding technology, which is currently compatible with our Single Cell Gene Expression and Immune Profiling solutions, allows researchers to simultaneously measure multiple analytes, such as protein and RNA, within the same set of cells or tissues.

Collectively, our solutions enable researchers to interrogate, understand and master biology at the appropriate resolution and scale.

We believe our solutions, which enable a comprehensive view of biology, target numerous market opportunities across the more than $50 billion global life sciences research tools market. We view much of this total market opportunity as ultimately accessible to us due to our ability to answer a broad diversity of biological questions. Based on the capabilities of our current solutions, and focusing solely on cases where our current solutions offer alternative or complementary approaches to existing tools, we believe, based on our internal estimates, we could access approximately $13 billion of the global life sciences research tools market. We believe we can further drive growth across our current and adjacent markets by improving or enabling new uses and applications of existing tools and technologies, as our solutions allow researchers to answer questions that may be impractical or impossible to address using existing tools.

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As of June 30, 2019, we employed a commercial team of over 190 employees, many of whom hold Ph.D. degrees, who help drive adoption of our products and support our vision. We prioritize creating a superior user experience from pre-sales to onboarding through the generation of novel publishable discoveries, which drive awareness and adoption of our products. We have a scalable, multi-channel commercial infrastructure including a direct sales force in North America and certain regions of Europe and distribution partners in Asia, certain regions of Europe, South America, the Middle East and Africa that drives our customer growth. This is supplemented with an extensive and highly specialized customer service infrastructure with Ph.D.-level specialists. We currently have customers in approximately 40 countries.

Our revenue was $71.1 million and $146.3 million for 2017 and 2018, respectively, representing an annual growth rate of 106%, and $59.2 million and $109.4 million for the six months ended June 30, 2018 and 2019, respectively, representing an annual growth rate of 85%. We generated net losses of $18.8 million and $112.5 million for 2017 and 2018. Our 2018 net loss resulted substantially from charges of $62.4 million associated with intellectual property acquisitions for research and development in addition to the litigation contingency accrual of $38.0 million which was recorded in the fourth quarter of 2018. We generated net losses of $21.6 million and $14.5 million for the six months ended June 30, 2018 and 2019, respectively. The $14.5 million net loss included a $15.9 million accrual for estimated royalties related to ongoing litigation.

The complexity of biology

Biology is staggeringly complex. The cell is the basic, fundamental organizational unit of all biological organisms. A human being starts from a single cell, which divides into over 40 trillion cells–such as blood cells, skin cells, muscle cells, bone cells, stem cells and neurons–to create the tissues that enable all necessary functions in the human body. These cells utilize the basic building blocks of DNA, RNA and protein, configured in cell-specific ways.

DNA, the hereditary material of living organisms, is the foundation for a series of biological processes that form the basis for biology and how cells function. DNA is transcribed into messenger RNA (“mRNA”) in a process referred to as transcription or, alternatively, gene expression. Information from the mRNA molecules is then translated into protein in a process called translation. Each gene has the ability to create multiple different mRNAs, resulting in the production of over 100,000 different mRNAs from about 30,000 genes. The complete collection of all of the DNA, mRNA and proteins are called the genome, transcriptome or gene expression profile, and the proteome, respectively. The epigenome includes molecular configurations and chemical DNA modifications that affect how genes are regulated. The genome, epigenome, transcriptome and proteome can be distinct for each of the trillions of cells in the human body and collectively constitute a rich architecture of biology.

Industry direction

The 20th century discovery of DNA, RNA, protein and the basic molecular and cellular mechanisms of their function paved early foundations for humanity to understand our own biology. In the early 2000s, the study of biology shifted from focusing on individual genes and their products to a more global level of characterizing the full collection of DNA, RNA and proteins and how they interact, giving rise to the field of genomics. Genomics is a broad, highly interdisciplinary field that approaches the study of biology at a system-wide level. We believe that genomics-based approaches will encompass much of biology and medical applications in the coming decades.

The Human Genome Project, which was completed in 2003, determined a reference sequence of the three billion nucleotides of the human genome as a composite over several individuals. This reference sequence

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provided an initial “parts list” of genes, enabling researchers to begin understanding human biology at a global molecular level.

The subsequent two decades of genomic research in many ways have been defined by genome-wide association studies (“GWAS”) and large-scale sequencing of individuals and populations. The goal was to compile all of the genetic variants in human populations and to link those variants to different conditions, traits and diseases. These associations would serve to generate clues and hypotheses that can be tested by subsequent experimentation to understand the detailed biology of each gene and variant.

Both of these efforts have provided substantial value and have been foundational in enabling multiple new research and clinical applications. However, much of the initial promise of the Human Genome Project and subsequent GWAS projects remains unfulfilled. We believe this is ultimately due to the tremendous underlying complexity of biology. The human genome project provided a list of parts and subsequent GWAS projects looked for statistical links between these parts and various diseases and traits. Going forward we need to understand the biological function of each gene and all the molecular and cellular networks they encode. Genomics needs to expand from its focus on the genome and statistical associations to the study of biology more broadly.

This presents an enormous challenge because of the limited capabilities of existing tools for accessing biology at the molecular and cellular level. Some of these limitations are:

- Average, or “bulk”, measurements obscure underlying differences between different biological units, such as individual cells;
- Low throughput prevents requisite sampling of the underlying complexity—for example, when only a few hundred cells can be evaluated at a time;
- Limited number of biological analytes are interrogated, giving a myopic view of only a few biological processes;
- Limited ability for multi-omic interrogation;
- Inefficient use of sample to generate a signal of sufficient strength to analyze the biological molecules of interest; and
- Inadequate bioinformatics and software tools.

We believe technologies that address these limitations will serve large and unmet market needs by providing a better understanding of molecular and cellular function, the origin of disease and how to improve of treatment.

Measure the full complexity of biology. A major need is for an in-depth cataloguing of biological complexity. This will involve going from a basic biological parts list to a detailed map of exactly how all of these parts are used and interact in both healthy and disease states. Researchers and clinicians need to characterize every cell in the human body, to understand how cell-to-cell variations in genomes, epigenomes, transcriptomes and proteomes give rise to function or dysfunction. They also need to characterize every tissue at a full molecular and cellular level, including how cells are arranged together into spatial patterns that affect function, give rise to disease, or impact treatment. For example, in the context of cancer biology, many tumors consist of a heterogeneous population of healthy and cancerous cells, the latter of which may consist of genetically distinct subpopulations that are susceptible to different therapeutics. Furthermore, different spatial patterns of cancer antigens may require different treatment approaches. Without being able to see cells and molecules in their spatial context it is difficult to fully understand tumor resistance and how cells interact with one another within the tumor microenvironment and enable targeted therapies.

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Massively parallelize experimentation. Mastering biology will require moving beyond the cataloguing of biological complexity and into performing experiments to understand the impact of active changes to biological systems. We believe technologies that enable measurement of massively parallel perturbation and the impact of these perturbations will be important for accelerating biological and medical discovery. For example, an unmet goal of researchers has been to compile all of the genetic variations in human populations and link those variations to different conditions, traits and diseases. Linking these variations to disease requires the analysis of the impact of these variations within different systems, alone and in various combinations. Technologies that enable these variations to be created in arbitrary combinations within various biological contexts and the impact of these combinations measured in a massively parallel fashion will highly accelerate this work. In another example, a longstanding need of researchers has been to predict the interactions between immune cells and the target molecules they can recognize. The human body can make over a trillion different immune cells that are collectively capable of recognizing and mounting a response to nearly any conceivable antigen. We believe that understanding, and ultimately harnessing, this targeting will require technologies that can enable the massively parallel screening of interactions between a set of recognizing immune cells and a set of synthetic antigen target molecules.

We believe technologies that address these needs will redefine biological discovery and power a Century of Biology in which many of humanity's most pressing health challenges will be solved by precision diagnostics, targeted therapies and cures to currently intractable diseases.

Our solutions

We have built and commercialized multiple product lines that allow researchers to interrogate, understand and master biological systems at a resolution and scale commensurate with the complexity of biology. We believe that our products overcome the limitations of existing tools. Our vision, discipline and multidisciplinary approach have allowed us to continuously innovate to develop the platforms, molecular assays and software that underlie our solutions.

Our technological imperatives: resolution and scale

Resolution and Scale are the imperatives that underlie our products and technology. First, our solutions enable understanding biology at the right level of biological resolution, such as at the level of the single cell or at high spatial resolution of tissues and organs. Second, we believe that high resolution tools only become truly powerful when they are built into technologies with tremendous scale. Measuring individual cells, spatial portions of tissues, or molecular interactions in small numbers is insufficient. Our products enable measuring and manipulating up to millions of single cells or thousands of tissue sample positions. Thus, our products provide the appropriate levels of both resolution and scale in a manner that allows researchers to easily sift through the complexity to access the underlying biology.

Our platforms, molecular assays and software

Our Chromium platform, recently announced Visium platform, molecular assays and software constitute the building blocks of our integrated solutions. These shared building blocks allow us to rapidly build and improve our solutions for studying biology at the appropriate resolution and scale:

Our Chromium platform enables high-throughput analysis of individual biological components. It is a precisely engineered reagent delivery system that divides a sample into individual components in up to a million or more partitions, enabling large numbers of parallel micro-reactions. In this manner, for example, the individual single cells of a large population of cells can be segregated so that each cell resides in its own partition. Each partition then behaves as a micro-scale reaction vessel in which its contents are barcoded with a DNA sequence that

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specifically identifies those contents as being distinct from the contents of other partitions. Once biological material in each partition is barcoded, they can then be pooled and sequenced together. Finally, the barcode sequences can be used to easily tease apart information originating from different partitions. Our paradigm of partitioning and barcoding gives researchers the ability to measure many discrete biological materials and/or perform many different experiments in parallel, providing tremendous resolution and scale.

We have leveraged our Chromium platform to create a suite of solutions that measure biological analytes at the resolution of the single cell, the most fundamental organizational unit of biology. We believe that, in this sense, all of biology is single cell biology and that our single cell solutions can enhance and sharpen a wide array of scientific work in genetics, developmental biology, molecular biology and cell biology.

Our Visium platform is being designed to identify where biological components are located and how they are arranged with respect to each other, otherwise referred to as “spatial analysis”. Our spatial platform will use high density DNA arrays which will have DNA barcode sequences that will encode the physical location of biological analytes within a sample, such as a tissue section. This should allow the spatial location of the analytes to be “read out” using sequencing to constitute a visual map of the analytes across the sample. Similarly to partitioning, spatial barcoding should gain tremendous power with large numbers of probes on an array, providing high resolution visualize patterns across biological tissues.

Our molecular assays are used with our Chromium platform, and with our planned Visium platform, to provide sensitive and robust biochemistries that convert minute amounts of biological analytes into detectable signals. We have created a wide variety of proprietary assays compatible with our platforms for measuring the genome, epigenome, transcriptome and proteome. For example:

- Our GEM-RT assay is a highly sensitive technique for detecting mRNA molecules that are in low abundance in single cells. Less sensitive methods easily miss low abundance mRNA molecules, resulting in loss of information about the activities of many important genes that are detectable using our assay.
- Our ATAC-seq assay can be used to determine whether particular genes are active or dormant on a system-wide basis and is tremendously useful in studying gene regulation.
- Our Feature Barcoding assay allows simultaneous multi-omic interrogation of different classes of biological analytes in a sample. Feature Barcoding is highly versatile and can be customized to analyze many different classes of analytes for a wide variety of applications.

Our software is essential to our mission of accelerating the mastery of biology. Since it enables new levels of resolution and scale, our platforms and molecular assays produce entirely new types of data and at much larger scales than previously achievable. To that end, we have developed sophisticated and scalable software that completes our solutions which we provide to researchers free of charge. Our analysis software transforms large amounts of raw data into usable results, giving researchers user friendly tools to dynamically explore these results. As larger and larger amounts of biological data are generated with greater ease, we believe that software tools will become increasingly critical for progress in biology.

Since our founding, we have committed to making software engineering and computational biology world-class, core internal competencies. We believe this deep investment distinguishes us from our competition and is worthwhile because it:

- *Removes barriers to adoption.* With our software, our customers can immediately begin making sense of their experimental data. Without it, they would be forced to develop their own software or wait for the community to do so, slowing down adoption of our products by months or even years;

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- *Accelerates pull-through.* Easy-to-use, efficient software helps our customers analyze their data and complete their experiments and studies faster, enabling them to move on to their next experimental questions sooner;
- *Increases scale.* Reliable, scalable software helps to remove analysis as a bottleneck as our customers plan larger and more ambitious experimental designs;
- *Expands the user base.* While early adopters are more likely to have access to bioinformatics expertise, our software enables a broader range of customers to take advantage of our solutions;
- *Enables better understanding of our customers' needs.* By supplying analysis software for our customers, we gain much greater insight into their use cases, helping us to design future products that best meet their needs; and
- *Enhances and accelerates product development.* The software we ship to customers is the same software we use to develop and optimize our platforms and chemistry. This aligns us closely with the needs of our customers and reduces our time-to-market.

Our product development approach

The success of our products is founded on how we approach product development. Our employees are deeply scientifically oriented, having the relevant scientific expertise embedded not only within research and development, but also within the management team and throughout the company. We are ambitious and focus on fundamentals. We strive to solve big challenges to enable new fundamental biology and to build technological capabilities with potential for exponential impact. We work closely with our customers, many of whom are thought leaders in genomics and medicine, to identify future frontiers and unmet needs. Once we identify the correct opportunities, we have the discipline to focus on execution and have a track record of bringing successful products to market. Since 2015, we have launched solutions in six major application areas, including significant version upgrades, which are supported by the launches of two instruments and a continuous stream of software releases.

Multidisciplinary collaboration and technological innovation are central to our product development process. We have built teams with deep expertise across diverse disciplines including chemistry, molecular biology, microfluidics, hardware, computational biology and software engineering. This multidisciplinary expertise forms the basis of our innovation engine, which allows us to introduce new products at a rapid pace as well as continuously launch improved versions of our existing products.

Our solutions enable our customers to focus on biology by providing them with intuitive user interfaces and software. Our products guide customers through the workflow, from preparing samples, to reading sample information on a third-party sequencer, through analyzing and visualizing this information, to make obtaining biological answers as easy as possible. Our workflows operate with existing sequencers that are widely available in research settings.

Our market opportunity

According to industry sources, the worldwide life sciences research tools market totaled more than $50 billion in 2017. Our diverse products and solutions allow biologists to interrogate and understand biological systems at exceptional resolution and scale. Our focus on enabling a comprehensive view of biology, and not narrowly focused on a particular analyte such as DNA alone, has produced products which we believe have broad applications and target numerous market opportunities across different areas of life sciences research. Because we provide solutions to answer a broad diversity of biological questions, we view much of this total market as ultimately accessible to us.

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Markets in which our current solutions offer alternative or complementary approaches to existing tools represented a total market opportunity of approximately $13 billion of the more than $50 billion global life sciences research tools market in 2017. This $13 billion market includes flow cytometry, next generation sequencing, laboratory automation, microscopy and sample preparation, among other tools. In many cases, our current solutions offer alternative approaches to existing tools, where the advantages of our solutions can provide more precise answers to existing biological questions than existing tools and technologies. Our tools may also complement, enhance and enable new applications of these technologies. Within this market, and more broadly within the entire life science research tools market, we believe we will compete for research spending and capture increasing share of research budgets as our solutions deliver new capabilities, enable new applications and lead to new discoveries. We also expect to enter additional markets in the future that will further expand our market opportunity.

We believe a strong benchmark of the potential adoption of our solutions is the installed base of real-time polymerase chain reaction ("RT-PCR") units, which is approximately 50,000 units globally. We also believe, based on industry sources, that there are over 15,000 next generation sequencers installed globally. While owners of next-generation sequencing instruments are one of several potential constituencies for buying our solutions, many of our customers do not own a sequencer and, as our installed base has grown, many of our customers have purchased multiple Chromium instruments. We believe that our market opportunity for placements of our instruments is meaningfully larger than the installed base of next generation sequencers.

Growth of our market opportunity is also driven by a broad and increasing range of applications for our solutions. Our solutions can be used in many different applications, including basic biology, oncology and immuno-oncology, genetic disease, neurological disease, autoimmunity, infectious disease, the human microbiome and many others. As we enter the "Century of Biology", we believe that the mastery of biology will create advances and benefits for a broad and growing range of industries including broader segments of the healthcare industry and beyond.

Our competitive strengths

We believe our continued growth will be driven by the following competitive strengths:

Our position as a leader in a large and growing market. Since launching our first product in mid-2015 through June 30, 2019, we have sold 1,284 instruments and we serve thousands of researchers globally. We have fostered deep relationships with many key opinion leaders and as of June 30, 2019, our customers included 93 of the top 100 global research institutions by publications, and 13 of the top 15 global pharmaceutical companies by 2018 revenue. Our products are entrenched within our customers' workflow and a significant portion of them utilize more than one of our solutions. Our technologies have become a vital tool for biological research. To date, more than 500 peer-reviewed articles have been published based on data generated using our products, with more than 200 of these published in 2018 and more than 200 published so far in 2019. Our position as a leader in this market allows us to form deep partnerships with our customers who help us stay on the frontiers of biology, giving us insight on industry needs that inform our product strategy and providing us with a strong competitive advantage.

Our proprietary technologies. Through multiple years of development, acquisition and licensing, we have amassed a core set of technologies that form the foundation of our growing suite of products and solutions. These technologies, including instruments, assays and software, combine a diverse set of disciplines, including chemistry, molecular biology, microfluidics, hardware, computational biology and software engineering. Our technologies underlie features and performance that differentiate our products from the competition. Further, many of these technological elements can be utilized across multiple products, enabling us to leverage our existing infrastructure and investment when building future products, increasing the speed of product

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development and product performance. As of June 30, 2019, worldwide we owned or exclusively licensed over 175 issued or allowed patents and 470 pending patent applications. We also license additional patents on a non-exclusive and/or territory restricted basis. Our intellectual property portfolio includes foundational patents in single cell analysis, epigenomics, spatial analysis and multi-omics.

Our rigorous product development processes and scalable infrastructure. We have implemented a rigorous and systematic product development process by which our vision can be efficiently translated into commercial products. We develop our products over a set of defined phases delineated by validating multifunctional reviews, which ensure our teams remain focused on quality, efficiency and profitability. This process allows many highly focused teams to execute on separate product development efforts in parallel while drawing effectively on the resources and capabilities of the company. We have also built extensive technological and operational infrastructure to support the efficient execution of these teams. This infrastructure includes multiple technological investments across a range of areas, including custom barcoded gel bead production, microfluidic chip manufacturing, scalable high-performance computation and automated software productization and testing tools. This infrastructure can be drawn on to develop new products and improved versions of our existing products with high quality at a rapid pace.

Our customer experience and broad commercial reach. We believe in providing our customers with a high-quality experience from start to finish: starting with a collection of validated methods for preparation of samples to be run on our systems and ending with extensive software to aid in analysis and visualization of the data generated. We have also built comprehensive product testing and quality control into our culture and processes to help guarantee the performance of our products in customer hands. As of June 30, 2019, we employed a commercial team of over 190 full time employees. This includes an extensive and highly specialized customer service infrastructure with technical specialists covering multiple areas of expertise, including both experimental biology and software. Many members of our sales and customer service teams have a Ph.D. degree in the relevant scientific field. Both our sales and customer service teams help ensure our customers have a positive experience with our products.

Our experienced multidisciplinary team. At 10x, our success begins with our people. We have built a multidisciplinary team with expertise across a diverse set of areas such as chemistry, molecular biology, microfluidics, hardware, computational biology and software engineering who are committed to identifying and addressing problems at the forefront of biology. We have supplemented our diverse technical experience by assembling an operational team with expertise in manufacturing, legal, sales, marketing, customer service and finance. We believe this confluence of talent from multiple disciplines at 10x allows us to stay ahead of our competitors by identifying highly impactful opportunities and building products and solutions that address these opportunities.

Our growth strategy

Our growth strategy includes the following key elements:

Develop critical enabling technologies. Just as our past success is attributable to our innovative technologies, we believe that our future growth will be driven in large part by our significant continued investment in research and development. We aim to build new platforms, consumables and software that further our goals of interrogating, understanding and mastering biological systems at the needed resolution and scale. We prioritize innovations that meet large unmet market needs, such as measuring novel biological analytes with key functional impact at the single cell or spatial level. We expect that these investments in research and development will allow us to increase our penetration of our accessible market.

Expand the installed base of our Chromium instruments. Since our commercial launch in mid-2015 through June 30, 2019, we have placed 1,284 instruments and serve thousands of researchers globally. Utilizing our

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multi-channel sales and distribution, we will continue to engage with researchers to increase our installed base of Chromium instruments. We will target new customers in addition to expanding the number of instruments within institutions that have already recognized the significant value of our technology. A portion of our current laboratory customers do not yet own a Chromium instrument, but rather gain access to one of our instruments through an adjacent lab or core facility within the institution. These customers are substantial and easily accessible and therefore represents an opportunity for future instrument sales. We also intend to expand our existing geographic reach, both directly and through distributors.

Strengthen use and adoption of our consumables. Our instruments are designed to be used exclusively with our consumables. This closed system generates recurring revenue from consumables tied to each instrument we sell. We plan to drive wider adoption of our products within the workflows of our existing customers. For example, although most of the biopharmaceutical companies using our products use them at multiple sites, we believe that as our applications are increasingly incorporated into the validation steps in the drug development process, the amount of our consumables used will grow. We have built a dedicated global strategic sales, marketing and business development team to support the adoption cycle by biopharmaceutical companies. The launch of our Chromium Connect instrument next year is also aimed at driving higher consumable revenue growth, as the fully automated workflow will reduce bottlenecks caused by manual processes. We also plan to demonstrate new applications using our current solutions, including applications making synergistic use of multiple solutions.

Identify the most relevant technologies, create or acquire such technologies and develop them into new products. Over the years, we have developed, acquired and licensed a core set of technologies and associated intellectual property across a broad range of emerging areas within biology and life sciences. The ability to identify these core technologies and capabilities has complemented our internal product development process and enhanced the foundation of our growing suite of products and solutions. We will continue to identify and acquire or license foundational technologies and intellectual property that accelerate the development of new products or complement our existing products and technologies. For instance, we acquired Epinomics and Spatial Transcriptomics in 2018, obtaining technology and intellectual property that formed the foundation of our ATAC-seq assay and spatial platform, respectively.

Promote our platforms as the standard for single and spatial cell analysis. We believe many key opinion leaders have recognized our Chromium platform as the standard for single cell analysis. One of our strategies is to broaden this recognition and promote the breadth of scientific achievements enabled by our products. To date, more than 500 peer-reviewed articles have been published using data generated by our portfolio of Chromium solutions. We also plan to highlight successful instances where our recently announced Visium platform is used to analyze biological samples within their spatial context. Further research and discoveries will unfold as our solutions are utilized as the global standard.

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Our products and technology

Our products are integrated solutions comprised of instruments, consumables and software. They are built with our expertise in chemistry, molecular biology, microfluidics, hardware, computational biology and software engineering. Our products begin with a researcher's sample (such as a collection of thousands to millions of cells) and perform high-throughput barcoding to construct libraries that are compatible with standard sequencers. Our proprietary software then provides turn-key analysis pipelines and intuitive visualization tools that allow researchers to easily interpret the biological data from the samples. A summary of our solutions is as follows:

	10x SOLUTION	INTERROGATES	KEY EXAMPLE APPLICATIONS
Single Cell	*Chromium Single Cell* **Gene Expression Solution** (with Feature Barcoding)	• RNA • Cell surface protein • CRISPR screening	• Developmental Biology, Oncology, Immunology, Neuroscience and BioPharma
Single Cell	*Chromium Single Cell* **Immune Profiling Solution** (with Feature Barcoding)	• Immune cell RNA • Immune cell paired receptor RNA • Immune cell surface protein and antigen specificity	• Immunology, Oncology and BioPharma
Single Cell	*Chromium Single Cell* **ATAC-seq Solution**	• Epigenetics (chromatin accessibility)	• Developmental Biology, Oncology and Immunology
Single Cell	*Chromium Single Cell* **CNV Solution**	• DNA copy number variations	• Oncology and Neuroscience
Single Cell	*Chromium* **Linked-Read Solution**	• Long read genome information	• Human Genetics, Oncology, Population Genetics and AgBio
Spatial	*Visium Spatial* **Gene Expression Solution** (expected launch in late 2019)	• RNA locations	• Pathology and Oncology

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Our Chromium Platform

Our Chromium platform, which includes our Chromium Controllers, microfluidic chips and related consumables, enables high-throughput analysis of individual biological components. It is a precisely engineered reagent delivery system that divides a sample into individual components in up to a million or more partitions, enabling large numbers of parallel micro-reactions. The Chromium platform can be used to partition not only single cells, but also other biological materials such as cell nuclei and DNA molecules. The large numbers of partitions generated using our Chromium products can be used for analyzing samples at high resolutions and at large scales. We pair a partitioned sample with our proprietary gel beads bearing barcodes that allow researchers to uniquely identify the contents of each partition and distinguish them from contents of other partitions. We refer to the partitions that are generated on our Chromium platform as “GEMs”, which stands for Gel beads in EMulsion. We collectively refer to our partitioning and barcoding technologies as our GemCode technology.

Our Chromium Controller and microfluidic chips. All of our Chromium consumables run on our Chromium Controller instrument. We have designed our instrument to be widely accessible to researchers with a list price of $75,000 and a form factor that easily fits on a standard laboratory bench. Our Chromium Controller operates exclusively with our microfluidic chips, which are highly engineered single-use devices that process sample and reagents. During our Chromium workflows, the researcher loads sample onto the microfluidic chip along with our proprietary gel beads and oils. The loaded chip is inserted into the Chromium Controller, which facilitates the generation of GEMs that contain sample and gel beads. We plan to launch the Chromium Connect next year, a high-throughput version of our Chromium instrument that incorporates liquid handling robotics to automate our workflow.

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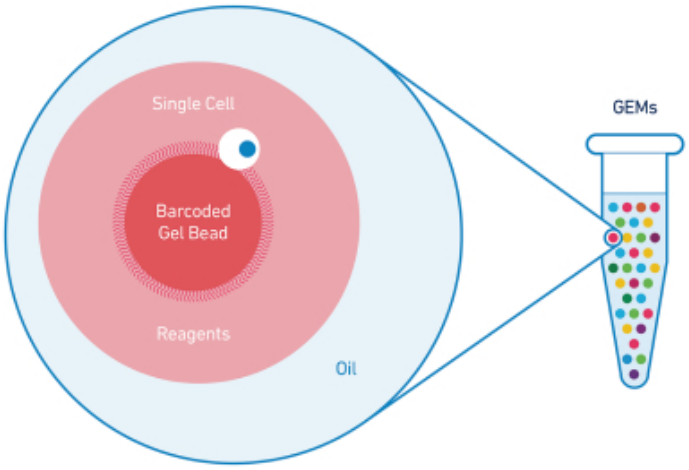

Our Gel Beads. Within each GEM, the sample is co-encapsulated with one of our proprietary gel beads which are designed to contain a unique, identifying DNA barcode for subsequent sequencing and analysis. Our gel beads, which we manufacture in-house using proprietary methods, incorporate barcoded DNA molecules that are designed to react with the sample inside each GEM. The GEMs act as individual reaction vessels to generate barcoded molecules. We have developed various molecular assays that can be used to perform barcoding reactions with different types of biological analytes—for example, our proprietary GEM-RT assay incorporates sequences of mRNA into barcoded molecules. Once those barcoded molecules are generated inside individual GEMs, the GEMs can be broken and their contents pooled to generate libraries that can be analyzed by widely available third-party sequencers. Critically, because different GEMs have different DNA barcodes, each sequencing read can be traced back to its GEM of origin, allowing identification of the biological source or context of the contents of the GEM. This barcoding paradigm enables multiplexing across very large numbers of cells or other biological material.

Key GemCode advantages. Our GemCode technology has a number of technological advantages over alternative tools. For example, our gel beads are composed of proprietary materials that permit their incorporation into GEMs at high efficiency. This efficiency increases the number of partitions that include one and only one barcoded gel bead and avoids loss of information from samples that are not paired with barcodes. Furthermore, the chemical structure of our gel beads allows them to not only encapsulate hundreds of millions of copies of DNA barcode oligonucleotides, but also permit their controlled release at precise times during our workflow. Similarly, our microfluidic chips are engineered to highly precise dimensions and consist of materials that optimize the partitioning of biological materials into GEMs. Such features enable our Chromium platform to provide a combination of superior performance characteristics for single cell analyses:

- *High cell throughput*: How many cells can be measured at once? Measuring more cells with resolution allows researchers to look for rare cells in a population. If a disease-causing cell occurs in only 1 in 10,000 cells in a sample, then measuring just 1,000 cells will be unlikely to find a single copy of the disease-causing cell. Our Single Cell Gene Expression and Immune Profiling solutions, on the other hand, have cell throughputs of up to 80,000 cells per run using one microfluidic chip which increases the likelihood of finding a copy of the disease-causing cell.
- *High cell capture rate*: What fraction of the researcher's sample cells are measured rather than lost? A high cell capture rate is important in many cases where researchers start with only a limited number of rare cells, such as a tumor biopsy from a patient. Our Single Cell Gene Expression and Immune Profiling solutions, for

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example, have typical cell capture rates of about 65%, which is significantly higher than those achieved by many competing solutions.

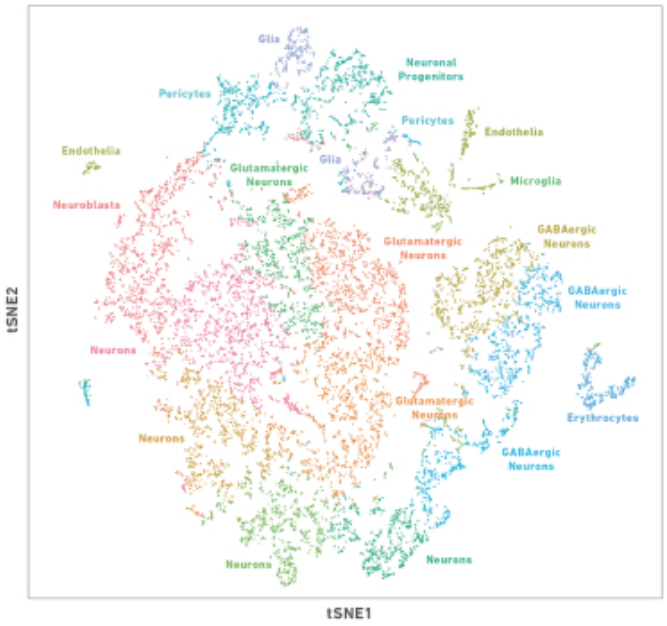
- *Low doublet rate*: How often do researchers avoid doublets—artifacts where two or more cells are read as one? Doublets result in loss of cell information, inaccurate information, and wasted sequencing. Researchers seek products with low doublet rates. Our Single Cell Gene Expression and Immune Profiling solutions, for example, have doublet rates of less than 1% per 1,000 cells.

Our Chromium platform currently provides researchers with solutions in five major application areas:

Single Cell Gene Expression

Our Chromium Single Cell Gene Expression solution provides customers with the ability to measure the transcriptome of single cells, revealing gene activity and networks on a cell-by-cell basis. This approach enables customers to identify and characterize rare cell types in a population of cells, characterize cell populations without prior knowledge of cell subtypes or cell markers, define novel cell types and cell states, discover new biomarkers for specific cell populations and analyze and understand cellular heterogeneity and its effects on biological systems.

For this solution, customers run their samples of interest on the Chromium Controller to generate GEMs containing single cells and prepare single cell libraries using our reagents. Researchers can sequence these single cell libraries on compatible third-party sequencers, analyze their data using our Cell Ranger analysis pipeline software and visualize their data using our Loupe Cell Browser software. The browser displays a visual representation of the data in which cells having similar gene expression profiles are colored and clustered together. Researchers can explore their data by cluster or gene(s) of interest to derive biological meaning from the visualizations. The following visualization is an example showing single cell profiling of approximately 10,000 mouse brain cells that reveals multiple types of neurons.



t-SNE projection of approximately 10,000 mouse brain cells derived from the combined cortex, hippocampus and ventricular zones of embryonic day 18 brain tissue. Major subpopulations were identified based on gene markers that are enriched in each class.

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Our Single Cell Gene Expression solution uses our proprietary biochemistry, GEM-RT to capture mRNA molecules with high sensitivity. Sensitivity is the number of different mRNA transcripts that can be detected. Higher sensitivities are required to detect mRNA molecules that are present in low abundance in a cell. Our latest version of this solution uses a new GEM-RT biochemistry that now has an increased sensitivity of up to 8,500 unique transcripts per cell.

Furthermore, our Single Cell Gene Expression solution can be used with our Feature Barcoding technology to simultaneously measure multiple analytes in the same cells. Our Feature Barcoding is highly customizable, allowing our customers to add a barcode to any biological feature they want to analyze in conjunction with gene expression and other biological data. Feature Barcoding can currently be used to:

- Measure cell surface proteins simultaneously with gene expression, giving a far fuller picture of the states of single cells that includes the transcriptional profile inside the cells as well as the proteins on the outside of the cells; and
- Measure a set of CRISPR genetic perturbations that have been applied to a cell simultaneously with the resulting changes to gene expression and/or surface protein characterization, allowing users to interrogate the impact of actively perturbing many different aspects of a biological system in a massively parallel fashion.

Our Single Cell Gene Expression solution, along with our other single cell solutions, are currently used by the Human Cell Atlas (“HCA”). The HCA is an international consortium of prominent genomics researchers that has emerged as the first and largest project aiming to develop reference maps for all cell types in all tissues of the human body. In 2017, we announced a collaboration with the HCA to enable pilot research projects. Under the terms of this collaboration, we provide members of the Human Cell Atlas consortium with discounts on our instruments and consumables. Sales to members of the Human Cell Atlas consortium accounted for less than 10% of our revenue for the year ended December 31, 2018. To our knowledge, none of our competitors have similar arrangements with the Human Cell Atlas. Our collaboration agreement with the Human Cell Atlas can be terminated at any time by either party. In much the same way that the standardized reference human genome generated by the Human Genome Project in 2003 paved the way for significant leaps in genomics, we believe that creation of a standardized reference of human cell types is critical for future advances. We believe that our partnership with the HCA is a recognition of the quality of our products and may accelerate their adoption by the wider research community.

To date, more than 350 peer-reviewed scientific publications have been published using data generated by our Single Cell Gene Expression solution with the top research areas being developmental biology, immunology and oncology. This body of work is already yielding insights into diseases. For example, in 2018 researchers used our solution to identify all of the cell types present in the mouse trachea. They found the seven previously known lung cell types, but also found evidence for an eighth rare cell type that was previously unknown. This rare cellular population, comprising less than 1% of all lung cells in both mouse and human, was found to express more than 50% of the lung Cftr protein. Loss of Cftr protein in humans is known to cause cystic fibrosis, a relatively common inherited disorder for which carrier screening is routinely performed. Although the gene underlying cystic fibrosis has been known for nearly 30 years, the cells that may be most critical to understanding the progression of disease were unknown until single cell expression analysis became available.

Single Cell Immune Profiling

Our Chromium Single Cell Immune Profiling solution is used to study the immune system, which is the body’s natural diagnostic and therapeutic system. The immune system has a vast network of T-cells and B-cells that recognize pathogens using receptor molecules that bind to foreign molecules, or antigens. T-cells and B-cells can generate an immense diversity of receptors that are each specific to a different potential antigen, making it possible for the human body to recognize nearly any conceivable antigen. Our Single Cell Immune Profiling

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solution enables researchers to study these receptor molecules at the single cell level in conjunction with the transcriptome of the immune cell. Through this, researchers can measure both the T-cell or B-cell receptors while also determining whether the cell has been activated to attack its target or is quiescent and waiting for a threat to emerge. Importantly, because our analysis is performed at the single cell level, we obtain information regarding the pairing of the sequences of the alpha and beta chains of T-cell receptors or the heavy and light chains of B-cell receptors. This paired receptor information is unavailable from traditional bulk approaches for analyzing immune cells and is critical as it is the pair of receptors that defines the targets of each immune cell. By enabling paired immune receptor and transcriptome analysis in massive numbers of immune cells, our Single Cell Immune Profiling solution sheds insight on the clonality, diversity and cellular context of the immune repertoire.

The workflow of this solution, which is similar to that of the Single Cell Gene Expression solution, utilizes our Chromium Controller to generate GEMs, followed by single cell library preparation and sequencing. In contrast to Gene Expression, our Single Cell Immune Profiling solution uses a different biochemistry that obtains sequence information from the 5' end of mRNA molecules, rather than their 3' end. This biochemistry allows researchers to capture the more information-rich regions of immune receptor transcripts. Our Single Cell Immune Profiling solution also includes a step of enriching for immune receptor transcripts using specific primers to create an immune-specific library that can be sequenced separately from gene expression. We have also developed specialized pipelines within our Cell Ranger software and a specialized visualization software, Loupe V(D)J Browser, for visualizing the paired immune receptor information derived from this product. This software allows researchers to identify cell type clusters based on gene expression and then layer T-cell and/or B-cell receptor sequence diversity directly onto that visualization, enabling users to easily derive biological meaning from these two different data types. The following visualization is an example showing the simultaneous assessment of paired immune cell receptor information and gene expression in colorectal cancer cells.

	V	D	J	C
H	IGHV1-3	IGH10R15-1A	IGHJ5	IGHA1
λ	IGLV2-14	-	IGLJ1	IGLC1

Overlay of gene expression and Ig clonotypes for colorectal cancer cells visualized using Loupe Cell Browser. Light blue dots indicate an Ig clonotype call. Dark blue dots show the location of the most prevalent Ig clonotype in the plasma cell cluster, with the table outlining the gene calls for the heavy (H) and lambda λ light chain. The paired H and λ chain V(D)J sequences are shown to the right and corresponding V(D)J nucleotides are color-coded (5'UTR: gray, V: red, D: yellow, J: green, C: purple).

Feature Barcoding can be used in combination with our Single Cell Immune Profiling solution, adding significant multi-omic functionality. Importantly, this functionality allows users to determine the antigen that is bound by

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immune cells simultaneously with their gene expression. This capability allows researchers to determine both the receptor sequences of individual immune cells as well as an antigen that the receptor targets and makes this analysis practical to perform for millions of immune cells. We believe that the capability to understand immune receptor-antigen interactions at a high-throughput single cell level is tremendously valuable for elucidating the rules of immune cell targeting and can be used to understand disease and identify leads for immunotherapies.

We believe our technology can assist researchers in constructing an immune map of receptor-antigen targeting rules. Such a map would allow for the prediction of the antigens recognized by a given receptor, or conversely, the prediction of receptors that bind to a given antigen. Due to the large number of potential receptor sequences and the large number of possible antigens, researchers previously assumed that computational prediction of the cognate antigen from receptor sequence alone would be impractical. However, recent work demonstrated that T-cell receptor sequences that recognize the same antigen shared enough sequence features that a computational prediction framework for mapping T-cell receptors to antigens is feasible. We believe that our Single Cell Immune Profiling Solution combined with Feature Barcoding will enable extending this work at far higher scales.

As a proof of concept for the immune map, we presented at the Advances of Genomes, Biology and Technology (“AGBT”) meeting in February 2019 results from a single experiment utilizing our Single Cell Immune Profiling Solution on approximately 200,000 T-cells from four individuals and 44 feature-barcoded antigens to identify T-cell receptor-antigen pairs. This experiment, which took place over approximately one week, generated a paired receptor-antigen dataset six times larger than the collection of all previously published receptor-antigen pairings. This leap was made possible by the tremendous resolution and scale with which the immune system can be analyzed using our solutions.

Single Cell ATAC

Our Chromium Single Cell ATAC solution enables customers to understand the epigenetic state—including how the genome and its surroundings are modified to “open” and “closed” states, affecting how genes are regulated—in up to millions of cells. While our Single Cell Gene Expression solution answers the “what” of what makes two cells different from each other, our Single Cell ATAC solution answers the “how”. These two products are highly complementary and can be used as a powerful combination to understand both the cause and effect of gene regulation.

ATAC-seq stands for “Assay for Transposase Accessible Chromatin using sequencing”. This technique uses an engineered transposase enzyme to insert nucleic acids tags into the genome while also excising the tagged sequences from its surroundings. ATAC-seq is based on the fact that the transposase enzyme will preferentially tag and excise regions of the genome that have an “open” chromatin state that is unimpeded by proteins bound to genomic DNA. The tagged sequences can be sequenced to infer genomic regions of increased chromatin accessibility as well as map regions that are bound by transcription factor proteins responsible for regulating gene expression. ATAC–seq was pioneered by researchers at Stanford University and is exclusively licensed to us. ATAC-seq has now become an important tool in epigenetics and genome-regulation research.

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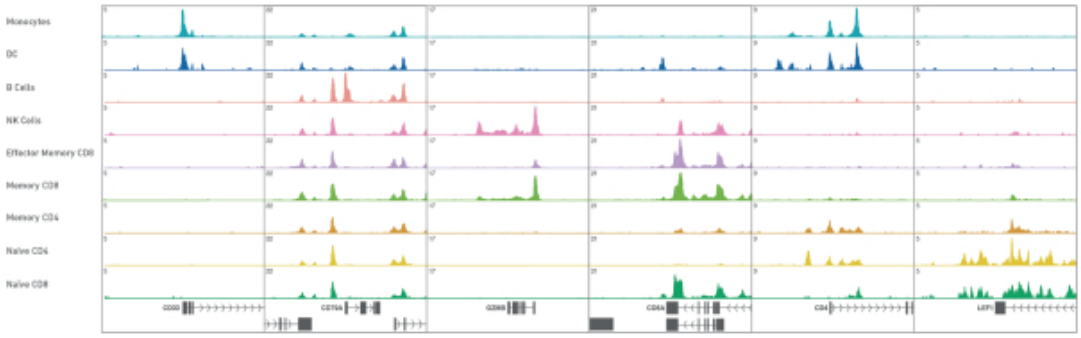
Our Single Cell ATAC solution uses the ATAC-seq assay in conjunction with our Chromium platform to create a product for high-throughput epigenetic interrogation at single cell resolution. In the workflow, users treat cell nuclei with transposase enzyme and then use our Chromium Controller to encapsulate these nuclei in GEMs. The tagged sequences from the nuclei are barcoded inside GEMs and then processed to generate sequencing libraries. Sequencing reads are analyzed using our Cell Ranger ATAC software, and visualized using our Loupe Cell Browser, which has been especially configured to display epigenetic data. The following visualization is an example of plots showing open chromatin around genes that are specifically associated with certain cell types.



Open chromatin signals around marker genes are specifically associated with the cell type of expression. Plots show aggregate chromatin accessibility profiles for each cluster at several marker gene loci.

Though we only launched this product in October 2018, our Single Cell ATAC solution has already been adopted by a number of key opinion leaders. In one example, researchers used a combination of single cell transcriptome profiling and single cell ATAC-seq to identify enhancer elements that mark specific sub-classes of cells in the mouse brain. Once these elements are identified they can be targeted in order to generate mice with specific cell types labeled or perturbed at a level of specificity not usually achievable using gene expression alone. The ability to specifically target new cell types of interest allows in-depth investigations of the functions of those targeted cells.

Single Cell Copy Number Variation (CNV)

Our Chromium Single Cell CNV solution enables the measurement of DNA changes—specifically changes in the number of copies of DNA segments—on a genome-wide basis at single cell resolution. This product is particularly useful for cancer research. Tumor cells frequently mutate and change such that a single “tumor” is actually comprised of many different types of tumor cells having different DNA mutations. This tumor heterogeneity allows different tumor cell types to evolve separately and respond differently to treatments. Our Single Cell CNV solution product enables researchers to systematically measure genomic differences between cells, providing information that is crucial in understanding how cancers evolve and can provide valuable insights into cancer treatment.

Our Single Cell CNV solution leverages a two-step process in which we first encapsulate cellular contents into cell beads, which are composed of a synthetic material that renders the genomic contents of individual cells accessible to our assays’ biochemistries. Once cell beads are formed, they are encapsulated into GEMs along with barcoded gel beads and undergo a reaction to generate barcoded sequencing libraries. Our Single Cell CNV solution has a cell throughput of up to 20,000 cells per run, cell capture rates of approximately 15% and doublet rates of less than 1% per 1,000 cells. Sequencing data is analyzed using our Cell Ranger DNA pipelines software, and visualized using our Loupe scDNA Browser, which offers intuitive visualization of DNA copy

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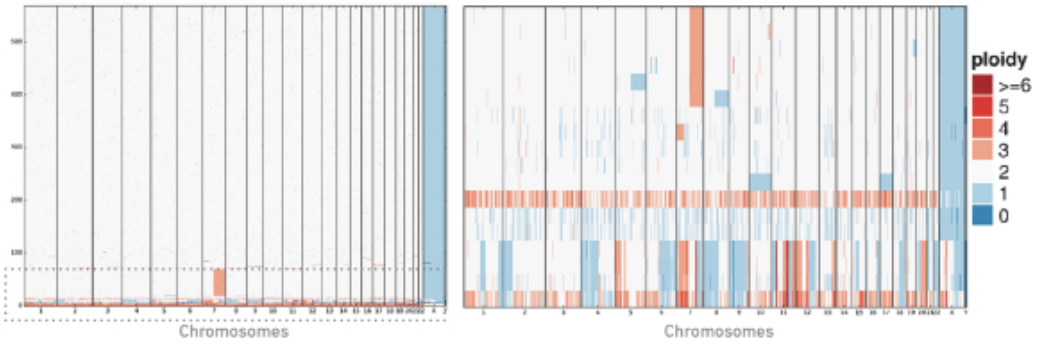
number changes along each human chromosome in the genome. The following visualization is an example of the detection of rare clones of a cell population having a particular DNA copy number variation.



Left: Heatmap showing the CNV profiles of 569 cells after 1% spike-in of MKN-45 cells (cancer cell line) into a BJ diploid cell line sample. The CNV profiles primarily correspond to the diploid cell line, while the bottom region of the heatmap corresponds to the MKN-45 cells.

Right: Enlargement of the bottom region of the heatmap highlighting the heterogeneous, non-diploid CNV profiles of the MKN-45 cells and an amplification in chromosome 7 of the diploid cell line, demonstrating that cell lines may not always be homogeneous.

This product, which became widely available in the third quarter of 2018, is yielding insights into disease states. For example, in a study undertaken by a major research university utilizing our products, gastric cancer samples were subjected to both single cell gene expression profiling and single cell CNV profiling. This combined approach allowed the direct comparison of sub-clonal structure revealed by DNA and RNA profiling. This study revealed that the use of both assays provided a more complete picture of the structure of the different cancerous and non-cancerous cells in their sample. This solution provides more resolution to researchers, enabling them to better understand the variations between the DNA in cloned cells.

For information relating to limitations on our ability to sell our Single Cell CNV solution, see the section titled *"Risk factors—Risks related to litigation and our intellectual property—We are involved in significant litigation which has consumed significant resources and management time and adverse resolution of these lawsuits could require us to pay significant damages, and prevent us from selling our products, which would severely adversely impact our business, financial condition or results of operations"*.

Our Visium platform

We are designing our Visium platform to enable researchers to understand the spatial positions of biological analytes within tissues at high resolution. Such spatial analysis can be critically important in understanding tissue function in both healthy and disease states. For example, in the context of neurobiology, neuronal degeneration in the *substantia nigra*, an area of the brain associated with movement, results in Parkinson's disease, while degeneration of upper and lower motor neurons results in amyotrophic lateral sclerosis, or Lou Gehrig's disease. In the context of cancer treatment, the knowledge of whether T-cells have infiltrated inside of a tumor, rather than merely surrounding the tumor, is an important prognostic indicator. Understanding the spatial relationship of the biological analytes in tissues may hold the key to unlocking the underlying causes and identifying cures for such diseases.

Our Visium products will be based on technology that we acquired from Spatial Transcriptomics in 2018. Spatial Transcriptomics utilized arrays having specialized probes on their surfaces that are encoded with the spatial position of the probe. In the Visium product workflow, a tissue sample will be placed onto the array. Reagents will be added by the user to create barcoded molecules from these probes and the biological material in the tissues. This barcoded material will encode the spatial information that was contained in the probes. Users will

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then pool the material from the array and follow a protocol to create libraries of molecules that can be sequenced using a standard sequencer. After sequencing, analysis software will assign each sequencing read to its spatial position of origin. Collectively, the spatially defined reads will provide a visual depiction of the locations and patterns of large numbers of biological analytes simultaneously in the tissue sample.

The Spatial Transcriptomics product performed spatial analysis of mRNAs using arrays that had 1,000 probes with distances of approximately 200 microns between probes. This product was used to identify heterogeneity in metastatic melanoma and to demonstrate that there was significantly more heterogeneity than could be predicted by manual pathology annotation. In an independent study of mouse and human amyotrophic lateral sclerosis samples researchers were able to observe changes in RNA expression over the disease course, while preserving the understanding of those changes in the spatial context. This allowed them to visualize the key changes that occur in brain regions before and during neuronal degeneration.

We are developing our Visium solution for spatial gene expression analysis, which we expect to launch in late 2019. Our Visium gene expression product is expected to have significant improvements over the Spatial Transcriptomics product, including increased spatial resolution, increase gene sensitivity, a simpler workflow and fully developed analysis and visualization software. Past this launch, we intend to continuously innovate to provide enhanced resolution, performance, throughput and efficiency. We also intend to develop additional Visium spatial products using our other assays which, analogously to the Chromium platform, allows spatial interrogation of a broader range of biological analytes including DNA, immune molecules, epigenetics and protein.

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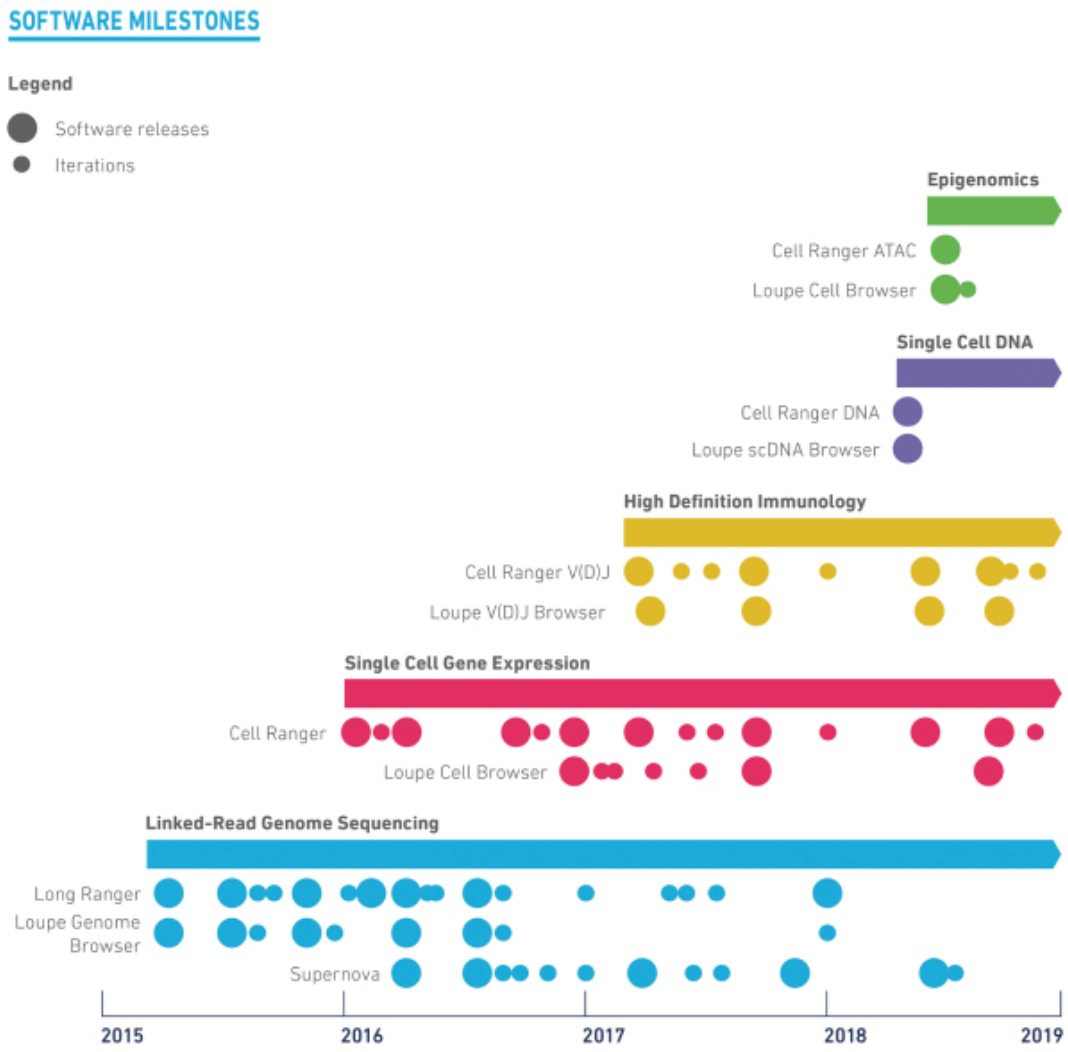
Our analysis and visualization software

Our software is a fundamental part of our integrated solutions and is comprised of two parts, analysis and visualization. Our analysis pipeline software tools, including Cell Ranger, Long Ranger and Supernova, take raw sequencing data as input and transform them into biologically meaningful results. Customers can further analyze these results in their own or third-party tools, or take them into our Loupe family of visualization software tools, which allow users to draw insights using an intuitive user interface without writing code. Our analysis and visualization software is generally available to researchers free of charge, so as to accelerate the adoption of our products and software as a standard for genome and single cell analysis.



Since our launch, we have shipped almost 50 major releases of our software. We believe that the main factors that differentiate our software include:

- **Ease of installation and use.** Much of the software typically used in bioinformatics analysis requires substantial programming expertise to use and even just to install. We invest substantial effort in making our

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software both easy to install and use, so researchers can focus on their experiments rather than installation requirements.

- **Advanced algorithms and methods.** Our software makes the latest analytical methods easily accessible to researchers and we are constantly working to improve our software's ability to realize the maximum value and benefit of the data produced by our chemistries and platforms.
- **Scalable from workstation to cluster to cloud.** A robust, common architecture underlying our software tools gives researchers maximum flexibility to run our software on-premises on individual workstations or servers, on large high-performance compute clusters and in private and public clouds.

Peer-reviewed scientific publications using our products

To date, more than 500 peer-reviewed articles have been published based on data generated using our products. More than 90 of these articles were published in three of the most highly-regarded journals: *Cell*, *Nature* and *Science*. Underscoring the reach of our products, these publications cover a wide range of research and applied areas from cell biology to genetic health to neuroscience with the top three areas of publication being developmental biology, immunology and cancer research.

Research area	Number of articles	Percentage
Developmental Biology	112	17%
Immunology	88	14%
Cancer Research	72	11%
Computational Method	62	10%
Neuroscience	51	8%
Genome Assembly	41	6%
Cell Biology	37	6%
Other	33	5%
Assay Method	31	5%
Cell Atlas	31	5%
Genetic Health	29	5%
Microbiology	13	2%
Population Genetics	13	2%
Agrigenomics	11	2%
Conservation Biology	10	2%
Reproductive Biology	7	1%

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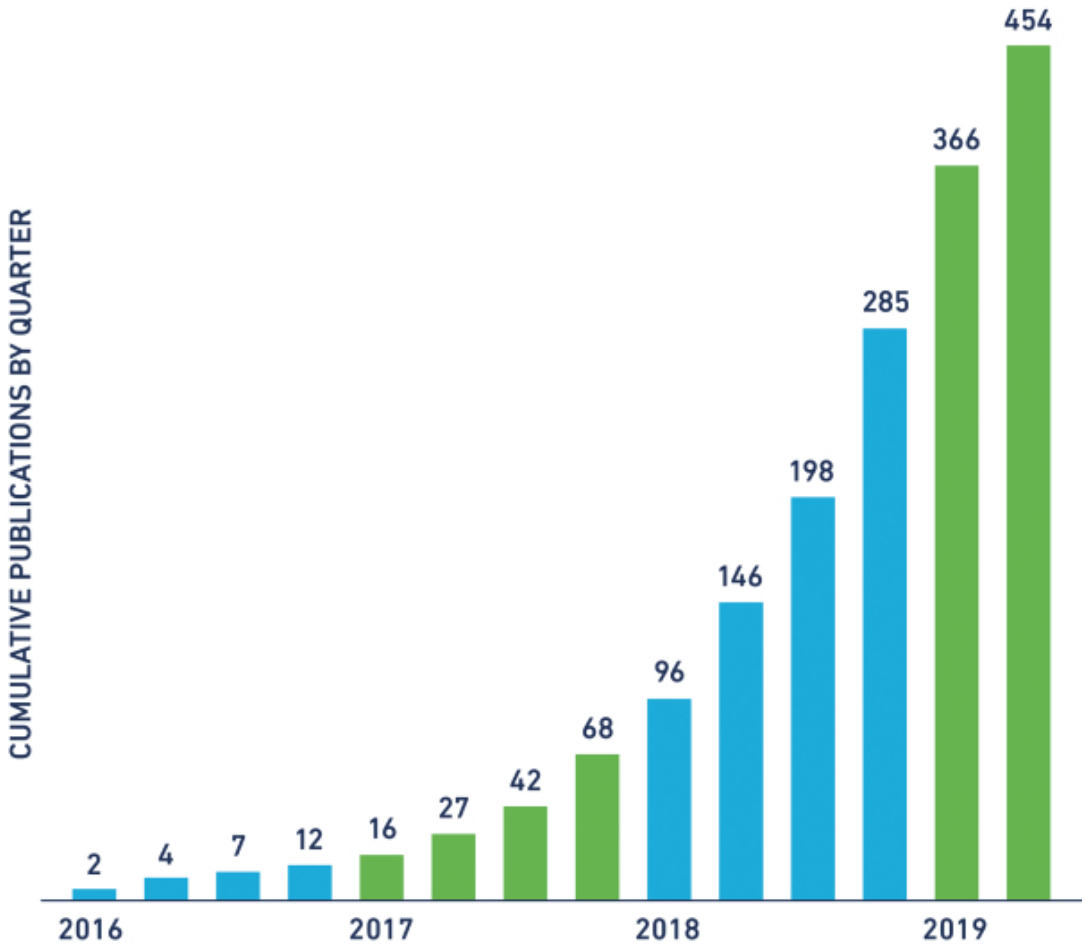
We have seen robust quarter-over-quarter growth in the number of publications commensurate with our commercial growth and success:



These publications describe, for example, the use of our products to:

- Construct a molecular map of cellular differentiation during early development in mice;
- Understand kidney tumors by studying cell types and compositions in malignant versus normal cells;
- Track patients with aggressive skin cancer undergoing immunotherapy to understand how the body develops resistance to immunotherapy;
- Understand why multiple myeloma, a cancer originating from plasma cells, is symptomatic or asymptomatic depending on underlying cell types, and identify rare circulating tumor cells as a potential early diagnostic indicator;
- Demonstrate that transcriptional diversity in cutaneous T cell lymphomas can be used to predict disease stage and guide treatment;
- Identify non-essential genes in humans;

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- Identify structural rearrangements in cancer; and
- Study the microbiome.

Research and development

Our research and development teams have designed and developed our proprietary products using an interdisciplinary approach that combines expertise across the fields of chemistry, molecular biology, microfluidics, hardware, computational biology and software engineering. Our research and development groups work together in cross-functional project teams; an approach that has been key to our success to date. Our research and development teams are currently located in our headquarters in Pleasanton, California and in Stockholm, Sweden.

The overarching goals of our research and development programs are to continue to bring new technologies to market that address the most pressing questions in biology and to provide exponential advances in human health. To this end, we plan to focus our research and development efforts on the following areas:

Improve the performance of our existing solutions. We plan to improve our existing assays and software. These improvements may provide increased sensitivity to capture greater amounts of signal from biological analytes, broader types of biological samples that can be interrogated with our solutions and larger amounts of biological information that can be obtained using our software.

Develop new solutions for our Chromium platform. We plan to expand the range of solutions that are available on our Chromium platform to allow researchers access to new types of biological information. For example, we are planning to develop additional *multi-omics* solutions on our Chromium platform for simultaneous interrogation of different classes of analytes.

Develop our Visium platform. We plan to introduce a product that offers high spatial resolution, high sensitivity, efficient workflow and analysis and visualization software. We are working to develop new technologies for our Visium platform that will further enhance the spatial resolution, usability and automation of our platform.

Improve and develop new capabilities for our Chromium instruments. We plan to develop new capabilities that would improve the usability and increase the performance of our Chromium instruments by increasing automation, throughput, workflow visibility or troubleshooting capabilities.

Develop combined software and workflows across multiple solutions. We plan to develop workflows that enable users to run multiple assays on the same biological samples and software that simultaneously analyzes the data generated from these multiple assays. We plan to do this for key solution combinations where the information obtained from the two solutions is highly complementary.

Investigate new technologies. We will seek to both develop and acquire new technologies that could be additive to or complementary with our current portfolio.

Our research and development costs were $32.2 million and $47.5 million for the years ended December 31, 2017 and 2018, respectively, and $23.3 million and $33.0 million for the six months ended June 30, 2018 and 2019, respectively. In-process research and development costs, consisting of costs incurred to acquire intellectual property for research and development were $62.4 million for the year ended December 31, 2018 and $0 for the six months ended June 30, 2019. As of June 30, 2019, we employed 192 employees in research and development. Looking forward, we will continue to invest in efforts to support the ongoing development of our instruments, consumables and software, as well as enhance the overall performance of our solutions.

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Commercial

Commercial team

We began the full launch of our first product in mid-2015 and have sold thousands of products globally. Our customers primarily include academic, government, biopharmaceutical, biotechnology and other institutions focused on life sciences research. We sell our products primarily through our own direct sales force in North America and certain regions of Europe. As of June 30, 2019, our commercial organization consisted of 192 full time employees, including 78 commissioned sales representatives, many with Ph.D. degrees and many with significant industry experience. We sell our products through third-party distributors in Asia, certain regions of Europe, South America, the Middle East and Africa. We have sold products in approximately 40 countries.

For both the year ended December 31, 2018 and the six months ended June 30, 2019, no single customer, including distributors, represented greater than 10% of our business. For both the year ended December 31, 2018 and the six months ended June 30, 2019, sales to academic institutions represented approximately 70% of our direct sales revenue. We expect that sales to biopharmaceutical companies will represent a growing proportion of our revenue in the future.

Commercial strategy

Our products are integrated solutions comprised of instruments, consumables and software. We aim to drive customer adoption and the installed base of our Chromium instruments which then forms a base of users who drive revenue by purchasing our consumables. Our products are designed to be easy to install and use without the need for extensive training.

Our customers primarily include academic, government, biopharmaceutical, biotechnology and other institutions. Our strategy typically involves targeting key opinion leaders during the initial phase of our product launches, after which we aim to expand adoption of our products across a broader base of customers. As our customer base has grown, we have been able to leverage our larger installed base of instruments to accelerate the adoption of new solutions. Approximately half of our customers purchased our consumables relating to more than one of our solutions in both the year ended December 31, 2018 and the six months ended June 30, 2019.

Our commercial strategy focuses on ensuring our customers are successful with our products. These successes often result in publications which can drive increased public awareness and further market adoption. Since our first product launch in 2015, there have been more than 500 publications by researchers using data generated by our products.

Our direct sales and marketing efforts are targeted at the principal investigators, research scientists, department heads, research laboratory directors and core facility directors at leading academic institutions, biopharmaceutical companies and publicly and privately-funded research institutions who control the buying decision. Due to the pricing of our instruments and consumables, the buying decision is typically made by the principal investigator rather than by committee or department chair, which we believe simplifies the purchasing decision and has helped accelerate adoption of our products. The sales cycle of our Chromium Controller instrument is typically between four and six months.

We also target researchers who do not own their own Chromium Controller instrument, but who have access to one, which we refer to as “halo users”. By sharing one instrument across groups within an institution, multiple halo users are able to utilize the instrument for their own research and experiments, contributing meaningfully to consumable pull-through on just one instrument. Halo users help drive consumable revenue and utilization of our consumable products and may become future purchasers of a Chromium instrument.

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The use of our products requires the access to, but not necessarily the ownership of a third-party next-generation sequencer, since sequencers are often accessible as a shared resource. This broadens our target customer base beyond those who own a next-generation sequencer.

We increase awareness of our products among our target customers through direct sales calls, trade shows, seminars, academic conferences, web presence, social media and other forms of internet marketing. We supplement these traditional marketing efforts by fostering an active online community of users of our products consisting of communities, forums and blogs with internally generated and user-generated content. We also provide education and training resources, both online and in person.

Suppliers and manufacturing

Consumables

The majority of our consumable products are manufactured in-house at our facilities in Pleasanton, California. These manufacturing operations include: gel bead generation, surfactant synthesis and emulsion oil formulation, reagent formulation and tube filling, microfluidic chip manufacturing, kit assembly and packaging as well as analytical and functional quality control testing. We achieved ISO 9001:2015 certification in the fourth quarter of 2017, which covers design, development, manufacturing, distribution, service and sales.

We obtain some components of our consumables from third-party suppliers. While some of these components are sourced from a single supplier, we have qualified second sources for several of our critical reagents, including microfluidic chips, arrays and oligonucleotides. We believe that having dual sources for our components helps reduce the risk of a production delay caused by a disruption in the supply of a critical component. For further discussion of the risks relating to our third-party suppliers, see the section titled "*Risk factors—Risks related to our business and industry—We are dependent on single source and sole source suppliers for some of the components and materials used in our products and the loss of any of these suppliers could harm our business*".

Instruments

We outsource manufacturing for our Chromium Controller to two qualified contract manufacturers. These manufacturers have represented to us that they each maintain ISO 9001 and ISO 13485 certification. Our recently announced Chromium Connect will include an automated workflow liquid handling robot which will be manufactured by our partner.

Employees

As of June 30, 2019, we had 500 employees, including 192 in research and development, 192 in sales, marketing, support and business development, 74 in general and administrative and 42 in manufacturing. None of our United States employees are represented by a labor union or covered under a collective bargaining agreement and we consider our relationship with our employees to be positive. As of June 30, 2019, 426 of our employees were employed in the United States and 74 were employed outside the United States.

Facilities

Our corporate headquarters, research and development facilities and manufacturing and distribution centers are located in Pleasanton, California, where we lease approximately 200,000 square feet of space under leases expiring between December 2020 and September 2029. These leases include our global headquarters and research and development center occupying approximately 150,000 square feet in Pleasanton, California. We do not own any real property and believe that our current facilities, together with our global headquarters and research and development center, are sufficient to meet our ongoing needs and that, if we require additional space, we will be able to obtain additional facilities on commercially reasonable terms.

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Competition

The life sciences market is highly competitive. There are other companies, both established and early-stage, that have indicated that they are designing, manufacturing and marketing products for, among other things, genomics analysis, single cell analysis and spatial analysis. These companies include Becton, Dickinson and Company, Bio-Rad Laboratories, Inc. and Nanostring Technologies, Inc., each of which has products that compete to varying degrees with some but not all of our product solutions, as well as a number of other emerging and established companies. Some of these companies may have substantially greater financial and other resources than us, including larger research and development staff or more established marketing and sales forces. Other competitors are in the process of developing novel technologies for the life sciences market which may lead to products that rival or replace our products.

However, we believe we are substantially differentiated from our competitors for many reasons, including our position as a leader in a large and growing market, proprietary technologies, rigorous product development processes and scalable infrastructure, customer experience and multidisciplinary teams. We believe our customers favor our products and company because of these differentiators.

For further discussion of the risks we face relating to competition, see the section titled "*Risk factors—Risks related to our business and industry—The life sciences technology market is highly competitive. If we fail to compete effectively, our business and operating results will suffer*".

Government regulation

The development, testing, manufacturing, marketing, post-market surveillance, distribution, advertising and labeling of certain of medical devices are subject to regulation in the United States by the Center for Devices and Radiological Health of the U.S. Food and Drug Administration ("FDA") under the Federal Food, Drug, and Cosmetic Act ("FDC Act") and comparable state and international agencies. A medical device is an instrument, apparatus, implement, machine, contrivance, implant, *in vitro* reagent or other similar or related article, including any component part or accessory, which is (1) intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in man or other animals, or (2) intended to affect the structure or any function of the body of man or other animals and which does not achieve any of its primary intended purposes through chemical action within or on the body of man or other animals and which is not dependent upon being metabolized for the achievement of any of its primary intended purposes. Medical devices to be commercially distributed in the United States must receive from the FDA either clearance of a premarket notification, known as 510(k), or premarket approval pursuant to the FDC Act prior to marketing, unless subject to an exemption. None of our products are currently medical devices and all of our products are currently designed "For Research Use Only. Not for use in diagnostic procedures" ("RUO") products, as they are not meant for clinical applications. RUO products are not regulated as medical devices and are therefore not subject to the regulatory requirements enforced by the FDA. The products must bear the statement: "For Research Use Only. Not for Use in Diagnostic Procedures". RUO products cannot make any claims related to safety, effectiveness or diagnostic utility and they cannot be intended for human clinical diagnostic use. In November 2013, the FDA issued a final guidance on products labeled RUO, which, among other things, reaffirmed that a company may not make any clinical or diagnostic claims about an RUO product. The FDA will also evaluate the totality of the circumstances to determine if the product is intended for diagnostic purposes. If FDA were to determine, based on the totality of circumstances, that our products labeled and marketed for RUO are intended for diagnostic purposes, they would be considered medical devices that will require clearance or approval prior to commercialization. Further, sales of devices for diagnostic purposes may subject us to additional healthcare regulation. We continue to monitor the changing legal and regulatory landscape to ensure our compliance with any applicable rules, laws and regulations.

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For further discussion of the risks we face relating to regulation, see the section titled "*Risk factors—Risks related to our business and industry—Our products could become subject to government regulation and the regulatory approval and maintenance process for such products may be expensive, time-consuming and uncertain both in timing and in outcome*".

Intellectual property

Our success depends in part on our ability to obtain and maintain intellectual property protection for our products and technology. We utilize a variety of intellectual property protection strategies, including patents, trademarks, trade secrets and other methods of protecting proprietary information.

As of June 30, 2019, worldwide we owned or exclusively licensed over 175 issued or allowed patents and 470 pending patent applications. We also license additional patents on a non-exclusive and/or territory restricted basis. Patent rights generally have a term of twenty years from the date in which they were filed. We own registered trademarks on 10X GENOMICS and product related brand names in the United States and worldwide.

We license certain U.S. and foreign patents and patent applications from various third parties for use in our products and technology. Some of these license agreements provide use the exclusive right to practice the licensed intellectual property subject to specific field or territory restrictions and certain fee and royalty arrangements. Subject to common termination rights, these exclusive license agreements typically are in force until the last of the licensed patents expires or, in some cases, upon our failure to achieve specified sales volume thresholds. Certain of these agreements also require that any products related to the licensed patents be substantially manufactured in the United States.

In connection with our acquisition of Spatial Transcriptomics, we are required to make contingent payments to the sellers based on revenue from sales of Spatial Transcriptomics products and our soon to be introduced Visium products, for the years ended December 31, 2019 through December 31, 2022. These contingent payments are equal to a percentage in the teens multiplied by such revenue. Pursuant to the license agreement we entered into with The Board of Trustees of the Leland Stanford Junior University ("Stanford"), we are required to pay Stanford a low single-digit royalty percentage based on the net revenue of certain ATAC-seq products during the applicable term of the licensed patents. Pursuant to the license agreement we entered into with the President and Fellows of Harvard University ("Harvard"), we are required to pay Harvard a low single-digit royalty percentage based on the net revenue of certain products covered by the licensed patents during the applicable term of those patents. For the years ended December 31, 2017 and 2018 and during the six months ended June 30, 2019, we made aggregate contingent and royalty payments under the Spatial Transcriptomics acquisition agreement, Stanford license agreement and Harvard license agreement, collectively, of less than $2.0 million, less than $4.0 million and less than $4.0 million, respectively. We expect the size of these payments to grow as our business grows and particularly as we launch our anticipated Visium products in late 2019.

The patents we own expire beginning in 2033 and the patents we exclusively license expire beginning in 2028. The Harvard license is exclusive in the field of sequencing sample preparation and single cell analysis and is projected to terminate in 2034. The Stanford license is exclusive in all fields and the initial exclusivity period of the license terminates in 2025, however we have the option to extend the exclusivity period for additional one-year terms if we meet certain minimum sales thresholds beginning in 2025. If the exclusivity period ends or we fail to extend the exclusivity period, we retain a non-exclusive license to the applicable patents. The Stanford license is projected to terminate in 2038. Both the Harvard and Stanford licenses are worldwide licenses.

We intend to pursue additional intellectual property protection to the extent we believe it would be beneficial and cost-effective. We cannot provide any assurance that any of our current or future patent applications will result in the issuance of patents, or that any of our current or future issued patents will effectively protect any of our products or technology from infringement or prevent others from commercializing infringing products or technology.

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For further discussion of the risks relating to intellectual property, see the section titled "*Risk Factors—Risks related to litigation and our intellectual property*".

Legal proceedings

We are regularly subject to claims, lawsuits, arbitration proceedings, administrative actions and other legal and regulatory proceedings involving commercial disputes, competition, intellectual property disputes and other matters, and we may become subject to additional types of claims, lawsuits, arbitration proceedings, administrative actions, government investigations and legal and regulatory proceedings in the future and as our business grows, including proceedings related to product liability or our acquisitions, securities issuances or our business practices, including public disclosures about our business. Our success depends in part on our non-infringement of the patents or proprietary rights of third parties. Third parties have asserted and may in the future assert that we are employing their proprietary technology without authorization. As we enter new markets or introduce new products, we expect that competitors will likely claim that our products infringe their intellectual property rights, including as part of a business strategy to impede our successful competition. There are inherent uncertainties in these legal matters, some of which are beyond management's control, making the ultimate outcomes difficult to predict. We are currently involved in the following litigation matters:

The 2015 Delaware Action

In February 2015, Raindance Technologies, Inc. ("Raindance") and the University of Chicago filed suit against us in the U.S. District Court for the District of Delaware, accusing that substantially all of our products that use our GEM microfluidic chips are infringing seven U.S. patents owned by or exclusively licensed to Raindance (the "Delaware Action"). In May 2017, Bio-Rad was substituted as the plaintiff following its acquisition of Raindance. A jury trial was held in November 2018. The jury found that all of our accused products infringed one or more of U.S. Patent Nos. 8,304,193, 8,329,407 and 8,889,083. The jury also concluded that our infringement was willful and awarded Bio-Rad approximately $24 million in damages. Post-trial, Bio-Rad moved for a permanent injunction, treble damages for willful infringement, attorneys' fees, supplemental damages for the period from the second quarter of 2018 through the end of the trial as well as pre- and post-judgment interest.

The Court denied Bio-Rad's request for attorneys' fees and enhanced damages for willful infringement. The Court awarded supplemental damages for the period from the second quarter of 2018 through the end of trial as well as pre- and post-judgment interest. The Court entered final judgment against us in the amount of approximately $35 million in August 2019. There could be additional future damages from the final judgment until the patents in suit expire in 2023.

In the fourth quarter of 2018, we began recording an accrual for estimated royalties as cost of revenue. This accrual is based on an estimated royalty rate of 15% of worldwide sales of our Chromium instruments operating our GEM microfluidic chips and associated consumables. As of June 30, 2019, we had accrued a total of $55.3 million relating to this matter which includes the $35 million judgment and our estimated 15% royalty for sales through that date.

The Court also granted Bio-Rad a permanent injunction against our GEM microfluidic chips and associated consumables that were found to infringe the Bio-Rad patents, which have historically constituted substantially all of our product sales. However, under the injunction, we are permitted to continue to sell our GEM microfluidic chips and associated consumables for use with our historical installed base of instruments provided that we pay a royalty of 15% into escrow on our net revenue related to such sales. We have appealed the injunction to the Federal Circuit and expect that it will not take effect until the Federal Circuit rules on our request for a stay of the injunction.

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We have dedicated significant resources to designing and manufacturing our Next GEM microfluidic chips which use fundamentally different physics from our GEM microfluidic chips. Neither the jury verdict nor the injunction relate to our Next GEM microfluidic chips based on our new proprietary design and associated consumables which we launched in May 2019 for three of our single cell solutions – Single Cell Gene Expression, Single Cell Immune Profiling and Single Cell ATAC. We currently expect that, by the end of the third quarter of 2019, all Chromium instruments that we sell will operate exclusively with our Next GEM solutions and that our Chromium products utilizing our Next GEM microfluidic chips will constitute substantially all of our Chromium sales by the end of 2020.

The ITC 1068 Action

On July 31, 2017, Bio-Rad and Lawrence Livermore National Security, LLC filed a complaint against us in the U.S. International Trade Commission ("ITC") pursuant to Section 337 of the Tariff Act of 1930, accusing substantially all of our products of infringing U.S. Patents Nos. 9,089,844, 9,126,160, 9,500,664, 9,636,682 and 9,649,635 (the "ITC 1068 Action"). Bio-Rad is seeking an exclusion order preventing us from importing the accused microfluidic chips, including (1) our GEM microfluidic chip, (2) our gel bead manufacturing microfluidic chip and (3) our Next GEM microfluidic chip, into the United States and a cease and desist order preventing us from selling such imported chips. Prior to the introduction of our Next GEM microfluidic chips and related products, substantially all of our product sales used GEM microfluidic chips. An evidentiary hearing for the ITC 1068 Action was held in May of 2018 and the presiding judge issued an Initial Determination in September 2018, finding that our GEM microfluidic chips infringe the '664, '682 and '635 patents but not the '160 patent. The judge further found that our gel bead manufacturing microfluidic chip and our Next GEM microfluidic chip do not infringe any claims asserted against them.

The judge recommended entry of an exclusion order against our GEM microfluidic chips, which are currently being imported into the United States. If the ITC were to adopt the judge's recommendation regarding the exclusion order, we would be prevented from importing such chips, which are used in substantially all of our products, into the United States. The judge also recommended a cease and desist order that would prevent us from selling such imported chips. The ITC is not reviewing the judge's findings that our GEM microfluidic chips directly infringe the '664, '682 and '635 patents. The ITC is currently reviewing the judge's findings that (1) we indirectly infringe the '682 and '635 patents, (2) our gel bead manufacturing microfluidic chip does not infringe certain claims in the '664 patent and (3) our Next GEM microfluidic chip does not infringe certain claims in the '160 and '664 patents. A Final Determination is expected to be issued in late September 2019. The Final Determination is subject to a 60-day presidential review period before taking effect. If the Initial Determination were to be upheld, then we would be unable to import our GEM microfluidic chips and sell such imported chips, which are used in substantially all of our products. The judge recommended a bond of 100% of the entered value of accused products imported during the Presidential review period.

In order to allow our customers to continue their important research, we have dedicated significant resources to developing the capabilities to manufacture our microfluidic chips in the United States prior to the entry of an exclusion order or cease and desist order which could take effect in late November 2019. Prior to the second quarter of 2019, all of our microfluidic chips were manufactured outside of the United States. We expect our United States manufacturing facilities to achieve volume production of certain of our GEM microfluidic chips accounting for the majority of our United States consumable revenue beginning in the third quarter of 2019.

The Northern District of California Action

On July 31, 2017, Bio-Rad and Lawrence Livermore National Security, LLC also filed suit against us in the U.S. District Court for the Northern District of California, alleging that substantially all of our products infringe U.S. Patents Nos. 9,216,392, 9,347,059 and the five patents asserted in the ITC 1068 Action. The complaint seeks

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injunctive relief, unspecified monetary damages, costs and attorneys' fees. This litigation has been stayed pending resolution of the ITC 1068 Action.

The Germany Action

On February 13, 2018, Bio-Rad filed suit against us in Germany in the Munich Region Court alleging that our Chromium instruments, GEM microfluidic chips and certain accessories infringe German Utility Model No. DE 20 2011 110 979. Bio-Rad seeks unspecified damages and an injunction prohibiting sales of these products in Germany and requiring us to recall these products sold in Germany subsequent to February 11, 2018. The accused GEM microfluidic chips are currently manufactured in Germany and are currently used in substantially all of our solutions. An initial hearing was held on November 27, 2018, and a subsequent hearing was held on May 15, 2019. The Court has not yet issued a ruling on the merits.

The 2018 Delaware Action

On October 25, 2018, Bio-Rad filed suit against us in the U.S. District Court for the District of Delaware alleging that substantially all of our products infringe U.S. Patent Nos. 9,562,837 and 9,896,722. Bio-Rad seeks injunctive relief, unspecified monetary damages, costs and attorneys' fees. Discovery is in progress.

The Becton Dickinson Action

On November 15, 2018, Becton, Dickinson and Company and Cellular Research, Inc. filed suit against us in the U.S. District Court for the District of Delaware, alleging that we infringe U.S. Patent Nos. 8,835,358, 9,845,502, 9,315,857, 9,816,137, 9,708,659, 9,290,808, 9,290,809, 9,567,645, 9,567,646, 9,598,736 and 9,637,799. The complaint asserted that our instruments, consumables and software that comprise our Single Cell Gene Expression solution, Single Cell Immune Profiling solution and Spatial Transcriptomics product infringe these patents. Plaintiffs seek injunctive relief, unspecified monetary damages, costs and attorneys' fees. On January 18, 2019, we filed a motion to dismiss certain of the asserted claims on the grounds that they are directed to patent ineligible abstract ideas. The Court has not yet ruled on or set a hearing date for the motion. Discovery is in progress.

The ITC 1100 Action

On January 11, 2018, we filed a complaint against Bio-Rad at the ITC pursuant to Section 337 of the Tariff Act of 1930 alleging that Bio-Rad infringes our U.S. Patent Nos. 9,644,204, 9,689,024, 9,695,468 and 9,856,530 (the "ITC 1100 Action"). The judge issued an Initial Determination on July 12, 2019 finding that Bio-Rad infringes the '024, '468 and '530 patents. The judge also found all of our asserted patents to be valid and rejected Bio-Rad's claim of ownership in all of the asserted patents. The Target Date for the Final Determination is scheduled for November 12, 2019, when we expect the ITC to issue an exclusion order preventing Bio-Rad from importing into the United States infringing microfluidic devices, components thereof and products containing same, including the ddSEQ single cell analysis products. We also expect the ITC to issue a cease and desist order preventing Bio-Rad from selling such imported products in the United States.

For further discussion of the risks relating to intellectual property and our pending litigation, see the section titled "*Risk factors—Risks related to litigation and our intellectual property*".

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Management

The following table sets forth information regarding our executive officers and directors as of August 16, 2019:

Name	Age	Position
Serge Saxonov	42	Chief Executive Officer and Director
Benjamin J. Hindson	44	Chief Scientific Officer, President and Director
Bradford J. Crutchfield	57	Chief Commercial Officer
Justin J. McAnear	44	Chief Financial Officer
Jean M. Philibert	59	Chief People Officer
Eric S. Whitaker	52	General Counsel
John R. Stuelpnagel(1)(2)	61	Chairman of our board of directors
Paul A. Conley(3)	51	Director
Sridhar Kosaraju(1)	41	Director
Mathai Mammen(3)	52	Director
Bryan E. Roberts(1)(2)	52	Director
Shehnaaz Suliman(3)	48	Director

(1) Member of the audit committee.

(2) Member of the compensation committee.

(3) Member of the nominating and corporate governance committee.

Executive officers

Serge Saxonov, Ph.D. co-founded 10x Genomics and has served as our Chief Executive Officer and on our board of directors since July 2012. Dr. Saxonov also served as our President from July 2012 until October 2012. Prior to co-founding our company, Dr. Saxonov was Vice President of Applications at QuantaLife, a privately-held life sciences company that developed and commercialized a droplet digital polymerase chain reaction platform, from May 2010 to April 2012. Dr. Saxonov was Founding Architect and Director of research and development at 23andMe, a privately held personal genomics and biotechnology company, from June 2006 until May 2010. Dr. Saxonov received a Ph.D. in biomedical informatics from Stanford University and an A.B. in applied mathematics from Harvard College.

We believe that Dr. Saxonov is qualified to serve on our board of directors because of his experience as our co-founder and Chief Executive Officer, industry knowledge, previous experience and extensive academic training.

Benjamin J. Hindson, Ph.D. co-founded 10x Genomics in July 2012 and has served as our Chief Scientific Officer and President since October 2012 and has served on our board of directors since July 2012. Dr. Hindson served as our President of Technology and Treasurer from July 2012 until October 2012 and Secretary from October 2012 until April 2014. Prior to co-founding our company, Dr. Hindson was Co-founder and Chief Scientific Officer of QuantaLife from August 2008 until its sale to Bio-Rad Laboratories in October 2011. From 2002 to 2008, Dr. Hindson served in various positions at Lawrence Livermore National Laboratory, in the Chemical and Biological Weapons Non-proliferation Program. Dr. Hindson earned his B.Sc. in Chemistry and his Ph.D. in Chemistry from Deakin University, Australia.

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We believe that Dr. Hindson is qualified to serve on our board of directors because of his experience as our co-founder, President and Chief Scientific Officer, industry knowledge, previous experience and extensive academic training.

Bradford J. Crutchfield has served as our Chief Commercial Officer since February 2017. From June 2015 to February 2017, Mr. Crutchfield served as Qiagen's senior vice president, life sciences business area. Prior to that, from October 2014 to April 2015, Mr. Crutchfield served as vice president and general manager, Europe, Middle East & Africa, for Illumina. From 1985 to 2014, Mr. Crutchfield held positions with Bio-Rad Laboratories including executive vice president and president of the Life Science Group. From June 2013 until October 2014, he was a director of Nanostring Technologies. Mr. Crutchfield holds a B.S. in Physiology from the University of California, Davis.

Justin J. McAnear has served as our Chief Financial Officer since October 2018. From August 2015 to October 2018, Mr. McAnear was the Vice President of Worldwide Finance and Operations at Tesla. From September 2013 to August 2015, Mr. McAnear served as a Finance Director at Apple in Corporate FP&A and Worldwide Operations and from February 2011 to September 2013, Mr. McAnear served as a Senior Finance Manager in Worldwide Operations. Mr. McAnear began his corporate finance career at Johnson & Johnson in August 2006 and left in February 2011. Mr. McAnear served over nine years in the U.S. Navy as an aviator and is a graduate of the U.S. Naval Academy in Annapolis, MD, where he earned his B.S. degree in Systems Engineering. He also holds an M.B.A. in Finance from the University of San Diego.

Jean M. Philibert has served as our Chief People Officer since April 2018. From January 2016 until March 2018, Ms. Philibert served as Senior Vice President of Human Resources at Analog Devices. From December 2014 to December 2015, Ms. Philibert served as the Chief People Officer at KIXEYE. In 1999, Ms. Philibert joined EMC and served as a Senior Director of Human Resources until November 2014. She earned a M.S. in Industrial Relations and Human Resources from Loyola University and a B.A. in French and Art History from the University of Iowa.

Eric S. Whitaker has served as our General Counsel since July 2017. Prior to joining our company, Mr. Whitaker served as the Chief Legal Officer of Nutanix from September 2014 to May 2017, the Chief Legal Officer of SanDisk from January 2013 to September 2014, and General Counsel of Tesla from October 2010 to November 2012. Prior to these roles, Mr. Whitaker served as General Counsel for a number of technology companies since 1999. Mr. Whitaker also worked as an attorney at Latham & Watkins LLP. Mr. Whitaker holds a J.D. from Stanford Law School and a B.A. in Politics from Princeton University.

Non-employee directors

John R. Stuelpnagel, D.V.M. has been Chairman of our board of directors since August 2013. In addition, Dr. Stuelpnagel co-founded and was Executive Chairman of Ariosa Diagnostics from October 2009 to January 2015 when that company was sold to Roche. He was also the Chairman of Sequenta from November 2010 to January 2015 when that company was merged with Adaptive Biotechnologies where he continued as a member of their board of directors from January 2015 to November 2017. Dr. Stuelpnagel is the Chairman of Fabric Genomics since August 2009, the Chairman of Inscripta since April 2017, the Chairman of Element Biosciences since September 2017, and a member of the board of directors for Encoded Therapeutics since May 2017. Previously, Dr. Stuelpnagel co-founded Illumina in 1998 where he worked until March 2009. Prior to Illumina, Dr. Stuelpnagel was an associate at CW Group from 1997 to 1998. Dr. Stuelpnagel received his B.S. in Biochemistry and his Doctorate in Veterinary Medicine from the University of California, Davis and his M.B.A. from the University of California, Los Angeles.

We believe that Dr. Stuelpnagel is qualified to serve on our board of directors because of his experience as a co-founder of life sciences and pharmaceutical companies, previous and current experience serving as a director and executive officer of other life sciences companies and his extensive experience in business.

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Paul A. Conley, Ph.D. has served on our board of directors since November 2013. Dr. Conley is a Partner and Managing Director at Paladin Capital Group, a prominent venture capital investment firm, a position he has held since November 2007. Dr. Conley also serves on the board of directors of many companies, several in the biotechnology and related fields, including Twist Bioscience, TOMA Bioscience and General Automation Laboratory Technologies. Dr. Conley holds a B.S. in Mechanical Engineering and an M.S. in Mechanical & Aerospace from the University of Virginia, an M.S. in Bioengineering and a Ph.D. in Engineering Sciences in Mechanical Engineering from the University of California, San Diego.

We believe that Dr. Conley is qualified to serve on our board of directors because of his extensive experience in the biotechnology industry, his service on a number of boards which provides an important perspective on operations and corporate governance matters, and his education in biotechnology.

Sridhar Kosaraju has served on our board of directors since April 2019. Mr. Kosaraju has served as the Chief Financial Officer and Head of Strategy of Penumbra since March 2015. Prior to joining Penumbra, he worked in investment banking for J.P. Morgan Securities LLC ("J.P. Morgan") from 1999 to May 2015, where he held a variety of positions with successively greater responsibility, most recently Managing Director of Equity Capital Markets, Head of Healthcare Equity Capital Markets and co-Head of Technology, Media, Telecom Equity Capital Markets. Prior to entering J.P. Morgan's equity capital markets group in 2006, Mr. Kosaraju served in various practice groups at J.P. Morgan, including Equity Derivatives from 2003 to 2006 and Technology, Media, Telecom Investment Banking Coverage from 1999 to 2003. He received a B.S. from Massachusetts Institute of Technology in 1999.

We believe that Mr. Kosaraju is qualified to serve on our board of directors because of his experiences in finance and the healthcare sector, including serving as an executive at a public healthcare technology company.

Mathai Mammen, M.D., Ph.D. has served on our board of directors since August 2017. Dr. Mammen currently serves as global head, science and development at the Janssen Pharmaceutical Companies of Johnson & Johnson. Prior to joining Janssen Pharmaceutical Companies in June 2017, Dr. Mammen was Senior Vice President at Merck Research Laboratories from March 2016 to June 2017. Prior to Merck, Dr. Mammen led research and development at Theravance, a company he co-founded in 1997 until March 2016. In 2014, he and the Theravance Leadership Team separated Theravance into two publicly traded companies: Innoviva and Theravance Biopharma. Dr. Mammen received his M.D. from Harvard Medical School/Massachusetts Institute of Technology (HST program) and his Ph.D. in Chemistry from Harvard University's Department of Chemistry. He received his B.Sc. in Chemistry and Biochemistry from Dalhousie University in Halifax, Nova Scotia.

We believe Dr. Mammen is qualified to serve on our board of directors because of his significant academic training and current and previous experience serving as a director and co-founder of another life sciences company, as well as his operating experience with several life sciences companies.

Bryan E. Roberts, Ph.D. has served on our board of directors since November 2013. Dr. Roberts joined Venrock, a venture capital firm, in 1997, where he currently serves as a Partner. Dr. Roberts currently serves as the Chairman of the board of directors of Achaogen and Castlight Health, which he co-founded, as well as a director on the boards of several private companies. Dr. Roberts previously served on the board of directors of athenahealth from 1999 to 2009, XenoPort from 2000 to 2007, Sirna Therapeutics from 2003 to 2007, Vitae Pharmaceuticals from 2001 to 2016, Zeltiq Aesthetics from 2008 to 2016, Ironwood Pharmaceuticals from 2001 to 2016 and Hua Medicine from 2010 to 2018. From 1989 to 1992, Dr. Roberts worked in the corporate finance department of Kidder, Peabody & Co., a brokerage company. Dr. Roberts received a B.A. in Chemistry from Dartmouth College and a Ph.D. in Chemistry and Chemical Biology from Harvard University.

We believe that Dr. Roberts is qualified to serve on our board of directors because of his experiences with facilitating the growth of health care, health care IT and biotechnology companies.

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Shehnaaz Suliman, M.D., M.Phil., M.B.A. has served on our board or directors since August 2019. In addition, Dr. Suliman has served on the board of directors for Ultragenyx Pharmaceutical Inc. since January 2019. Dr. Suliman served as Senior Vice President, Corporate Development and Strategy of Theravance Biopharma, Inc, from July 2017 to March 2019. Prior to her position at Theravance, Dr. Suliman worked for Roche and Genentech, Inc., as Group Leader and Project Team Leader in the R&D Portfolio Management and Operations Group at Genentech from September 2010 to May 2015 and then as Vice President and Global Therapeutic Area Head, Roche Partnering from June 2015 to July 2017. Prior to Genentech, Dr. Suliman held various management roles of increasing responsibility at Gilead Sciences, Inc., between January 2005 and September 2010. Prior to Gilead, Dr. Suliman was an investment banker with Lehman Brothers and Petkevich & Partners. She has previously served as a member of the board of directors of Parvus Therapeutics, Inc., a private biopharmaceutical company from October 2017 to July 2019. Dr. Suliman received her M.D. (MB, ChB) at the University of Cape Town Medical School, South Africa, and holds an M.B.A, with distinction, and M.Phil. in Development Studies degrees from Oxford University, where she was a Rhodes Scholar.

We believe that Dr. Suliman is qualified to serve on our board of directors due to her extensive operational experience with global biopharmaceutical and life sciences companies, and particularly her expertise in business development and corporate strategy.

Family relationships

There are no family relationships among any of our executive officers or directors.

Board of directors structure

Upon completion of this offering, our board of directors will consist of eight members. Our board of directors has determined that each of Messrs. Stuelpnagel, Conley, Kosaraju, Mammen and Roberts and Ms. Suliman is independent under the applicable Nasdaq listing rules.

Our directors will be divided into three classes serving staggered three-year terms. Class I, Class II and Class III directors will serve until our annual meetings of stockholders in 2020, 2021 and 2022, respectively. The class I directors will consist of Messrs. Hindson, Saxonov and Stuelpnagel. The class II directors will consist Messrs. Conley and Roberts. The class III directors will consist of Messrs. Kosaraju and Mammen and Ms. Suliman. At each annual meeting of stockholders, directors will be elected to succeed the class of directors whose terms have expired. This classification of our board of directors could have the effect of increasing the length of time necessary to change the composition of a majority of the board of directors.

Board of directors committees

Our board of directors has three standing committees: the audit committee; the compensation committee; and the nominating and corporate governance committee. Each committee is governed by a charter which will be available on our website following completion of this offering.

Audit committee

The members of our audit committee are Messrs. Kosaraju, Roberts and Stuelpnagel. Mr. Kosaraju is the chairperson of our audit committee. The composition of our audit committee meets the requirements for independence under the current Nasdaq listing rules and Rule 10A-3 of the Exchange Act. Each member of our audit committee is financially literate and a person with “appropriate accounting or related financial management expertise” under Rule 3.10(2) and 3.21 of the Nasdaq listing rules. In addition, our board of directors has determined that Mr. Kosaraju is an “audit committee financial expert” within the meaning of the

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SEC rules. This designation does not impose on such directors any duties, obligations or liabilities that are greater than are generally imposed on members of our audit committee and our board of directors. Our audit committee is directly responsible for, among other things:

- appointing, retaining, compensating and overseeing the work of our independent registered public accounting firm;
- assessing the independence and performance of the independent registered public accounting firm;
- reviewing with our independent registered public accounting firm the scope and results of the firm's annual audit of our financial statements;
- overseeing the financial reporting process and discussing with management and our independent registered public accounting firm the interim and annual financial statements that we will file with the SEC;
- pre-approving all audit and permissible non-audit services to be performed by our independent registered public accounting firm;
- reviewing policies and practices related to risk assessment and management;
- reviewing our accounting and financial reporting policies and practices and accounting controls, as well as compliance with legal and regulatory requirements;
- reviewing, overseeing, approving or disapproving any related-person transactions;
- reviewing with our management the scope and results of management's evaluation of our disclosure controls and procedures and management's assessment of our internal control over financial reporting, including the related certifications to be included in the periodic reports we will file with the SEC; and
- establishing procedures for the confidential anonymous submission of concerns regarding questionable accounting, internal controls or auditing matters, or other ethics or compliance issues.

Compensation committee

The members of our compensation committee are Messrs. Roberts and Stuelpnagel. Mr. Stuelpnagel is the chairperson of our compensation committee. Each of Messrs. Roberts and Stuelpnagel is a non-employee director, as defined by Rule 16b-3 promulgated under the Exchange Act and will meet the requirements for independence under the current Nasdaq listing standards and SEC rules and regulations. Our compensation committee is responsible for, among other things:

- reviewing and approving, or recommending that our board of directors approve, the compensation of our executive officers;
- acting as an administrator of our equity incentive plans;
- reviewing and approving, or making recommendations to our board of directors with respect to, incentive compensation and equity plans; and
- establishing and reviewing general policies relating to compensation and benefits of our employees.

Nominating and corporate governance committee

The members of our nominating and corporate governance committee are Messrs. Conley and Mammen and Ms. Suliman. Ms. Suliman is the chairperson of our nominating and corporate governance committee. Our nominating and corporate governance committee is responsible for, among other things:

- identifying and recommending candidates for membership on our board of directors, including the consideration of nominees submitted by stockholders, and to each of the board's committees;

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- reviewing and recommending our corporate governance guidelines and policies;
- reviewing proposed waivers of the code of business conduct and ethics for directors and executive officers;
- overseeing and setting compensation for our directors, including approval of performance-based compensation by reference to corporate goals and objectives resolved by the board of directors from time to time;
- overseeing the process of evaluating the performance of our board of directors; and
- assisting our board of directors on corporate governance matters.

Code of business conduct and ethics

Our board of directors has adopted a code of business conduct and ethics that applies to all of our employees, officers and directors, including our Chief Executive Officer, President, Chief Financial Officer and other executive and senior financial officers. Upon completion of this offering, the full text of our code of business conduct and ethics will be posted on the investor relations section of our website. We intend to disclose future amendments to our code of business conduct and ethics, or any waivers of such code, on our website or in public filings.

Compensation committee interlocks and insider participation

None of the members of our compensation committee is or has been an officer or employee of our company. None of our executive officers currently serves, or in the past year has served, as a member of the board of directors or compensation committee (or other board committee performing equivalent functions) of any entity that has one or more of its executive officers serving on our board of directors or compensation committee. See the section titled "*Certain relationships and related party transactions*" for information about related party transactions involving members of our compensation committee or their affiliates.

Compensation of directors

For information on director compensation, see the section titled "*Executive compensation—Director compensation*".

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Executive compensation

The following table sets forth information concerning the compensation paid to our named executive officers ("NEO"), during our fiscal year ended December 31, 2018 ("fiscal year 2018"). Our NEOs for the summary compensation table include our Chief Executive Officer ("CEO") and our next two most highly compensated executive officers.

Summary compensation table

Name and principal position	Year	Salary ($)(1)	Bonus ($)(2)	Stock option awards ($)(3)	Non-equity incentive plan compensation ($)(4)	All other compensation ($)	Total ($)
Serge Saxonov *Chief Executive Officer*	2018	365,833	—	601,010	117,432	—	1,084,275
Justin J. McAnear *Chief Financial Officer*	2018	71,737	—	1,455,118	—	—	1,526,855
Eric S. Whitaker *General Counsel*	2018	316,340	21,748	480,845	55,850	—	874,783

(1) The amounts shown represent the base salaries earned by our NEOs in fiscal year 2018. The amount for Mr. McAnear reflects the prorated portion of his annual base salary of $310,000 earned after commencing employment with us on October 8, 2018. The amount for each of Dr. Saxonov and Mr. Whitaker reflects the increase in their annual base salary to $375,000 effective March 1, 2018 and $319,608 effective March 1, 2018, respectively.

(2) The amount shown for Mr. Whitaker represents the portion of his annual bonus earned based on his individual performance in fiscal year 2018. 25% of Mr. Whitaker's bonus payout was tied to individual performance. Dr. Saxonov's bonus payout did not depend on his individual performance and was tied solely to corporate performance as described in footnote 4 below. For a discussion of the determination of the bonus amounts, see the section titled "*—Other elements of compensation—Fiscal year 2018 annual bonus*".

(3) The amounts shown represent the grant date fair values of stock options to purchase shares of our Historical Class B common stock granted in fiscal year 2018 as computed in accordance with Financial Accounting Standards Board ("FASB") Accounting Standard Codification ("ASC") Topic 718. For a discussion of valuation assumptions used to determine the grant date fair values of equity awards made to NEOs in fiscal year 2018, see the section titled "*Management's discussion and analysis of financial condition and results of operations—Critical accounting policies and estimates—Stock-based compensation*". For a discussion of the terms and conditions of the stock option grants, see the section titled "*—Outstanding equity awards at 2018 fiscal year end*". As a result of the reclassification of our Historical Class B common stock into shares of Class A common stock prior to the closing of this offering as described under "*Description of capital stock—Reclassification of common stock and conversion of Convertible Preferred Stock*", to the extent such stock options remain unexercised at the time of the reclassification, such stock options will become stock options to purchase shares of our Class A common stock.

(4) The amounts shown for each of Dr. Saxonov and Mr. Whitaker represent the portion of their annual bonus earned based on our achievement of certain corporate performance goals established for fiscal year 2018. Dr. Saxonov's bonus payout was tied entirely to corporate performance and 75% of Mr. Whitaker's bonus payout was tied to corporate performance. Mr. McAnear was not eligible to receive a bonus for fiscal year 2018 because he commenced employment with us after the bonus eligibility cutoff date. For a discussion of the determination of these amounts, see the section titled "*—Other elements of compensation— Fiscal year 2018 annual bonus*".

Employment arrangements

This section contains a description of the material terms of the employment arrangements with our NEOs. Our NEOs, other than Dr. Saxonov, signed an offer letter with us, which provides for at-will employment and sets forth other terms of employment, including the initial base salary, target incentive opportunity, the terms of the initial equity grant and, in the case of Mr. McAnear, severance protections upon a qualifying termination. In addition, each of our NEOs executed a form of our standard at-will employment, confidential information, invention assignment and arbitration agreement, which includes a non-solicit of employees covenant during employment and for one year following termination.

Dr. Saxonov

Dr. Saxonov co-founded our company in 2012 and has not been party to an offer letter with us since our inception. In fiscal year 2018, Dr. Saxonov was entitled to an annual base salary of $320,000 (increased to

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$375,000 effective March 1, 2018 and to $400,000 effective April 1, 2019) and was eligible to earn an annual target bonus equal to 30% of his eligible base salary.

Mr. McAnear

Mr. McAnear signed an offer letter with us on August 17, 2018, under which he was entitled to an annual base salary of $310,000 (increased to $330,000 effective April 1, 2019), and at the time of execution, was eligible to earn an annual target bonus equal to 21% of his eligible base salary. However, because Mr. McAnear commenced employment with us after the bonus eligibility cutoff date of August 31, 2018, he was not eligible to receive a bonus for fiscal year 2018. Mr. McAnear's offer letter provides that he is eligible to participate in employee benefit plans that are generally available to other senior executives of our company located in the United States and is entitled to a lump sum severance payment of $500,000 if he is terminated by us without cause (as defined below) prior to October 8, 2019, subject to his execution and non-revocation of a release of claims against us. Pursuant to Mr. McAnear's offer letter, "cause" means our good faith determination that Mr. McAnear has (i) committed either a felony or other crime involving moral turpitude or any other act or omission involving theft, dishonesty, disloyalty or fraud; (ii) substantially and repeatedly failed to follow our policies, procedures and guidelines or substantially and repeatedly failed to perform his duties as reasonably directed by us; (iii) committed a breach of fiduciary duty, gross negligence or willful misconduct with respect to us; or (iv) committed any material breach of his offer letter. If Mr. McAnear's employment is terminated other than for cause, but at a time when we had cause to terminate him (or would have cause if we knew all of the relevant facts), his termination is to be treated as a termination for cause. In connection with his employment, we granted Mr. McAnear stock options to purchase 600,000 shares of our Historical Class B common stock. For a description of the material terms of this stock option grant, see footnote 10 to the outstanding equity awards at 2018 fiscal year end table.

Mr. Whitaker

Mr. Whitaker signed an offer with us on June 12, 2017, under which he was entitled to an annual base salary of $300,000 (increased to $319,608 effective March 1, 2018 and to $340,000 effective April 1, 2019) and was eligible to earn a discretionary annual target bonus equal to 16% (increased to 22% effective March 1, 2018) of his eligible base salary. In addition, Mr. Whitaker is eligible to participate in employee benefit plans that are generally available to other senior executives of our company located in the United States. In connection with his employment, we granted Mr. Whitaker stock options to purchase 525,000 shares of our Historical Class B common stock. For a description of the material terms of this stock option grant, see footnote 11 to the outstanding equity awards at 2018 fiscal year end table.

Other elements of compensation

Fiscal year 2018 annual bonus

We provide our executives an opportunity to earn annual cash bonuses to motivate and reward achievements of certain corporate and individual performance goals for each fiscal year. Because Mr. McAnear commenced employment with us after the bonus eligibility cutoff date for fiscal year 2018, he did not receive a bonus. The target bonus, expressed as a percentage of eligible base salary, for Dr. Saxonov and Mr. Whitaker was 30% and 22%, respectively, for fiscal year 2018. The payout of Dr. Saxonov's bonus was tied entirely to corporate performance and not dependent on individual performance, and the payout of Mr. Whitaker's bonus was tied 75% to corporate performance and 25% to individual performance.

Based on our achievement of net income and revenue targets established by our board of directors for fiscal year 2018, our compensation committee determined that Dr. Saxonov's and Mr. Whitaker's bonus amount relating to corporate performance would be paid out at 107%. Based on the assessment of Mr. Whitaker's

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performance during fiscal year 2018, our compensation committee (with input from Dr. Saxonov) determined that the bonus amount relating to his individual performance would be paid out at 125%.

Health benefits

We provide customary employee benefits to eligible employees, including to our NEOs, including medical, dental and vision benefits, short-term and long-term disability insurance, basic and supplemental life insurance and basic and supplemental accidental death and dismemberment insurance.

Retirement benefits

We maintain a defined contribution plan (the "401(k) Plan") for all full-time United States employees, including our NEOs. The 401(k) Plan is intended to qualify as a tax-qualified plan under Section 401(a) of the Code. Each participant may contribute between 1% to 100% of such participant's eligible compensation to the 401(k) Plan subject to annual limitations. For fiscal year 2018, we did not make matching contributions to the 401(k) Plan on behalf of our employees.

Nonqualified deferred compensation

We do not maintain nonqualified defined contribution plans or other nonqualified deferred compensation plans.

Perquisites

We generally do not provide perquisites or personal benefits to our NEOs.

Outstanding equity awards at fiscal year end

The following table sets forth the number of securities underlying the equity awards held by each of our NEOs as of December 31, 2018.

Outstanding equity awards at 2018 fiscal year end

		Stock option awards[1]				
Name	**Grant date**	**Numbers of securities underlying unexercised stock options exercisable (#)(2)**	**Numbers of securities underlying unexercised stock options unexercisable (#)(3)**	**Equity incentive plan awards: number of securities underlying unexercised unearned stock options (#)(4)**	**Stock option exercise price ($)**	**Stock option expiration date**
Serge Saxonov	10/27/2015(5)	—	83,334	—	0.88	10/27/2025
	11/18/2016(6)	—	83,334	—	1.07	11/18/2026
	10/18/2017(7)	—	206,250	—	1.20	10/18/2027
	10/18/2017(8)	—	150,000	150,000	1.20	10/18/2027
	11/2/2018(9)	—	234,375	—	5.04	11/2/2028
Justin J. McAnear	11/2/2018(10)	600,000	—	—	5.04	11/2/2028
Eric S. Whitaker	7/28/2017(11)	441,000	—	—	1.20	7/28/2027
	11/2/2018(12)	12,500	187,500	—	5.04	11/2/2028

(1) As a result of the reclassification of our Historical Class B common stock into shares of Class A common stock prior to the closing of this offering as described under "*Description of capital stock—Reclassification of common stock and conversion of Convertible Preferred Stock*", (a) any

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outstanding restricted stock awards at the time of the reclassification will become restricted stock awards for shares of Class A common stock and (b) to the extent any stock options remain unexercised at the time of the reclassification, such stock options will become stock options to purchase shares of our Class A common stock.

(2) The amounts shown represent stock options to purchase shares of our Historical Class B common stock that are (i) early-exercisable (meaning that the stock options may be exercised before they vest in exchange for a restricted stock award for shares of our Historical Class B common stock) and have vested or have not yet vested and (i) not early-exercisable and have vested.

(3) The amounts shown represent stock options to purchase shares of our Historical Class B common stock that are not early-exercisable and have not yet vested, including those stock options discussed in footnote 4 below that have satisfied the performance-based portion of the vesting condition.

(4) The amount shown represents stock options to purchase shares of our Historical Class B common stock that remain subject to both time- and performance-based vesting conditions and have not yet vested.

(5) 12/48th of the stock options vested on the 12-month anniversary of August 1, 2015, and 1/48th of the stock options was scheduled to vest in equal monthly installments on the same day of each month thereafter over the following three years, subject to Dr. Saxonov's continued service through each applicable vesting date.

(6) 12/48th of the stock options vested on the 12-month anniversary of August 1, 2016, and 1/48th of the stock options was scheduled to vest in equal monthly installments on the same day of each month thereafter over the following three years, subject to Dr. Saxonov's continued service through each applicable vesting date.

(7) 1/48th of the stock options vested on the one month anniversary of September 1, 2017, and 1/48th of the stock options was scheduled to vest in equal monthly installments on the same day of each month thereafter, subject to Dr. Saxonov's continued service through each applicable vesting date.

(8) (i) 1/36th of 150,000 stock options was scheduled to vest in equal monthly installments beginning on January 1, 2019 over the following three years because our board of directors determined that our total revenue target of $140 million was achieved for fiscal year 2018, and (ii) 1/36th of an additional 150,000 stock options are eligible to vest in equal monthly installments beginning on January 1, 2020 if our board of directors determines that that our total revenue target of $230 million is achieved for fiscal year 2019, in each case of clauses (i) and (ii), subject to Dr. Saxonov's continued service through each applicable vesting date.

(9) 1/48th of the stock options vested on the one-month anniversary of September 1, 2018, and 1/48th of the stock options was scheduled to vest in equal monthly installments on the same day of each month thereafter, subject to Dr. Saxonov's continued service through each applicable vesting date.

(10) 12/48th of the early-exercisable stock options are eligible to vest on the 12-month anniversary of October 8, 2018, and 1/48th of the early-exercisable stock options are scheduled to vest in equal monthly installments on the same day of each month thereafter over the following three years, subject to Mr. McAnear's continued service through each applicable vesting date.

(11) 12/48th of the early-exercisable stock options vested on the 12-month anniversary of July 18, 2017, and 1/48th of the early-exercisable stock options was scheduled to vest in equal monthly installments on the 18th day of each month thereafter over the following three years, subject to Mr. Whitaker's continued service through each applicable vesting date. Out of the unexercised stock options exercisable, 339,063 stock options remained unvested as of December 31, 2018. Mr. Whitaker early-exercised 84,000 of his stock options in fiscal year 2018 and received a restricted stock award subject to our right of repurchase as to the unvested portion in the event Mr. Whitaker's service with us terminates for any reason. As of December 31, 2018, all of the shares subject to his restricted stock award were vested.

(12) 1/48th of the stock options vested on the one-month anniversary of September 1, 2018, and 1/48th of the stock options was scheduled to vest in equal monthly installments on the same day of each month thereafter, subject to Mr. Whitaker's continued service through each applicable vesting date.

Fiscal 2019 equity awards to Named Executive Officers

In May 2019, our board of directors approved option grants to employees and certain service providers, including Messrs. Saxonov, McAnear and Whitaker. The options granted to our NEOs in May 2019 included a 145,786 share option grant for Dr. Saxonov, a 20,000 share option grant for Mr. McAnear and an 85,000 share grant for Mr. Whitaker. 1/48th of the each of the options granted to our NEOs in May 2019 vested on the one month anniversary of April 1, 2019, and 1/48th of the stock options vested, and shall vest, in equal monthly installments on the same day of each month thereafter, subject to each NEO's continued service through each applicable vesting date. In addition, our NEOs are eligible to receive accelerated vesting of their unvested stock options granted in May 2019, such that 50% of the then-unvested stock options subject to an award will vest immediately prior to a change of control (as defined in the 2012 Stock Plan), and 100% of the then unvested stock options subject to such award will vest if the NEO's service is terminated without cause (as defined in the 2012 Stock Plan) in connection with or following a change of control.

Equity incentive plans

Amended and Restated 2012 Stock Plan

Our board of directors adopted, and our stockholders approved, the 10x Genomics, Inc. 2012 Stock Plan on October 2, 2012, which has been periodically amended and/or restated from time to time (such Amended and

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Restated 2012 Stock Plan, the "2012 Stock Plan"). When the Omnibus Incentive Plan (as defined below) becomes effective upon the completion of this offering, no further awards may be granted under the 2012 Stock Plan.

Purpose. The purpose of the 2012 Stock Plan is to attract and retain the best available personnel for positions of substantial responsibility, to provide additional incentive to our employees and service providers and to promote the success of our business.

Administration. Our board of directors, a committee appointed by our board of directors, or any combination thereof, as determined by our board of directors, acts as the administrator of the 2012 Stock Plan. Subject to the terms of the 2012 Stock Plan, the administrator determines the recipients, the number and type of stock awards to be granted and the terms and conditions of the stock awards, including the exercise price, period of exercisability and vesting schedule applicable to a stock award. The administrator has sole discretion to interpret and to make all decisions and determinations related to the 2012 Stock Plan and any stock award granted thereunder.

Awards subject to the 2012 Stock Plan. The 2012 Stock Plan provides for the grant of stock options (both incentive stock options and nonstatutory stock options) and restricted stock awards. Incentive stock options may be granted only to our employees, exclusive of employees of our affiliates. Nonstatutory stock options and restricted stock awards may be granted to our employees, non-employee directors and other service providers, including those of our affiliates.

Authorized shares. We previously reserved 24,782,088 shares of our Historical Class B common stock for issuance under the 2012 Stock Plan. Upon the effectiveness of the Omnibus Incentive Plan, no additional stock awards may be granted under the 2012 Stock Plan. Any stock awards granted under the 2012 Stock Plan will remain subject to the terms of the 2012 Stock Plan and applicable award agreement, until such outstanding awards that are stock options are exercised, terminate or expire by their terms, and until any restricted stock awards become vested, terminate or are forfeited.

Adjustments upon certain events. In the event of certain changes in our corporate structure, the 2012 Stock Plan provides that the administrator will make such adjustments to outstanding stock awards as required under the 2012 Stock Plan or in such manner as the administrator may deem appropriate. In the event of a change of control (as defined in the 2012 Stock Plan), the 2012 Stock Plan provides that the administrator will determine the treatment of each outstanding award, which determination will be made without the consent of any award holder, including: the continuation, assumption or substitution of such outstanding awards by the surviving corporation or its parent entity; the cancellation of such outstanding awards for consideration or no consideration; or the acceleration of vesting of such outstanding awards, as applicable. Such treatment determined by the administrator may be provided for in an award agreement.

Transferability of awards. Stock awards are generally not transferable other than by will or the laws of descent and distribution, except as otherwise provided under the 2012 Stock Plan.

Reclassification of common stock. As a result of the reclassification of our Historical Class B common stock into shares of Class A common stock prior to the closing of this offering as described under "*Description of capital stock—Reclassification of common stock and conversion of Convertible Preferred Stock*", all shares of our Historical Class B common stock reserved for issuance under the 2012 Stock Plan will become reserved shares of Class A common stock and all outstanding stock options to purchase shares of our Historical Class B common stock will become stock options to purchase shares of our Class A common stock.

Amendment and termination. Our board of directors has authority to amend or terminate the 2012 Stock Plan at any time, although certain amendments require the approval of our stockholders, and amendments or terminations that would materially and adversely affect the rights of any award holder require such award holder's consent.

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2019 Omnibus Incentive Plan

Our board of directors has adopted, and our stockholders have approved, our 10x Genomics, Inc. 2019 Omnibus Incentive Plan (the "Omnibus Incentive Plan") which will be effective upon the completion of the offering. The Omnibus Incentive Plan is intended to replace the 2012 Stock Plan, but any awards outstanding under the 2012 Stock Plan will continue to be governed by their existing terms. The following summary is qualified in its entirety by reference to the Omnibus Incentive Plan that has been adopted by our board of directors.

Purpose. The purpose of the Omnibus Incentive Plan is to provide a means through which to attract and retain key personnel and to provide a means whereby our directors, officers, employees, consultants and advisors (and those of our subsidiaries or affiliates) can acquire and maintain an equity interest in us, or be paid incentive compensation, including incentive compensation measured by reference to the value of our shares of our Class A common stock, thereby strengthening their commitment to our welfare and aligning their interests with those of our stockholders.

Administration. The Omnibus Incentive Plan will be administered by the compensation committee of our board of directors, or such other committee of our board of directors to which it has properly delegated power, or if no such committee or subcommittee exists, our board of directors (such administering body referred to herein, for purposes of this description of the Omnibus Incentive Plan, as the committee). Except to the extent prohibited by applicable law or the applicable rules and regulations of any securities exchange or interdealer quotation system on which our securities are listed or traded, the committee may allocate all or any portion of its responsibilities and powers to any one or more of its members and may delegate all or any part of its responsibilities and powers to any person or persons selected by it in accordance with the terms of the Omnibus Incentive Plan.

The committee is authorized to: (i) designate participants; (ii) determine the type or types of awards to be granted to a participant; (iii) determine the number of shares of our Class A common stock to be covered by, or with respect to which payments, rights or other matters are to be calculated in connection with, awards; (iv) determine the terms and conditions of any award; (v) determine whether, to what extent and under what circumstances awards may be settled in, or exercised for, cash, shares of our Class A common stock, other securities, other awards or other property, or canceled, forfeited or suspended and the method or methods by which awards may be settled, exercised, canceled, forfeited or suspended; (vi) determine whether, to what extent, and under what circumstances the delivery of cash, shares of our Class A common stock, other securities, other awards or other property and other amounts payable with respect to an award will be deferred either automatically or at the election of the participant or of the committee; (vii) interpret, administer, reconcile any inconsistency in, correct any defect in, and/or supply any omission in the Omnibus Incentive Plan and any instrument or agreement relating to, or award granted under, the Omnibus Incentive Plan; (viii) establish, amend, suspend or waive any rules and regulations and appoint such agents as the committee may deem appropriate for the proper administration of the Omnibus Incentive Plan; (ix) adopt sub-plans and (x) make any other determination and take any other action that the committee deems necessary or desirable for the administration of the Omnibus Incentive Plan. Unless otherwise expressly provided in the Omnibus Incentive Plan, all designations, determinations, interpretations and other decisions under or with respect to the Omnibus Incentive Plan or any award or any documents evidencing awards granted pursuant to the Omnibus Incentive Plan are within the sole discretion of the committee, may be made at any time and are final, conclusive and binding upon all persons or entities, including, without limitation, us, any participant, any holder or beneficiary of any award and any of our stockholders. The committee may make grants of awards to eligible persons pursuant to the terms and conditions set forth in the applicable award agreement, including subjecting such awards to performance criteria listed in the Omnibus Incentive Plan.

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Term. The Omnibus Incentive Plan will have a term of ten years unless earlier terminated (i) on the date on which all the shares of our Class A common stock available for issuance under the Omnibus Incentive Plan have been issued or (ii) by the committee in accordance with the terms of the Omnibus Incentive Plan.

Awards subject to the Omnibus Incentive Plan. The Omnibus Incentive Plan provides that the total number of shares of our Class A common stock that may be issued under the Omnibus Incentive Plan is 11,000,000, which includes an aggregate of 1,323,858 shares of our Historical Class B common stock reserved for future grants under the 2012 Stock Plan, as of June 30, 2019, which will be added to the total number of shares of reserved under the Omnibus Incentive Plan upon the effectiveness of the Omnibus Incentive Plan (such share limit as increased from time to time, the "Absolute Share Limit"). However, the Absolute Share Limit shall be increased on the first day of each calendar year commencing on January 1, 2021 and ending on January 1, 2029 in an amount equal to the lesser of (i) 5% of the total number of shares of common stock outstanding on the last day of the immediately preceding fiscal year and (ii) such number of shares of Class A common stock as determined by our board of directors. However, if on January 1 of a calendar year, our board of directors has not either confirmed the 5% increase described in clause (i) or approved a lesser number of shares for such calendar year, then our board of directors will be deemed to have waived the automatic increase, and no such increase will occur for such calendar year. Of the Absolute Share Limit, no more than 11,000,000 shares of Class A common stock may be issued in the aggregate pursuant to the exercise of incentive stock options granted under the Omnibus Incentive Plan. During a single fiscal year, each non-employee director shall be granted a number of shares of Class A common stock subject to awards under the Omnibus Incentive Plan, taken together with any cash fees paid to such non-employee director during such fiscal year, equal to $1,000,000 or such lower amount as determined by our board of directors. Except for substitute awards (as described below) (or the equivalent thereof under the 2012 Stock Plan), to the extent that an award (or an award under the 2012 Stock Plan) expires or is canceled, forfeited, terminated, settled in cash or otherwise is settled without issuance to the participant of the full number of shares our Class A common stock to which the award (or the award under the 2012 Stock Plan) related, the unissued shares will again be available for grant under the Omnibus Incentive Plan. Shares of our Class A common stock withheld in payment of the exercise price, or taxes relating to an award, and shares equal to the number of shares surrendered in payment of any exercise price, or taxes relating to an award, shall be deemed to constitute shares not issued. However, such shares shall not become available for issuance if either: (i) the applicable shares are withheld or surrendered following the termination of the Omnibus Incentive Plan or (ii) at the time the applicable shares are withheld or surrendered, it would constitute a material revision of the Omnibus Incentive Plan subject to stockholder approval under any then-applicable rules of the national securities exchange on which our Class A common stock is listed. No award may be granted under the Omnibus Incentive Plan after the tenth anniversary of the Effective Date (as defined in the Omnibus Incentive Plan), but awards granted before then may extend beyond that date. Awards may, in the sole discretion of the committee, be granted in assumption of, or in substitution for, outstanding awards previously granted by an entity directly or indirectly acquired by us or with which we combine, or substitute awards, and such substitute awards will not be counted against the Absolute Share Limit, except that substitute awards intended to qualify as "incentive stock options" will count against the limit on incentive stock options described above.

Stock options. Under the Omnibus Incentive Plan, the committee may grant nonqualified stock options and incentive stock options with terms and conditions determined by the committee that are not inconsistent with the Omnibus Incentive Plan, except that all stock options granted under the Omnibus Incentive Plan are required to have a per share exercise price that is not less than 100% of the fair market value of our shares of our Class A common stock underlying such stock options on the date such stock options are granted (other than in the case of stock options that are substitute awards), and all stock options that are intended to qualify as incentive stock options must be granted pursuant to an award agreement expressly stating that the stock options are intended to qualify as incentive stock options, and will be subject to the terms and conditions that

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comply with the rules as may be prescribed by Section 422 of the Code. The maximum term for stock options granted under the Omnibus Incentive Plan will be ten years from the initial date of grant, or with respect to any stock options intended to qualify as incentive stock options, such shorter period as prescribed by Section 422 of the Code. However, if a nonqualified stock option would expire at a time when trading of shares of our Class A common stock is prohibited by our insider trading policy (or "blackout period" imposed by us), the term will automatically be extended to the 30th day following the end of such period. The purchase price for the shares of our Class A common stock as to which a stock option is exercised may be paid to us, to the extent permitted by law (i) in cash, check, cash equivalent and/or shares of our Class A common stock valued at the fair market value at the time the stock option is exercised equal to the aggregate exercise price for the shares of our Class A common stock being purchased and satisfying any requirements as may be imposed by the committee, except that such shares of our Class A common stock are not subject to any pledge or other security interest and have been held by the participant for at least six months (or such other period as established from time to time by the committee in order to avoid adverse accounting treatment applying generally accepted accounting principles in the United States ("GAAP")) or (ii) by such other method as the committee may permit in its sole discretion, including, without limitation: (a) in other property having a fair market value on the date of exercise equal to the exercise price, (b) if there is a public market for the shares of our Class A common stock at such time, by means of a broker-assisted "cashless exercise" pursuant to which we are delivered (including telephonically to the extent permitted by the committee) a copy of irrevocable instructions to a stockbroker to sell the shares of our Class A common stock otherwise issuable upon the exercise of the stock option and to deliver promptly to us an amount equal to the aggregate exercise price for the shares of our Class A common stock being purchased or (c) a "net exercise" procedure effected by withholding the minimum number of shares of our Class A common stock otherwise issuable in respect of a stock option that is needed to pay the exercise price. Any fractional shares of our Class A common stock will be settled in cash.

Stock appreciation rights. The committee may grant stock appreciation rights ("SARs") under the Omnibus Incentive Plan, with terms and conditions determined by the committee that are not inconsistent with the Omnibus Incentive Plan. The committee may grant SARs in tandem with a stock option, but the committee may also award SARs independent of any stock option. Generally, each SAR will entitle the participant upon exercise to an amount (in cash, shares of our Class A common stock or a combination of cash and shares, as determined by the committee) equal to the product of (i) the excess of (a) the fair market value on the exercise date of one share of our Class A common stock over (b) the strike price per share of our Class A common stock covered by the SAR, times (ii) the number of shares of our Class A common stock covered by the SAR, less any taxes required to be withheld. The strike price per share of our Class A common stock covered by a SAR will be determined by the committee at the time of grant but in no event may such amount be less than 100% of the fair market value of a share of our Class A common stock on the date the SAR is granted (other than in the case of SARs that are substitute awards).

Restricted stock and restricted stock units. The committee may grant restricted stock awards for shares of our Class A common stock or may grant restricted stock units ("RSUs") representing the right to receive, upon vesting and the expiration of any applicable restricted period, one share of our Class A common stock for each RSU, or, in the sole discretion of the committee, the cash value thereof (or any combination thereof). As to restricted stock awards, subject to the other provisions of the Omnibus Incentive Plan, the holder will generally have the rights and privileges of a stockholder as to such restricted stock, including, without limitation, the right to vote. Participants have no rights or privileges as a stockholder with respect to RSUs.

Other equity-based awards and other cash-based awards. The committee may grant other equity-based or cash-based awards under the Omnibus Incentive Plan, with terms and conditions determined by the committee that are not inconsistent with the Omnibus Incentive Plan.

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Effect of certain events on the Omnibus Incentive Plan and awards. In the event of certain changes to our capital structure or a change in control (as defined in the Omnibus Incentive Plan) (each, an "Adjustment Event"), the committee will, in respect of any such Adjustment Event, make such proportionate substitution or adjustment, if any, as it deems equitable, to any or all of (i) the Absolute Share Limit, or any other limit applicable under the Omnibus Incentive Plan with respect to the number of awards which may be granted thereunder; (ii) the number of shares of our Class A common stock or other of our securities (or number and kind of other securities or other property) which may be issued in respect of awards or with respect to which awards may be granted under the Omnibus Incentive Plan or any sub-plan; and (iii) the terms of any outstanding award, including, without limitation, (a) the number of shares of our Class A common stock or other of our securities (or number and kind of other securities or other property) subject to outstanding awards or to which outstanding awards relate; (b) the exercise price or strike price with respect to any award; or (c) any applicable performance measures, except in the case of any "equity restructuring" (within the meaning of the FASB ASC Topic 718 (or any successor pronouncement thereto)), the committee will make an equitable or proportionate adjustment to outstanding awards to reflect such equity restructuring.

In connection with a change in control, the committee may, in its sole discretion, provide for any one or more of the following: (i) substitution or assumption of awards, or to the extent not substituted or assumed, acceleration of the vesting of, exercisability of, or lapse of restrictions on, as applicable, any awards immediately prior to, and contingent upon, such change in control. However, with respect to any performance-vesting awards, any such acceleration of vesting, exercisability, or lapse of restriction, shall be based on actual performance through the date of such change in control; and (ii) subject to any limitations or reductions as may be necessary to comply with Section 409A of the Code, cancellation of any one or more outstanding awards and payment to the holders of such awards that are vested as of such cancellation (including, without limitation, any awards that would vest as a result of the occurrence of such event but for such cancellation or for which vesting is accelerated by the committee in connection with such event) the value of such awards, if any, as determined by the committee (which value, if applicable, may be based upon the price per share of our Class A common stock received or to be received by other holders of shares of our Class A common stock in such event), including, without limitation, in the case of stock options and SARs, a cash payment equal to the excess, if any, of the fair market value of the shares of our Class A common stock subject to the stock option or SAR over the aggregate exercise price or strike price thereof.

Nontransferability of awards. No award will be permitted to be assigned, alienated, pledged, attached, sold or otherwise transferred or encumbered by a participant (unless such transfer is specifically required pursuant to a domestic relations order by applicable law) other than by will or by the laws of descent and distribution and any such purported assignment, alienation, pledge, attachment, sale, transfer or encumbrance will be void and unenforceable against us or any of our subsidiaries. However, the committee may, in its sole discretion, permit awards (other than incentive stock options) to be transferred, including transfers to a participant's family members, any trust established solely for the benefit of a participant or such participant's family members, any partnership or limited liability company of which a participant, or such participant and such participant's family members, are the sole member(s), and a beneficiary to whom donations are eligible to be treated as "charitable contributions" for tax purposes.

Amendment and termination. Our board of directors may amend, alter, suspend, discontinue or terminate the Omnibus Incentive Plan or any portion thereof at any time, except that no such amendment, alteration, suspension, discontinuance or termination may be made without stockholder approval if (i) such approval is necessary to comply with any regulatory requirement applicable to the Omnibus Incentive Plan or for changes in GAAP to new accounting standards; (ii) it would materially increase the number of securities which may be issued under the Omnibus Incentive Plan (except for adjustments in connection with certain corporate events); or (iii) it would materially modify the requirements for participation in the Omnibus Incentive Plan. In addition,

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any such amendment, alteration, suspension, discontinuance or termination that would materially and adversely affect the rights of any participant or any holder or beneficiary of any award will not to that extent be effective without such individual's consent.

The committee may, to the extent consistent with the terms of any applicable award agreement, waive any conditions or rights under, amend any terms of, or alter, suspend, discontinue, cancel or terminate, any award granted or the associated award agreement, prospectively or retroactively (including after a termination of employment or services, as applicable), but, except as otherwise permitted in the Omnibus Incentive Plan, any such waiver, amendment, alteration, suspension, discontinuance, cancellation or termination that would materially and adversely affect the rights of any participant with respect to such award will not to that extent be effective without such individual's consent. In addition, without stockholder approval, except as otherwise permitted in the Omnibus Incentive Plan, (i) no amendment or modification may reduce the exercise price of any stock option or the strike price of any SAR; (ii) the committee may not cancel any outstanding stock option or SAR and replace it with a new stock option or SAR (with a lower exercise price or strike price, as the case may be) or other award or cash payment that is greater than the value of the cancelled stock option or SAR; and (iii) the committee may not take any other action which is considered a "repricing" for purposes of the stockholder approval rules of any securities exchange or inter-dealer quotation system on which our securities are listed or quoted.

Dividends and dividend equivalents. The committee in its sole discretion may provide as part of an award dividends or dividend equivalents, on such terms and conditions as may be determined by the committee in its sole discretion. Unless otherwise provided in the award agreement, any dividends payable in respect of restricted stock awards that remain subject to vesting conditions at the time of payment shall be retained by us and remain subject to the same vesting conditions as the restricted stock awards to which the dividend relates and delivered to the participant within 15 days following the date on which such restrictions on such restricted stock awards lapse and, if such restricted stock is forfeited, the participant shall have no right to such dividends. Dividend equivalent payments attributable to RSUs shall be distributed to the participant in cash or, in the sole discretion of the committee, in shares of our Class A common stock having a fair market value equal to the amount of dividends paid on shares of our Class A common stock, upon the settlement of the RSUs and, if such RSUs are forfeited, the participant shall have no right to such dividend equivalent payments (or interest thereon, if applicable).

Clawback/repayment. All awards are subject to reduction, cancellation, forfeiture or recoupment to the extent necessary to comply with (i) any clawback, forfeiture or other similar policy adopted by our board of directors or the committee and as in effect from time to time and (ii) applicable law. To the extent that a participant receives any amount in excess of the amount that the participant should otherwise have received under the terms of the award for any reason (including, without limitation, by reason of a financial restatement, mistake in calculations or other administrative error), the participant will be required to repay us any such excess amount.

Detrimental activity. If a participant has engaged in any detrimental activity, as defined in the Omnibus Incentive Plan, as determined by the committee, the committee may, in its sole discretion, provide for one or more of the following: (i) cancellation of any or all of such participant's outstanding awards or (ii) forfeiture and repayment to us on any gain realized on the vesting, exercise or settlement of any awards previously granted to such participant.

2019 Employee Stock Purchase Plan

Our board of directors has adopted, and our stockholders have approved, our 10x Genomics, Inc. 2019 Employee Stock Purchase Plan (the "ESPP") which will be effective upon the completion of this offering. The following

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summary is qualified in its entirety by reference to the ESPP that has been adopted by our board of directors.

Purpose. The ESPP is intended to give eligible employees an opportunity to purchase shares of our Class A common stock. We believe that allowing our employees to participate in the ESPP provides them with a further incentive towards ensuring our success and accomplishing our corporate goals. The ESPP is intended to qualify as an "employee stock purchase plan" under Section 423 of the Code. We may authorize offerings under the ESPP that are not intended to comply with Section 423 of the Code, which offerings will be made pursuant to any rules, procedures or sub-plans adopted by the committee for such purpose.

Authorized shares. The ESPP provides that the maximum number of shares of our Class A common stock made available for sale thereunder will be 2,000,000, which number will be automatically increased on the first day of each calendar year commencing on January 1, 2021 and ending on January 1, 2029 in an amount equal to the lesser of (i) 1% of the total number of shares of common stock outstanding on the last day of the immediately preceding fiscal year and (ii) such number of shares of our Class A common stock as determined by our board of directors. However, if on January 1 of a calendar year our board of directors has not either confirmed the 1% described in clause (i) or approved a lesser number of shares of our Class A common stock for such calendar year, our board of directors will be deemed to have waived the automatic increase and no such increase will occur for such calendar year. The maximum number of shares available under the ESPP (and any share limitations thereunder, as applicable) will automatically be adjusted upon certain changes to our capital structure.

Administration. The ESPP will be administered by the compensation committee, or such other committee as may be designated by our board of directors, or our board of directors (such administering body, the "Administrator"). The Administrator will have full authority to make, administer and interpret such terms, rules and regulations regarding administration of the ESPP as it may deem advisable, and such decisions are final and binding.

Term. The ESPP will have a term of ten years unless earlier terminated (i) on the date on which all the shares of Class A common stock available for issuance under the ESPP have been issued or (ii) by the Administrator in accordance with the terms of the ESPP.

Eligible employees. Subject to the Administrator's ability to exclude certain groups of employees on a uniform and nondiscriminatory basis, including section 16 officers, generally, all of our employees will be eligible to participate if they are employed by us or any participating subsidiary or affiliate for at least 90 days or any lesser number of hours per week and/or number of days established by the Administrator. In no event will an employee who is deemed to own 5% or more of the total combined voting power or value of all classes of our capital stock or the capital stock of any parent or subsidiary be eligible to participate in the ESPP, and no participant in the ESPP may purchase shares of our Class A common stock under any employee stock purchase plans of our company to the extent the option to purchase shares accrue at a rate that exceeds $25,000 of the fair market value of such shares of our Class A common stock, determined as of the first day of the offering period, for each calendar year in which such option is outstanding.

Offering periods and purchase periods. Offering periods under the ESPP will be 6 months long, except that the first offering period will run from the date of completion of this offering and end on May 14, 2020. Following the end of the first offering period, a new offering period will commence on each of May 15 and November 15 of each calendar year. The Administrator may choose to start a new offering period as it may determine from time to time as appropriate and offering periods may overlap or be consecutive. During each offering period, there will be one 6 month purchase period, which will have the same duration and coincide with the length of the offering period.

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Purchase price. Eligible employees who participate will receive an option to purchase shares of our Class A common stock at a purchase price equal to the lower of 85% of (i) the closing price per share of our Class A common stock on the date of purchase or (ii) the closing price per share of our Class A common stock on the first day of the applicable offering period (or, in the case of the first offering period, the price per share at which shares of our Class A common stock are first sold to the public in connection with this offering). Eligible employees participate by authorizing payroll deductions before the beginning of an offering period, which deduction may not exceed 15% of such employee's cash compensation.

Contributions and grants. Eligible employees participate by authorizing payroll deductions before the beginning of an offering period, which deduction may not exceed 15% of such employee's cash compensation. In addition, the maximum number of shares of our Class A common stock that may be purchased by all participants in any particular purchase period is limited to 2,000 shares (subject to adjustment as provided in the ESPP), and the maximum number of shares of our Class A common stock that may be purchased by any participant during any one year period is limited to 4,000 shares. The Administrator may modify this limit from time to time by resolution or otherwise.

Cancellation of election to purchase. A participant may cancel his or her participation entirely at any time by withdrawing all, but not less than all, of his or her contributions credited to his or her account and not yet used to exercise his or her option under the ESPP. Participation will end automatically upon termination of employment with us.

Effect of a change in control. In the event of a change in control (as defined under the ESPP), the Administrator may in its discretion provide, without limitation, that each outstanding option be assumed, or an equivalent option be substituted by the successor corporation or a parent or subsidiary of the successor corporation and, if not so assumed or substituted, the offering period for that option be shortened by setting a new exercise date on which the offering period will end; terminate outstanding options and refund accumulated contributions to participants; or continue outstanding options unchanged.

Rights as stockholder. A participant will have no rights as a stockholder with respect to the shares of our Class A common stock that the participant has an option to purchase in any offering until those shares have been issued to the participant.

Options not transferable and restrictions on sale. A participant's option under the ESPP will be exercisable only by the participant and may not be sold, transferred, pledged or assigned in any manner other than by will or the laws of descent and distribution. Unless otherwise determined by the Administrator, a participant may not sell, transfer or otherwise dispose of any shares of our Class A common stock purchased under the ESPP for 12 months following the applicable exercise date.

Amendment or termination. The Administrator, in its sole discretion, may amend, alter, suspend or terminate the ESPP, or any option subject thereto, at any time and for any reason as long as such amendment or termination of an option does not materially adversely affect the rights of a participant with respect to the option without the written consent of such participant.

Potential payments upon a change of control or termination of employment

Each of our NEOs is eligible to receive accelerated vesting of their unvested stock options granted under the 2012 Stock Plan, such that 50% of the then-unvested stock options subject to an award will vest and become exercisable immediately prior to a change of control (as defined in the 2012 Stock Plan), and 100% of the then-unvested stock options subject to an award will vest and become exercisable if the NEO's employment is terminated without cause (as defined in the 2012 Stock Plan) in connection with or following a change of control. For a description of the severance protection provided to Mr. McAnear under his offer letter, see the section titled "*—Employment arrangements—Mr. McAnear*".

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Director compensation

This following table sets forth information concerning the compensation paid to our non-employee directors during fiscal year 2018. Our employee directors receive no additional compensation for serving on our board of directors. The compensation paid to Dr. Saxonov for serving as our CEO is set forth in the Summary compensation table above.

Name(3)	Stock option awards ($)(1)(2)	Total ($)
Paul A. Conley	—	—
Mathai Mammen	—	—
Bryan E. Roberts	—	—
John R. Stuelpnagel	240,442	240,442

(1) As a result of the reclassification of our Historical Class B common stock into shares of Class A common stock prior to the closing of this offering as described under "*Description of capital stock—Reclassification of common stock and conversion of Convertible Preferred Stock*", (i) any outstanding restricted stock awards at the time of the reclassification will become restricted stock awards for shares of Class A common stock and (ii) to the extent any stock options remain unexercised at the time of the reclassification, such stock options will become stock options to purchase shares of our Class A common stock.

(2) The amount shown represents the grant date fair value of stock options to purchase shares of our Historical Class B common stock granted to Dr. Stuelpnagel in fiscal year 2018, as computed in accordance with FASB ASC Topic 718. We did not grant stock options to any other non-employee director in fiscal year 2018. For a discussion of valuation assumptions used to determine the grant date fair value of the stock options granted to Dr. Stuelpnagel in fiscal year 2018, see the section titled "*Management's discussion and analysis of financial condition and results of operations—Critical accounting policies and estimates—Stock-based compensation*". As of December 31, 2018, the aggregate number of outstanding stock options to purchase shares of our Historical Class B common stock held by each of our non-employee directors was as follows: Dr. Conley, 0; Dr. Mammen, 200,000; Dr. Roberts, 0; and Dr. Stuelpnagel, 0. For Dr. Mammen, 1/48th of his early-exercisable stock options vested on the one-month anniversary of June 12, 2017, and 1/48th of such stock options was scheduled to vest in equal monthly installments on the same day of each month thereafter, subject to Dr. Mammen's continued service through each applicable vesting date. For Dr. Stuelpnagel, 1/48th of his early-exercisable stock options vested on the one-month anniversary of September 1, 2018, and 1/48th of such stock options was scheduled to in equal monthly installments on the same day of each month thereafter, subject to Dr. Stuelpnagel's continued service through each applicable vesting date. Dr. Stuelpnagel early-exercised all 100,000 stock options subject to his award in fiscal year 2018 and received a restricted stock award for shares of our Historical Class B common stock, subject to our right of repurchase as to the unvested portion in the event Dr. Stuelpnagel's service with us terminates for any reason. As of December 31, 2018, 218,750 shares subject to his restricted stock award remained unvested.

(3) Mr. Kosaraju and Dr. Suliman were appointed to our board of directors in April 2019 and August 2019, respectively.

Description of director compensation

Prior to this offering, we did not have a formal director compensation policy and did not pay our non-employee directors compensation for their services as a director, other than reimbursing them for reasonable out-of-pocket travel expenses incurred while attending meetings of our board of directors or occasionally granting an equity award to certain of our non-employee directors upon their initial appointment to our board of directors. In connection with this offering, each of Messrs. Conley and Roberts will receive a grant of nonqualified stock options to purchase 40,000 shares of Class A common stock with an exercise price per share equal to the initial public offering price. These option grants will vest in equal monthly installments for three years following the date of grant, subject to Messrs. Conley's or Roberts's continued service as a non-employee director through each such vesting date. Notwithstanding the foregoing, each such option grant will vest as to 50% upon the occurrence of a change in control (as defined in the Omnibus Incentive Plan) and as to 100% upon Messrs. Conley's or Roberts's respective termination without cause (as defined in the Omnibus Incentive Plan) in connection with or after such change in control. Following the completion of this offering, each of our non-employee directors will be entitled to annual compensation in accordance with the following non-employee director compensation policy:

- an annual cash retainer of $40,000, payable quarterly in arrears;

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- the non-employee director serving as chair of our board of directors and each non-employee director serving as a member or chair, as applicable, of the following committees of our board of directors will receive the following additional annual retainers, each of which is also payable quarterly in arrears:

  Chair of the Board: $40,000

  Audit Committee Chair: $20,000

  Audit Committee Member: $10,000

  Compensation Committee Chair: $15,000

  Compensation Committee Member: $7,500

  Nominating and Corporate Governance Chair: $10,000

  Nominating and Corporate Governance Member: $5,000;

- upon becoming a member of our board of directors, a one-time grant of options having a grant date fair value equal to $400,000 (with the number of options to be granted calculated using the Black-Scholes or similar established formula) and an exercise price per share equal to the fair market value (as defined in the Omnibus Incentive Plan) of a share of Class A common stock on the date of grant, which will vest as to one-third of such grant on the first anniversary of the date of grant and thereafter in equal monthly installments for the following two years, subject to the non-employee director continuing in service though each such vesting date; and

- an annual grant of options having a grant date fair market value equal to $200,000 (with the number of options to be granted calculated using the Black-Scholes or a similar established formula) and an exercise price per share equal to the fair value of a share of Class A common stock on the date of grant, to be granted on the date of our annual meeting of stockholders and which will vest monthly over the 12 month period following the date of grant, subject to the non-employee director continuing in service through each such vesting date.

In each case, the options granted will vest in full upon the occurrence of a change in control.

Our directors will not be paid any fees for attending meetings. However, our directors will be reimbursed for travel and lodging expenses associated with attendance at board or committee meetings.

Fiscal 2019 Equity Awards to Our Directors

Other than the grants to Messrs. Conley and Roberts described above which will be effective upon this offering, our board of directors has approved option grants for two of our non-employee directors to date in 2019. The options granted to our non-employee directors included a 130,000 share option grant in May 2019 for Mr. Kosaraju and a 100,000 share option grant in August 2019 for Dr. Suliman. 1/48th of the each of the options granted to each of Mr. Kosaraju and Dr. Suliman vested on the one month anniversary of each director's vesting commencement dates of May 10, 2019 and August 7, 2019, respectively, and 1/48th of the stock options vested, and shall vest, in equal monthly installments on the same day of each month thereafter, subject to each director's continued service through each applicable vesting date. The options granted to Mr. Kosaraju and Dr. Suliman are early exercisable and are eligible to receive accelerated vesting of their unvested stock options, such that 50% of the then-unvested stock options subject to an award will vest immediately prior to a change of control (as defined in the 2012 Stock Plan), and 100% of the then unvested stock options subject to such award will vest if the director's service is terminated without cause (as defined in the 2012 Stock Plan) in connection with or following a change of control.

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Certain relationships and related party transactions

In addition to the compensation arrangements, including employment, termination of employment and change in control arrangements discussed in the sections titled “Management” and “Executive compensation” and the registration rights described in the section titled “Description of capital stock—Registration rights”, the following is a description of each transaction since January 1, 2016 and each currently proposed transaction in which:

- we have been or are to be a participant;
- the amount involved exceeded or exceeds $120,000; and
- any of our directors, executive officers or holders of more than 5% of any class of our outstanding capital stock, or any immediate family member of, or person sharing the household with, any of these individuals or entities had or will have a direct or indirect material interest.

Other than as described below, there have not been, nor are there any currently proposed, transactions or series of similar transactions meeting this criteria to which we have been or will be a party other than compensation arrangements, which are described where required under “*Executive compensation*”. We believe the terms of the transactions described below were comparable to terms we could have obtained in arm’s-length dealings with unrelated third parties.

Convertible preferred stock financings

The following table summarizes purchases of our Convertible Preferred Stock by our directors and holders of more than 5% percent of any class of our capital stock and their affiliated entities. None of our executive officers purchased shares of Convertible Preferred Stock since January 1, 2016.

Name	Shares of Series C convertible preferred stock(1)	Shares of Series D convertible preferred stock(2)	Shares of Series D-1 convertible preferred stock(3)	Aggregate purchase price
				(in thousands)
Paladin Capital Group and affiliated entities(4)	1,004,868	653,082	—	$ 10,750
John R. Stuelpnagel Trust(5)	223,304	49,634	—	$ 1,475
Fidelity and affiliated entities(6)	7,034,076	764,890	785,545	$ 48,820
Foresite Capital Management and affiliated entities(7)	1,770,803	—	—	$ 7,930
Venrock and affiliated entities(8)	1,786,431	—	—	$ 8,000

(1) Our Series C Convertible Preferred Stock was issued between February 2016 and March 2017.

(2) Our Series D Convertible Preferred Stock was issued in April 2018.

(3) Our Series D-1 Convertible Preferred Stock was issued in October 2018.

(4) Paladin Capital Group is one of our principal stockholders. See the section titled “*Principal stockholders*” for more information. Dr. Conley, a member of our board of directors, is affiliated with Paladin Capital Group.

(5) Dr. Stuelpnagel, the chairman of our board of directors, is trustee of the John R. Stuelpnagel Trust.

(6) Fidelity is one of our principal stockholders. See the section titled “*Principal stockholders*” for more information.

(7) Foresite Capital Management is one of our principal stockholders. See the section titled “*Principal stockholders*” for more information.

(8) Venrock is one of our principal stockholders. See the section titled “*Principal stockholders*” for more information. Dr. Roberts, a member of our board of directors, is affiliated with Venrock.

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Amended and Restated Investors' Rights Agreement

We are party to our Amended and Restated Investors' Rights Agreement (the "IRA"), dated as of October 18, 2018, which provides, among other things, that certain holders of our capital stock, including Dr. Saxonov, our Chief Executive Officer, Dr. Hindson, our Chief Scientific Officer, the John R. Stuelpnagel Trust and entities affiliated with each of Fidelity, Foresite Capital Management, Venrock and Paladin Capital Group and certain of their transferees, be covered by a registration statement that we are otherwise filing and receive certain registration rights. Dr. Conley, a member of our board of directors, is affiliated with Paladin Capital Group. Dr. Roberts, a member of our board of directors, is affiliated with Venrock. Dr. Stuelpnagel, the chairman of our board of directors, is trustee of the John R. Stuelpnagel Trust. The registration and associated rights set forth in the IRA will expire no later than two years following the completion of this offering. See the section titled "*Description of capital stock—Registration rights*" for additional information regarding these registration rights. All other rights set forth in the IRA will terminate immediately prior to the completion of this offering.

Amended and Restated Right of First Refusal and Co-Sale Agreement

Pursuant to certain of our bylaws, equity compensation plans and certain agreements with our stockholders, including our Amended and Restated Right of First Refusal and Co-Sale Agreement, dated October 18, 2018, we or our assignees have a right to purchase shares of our capital stock which certain stockholders propose to sell to other parties. Should we chose not to exercise this right, certain holders of our capital stock, including Dr. Saxonov, our Chief Executive Officer, Dr. Hindson, our Chief Scientific Officer, the John R. Stuelpnagel Trust and entities affiliated with each of Fidelity, Foresite Capital Management, Venrock and Paladin Capital Group, have a right to purchase such shares of our capital stock. Immediately prior to the completion of this offering, our Amended and Restated Right of First Refusal and Co-Sale Agreement will terminate and none of our stockholders will have any right of first refusal or co-sale rights.

Amended and Restated Voting Agreement

We are party to our Amended and Restated Voting Agreement (the "Voting Agreement"), dated as of October 18, 2018, under which certain holders of our capital stock, including Dr. Saxonov, our Chief Executive Officer, Dr. Hindson, our Chief Scientific Officer, the John R. Stuelpnagel Trust and entities affiliated with each of Fidelity, Foresite Capital Management, Venrock and Paladin Capital Group, have agreed to vote their shares of our capital stock with respect to the election, appointment and removal of directors. In accordance with our Seventh Amended and Restated Certificate of Incorporation (the "Pre-IPO Charter") and the terms of the Voting Agreement: Dr. Conley and Dr. Roberts were elected as the Preferred Directors (as defined in the Pre-IPO Charter), which are elected by the holders of at least a majority of the outstanding shares of our Series A-1 and Series A-2 Convertible Preferred Stock, respectively, that are party to the Voting Agreement); Dr. Saxonov and Dr. Hindson were elected as the Common Directors (as defined in the Pre-IPO Charter), with Dr. Saxonov being elected because he is our current Chief Executive Officer and Dr. Hindson being elected by the holders of at least a majority of the voting power of the Historical Common Stock (excluding the voting power of shares of Historical Common Stock issuable upon conversion of Convertible Preferred Stock) held by holders party to the Voting Agreement; and Dr. Stuelpnagel and Dr. Mammen were elected as At-Large Directors (as defined in the Pre-IPO Charter), one of which is elected by a majority of the voting power of the Historical Common Stock (excluding the voting power of shares of Historical Common Stock issuable upon conversion of Convertible Preferred Stock) held by holders party to the Voting Agreement, voting as a single class, and one of which is elected by a majority of the outstanding Convertible Preferred Stock held by holders party to the Voting Agreement, voting as a single class. Dr. Conley is affiliated with Paladin Capital Group, Dr. Roberts is affiliated with Venrock and Dr. Stuelpnagel is trustee of the John R. Stuelpnagel Trust. Immediately prior to the

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completion of this offering, the Voting Agreement will terminate and none of our stockholders will have any special rights regarding the election, appointment or removal of members of our board of directors.

Stock option grants to directors and executive officers

We have granted stock options to our certain of our directors and executive officers. For more information regarding the stock options and stock awards granted to our directors and named executive officers see the section titled "*Executive Compensation*".

Limitation of liability and indemnification of directors and officers

Our amended and restated certificate of incorporation will provide that no director will be personally liable to us or our stockholders for monetary damages for breach of fiduciary duty as a director, except as required by applicable law, as in effect from time to time. For more information regarding the limitations of liability and indemnification see the section titled "*Description of capital stock*".

Related-party transaction policy

We have adopted a formal written policy that applies to our executive officers, directors, holders of more than five percent of any class of our voting securities and any member of the immediate family of, and any entity affiliated with, any of the foregoing persons. Such persons will not be permitted to enter into a related-party transaction with us without the prior consent of our audit committee, or other independent members of our board of directors in the event it is inappropriate for our audit committee to review such transaction due to a conflict of interest. Any request for us to enter into a transaction with an executive officer, director, principal stockholder or any of their immediate family members or affiliates in which the amount involved exceeds $120,000 must first be presented to our audit committee for review, consideration and approval. In approving or rejecting any such proposal, our audit committee will consider the relevant facts and circumstances available and deemed relevant to our audit committee, including, but not limited to, whether the transaction will be on terms no less favorable than terms generally available to an unaffiliated third-party under the same or similar circumstances and the extent of the related-party's interest in the transaction.

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Principal stockholders

The following table sets forth information regarding beneficial ownership of our common stock as of August 14, 2019, by:

- each of our named executive officers and directors individually;
- all directors and executive officers as a group; and
- each person whom we know to beneficially own more than 5% of any class our common stock.

In accordance with the rules of the SEC, beneficial ownership includes voting or investment power with respect to securities and includes the shares issuable pursuant to stock options and warrants that are exercisable within 60 days of August 14, 2019. Shares issuable pursuant to stock options and warrants are deemed outstanding for computing the percentage of the person holding such options or warrants, as applicable, but are not outstanding for computing the percentage of any other person.

We have based our calculation of the percentage of beneficial ownership prior to this offering on 8,344,223 shares of our Class A common stock and 75,754,278 shares of our Class B common stock outstanding and reflected:

- the filing and effectiveness of our amended and restated certificate of incorporation to be in effect at the closing of this offering;
- the reclassification of all outstanding shares of our Historical Class A common stock into Class B common stock and of our Historical Class B common stock (including outstanding options and warrants to purchase such shares) into Class A common stock prior to the closing of this offering as described under "*Description of capital stock—Reclassification of common stock and conversion of Convertible Preferred Stock*"; and
- the automatic conversion of all shares of our Convertible Preferred Stock outstanding as of August 14, 2019 into 67,704,278 shares of Class B common stock prior to the closing of the offering as described under "*Description of capital stock—Reclassification of common stock and conversion of Convertible Preferred Stock*".

We have based our calculation of the percentage of beneficial ownership after this offering on shares of our Class A common stock issued by us in this offering and shares of Class A common stock outstanding immediately after the completion of this offering, assuming that the underwriters will not exercise their option to purchase up to an additional shares of our Class A common stock from us in full.

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Unless otherwise indicated, the address for each listed stockholder is: c/o 10x Genomics, Inc., 6230 Stoneridge Mall Road, Pleasanton, California 94588. To our knowledge, except as indicated in the footnotes to this table and pursuant to applicable community property laws, the persons named in the table have sole voting and investment power with respect to all shares of common stock.

	Shares beneficially owned before the offering(1)				Shares beneficially owned after the offering(1)			
	Class A common stock		Class B common stock		Class A common stock		Class B common stock	
Name and address of beneficial owner	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Named Executive Officers and Directors:								
Serge Saxonov(2)	946,347	11.2%	3,031,865	4.0%		%	3,031,865	4.0%
Benjamin J. Hindson(3)	710,059	7.8%	3,000,000	4.0%		%	3,000,000	4.0%
Justin J. McAnear(4)	602,499	6.7%	—	—		%	—	—
Eric S. Whitaker(5)	589,791	6.7%	—	—			—	—
John R. Stuelpnagel(6)	723,991	8.7%	2,105,736	2.8%		%	2,105,736	2.8%
Paul A. Conley(7)	—	—	—	—		%	—	—
Sridhar Kosaraju(8)	130,000	1.5%	—	—		%	—	—
Mathai Mammen(9)	200,000	2.3%	—	—		%	—	—
Bryan E. Roberts(10)	—	—	—	—		%	—	—
Shehnaaz Suliman(11)	100,000	1.2%	—	—		%	—	—
All executive officers and directors as a group (12 persons)(12)	4,967,061	56.8%	8,105,736	10.8%		%	8,105,736	10.8%
5% Stockholders:								
Foresite Capital Management and affiliated entities(13)	—	—	13,688,762	18.1%	—	—	13,688,762	18.1%
Venrock and affiliated entities(14)	—	—	12,362,861	16.3%	—	—	12,362,861	16.3%
Paladin Capital Group and affiliated entities(15)	—	—	8,675,167	11.5%	—	—	8,675,167	11.5%
Fidelity and affiliated entities(16)	—	—	8,584,511	11.3%	—	—	8,584,511	11.3%

* Less than 1%.

(1) Assumes no exercise by underwriters of their option to purchase additional shares. See the section titled "*Underwriting*".

(2) Consists of (a) 892,356 shares of Class A common stock, (b) 53,991 shares of Class A common stock issuable pursuant to stock options exercisable within 60 days of August 14, 2019, (c) 1,281,865 shares of Class B common stock and (d) 1,750,000 shares of Class B common stock held by Polaris 2018 Irrevocable Trust, Antares 2018 Irrevocable Trust, Arcturus 2018 Irrevocable Trust, FLY 2018 Irrevocable Trust, LY 2018 Irrevocable Trust, MS 2018 Irrevocable Trust and NS 2018 Irrevocable Trust of which Dr. Saxonov is the sole trustee.

(3) Consists of (a) 3,000,000 shares of Class B common stock and (b) 688,020 shares of Class A common stock issuable pursuant to stock options exercisable within 60 days of August 14, 2019.

(4) Consists of (a) 600,000 shares of Class A common stock issuable pursuant to stock options that may be early exercised at any time (no shares of which were vested as of August 14, 2019) and (b) 2,499 shares of Class A common stock issuable pursuant to stock options exercisable within 60 days of August 14, 2019.

(5) Consists of (a) 84,000 shares of Class A common stock, (b) 441,000 shares of Class A common stock issuable pursuant to stock options that may be early exercised at any time (178,500 shares of which were vested as of August 14, 2019) and (c) 64,791 shares of Class A common stock issuable pursuant to stock options exercisable within 60 days of August 14, 2019.

(6) Consists of (a) 2,105,736 shares of Class B common stock held by the John R. Stuelpnagel Trust of which Dr. Stuelpnagel is the sole trustee and (b) 723,991 shares of Class A common stock (152,084 shares of which were subject to our right of repurchase as of August 14, 2019).

(7) Dr. Conley does not have voting and dispositive power over the shares held of record by the Paladin Funds (as defined below) and Paladin III Co-Investment LLC.

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(8) Consists of 130,000 shares of Class A common stock issuable pursuant to stock options that may be early exercised at any time (10,833 shares of which were vested as of August 14, 2019).

(9) Consists of 200,000 shares of Class A common stock issuable pursuant to stock options that may be early exercised at any time (108,333 shares of which were vested as of August 14, 2019).

(10) Dr. Roberts does not have voting and dispositive power over the shares held by Venrock and affiliated entities.

(11) Consists of 100,000 shares of Class A common stock issuable pursuant to stock options that may be early exercised at any time (no shares of which were vested as of August 14, 2019).

(12) Consists of (a) 1,788,680 shares of Class A common stock beneficially owned by our named executive officers, current directors and other executive officers (152,084 shares of which were subject to our right of repurchase as of August 14, 2019), (b) 2,271,000 shares of Class A common stock issuable pursuant to stock options that may be early exercised at any time (797,666 shares of which were vested as of August 14, 2019) by our named executive officers, current directors and other executive officers, (c) 846,307 shares of Class A common stock subject to outstanding stock options that are exercisable within 60 days of August 14, 2019 by our named executive officers, current directors and other executive officers and (d) 8,105,736 shares of Class B common stock beneficially owned by our named executive officers, current directors and other executive officers.

(13) Consists of (a) 6,851,509 shares of Class B common stock owned directly by Foresite Capital Fund I, L.P. ("FCF I") and (b) 6,837,253 shares of Class B common stock owned directly by Foresite Capital Fund II, L.P. ("FCF II"). Foresite Capital Management I, LLC ("FCM I"), the general partner of FCF I, may be deemed to have sole voting and dispositive power over such shares. Foresite Capital Management II, LLC ("FCM II"), the general partner of FCF II, may be deemed to have sole voting and dispositive power over such shares. James B. Tananbaum ("Mr. Tananbaum"), in his capacity as managing member of FCM I and FCM II, may be deemed to have sole voting and dispositive power over these shares. Each Reporting Person disclaims the existence of a "group". Each of FCM I, FCM II and its members and Mr. Tananbaum disclaim beneficial ownership of any of these shares except to the extent of any pecuniary interest therein, and the filing of this report is not an admission that FCM I, FCM II and its members or Mr. Tananbaum is the beneficial owner of these shares for purposes of Section 16 or any other purpose. The address for Mr. Tananbaum and each of the entities identified in this footnote is c/o Foresight Capital Management, 600 Montgomery Street, Suite 4500, San Francisco CA, 94111.

(14) Consists of (a) 10,284,332 shares of Class B common stock held by Venrock Associates VI, L.P. ("VA VI"), (b) 1,271,045 shares of Class B common stock held by Venrock Healthcare Capital Partners II, L.P. ("VHCP II"), (c) 807,484 shares of Class B common stock held by Venrock Partners VI, L.P. ("VP VI") and (d) 515,386 shares of Class B common stock held by VHCP Co-Investment Holdings II, LLC ("VHCP II Co"). Venrock Management VI, LLC ("VM VI"), is the sole general partner of VA VI. Venrock Partners Management VI, LLC ("VPM VI"), is the sole general partner of VP VI. VHCP Management II, LLC ("VHCPM II"), is the sole general partner of VHCP II and the sole manager of VHCP II Co. VM VI, VPM VI and (VHCPM II) expressly disclaim beneficial ownership over all shares held by VA VI, VP VI, VHCP II and VHCP II Co, except to the extent of their indirect pecuniary interest therein. Dr. Roberts is a member of VM VI and VPM VI and disclaims beneficial ownership over all shares held by VA VI, and VP VI, except to the extent of his indirect pecuniary interests therein. Dr. Bong Koh and Nimish Shah are the sole managers of (VHCPM II) and disclaim beneficial ownership over all shares held by VHCP II and VHCP II Co, except to the extent of their indirect pecuniary interests therein. The address of each of the entities and individuals identified in this footnote is c/o Venrock, 3340 Hillview Avenue, Palo Alto, CA 94304.

(15) Consists of (a) 2,176,409 shares of Class B common stock held by Paladin III, LP, (b) 1,836,875 shares of Class B common stock held by Paladin III (NY City), LP, (c) 1,647,342 shares of Class B common stock held by Paladin III Co-Investment, LLC, (d) 1,261,564 shares of Class B common stock held by Paladin III (Cayman Islands), LP, (e) 626,602 shares of Class B common stock held by Paladin III (CA), LP, (f) 626,602 shares of Class B common stock held by Paladin III (HR), LP and (g) 499,773 shares of Class B common stock held by Paladin International, LLC, collectively except for Paladin III Co-Investment, LLC and Paladin International, LLC (the "Paladin Funds"). Paladin Holdings III, L.P. is the general partner of the Paladin Funds and Managing Member of Paladin III Co-Investment, LLC and Paladin Cyber Holdings, L.P. is the Managing Member of Paladin Intentional, LLC and its Investment Committee, led by Michael Steed as its Chairman, may be deemed to have voting and dispositive power over the shares held of record by the Paladin Funds, Paladin III Co-Investment LLC and Paladin International, LLC. In addition, Dr. Paul Conley is a Managing Director of Paladin Capital Management, LLC and is a member of our board of directors as Paladin's designee representing the Paladin Funds, Paladin III Co-Investment, LLC and Paladin International, LLC, but does not have voting and dispositive power over the shares held of record for the Paladin entities heretofore defined. The address for each of the entities and individuals identified in this footnote is c/o Paladin Capital Management, LLC, 2020 K Street, NW—Suite 620, Washington, DC 20006.

(16) Consists of (a) 3,018,778 shares of Class B common stock held by Mag & Co fbo Fidelity Select Portfolios: Health Care Portfolio, (b) 2,870,040 shares of Class B common stock held by Powhatan & Co., LLC fbo Fidelity Mt. Vernon Street Trust: Fidelity Growth Company Fund, (c) 1,487,064 shares of Class B common stock held by Mag & Co fbo Fidelity Growth Company Commingled Pool, (d) 815,857 shares of Class B common stock held by WAVELENGTH + CO fbo Fidelity Mt. Vernon Street Trust: Fidelity Series Growth Company Fund and (e) 392,772 shares of Class B common stock held by Fidelity Select Portfolios: Medical Technology and Devices Portfolio. These accounts are managed by direct or indirect subsidiaries of FMR LLC. Abigail P. Johnson is a Director, the Chairman, the Chief Executive Officer and the President of FMR LLC. Members of the Johnson family, including Abigail P. Johnson, are the predominant owners, directly or through trusts, of Series B voting common shares of FMR LLC, representing 49% of the voting power of FMR LLC. The Johnson family group and all other Series B shareholders have entered into a shareholders' voting agreement under which all Series B voting common shares will be voted in accordance with the majority vote of Series B voting common shares. Accordingly, through their ownership of voting common shares and the execution of the shareholders' voting agreement, members of the Johnson family may be deemed, under the Investment Company Act of 1940, to form a controlling group with respect to FMR LLC. Neither FMR LLC nor Abigail P. Johnson has the sole power to vote or direct the voting of the shares owned directly by the various investment companies registered under the Investment Company Act ("Fidelity Funds") advised by Fidelity Management & Research Company ("FMR Co"), a wholly owned subsidiary of FMR LLC, which power resides with the Fidelity Funds' Boards of Trustees. Fidelity Management & Research Company carries out the voting of the shares under written guidelines established by the Fidelity Funds' Boards of Trustees. The address for FMR LLC is 200 Seaport Blvd. V12G, Boston, MA 02210.

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Description of capital stock

The following description summarizes certain important terms of our capital stock, as they are expected to be in effect immediately prior to the completion of this offering. Our board of directors has adopted, and our stockholders have approved, an amended and restated certificate of incorporation and amended and restated bylaws that will become effective immediately prior to the completion of this offering and this description summarizes the provisions that are expected to be included in such documents. Because it is only a summary, it does not contain all the information that may be important to you. For a complete description of the matters set forth in this section titled "*Description of capital stock*", you should refer to our amended and restated certificate of incorporation, amended and restated bylaws and amended and restated investors' rights agreement, which are included as exhibits to the registration statement of which this prospectus forms a part, and to the applicable provisions of Delaware law.

Reclassification of common stock and conversion of Convertible Preferred Stock

Our Seventh Amended and Restated Certificate of Incorporation authorizes the issuance of three classes of stock, designated as "Class A common stock" (the "Historical Class A common stock"), "Class B common stock" (the "Historical Class B common stock") and "Preferred Stock", which has been divided into six series (all six series of which are collectively referred to herein as the "Convertible Preferred Stock").

Pursuant to the terms of our Seventh Amended and Restated Certificate of Incorporation, the rights of the holders of Historical Class A common stock and Historical Class B common stock are identical, except with respect to voting and conversion. Holders of Historical Class A common stock are entitled to one hundred votes for each share held on all matters submitted to a vote of stockholders and holders of Historical Class B common stock are entitled to one vote for each share held. In addition, each share of Historical Class A common stock is convertible at any time at the option of the holder into one share of Historical Class B common stock.

Shares of Convertible Preferred Stock are convertible into shares of Historical Class A common stock pursuant to the terms of our Seventh Amended and Restated Certificate of Incorporation and convert automatically upon the occurrence of specified events, including in connection with a qualifying public offering registered under the Securities Act.

Prior to the completion of this offering, we will amend our certificate of incorporation to provide for the reclassification of our (i) Historical Class A common stock into our Class B common stock and (ii) Historical Class B common stock into our Class A common stock. The terms of the Class A common stock and Class B common stock are summarized below. As a result of this reclassification, all outstanding shares of Convertible Preferred Stock will, immediately prior to the completion of this offering, automatically convert into shares of Class B common stock, all shares of our Historical Class B common stock reserved for issuance under the 2012 Stock Plan will become reserved shares of Class A common stock, all outstanding stock options to purchase shares of our Historical Class B common stock will become stock options to purchase shares of our Class A common stock and all outstanding warrants to purchase shares of our Historical Class B common stock will become warrants to purchase shares of our Class A common stock.

In addition, we will further amend our certificate of incorporation and bylaws to include the provisions described below.

Authorized and outstanding capital stock

Immediately following the completion of this offering, our authorized capital stock will consist of 1,200,000,000 shares of capital stock, $0.00001 par value per share, of which:

- 1,000,000,000 shares are designated as Class A common stock;

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- 100,000,000 shares are designated as Class B common stock; and
- 100,000,000 shares are designated as preferred stock.

Assuming the conversion of all outstanding shares of our Convertible Preferred Stock into shares of our Class B common stock, which will occur immediately prior to the completion of this offering, as of June 30, 2019 there were 8,095,382 shares of our Class A common stock outstanding, held by 170 stockholders of record and 75,754,278 shares of our Class B common stock outstanding, held by 62 stockholders of record. The number of shares of our authorized capital stock designated as Class B common stock are equal to the number of shares of Class B common stock outstanding immediately following the completion of this offering. As such, we will not be able to issue any additional shares of Class B common stock following the completion of this offering unless we obtain stockholder approval to amend our amended and restated certificate of incorporation. Pursuant to our amended and restated certificate of incorporation and amended and restated bylaws, our board of directors will have the authority, without stockholder approval except as required by the listing standards of Nasdaq, to issue additional shares of our capital stock.

Common stock

We have two classes of authorized common stock, Class A common stock and Class B common stock. The rights of the holders of Class A common stock and Class B common stock are identical except with respect to voting and conversion.

Voting rights. Holders of our Class A common stock are entitled to one vote for each share held on all matters submitted to a vote of stockholders and holders of our Class B common stock are entitled to ten votes for each share held. The holders of our Class A common stock and Class B common stock vote together as a single class, unless otherwise required by law. Delaware law could require either holders of our Class A common stock or our Class B common stock to vote separately as a single class in the following circumstances:

- if we were to seek to amend our amended and restated certificate of incorporation to increase the authorized number of shares of a class of stock, or to increase or decrease the par value of a class of stock, then that class would be required to vote separately to approve the proposed amendment; and
- if we were to seek to amend our amended and restated certificate of incorporation in a manner that alters or changes the powers, preferences, or special rights of a class of stock in a manner that affected its holders adversely, then that class would be required to vote separately to approve the proposed amendment.

Stockholders do not have the ability to cumulate votes for the election of directors. Our amended and restated certificate of incorporation and amended and restated bylaws that will be in effect at the closing of our initial public offering will provide for a classified board of directors consisting of three classes of approximately equal size, each serving staggered three-year terms. Only the directors in one class will be subject to election by a plurality of the votes cast at each annual meeting of stockholders, with the directors in the other classes continuing for the remainder of their respective three year terms.

Dividend rights. Subject to preferences that may apply to any shares of preferred stock outstanding at the time, the holders of our common stock are entitled to receive dividends out of funds legally available if our board of directors, in its discretion, determines to issue dividends and then only at the times and in the amounts that our board of directors may determine. See the section titled "*Dividend policy*" for additional information.

Rights upon liquidation. If we become subject to a liquidation, dissolution, or winding-up, the assets legally available for distribution to our stockholders would be distributable ratably among the holders of our common stock and any participating preferred stock outstanding at that time, subject to prior satisfaction of all

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outstanding debt and liabilities and the preferential rights of and the payment of liquidation preferences, if any, on any outstanding shares of preferred stock; provided, however, that holders of common stock may receive, or have the right to elect to receive, different or disproportionate consideration if the only difference is that any securities distributed to the holder of a share of Class B common stock have ten times the voting power of any securities distributed to the holder of a share of Class A common stock.

No preemptive or similar rights. Our common stock is not entitled to preemptive rights and is not subject to conversion (other than as described below), redemption or sinking fund provisions.

Conversion of Class B common stock. Each share of Class B common stock is convertible at any time at the option of the holder into one share of Class A common stock. Following the completion of this offering, shares of Class B common stock will automatically convert into shares of Class A common stock upon sale or transfer (other than certain transfers described in our amended and restated certificate of incorporation, including estate planning transfers where sole dispositive power and exclusive voting control with respect to the shares of Class B common stock are retained by the transferring holder and transfers between our co-founders. In addition, each outstanding share of Class B common stock held by a stockholder who is a natural person, or held by the permitted entities of such natural person (as described in our amended and restated certificate of incorporation), will convert automatically into one share of Class A common stock upon the death of such natural person. In the event of the death or permanent and total disability of a co-founder, shares of Class B common stock held by such co-founder or his permitted entities will convert to Class A common stock, provided that the conversion will be deferred for nine months, or up to 18 months if approved by a majority of our independent directors, following his death or permanent and total disability. Transfers between our co-founders are permitted transfers and will not result in conversion of the shares of Class B common stock that are transferred. Each share of Class B common stock will convert automatically into one share of Class A common stock upon (i) a date on or after the one year anniversary of the closing of our initial public offering that is specified by affirmative vote of the holders of a majority of the then outstanding shares of Class B common stock, (ii) the date on which the outstanding shares of Class B common stock represent less than two percent of the aggregate number of shares of the then outstanding Class A common stock and Class B common stock, or (iii) nine months after the death or total disability of both of our co-founders, or such later date not to exceed a total period of 18 months after such death or disability as may be approved by a majority of our independent directors.

Fully paid and non assessable. In connection with this offering, our legal counsel will opine that the shares of our Class A common stock to be issued in this offering will be fully paid and non-assessable.

Preferred stock

Our board of directors is authorized, subject to limitations prescribed by Delaware law, to issue preferred stock in one or more series, to establish from time to time the number of shares to be included in each series, and to fix the designation, powers, preferences and rights of the shares of each series and any of its qualifications, limitations or restrictions, in each case without further vote or action by our stockholders. Our board of directors can also increase or decrease the number of shares of any series of preferred stock, but not below the number of shares of that series then outstanding, without any further vote or action by our stockholders. Our board of directors may authorize the issuance of preferred stock with voting or conversion rights that could adversely affect the voting power or other rights of the holders of our Class A common stock. The issuance of preferred stock, while providing flexibility in connection with possible acquisitions and other corporate purposes, could, among other things, have the effect of delaying, deferring or preventing a change in control of our company and might adversely affect the market price of our Class A common stock and the voting and other rights of the holders of our common stock. We have no current plan to issue any shares of preferred stock.

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Warrants

As of June 30, 2019, warrants to purchase an aggregate of 266,099 shares of Historical Class B common at a weighted-average exercise price of $1.17 per share were outstanding. Upon the closing of this offering, these warrants will become exercisable for the same number of shares of Class A common stock.

All of these warrants have a net exercise provision under which its holder may, in lieu of payment of the exercise price in cash, surrender the warrant and receive a net amount of shares based on the fair market value of our Class A common stock at the time of exercise of the warrant after deduction of the aggregate exercise price. Each warrant contains provisions for the adjustment of the exercise price and the number of shares issuable upon the exercise of the warrant in the event of certain stock dividends, stock splits, reorganizations, reclassifications and consolidations.

Stock options

As of June 30, 2019, we had outstanding stock options to purchase an aggregate of 15,634,182 shares of our Historical Class B common stock, with a weighted-average exercise price of $3.61 per share, under our equity compensation plans. Upon the closing of this offering, these options will become exercisable for the same number of shares of Class A common stock.

Amended and Restated Certificate of Incorporation and Amended and Restated Bylaw provisions

Our amended and restated certificate of incorporation and our amended and restated bylaws will include a number of provisions that could deter hostile takeovers or delay or prevent changes in control of our board of directors or management team, including the following:

Multi-class stock. As described above in "*—Common stock—Voting rights*", our amended and restated certificate of incorporation provides for a multi-class common stock structure, which will provide our pre-offering investors, which includes our executive officers, employees, directors and their affiliates, with significant influence over matters requiring stockholder approval, including the election of directors and significant corporate transactions, such as a merger or other sale of our company or its assets.

Board of directors vacancies. Our amended and restated certificate of incorporation and amended and restated bylaws will authorize only our board of directors to fill vacant directorships, including newly created seats. In addition, the number of directors constituting our board of directors will be permitted to be set only by a resolution adopted by a majority vote of our entire board of directors. These provisions would prevent a stockholder from increasing the size of our board of directors and then gaining control of our board of directors by filling the resulting vacancies with its own nominees. This will make it more difficult to change the composition of our board of directors and will promote continuity of management.

Classified board of directors. Our amended and restated certificate of incorporation and amended and restated bylaws will provide that our board of directors is classified into three classes of directors. A third-party may be discouraged from making a tender offer or otherwise attempting to obtain control of us as it is more difficult and time consuming for stockholders to replace a majority of the directors on a classified board of directors. See the section titled "*Management—Classified board of directors*".

Stockholder action; special meeting of stockholders. Our amended and restated certificate of incorporation and amended and restated bylaws will provide that our stockholders may not take action by written consent, but may only take action at annual or special meetings of our stockholders. As a result, a holder controlling a majority of our capital stock would not be able to amend our amended and restated bylaws or remove directors

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without holding a meeting of our stockholders called in accordance with our amended and restated bylaws. Our amended and restated bylaws will further provide that special meetings of our stockholders may be called only by a majority of our board of directors, the Chairman of our board of directors, or our Chief Executive Officer, thus prohibiting a stockholder from calling a special meeting. These provisions might delay the ability of our stockholders to force consideration of a proposal or for stockholders controlling a majority of our capital stock to take any action, including the removal of directors.

Advance notice requirements for stockholder proposals and director nominations. Our amended and restated bylaws will provide advance notice procedures for stockholders seeking to bring business before our annual meeting of stockholders or to nominate candidates for election as directors at our annual meeting of stockholders. Our amended and restated bylaws will also specify certain requirements regarding the form and content of a stockholder's notice. These provisions might preclude our stockholders from bringing matters before our annual meeting of stockholders or from making nominations for directors at our annual meeting of stockholders if the proper procedures are not followed. We expect that these provisions may also discourage or deter a potential acquirer from conducting a solicitation of proxies to elect the acquirer's own slate of directors or otherwise attempting to obtain control of our company.

No cumulative voting. The Delaware General Corporation Law provides that stockholders are not entitled to cumulate votes in the election of directors unless a corporation's certificate of incorporation provides otherwise. Our amended and restated certificate of incorporation does not provide for cumulative voting.

Directors removed only for cause. Our amended and restated certificate of incorporation will provide that stockholders may remove directors only for cause by the affirmative vote of holders of at least two-thirds of the voting power of our then outstanding capital stock.

Amendment of charter and bylaws provisions. Any amendment of the above provisions in our amended and restated certificate of incorporation and amended and restated bylaws would require approval by holders of at least two-thirds of the voting power of our then outstanding capital stock.

Issuance of undesignated preferred stock. Our board of directors will have the authority, without further action by our stockholders, to issue up to 100,000,000 shares of undesignated preferred stock with rights and preferences, including voting rights, designated from time to time by our board of directors. The existence of authorized but unissued shares of preferred stock would enable our board of directors to render more difficult or to discourage an attempt to obtain control of us by means of a merger, tender offer, proxy contest or other means.

Delaware Law

We will be governed by the provisions of Section 203 of the Delaware General Corporation Law. In general, Section 203 prohibits a public Delaware corporation from engaging in a "business combination" with an "interested stockholder" for a period of three years after the date of the transaction in which the person became an interested stockholder, unless:

- any breach of the director's duty of loyalty to our company or our stockholders;
- the transaction was approved by the board of directors prior to the time that the stockholder became an interested stockholder;
- upon consummation of the transaction which resulted in the stockholder becoming an interested stockholder, the interested stockholder owned at least 85% of the voting stock of the corporation outstanding at the time the transaction commenced, excluding shares owned by directors who are also

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officers of the corporation and shares owned by employee stock plans in which employee participants do not have the right to determine confidentially whether shares held subject to the plan will be tendered in a tender or exchange offer; or

- at or subsequent to the time the stockholder became an interested stockholder, the business combination was approved by the board of directors and authorized at an annual or special meeting of the stockholders, and not by written consent, by the affirmative vote of at least two-thirds of the outstanding voting stock which is not owned by the interested stockholder.

In general, Section 203 defines a "business combination" to include mergers, asset sales and other transactions resulting in financial benefit to a stockholder and an "interested stockholder" as a person who, together with affiliates and associates, owns, or within three years did own, 15% or more of the corporation's outstanding voting stock. These provisions may have the effect of delaying, deferring, or preventing changes in control of our company.

Limitation of liability of directors and officers

Our amended and restated certificate of incorporation will provide that no director will be personally liable to us or our stockholders for monetary damages for breach of fiduciary duty as a director, except as required by applicable law, as in effect from time to time. Section 102(b)(7) of the Delaware General Corporation Law permits a corporation to provide in its certificate of incorporation that a director of the corporation shall not be personally liable to the corporation or its stockholders for monetary damages for breach of fiduciary duty as a director, except for liability:

- any breach of the director's duty of loyalty to our company or our stockholders;
- any act or omission not in good faith or which involved intentional misconduct or a knowing violation of law;
- unlawful payments of dividends or unlawful stock repurchases or redemptions as provided in Section 174 of the Delaware General Corporation Law; and
- any transaction from which the director derived an improper personal benefit.

As a result, neither we nor our stockholders have the right, through stockholders' derivative suits on our behalf, to recover monetary damages against a director for breach of fiduciary duty as a director, including breaches resulting from grossly negligent behavior, except in the situations described above.

Our amended and restated bylaws will also provide that, to the fullest extent permitted by law, we will indemnify any officer or director of our company against all damages, claims and liabilities arising out of the fact that the person is or was our director or officer, or served any other enterprise at our request as a director or officer. Amending this provision will not reduce our indemnification obligations relating to actions taken before an amendment.

Forum selection

Our amended and restated bylaws will provide that, unless we consent in writing to the selection of an alternative forum, the sole and exclusive forum for the following types of actions or proceedings under Delaware statutory or common law: (i) any derivative action or proceeding brought on our behalf, (ii) any action asserting a claim of breach of a fiduciary duty owed by any of our directors, officers or other employees to us or our stockholders, (iii) any action asserting a claim against us or any of our directors or officers arising pursuant to any provision of the Delaware General Corporation Law, our certificate of incorporation or our amended and

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restated bylaws or (iv) any other action asserting a claim that is governed by the internal affairs doctrine shall be a state or federal court located within the State of Delaware, in all cases subject to the courts having jurisdiction over indispensable parties named as defendants. Although we believe these provisions benefit us by providing increased consistency in the application of Delaware law for the specified types of actions and proceedings, the provisions may have the effect of discouraging lawsuits against us or our directors and officers. Nothing in our amended and restated bylaws will preclude stockholders that assert claims under the Securities Act or the Exchange Act from bringing such claims in state or federal court, subject to applicable law. Any person or entity purchasing or otherwise acquiring any interest in shares of our capital stock shall be deemed to have notice of and consented to the foregoing forum selection provisions.

Anti-takeover effects of some provisions

Certain provisions of Delaware law, our amended and restated certificate of incorporation and our amended and restated bylaws, which are summarized below, may have the effect of delaying, deferring or discouraging another person from acquiring control of us. They are also designed, in part, to encourage persons seeking to acquire control of us to negotiate first with our board of directors. We believe that the benefits of increased protection of our potential ability to negotiate with an unfriendly or unsolicited acquirer outweigh the disadvantages of discouraging a proposal to acquire us because negotiation of these proposals could result in an improvement of their terms.

Registration rights

After the completion of this offering, certain holders of our Class B common stock will be entitled to rights with respect to the registration of their shares under the Securities Act. These registration rights are contained in our Amended and Restated Investors' Rights Agreement (the "IRA"). We and certain holders of our Convertible Preferred Stock are parties to the IRA. Immediately prior to the completion of this offering, each share of outstanding Convertible Preferred Stock will convert automatically into one share of Class B common stock. The registration rights set forth in the IRA will expire two years following the completion of this offering, or, with respect to any particular stockholder, when such stockholder is able to sell all of its shares on any one day pursuant to Rule 144 of the Securities Act or a similar exemption. We will pay the registration expenses (other than underwriting discounts, selling commissions and transfer taxes) of the holders of the shares registered pursuant to the registrations described below. In an underwritten offering, the managing underwriter, if any, has the right, subject to specified conditions, to limit the number of shares such holders may include. We expect that our stockholders will waive their rights under the IRA (i) to notice of this offering and (ii) to include their registrable shares in this offering. In addition, in connection with this offering, we expect that each stockholder that has registration rights will agree not to sell or otherwise dispose of any securities without the prior written consent of the company and J.P. Morgan Securities LLC and Goldman Sachs & Co. LLC through and including , 2020, subject to certain terms and conditions and early release of certain holders in specified circumstances. See the section titled "*Shares eligible for future sale—Lock-up agreements*" for additional information regarding such restrictions.

Certain stockholders who are party to the IRA have waived their registration rights and the registration rights of the other stockholders who are party to the IRA, in each case, with respect to this offering and have entered into contractual lock-up agreements with the underwriters. See the sections titled "*Shares eligible for future sale*" and "*Underwriting*" for more information.

Demand registration rights. After the completion of this offering, the holders of up to shares of our Class B common stock and shares of our Class A common stock will be entitled to certain demand registration rights. At any time beginning six months after the effective date of this offering, the holders of at

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least 50% of these shares then outstanding can request that we register the offer and sale of their shares, or such request must cover securities in which the anticipated aggregate public offering price, before payment of underwriting discounts and commissions, is at least $10,000,000. We are obligated to effect only two such registrations. If we determine that it would be seriously detrimental to us and our stockholders to effect such a demand registration, we have the right to defer such registration, not more than once in any 12-month period, for a period of up to 90 days.

S-3 registration rights. After the completion of this offering, the holders of up to shares of our Class B common stock and shares of our Class A common stock will be entitled to certain Form S-3 registration rights. The holders of at least 20% of these shares then outstanding may make a written request that we register the offer and sale of their shares on a registration statement on Form S-3 if we are eligible to file a registration statement on Form S-3 so long as the request covers securities the anticipated aggregate public offering price of which, before payment of underwriting discounts and commissions, is at least $1,000,000. These stockholders may make an unlimited number of requests for registration on Form S-3; however, we will not be required to effect a registration on Form S-3 if we have effected two such registrations within the 12-month period preceding the date of the request. Additionally, if we determine that it would be seriously detrimental to us and our stockholders to effect such a registration, we have the right to defer such registration, not more than once in any 12-month period, for a period of up to 120 days.

Piggyback registration rights. After the completion of this offering, if we propose to register the offer and sale of our Class A common stock under the Securities Act, in connection with the public offering of such Class A common stock, the holders of up to shares of our Class B common stock and shares of our Class A common stock will be entitled to certain “piggyback” registration rights allowing the holders to include their shares in such registration, subject to certain marketing and other limitations. As a result, whenever we propose to file a registration statement under the Securities Act, other than with respect to (i) a registration in which the only Class A common stock being registered is Class A common stock issuable upon conversion of debt securities that are also being registered, (ii) a registration related to any employee benefit plan or a corporate reorganization or other transaction covered by Rule 145 promulgated under the Securities Act, or (iii) a registration on any registration form which does not include substantially the same information as would be required to be included in a registration statement covering the public offering of our Class A common stock, the holders of these shares are entitled to notice of the registration and have the right, subject to certain limitations, to include their shares in the registration.

Listing

We have applied for the listing of our Class A common stock on Nasdaq under the symbol “TXG”.

Transfer agent and registrar

Upon completion of this offering, the transfer agent and registrar for our common stock will be American Stock Transfer & Trust Company, LLC. The transfer agent and registrar’s address is 6201 15th Avenue, Brooklyn, New York 11219.

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Shares eligible for future sale

Prior to this offering, there has been no public market for our Class A common stock. Future sales of substantial amounts of our Class A common stock in the public market could adversely affect market prices prevailing from time to time. Furthermore, because only a limited number of shares will be available for sale shortly after this offering due to existing contractual and legal restrictions on resale as described below, there may be sales of substantial amounts of our Class A common stock in the public market after the restrictions lapse. This may adversely affect the prevailing market price and our ability to raise equity capital in the future.

Upon completion of this offering, we will have shares of Class A common stock outstanding assuming no exercise by the underwriters of their stock option to purchase additional shares and no exercise of any stock options or warrants after June 30, 2019 and shares of Class B common stock outstanding. Of these shares, shares, or shares of our Class A common stock if the underwriters exercise their stock option to purchase additional shares in full, sold in this offering will be freely transferable without restriction or registration under the Securities Act, except for any shares purchased by one of our existing "affiliates", as that term is defined in Rule 144 under the Securities Act. The remaining shares of Class A common stock outstanding and all outstanding shares of Class B common stock (including the shares of Class A common stock into which such shares are convertible) are "restricted shares" as defined in Rule 144 may be sold in the public market only if registered or if they qualify for an exemption from registration under Rules 144 or 701 of the Securities Act. As a result of the contractual lock-up period through and including , 2020 and the provisions of Rules 144 and 701, these shares will be available for sale in the public market as follows:

Number of Shares	Date
	On the date of this prospectus
	After 91 days from the date of this prospectus
	Beginning , 2020 (subject, in some cases, to volume limitations)
	At various times after , 2020 (subject, in some cases, to volume limitations)

Rule 144

In general, a person who has beneficially owned restricted shares of our Class A common stock for at least six months would be entitled to sell such securities, provided that (i) such person is not deemed to have been one of our affiliates at the time of, or at any time during the 90 days preceding, a sale and (ii) we are subject to the Exchange Act periodic reporting requirements for at least 90 days before the sale. Persons who have beneficially owned restricted shares of our Class A common stock for at least six months but who are our affiliates at the time of, or any time during the 90 days preceding, a sale, would be subject to additional restrictions, by which such person would be entitled to sell within any three-month period only a number of securities that does not exceed the greater of either of the following:

- 1% of the number of shares of our Class A common stock then outstanding, which will equal approximately shares immediately after this offering, assuming no exercise by the underwriters of their stock option to purchase additional shares; or
- the average weekly trading volume of our Class A common stock on Nasdaq during the four calendar weeks preceding the filing of a notice on Form 144 with respect to the sale;

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provided, in each case, that we are subject to the Exchange Act periodic reporting requirements for at least 90 days before the sale. Such sales both by affiliates and by non-affiliates must also comply with the manner of sale, current public information and notice provisions of Rule 144 to the extent applicable.

Rule 701

In general, under Rule 701, any of our employees, directors, officers, consultants or advisors who purchases shares from us in connection with a compensatory stock or stock option plan or other written agreement before the effective date of this offering is entitled to resell such shares 90 days after the effective date of this offering in reliance on Rule 144, without having to comply with the holding period requirements or other restrictions contained in Rule 701.

The SEC has indicated that Rule 701 will apply to typical stock options granted by an issuer before it becomes subject to the reporting requirements of the Exchange Act, along with the shares acquired upon exercise of such stock options, including exercises after the date of this prospectus. Securities issued in reliance on Rule 701 are restricted securities and, subject to the contractual restrictions described above, beginning 90 days after the date of this prospectus, may be sold by persons other than "affiliates", as defined in Rule 144, subject only to the manner of sale provisions of Rule 144 and by "affiliates" under Rule 144 without compliance with its one-year minimum holding period requirement.

Registration rights

Upon completion of this offering, the holders of shares of Class A common stock, including shares of Class A common stock issuable upon the exercise of outstanding stock options or their transferees, and the holders of shares of Class B common stock, as converted into an equivalent number of shares of our Class A common stock upon such offer and sale, will be entitled to various rights with respect to the registration of these shares under the Securities Act. Registration of these shares under the Securities Act would result in these shares becoming freely tradable without restriction under the Securities Act immediately upon the effectiveness of the registration, except for shares purchased by affiliates. See the sections titled "*Description of capital stock—Registration rights*" and "*—Lock-up agreements*" of such shares are covered by lock-up agreements. Following the expiration of the lock-up period, registration of these shares under the Securities Act would result in the shares becoming freely tradable without restriction under the Securities Act immediately upon the effectiveness of the registration.

Stock options

As of June 30, 2019, stock options to purchase a total of 15,634,182 shares of Class A common stock were outstanding. All of the shares subject to stock options are subject to lock-up agreements. An additional 1,323,858 shares of Class A common stock were available for future grants under our stock plans at such date. See the section titled "*Executive Compensation—Equity incentive plans*" for more information regarding the 2012 Stock Plan and our Omnibus Incentive Plan.

Upon completion of this offering, we intend to file a registration statement under the Securities Act covering all shares of Class A common stock subject to outstanding stock options or issuable pursuant to the 2012 Stock Plan, our Omnibus Incentive Plan and our ESPP. Subject to Rule 144 volume limitations applicable to affiliates, shares registered under any registration statements will be available for sale in the open market, except to the extent that the shares are subject to vesting restrictions with us or the contractual lock-up restrictions described below.

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Lock-up agreements

All of our directors, executive officers and the holders of substantially all of our capital stock and securities convertible or exchangeable for our Class A common stock will agree, subject to certain exceptions, from the date of this prospectus through and including , 2020, that they will not, without the consent of J.P. Morgan Securities LLC and Goldman Sachs & Co. LLC (i) offer, pledge, sell, contract to sell, sell any option or contract to purchase, purchase any option or contract to sell, grant any option, right or warrant to purchase, hedge, lend, or otherwise transfer or dispose of, directly or indirectly, any shares of Class A common stock or any securities convertible into or exercisable or exchangeable for shares of Class A common stock (including, without limitation, shares of Class A common stock or such other securities which may be deemed to be beneficially owned by the undersigned in accordance with the rules and regulations of the SEC and securities which may be issued upon exercise of a stock option or warrant), or publicly disclose the intention to undertake any of the foregoing, (ii) enter into any swap or other agreement that transfers, in whole or in part, any of the economic consequences of ownership of shares of Class A common stock or such other securities, whether any such transaction described in clause (i) or (ii) above is to be settled by delivery of shares of Class A common stock or such other securities, in cash or otherwise or (iii) make any demand for or exercise any right with respect to the registration of any shares of Class A common stock or any security convertible into or exercisable or exchangeable for shares of Class A common stock. See the section titled "*Underwriting*".

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Material United States federal income and estate tax consequences to non-U.S. holders

The following is a summary of material United States federal income and estate tax consequences of the purchase, ownership and disposition of our Class A common stock as of the date hereof. Except where noted, this summary deals only with Class A common stock that is held as a capital asset by a non-U.S. holder (as defined below).

A “non-U.S. holder” means a beneficial owner of our Class A common stock (other than an entity treated as a partnership for United States federal income tax purposes) that is not, for United States federal income tax purposes, any of the following:

- an individual citizen or resident of the United States;
- a corporation (or any other entity treated as a corporation for United States federal income tax purposes) created or organized in or under the laws of the United States, any state thereof or the District of Columbia;
- an estate the income of which is subject to United States federal income taxation regardless of its source; or
- a trust if it (i) is subject to the primary supervision of a court within the United States and one or more United States persons have the authority to control all substantial decisions of the trust or (ii) has a valid election in effect under applicable United States Treasury regulations to be treated as a United States person.

This summary is based upon provisions of the Code, regulations, rulings and judicial decisions as of the date hereof. Those authorities may be changed, perhaps retroactively, so as to result in United States federal income and estate tax consequences different from those summarized below. This summary does not address all aspects of United States federal income and estate taxes and does not deal with foreign, state, local or other tax considerations that may be relevant to non-U.S. holders in light of their particular circumstances. In addition, it does not represent a detailed description of the United States federal income and estate tax consequences applicable to you if you are subject to special treatment under the United States federal income tax laws (including if you are a United States expatriate, foreign pension fund, “controlled foreign corporation”, “passive foreign investment company” or a partnership or other pass-through entity for United States federal income tax purposes). We cannot assure you that a change in law will not alter significantly the tax considerations that we describe in this summary.

If a partnership (or other entity treated as a partnership for United States federal income tax purposes) holds our Class A common stock, the tax treatment of a partner will generally depend upon the status of the partner and the activities of the partnership. If you are a partner of a partnership holding our Class A common stock, you should consult your tax advisors.

If you are considering the purchase of our Class A common stock, you should consult your own tax advisors concerning the particular United States federal income and estate tax consequences to you of the purchase, ownership and disposition of our Class A common stock, as well as the consequences to you arising under other United States federal tax laws and the laws of any other taxing jurisdiction.

Dividends

In the event that we make a distribution of cash or other property (other than certain pro rata distributions of our stock) in respect of our Class A common stock, the distribution generally will be treated as a dividend for United States federal income tax purposes to the extent it is paid from our current or accumulated earnings and profits, as determined under United States federal income tax principles. Any portion of a distribution that

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exceeds our current and accumulated earnings and profits generally will be treated first as a tax-free return of capital, causing a reduction in the adjusted tax basis of a non-U.S. holder's Class A common stock, and to the extent the amount of the distribution exceeds a non-U.S. holder's adjusted tax basis in our Class A common stock, the excess will be treated as gain from the disposition of our Class A common stock (the tax treatment of which is discussed below under "—*Gain on disposition of Class A common stock*").

Dividends paid to a non-U.S. holder generally will be subject to withholding of United States federal income tax at a 30% rate or such lower rate as may be specified by an applicable income tax treaty. However, dividends that are effectively connected with the conduct of a trade or business by the non-U.S. holder within the United States (and, if required by an applicable income tax treaty, are attributable to a United States permanent establishment) are not subject to the withholding tax, provided certain certification and disclosure requirements are satisfied. Instead, such dividends are subject to United States federal income tax on a net income basis in the same manner as if the non-U.S. holder were a United States person as defined under the Code. Any such effectively connected dividends received by a foreign corporation may be subject to an additional "branch profits tax" at a 30% rate or such lower rate as may be specified by an applicable income tax treaty.

A non-U.S. holder who wishes to claim the benefit of an applicable treaty rate and avoid backup withholding, as discussed below, for dividends will be required (i) to provide the applicable withholding agent with a properly executed Internal Revenue Service ("IRS") Form W-8BEN or Form W-8BEN-E (or other applicable form) certifying under penalty of perjury that such holder is not a United States person as defined under the Code and is eligible for treaty benefits or (ii) if our Class A common stock is held through certain foreign intermediaries, to satisfy the relevant certification requirements of applicable United States Treasury regulations. Special certification and other requirements apply to certain non-U.S. holders that are pass-through entities rather than corporations or individuals.

A non-U.S. holder eligible for a reduced rate of United States federal withholding tax pursuant to an income tax treaty may obtain a refund of any excess amounts withheld by timely filing an appropriate claim for refund with the IRS.

Gain on disposition of Class A common stock

Subject to the discussion of backup withholding below, any gain realized by a non-U.S. holder on the sale or other disposition of our Class A common stock generally will not be subject to United States federal income tax unless:

- the gain is effectively connected with a trade or business of the non-U.S. holder in the United States (and, if required by an applicable income tax treaty, is attributable to a United States permanent establishment of the non-U.S. holder);
- the non-U.S. holder is an individual who is present in the United States for 183 days or more in the taxable year of that disposition and certain other conditions are met; or
- we are or have been a "United States real property holding corporation" for United States federal income tax purposes and certain other conditions are met.

A non-U.S. holder described in the first bullet point immediately above will be subject to tax on the gain derived from the sale or other disposition in the same manner as if the non-U.S. holder were a United States person as defined under the Code. In addition, if any non-U.S. holder described in the first bullet point immediately above is a foreign corporation, the gain realized by such non-U.S. holder may be subject to an additional "branch profits tax" at a 30% rate or such lower rate as may be specified by an applicable income tax treaty. An

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individual non-U.S. holder described in the second bullet point immediately above will be subject to a 30% (or such lower rate as may be specified by an applicable income tax treaty) tax on the gain derived from the sale or other disposition, which gain may be offset by United States source capital losses even though the individual is not considered a resident of the United States (provided such individual has timely filed United States federal income tax returns with respect to such losses).

Generally, a corporation is a "United States real property holding corporation" if the fair market value of its United States real property interests equals or exceeds 50% of the sum of the fair market value of its worldwide real property interests and its other assets used or held for use in a trade or business (all as determined for United States federal income tax purposes). We believe we are not and do not anticipate becoming a "United States real property holding corporation" for United States federal income tax purposes.

Federal estate tax

Class A common stock held by an individual non-U.S. holder at the time of death will be included in such holder's gross estate for United States federal estate tax purposes, unless an applicable estate tax treaty provides otherwise.

Information reporting and backup withholding

Distributions paid to a non-U.S. holder and the amount of any tax withheld with respect to such distributions generally will be reported to the IRS. Copies of the information returns reporting such distributions and any withholding may also be made available to the tax authorities in the country in which the non-U.S. holder resides under the provisions of an applicable income tax treaty.

A non-U.S. holder will not be subject to backup withholding on dividends received if such holder certifies under penalty of perjury that it is a non-U.S. holder (and the payor does not have actual knowledge or reason to know that such holder is a United States person as defined under the Code), or such holder otherwise establishes an exemption.

Information reporting and, depending on the circumstances, backup withholding will apply to the proceeds of a sale or other disposition of our Class A common stock made within the United States or conducted through certain United States-related financial intermediaries, unless the beneficial owner certifies under penalty of perjury that it is a non-U.S. holder (and the payor does not have actual knowledge or reason to know that the beneficial owner is a United States person as defined under the Code), or such owner otherwise establishes an exemption.

Backup withholding is not an additional tax and any amounts withheld under the backup withholding rules will be allowed as a refund or a credit against a non-U.S. holder's United States federal income tax liability provided the required information is timely furnished to the IRS.

Additional withholding requirements

Under Sections 1471 through 1474 of the Code (such Sections commonly referred to as "FATCA"), a 30% United States federal withholding tax may apply to any dividends paid on our Class A common stock to (i) a "foreign financial institution" (as specifically defined in the Code) which does not provide sufficient documentation, typically on IRS Form W-8BEN-E, evidencing either (a) an exemption from FATCA, or (b) its compliance (or deemed compliance) with FATCA (which may alternatively be in the form of compliance with an intergovernmental agreement with the United States) in a manner which avoids withholding, or (ii) a "non-financial foreign entity" (as specifically defined in the Code) which does not provide sufficient documentation, typically on IRS Form W-8BEN-E,

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evidencing either (x) an exemption from FATCA, or (y) adequate information regarding certain substantial United States beneficial owners of such entity (if any). If a dividend payment is both subject to withholding under FATCA and subject to the withholding tax discussed above under "*—Dividends*", the withholding under FATCA may be credited against, and therefore reduce, such other withholding tax. Under applicable Treasury regulations and administrative guidance, withholding under FATCA would have applied to payments of gross proceeds from the sale or other disposition of stock on or after January 1, 2019, although under recently proposed regulations (the preamble to which specifies that taxpayers are permitted to rely on such proposed regulations pending finalization), no withholding would apply with respect to payments of gross proceeds. You should consult your own tax advisors regarding these requirements and whether they may be relevant to your ownership and disposition of our Class A common stock.

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Underwriting

We are offering the shares of Class A common stock described in this prospectus through a number of underwriters. J.P. Morgan Securities LLC, Goldman Sachs & Co. LLC and BofA Securities, Inc. are acting as joint book-running managers of the offering and as representatives of the underwriters. We have entered into an underwriting agreement with the underwriters. Subject to the terms and conditions of the underwriting agreement, we have agreed to sell to the underwriters, and each underwriter has severally agreed to purchase, at the public offering price less the underwriting discounts and commissions set forth on the cover page of this prospectus, the number of shares of Class A common stock listed next to its name in the following table:

Name	Number of shares
J.P. Morgan Securities LLC	
Goldman Sachs & Co. LLC	
BofA Securities, Inc.	
Cowen and Company, LLC	
Total	

The underwriters are committed to purchase all the shares of Class A common stock offered by us if they purchase any shares. The underwriting agreement also provides that if an underwriter defaults, the purchase commitments of non-defaulting underwriters may also be increased or the offering may be terminated.

The underwriters propose to offer the shares of Class A common stock directly to the public at the initial public offering price set forth on the cover page of this prospectus and to certain dealers at that price less a concession not in excess of $ per share. After the initial offering of the shares of Class A common stock to the public, the underwriters may change the offering price and the other selling terms. Sales of shares made outside of the United States may be made by affiliates of the underwriters. The offering of the shares by the underwriters is subject to receipt and acceptance and subject to the underwriters' right to reject any order in whole or in part.

The underwriters have an option to buy up to additional shares of Class A common stock from us to cover sales of shares by the underwriters which exceed the number of shares specified in the table above. The underwriters have 30 days from the date of this prospectus to exercise this option to purchase additional shares. If any shares are purchased with this option to purchase additional shares, the underwriters will purchase shares in approximately the same proportion as shown in the table above. If any additional shares of Class A common stock are purchased, the underwriters will offer the additional shares on the same terms as those on which the shares are being offered.

The underwriting fee is equal to the public offering price per share of Class A common stock less the amount paid by the underwriters to us per share of Class A common stock. The underwriting fee is $ per share. The following table shows the per share and total underwriting discounts and commissions to be paid to the underwriters assuming both no exercise and full exercise of the underwriters' option to purchase additional shares.

	Without exercise of option to purchase additional shares	With full exercise of option to purchase additional shares
Per share	$	$
Total	$	$

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We estimate that the total expenses of this offering, including registration, filing and listing fees, printing fees and legal and accounting expenses, but excluding the underwriting discounts and commissions, will be approximately $. We have agreed to reimburse the underwriters for expenses relating to the clearance of this offering with the Financial Industry Regulatory Authority, Inc. in an amount up to $.

A prospectus in electronic format may be made available on the websites maintained by one or more underwriters, or selling group members, if any, participating in the offering. The underwriters may agree to allocate a number of shares to underwriters and selling group members for sale to their online brokerage account holders. Internet distributions will be allocated by the representatives to underwriters and selling group members that may make internet distributions on the same basis as other allocations.

We have agreed that we will not, subject to certain exceptions, for a period of 180 days after the date of this prospectus (i) offer, pledge, sell, contract to sell, sell any option or contract to purchase, purchase any option or contract to sell, grant any option, right or warrant to purchase, or otherwise transfer or dispose of, directly or indirectly, or file with, or submit to, the SEC a registration statement under the Securities Act relating to, any shares of Class A common stock or any securities convertible into or exercisable or exchangeable for shares of Class A common stock, or publicly disclose the intention to make any offer, sale, pledge, disposition, submission or filing, or (ii) enter into any swap, hedging, or other agreement that transfers, in whole or in part, any of the economic consequences of ownership of shares of Class A common stock or any such other securities, whether any such transaction described in clause (i) or (ii) above is to be settled by delivery of shares of Class A common stock or such other securities, in cash or otherwise, without the prior written consent of J.P. Morgan Securities LLC and Goldman Sachs & Co. LLC. Notwithstanding the foregoing, we may issue shares of our Class A common stock or securities convertible into or exercisable or exchangeable for shares of our Class A common stock in amount equal to up to % of the total number of outstanding shares of our Class A common stock outstanding immediately following the issuance of the shares of Class A common stock to be sold in this offering plus the shares of Class A common stock reserved for issuance under our 2012 Stock Plan, Omnibus Incentive Plan and ESPP, in connection with mergers, acquisitions or commercial or strategic transactions (including, without limitation, entry into joint ventures, marketing or distribution agreements or collaboration agreements or acquisitions of technology, assets or intellectual property licenses) provided that the recipient execute a lockup agreement with respect to such shares.

All of our directors, executive officers and the holders of substantially all of our capital stock and securities convertible or exchangeable for our Class A common stock will agree, subject to certain exceptions, from the date of this prospectus through and including , 2020, that they will not, without the consent of J.P. Morgan Securities LLC and Goldman Sachs & Co. LLC (i) offer, pledge, sell, contract to sell, sell any option or contract to purchase, purchase any option or contract to sell, grant any option, right or warrant to purchase, hedge, lend, or otherwise transfer or dispose of, directly or indirectly, any shares of Class A common stock or any securities convertible into or exercisable or exchangeable for shares of Class A common stock (including, without limitation, shares of Class A common stock or such other securities which may be deemed to be beneficially owned by the undersigned in accordance with the rules and regulations of the SEC and securities which may be issued upon exercise of a stock option or warrant), or publicly disclose the intention to undertake any of the foregoing, (ii) enter into any swap or other agreement that transfers, in whole or in part, any of the economic consequences of ownership of shares of Class A common stock or such other securities, whether any such transaction described in clause (i) or (ii) above is to be settled by delivery of shares of Class A common stock or such other securities, in cash or otherwise or (iii) make any demand for or exercise any right with respect to the registration of any shares of Class A common stock or any security convertible into or exercisable or exchangeable for shares of Class A common stock.

We have agreed to indemnify the underwriters against certain liabilities, including liabilities under the Securities Act.

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We have applied for the listing of our Class A common stock on Nasdaq under the symbol “TXG”.

In connection with this offering, the underwriters may engage in stabilizing transactions, which involves making bids for, purchasing and selling shares of Class A common stock in the open market for the purpose of preventing or retarding a decline in the market price of the Class A common stock while this offering is in progress. These stabilizing transactions may include making short sales of the Class A common stock, which involves the sale by the underwriters of a greater number of shares of Class A common stock than they are required to purchase in this offering and the purchasing of shares of Class A common stock on the open market to cover positions created by short sales. Short sales may be “covered” shorts, which are short positions in an amount not greater than the underwriters’ option to purchase additional shares referred to above, or may be “naked” shorts, which are short positions in excess of that amount. The underwriters may close out any covered short position either by exercising their option to purchase additional shares, in whole or in part, or by purchasing shares in the open market. In making this determination, the underwriters will consider, among other things, the price of shares available for purchase in the open market compared to the price at which the underwriters may purchase shares through the option to purchase additional shares. A naked short position is more likely to be created if the underwriters are concerned that there may be downward pressure on the price of the Class A common stock in the open market that could adversely affect investors who purchase in this offering. To the extent that the underwriters create a naked short position, they will purchase shares in the open market to cover the position.

The underwriters have advised us that, pursuant to Regulation M of the Securities Act, they may also engage in other activities that stabilize, maintain or otherwise affect the price of the Class A common stock, including the imposition of penalty bids. This means that if the representatives of the underwriters purchase Class A common stock in the open market in stabilizing transactions or to cover short sales, the representatives can require the underwriters that sold those shares as part of this offering to repay the underwriting discount received by them.

These activities may have the effect of raising or maintaining the market price of the Class A common stock or preventing or retarding a decline in the market price of the Class A common stock and, as a result, the price of the Class A common stock may be higher than the price that otherwise might exist in the open market. If the underwriters commence these activities, they may discontinue them at any time. The underwriters may carry out these transactions on Nasdaq, in the over-the-counter market or otherwise.

Prior to this offering, there has been no public market for our Class A common stock. The initial public offering price will be determined by negotiations between us and the representatives of the underwriters. In determining the initial public offering price, we and the representatives of the underwriters expect to consider a number of factors including:

- the information set forth in this prospectus and otherwise available to the representatives;
- our prospects and the history and prospects for the industry in which we compete;
- an assessment of our management;
- our prospects for future earnings;
- the general condition of the securities markets at the time of this offering;
- the recent market prices of, and demand for, publicly traded common stock of generally comparable companies; and
- other factors deemed relevant by the underwriters and us.

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Neither we nor the underwriters can assure investors that an active trading market will develop for shares of our Class A common stock, or that the shares will trade in the public market at or above the initial public offering price.

Other relationships

Certain of the underwriters and their affiliates have provided in the past to us and our affiliates and may provide from time to time in the future certain commercial banking, financial advisory, investment banking and other services for us and such affiliates in the ordinary course of their business, for which they have received and may continue to receive customary fees and commissions. In addition, from time to time, certain of the underwriters and their affiliates may effect transactions for their own account or the account of customers, and hold on behalf of themselves or their customers, long or short positions in our debt or equity securities or loans, and may do so in the future.

Selling restrictions

Other than in the United States, no action has been taken by us or the underwriters that would permit a public offering of the securities offered by this prospectus in any jurisdiction where action for that purpose is required. The securities offered by this prospectus may not be offered or sold, directly or indirectly, nor may this prospectus or any other offering material or advertisements in connection with the offer and sale of any such securities be distributed or published in any jurisdiction, except under circumstances that will result in compliance with the applicable rules and regulations of that jurisdiction. Persons into whose possession this prospectus comes are advised to inform themselves about and to observe any restrictions relating to the offering and the distribution of this prospectus. This prospectus does not constitute an offer to sell or a solicitation of an offer to buy any securities offered by this prospectus in any jurisdiction in which such an offer or a solicitation is unlawful.

Notice to prospective investors in the European Economic Area

In relation to each Member State of the European Economic Area which has implemented the Prospectus Directive (each, a “Relevant Member State”), with effect from and including the date on which the Prospectus Directive is implemented in that Relevant Member State, no offer of shares may be made to the public in that Relevant Member State other than:

(i) to any legal entity which is a qualified investor as defined in the Prospectus Directive;

(ii) to fewer than 150 natural or legal persons (other than qualified investors as defined in the Prospectus Directive), subject to obtaining the prior consent of the underwriters; or

(iii) in any other circumstances falling within Article 3(2) of the Prospectus Directive,

provided that no such offer of shares shall require the Company or any underwriter to publish a prospectus pursuant to Article 3 of the Prospectus Directive or supplement a prospectus pursuant to Article 16 of the Prospectus Directive and each person who initially acquires any shares or to whom any offer is made will be deemed to have represented, acknowledged and agreed to and with each of the underwriters and the Company that it is a “qualified investor” within the meaning of the law in that Relevant Member State implementing Article 2(1)(e) of the Prospectus Directive.

In the case of any shares being offered to a financial intermediary as that term is used in Article 3(2) of the Prospectus Directive, each such financial intermediary will be deemed to have represented, acknowledged and

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agreed that the shares acquired by it in the offer have not been acquired on a non-discretionary basis on behalf of, nor have they been acquired with a view to their offer or resale to, persons in circumstances which may give rise to an offer of any shares to the public other than their offer or resale in a Relevant Member State to qualified investors as so defined or in circumstances in which the prior consent of the representatives has been obtained to each such proposed offer or resale.

For the purposes of this provision, the expression an "offer of shares to the public" in relation to any shares in any Relevant Member State means the communication in any form and by means of sufficient information on the terms of the offer and the shares to be offered so as to enable an investor to decide to purchase shares, as the same may be varied in that Member State by any measure implementing the Prospectus Directive in that Member State, the expression "Prospectus Directive" means Directive 2003/71/EC (as amended, including by Directive 2010/73/EU), and includes any relevant implementing measure in the Relevant Member State.

Notice to prospective investors in the United Kingdom

In addition, in the United Kingdom, this document is being distributed only to, and is directed only at, and any offer subsequently made may only be directed at, persons who are "qualified investors" (as defined in the Prospectus Directive) (i) who have professional experience in matters relating to investments falling within Article 19(5) of the Financial Services and Markets Act 2000 (Financial Promotion) Order 2005, as amended (the "Order") and/or (ii) who are high net worth companies (or persons to whom it may otherwise be lawfully communicated) falling within Article 49(2)(a) to (d) of the Order (all such persons together being referred to as "relevant persons") or otherwise in circumstances which have not resulted and will not result in an offer to the public of the shares in the United Kingdom within the meaning of the Financial Services and Markets Act 2000.

Any person in the United Kingdom that is not a relevant person should not act or rely on the information included in this document or use it as basis for taking any action. In the United Kingdom, any investment or investment activity that this document relates to may be made or taken exclusively by relevant persons.

Notice to prospective investors in Canada

The shares may be sold only to purchasers purchasing, or deemed to be purchasing, as principal that are accredited investors, as defined in National Instrument 45-106 Prospectus Exemptions or subsection 73.3(1) of the Securities Act (Ontario), and are permitted clients, as defined in National Instrument 31-103 Registration Requirements, Exemptions and Ongoing Registrant Obligations. Any resale of the shares must be made in accordance with an exemption from, or in a transaction not subject to, the prospectus requirements of applicable securities laws.

Securities legislation in certain provinces or territories of Canada may provide a purchaser with remedies for rescission or damages if this prospectus (including any amendment thereto) contains a misrepresentation, provided that the remedies for rescission or damages are exercised by the purchaser within the time limit prescribed by the securities legislation of the purchaser's province or territory. The purchaser should refer to any applicable provisions of the securities legislation of the purchaser's province or territory for particulars of these rights or consult with a legal advisor.

Pursuant to section 3A.3 of National Instrument 33-105 Underwriting Conflicts ("NI 33-105"), the underwriters are not required to comply with the disclosure requirements of NI 33-105 regarding underwriter conflicts of interest in connection with this offering.

Notice to prospective investors in Switzerland

The shares may not be publicly offered in Switzerland and will not be listed on the SIX Swiss Exchange ("SIX") or on any other stock exchange or regulated trading facility in Switzerland. This document does not constitute a

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prospectus within the meaning of, and has been prepared without regard to the disclosure standards for, issuance prospectuses under art. 652a or art. 1156 of the Swiss Code of Obligations or the disclosure standards for listing prospectuses under art. 27 ff. of the SIX Listing Rules or the listing rules of any other stock exchange or regulated trading facility in Switzerland. Neither this document nor any other offering or marketing material relating to the shares or the offering may be publicly distributed or otherwise made publicly available in Switzerland.

Neither this document nor any other offering or marketing material relating to the offering, the Company or the shares has been or will be filed with or approved by any Swiss regulatory authority. In particular, this document will not be filed with, and the offer of shares will not be supervised by, the Swiss Financial Market Supervisory Authority FINMA, and the offer of shares has not been and will not be authorized under the Swiss Federal Act on Collective Investment Schemes ("CISA"). The investor protection afforded to acquirers of interests in collective investment schemes under the CISA does not extend to acquirers of shares.

Notice to prospective investors in the Dubai International Financial Centre

This document relates to an Exempt Offer in accordance with the Markets Rules 2012 of the Dubai Financial Services Authority ("DFSA"). This document is intended for distribution only to persons of a type specified in the Markets Rules 2012 of the DFSA. It must not be delivered to, or relied on by, any other person. The DFSA has no responsibility for reviewing or verifying any documents in connection with Exempt Offers. The DFSA has not approved this prospectus supplement nor taken steps to verify the information set forth herein and has no responsibility for this document. The securities to which this document relates may be illiquid and/or subject to restrictions on their resale. Prospective purchasers of the securities offered should conduct their own due diligence on the securities. If you do not understand the contents of this document you should consult an authorized financial advisor.

In relation to its use in the Dubai International Financial Centre ("DIFC"), this document is strictly private and confidential and is being distributed to a limited number of investors and must not be provided to any person other than the original recipient and may not be reproduced or used for any other purpose. The interests in the securities may not be offered or sold directly or indirectly to the public in the DIFC.

Notice to prospective investors in the United Arab Emirates

The shares have not been, and are not being, publicly offered, sold, promoted or advertised in the United Arab Emirates (including the DIFC) other than in compliance with the laws of the United Arab Emirates (and the DIFC) governing the issue, offering and sale of securities. Further, this prospectus does not constitute a public offer of securities in the United Arab Emirates (including the DIFC) and is not intended to be a public offer. This prospectus has not been approved by or filed with the Central Bank of the United Arab Emirates, the Securities and Commodities Authority or the DFSA.

Notice to prospective investors in Australia

This prospectus:

- does not constitute a product disclosure document or a prospectus under Chapter 6D.2 of the Corporations Act 2001 (Cth) (the "Corporations Act");
- has not been, and will not be, lodged with the Australian Securities and Investments Commission ("ASIC"), as a disclosure document for the purposes of the Corporations Act and does not purport to include the information required of a disclosure document under Chapter 6D.2 of the Corporations Act;

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- does not constitute or involve a recommendation to acquire, an offer or invitation for issue or sale, an offer or invitation to arrange the issue or sale, or an issue or sale, of interests to a "retail client" (as defined in section 761G of the Corporations Act and applicable regulations) in Australia; and
- may only be provided in Australia to select investors who are able to demonstrate that they fall within one or more of the categories of investors ("Exempt Investors") available under section 708 of the Corporations Act.

The shares may not be directly or indirectly offered for subscription or purchased or sold, and no invitations to subscribe for or buy the shares may be issued, and no draft or definitive offering memorandum, advertisement or other offering material relating to any shares may be distributed in Australia, except where disclosure to investors is not required under Chapter 6D of the Corporations Act or is otherwise in compliance with all applicable Australian laws and regulations. By submitting an application for the shares, you represent and warrant to us that you are an Exempt Investor.

As any offer of shares under this document will be made without disclosure in Australia under Chapter 6D.2 of the Corporations Act, the offer of those securities for resale in Australia within 12 months may, under section 707 of the Corporations Act, require disclosure to investors under Chapter 6D.2 if none of the exemptions in section 708 applies to that resale. By applying for the shares you undertake to us that you will not, for a period of 12 months from the date of issue of the shares, offer, transfer, assign or otherwise alienate those securities to investors in Australia except in circumstances where disclosure to investors is not required under Chapter 6D.2 of the Corporations Act or where a compliant disclosure document is prepared and lodged with ASIC.

Notice to prospective investors in Japan

The shares have not been and will not be registered pursuant to Article 4, Paragraph 1 of the Financial Instruments and Exchange Act. Accordingly, none of the shares nor any interest therein may be offered or sold, directly or indirectly, in Japan or to, or for the benefit of, any "resident" of Japan (which term as used herein means any person resident in Japan, including any corporation or other entity organized under the laws of Japan), or to others for re-offering or resale, directly or indirectly, in Japan or to or for the benefit of a resident of Japan, except pursuant to an exemption from the registration requirements of, and otherwise in compliance with, the Financial Instruments and Exchange Act and any other applicable laws, regulations and ministerial guidelines of Japan in effect at the relevant time.

Notice to prospective investors in Hong Kong

The shares have not been offered or sold and will not be offered or sold in Hong Kong, by means of any document, other than (i) to "professional investors" as defined in the Securities and Futures Ordinance (Cap. 571) of Hong Kong and any rules made under that Ordinance; or (ii) in other circumstances which do not result in the document being a "prospectus" as defined in the Companies (Winding Up and Miscellaneous Provisions) Ordinance (Cap. 32) of Hong Kong or which do not constitute an offer to the public within the meaning of that Ordinance. No advertisement, invitation or document relating to the shares has been or may be issued or has been or may be in the possession of any person for the purposes of issue, whether in Hong Kong or elsewhere, which is directed at, or the contents of which are likely to be accessed or read by, the public of Hong Kong (except if permitted to do so under the securities laws of Hong Kong) other than with respect to shares which are or are intended to be disposed of only to persons outside Hong Kong or only to "professional investors" as defined in the Securities and Futures Ordinance and any rules made under that Ordinance.

Notice to prospective investors in Singapore

This prospectus has not been registered as a prospectus with the Monetary Authority of Singapore. Accordingly, this prospectus and any other document or material in connection with the offer or sale, or invitation for

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subscription or purchase, of shares may not be circulated or distributed, nor may the shares be offered or sold, or be made the subject of an invitation for subscription or purchase, whether directly or indirectly, to persons in Singapore other than (i) to an institutional investor under Section 274 of the Securities and Futures Act, Chapter 289 of Singapore (the "SFA"), (ii) to a relevant person pursuant to Section 275(1), or any person pursuant to Section 275(1A), and in accordance with the conditions specified in Section 275 of the SFA or (iii) otherwise pursuant to, and in accordance with the conditions of, any other applicable provision of the SFA.

Where the shares are subscribed or purchased under Section 275 of the SFA by a relevant person which is:

(i) a corporation (which is not an accredited investor (as defined in Section 4A of the SFA)) the sole business of which is to hold investments and the entire share capital of which is owned by one or more individuals, each of whom is an accredited investor; or

(ii) a trust (where the trustee is not an accredited investor) whose sole purpose is to hold investments and each beneficiary of the trust is an individual who is an accredited investor,

securities (as defined in Section 239(1) of the SFA) of that corporation or the beneficiaries' rights and interest (howsoever described) in that trust shall not be transferred within six months after that corporation or that trust has acquired the shares pursuant to an offer made under Section 275 of the SFA except:

(i) to an institutional investor or to a relevant person defined in Section 275(2) of the SFA, or to any person arising from an offer referred to in Section 275(1A) or Section 276(4)(i)(B) of the SFA;

(ii) where no consideration is or will be given for the transfer;

(iii) where the transfer is by operation of law;

(iv) as specified in Section 276(7) of the SFA; or

(v) as specified in Regulation 32 of the Securities and Futures (Offers of Investments) (Shares and Debentures) Regulations 2005 of Singapore.

Solely for the purposes of its obligations pursuant to Section 309B of the SFA, we have determined, and hereby notify all relevant persons (as defined in the CMP Regulations 2018), that the shares are "prescribed capital markets products" (as defined in the CMP Regulations 2018) and Excluded Investment Products (as defined in MAS Notice SFA 04-N12: Notice on the Sale of Investment Products and MAS Notice FAA-N16: Notice on Recommendations on Investment Products).

Notice to prospective investors in Bermuda

Shares may be offered or sold in Bermuda only in compliance with the provisions of the Investment Business Act of 2003 of Bermuda which regulates the sale of securities in Bermuda. Additionally, non-Bermudian persons (including companies) may not carry on or engage in any trade or business in Bermuda unless such persons are permitted to do so under applicable Bermuda legislation.

Notice to prospective investors in Saudi Arabia

This document may not be distributed in the Kingdom of Saudi Arabia except to such persons as are permitted under the Offers of Securities Regulations as issued by the board of the Saudi Arabian Capital Market Authority ("CMA") pursuant to resolution number 2-11-2004 dated 4 October 2004 as amended by resolution number 1-28-2008, as amended (the "CMA Regulations"). The CMA does not make any representation as to the accuracy or completeness of this document and expressly disclaims any liability whatsoever for any loss arising from, or

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incurred in reliance upon, any part of this document. Prospective purchasers of the securities offered hereby should conduct their own due diligence on the accuracy of the information relating to the securities. If you do not understand the contents of this document, you should consult an authorized financial adviser.

Notice to prospective investors in the British Virgin Islands

The shares are not being and may not be offered to the public or to any person in the British Virgin Islands for purchase or subscription by or on behalf of the Company. The Company may be offered to companies incorporated under the BVI Business Companies Act, 2004 (British Virgin Islands) ("BVI Companies"), but only where the offer will be made to, and received by, the relevant BVI Company entirely outside of the British Virgin Islands. This prospectus has not been, and will not be, registered with the Financial Services Commission of the British Virgin Islands. No registered prospectus has been or will be prepared in respect of the shares for the purposes of the Securities and Investment Business Act, 2010 or the Public Issuers Code of the British Virgin Islands.

Notice to prospective investors in China

This prospectus does not constitute a public offer of shares, whether by sale or subscription, in the People's Republic of China (the "PRC"). The shares are not being offered or sold directly or indirectly in the PRC to or for the benefit of, legal or natural persons of the PRC.

Further, no legal or natural persons of the PRC may directly or indirectly purchase any of the shares or any beneficial interest therein without obtaining all prior PRC's governmental approvals that are required, whether statutorily or otherwise. Persons who come into possession of this document are required by the issuer and its representatives to observe these restrictions.

Notice to prospective investors in Korea

The shares have not been and will not be registered under the Financial Investments Services and Capital Markets Act of Korea and the decrees and regulations thereunder (the "FSCMA") and the shares have been, and will be, offered in Korea as a private placement under the FSCMA. None of the shares may be offered, sold or delivered directly or indirectly, or offered or sold to any person for re-offering or resale, directly or indirectly, in Korea or to any resident of Korea except pursuant to the applicable laws and regulations of Korea, including the FSCMA and the Foreign Exchange Transaction Law of Korea and the decrees and regulations thereunder (the "FETL"). Furthermore, the purchaser of the shares shall comply with all applicable regulatory requirements (including but not limited to requirements under the FETL) in connection with the purchase of the shares. By the purchase of the shares, the relevant holder thereof will be deemed to represent and warrant that if it is in Korea or is a resident of Korea, it purchased the shares pursuant to the applicable laws and regulations of Korea.

Notice to prospective investors in Malaysia

No prospectus or other offering material or document in connection with the offer and sale of the shares has been or will be registered with the Securities Commission of Malaysia ("Commission") for the Commission's approval pursuant to the Capital Markets and Services Act 2007. Accordingly, this prospectus and any other document or material in connection with the offer or sale, or invitation for subscription or purchase, of the shares may not be circulated or distributed, nor may the shares be offered or sold, or be made the subject of an invitation for subscription or purchase, whether directly or indirectly, to persons in Malaysia other than (i) a closed end fund approved by the Commission; (ii) a holder of a Capital Markets Services License; (iii) a person

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who acquires the shares, as principal, if the offer is on terms that the shares may only be acquired at a consideration of not less than RM250,000 (or its equivalent in foreign currencies) for each transaction; (iv) an individual whose total net personal assets or total net joint assets with his or her spouse exceeds RM3 million (or its equivalent in foreign currencies), excluding the value of the primary residence of the individual; (v) an individual who has a gross annual income exceeding RM300,000 (or its equivalent in foreign currencies) per annum in the preceding twelve months; (vi) an individual who, jointly with his or her spouse, has a gross annual income of RM400,000 (or its equivalent in foreign currencies), per annum in the preceding twelve months; (vii) a corporation with total net assets exceeding RM10 million (or its equivalent in a foreign currencies) based on the last audited accounts; (viii) a partnership with total net assets exceeding RM10 million (or its equivalent in foreign currencies); (ix) a bank licensee or insurance licensee as defined in the Labuan Financial Services and Securities Act 2010; (x) an Islamic bank licensee or takaful licensee as defined in the Labuan Financial Services and Securities Act 2010; and (xi) any other person as may be specified by the Commission; provided that, in the each of the preceding categories (i) to (xi), the distribution of the shares is made by a holder of a Capital Markets Services License who carries on the business of dealing in securities. The distribution in Malaysia of this prospectus is subject to Malaysian laws. This prospectus does not constitute and may not be used for the purpose of public offering or an issue, offer for subscription or purchase, invitation to subscribe for or purchase any securities requiring the registration of a prospectus with the Commission under the Capital Markets and Services Act 2007.

Notice to prospective investors in Taiwan

The shares have not been and will not be registered with the Financial Supervisory Commission of Taiwan pursuant to relevant securities laws and regulations and may not be sold, issued or offered within Taiwan through a public offering or in circumstances which constitutes an offer within the meaning of the Securities and Exchange Act of Taiwan that requires a registration or approval of the Financial Supervisory Commission of Taiwan. No person or entity in Taiwan has been authorized to offer, sell, give advice regarding or otherwise intermediate the offering and sale of the shares in Taiwan.

Notice to prospective investors in South Africa

Due to restrictions under the securities laws of South Africa, the shares are not offered, and the offer shall not be transferred, sold, renounced or delivered, in South Africa or to a person with an address in South Africa, unless one or other of the following exemptions applies:

(i) the offer, transfer, sale, renunciation or delivery is to:

- (a) persons whose ordinary business is to deal in securities, as principal or agent;
- (b) the South African Public Investment Corporation;
- (c) persons or entities regulated by the Reserve Bank of South Africa;
- (d) authorized financial service providers under South African law;
- (e) financial institutions recognized as such under South African law;
- (f) a wholly-owned subsidiary of any person or entity contemplated in (c), (d) or (e), acting as agent in the capacity of an authorized portfolio manager for a pension fund or collective investment scheme (in each case duly registered as such under South African law); or
- (g) any combination of the person in (a) to (f); or

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(ii) the total contemplated acquisition cost of the securities, for any single addressee acting as principal is equal to or greater than ZAR1,000,000.

No “offer to the public” (as such term is defined in the South African Companies Act, No. 71 of 2008 (as amended or re-enacted) (the “South African Companies Act”)) in South Africa is being made in connection with the issue of the shares. Accordingly, this document does not, nor is it intended to, constitute a “registered prospectus” (as that term is defined in the South African Companies Act) prepared and registered under the South African Companies Act and has not been approved by, and/or filed with, the South African Companies and Intellectual Property Commission or any other regulatory authority in South Africa. Any issue or offering of the shares in South Africa constitutes an offer of the shares in South Africa for subscription or sale in South Africa only to persons who fall within the exemption from “offers to the public” set out in section 96(1)(a) of the South African Companies Act. Accordingly, this document must not be acted on or relied on by persons in South Africa who do not fall within section 96(1)(a) of the South African Companies Act (such persons being referred to as “SA Relevant Persons”). Any investment or investment activity to which this document relates is available in South Africa only to SA Relevant Persons and will be engaged in South Africa only with SA relevant persons.

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Legal matters

The validity of the issuance of the shares of Class A common stock offered hereby will be passed upon for 10x Genomics, Inc. by Simpson Thacher & Bartlett LLP. Cooley LLP is representing the underwriters.

Experts

Ernst & Young LLP, independent registered public accounting firm, has audited our consolidated financial statements at December 31, 2017 and 2018, and for each of the two years in the period ended December 31, 2018, as set forth in their report. We have included our financial statements in the prospectus and elsewhere in the registration statement in reliance of Ernst & Young LLP's report, given on their authority as experts in accounting and auditing.

Where you can find more information

We have filed with the SEC a registration statement on Form S-1 under the Securities Act with respect to the shares of Class A common stock offered hereby. This prospectus does not contain all of the information set forth in the registration statement and the exhibits and schedules thereto. For further information with respect to the company and its Class A common stock, reference is made to the registration statement and the exhibits and any schedules filed therewith. Statements contained in this prospectus as to the contents of any contract or other document referred to are not necessarily complete and in each instance, if such contract or document is filed as an exhibit, reference is made to the copy of such contract or other document filed as an exhibit to the registration statement, each statement being qualified in all respects by such reference. The SEC maintains a website at www.sec.gov, from which interested persons can electronically access the registration statement, including the exhibits and any schedules thereto and which contains reports, proxy and information statements and other information regarding issuers that file electronically with the SEC.

As a result of the offering, we will be required to file periodic reports and other information with the SEC. We also maintain a website at https://www.10xgenomics.com. Our website and the information contained therein or connected thereto shall not be deemed to be incorporated into this prospectus or the registration statement of which it forms a part.

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10x Genomics, Inc.

Index to Consolidated Financial Statements

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Report of Independent Registered Public Accounting Firm

To the Stockholders and the Board of Directors of 10x Genomics, Inc.

Opinion on the Financial Statements

We have audited the accompanying consolidated balance sheets of 10x Genomics, Inc. (the "Company") as of December 31, 2017 and 2018, the related consolidated statements of operations and comprehensive loss, convertible preferred stock and stockholders' equity (deficit) and cash flows for the years then ended, and the related notes (collectively referred to as the "consolidated financial statements"). In our opinion, the consolidated financial statements present fairly, in all material respects, the financial position of the Company as of December 31, 2017 and 2018, and the results of its operations and its cash flows for the years then ended in conformity with U.S. generally accepted accounting principles.

Basis for Opinion

These financial statements are the responsibility of the Company's management. Our responsibility is to express an opinion on the Company's financial statements based on our audits. We are a public accounting firm registered with the Public Company Accounting Oversight Board (United States) (PCAOB) and are required to be independent with respect to the Company in accordance with the U.S. federal securities laws and the applicable rules and regulations of the Securities and Exchange Commission and the PCAOB.

We conducted our audits in accordance with the standards of the PCAOB. Those standards require that we plan and perform the audit to obtain reasonable assurance about whether the financial statements are free of material misstatement, whether due to error or fraud. The Company is not required to have, nor were we engaged to perform, an audit of its internal control over financial reporting. As part of our audits we are required to obtain an understanding of internal control over financial reporting but not for the purpose of expressing an opinion on the effectiveness of the Company's internal control over financial reporting. Accordingly, we express no such opinion.

Our audits included performing procedures to assess the risks of material misstatement of the financial statements, whether due to error or fraud, and performing procedures that respond to those risks. Such procedures included examining, on a test basis, evidence regarding the amounts and disclosures in the financial statements. Our audits also included evaluating the accounting principles used and significant estimates made by management, as well as evaluating the overall presentation of the financial statements. We believe that our audits provide a reasonable basis for our opinion.

/s/ Ernst & Young LLP

We have served as the Company's auditor since 2015.

Redwood City, California
May 10, 2019

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10x Genomics, Inc.

Consolidated Balance Sheets
(in thousands, except share and per share data)

	December 31, 2017	December 31, 2018	June 30, 2019 (unaudited)	Pro forma June 30, 2019 (unaudited) (Note 2)
Assets				
Current assets:				
Cash and cash equivalents	$ 47,857	$ 65,080	$ 56,034	
Accounts receivable, net	13,341	28,088	26,803	
Inventory	4,838	8,570	12,325	
Tenant allowance receivable	—	1,478	7,944	
Prepaid expenses and other current assets	2,071	3,020	4,120	
Total current assets	68,107	106,236	107,226	
Property and equipment, net	6,925	11,127	38,337	
Restricted cash	—	5,008	5,008	
Other assets	577	1,939	5,023	
Total assets	$ 75,609	$ 124,310	$ 155,594	
Liabilities, Convertible Preferred Stock and Stockholders' Equity (Deficit)				
Current liabilities:				
Accounts payable	$ 5,443	$ 8,792	$ 9,429	
Accrued compensation and related benefits	4,477	7,047	6,399	
Accrued expenses and other current liabilities	3,662	8,172	16,434	
Term loans, current portion	4,224	4,187	4,887	
Accrued legal expenses	3,223	1,769	3,321	
Deferred revenue, current	1,112	2,395	2,757	
Total current liabilities	22,141	32,362	43,227	
Term loans, noncurrent portion	6,335	25,489	24,777	
Accrued contingent liabilities	—	38,000	55,255	
Deferred revenue, noncurrent	713	1,102	1,131	
Deferred rent, noncurrent	1	3,329	15,019	
Other noncurrent liabilities	514	771	889	
Total liabilities	29,704	101,053	140,298	
Commitments and contingencies (Note 7)				
Convertible preferred stock, $0.00001 par value, 59,730,213 shares authorized, issued and outstanding as of December 31, 2017, 67,904,871 shares authorized and 67,704,278 shares issued and outstanding as of December 31, 2018 and June 30, 2019 (unaudited); aggregate liquidation preference of $242,588 as of December 31, 2018 and June 30, 2019 (unaudited); no shares issued and outstanding, pro forma (unaudited)	158,414	243,244	243,244	
Stockholders' equity (deficit):				
Historical common stock, $0.00001 par value; 170,000,000 shares authorized as of December 31, 2017, 12,883,930 shares issued and outstanding as of December 31, 2017; 190,955,000 shares authorized as of December 31, 2018 and June 30, 2019 (unaudited), 14,549,801 shares issued and outstanding as of December 31, 2018, 16,145,382 shares issued and outstanding as of June 30, 2019 (unaudited); shares issued and outstanding, pro forma (unaudited)	1	1	1	
Additional paid-in capital	6,136	11,165	17,715	
Accumulated deficit	(118,631)	(231,116)	(245,630)	
Accumulated other comprehensive loss	(15)	(37)	(34)	
Total stockholders' equity (deficit)	(112,509)	(219,987)	(227,948)	
Total liabilities, convertible preferred stock and stockholders' equity (deficit)	$ 75,609	$ 124,310	$ 155,594	

The accompanying notes are an integral part of these consolidated financial statements.

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10x Genomics, Inc.

Consolidated Statements of Operations and Comprehensive Loss
(in thousands, except share and per share data)

	Year Ended December 31,		Six Months Ended June 30,	
	2017	2018	2018	2019
			(unaudited)	
Revenue	$ 71,085	$ 146,313	$ 59,152	$ 109,397
Cost of revenue	10,560	28,661	8,520	28,971
Gross profit	60,525	117,652	50,632	80,426
Operating expenses:				
Research and development	32,164	47,537	23,372	32,999
In-process research and development	—	62,363	6,206	—
Selling, general and administrative	46,736	87,936	41,920	59,464
Accrued contingent liabilities	—	30,580	—	1,360
Total operating expenses	78,900	228,416	71,498	93,823
Loss from operations	(18,375)	(110,764)	(20,866)	(13,397)
Other income (expense):				
Interest income	308	1,024	461	505
Interest expense	(811)	(2,409)	(1,062)	(1,379)
Other income (expense), net	137	(249)	(120)	(141)
Total other income (expense)	(366)	(1,634)	(721)	(1,015)
Loss before provision for income taxes	(18,741)	(112,398)	(21,587)	(14,412)
Provision for income taxes	21	87	29	102
Net loss	$ (18,762)	$ (112,485)	$ (21,616)	(14,514)
Other comprehensive income (loss):				
Foreign currency translation adjustment	(15)	(22)	16	3
Comprehensive loss	$ (18,777)	$ (112,507)	$ (21,600)	(14,511)
Net loss per share attributable to Historical common stockholders, basic and diluted	$ (1.62)	$ (8.40)	$ (1.66)	$ (0.96)
Weighted-average shares used to compute net loss per share attributable to Historical common stockholders, basic and diluted	11,587,751	13,392,273	12,985,535	15,187,258
Pro forma net loss per share attributable to Historical common stockholders, basic and diluted (unaudited)		$ (1.45)		$ (0.18)
Weighted-average shares used to compute pro forma net loss per share attributable to Historical common stockholders, basic and diluted (unaudited)		77,494,992		82,891,536

The accompanying notes are an integral part of these consolidated financial statements.

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10x Genomics, Inc.

Consolidated Statements of Convertible Preferred Stock and Stockholders' Equity (Deficit)
(in thousands, except share data)

	Convertible Preferred Stock		Historical Common Stock		Additional Paid-in Capital	Accumulated Deficit	Accumulated Other Comprehensive Loss	Total Stockholders' Deficit
	Shares	Amount	Shares	Amount				
Balance as of January 1, 2017	55,264,133	$ 138,450	11,330,679	$ 1	$ 3,437	$ (99,869)	$ —	$ (96,431)
Issuance of Series C convertible preferred stock, net of issuance costs	4,466,080	19,964	—	—	—	—	—	—
Issuance of Historical Class B common stock upon exercise of options	—	—	1,628,251	—	926	—	—	926
Repurchase of unvested Historical Class B common stock related to early exercised options	—	—	(75,000)	—	—	—	—	—
Vesting of shares subject to repurchase, including early exercised options	—	—	—	—	112	—	—	112
Stock-based compensation	—	—	—	—	1,661	—	—	1,661
Net loss	—	—	—	—	—	(18,762)	—	(18,762)
Other comprehensive income (loss)	—	—	—	—	—	—	(15)	(15)
Balance as of December 31, 2017	59,730,213	158,414	12,883,930	1	6,136	(118,631)	(15)	(112,509)
Issuance of Series D convertible preferred stock, net of issuance costs	5,224,658	49,878	—	—	—	—	—	—
Issuance of Series D-1 convertible preferred stock, net of issuance costs	2,749,407	34,952	—	—	—	—	—	—
Issuance of Historical Class B common stock upon exercise of options	—	—	1,508,762	—	1,173	—	—	1,173
Issuance of Historical Class B common stock for in-process research and development	—	—	157,109	—	792	—	—	792
Vesting of shares subject to repurchase, including early exercised options	—	—	—	—	256	—	—	256
Issuance of warrants to purchase Historical common stock	—	—	—	—	150	—	—	150
Stock-based compensation	—	—	—	—	2,658	—	—	2,658
Net loss	—	—	—	—	—	(112,485)	—	(112,485)
Other comprehensive income (loss)	—	—	—	—	—	—	(22)	(22)
Balance as of December 31, 2018	67,704,278	243,244	14,549,801	1	11,165	(231,116)	(37)	(219,987)
Issuance of Historical Class B common stock upon exercise of options (unaudited)	—	—	1,595,581	—	2,005	—	—	2,005
Vesting of shares subject to repurchase, including early exercised options (unaudited)	—	—	—	—	161	—	—	161
Stock-based compensation (unaudited)	—	—	—	—	4,384	—	—	4,384
Net loss (unaudited)	—	—	—	—	—	(14,514)	—	(14,514)
Other comprehensive income (loss) (unaudited)	—	—	—	—	—	—	3	3
Balance as of June 30, 2019 (unaudited)	67,704,278	$ 243,244	16,145,382	$ 1	$ 17,715	$ (245,630)	$ (34)	$ (227,948)
Balance as of December 31, 2017	59,730,213	158,414	12,883,930	1	6,136	(118,631)	(15)	(112,509)
Issuance of Series D convertible preferred stock, net of issuance costs (unaudited)	5,224,658	49,878	—	—	—	—	—	—
Issuance of Historical Class B common stock upon exercise of options (unaudited)	—	—	648,175	—	461	—	—	461
Vesting of shares subject to repurchase, including early exercised options (unaudited)	—	—	—	—	18	—	—	18
Issuance of warrants to purchase Historical common stock (unaudited)	—	—	—	—	150	—	—	150
Stock-based compensation (unaudited)	—	—	—	—	1,006	—	—	1,006
Net loss (unaudited)	—	—	—	—	—	(21,616)	—	(21,616)
Other comprehensive income (loss) (unaudited)	—	—	—	—	—	—	16	16
Balance as of June 30, 2018 (unaudited)	64,954,871	208,292	13,532,105	1	7,771	(140,247)	1	(132,474)

The accompanying notes are an integral part of these consolidated financial statements.

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10x Genomics, Inc.

Consolidated Statements of Cash Flows
(in thousands)

	Year Ended December 31,		Six Months Ended June 30,	
	2017	2018	2018	2019
			(unaudited)	
Operating activities				
Net loss	$(18,762)	$(112,485)	$(21,616)	$(14,514)
Adjustments to reconcile net loss to net cash used in operating activities:				
Depreciation and amortization	4,305	3,905	2,167	2,180
Stock-based compensation	1,661	2,658	1,006	4,384
Historical Class B common stock issued for in-process research and development	—	792	—	—
Loss on disposal of property and equipment	—	251	12	614
Accretion of discount on term loans	149	455	206	46
Changes in operating assets and liabilities:				
Accounts receivable	(5,131)	(14,747)	(3,957)	1,360
Inventory	(1,995)	(3,732)	(1,600)	(3,755)
Tenant allowance receivable	—	(1,478)	—	(6,466)
Prepaid expenses and other current assets	(702)	(951)	(406)	(1,176)
Other assets	33	(999)	(337)	(73)
Accounts payable	3,034	2,587	5,118	(986)
Accrued compensation and other related benefits	3,498	2,600	(1,267)	(645)
Deferred revenue	1,317	1,673	677	390
Accrued contingent liabilities	—	38,000	—	17,255
Accrued expenses and other current liabilities	1,844	1,701	(367)	2,978
Deferred rent, noncurrent	(68)	3,328	4	11,690
Other noncurrent liabilities	118	33	134	119
Net cash provided by (used in) operating activities	(10,699)	(76,409)	(20,226)	13,401
Investing activities				
Purchases of property and equipment	(3,756)	(6,284)	(3,261)	(22,508)
Purchase of intangible assets	—	(425)	—	—
Net cash used in investing activities	(3,756)	(6,709)	(3,261)	(22,508)
Financing activities				
Proceeds from term loans	—	19,512	19,512	—
Payments on term loans	—	(704)	(704)	—
Payments on capital lease obligations	(393)	(69)	(69)	—
Proceeds from issuance of preferred stock, net of issuance costs	19,964	84,830	49,878	—
Repurchase of unvested Historical Class B common stock related to early exercised shares	(80)	—	—	
Proceeds from issuance of Historical Class B common stock upon exercise of stock options	1,092	1,798	461	2,005
Deferred offering costs for initial public offering	—	—	—	(1,946)
Net cash provided by financing activities	20,583	105,367	69,078	59
Effect of exchange rates on changes on cash, cash equivalents, and restricted cash	(14)	(18)	11	2
Net increase (decrease) in cash, cash equivalents, and restricted cash	6,114	22,231	45,602	(9,046)
Cash, cash equivalents, and restricted cash at beginning of year	41,743	47,857	47,857	70,088
Cash, cash equivalents, and restricted cash at end of year	$ 47,857	$ 70,088	$ 93,459	$ 61,042
Supplemental disclosures of cash flow information				
Cash paid for interest	$ 658	$ 1,824	$ 745	$ 1,134
Cash paid for taxes	$ —	$ 6	$ —	$ 22
Noncash investing and financing activities				
Purchases of property and equipment included in accounts payable and accrued expenses and other current liabilities	$ 250	$ 2,260	$ 294	$ 9,679
Deferred offering costs in accounts payable and accrued expenses and other current liabilities	$ —	$ —	$ —	$ 1,142
Debt discount included in accrued expenses and other current liabilities	$ —	$ —	$ —	$ 58

The accompanying notes are an integral part of these consolidated financial statements.

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10x Genomics, Inc.

Notes to Consolidated Financial Statements

1. Description of Business and Basis of Presentation

Organization and Description of Business

10x Genomics, Inc. (the "Company") was incorporated in the state of Delaware on July 2, 2012. The Company's integrated solutions include the Company's Chromium instruments, which are referred to as "instruments", its enzymes, reagents, microfluidic chips and other consumable products, which are referred to as "consumables", and software for analyzing biological systems. These solutions guide customers through the workflow from sample preparation to next-generation sequencing to subsequent analysis and visualization. Each of the Company's solutions is designed to interrogate a major class of biological information that is impactful to researchers. The Company began commercial and manufacturing operations and selling its instruments and consumables in 2015. The Company's headquarters is located in Pleasanton, California and has wholly-owned subsidiaries in Sweden, Netherlands, Singapore, Germany and China.

Since inception, the Company has incurred net losses. During the years ended December 31, 2017 and 2018, the Company incurred net losses of $18.8 million and $112.5 million, respectively, and net losses of $21.6 million and $14.5 million during the six months ended June 30, 2018 and 2019 (unaudited), respectively. As of December 31, 2018 and June 30, 2019 (unaudited), the Company had an accumulated deficit of $231.1 million and $245.6 million, respectively. The Company has historically financed its operations primarily through the issuance and sale of convertible preferred stock and Historical common stock and the issuance of debt. Management expects to continue to incur significant expenses for the foreseeable future and to incur operating losses in the near term while the Company makes investments to support its anticipated growth. While management believes that the Company's existing cash and cash equivalents, cash generated from sales of its products and available borrowing capacity under existing credit agreements will be sufficient to meet its anticipated cash needs for the next 12 months from the date these financial statements are issued, the Company may need to raise additional financing in the future to fund its operations. The Company has evaluated and concluded there are no conditions or events, considered in the aggregate, that raise substantial doubt about its ability to continue as a going concern for a period of one year following the date that these financial statements were issued. The accompanying financial statements have been prepared assuming the Company will continue as a going concern.

Basis of Presentation

The consolidated financial statements include the Company's accounts and the accounts of its wholly-owned subsidiaries. All intercompany transactions and balances have been eliminated. The consolidated financial statements have been prepared in conformity with U.S. generally accepted accounting principles (or "GAAP"). The Company has issued shares of Class A common stock herein referred to as "Historical Class A common stock" or "Historical Class A" and Class B common stock herein referred to as "Historical Class B common stock" or "Historical Class B", and collectively as "Historical common stock".

2. Summary of Significant Accounting Policies

Use of Estimates

The preparation of financial statements in conformity with GAAP requires management to make judgments, estimates and assumptions that affect the reported amounts of assets and liabilities at the date of the financial statements, disclosure of contingent assets and liabilities and the reported amounts of revenue and expense. These judgments, estimates and assumptions are used for, but not limited to, revenue recognition, inventory valuation and write-downs, loss contingencies, accounting for asset acquisitions and the fair value of common stock and stock option awards. The Company bases its estimates on various factors and information, which may include, but are not limited to, history and prior experience, the Company's forecasts

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10x Genomics, Inc.

Notes to Consolidated Financial Statements—(Continued)

and future plans, current economic conditions and information from third-party professionals that management believes to be reasonable under the circumstances, the results of which form the basis for making judgments about the carrying value of assets and liabilities and recorded amounts of expenses that are not readily apparent from other sources. Actual results may differ from those estimates and the differences may be material.

Reclassifications

Certain prior year amounts have been reclassified in the consolidated balance sheets and consolidated statements of cash flows to conform to the current year presentation. These reclassifications from prepaid expenses and other current assets to tenant allowance receivable and from other noncurrent liabilities to deferred rent, noncurrent had no impact on total assets, total liabilities or net cash provided by (used in) operating activities.

Unaudited Pro Forma Information

Unaudited Pro Forma Balance Sheet

The unaudited pro forma balance sheet information as of June 30, 2019, assumes all shares of convertible preferred stock had automatically converted into an aggregate of 67,704,278 shares of the Company's Historical Class A common stock upon the completion of a qualifying initial public offering ("IPO"). The shares of Historical common stock issuable and the proceeds expected to be received upon the completion of a qualifying IPO are excluded from such pro forma financial information.

Unaudited Pro Forma Net Loss Per Share

Unaudited pro forma basic and diluted net loss per share attributable to Historical common stockholders is computed, using the if-converted method, to give effect to the automatic conversion of all outstanding shares of the Company's convertible preferred stock into 67,704,278 shares of Historical Class A common stock.

Unaudited Interim Financial Information

The accompanying interim consolidated balance sheet as of June 30, 2019, the interim consolidated statements of operations and comprehensive loss, cash flows, convertible preferred stock and stockholders' equity (deficit) for the six months ended June 30, 2018 and 2019 and the related footnote disclosures are unaudited. The unaudited interim consolidated financial statements have been prepared on the same basis as the annual consolidated financial statements and include, in the opinion of management, all adjustments, consisting of normal recurring adjustments, that are necessary for the fair presentation of the Company's financial position as of June 30, 2019 and results of operations and cash flows for the six months ended June 30, 2018 and 2019. The results as of and for the six months ended June 30, 2019 are not necessarily indicative of the results to be expected for the year ending December 31, 2019 or for any other future periods.

Cash Equivalents and Restricted Cash

The Company considers all highly liquid investments with an original maturity of three months or less from the date of purchase to be cash equivalents. Cash equivalents consist primarily of amounts invested in money market funds and are stated at fair value.

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10x Genomics, Inc.

Notes to Consolidated Financial Statements—(Continued)

The following table provides a reconciliation of cash, cash equivalents, and restricted cash reported on the consolidated balance sheets that sum to the total of the same amounts shown in the consolidated statements of cash flows (in thousands):

	December 31,		June 30,
	2017	2018	2019 (unaudited)
Cash and cash equivalents	$47,857	$65,080	$56,034
Restricted cash	–	5,008	5,008
Total cash, cash equivalents, and restricted cash	$47,857	$70,088	$61,042

Segment Information

The Company operates as a single operating segment. The Company's chief operating decision maker, its Chief Executive Officer, manages the Company's operations on a consolidated basis for the purposes of allocating resources, making operating decisions and evaluating financial performance.

Fair Value of Financial Instruments

The Company determines the fair value of an asset or liability based on the assumptions that market participants would use in pricing the asset or liability in an orderly transaction between market participants at the measurement date. The identification of market participant assumptions provides a basis for determining what inputs are to be used for pricing each asset or liability.

A fair value hierarchy has been established which gives precedence to fair value measurements calculated using observable inputs over those using unobservable inputs. This hierarchy prioritized the inputs into three broad levels as follows:

Level 1: Quoted prices in active markets for identical instruments;

Level 2: Other significant observable inputs (including quoted prices in active markets for similar instruments); and

Level 3: Significant unobservable inputs (including assumptions in determining the fair value of certain investments).

Money market funds are highly liquid investments and are actively traded. The pricing information on the Company's money market funds are readily available and can be independently validated as of the measurement date. This approach results in the classification of these securities as Level 1 of the fair value hierarchy. There were no transfers between Levels 1, 2 or 3 for any of the periods presented. As of December 31, 2017 and 2018 and June 30, 2019 (unaudited), the Company held $43.3 million, $44.5 million and $33.4 million, respectively, in money market funds with no unrealized gains or losses.

The Company has issued common stock warrants for which fair value is determined using Level 3 inputs, see discussion in Note 8.

Accounts Receivable, Net

Accounts receivable consist of amounts due from customers for the sales of products and services. The Company reviews its accounts receivable and provides allowances of specific amounts if collectability is no

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10x Genomics, Inc.

Notes to Consolidated Financial Statements—(Continued)

longer reasonably assured based on historical experience and specific customer collection issues. The allowance for doubtful accounts was $0.3 million as of December 31, 2018 and $0.4 million as of June 30, 2019 (unaudited). There was no allowance for doubtful accounts as of December 31, 2017.

Business Concentrations

The Company's instruments are currently assembled and tested by a single contract manufacturer in the United States. The Company's agreement with the contract manufacturer expires in 2020 and may be terminated by either party for any reason by providing the other party with at least 30 days written notice. The Company's agreement with the contract manufacturer contains purchase commitments. In addition, the Company is reliant on several suppliers for key components for its consumables. A significant disruption in the operations of the contract manufacturer or suppliers may impact the production of the Company's products for a substantial period of time, which could have a material adverse effect on its business, financial condition and results of operations.

Concentrations

Financial instruments that potentially subject the Company to credit risk consist of cash equivalents and accounts receivable. The Company's cash and cash equivalents are primarily held with a large financial institution in the U.S. and deposits exceed the Federal Deposit Insurance Corporation's insurance limit. The Company's debt is with this same financial institution. The Company performs periodic evaluations of the risks associated with its investments and the relative credit standing of this financial institution.

The Company performs ongoing credit evaluations of its customers' financial condition. The Company does not require collateral from its customers but may require upfront payments from certain customers. The Company has not experienced significant credit losses to date. For the years ended December 31, 2017 and 2018 and the six months ended June 30, 2018 and 2019 (unaudited), no single customer, including distributors, represented more than 10% of revenue. As of December 31, 2017 and 2018 and June 30, 2019 (unaudited), no single customer, including distributors, represented more than 10% of the Company's outstanding accounts receivable.

Substantially all of the Company's long-lived assets are located in the United States.

Inventory

Inventory is recorded at the lower of cost, determined on a first-in, first-out basis, or net realizable value. The Company uses judgment to analyze and determine if the composition of its inventory is obsolete, slow-moving or unsalable and frequently reviews such determinations. The Company writes down specifically identified unusable, obsolete, slow-moving or known unsalable inventory in the period that it is first recognized by using a number of factors including product expiration dates, open and unfulfilled orders and sales forecasts. Any write-down of its inventory to net realizable value establishes a new cost basis and will be maintained even if certain circumstances suggest that the inventory is recoverable in subsequent periods. Costs associated with the write-down of inventory are recorded to cost of revenue on the Company's consolidated statements of operations

Property and Equipment, Net

Property and equipment, net is stated at cost, net of accumulated depreciation. Depreciation is computed using the straight-line method based on the estimated useful lives of the assets. Assets held under capital leases are recorded at the lower of the net present value of the minimum lease payments or the fair value of

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10x Genomics, Inc.

Notes to Consolidated Financial Statements—(Continued)

the leased assets at the inception of the lease. Amortization expense is computed using the straight-line method over the shorter of the estimated useful lives of the leased assets or the period of the related lease. Amortization of assets under capital leases is included in depreciation expense. The estimated useful lives of the Company's property and equipment are as follows:

	Useful Life
Laboratory equipment and machinery	3 – 5 years
Computer equipment	2 – 3 years
Furniture and fixtures	3 years
Leasehold improvements	1 – 3 years

Impairment of Long-Lived Assets

The Company evaluates long-lived assets, such as property and equipment and intangible assets, for impairment whenever events or changes in circumstances indicate that the carrying value of an asset may not be recoverable. If indicators of impairment exist and the undiscounted future cash flows that the assets are expected to generate are less than the carrying value of the assets, the Company reduces the carrying amount of the assets to their estimated fair values based on a discounted cash flow approach or, when available and appropriate, to comparable market values. There were no impairment losses recorded for the years ended December 31, 2017 and 2018 and the six months ended June 30, 2018 and 2019 (unaudited).

Product Warranties

The Company generally provides a one-year warranty on its instruments. The Company reviews its exposure to estimated warranty obligations associated with instrument sales and establishes an accrual based on historical product failure rates and actual warranty costs incurred. This expense is recorded as a component of cost of revenue in the consolidated statements of operations and comprehensive loss.

Deferred Revenue

Deferred revenue consists of payments received in advance of revenue recognition primarily related to instrument service agreements, also referred to as extended warranties. Revenue under these agreements is recognized over the related service period. Deferred revenue that will be recognized during the 12 months following the balance sheet date is recorded as current portion of deferred revenue and the remaining portion is recorded as long term.

Accrued Contingent Liabilities

Accrued contingent liabilities represents the Company's estimates of possible losses on pending litigations, including related accrued royalties that are both probable and reasonably estimable. See Note 7.

Revenue Recognition

The Company generates revenue from sales of products and services. The Company's products consist of instruments and consumables. The Company also sells instrument service agreements which relate to extended warranties.

The revenue recognition accounting policy described below relates to revenue transactions from January 1, 2019 and onward, which are accounted for in accordance with Accounting Standards Codification Topic 606 – Revenue from Contracts with Customers.

The Company recognizes revenue when control of the products and services is transferred to its customers in an amount that reflects the consideration it expects to receive from its customers in exchange for those

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Notes to Consolidated Financial Statements—(Continued)

products and services. This process involves identifying the contract with a customer, determining the performance obligations in the contract, determining the contract price, allocating the contract price to the distinct performance obligations in the contract, and recognizing revenue when the performance obligations have been satisfied. A performance obligation is considered distinct from other obligations in a contract when it provides a benefit to the customer either on its own or together with other resources that are readily available to the customer and is separately identified in the contract. The Company considers a performance obligation satisfied once it has transferred control of a good or service to the customer, meaning the customer has the ability to use and obtain the benefit of the good or service.

Revenue from product sales is recognized when control of the product is transferred, which is generally upon shipment to the customer. In instances where right of payment or transfer of title is contingent upon the customer's acceptance of the product, revenue is deferred until all acceptance criteria have been met. Instrument service agreements, which relate to extended warranties, are typically entered into for one-year terms, following the expiration of the standard one-year warranty period Revenue for extended warranties is recognized ratably over the term of the extended warranty period as a stand ready performance obligation. Revenue is recorded net of discounts, distributor commissions, and sales taxes collected on behalf of governmental authorities. Customers are invoiced generally upon shipment, or upon order for services, and payment is typically due within 45 days. Cash received from customers in advance of product shipment or providing services is recorded as a contract liability. The Company's contracts with its customer generally do not include rights of return or a significant financing component.

The Company regularly enters into contracts that include various combinations of products and services which are generally distinct and accounted for as separate performance obligations. The transaction price is allocated to each performance obligation in proportion to its standalone selling price. The Company determines standalone selling price using average selling prices with consideration of current market conditions. If the product or service has no history of sales or if the sales volume is not sufficient, the Company relies upon prices set by management, adjusted for applicable discounts.

The revenue recognition accounting policy described below relates to revenue transactions prior to January 1, 2019, which are accounted for in accordance with Accounting Standards Codification Topic 605 – Revenue Recognition.

The Company recognizes revenue when persuasive evidence of an arrangement exists, delivery has occurred or services have been rendered, the price to the customer is fixed or determinable, and collectability is reasonably assured. The Company assesses collectability based on factors such as the customer's creditworthiness and past collection history, if applicable. If collection is not reasonably assured, revenue recognition is deferred until receipt of payment. The Company also assesses whether a price is fixed or determinable by, among other things, reviewing contractual terms and conditions related to payment. Delivery occurs when there is a transfer of title and risk of loss passes to the customer.

Certain of the Company's sales arrangements involve the delivery of multiple products and services within contractually binding arrangements. Multiple-deliverable sales transactions typically consist of the sale and delivery of one or more instruments and consumables together and may include an instrument service agreement.

For sales arrangements that include multiple deliverables, the Company uses the stated contractual price for its instrument service agreements as its best estimate of selling price, if and when sold, and allocates the remaining contract consideration at the inception of the contract to the other units of accounting based upon

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10x Genomics, Inc.

Notes to Consolidated Financial Statements—(Continued)

their relative selling price. The Company may use its best estimate of selling price for individual deliverables when vendor specific objective evidence or third-party evidence is unavailable. A delivered item is considered to be a separate unit of accounting when it has value to the customer on a stand-alone basis.

The Company's products are typically delivered together or within a short time frame, generally within one to three months of the contract date. Instrument service agreements, which relate to extended warranties, are typically entered into for one-year terms, following the expiration of the standard one-year warranty period. The Company's products are generally sold without the right of return. Amounts received before revenue recognition criteria are met are classified on the consolidated balance sheets as deferred revenue.

Contract Costs

Beginning January 1, 2019, sales commissions earned by the Company's sales force are considered incremental and recoverable costs of obtaining a contract with a customer. Sale commissions related to the sale of extended warranties are deferred and amortized on a straight-line basis over the service term, which is typically greater than one year from the contract date. Amortization of deferred commissions is included in sales and marketing expenses in the accompanying consolidated statements of operations and comprehensive loss. As of June 30, 2019, unamortized deferred commissions were immaterial.

Cost of Revenue

Costs of revenue primarily consist of manufacturing costs incurred in the production process, including personnel and related costs, component materials, labor and overhead, packaging and delivery costs and allocated costs including facilities and information technology. In addition, costs of product revenue includes royalty costs for licensed technologies included in the Company's products, warranty costs and provisions for slow-moving and obsolete inventory. In addition, cost of revenue includes estimated accrued royalties related to the Bio-Rad litigation. See Note 7.

Shipping and Handling Costs

Shipping and handling charged to customers are recorded as revenue. Shipping and handling costs are included in the Company's cost of revenue.

Research and Development

Research and development costs are expensed in the period incurred. Research and development expense consists of personnel and related costs, independent contractor costs, laboratory supplies, equipment maintenance, prototype and materials expenses, amortization of developed technology and intangibles and allocated costs including facilities and information technology.

See Note 3 for discussion of in-process research and development included on the consolidated statements of operations.

Advertising Costs

Advertising costs are expensed as incurred. The Company incurred advertising costs of $0.2 million and $0.7 million for the years ended December 31, 2017 and 2018, respectively, and $0.2 million and $0.4 million for the six months ended June 30, 2018 and 2019 (unaudited), respectively.

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10x Genomics, Inc.

Notes to Consolidated Financial Statements—(Continued)

Stock-Based Compensation

The Company estimates the fair value of share-based payment awards granted to employees and directors on the grant date using the Black-Scholes option-pricing model. The fair value of share-based payment awards is recognized as compensation expense on a straight-line basis over the requisite service period in which the awards are expected to vest and forfeitures are recognized as they occur. Share-based payment awards that include a service condition and a performance condition are considered expected to vest when the performance condition is probable of being met.

The Black-Scholes model considers several variables and assumptions in estimating the fair value of stock-based awards. These variables include the per share fair value of the underlying Historical common stock, exercise price, expected term, risk-free interest rate, expected annual dividend yield and the expected stock price volatility over the expected term. For all stock options granted, the Company calculated the expected term using the simplified method for “plain vanilla” stock option awards. The Company has no publicly available stock information and therefore, the Company has used the historical volatility of the stock price of similar publicly traded peer companies. The risk-free interest rate is based on the yield available on U.S. Treasury zero-coupon issues similar in duration to the expected term of the equity-settled award.

Stock-based compensation expense for nonemployee stock options is measured based on fair market value using the Black-Scholes option pricing model and is recorded as the options vest. Prior to January 1, 2019, nonemployee stock options subject to vesting were revalued periodically over the requisite service period, which was generally the same as the vesting term of the award. From January 1, 2019, the grant date fair market value of nonemployee stock options is recognized in the consolidated statements of operations on a straight-line basis over the requisite service period and forfeitures are recognized as they occur.

Foreign Currency

For foreign subsidiaries where the functional currency is the local currency, assets and liabilities are translated to the U.S. dollar using month-end exchange rates, and revenue and expenses using average exchange rates. The adjustments resulting from these foreign currency translations are recorded in accumulated other comprehensive loss.

For foreign subsidiaries where the functional currency is the U.S. dollar, monetary assets and liabilities are remeasured using exchange rates in effect at the balance sheet dates and non-monetary assets and liabilities are remeasured at historical exchange rates. Revenue and expenses are remeasured at the average exchange rates for the period. Gains or losses from foreign currency remeasurement are included in other income (expense), net in the consolidated statements of operations and comprehensive loss. The Company recognized foreign currency transaction gains of $0.1 million for the year ended December 31, 2017, foreign currency transaction losses of $0.3 million for the year ended December 31, 2018, and foreign currency transaction losses of $0.1 million and $0.1 million for the six months ended June 30, 2018 and 2019 (unaudited), respectively.

Income Taxes

The Company uses the asset and liability method of accounting for income taxes, in which deferred tax assets and liabilities are recognized for the future tax consequences attributable to the differences between the financial statement carrying amounts of existing assets and liabilities and their respective tax bases. Deferred tax assets and liabilities are measured using the enacted tax rates expected to apply to taxable income in the years in which those temporary differences are expected to be reversed. The effect on deferred tax assets and liabilities of a change in tax rates is recognized as income in the period that includes the enactment date. A valuation allowance is established if it is more likely than not that all or a portion of the deferred tax asset will not be realized.

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10x Genomics, Inc.

Notes to Consolidated Financial Statements—(Continued)

The Company's tax positions are subject to income tax audits. The Company recognizes the tax benefit of an uncertain tax position only if it is more likely than not that the position is sustainable upon examination by the taxing authority, based on the technical merits. The tax benefit recognized is measured as the largest amount of benefit which is more likely than not (greater than 50% likely) to be realized upon settlement with the taxing authority. The Company recognizes interest accrued and penalties related to unrecognized tax benefits in its tax provision.

The Company calculates the current and deferred income tax provision based on estimates and assumptions that could differ from the actual results reflected in income tax returns filed in subsequent years. Adjustments based on filed income tax returns are recorded when identified. The amount of income tax paid is subject to examination by U.S. federal and state tax authorities. The estimate of the potential outcome of any uncertain tax issue is subject to management's assessment of the relevant risks, facts and circumstances existing at that time. To the extent the assessment of such tax position changes, the change in estimate is recorded in the period in which the determination is made.

Net Loss Per Share Attributable to Common Stockholders

Net loss per share of Historical common stock is computed using the two-class method required for multiple classes of common stock and participating securities. The rights, including the liquidation and dividend rights and sharing of losses, of the Historical Class A common stock and Historical Class B common stock are identical, other than voting rights. As the liquidation and dividend rights and sharing of losses are identical, the undistributed earnings are allocated on a proportionate basis and the resulting net loss per share attributed to common stockholders will, therefore, be the same for both Historical Class A and Historical Class B common stock on an individual or combined basis.

The Company's participating securities include the Company's convertible preferred stock, as the holders are entitled to receive noncumulative dividends on a pari passu basis in the event that a dividend is paid on Historical common stock. The Company also considers any shares issued on the early exercise of stock options subject to repurchase to be participating securities because holders of such shares have non-forfeitable dividend rights in the event a dividend is paid on Historical common stock. The holders of convertible preferred stock, as well as the holders of early exercised shares subject to repurchase, do not have a contractual obligation to share in losses.

Basic net loss per share is computed by dividing net loss attributable to Historical common stockholders by the weighted-average number of shares of Historical common stock outstanding during the period, adjusted for outstanding shares that are subject to repurchase.

For the calculation of diluted net loss per share, basic net loss per share attributable to Historical common stockholders is adjusted by the effect of dilutive securities, including convertible preferred stock, awards under the Company's equity compensation plan and common stock warrants. Diluted net loss per share attributable to Historical common stockholders is computed by dividing net loss attributable to Historical common stockholders by the weighted-average number of shares of Historical common stock outstanding. For periods in which the Company reports net losses, diluted net loss per share attributable to Historical common stockholders is the same as basic net loss per share attributable to Historical common stockholders because potentially dilutive shares of Historical common stock are not assumed to have been issued if their effect is anti-dilutive.

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10x Genomics, Inc.

Notes to Consolidated Financial Statements—(Continued)

Acquisitions of Assets

The Company evaluates acquisitions of assets and other similar transactions to assess whether or not the transaction should be accounted for as a business combination or asset acquisition by first applying a screen to determine if substantially all of the fair value of the gross assets acquired is concentrated in a single identifiable asset or group of similar identifiable assets. If the screen is met the transaction is accounted for as an asset acquisition. If the screen is not met, further determination is required as to whether or not the Company has acquired inputs and processes that have the ability to create outputs which would meet the requirements of a business.

The Company accounts for an asset acquisition under Accounting Standards Codification ("ASC"), *Business Combinations Topic 805, Subtopic 50*, which requires the acquiring entity in an asset acquisition to recognize net assets based on the cost to the acquiring entity on a relative fair value basis, which includes transaction costs in addition to consideration given. Goodwill is not recognized in an asset acquisition; any excess consideration transferred over the fair value of the net assets acquired is allocated to the non-monetary identifiable assets based on relative fair values. In-process research and development expense is expensed as incurred provided there is no alternative future use.

Contingent consideration payments in asset acquisitions are recognized when the contingency is resolved and the consideration is paid or becomes payable (unless the contingent consideration meets the definition of a derivative, in which case the amount becomes part of the basis in the asset acquired). Upon recognition of the contingent consideration payment, the amount is included in the cost of the acquired asset or group of assets.

Recently Adopted Accounting Pronouncements

In January 2017, the Financial Accounting Standards Board ("FASB") issued Accounting Standards Update ("ASU") No. 2017-01, *Business Combinations (Topic 805): Clarifying the Definition of a Business*. This standard provides guidance to evaluate whether transactions should be accounted for as acquisitions (or disposals) of assets or businesses. If substantially all of the fair value of the gross assets acquired (or disposed of) is concentrated in a single asset or a group of similar assets, the assets acquired (or disposed of) are not considered a business. The Company early adopted the standard as of January 1, 2018 on a prospective basis. As such, the Company applied this standard to its transactions beginning January 1, 2018.

In March 2016, the FASB issued ASU No. 2016-09, *Compensation-Stock Compensation (Topic 718): Improvements to Employee Share-Based Payment Accounting*. This standard relates to the simplification of share-based payments accounting and requires companies to record excess tax benefits and tax deficiencies as an income tax benefit or expense in the consolidated statements of operations and comprehensive loss when the awards vest or are settled, eliminates the requirement to reclassify cash flows related to excess tax benefits from operating activities to financing activities on the consolidated statements of cash flows and provides the option to recognize gross share-based compensation expense with actual forfeitures recognized as they occur. This standard is effective for annual periods beginning after December 15, 2017. The Company adopted this standard as of January 1, 2018 on a prospective basis and elected to account for forfeitures as they occur, rather than estimate expected forfeitures. The adoption of this standard did not have a material impact on the Company's consolidated financial statements.

In November 2016, the FASB issued ASU No. 2016-18, *Statement of Cash Flows, Restricted Cash (Topic 230)*. This standard requires entities to show the changes in total of cash, cash equivalents, restricted cash, and restricted cash equivalents in their statement of cash flows. As a result, entities will no longer present transfers between cash and cash equivalents and restricted cash and restricted cash equivalents in the statement of cash

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flows. This standard is effective for annual periods beginning after December 15, 2018, is applied retrospectively, and early adoption is permitted. The Company early adopted this standard as of January 1, 2018, which did not have an impact on its consolidated financial statements for the year ended December 31, 2017.

In May 2014, the FASB issued ASU No. 2014-09, *Revenue from Contracts with Customers (Topic 606)*, which supersedes the revenue recognition requirements in ASC 605, *Revenue Recognition*. This standard is based on the principle that revenue is recognized to depict the transfer of goods or services to customers in an amount that reflects the consideration to which the entity expects to be entitled in exchange for those goods or services. The standard also requires additional disclosure about the nature, amount, timing and uncertainty of revenue and cash flows arising from customer contracts, including significant judgments and changes in judgments and assets recognized from costs incurred to obtain or fulfill a contract. The Company adopted this standard as of January 1, 2019 using the modified retrospective approach, which did not have a material impact on its consolidated financial statements as of the adoption date and for the six months ended June 30, 2019. See *Revenue Recognition* for further details of the Company's revenue recognition policy under this standard.

In June 2018, the FASB issued ASU 2018-07, *Compensation-Stock Compensation (Topic 718): Improvements to Nonemployee Share-Based Payment Accounting*. This standard expands the scope of Topic 718, *Compensation—Stock Compensation* (which currently only includes share-based payments to employees) to include share-based payments issued to nonemployees for goods or services. Consequently, the accounting for share-based payments to nonemployees and employees will be substantially aligned. This standard is effective for annual periods beginning after December 15, 2019. The Company early adopted this standard on January 1, 2019 which did not have a material impact on the Company's consolidated financial statements.

Recently Issued Accounting Pronouncements

In February 2016, the FASB issued ASU No. 2016-02*, Leases (Topic 842)*, which supersedes the guidance in former ASC 840, *Leases*. This standard requires lessees to apply a dual approach, classifying leases as either finance or operating leases based on the principle of whether or not the lease is effectively a financed purchase by the lessee. The classification will determine whether lease expense is recognized based on an effective interest method or on a straight-line basis over the term of the lease. A lessee is also required to record a right-of-use asset and a lease liability for all leases with a term of greater than 12 months regardless of their classification. Leases with a term of 12 months or less will be accounted for similar to existing guidance for operating leases. This standard is effective for interim and annual periods beginning after December 15, 2019, with early adoption permitted. The Company is currently evaluating adoption methods and whether this standard will have a material impact on its consolidated financial statements.

In October 2016, the FASB issued ASU No. 2016-16, *Income Taxes (Topic 740): Intra-Entity Transfers of Assets Other Than Inventory.* This standard will require entities to recognize the income tax consequences of an intra-entity transfer of an asset other than inventory when the transfer occurs instead of when the asset is sold. This standard is effective for annual periods beginning after December 15, 2018. The Company is currently assessing the impact of this standard to the consolidated financial statements but does not anticipate a material impact on the adoption due to the valuation allowance.

In August 2018, the FASB issued ASU 2018-15, *Intangibles – Goodwill and Other – Internal Use Software (Subtopic 350-40) – Customer's Accounting for Implementation Costs Incurred in a Cloud Computing Arrangement That Is a Service Contract*, which aligns the accounting for implementation costs incurred in a hosting arrangement that is a service contract with the accounting for implementation costs incurred to develop or

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obtain internal-use software under ASC 350-40, in order to determine which costs to capitalize and recognize as an asset and which costs to expense. This standard is effective for annual periods beginning after December 15, 2020, and interim periods within annual periods beginning after December 15, 2021. Early adoption is permitted, including adoption in any interim period. This standard can be applied either retrospectively or prospectively to all implementation costs incurred after the date of adoption. The Company is currently evaluating the impact of adopting this standard on its consolidated financial statements.

3. Asset Acquisitions

In March 2018, the Company acquired all of the outstanding shares of Epinomics, Inc. for $22.2 million inclusive of acquisition costs of $0.3 million. The technology licenses acquired in this transaction will enable the Company to develop epigenetics products. The transaction was accounted for as an asset acquisition. The Company recognized a charge of $22.2 million related to the transaction which is included as a component of in-process research and development on the consolidated statements of operations and comprehensive loss.

In November 2018, the Company purchased all of the outstanding shares of Spatial Transcriptomics Holdings AB (“Spatial”), for $38.6 million inclusive of acquisition costs of $0.5 million. The patents acquired in this transaction will enable the Company to develop spatial products. The transaction was accounted for as an asset acquisition. In connection with this acquisition, the Company acquired patents, trademarks and customer relationships. The patents acquired were allocated a value of $36.9 million. Accordingly, the Company recognized a charge of $36.9 million related to the transaction which is included as a component of in-process research and development on the consolidated statements of operations and comprehensive loss. The Company recognized a total of $0.4 million in intangible assets related to acquired trademarks and customer relationships which are included in other assets on the consolidated balance sheets. The Company must also make contingent payments to the sellers of Spatial based on revenue from certain spatial-related technology sales for the years ended December 31, 2019 through December 31, 2022, which are subject to continuing service requirements. These contingent payments are equal to a percentage in the teens multiplied by such revenue. Due to continuing service requirements pertaining to earn the contingent payments, the contingent payments have been deemed to be a compensation arrangement which will be accounted for if and when earned.

The following table summarizes the value of assets acquired and liabilities assumed (in thousands):

Assets Acquired and Liabilities Assumed	
In-process research and development	$36,899
Intangible assets	425
Other assets and liabilities, net	1,237
Total net assets acquired	$38,561

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4. Other Financial Statement Information

Inventory

Inventory was comprised of the following as of the dates indicated (in thousands):

	December 31, 2017	December 31, 2018	June 30, 2019 (unaudited)
Purchased materials	$1,618	$3,052	$ 3,807
Work in progress	1,895	2,553	3,982
Finished goods	1,325	2,965	4,536
Inventory	$4,838	$8,570	$12,325

Property and Equipment, Net

Property and equipment, net consisted of the following as of the dates indicated (in thousands):

	December 31, 2017	December 31, 2018	June 30, 2019 (unaudited)
Laboratory equipment and machinery	$ 11,634	$ 14,616	$ 16,315
Computer equipment	2,472	3,303	3,562
Furniture and fixtures	947	1,002	3,430
Leasehold improvements	2,291	3,342	14,342
Construction in progress	614	2,947	15,180
Total property and equipment	17,958	25,210	52,829
Less: accumulated depreciation and amortization	(11,033)	(14,083)	(14,492)
Property and equipment, net	$ 6,925	$ 11,127	$ 38,337

Depreciation expense was $4.3 million and $3.8 million for the years ended December 31, 2017 and 2018, respectively, and $2.1 million and $2.1 million for the six months ended June 30, 2018 and 2019 (unaudited), respectively. Included in property and equipment were capital leases of $1.7 million as of December 31, 2017, and accumulated depreciation related to the capital leases of $1.6 million as of December 31, 2017. There were no capital leases as of December 31, 2018 and June 30, 2019 (unaudited). Depreciation expense related to capital leases was $0.6 million and $0.1 million for the years ended December 31, 2017 and 2018, respectively and $0.1 million for the six months ended June 30, 2018 (unaudited). No depreciation expense related to capital leases was recognized related in the six months ended June 30, 2019 (unaudited).

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Accrued Compensation and Related Benefits

Accrued compensation and related benefits was comprised of the following as of the dates indicated (in thousands):

	December 31, 2017	December 31, 2018	June 30, 2019 (unaudited)
Accrued bonus	$2,216	$3,545	$2,992
Accrued commissions	1,903	2,299	1,390
Other	358	1,203	2,017
Accrued compensation and related benefits	$4,477	$7,047	$6,399

Accrued Expenses and Other Current Liabilities

Accrued expenses and other current liabilities was comprised of the following as of the dates indicated (in thousands):

	December 31, 2017	December 31, 2018	June 30, 2019 (unaudited)
Accrued royalties for licensed technologies	$1,099	$1,571	$ 1,887
Accrued property and equipment	–	990	7,227
Accrued consulting	160	741	979
Accrued offering costs	–	–	701
Product warranties	174	804	447
Customer deposits	715	381	479
Taxes payable	163	738	797
Other	1,351	2,947	3,917
Accrued expenses and other current liabilities	$3,662	$8,172	$16,434

Product Warranties

Changes in the reserve for product warranties were as follows for the periods indicated (in thousands):

	December 31, 2017	December 31, 2018	June 30, 2019 (unaudited)
Beginning of period	$ 35	$ 174	$ 804
Additions charged to cost of revenue	476	1,685	201
Repairs and replacements	(337)	(1,055)	(558)
End of period	$ 174	$ 804	$ 447

Revenue and Deferred Revenue

As of June 30, 2019, the aggregate amount of the transaction price allocated to remaining performance obligations was $3.9 million, of which approximately 71% is expected to be recognized to revenue in the next twelve months, with the remainder thereafter.

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As of June 30, 2019, contract liabilities, which consisted of deferred revenue related to extended warranty service agreements' were $3.9 million, of which the short-term portions were $2.8 million. Revenue recorded during the six months ended June 30, 2019 included $1.4 million of previously deferred revenue that was included in contract liabilities as of the adoption date of January 1, 2019. Contract assets as of the adoption date of January 1, 2019 and June 30, 2019 were immaterial.

The following table presents revenue by source for the periods indicated (in thousands):

	Year Ended December 31,		Six Months Ended June 30,	
	2017	2018	2018	2019
			(unaudited)	
Instruments	$24,467	$ 36,540	$15,864	$ 15,150
Consumables	46,192	107,616	42,482	92,389
Services	426	2,157	806	1,858
Total revenue	$71,085	$146,313	$59,152	$109,397

The following table presents revenue by geography based on the location of the customer for the periods indicated (in thousands):

	Year Ended December 31,		Six Months Ended June 30,	
	2017	2018	2018	2019
			(unaudited)	
North America	$43,622	$ 85,132	$35,541	$ 61,455
Europe, the Middle East and Africa	18,602	35,812	13,725	24,498
China	3,171	15,075	5,462	15,407
Asia Pacific	5,690	10,294	4,424	8,037
Total revenue	$71,085	$146,313	$59,152	$109,397

Revenue for the United States, which is included in North America in the table above, was 58% and 55% of consolidated revenue for the years ended December 31, 2017 and 2018, respectively, and 56% and 53% of consolidated revenue for the six months ended June 30, 2018 and 2019 (unaudited), respectively.

5. Debt

In September 2016, the Company entered into a loan and security agreement which includes a term loan and revolving line of credit facility. The Company initially borrowed $10.6 million as a term loan, known as Tranche A, which was originally scheduled to mature in June 2020. Monthly payments of interest were due through December 31, 2017, with equal monthly installments of principal and interest due for thirty months thereafter. The term loan accrued interest at a floating per annum rate equal to *The Wall Street Journal* prime rate plus 2.0%. The Company had an option to borrow an additional $10.0 million as a term loan, known as Tranche B, beginning July 1, 2017, which expired with no amounts borrowed as of December 31, 2017. Additionally, no amounts were borrowed under the revolving line of credit. The agreement required an end of term payment of $0.6 million upon maturity of Tranche A.

In February 2018, the loan and security agreement was amended. Under the terms of the amendment, amounts available under Tranche A were increased to $30.0 million (the "Amended Tranche A"). As of the date of modification, the balance outstanding under Tranche A was $10.5 million. After giving consideration to the

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end of term payment, the Company borrowed an additional $19.5 million under the Amended Tranche A. Under the amended agreement the Company has an option to borrow an additional $20.0 million as a term loan, known as the Amended Tranche B, beginning October 1, 2018 through June 30, 2019, or the date of an event of default if earlier, and the revolving line of credit facility was increased from $5.0 million to $25.0 million.

Monthly payments of interest are due under the Amended Tranche A term loan through June 30, 2019, with monthly installments of principal and interest due for 42 months thereafter. However, if the Amended Tranche B is borrowed, monthly installments of principal and interest will be reduced to 36 months. The term loan accrues interest at the greater of the floating per annum rate equal to the greater of *The Wall Street Journal* prime rate plus 2.0% or 6.25%. Additionally, an end of term payment is due to the lender in the amount of $1.8 million upon maturity, prepayment, or acceleration of the term loan, as amended. The end of term payment is being accreted as additional interest expense over the term of the debt using the effective interest method.

The term loan can be repaid prior to the maturity date, however, a prepayment fee of 3.0% of the outstanding principal balance will be due in addition to all outstanding principal and interest, if the prepayment is made before the first anniversary date of the loan closing date. This prepayment fee decreases to 2.0% if the prepayment is made on or after the first anniversary of the loan closing date but before the second anniversary of the loan closing date and the fee decreases to 1.0% of the outstanding principal amount if paid after the second anniversary and prior to the maturity date.

The loan and security agreement provides the Company with a revolving line of credit of up to $25.0 million through December 2022. The amount available on the revolving line of credit is based on 80% of eligible receivables and is subject to a borrowing base calculation. Principal amounts outstanding under the revolving line of credit accrue interest at the greater of a floating per annum rate equal to the greater of *The Wall Street Journal* prime rate plus 0.25% or 4.5% and are repayable monthly. Upon termination of the agreement for any reason prior to the revolving credit facility's maturity date, a termination fee of $250,000 will be due in addition to all outstanding principal and interest. Additionally, the revolving line of credit has a nonrefundable annual commitment fee of $62,500 payable on each anniversary date.

In connection with the amendment of the loan and security agreement and the Amended Tranche A term loan entered into in February 2018, the Company issued the lender a warrant to purchase 125,000 Historical Class B common shares with an exercise price per share of $1.62. If the Company borrows under the Amended Tranche B term loan, the Company is obligated to issue the lender a warrant to purchase an additional 133,000 Historical Class B common shares with an exercise price per share of $1.62. The warrants had an estimated fair value of $150,000 which has been recorded as a debt discount.

Amounts borrowed under the loan and security agreement are collateralized by all of the Company's assets, except for intellectual property, but including the proceeds from the sale of any of the Company's intellectual property. In addition, the Company has provided a negative pledge regarding its intellectual property and cannot encumber it without the lender's consent. The loan and security agreement contains various covenants for reporting, protecting and obtaining adequate insurance coverage for assets collateralized and for coverage of business operations, and complying with requirements, including the payment of all necessary taxes and fees for all federal, state and local government entities. Immediately upon the occurrence and during the continuance of an event of default, including the noncompliance with the above covenants, the lender may increase the interest rate per annum by 5.0% above the rate that is otherwise applicable; stop future loan advances; require the Company to deposit 105% of any undrawn letters of credit, or 110% if the letter of credit is denominated in a foreign currency; and take control over all assets collateralizing the loan and

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take necessary means to protect the collateral. The loan and security agreement contains a material adverse change clause, including terms for subjective acceleration.

As of December 31, 2018 and June 30, 2019 (unaudited), and as of the date of the issuance of these financial statements, the Company was in compliance with all loan covenants.

In June 2019, the Company's loan and security agreement was amended to extend the Company's option to borrow an additional $20.0 million as a term loan (the "Amended Tranche B") through December 31, 2019. Monthly payments of interest are due through December 31, 2019, with monthly installments of principal and interest due for 36 months thereafter. In connection with the amendment, the Company paid a one-time fee of $50,000 to the lender. As a result, annual payments due on the term loan decreased by approximately $4.2 million in 2019 and increased by $1.7 million, $1.6 million and $1.5 million in 2020, 2021 and 2022, respectively.

Aggregate annual payments due on the term loan as of December 31, 2018, are as follows (in thousands):

2019	$ 6,495
2020	10,233
2021	9,576
2022	10,724
Total payments	37,028
Less: amount representing interest	(7,028)
Total term loan	30,000
Less: unamortized debt discount	(324)
Total term loan, net of debt discount	29,676
Less: current portion	(4,187)
Non-current portion	$25,489

6. Income Taxes

Loss before provision for income taxes were as follows for the periods indicated (in thousands):

	Year Ended December 31,	
	2017	2018
United States	$(17,275)	$ (77,517)
International	(1,466)	(34,881)
Total	$(18,741)	$(112,398)

The provision for income taxes was $0.1 million for the year ended December 31, 2018, which related to foreign and state income taxes. For the year ended December 31, 2017, the provision for income taxes was not material.

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A reconciliation of the federal statutory income tax provision to the effective income tax provision is as follows for the periods indicated (in thousands):

	Year Ended December 31,	
	2017	2018
Income tax provision at statutory rate	$ (6,372)	$(23,604)
State taxes, net	(1,180)	(4,479)
Tax credits	(1,213)	(1,631)
Foreign taxes	565	41
Stock-based compensation	481	421
Change in valuation allowance	(5,230)	19,133
Change in federal rate	12,912	–
Acquisition related expenses	–	10,143
Other	58	63
Total provision for income taxes	$ 21	$ 87

Deferred income taxes reflect the net tax effect of temporary differences between amounts recorded for financial reporting purposes and amounts used for tax purposes. The major components of deferred tax assets and liabilities are as follows as of the dates indicated (in thousands):

	December 31,	
	2017	2018
Deferred tax assets		
Net operating loss carryforwards	$ 27,438	$ 31,031
Research and development tax credits	7,199	10,874
Fixed assets	140	–
Accruals and reserves	1,520	12,612
Other	125	1,616
Total deferred tax assets	36,422	56,133
Valuation allowance	(36,422)	(55,673)
Net deferred tax assets	–	460
Deferred tax liabilities		
Fixed assets	–	(460)
Net deferred taxes	$ –	$ –

As of December 31, 2017 and 2018, the Company maintained a full valuation allowance on its net deferred tax assets. The deferred tax assets predominantly relate to operating losses and tax credits. The valuation allowance was determined in accordance with the provisions of ASC 740, *Accounting for Income Taxes*, which requires an assessment of both positive and negative evidence when determining whether it is more likely than not that deferred tax assets are recoverable. Such assessment is required on a jurisdiction-by-jurisdiction basis. The Company's history of cumulative losses, along with expected future U.S. losses, required that a full valuation allowance be recorded against all net deferred tax assets. The Company intends to maintain a full valuation allowance on net deferred tax assets until sufficient positive evidence exists to support a reversal of the valuation allowance. The valuation allowance increased by $19.3 million and decreased by $5.2 million for the years ended December 31, 2018 and 2017. The increase in the valuation allowance in the year ended

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December 31, 2018 primarily related to federal net operating losses ("NOLs") and accruals recorded for book purposes which are deductible for tax purposes when paid. The decrease in the valuation allowance in the year ended December 31, 2017 was primarily related to the change in the statutory tax rate as a result of tax reform.

As of December 31, 2018, the Company had federal NOLs carryforwards of approximately $116.1 million and federal tax credit carryforwards of approximately $8.3 million. The federal NOL carryforwards generated during and after fiscal 2018 totaling $5.5 million are carried forward indefinitely, while all others, along with the federal tax credit carryforwards, expire in years beginning in 2032. As of December 31, 2018, the Company had state net operating loss carryforwards of approximately $93.5 million, which begin to expire in 2032. In addition, the Company had state tax credit carryforwards of approximately $7.9 million, which do not expire.

The federal and state net operating losses and credit carryforwards are subject to change of ownership limitations provided by the Internal Revenue Code and similar state provisions. In general, if the Company experiences a greater than 50 percentage point aggregate change in ownership over a 3-year period (a "Section 382 ownership change"), utilization of its pre-change NOL and credit carryforwards are subject to an annual limitation. The Company completed a study in early 2019 to determine whether an ownership change had occurred and determined at that time that an ownership change occurred in 2013. As a result, the Company's net operating losses generated through November 1, 2013 may be subject to limitation under Section 382 of the Code. The amount of pre-change loss carryforwards which may be subject to this limitation is $4.8 million. Such limitations may result in expiration of a portion of the carryforwards before utilization. Depending on the timing and amount of any future taxable income, the Company may be limited as to the amount of carryforwards that can be utilized. The Company does not believe these limitations will cause a material amount of its federal credit carryforwards to expire prior to utilization. The ability of the Company to use its remaining NOL and credit carryforwards may be further limited as a result of future changes in its stock ownership.

The total balance of unrecognized gross tax benefits for the years ended December 31, 2017 and 2018 resulting primarily from research and development tax credits claimed on the Company's annual tax returns were as follows (in thousands):

	2017	2018
Unrecognized tax benefits at beginning of year	$1,859	$2,692
Additions based on prior year tax provisions	–	118
Additions based on current year tax provisions	833	1,359
Unrecognized tax benefits at end of year	$2,692	$4,169

The Company has not been audited by the Internal Revenue Service or any state income or franchise tax agency. As of December 31, 2018, its federal returns for the years ended 2012 through the current period and state returns for the years ended 2012 through the current period are still open to examination. In addition, all of the net operating losses and research and development credit carry-forwards that may be used in future years are still subject to inquiry given that the statute of limitation for these items would begin in the year of the utilization. The Company does not expect its unrecognized tax benefits to change significantly over the next 12 months.

No liability related to uncertain tax positions has been recorded in the Company's consolidated financial statements due to the fact that such liabilities have been netted against deferred attribute carryovers.

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The Company maintains undistributed earnings overseas as of December 31, 2018. At this time, the Company believes the funds held by all non-US subsidiaries will be permanently reinvested outside of the U.S. However, if these funds were repatriated to the U.S. or used for U.S. operations the Company may be subject to withholding taxes in the foreign countries. As a result of tax reform, the Company's unrepatriated earnings are no longer subject to income tax in the U.S. when distributed.

The Tax Cuts and Jobs Act (the "Tax Act"), was enacted on December 22, 2017, which reduced the U.S. federal corporate tax rate from 35% to 21%, among other changes, effective January 1, 2018. The Company's accounting for the elements of the Tax Act is completed and resulted in $21.9 million reduction in its net deferred tax assets as of December 31, 2017 to reflect the new statutory rate. The rate adjustment to the deferred tax assets was fully offset by a decrease in the valuation allowance, resulting in no rate impact to the Company. There were no changes from the original amount booked as of December 31, 2017.

The Tax Act created a new requirement that global intangible low-taxed income ("GILTI") earned by the Company's foreign subsidiaries must be included in gross U.S. taxable income. While the Tax Act provides for a modified territorial tax system, beginning in 2018, GILTI provisions will be applied providing an incremental tax on low taxed foreign income. The GILTI provisions require the Company to include in its U.S. income tax return foreign subsidiary earnings in excess of an allowable return on the foreign subsidiary's tangible assets. During 2018, the Company made an accounting policy election to treat taxes related to GILTI as a current period expense when incurred.

7. Commitments and Contingencies

Indemnification

From time to time, the Company has entered into indemnification provisions under certain agreements in the ordinary course of business, typically with business partners, customers and suppliers. Pursuant to these agreements, the Company may indemnify, hold harmless and agree to reimburse the indemnified parties on a case-by-case basis for losses suffered or incurred by the indemnified parties in connection with any patent or other intellectual property infringement claim by any third party with respect to the Company's products. The Company maintains product liability insurance coverage that would generally enable it to recover a portion of the amounts paid. The Company has also agreed to indemnify its directors and executive officers for costs associated with any fees, expenses, judgments, fines and settlement amounts incurred by them in any action or proceeding to which any of them are, or are threatened to be, made a party by reason of their service as a director or officer (see "*—Litigation*" below). The Company maintains director and officer insurance coverage that would generally enable it to recover a portion of the amounts paid. The Company also may be subject to indemnification obligations by law with respect to the actions of its employees under certain circumstances and in certain jurisdictions.

Non-cancelable Purchase Commitments

The Company's contract manufacturer makes advance purchases of components based on the instrument unit forecasts and purchase orders placed by the Company. To the extent these components are purchased by the contract manufacturer on the Company's behalf and cannot be used by their other customers, the Company is obligated to purchase these components. In addition, certain supplier agreements require that the Company make minimum annual purchases under the agreements which are not significant. To date, the Company has met the minimum purchase commitments.

As of December 31, 2018, the Company has entered into non-cancelable arrangements for subscription software services under which the Company has an obligation to make payments aggregating to $1.7 million over the next three years.

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Intellectual Property Licensing

In July 2018, the Company and The Board of Trustees of the Leland Stanford Junior University ("Stanford") entered into a license agreement pursuant to which the Company was granted an exclusive license to ATAC-seq. As the Company receives revenue related to products covered by these licenses, the Company is required to pay Stanford a low single-digit royalty percentage based on the net revenue of certain ATAC-seq products during the applicable term of the licensed patents.

In September 2013, the Company and the President and Fellows of Harvard College ("Harvard") entered into a license agreement pursuant to which the Company was granted a license to certain intellectual property from Harvard. The Company is required to pay Harvard a low single-digit royalty percentage based on the net revenue of certain products covered by certain licensed patents during their applicable term.

In November 2018, the Company and Prognosys Biosciences, Inc. ("Prognosys") entered into a license agreement pursuant to which the Company was granted an exclusive license to certain intellectual property relating to spatial analysis from Prognosys. As part of the agreement, the Company fully expensed total purchase consideration of $3.3 million comprised of cash consideration and shares of the Company's Historical Class B common stock.

The minimum commitments related to the above license arrangements aggregate to $5.0 million to be paid over the next 16 years.

Lease Obligations

The Company leases its facilities under noncancelable lease agreements. Certain of these arrangements have free rent, escalating rent payment provisions and tenant allowances. Under such arrangements, the Company recognizes rent expense on a straight-line basis over the noncancelable lease term and records the difference between cash rent payments and the recognition of rent expense as a deferred rent liability within other current liabilities (current portion) and other liabilities (noncurrent portion).

In August 2018, the Company entered into a new lease agreement for office and laboratory space which consists of approximately 150,000 square feet located in Pleasanton, California. The lease term commenced in September 2018 and ends in September 2029. In connection with the lease, the Company maintains a letter of credit for the benefit of the landlord in the amount of $5.0 million, which is secured by restricted cash classified as noncurrent restricted cash on the consolidated balance sheets based on the term of the underlying lease.

Rent expense related to noncancelable operating leases was $1.0 million and $3.5 million for the years ended December 31, 2017 and 2018, respectively, and $0.8 million and $3.5 million for the six months ended June 30, 2018 and 2019 (unaudited), respectively.

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Notes to Consolidated Financial Statements—(Continued)

Future minimum lease payments under the leases for facilities as of December 31, 2018, are as follows (in thousands):

	Operating Leases
2019	$ 2,847
2020	5,974
2021	6,621
2022	5,860
2023 and thereafter	44,757
Total future minimum lease commitments	$66,059

Litigation

The Company is currently a defendant in the lawsuits and proceedings described below. Other than with respect to the 2015 Delaware Action, losses are not probable or estimable for the described below.

The 2015 Delaware Action

In February 2015, Raindance Technologies, Inc. (“Raindance”) and the University of Chicago filed suit against the Company in the U.S. District Court for the District of Delaware, accusing substantially all of the Company’s products of infringing certain patents. In May 2017, Bio-Rad Laboratories, Inc. (“Bio-Rad”) was substituted as the plaintiff following its acquisition of Raindance. In November 2018, a jury found that the accused products willfully infringed one or more of the asserted patents and awarded Bio-Rad approximately $24 million in damages through June 30, 2018. Post-trial, Bio-Rad moved for a permanent injunction, treble damages for willful infringement, attorneys’ fees, supplemental damages as well as pre- and post-judgment interest.

In response to the jury award, the Company established an accrual of $30.6 million as of December 31, 2018, which was recorded as an operating expense on the consolidated statement of operations for the year ended December 31, 2018. Additionally, beginning in the fourth quarter of 2018, the Company also began recording an accrual for estimated royalties to Bio-Rad as a cost of revenue on the consolidated statements of operations based on an estimated royalty rate of 15% of sales of the Company’s Chromium instruments operating its GEM microfluidic chips and associated consumables. As a result, the Company recorded $7.4 million of royalties for the fourth quarter of 2018. As of December 31, 2018, the Company recorded a total accrual of $38 million related to this matter which represented the jury award plus the Company’s estimate of additional damages for the period from June 30, 2018 to the trial date in November 2018 and the royalties accrued in the fourth quarter of 2018.

During the six months ended June 30, 2019 (unaudited), the Company recorded royalties of $15.9 million as a cost of revenue and an additional $1.4 million as an operating expense for estimated pre- and post-judgment interest for the period from January 1, 2019 through June 30, 2019. As of June 30, 2019 (unaudited), the Company has accrued a total of $55.3 million related to this matter. To date the Company has not made any payments related to the judgment or royalties.

In July 2019, the Court awarded supplemental damages for the period from June 30, 2018 through the end of the trial in November 2018 and established the interest rates for pre- and post-judgment interest, which when combined with the original award, resulted in a $35 million preliminary judgment in favor of Bio-Rad for

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10x Genomics, Inc.

Notes to Consolidated Financial Statements—(Continued)

damages through November 2018 and interest. The Company's accrual of $55.3 million as of June 30, 2019 is comprised of this judgment, along with the Company's estimate of additional royalties and interest for the period from November 2018 through June 30, 2019. In July 2019, the Court denied Bio-Rad's other post-trial requests such as attorneys' fees and enhanced damages for willful infringement.

In July 2019, the Court also granted Bio-Rad a permanent injunction against the Company's GEM microfluidic chips and associated consumables that were found to infringe the Bio-Rad patents, which constitute substantially all of the Company's product sales. However, under the injunction, the Company is permitted to continue to sell its GEM microfluidic chips and associated consumables for use with its historical installed base of instruments provided that the Company pay a royalty of 15% into escrow on the Company's net revenue related to such sales commencing after the injunction effective date. These decisions were entered as a final judgment against the Company in August 2019, with the injunction effective date anticipated to be in late August 2019.

As a result, the Company has asked the Court to allow it to post a bond for the amount of the final judgment of approximately $35 million. The Company expects it will be required to provide cash collateral related to the bond in an amount between $30 and $35 million. The cash collateral will be held until the conclusion of the Company's appeal and will not be available to the Company to fund working capital or other corporate expenditures.

In addition, the Company will be required to place cash into escrow each quarter of an amount equal to 15% of net revenue from sales of the Company's GEM microfluidic chips and associated consumables subsequent to the effective date of the injunction, which is anticipated to be in late August 2019. The amounts will be held in escrow until the conclusion of the Company's appeal.

The Company intends to appeal the verdict.

The ITC 1068 Action

On July 31, 2017, Bio-Rad and Lawrence Livermore National Security, LLC filed a complaint against the Company in the U.S. International Trade Commission ("ITC") pursuant to Section 337 of the Tariff Act of 1930, accusing substantially all of the Company's products of infringing certain asserted patents (the "ITC 1068 Action"). In September 2018, the judge found that the Company's GEM microfluidic chips infringe certain of the asserted patents, but also that the Company's gel bead manufacturing microfluidic chip and Next GEM microfluidic chip do not infringe any claim asserted against them. The judge recommended entry of an exclusion order preventing the Company from importing its GEM microfluidic chips and a cease and desist order that would prevent the Company from selling such imported chips. A Final Determination is expected to be issued in late September 2019, which is subject to a 60-day presidential review period before taking effect. The Company believes this proceeding is without merit and intends to vigorously defend itself.

The Northern District of California Action

On July 31, 2017, Bio-Rad and Lawrence Livermore National Security, LLC also filed suit against the Company in the U.S. District Court for the Northern District of California, alleging that substantially all of its products infringe certain patents in addition to the patents asserted in the ITC 1068 Action. The complaint seeks injunctive relief, unspecified monetary damages, costs and attorneys' fees. The Company believes that this lawsuit is without merit and intends to vigorously defend itself.

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10x Genomics, Inc.

Notes to Consolidated Financial Statements—(Continued)

The Germany Action

On July 31, 2017, Bio-Rad filed suit against the Company in Germany in the Munich Regional Court alleging that the Company infringed a European patent. Bio-Rad dismissed this action in August 2018.

On February 13, 2018, Bio-Rad filed suit against the Company in Germany in the Munich Region Court alleging that its Chromium instruments, GEM microfluidic chips and certain accessories infringe a German utility model. Bio-Rad seeks unspecified damages and an injunction prohibiting sales of these products in Germany and requiring the Company to recall these products sold in Germany subsequent to February 11, 2018. The Company believes that this lawsuit is without merit and intends to vigorously defend itself.

The 2018 Delaware Action

On October 25, 2018, Bio-Rad filed suit against the Company in the U.S. District Court for the District of Delaware alleging that the Company infringed certain patents. Bio-Rad seeks injunctive relief, unspecified monetary damages, costs and attorneys' fees. The Company believes that this lawsuit is without merit and intends to vigorously defend itself.

The Becton Dickinson Action

On November 15, 2018, Becton, Dickinson and Company and Cellular Research, Inc. filed suit against the Company in the U.S. District Court for the District of Delaware, alleging that the Company infringed certain patents. Plaintiffs seek injunctive relief, unspecified monetary damages, costs and attorneys' fees. The Company believes that this lawsuit is without merit and intends to vigorously defend itself.

8. Capital Stock and Stockholders' Deficit

The Company's Amended and Restated Certificate of Incorporation authorizes it to issue 258,859,871 shares of capital stock consisting of 75,955,000 shares of Historical Class A common stock, 115,000,000 shares of Historical Class B common stock, and 67,904,871 shares of convertible preferred stock.

Convertible Preferred Stock

Convertible preferred stock authorized, issued and outstanding as of the date indicated below consisted of the following (in thousands, except share and per share data):

	As of December 31, 2017				
Series	Issue Price	Shares Authorized	Shares Issued and Outstanding	Liquidation Preference	Carrying Value
A-1	$0.85	5,523,394	5,523,394	$ 4,688	$ 5,882
A-2	$1.09	20,486,543	20,486,543	22,400	22,265
B	$3.27	16,972,477	16,972,477	55,500	55,415
C	$4.48	16,747,799	16,747,799	75,000	74,852
Total Convertible Preferred stock		59,730,213	59,730,213	$157,588	$158,414

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10x Genomics, Inc.

Notes to Consolidated Financial Statements—(Continued)

As of December 31, 2018 and June 30, 2019 (unaudited)

Series	Issue Price	Shares Authorized	Shares Issued and Outstanding	Liquidation Preference	Carrying Value
A-1	$ 0.85	5,523,394	5,523,394	$ 4,688	$ 5,882
A-2	$ 1.09	20,486,543	20,486,543	22,400	22,265
B	$ 3.27	16,972,477	16,972,477	55,500	55,415
C	$ 4.48	16,747,799	16,747,799	75,000	74,852
D	$ 9.57	5,224,658	5,224,658	50,000	49,878
D-1	$ 12.73	2,950,000	2,749,407	35,000	34,952
Total Convertible Preferred stock		67,904,871	67,704,278	$ 242,588	$ 243,244

Redemption

The holders of the Company's preferred stock have no voluntary rights to redeem shares. A liquidation or winding up of the Company, a change in control, or a sale of substantially all of the Company's assets would constitute a redemption event which may be outside of the Company's control. Accordingly, these shares are considered contingently redeemable and are classified as temporary equity on the consolidated balance sheets. The carrying value of the convertible preferred stock has not been adjusted to its redemption value because redemption was not probable as of the balance sheet dates presented. The carrying value of the convertible preferred stock will be adjusted to its redemption value if redemption becomes probable in the future.

Conversion

Each share of preferred stock is convertible at the right and option of its holder into such number of fully paid and nonassessable shares of Historical Class A common stock as is determined by dividing the original issue price per share by the applicable conversion price per share on the date of conversion. As of December 31, 2017 and 2018 and June 30, 2019 (unaudited), the conversion prices per share for all series of convertible preferred stock were equal to the original issue prices and the rate at which each share would convert into Historical Class A common stock was one-for-one.

In the event that the Company, at any time after the original issuance date of any series of preferred stock, issues additional shares of common stock (including convertible securities) without consideration or for consideration per share that is less than the conversion price of a particular series of preferred stock in effect on the date of and immediately prior to such issuance, then and in such event, the conversion price of that series shall be reduced, concurrently with such issuance (down round conversion provision).

Each share of preferred stock will automatically convert into a fully paid, nonassessable share of Historical Class A common stock at the then-effective conversion rate for such share (i) upon the closing of a firm commitment, underwritten initial public offering of the Company's common stock at an aggregate offering price of not less than $50.0 million; or (ii) upon the receipt by the Company of a written request for such conversion from (A) the holders of a majority of the then outstanding shares of convertible preferred stock (voting together as a single class on an as-converted basis), (B) the holders of a majority of the then outstanding shares of Series C convertible preferred stock (voting as a separate class) and (C) the holders of two-thirds of the then outstanding shares of Series D convertible preferred stock and Series D-1 convertible preferred stock (voting together as a separate class).

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10x Genomics, Inc.

Notes to Consolidated Financial Statements—(Continued)

Liquidation Preference

Each series of the Company's convertible preferred stock is contingently redeemable upon the occurrence of a liquidation transaction as defined in the Company's amended and restated certificate of incorporation. In the event of a liquidation, dissolution or winding up of the Company, either voluntary or involuntary, the holders of Series D and D-1 convertible preferred stock shall be entitled to receive, prior and in preference to any distribution of any of the Company's assets or funds to Series C convertible preferred stock, Series B convertible preferred stock, Series A-2 convertible preferred stock, Series A-1 convertible preferred stock, and Historical common stock, an amount per share equal to $9.57 per share for each outstanding share of Series D convertible preferred stock and $12.73 per share for each outstanding share of Series D-1 convertible preferred stock, plus any declared but unpaid dividends on such shares. The holders of Series C convertible preferred stock shall be entitled to receive, prior and in preference to any distribution of any of the Company's assets or funds to Series B convertible preferred stock, Series A-2 convertible preferred stock, Series A-1 convertible preferred stock, and Historical common stock, an amount per share equal to $4.4782 per share for each outstanding share of Series C convertible preferred stock, plus any declared but unpaid dividends on such shares. The holders of Series B convertible preferred stock shall be entitled to receive, prior and in preference to any distribution of any of the Company's assets or funds to Series A-2 convertible preferred stock, Series A-1 convertible preferred stock, and Historical common stock, an amount per share equal to $3.27 per share for each outstanding share of Series B convertible preferred stock, plus any declared but unpaid dividends on such shares. The holders of Series A-1 and Series A-2 convertible preferred stock shall be entitled to receive, prior and in preference to any distribution of any of the Company's assets or funds to Historical common stock, an amount per share equal to $0.8488 per share for each outstanding share of Series A-1 convertible preferred stock and $1.0934 per share for each outstanding share of Series A-2 convertible preferred stock, plus any declared but unpaid dividends on such shares. After liquidation preferences to Series A-1, Series A-2, Series B, Series C, Series D and Series D-1 convertible preferred stockholders have been paid, the remaining assets of the Company shall be distributed among the holders of Historical common stock. If, upon liquidation, the assets of the Company legally available for distribution or any other type of consideration payable to the stockholders are insufficient to permit the distribution or payment to such holders of the full amounts specified in the Company's amended and restated certificate of incorporation, then the entire assets of the Company legally available for distribution or consideration would be payable to the holders of preferred stock in proportion to the full amounts, with equal priority and pro rata among the holders, of the preferred stock in proportion to the full amounts they would otherwise be entitled to receive.

Voting Rights

Each share of Series A-1, A-2, B, C, D and D-1 convertible preferred stock has the right to vote on an as-converted to Historical Class A common stock basis, which equates to 100 votes for each share of Historical common stock into which such preferred stock could be converted, and with respect to such vote, such holder will have full voting rights and powers equal to the holders of Historical common stock.

Dividends

Each stockholder of Series A-1, A-2, B, C, D and D-1 convertible preferred stock is entitled to receive dividends at the rate of $0.068, $0.087, $0.262, $0.358, $0.7656 and $1.0184 per share, respectively, per annum, when and if declared by the Board of Directors, in accordance with the payment preference order set forth in *Liquidation Preference* discussed above, prior to payment of dividends on Historical common stock. Dividends are noncumulative and no dividends have been declared to date.

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10x Genomics, Inc.

Notes to Consolidated Financial Statements—(Continued)

Historical Common Stock

Historical common stock issued and outstanding was 12,883,930, 14,549,801, and 16,145,382 as of December 31, 2017 and 2018 and June 30, 2019 (unaudited), respectively. Historical Class A was 8,050,000 as of December 31, 2017 and 2018, and June 30, 2019 (unaudited). Historical Class B was 4,833,930 and 6,499,801 as of December 31, 2017 and 2018, respectively, and 8,095,382 as of June 30, 2019 (unaudited). The Company's Historical Class A common stock and Historical Class B common stock have a par value of $0.00001 per share. Each share of Historical Class A common stock has the right to 100 votes and each share of Historical Class B common stock has the right to one vote per share. All other rights and privileges of Historical Class A and Historical Class B common stock are equivalent. Historical Class A common shares are convertible to Historical Class B at any time upon written notification and all Historical Class A will convert upon the date specified by vote or written consent of the holders of a majority of the then outstanding Historical Class A common stock, voting together as a single class. The holders of common stock are also entitled to receive dividends whenever funds are legally available and when declared by the Board of Directors, subject to the prior rights of holders of all classes of stock outstanding having priority rights as to dividends.

Warrants to Purchase Common Stock

In connection with certain debt arrangements, the Company issued the lender warrants to purchase shares of Historical Class B common stock which have an exercise term of 10 years. The outstanding warrants as of December 31, 2018 and June 30, 2019 (unaudited) were as follows:

Issue Date	Exercise Price Per Share	Number of Shares
April 2014	$ 0.21	43,750
September 2015	$ 0.88	18,349
September 2016	$ 1.07	79,000
February 2018	$ 1.62	125,000

The Company's common stock warrants were recorded to additional paid-in capital at fair value as of the date of issuance using the Black-Scholes valuation model. The fair value of the warrants for 125,000 shares of Historical Class B common stock issued in February 2018 was estimated at $150,000 using the following assumptions: fair value of shares of Historical Class B common stock on the issuance date of $1.62, risk-free interest rate of 1.54%, contractual term of 10 years, no anticipated dividends, and estimated volatility of 68%. The initial amount allocated to the warrants are accounted for as a discount to the related debt and amortized to interest expense over the loan term using the effective interest method.

9. Equity Incentive Plans

2012 Stock Plan

In October 2012, the Company adopted the 10x Genomics, Inc. 2012 Stock Plan (the "2012 Stock Plan") which has been amended in subsequent years for increases in authorized shares. The 2012 Stock Plan allows for the issuance of incentive stock options ("ISOs"), non-statutory stock options ("NSOs") or restricted shares. ISOs may be granted only to the Company's employees (including officers and directors who are also considered employees). NSOs and restricted shares may be granted to the Company's employees and service providers. Unvested options that were not exercised as of an employee's termination date revert to the 2012 Stock Plan. As of December 31, 2018 and June 30, 2019 (unaudited), the number of shares of Historical Class B common stock issuable under the 2012 Stock Plan is 24,782,088. The 2012 Stock Plan does not allow for the issuance of shares of Historical Class A common stock.

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10x Genomics, Inc.

Notes to Consolidated Financial Statements—(Continued)

Options under the 2012 Stock Plan have a contractual term of 10 years. In the case of an ISO granted to an optionee who, at the time the option is granted, owns stock representing more than 10% of the voting power of all classes of stock of the Company, the term of the option is five years from the date of grant, or such shorter term as may be provided in the option agreement.

The exercise price of an ISO and NSO shall not be less than 100% of the estimated fair value of the shares on the date of grant, and the exercise price of an ISO granted to a 10% stockholder shall not be less than 110% of the estimated fair value of the shares on the date of grant. Stock options granted generally vest over a four-year period.

A summary of the Company's stock option activity from December 31, 2017 to June 30, 2019, under the 2012 Stock Plan is as follows:

	Outstanding Options	Weighted-Average Exercise Price	Weighted-Average Remaining Terms (Years)	Aggregate Intrinsic Value
Balance as of December 31, 2017	11,999,004	$0.99		
Granted	4,198,573	$4.21		
Exercised	(1,508,762)	$1.17		
Cancelled	(424,439)	$1.22		
Balance as of December 31, 2018	14,264,376	$1.91	8.2	$ 71,902,234
Granted (unaudited)	3,283,297	$9.91		
Exercised (unaudited)	(1,595,581)	$1.46		
Cancelled (unaudited)	(317,910)	$3.37		
Balance as of June 30, 2019 (unaudited)	15,634,182	$3.61	8.2	$351,527,047
Vested and exercisable as of December 31, 2018	5,614,120	$0.97	7.2	$ 33,595,289
Unvested and exercisable as of December 31, 2018	897,397	$1.18	8.3	$ 5,182,366
Vested and exercisable as of June 30, 2019 (unaudited)	6,012,392	$1.31	7.1	$148,964,420
Unvested and exercisable as of June 30, 2019 (unaudited)	1,555,939	$4.32	8.7	$ 33,879,218

The weighted-average grant date fair value of options granted during the years ended December 31, 2017 and 2018 was $0.66 and $2.04 per share, respectively, and was $1.31 and $10.22 per share for the six months ended June 30, 2018 and 2019 (unaudited), respectively. The total intrinsic value of stock options exercised was $0.8 million and $3.3 million during the years ended December 31, 2017 and 2018, respectively, and $0.9 million and $11.9 million during the six months ended June 30, 2018 and 2019 (unaudited), respectively. As of December 31, 2018 and June 30, 2019 (unaudited), the total unrecognized stock-based compensation related to stock options was $10.7 million and $39.2 million, respectively, which will be recognized over a weighted-average period of approximately 3.1 years and 3.4 years, respectively.

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10x Genomics, Inc.

Notes to Consolidated Financial Statements—(Continued)

Early Exercise of Options

Stock options granted under the 2012 Stock Plan provide for certain employee and director option holders the right to exercise unvested options in exchange for restricted shares of Historical Class B common stock which are subject to repurchase by the Company at the original issuance price in the event the optionee's employment is terminated either voluntarily or involuntarily prior to the applicable vesting date. The consideration received for the early exercised options is recorded as a liability on the consolidated balance sheets and reclassified to stockholders' deficit as the shares vest. As of December 31, 2017 and 2018 and June 30, 2019 (unaudited), the total repurchase liability related to the unvested early exercised options was $317,000, $652,000 and $825,000, respectively, which is included in other current and noncurrent liabilities on the consolidated balance sheets. A summary of these restricted shares issued under the 2012 Stock Plan is as follows:

	Number of Shares	Weighted-Average Exercise Price
Outstanding and unvested as of December 31, 2017	301,372	$ 1.05
Exercised	124,000	$ 4.76
Vested	(192,622)	$ 1.33
Outstanding and unvested as of December 31, 2018	232,750	$ 2.80
Exercised (unaudited)	29,000	$ 11.48
Vested (unaudited)	(63,500)	$ 2.52
Outstanding and unvested as of June 30, 2019 (unaudited)	198,250	$ 4.16

The fair value of each employee option grant was estimated on the date of grant using the following assumptions for the periods indicated:

	Year Ended December 31,		Six Months Ended June 30,	
	2017	2018	2018	2019
			(unaudited)	
Expected volatility	45% – 48%	45% – 46%	45%	45%
Risk-free interest rate	1.9% – 2.3%	2.7% – 3.1%	2.7% – 2.8%	2.2% – 2.5%
Expected term	4.2 – 6.5 years	5.3 – 6.5 years	5.6 – 6.1 years	5.0 – 6.9 years
Expected dividend	–	–	–	–

Stock-Based Compensation for Nonemployees

The Company granted stock options to consultants in exchange for services performed for the Company. The stock options vest over terms ranging from 12 to 48 months. The stock options generally vest over the contractual period of the consulting arrangement and, therefore, the Company will revalue the options periodically and record additional compensation expense related to these options over the remaining vesting period.

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10x Genomics, Inc.

Notes to Consolidated Financial Statements—(Continued)

During the years ended December 31, 2017 and 2018 compensation expense related to these options was $33,000, $70,000, respectively, and $10,000 and $211,000, and during the months ended June 30, 2018 and 2019 (unaudited), respectively.

Stock-Based Compensation Expense

The following table sets forth the total stock-based compensation expense included in the Company's consolidated statements of operations and comprehensive loss for the periods indicated (in thousands):

	Year Ended December 31,		Six Months Ended June 30, 2018	
	2017	2018	2018	2019
			(unaudited)	
Cost of revenue	$ 44	$ 85	$ 36	$ 90
Research and development	801	1,030	440	1,798
Selling, general and administrative	816	1,543	530	2,496
Total stock-based compensation expense	$1,661	$2,658	$1,006	$4,384

10. Employee Benefit Plans

The Company has made available to all full-time United States employees a 401(k) retirement savings plan. Under this plan, employee and employer contributions and accumulated plan earnings qualify for favorable tax treatment under Section 401(k) of the Internal Revenue Code. The Company has not contributed to the plan.

11. Net Loss Per Share

The following table sets forth the computation of basic and diluted net loss per share for the periods indicated (in thousands, except share and per share data):

	Year Ended December 31,		Six Months Ended June 30,	
	2017	2018	2018	2019
			(unaudited)	
Net loss attributable to Historical common stockholders	$ (18,762)	$ (112,485)	$ (21,616)	$ (14,514)
Weighted-average shares used in computing net loss per share, basic and diluted	11,587,751	13,392,273	12,985,535	15,187,258
Net loss per share attributable to Historical common stockholders, basic and diluted	$ (1.62)	$ (8.40)	$ (1.66)	$ (0.96)

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10x Genomics, Inc.

Notes to Consolidated Financial Statements—(Continued)

The following outstanding shares of potentially dilutive securities were excluded from the computation of diluted net loss per share because including them would have had an anti-dilutive effect for the dates indicated:

	As of December 31,		As of June 30,	
	2017	2018	2018	2019
			(unaudited)	
Convertible preferred stock (on an if-converted basis)	59,730,213	67,704,278	64,954,871	67,704,278
Stock options to purchase Historical Class B common stock	11,949,004	14,264,376	12,402,386	15,634,182
Shares subject to repurchase	301,372	232,750	277,750	198,250
Common stock warrants to purchase Historical Class B common stock	141,099	266,099	266,099	266,099
Total	72,121,688	82,467,503	77,901,106	83,802,809

Unaudited Pro Forma Net Loss Per Share

The following table presents the calculation of pro forma basic and diluted net loss per share attributable to Historical common stockholders for the period indicated (in thousands, except share and per share data):

	Year Ended December 31, 2018	Six Months Ended June 30, 2019 (unaudited)
Numerator		
Net loss attributable to Historical common stockholders	$ (112,485)	$ (14,514)
Denominator		
Weighted-average shares used in computing net loss per share, basic and diluted	13,392,273	15,187,258
Pro forma adjustment to reflect the assumed conversion of the convertible preferred stock	64,102,719	67,704,278
Weighted-average shares used in computing pro forma net loss per share attributable to Historical common stockholders, basic and diluted	77,494,992	82,891,536
Pro forma net loss per share attributable to Historical common stockholders		
Basic and diluted	$ (1.45)	$ (0.18)

12. Subsequent Events

The Company evaluated events subsequent to December 31, 2018 through May 10, 2019, the date at which the consolidated financial statements were available to be issued.

13. Subsequent Events (unaudited)

The Company evaluated events subsequent to December 31, 2018 through August 19, 2019, which is the date the unaudited financial statements were issued.

In August 2019, the Court entered a final judgment in the amount of approximately $35 million in favor of Bio-Rad related to the Delaware Action (see Note 7).

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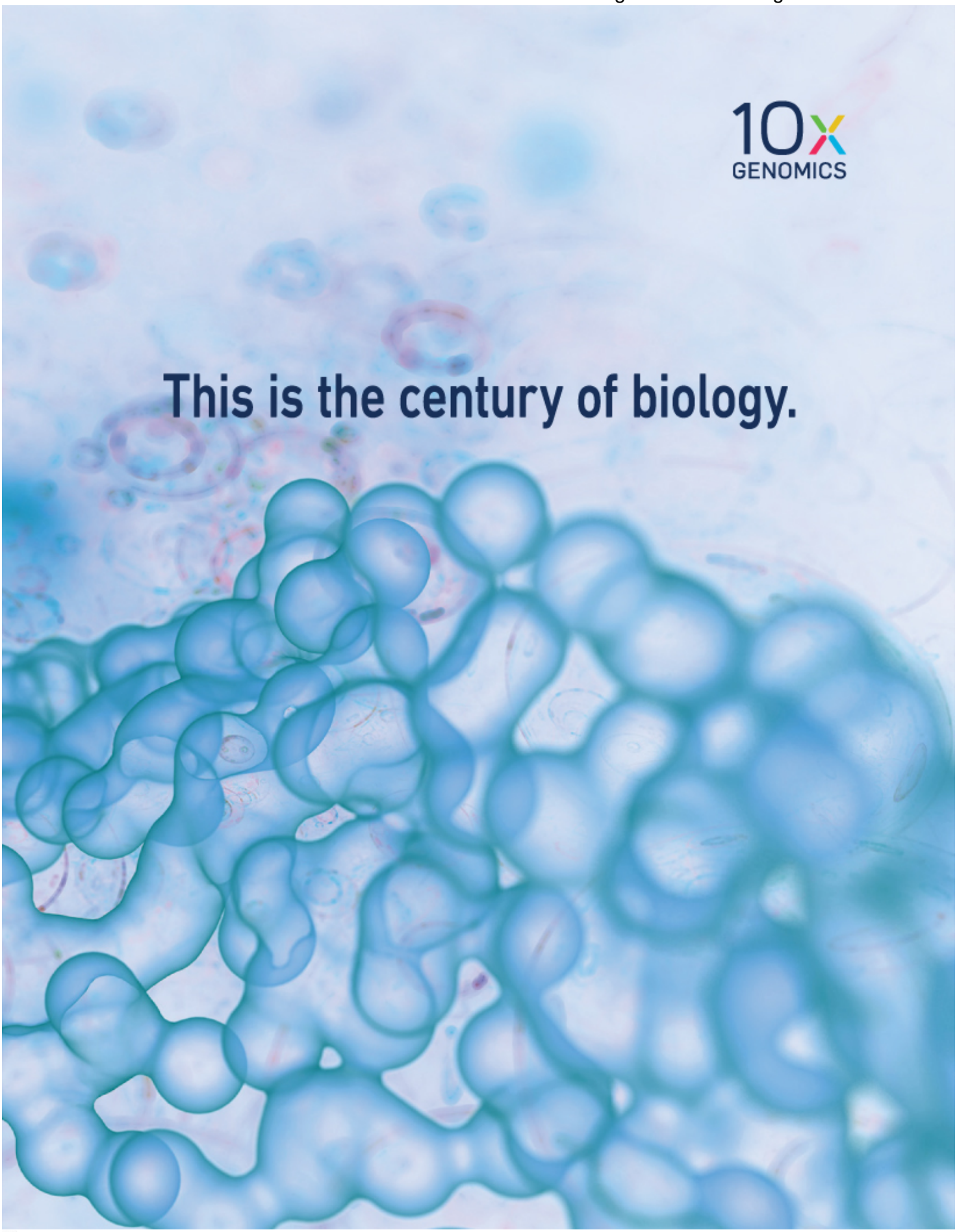
10x
GENOMICS
This is the century of biology.

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shares

Class A common stock

Prospectus

J.P. Morgan **Goldman Sachs & Co. LLC** **BofA Merrill Lynch**

Cowen

, 2019

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Part II

Information not required in prospectus

Item 13. Other expenses of issuance and distribution

The following table sets forth all costs and expenses, other than underwriting discounts and commissions, payable by us in connection with the sale of the Class A common stock being registered. All amounts shown are estimates except for the Securities and Exchange Commission (the "SEC") registration fee, the Financial Industry Regulatory Authority ("FINRA") filing fee and the Nasdaq Global Select Market ("Nasdaq") listing fee.

	Amount paid or to be paid
SEC registration fee	$ 12,120
FINRA filing fee	15,500
Nasdaq listing fee	*
Transfer agent's fees	*
Printing and engraving expenses	*
Legal fees and expenses	*
Accounting fees and expenses	*
Blue sky fees and expenses	*
Miscellaneous	*
Total	$ *

* To be completed by amendment.

Item 14. Indemnification of directors and officers

Section 145 of the Delaware General Corporation Law provides that a corporation may indemnify directors and officers as well as other employees and individuals against expenses (including attorneys' fees), judgments, fines and amounts paid in settlement actually and reasonably incurred by such person in connection with any threatened, pending, or completed actions, suits or proceedings in which such person is made a party by reason of such person being or having been a director, officer, employee or agent to the registrant. The Delaware General Corporation Law provides that Section 145 is not exclusive of other rights to which those seeking indemnification may be entitled under any bylaw, agreement, vote of stockholders or disinterested directors or otherwise. Section 7 of the registrant's amended and restated bylaws provides for indemnification by the registrant of its directors, officers and employees to the fullest extent permitted by the Delaware General Corporation Law. The registrant has entered into indemnification agreements with each of its current directors, executive officers and certain other officers to provide these directors and officers additional contractual assurances regarding the scope of the indemnification set forth in the registrant's amended and restated certificate of incorporation and amended and restated bylaws and to provide additional procedural protections. There is no pending litigation or proceeding involving a director or executive officer of the registrant for which indemnification is sought.

Section 102(b)(7) of the Delaware General Corporation Law permits a corporation to provide in its certificate of incorporation that a director of the corporation shall not be personally liable to the corporation or its stockholders for monetary damages for breach of fiduciary duty as a director, except for liability (i) for any breach of the director's duty of loyalty to the corporation or its stockholders, (ii) for acts or omissions not in good faith or which involve intentional misconduct or a knowing violation of law, (iii) for unlawful payments of

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dividends or unlawful stock repurchases, redemptions, or other distributions, or (iv) for any transaction from which the director derived an improper personal benefit. The registrant's amended and restated certificate of incorporation provides for such limitation of liability.

The registrant maintains policies of insurance under which coverage is provided to its directors and officers against loss rising from claims made by reason of breach of duty or other wrongful act.

The proposed form of underwriting agreement filed as Exhibit 1.1 to this registration statement provides for indemnification of directors and officers of the registrant by the underwriters against certain liabilities.

Item 15. Recent sales of unregistered securities

From January 1, 2016 through August 14, 2019, the registrant has issued and sold the following securities without registration under the Securities Act of 1933:

- 16,747,799 shares of Series C Convertible Preferred Stock to 25 accredited investors at a price of $4.48 per share, for aggregate proceeds of approximately $74,999,994;
- 5,224,658 shares of Series D Convertible Preferred Stock to 20 accredited investors at a price of $9.57 per share, for aggregate proceeds of approximately $49,999,977;
- 2,749,407 shares of Series D-1 Convertible Preferred Stock to 6 accredited investors at a price of $12.73 per share, for aggregate proceeds of approximately $34,999,951;
- Stock options to employees, directors, consultants and other service providers of the Registrant to purchase an aggregate of 15,357,125 shares of Historical Class B common stock under the Registrant's 2012 Stock Plan, with per share exercise prices ranging from $1.07 to $30.00;
- 5,993,292 shares of Historical Class B common stock to employees, directors, consultants and other service providers of the Registrant upon the exercise of stock options granted under the Registrant's 2012 Stock Plan, with per share purchase prices ranging from $0.21 to $11.48;
- 157,109 shares of Historical Class B common stock for in-process research and development; and
- Warrants to purchase an aggregate of 79,000 shares of our Historical Class B common stock, exercisable for a period of 10 years at an exercise price of $1.07 per share, to a lender in connection with our entry into a Loan and Security Agreement with Silicon Valley Bank in 2016 and an aggregate of 125,000 shares of our Historical Class B common stock, exercisable for a period of 10 years at an exercise price of $1.62 per share, to a lender in connection with the entry into the Second Amended and Restated Loan and Security Agreement with Silicon Valley Bank in 2018.

None of the foregoing transactions involved any underwriters, underwriting discounts or commissions, or any public offering. Unless otherwise stated, the sales of the above securities were deemed to be exempt from registration under the Securities Act in reliance upon Section 4(a)(2) of the Securities Act (or Regulation D or Regulation S promulgated thereunder) or Rule 701 promulgated under Section 3(b) of the Securities Act as transactions by an issuer not involving any public offering or pursuant to benefit plans and contracts relating to compensation as provided under Rule 701. The recipients of the securities in each of these transactions represented their intentions to acquire the securities for investment only and not with a view to or for sale in connection with any distribution thereof and appropriate legends were placed upon the stock certificates issued in these transactions.

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Item 16. Exhibits and financial statement schedules

See the Exhibit Index immediately following the signature page for a list of exhibits filed as part of this registration statement, which Exhibit Index is incorporated herein by reference.

Item 17. Undertakings

(a) Insofar as indemnification for liabilities arising under the Securities Act may be permitted to directors, officers and controlling persons of the registrant pursuant to the provisions referenced in Item 14 of this registration statement, or otherwise, the registrant has been advised that in the opinion of the Securities and Exchange Commission such indemnification is against public policy as expressed in the Securities Act and is, therefore, unenforceable. In the event that a claim for indemnification against such liabilities (other than the payment by the registrant of expenses incurred or paid by a director, officer, or controlling person of the registrant in the successful defense of any action, suit, or proceeding) is asserted by such director, officer, or controlling person in connection with the securities being registered hereunder, the registrant will, unless in the opinion of its counsel the matter has been settled by controlling precedent, submit to a court of appropriate jurisdiction the question of whether such indemnification by it is against public policy as expressed in the Securities Act and will be governed by the final adjudication of such issue.

(b) The undersigned registrant hereby undertakes that:

(1) For purposes of determining any liability under the Securities Act, the information omitted from the form of prospectus filed as part of this registration statement in reliance upon Rule 430A and contained in a form of prospectus filed by the registrant pursuant to Rule 424(b)(1) or (4) or 497(h) under the Securities Act shall be deemed to be part of this registration statement as of the time it was declared effective.

(2) For the purpose of determining any liability under the Securities Act, each post-effective amendment that contains a form of prospectus shall be deemed to be a new registration statement relating to the securities offered therein and the offering of such securities at that time shall be deemed to be the initial bona fide offering thereof.

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Exhibit index

Exhibit Number	Description
1.1†	Form of Underwriting Agreement.
3.1	Seventh Amended and Restated Certificate of Incorporation of the Registrant, as currently in effect.
3.2	Form of Amended and Restated Certificate of Incorporation of the Registrant, to be in effect immediately prior to the completion of this offering.
3.3	Amended and Restated Bylaws of the Registrant, as currently in effect.
3.4	Form of Amended and Restated Bylaws of the Registrant, to be in effect immediately prior to the completion of this offering.
4.1	Amended and Restated Investors' Rights Agreement, dated as of October 18, 2018, by and among the Registrant and the other parties thereto.
4.2	Form of Stock Certificate for Class A Common Stock of the Registrant.
5.1†	Opinion of Simpson Thacher & Bartlett LLP.
10.1	Second Amended and Restated Loan and Security Agreement, dated as of February 9, 2018, by and between the Registrant and Silicon Valley Bank.
10.2	First Amendment to Second Amended and Restated Loan and Security Agreement, dated June 26, 2019, by and between the Registrant and Silicon Valley Bank.
10.3	Lease Agreement, dated August 2, 2018, between the Registrant and 6200 Stoneridge Mall Road Investors LLC.
10.4	First Amendment to Lease Agreement, dated May 20, 2019, between the Registrant and 6200 Stoneridge Mall Road Investors LLC.
10.5*	License Agreement, dated September 26, 2013, between the Registrant and the President and Fellows of Harvard College.
10.6*	Amendment No. 1 to License Agreement, dated October 25, 2018, between the Registrant and President and Fellows of Harvard College.
10.7*	Exclusive (Equity) Agreement, dated October 15, 2015, between Epinomics, Inc. and The Board of Trustees of the Leland Stanford Junior University.
10.8	Amendment No. 1 to the License Agreement, dated February 1, 2017, between Epinomics and The Board of Trustees of the Leland Stanford Junior University.
10.9*	Amendment No. 2 to the License Agreement, dated July 27, 2018, between the Registrant and The Board of Trustees of the Leland Stanford Junior University.
10.10+	Amended and Restated 2012 Stock Plan and forms of award agreements thereunder.
10.11+	2019 Omnibus Incentive Plan and forms of award agreements thereunder, to be in effect upon the completion of this offering.
10.12+	2019 Employee Stock Purchase Plan, to be in effect upon the completion of this offering.
10.13+	Non-Employee Director Compensation Policy, to be in effect upon the completion of this offering.
10.14+	Employment Offer Letter by and between the Registrant and Eric S. Whitaker.
10.15+	Employment Offer Letter by and between the Registrant and Justin McAnear.
10.16+	Form of At-Will Employment, Confidential Information, Invention Assignment, and Arbitration Agreement.

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Exhibit Number	Description
23.1†	Consent of Simpson Thacher & Bartlett LLP (included in Exhibit 5.1).
23.2	Consent of Independent Registered Public Accounting Firm.
24.1	Power of Attorney (included in the signature page to this Registration Statement).

† To be filed by amendment

+ Management contract or compensatory plan or arrangement.

* Portions of this exhibit have been omitted pursuant to Item 601 of Regulation S-K promulgated under the Securities Act because the information (i) is not material and (ii) would be competitively harmful if publicly disclosed.

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Signatures

Pursuant to the requirements of the Securities Act of 1933, the registrant has duly caused this registration statement to be signed on its behalf by the undersigned, thereunto duly authorized, in the City of Pleasanton, State of California, on the nineteenth day of August, 2019.

10x Genomics, Inc.

By: /s/ Serge Saxonov
Name: Serge Saxonov
Title: Chief Executive Officer and Director

KNOW ALL PERSONS BY THESE PRESENTS, that each person whose signature appears below constitutes and appoints Serge Saxonov, Justin J. McAnear and Eric S. Whitaker, and each of them, his or her true and lawful attorneys-in-fact and agents, with full power of substitution and resubstitution, for him or her and in his or her name, place, and stead, in any and all capacities, to sign any and all amendments (including post-effective amendments) to this registration statement and any and all additional registration statements pursuant to Rule 462(b) of the Securities Act of 1933, and to file the same, with all exhibits thereto, and all other documents in connection therewith, with the Securities and Exchange Commission, granting unto each said attorney-in-fact and agents full power and authority to do and perform each and every act in person, hereby ratifying and confirming all that said attorneys-in-fact and agents or either of them or their or his or her substitute or substitutes may lawfully do or cause to be done by virtue hereof.

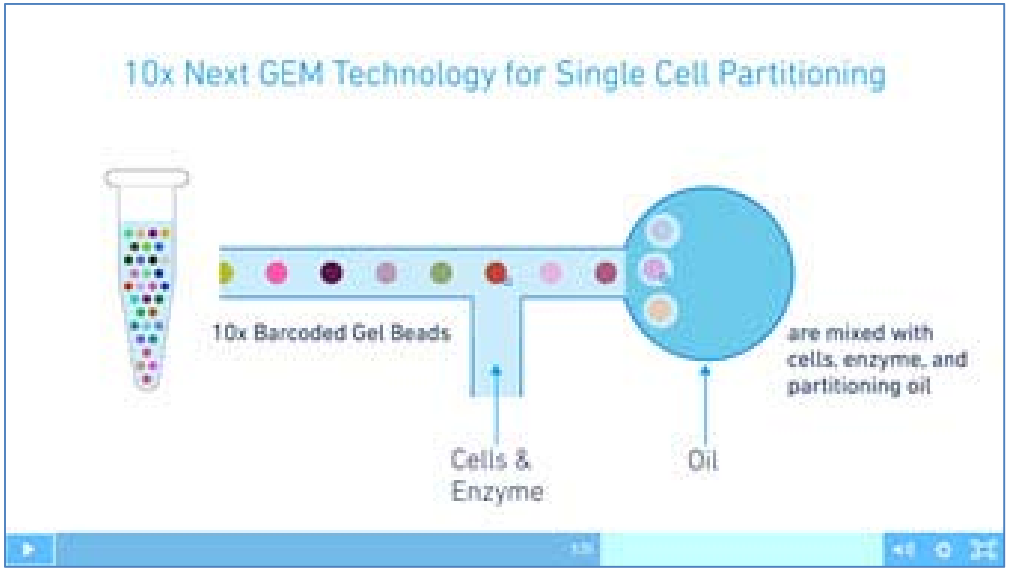
Pursuant to the requirements of the Securities Act of 1933, this registration statement has been signed by the following persons in the capacities and on the dates indicated.

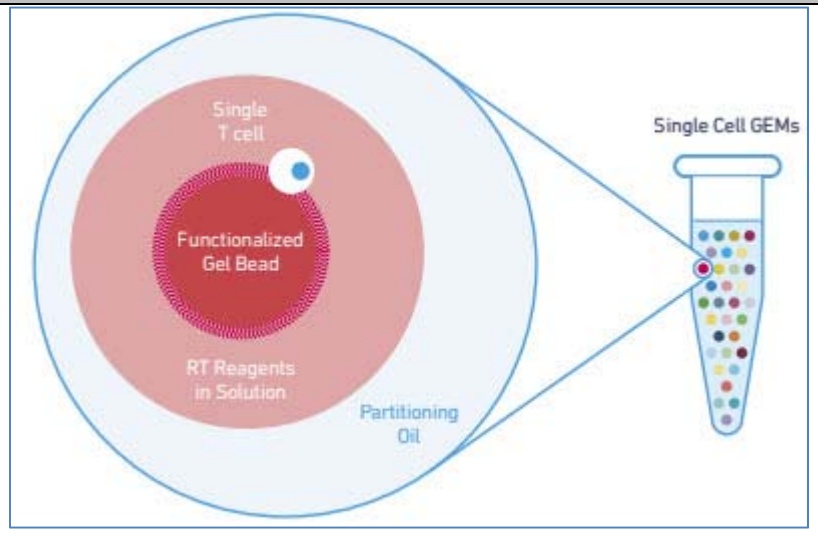
Signature	Title	Date
/s/ Serge Saxonov Serge Saxonov	Chief Executive Officer and Director (Principal Executive Officer)	August 19, 2019
/s/ Benjamin J. Hindson Benjamin J. Hindson	President and Director	August 19, 2019
/s/ Justin J. McAnear Justin J. McAnear	Chief Financial Officer (Principal Accounting and Financial Officer)	August 19, 2019
/s/ John R. Stuelpnagel John R. Stuelpnagel	Chairman of the board of directors	August 19, 2019
/s/ Paul A. Conley Paul A. Conley	Director	August 19, 2019
/s/ Sridhar Kosaraju Sridhar Kosaraju	Director	August 19, 2019
/s/ Mathai Mammen Mathai Mammen	Director	August 19, 2019
/s/ Bryan E. Roberts Bryan E. Roberts	Director	August 19, 2019
/s/ Shehnaaz Suliman Shehnaaz Suliman	Director	August 19, 2019

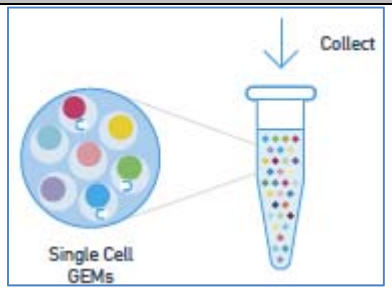
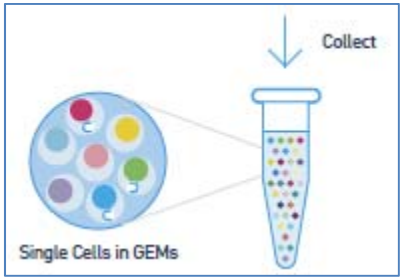
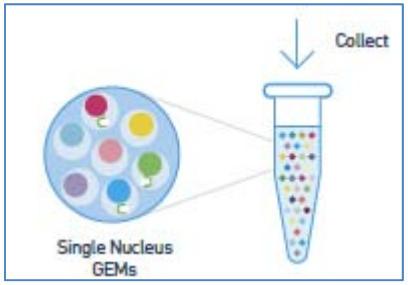
EXHIBIT 5

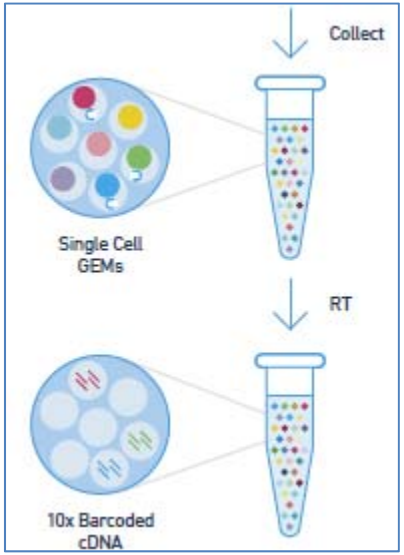
US 8,871,444
Infringement Analysis

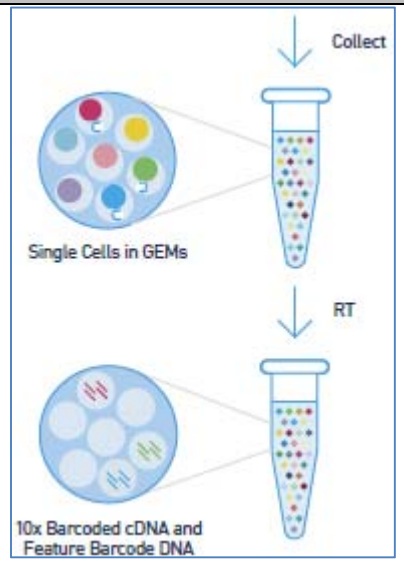
'444 Claim Language	**Infringement Support**
1. A method for detecting a product of an enzymatic reaction, comprising the steps of:	10X's Next GEM platform conducts enzymatic barcoding reactions in microfluidic droplets and detects the products of those reactions using DNA sequencing: Massive Partitioning and Barcoding Encapsulate your sample into hundreds to tens of thousands of uniquely addressable partitions in minutes, each containing an identifying barcode for downstream analysis. Each Gel Bead, infused with millions of unique oligonucleotide sequences, is mixed with a sample, which can be high molecular weight (HMW) DNA, individual cells, cells labeled using Feature Barcoding technology, nuclei, nuclei treated with transposase, or Cell Beads (see page 6). Gel Beads and samples are then added to an oil-surfactant solution to create Gel Beads in EMulsion (GEMs), which act as individual reaction vesicles in which the Gel Beads are dissolved and the sample is barcoded (Figure 1). Barcoded products are pooled for downstream reactions to create short-read sequencer compatible libraries. After sequencing, the resulting barcoded short read sequences are fed into turnkey analysis pipelines that use the identifying barcodes to map reads back to their original HMW DNA, single cell, or single nucleus of origin. Ex. 2 at 2.

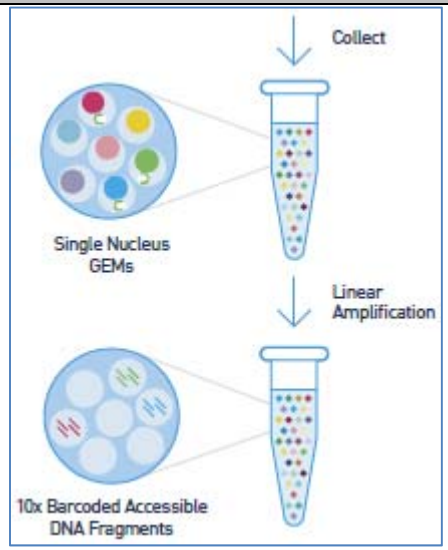
'444 Claim Language	**Infringement Support**
providing a droplet generator to produce, under microfluidic control, a plurality of aqueous microcapsules surrounded by an immiscible continuous phase that comprises a fluorinated oil that comprises a fluorinated polymer surfactant, each of the plurality of microcapsules comprising an enzyme, a genetic element, and reagents for the enzymatic reaction;	10X's Next GEM platform includes a microfluidic droplet generator, which creates aqueous microcapsules:  Ex. 6. (Video, available at: https://www.10xgenomics.com/solutions/single-cell/). As shown in 10X's technology brochure, each of its reagent kits that utilize the Next GEM platform use the approach immediately above to generate aqueous microcapsules. *See* Ex. 2 at 3 (single cell partitioning for transcriptome analysis); *id.* at 4 (single cell partitioning for immunology applications); *id.* at 5 (single cell partition for epigenome analysis). Additionally, the 10X technology brochure shows that the aqueous microcapsules include an enzyme (included with the "RT Reagents in Solution"), a genetic element (the sample nucleic acids), and reagents for the enzymatic reaction (the "RT Reagents in Solution" and the barcodes included with the "Functionalized Gel Bead"):

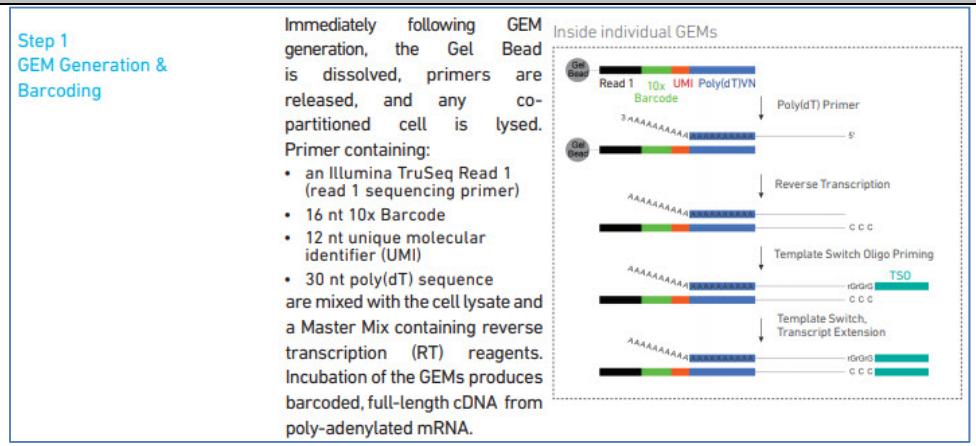
'444 Claim Language	Infringement Support
	 Figure 1 A GEM is a "Gel bead in EMulsion" droplet that encapsulates each micro-reaction within the Chromium System. Here we show a Single Cell GEM with a single T cell, reagents, and barcoded gel bead all partitioned within a single droplet. *Id.* at 1. As the image above shows, each aqueous microcapsule is surrounded by an immiscible continuous phase (the "Partitioning Oil"). As confirmed during 10X's November 2018 trial against Bio-Rad in this district, 10X's products all utilize fluorinated chemistry, including a fluorinated oil carrier fluid and a fluorinated polymer surfactant in the carrier fluid.
pooling the microcapsules into one or more common compartments such that a portion of the plurality of microcapsules contact each other but	10X's technology brochure confirms that "[b]arcoded products are pooled for downstream reactions to create short-read sequencer compatible libraries." Ex. 2 at 2. The brochure shows that for each of 10X's Next GEM applications the droplets are pooled in a compartment (e.g., a tube), and the droplets contact each other but do not fuse:

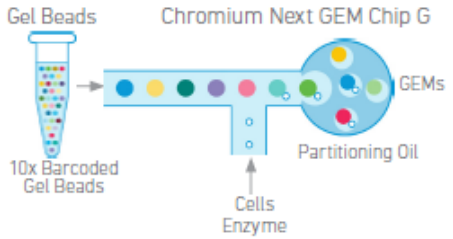
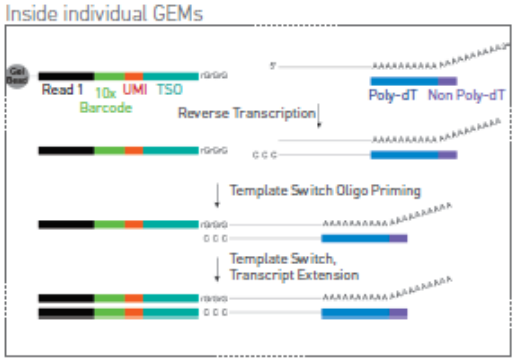
'444 Claim Language	**Infringement Support**
do not fuse with each other due to the presence of the surfactant;	 *Id.* at 3  *Id.* at 4.  *Id.* at 5.

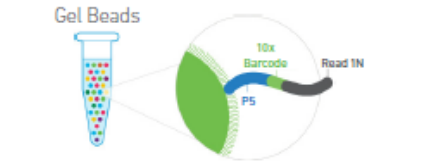
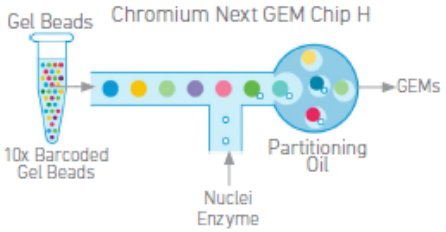
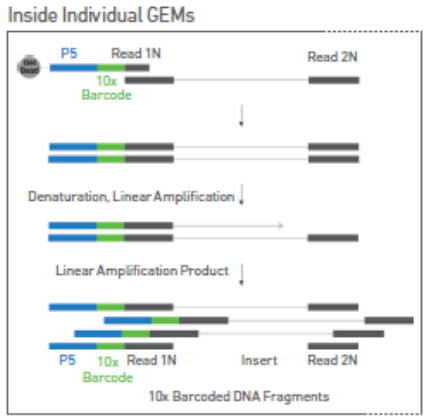
'444 Claim Language	**Infringement Support**
conducting the enzymatic reaction on the genetic element of at least one of the plurality of microcapsules within the one or more common compartments; and	In 10X's Next GEM platform, an enzymatic reaction is conducted within the microcapsule that is within the compartment. This is shown in the product brochure, which shows an enzymatic reaction based on the use of reverse transcriptase ("RT") enzyme or another enzyme for "Linear Amplification" to yield barcoded DNA fragments:  *Id.* at 3.

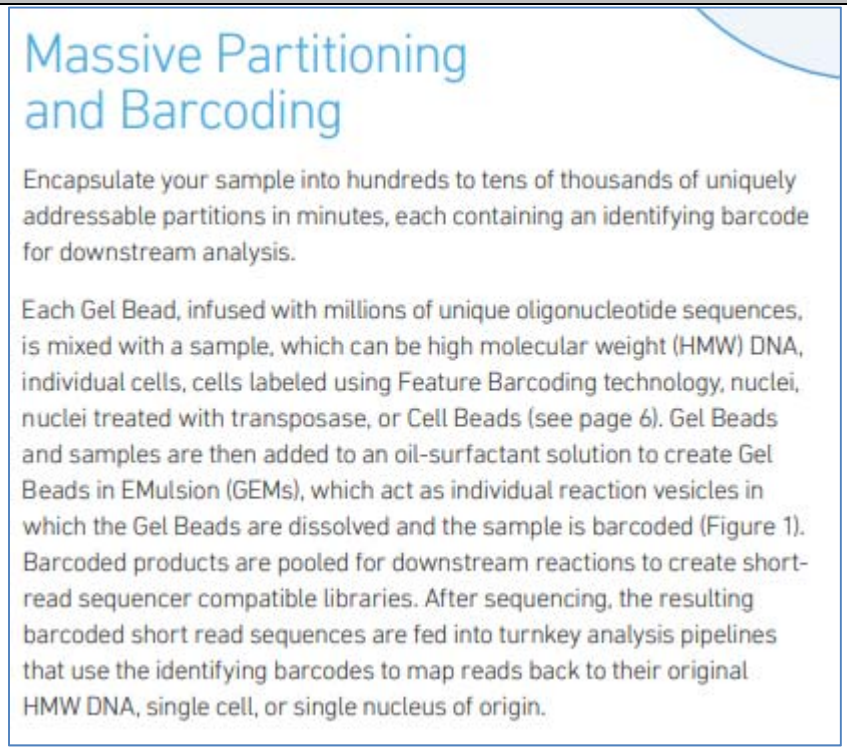
'444 Claim Language	**Infringement Support**
	 *Id.* at 4.

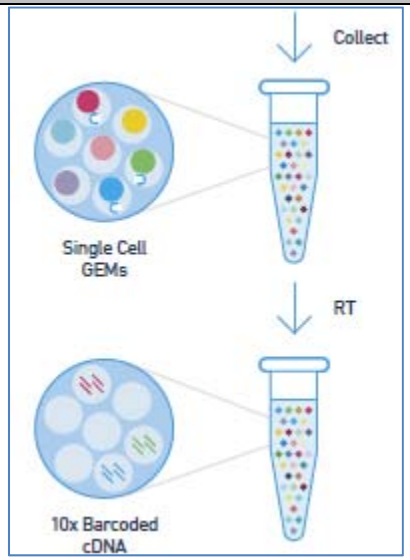
’444 Claim Language	Infringement Support
	 *Id.* at 5. The product manuals for each of 10X’s Next GEM products further depict the enzymatic reaction:

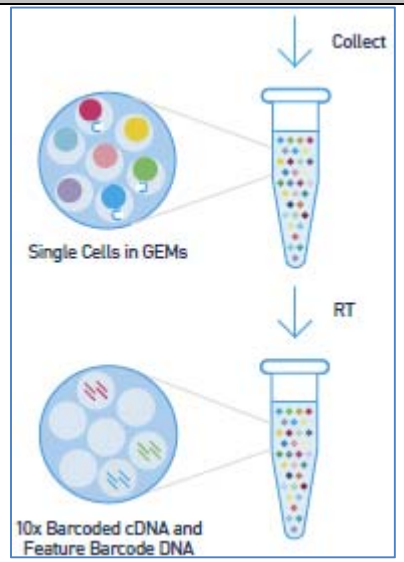
’444 Claim Language	Infringement Support
	 Ex. 7 at 15.

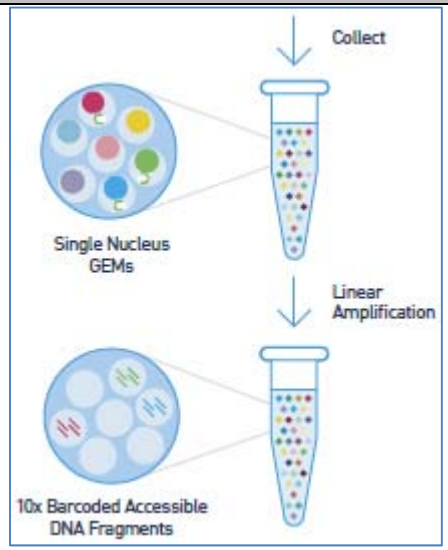
'444 Claim Language	Infringement Support
	Step 1 GEM Generation & Barcoding GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads v1.1, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell. Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.    Ex. 8 at 16.

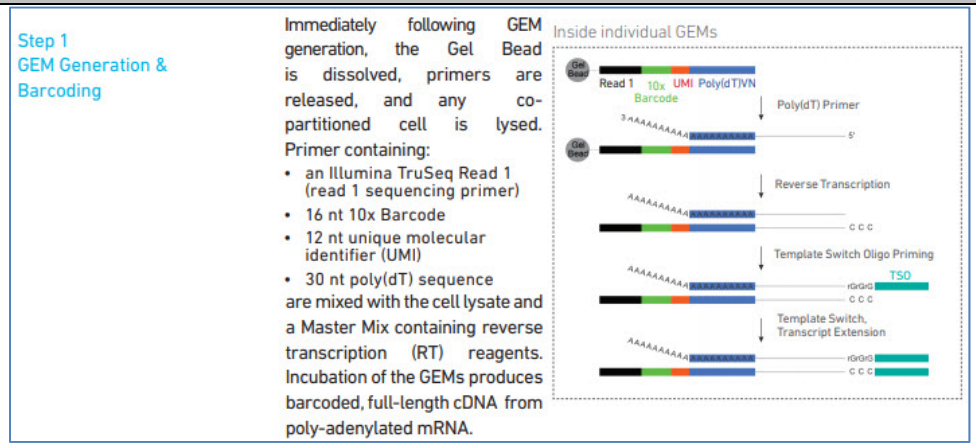
’444 Claim Language	Infringement Support
	Step 2 GEM Generation & Barcoding GEMs are generated by combining barcoded Gel Beads, transposed nuclei, a Master Mix, and Partitioning Oil on a Chromium Next GEM Chip H. To achieve single nuclei resolution, the nuclei are delivered at a limiting dilution, such that the majority (~90-99%) of generated GEMs contains no nuclei, while the remainder largely contain a single nucleus. Upon GEM generation, the Gel Bead is dissolved. Oligonucleotides containing (i) an Illumina® P5 sequence, (ii) a 16 nt 10x Barcode and (iii) a Read 1 (Read 1N) sequence are released and mixed with DNA fragments and Master Mix. Thermal cycling of the GEMs produces 10x barcoded single-stranded DNA. After incubation, the GEMs are broken and pooled fractions are recovered.    Ex. 9 at 13.
detecting the product of the enzymatic reaction.	In 10X’s Next GEM platform, the product of the enzymatic reaction is detected via sequencing:

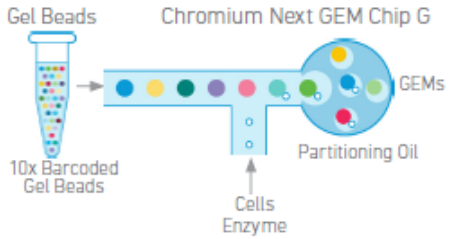
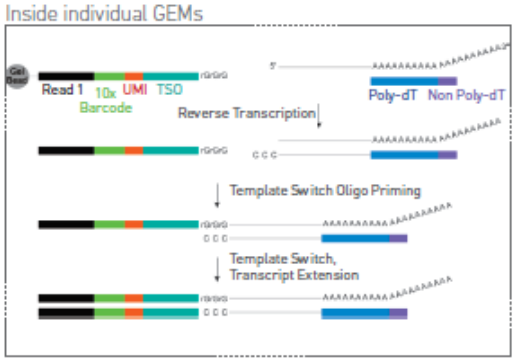
'444 Claim Language	**Infringement Support**
	 Ex. 2 at 2.
2. The method of claim 1, wherein the genetic elements are nucleic acids, proteins, or cells.	In 10X's Next GEM platform the genetic elements encompass at least nucleic acids. This is shown in the product brochure, which shows an enzymatic reaction based on the use of reverse transcriptase ("RT") enzyme or another enzyme for "Linear Amplification" to yield barcoded DNA fragments:

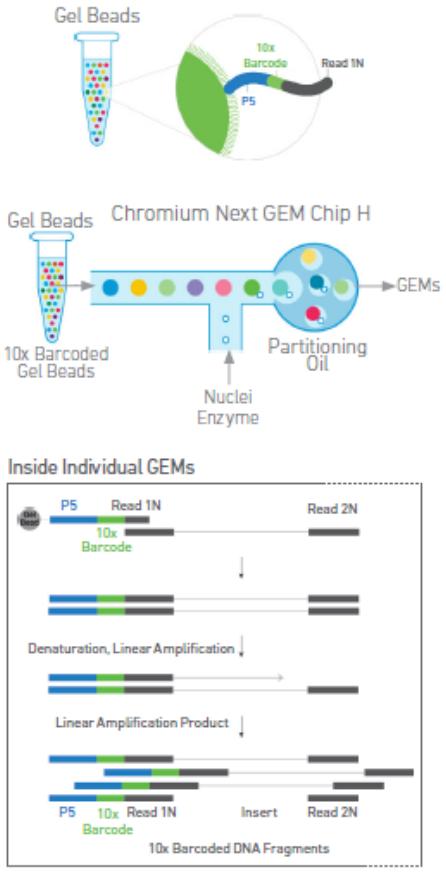
’444 Claim Language	Infringement Support
	 *Id.* at 3.

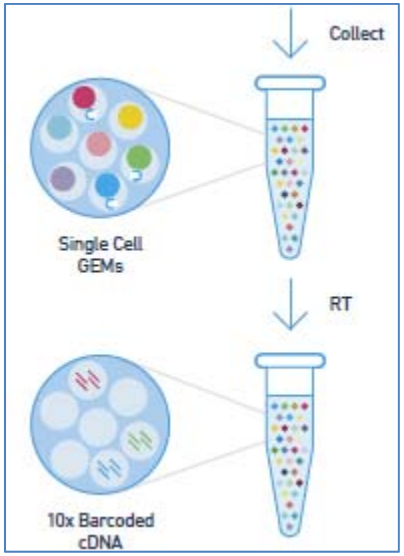
’444 Claim Language	Infringement Support
	 *Id.* at 4.

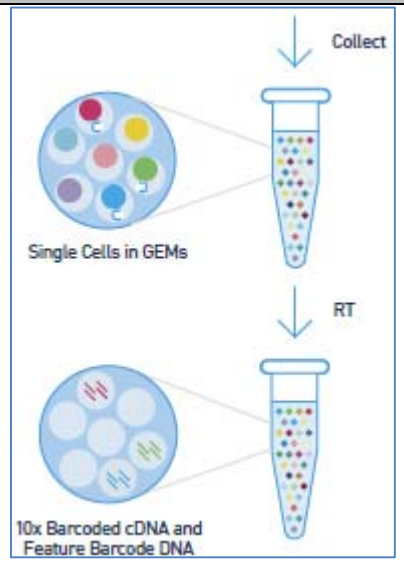
’444 Claim Language	Infringement Support
	 *Id.* at 5. The product manuals for each of 10X’s Next GEM products further depict the enzymatic reaction involving nucleic acids:

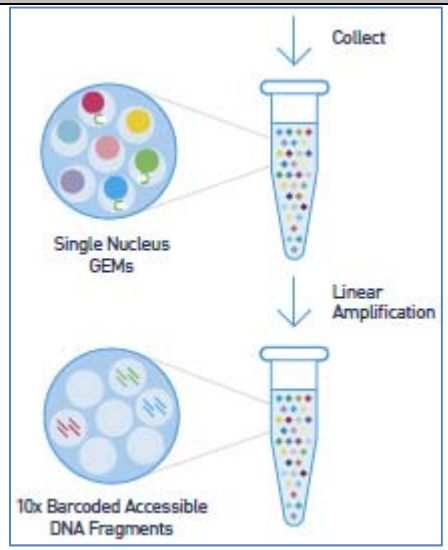
’444 Claim Language	Infringement Support
	 Ex. 7 at 15.

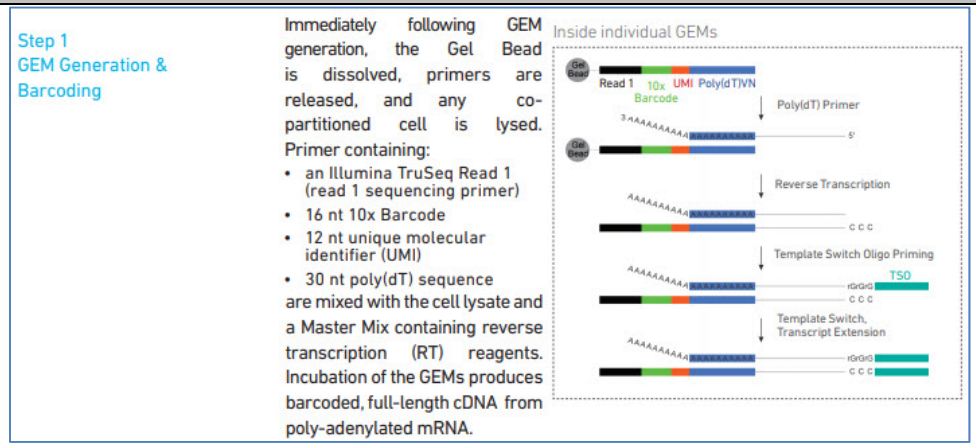
'444 Claim Language	Infringement Support
	Step 1 GEM Generation & Barcoding GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads v1.1, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell. Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.    Ex. 8 at 16.

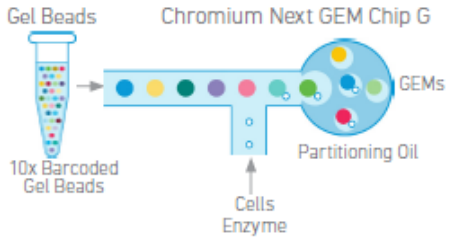
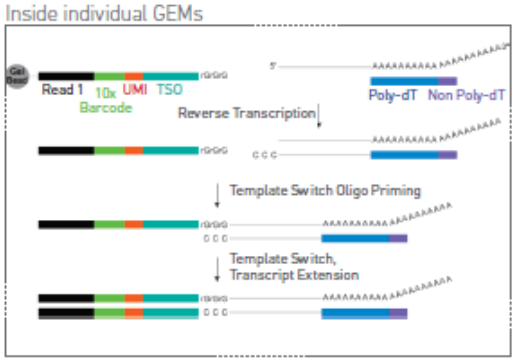
'444 Claim Language	Infringement Support
	Step 2 GEM Generation & Barcoding GEMs are generated by combining barcoded Gel Beads, transposed nuclei, a Master Mix, and Partitioning Oil on a Chromium Next GEM Chip H. To achieve single nuclei resolution, the nuclei are delivered at a limiting dilution, such that the majority (~90-99%) of generated GEMs contains no nuclei, while the remainder largely contain a single nucleus. Upon GEM generation, the Gel Bead is dissolved. Oligonucleotides containing (i) an Illumina® P5 sequence, (ii) a 16 nt 10x Barcode and (iii) a Read 1 (Read 1N) sequence are released and mixed with DNA fragments and Master Mix. Thermal cycling of the GEMs produces 10x barcoded single-stranded DNA. After incubation, the GEMs are broken and pooled fractions are recovered.  Ex. 9 at 13.
4. The method of claim 1, wherein the genetic elements are labeled.	In 10X's Next GEM platform the genetic elements encompass at least nucleic acids. This is shown in the product brochure, which shows an enzymatic reaction based on the use of

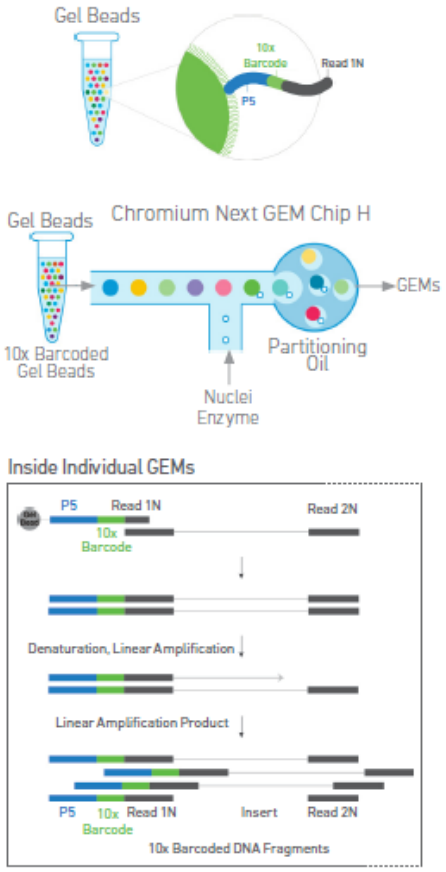
’444 Claim Language	Infringement Support
	reverse transcriptase (“RT”) enzyme or another enzyme for “Linear Amplification” to yield barcoded DNA fragments:  *Id.* at 3.

’444 Claim Language	Infringement Support
	 *Id.* at 4.

’444 Claim Language	Infringement Support
	 *Id.* at 5. The product manuals for each of 10X’s Next GEM products further depict the enzymatic reaction involving nucleic acids:

’444 Claim Language	Infringement Support
	 Ex. 7 at 15.

'444 Claim Language	Infringement Support
	Step 1 GEM Generation & Barcoding GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads v1.1, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell. Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.    Ex. 8 at 16.

'444 Claim Language	Infringement Support
	Step 2 GEM Generation & Barcoding GEMs are generated by combining barcoded Gel Beads, transposed nuclei, a Master Mix, and Partitioning Oil on a Chromium Next GEM Chip H. To achieve single nuclei resolution, the nuclei are delivered at a limiting dilution, such that the majority (~90-99%) of generated GEMs contains no nuclei, while the remainder largely contain a single nucleus. Upon GEM generation, the Gel Bead is dissolved. Oligonucleotides containing (i) an Illumina® P5 sequence, (ii) a 16 nt 10x Barcode and (iii) a Read 1 (Read 1N) sequence are released and mixed with DNA fragments and Master Mix. Thermal cycling of the GEMs produces 10x barcoded single-stranded DNA. After incubation, the GEMs are broken and pooled fractions are recovered.  Ex. 9 at 13.
8. The method of claim 1, wherein the microcapsules are monodisperse with respect to each other.	10X's product literature for its Next GEM platform depicts the microcapsules as being monodisperse with respect to each other:

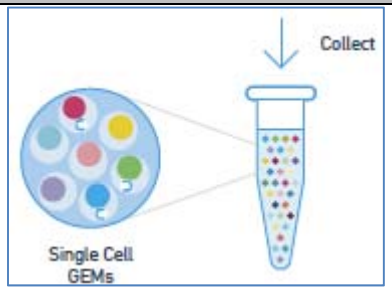
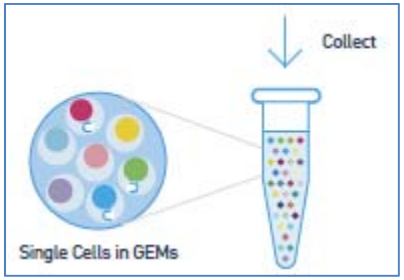
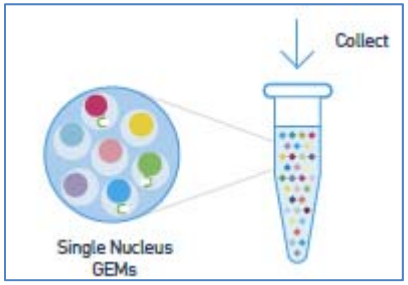
’444 Claim Language	Infringement Support
	 Ex. 2 at 3  *Id.* at 4.  *Id.* at 5.

EXHIBIT 6

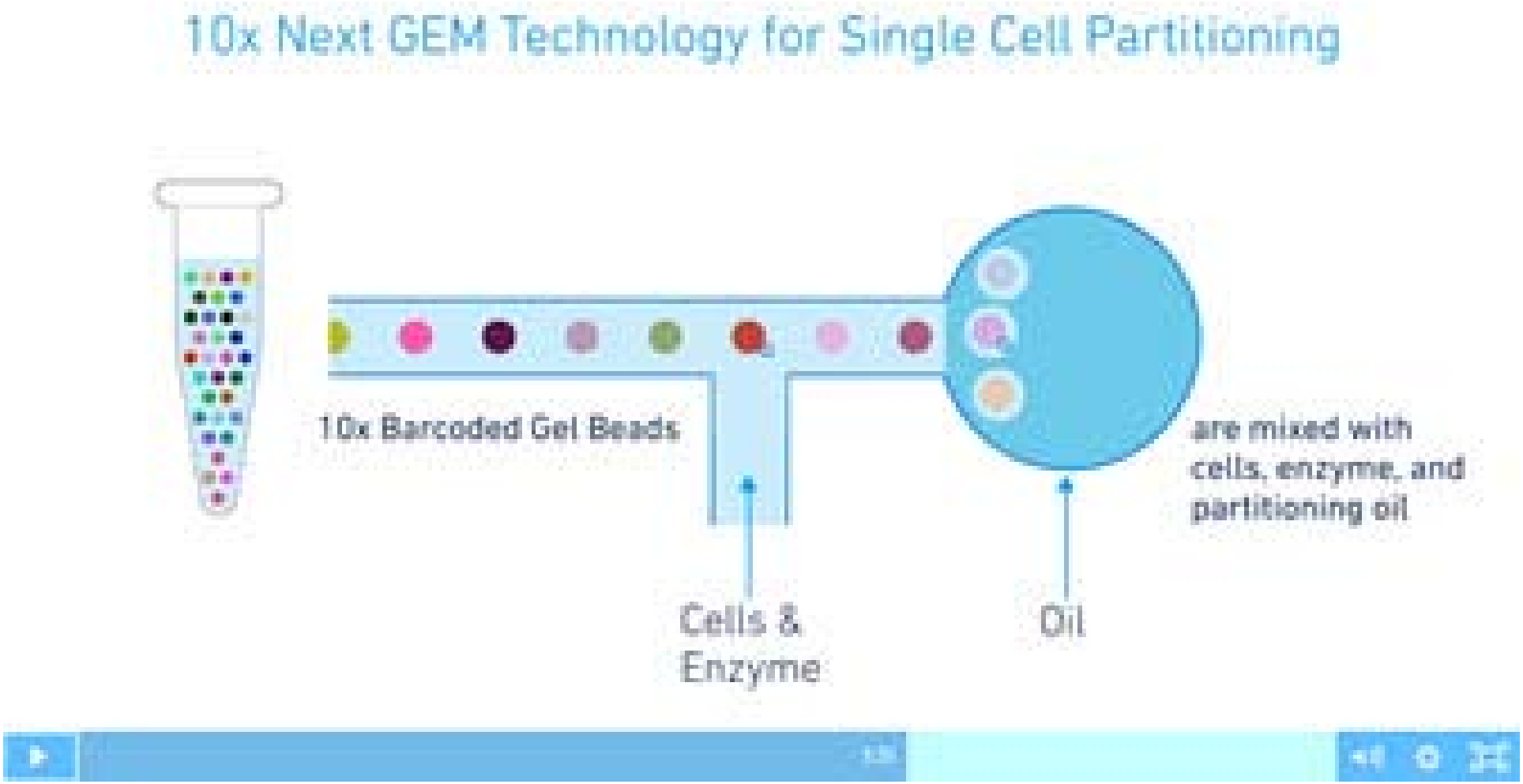

10x Next GEM Technology for Single Cell Partitioning
10x Barcoded Gel Beads
are mixed with cells, enzyme, and partitioning oil
Cells & Enzyme
Oil

EXHIBIT 7

CG000204 Rev C

USER GUIDE

Chromium Next GEM Single Cell 3' Reagent Kits v3.1

FOR USE WITH

Chromium Next GEM Single Cell 3' GEM, Library & Gel Bead Kit v3.1, 16 rxns PN-1000121
Chromium Next GEM Single Cell 3' GEM, Library & Gel Bead Kit v3.1, 4 rxns PN-1000128
Chromium Next GEM Chip G Single Cell Kit, 48 rxns PN-1000120
Chromium Next GEM Chip G Single Cell Kit, 16 rxns PN-1000127
Chromium i7 Multiplex Kit, 96 rxns PN-120262

Next GEM reagents are specific to Next GEM products and should not be used interchangeably with non-Next GEM reagents.

Notices

Document Number

CG000204 • Rev C

Legal Notices

Instrument & Licensed Software Updates Warranties

Updates to existing Instruments and Licensed Software may be required to enable customers to use new or existing products. In the event of an Instrument failure resulting from an update, such failed Instrument will be replaced or repaired in accordance with the 10x Limited Warranty, Assurance Plan or service agreement, only if such Instrument is covered by any of the foregoing at the time of such failure. Instruments not covered under a current 10x Limited Warranty, Assurance Plan or service agreement will not be replaced or repaired.

Support

Email: support@10xgenomics.com

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6230 Stoneridge Mall Road

Pleasanton, CA 94588 USA

Document Revision Summary

Document Number	CG000204
Title	Chromium Next GEM Single Cell 3' Reagent Kits v3.1 User Guide
Revision	Rev B to Rev C
Revision Date	Aug 2019

Specific Changes:

- Updated to state that Next GEM reagents are specific to Next GEM products.

General Changes:

- Updated for general minor consistency of language and terms throughout.

Table of Contents

Introduction

Chromium Next GEM Single Cell 3' Reagent Kits v3.1
Chromium Accessories
Recommended Thermal Cyclers
Additional Kits, Reagents & Equipment
Protocol Steps & Timing
Stepwise Objectives

Chromium Next GEM Single Cell 3' Reagent Kits v3.1

Chromium Next GEM Single Cell 3' GEM, Library & Gel Bead Kit v3.1, 16 rxns PN-1000121

Chromium Next GEM Single Cell 3' GEM Kit v3.1 16 rxns PN-1000123 (store at –20°C)

Chromium
Next GEM
Single Cell 3'
GEM Kit v3.1

	#	PN
RT Reagent B	1	2000165
RT Enzyme C	1	2000085
Template Switch Oligo	1	3000228
Reducing Agent B	1	2000087
Cleanup Buffer	2	2000088
cDNA Primers	1	2000089
Amp Mix	1	2000047

10xGenomics.com 10x GENOMICS

Chromium Next GEM Single Cell 3' Library Kit v3.1 16 rxns PN-1000157 (store at –20°C)

Chromium
Next GEM
Single Cell 3'
Library Kit v3.1

	#	PN
Fragmentation Enzyme	1	2000090
Fragmentation Buffer	1	2000091
Ligation Buffer	1	2000092
DNA Ligase	1	220110
Adaptor Oligos	1	2000094
SI Primer	1	2000095
Amp Mix	1	2000047

10xGenomics.com 10x GENOMICS

Chromium Next GEM Single Cell 3' Gel Bead Kit v3.1, 16 rxns PN-1000122 (store at –80°C)

Chromium
Next GEM
Single Cell 3'
v3.1 Gel Beads

	#	PN
Single Cell 3' v3.1 Gel Beads	2	2000164

10xGenomics.com 10x GENOMICS

Dynabeads™ MyOne™ SILANE PN-2000048 (store at 4°C)

	#	PN
Dynabeads MyOne SILANE	1	2000048

Chromium Next GEM Single Cell 3' GEM, Library & Gel Bead Kit v3.1, 4 rxns PN-1000128

Chromium Next GEM Single Cell 3'GEM Kit v3.1 4 rxns PN-1000130 (store at –20°C)

Chromium
Next GEM
Single Cell 3'
GEM Kit v3.1

	#	PN
RT Reagent B	1	2000165
RT Enzyme C	1	2000102
Template Switch Oligo	1	3000228
Reducing Agent B	1	2000087
Cleanup Buffer	1	2000088
cDNA Primers	1	2000089
Amp Mix	1	2000103

10xGenomics.com 10x GENOMICS

Chromium Next GEM Single Cell 3' Library Kit v3.1 4 rxns PN-1000158 (store at –20°C)

Chromium
Next GEM
Single Cell 3'
Library Kit v3.1

	#	PN
Fragmentation Enzyme	1	2000104
Fragmentation Buffer	1	2000091
Ligation Buffer	1	2000092
DNA Ligase	1	220131
Adaptor Oligos	1	2000094
SI Primer	1	2000095

10xGenomics.com 10x GENOMICS

Chromium Next GEM Single Cell 3' Gel Bead Kit v3.1, 4 rxns PN-1000129 (store at –80°C)

Chromium
Next GEM
Single Cell 3'
v3.1 Gel Beads

	#	PN
Single Cell 3' v3.1 Gel Beads (4 rxns)	1	2000164

10xGenomics.com 10x GENOMICS

Dynabeads™ MyOne™ SILANE PN-2000048 (store at 4°C)

	#	PN
Dynabeads MyOne SILANE	1	2000048

Chromium Next GEM Chip G Single Cell Kit, 48 rxns PN-1000120 (store at ambient temperature)

Chromium
Partitioning Oil

	#	PN
Partitioning Oil	6	2000190

Chromium
Recovery Agent

	#	PN
Recovery Agent	6	220016

Chromium
Next GEM
Chip G & Gaskets

	#	PN
Chromium Next GEM Chip G	6	2000177
Gasket, 6-pack	1	370017

10xGenomics.com

10x GENOMICS

Chromium Next GEM Chip G Single Cell Kit, 16 rxns PN-1000127 (store at ambient temperature)

Chromium
Partitioning Oil

	#	PN
Partitioning Oil	2	2000190

Chromium
Recovery Agent

	#	PN
Recovery Agent	2	220016

Chromium
Next GEM
Chip G & Gaskets

	#	PN
Chromium Next GEM Chip G	2	2000177
Gasket, 2-pack	1	3000072

10xGenomics.com

10x GENOMICS

Chromium i7 Multiplex Kit, 96 rxns PN-120262 (store at –20°C)

Chromium
i7 Multiplex Kit

	#	PN
Chromium i7 Sample Index Plate	1	220103

Chromium Accessories

Product	Part Number (Kit)	Part Number (Item)
10x Vortex Adapter	120251	330002
10x Magnetic Separator	120250	230003
Chromium Next GEM Secondary Holder	1000195	3000332

Recommended Thermal Cyclers

Thermal cyclers used must support uniform heating of 100 µl emulsion volumes.

Supplier	Description	Part Number
BioRad	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module	1851197
Eppendorf	MasterCycler Pro	North America 950030010 International 6321 000.019
Thermo Fisher Scientific	Veriti 96-Well Thermal Cycler	4375786

Additional Kits, Reagents & Equipment

The items in the table below have been validated by 10x Genomics and are highly recommended for the Single Cell 3' protocol. Substituting materials may adversely affect system performance.

Supplier	Description	Part Number (US)
Plastics		
Eppendorf	PCR Tubes 0.2 ml 8-tube strips DNA LoBind Tubes, 1.5 ml DNA LoBind Tubes, 2.0 ml	951010022 022431021 022431048
USA Scientific	TempAssure PCR 8-tube strip	1402-4700
Thermo Fisher Scientific	MicroAmp 8-Tube Strip, 0.2 ml MicroAmp 8 -Cap Strip, clear	N8010580 N8010535
Rainin	Tips LTS 200UL Filter RT-L200FLR Tips LTS 1ML Filter RT-L1000FLR Tips LTS 20UL Filter RT-L10FLR	30389240 30389213 30389226
Kits & Reagents		
Thermo Fisher Scientific	Nuclease-free Water	AM9937
	Low TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA)	12090-015
Millipore Sigma	Ethanol, Pure (200 Proof, anhydrous)	E7023-500ML
Beckman Coulter	SPRIselect Reagent Kit	B23318
Bio-Rad	10% Tween 20	1662404
Ricca Chemical Company	Glycerin (glycerol), 50% (v/v) Aqueous Solution	3290-32
Qiagen	Qiagen Buffer EB	19086
Equipment		
VWR	Vortex Mixer Divided Polystyrene Reservoirs	10153-838 41428-958
Eppendorf	Eppendorf ThermoMixer C Eppendorf SmartBlock 1.5 ml, Thermoblock for 24 reaction vessel (alternatively, use a temperature-controlled Heat Block)	5382000023 5360000038
Rainin	Pipet-Lite Multi Pipette L8-50XLS+ Pipet-Lite Multi Pipette L8-200XLS+ Pipet-Lite Multi Pipette L8-10XLS+ Pipet-Lite Multi Pipette L8-20XLS+ Pipet-Lite LTS Pipette L-2XLS+ Pipet-Lite LTS Pipette L-10XLS+ Pipet-Lite LTS Pipette L-20XLS+ Pipet-Lite LTS Pipette L-100XLS+ Pipet-Lite LTS Pipette L-200XLS+ Pipet-Lite LTS Pipette L-1000XLS+	17013804 17013805 17013802 17013803 17014393 17014388 17014392 17014384 17014391 17014382

Choose either Eppendorf, USA Scientific or Thermo Fisher Scientific PCR 8-tube strips.

Additional Kits, Reagents & Equipment

The items in the table below have been validated by 10x Genomics and are highly recommended for the Single Cell 3' protocol. Substituting materials may adversely affect system performance.

Supplier	Description	Part Number (US)
Quantification & Quality Control		
Agilent	2100 Bioanalyzer Laptop Bundle High Sensitivity DNA Kit 4200 TapeStation High Sensitivity D1000 ScreenTape/Reagents High Sensitivity D5000 ScreenTape/Reagents	G2943CA 5067-4626 G2991AA 5067-5592/ 5067-5593 5067-5584/ 5067-5585
Thermo Fisher Scientific	Qubit 4.0 Flourometer Qubit dsDNA HS Assay Kit	Q33226 Q32854
Advanced Analytical	Fragment Analyzer Automated CE System - 12 cap Fragment Analyzer Automated CE System - 48/96 cap High Sensitivity NGS Fragment Analysis Kit	FSv2-CE2F FSv2-CE10F DNF-474
KAPA Biosystems	KAPA Library Quantification Kit for Illumina Platforms	KK4824

Choose Bioanalyzer, TapeStation, or Qubit based on availability & preference.

Protocol Steps & Timing

Day	Steps		Timing	Stop & Store
2 h	**Cell Preparation**			
	Dependent on Cell Type		~1-1.5 h	
	Step 1 – GEM Generation & Barcoding			
4 h	1.1	Prepare Reaction Mix	20 min	
	1.2	Load Chromium Next GEM Chip G	10 min	
	1.3	Run the Chromium Controller	18 min	
	1.4	Transfer GEMs	3 min	
	1.5	GEM-RT Incubation	55 min	STOP 4°C ≤72 h or –20°C ≤1 week
	Step 2 – Post GEM-RT Cleanup & cDNA Amplification			
6 h	2.1	Post GEM RT-Cleanup – Dynabead	45 min	
	2.2	cDNA Amplification	40 min	STOP 4°C ≤72 h or –20°C ≤1 week
	2.3	cDNA Cleanup – SPRIselect	20 min	STOP 4°C ≤72 h –20°C ≤4 weeks
	2.4	cDNA QC & Quantification	50 min	
	Step 3 – 3' Gene Expression Library Construction			
8 h	3.1	Fragmentation, End Repair & A-tailing	50 min	
	3.2	Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect	30 min	
	3.3	Adaptor Ligation	25 min	
	3.4	Post Ligation Cleanup- SPRIselect	20 min	
	3.5	Sample Index PCR	40 min	STOP 4°C ≤72 h
	3.6	Post Sample Index PCR Double Sided Size Selection- SPRIselect	30 min	STOP 4°C ≤72 h or -20°C long term
	3.7	Post Library Construction QC	50 min	

Stepwise Objectives

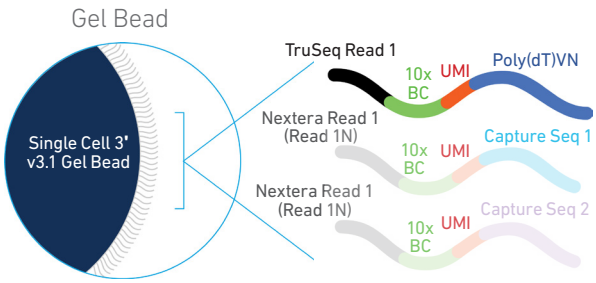
The Chromium Single Cell Gene Expression Solution upgrades short read sequencers to deliver a scalable microfluidic platform for 3' digital gene expression by profiling 500-10,000 individual cells per sample. A pool of ~3,500,000 10x Barcodes are sampled separately to index each cell's transcriptome. It is done by partitioning thousands of cells into nanoliter-scale Gel Beads-in-emulsion (GEMs), where all generated cDNA share a common 10x Barcode. Libraries are generated and sequenced from the cDNA and 10x Barcodes are used to associate individual reads back to the individual partitions.

This document outlines the protocol for generating Single Cell 3' Gene Expression libraries from single cells.

Single Cell 3' v3.1 Gel Beads

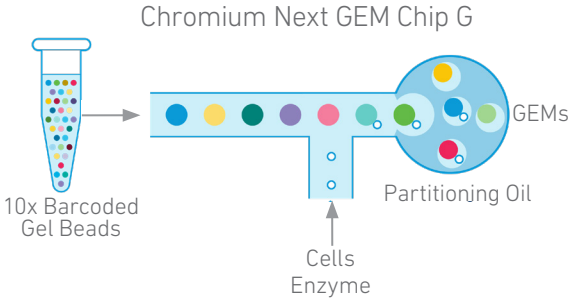
In addition to the poly(dT) primer that enables the production of barcoded, full-length cDNA from poly-adenylated mRNA, the Single Cell 3' v3.1 Gel Beads also include two additional primer sequences (Capture Sequence 1 and Capture Sequence 2), that enable capture and priming of Feature Barcoding technology compatible targets or analytes of interest.

Only the poly(dT) primers are used in this protocol for generating Single Cell 3' Gene Expression libraries.



Step 1 GEM Generation & Barcoding

GEMs are generated by combining barcoded Single Cell 3' v3.1 Gel Beads, a Master Mix containing cells, and Partitioning Oil onto Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90-99%) of generated GEMs contain no cell, while the remainder largely contain a single cell.



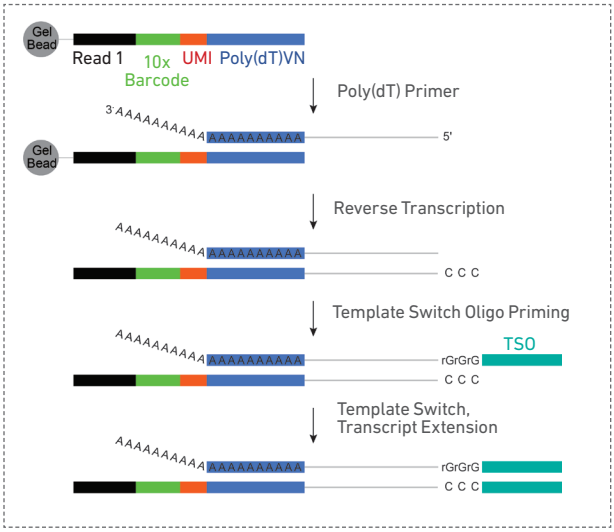
Step 1 GEM Generation & Barcoding

Immediately following GEM generation, the Gel Bead is dissolved, primers are released, and any co-partitioned cell is lysed. Primer containing:

- an Illumina TruSeq Read 1 (read 1 sequencing primer)
- 16 nt 10x Barcode
- 12 nt unique molecular identifier (UMI)
- 30 nt poly(dT) sequence

are mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents. Incubation of the GEMs produces barcoded, full-length cDNA from poly-adenylated mRNA.

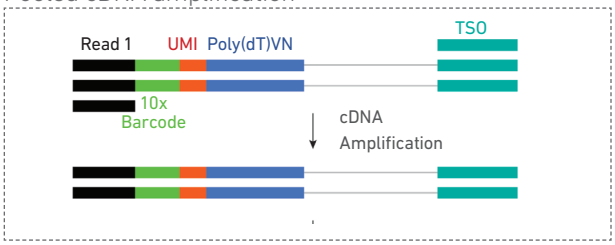
Inside individual GEMs



Step 2 Post GEM-RT Cleanup & cDNA Amplification

After incubation, GEMs are broken and pooled fractions are recovered. Silane magnetic beads are used to purify the first-strand cDNA from the post GEM-RT reaction mixture, which includes leftover biochemical reagents and primers. Barcoded, full-length cDNA is amplified via PCR to generate sufficient mass for library construction.

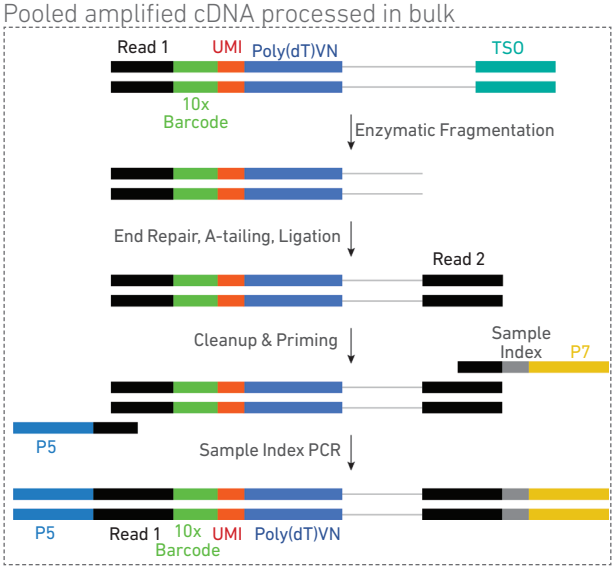
Pooled cDNA amplification



Step 3 3' Gene Expression Library Construction

Enzymatic fragmentation and size selection are used to optimize the cDNA amplicon size. TruSeq Read 1 (read 1 primer sequence) is added to the molecules during GEM incubation. P5, P7, a sample index, and TruSeq Read 2 (read 2 primer sequence) are added via End Repair, A-tailing, Adaptor Ligation, and PCR. The final libraries contain the P5 and P7 primers used in Illumina bridge amplification.

Pooled amplified cDNA processed in bulk

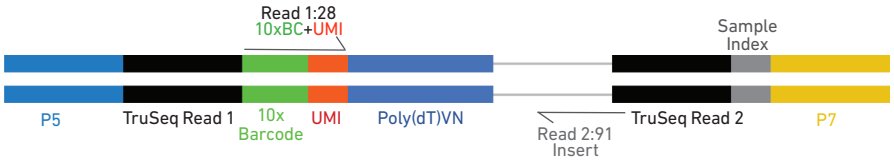


Step 4 Sequencing

A Chromium Single Cell 3' Gene Expression library comprises standard Illumina paired-end constructs which begin and end with P5 and P7. The 16 bp 10x Barcode and 12 bp UMI are encoded in Read 1, while Read 2 is used to sequence the cDNA fragment. Sample index sequences are incorporated as the i7 index read. TruSeq Read 1 and TruSeq Read 2 are standard Illumina sequencing primer sites used in paired-end sequencing.

Illumina sequencer compatibility, sample indices, library loading and pooling for sequencing are summarized in step 4.

Chromium Single Cell 3' Gene Expression Library



See Appendix for Oligonucleotide Sequences

Tips & Best Practices

Icons

Tips & Best Practices section includes additional guidance

Signifies critical step requiring accurate execution

Troubleshooting section includes additional guidance

Next GEM specific protocol step updates

Emulsion-safe Plastics

- Use validated emulsion-safe plastic consumables when handling GEMs as some plastics can destabilize GEMs.

Cell Concentration

- Recommended starting point is to load ~1,600 cells per reaction, resulting in recovery of ~1000 cells, and a multiplet rate of ~0.8%. The optimal input cell concentration is 700-1,200 cells/µl.
- The presence of dead cells in the suspension may also reduce the recovery rate. Consult the 10x Genomics Single Cell Protocols Cell Preparation Guide and the Guidelines for Optimal Sample Preparation flowchart (Documents CG00053 and CG000126 respectively) for more information on preparing cells.

Multiplet Rate (%)	# of Cells Loaded	# of Cells Recovered
~0.4%	~800	~500
~0.8%	~1,600	~1,000
~1.6%	~3,200	~2,000
~2.3%	~4,800	~3,000
~3.1%	~6,400	~4,000
~3.9%	~8,000	~5,000
~4.6%	~9,600	~6,000
~5.4%	~11,200	~7,000
~6.1%	~12,800	~8,000
~6.9%	~14,400	~9,000
~7.6%	~16,000	~10,000

General Reagent Handling

- Fully thaw and thoroughly mix reagents before use.
- Keep all enzymes and Master Mixes on ice during setup and use. Promptly move reagents back to the recommended storage.
- Calculate reagent volumes with 10% excess of 1 reaction values.
- Cover Partitioning Oil tubes and reservoirs to minimize evaporation.
- If using multiple chips, use separate reagent reservoirs for each chip during loading.
- Thoroughly mix samples with the beads during bead-based cleanup steps.

50% Glycerol Solution

- Purchase 50% glycerol solution from Ricca Chemical Company, Glycerin (glycerol), 50% (v/v) Aqueous Solution, PN-3290-32.
- Prepare 50% glycerol solution:
  i. Mix an equal volume of water and 99% Glycerol, Molecular Biology Grade.
  ii. Filter through a 0.2 µm filter.
  iii. Store at –20°C in 1-ml LoBind tubes. 50% glycerol solution should be equilibrated to room temperature before use.

Pipette Calibration

- Follow manufacturer's calibration and maintenance schedules.
- Pipette accuracy is particularly important when using SPRIselect reagents.

Chromium Next GEM Chip Handling

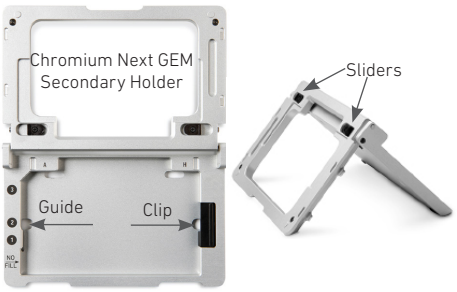
Next GEM

- Minimize exposure of reagents, chips, and gaskets to sources of particles and fibers, laboratory wipes, frequently opened flip-cap tubes, clothing that sheds fibers, and dusty surfaces.
- After removing the chip from the sealed bag, use in ≤ 24 h.
- Execute steps without pause or delay, unless indicated. When multiple chips are to be used, load, run, and collect the content from one chip before loading the next.
- Fill all unused input wells in rows labeled 1, 2, and 3 on a chip with an appropriate volume of 50% glycerol solution before loading the used wells. DO NOT add glycerol to the wells in the bottom NO FILL row.
- Avoid contacting the bottom surface of the chip with gloved hands and other surfaces. Frictional charging can lead to inadequate priming of the channels, potentially leading to either clogs or wetting failures.
- Minimize the distance that a loaded chip is moved to reach the Chromium Controller.
- Keep the chip horizontal to prevent wetting the gasket with oil, which depletes the input volume and may adversely affect the quality of the resulting emulsion.

Chromium Next GEM Secondary Holders

Next GEM

- Chromium Next GEM Secondary Holders encase Chromium Next GEM Chips.
- The holder lid flips over to become a stand, holding the chip at 45 degrees for optimal recovery well content removal.
- Squeeze the black sliders on the back side of the holder together to unlock the lid and return the holder to a flat position.



Chromium Next GEM Chip & Holder Assembly

Next GEM

- Align notch on the chip (upper left corner) and the holder.
- Insert the left-hand side of the chip under the guide. Depress the right-hand side of the chip until the spring-loaded clip engages.
- Close the lid before dispensing reagents into the wells.

Chromium Next GEM Chip Loading

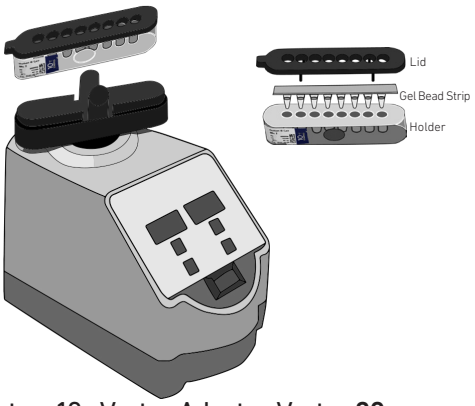
Next GEM

- Place the assembled chip and holder flat on the bench with the lid closed.
- Dispense at the bottom of the wells without introducing bubbles.
- When dispensing Gel Beads into the chip, wait for the remainder to drain into the bottom of the pipette tips and dispense again to ensure complete transfer.
- Refer to Load Chromium Next GEM Chip G for specific instructions.

Gel Bead Handling

Next GEM

- Use one tube of Gel Beads per sample. DO NOT puncture the foil seals of tubes not used at the time.
- Equilibrate the Gel Beads strip to room temperature before use.
- Store unused Gel Beads at –80°C and avoid more than 12 freeze-thaw cycles. DO NOT store Gel Beads at –20°C.



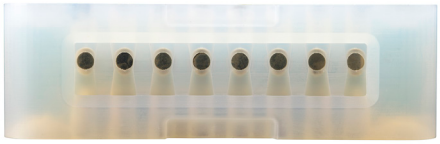
- Snap the tube strip holder with the Gel Bead strip into a 10x Vortex Adapter. Vortex **30 sec**.
- Centrifuge the Gel Bead strip for ~**5 sec** after removing from the holder. Confirm there are no bubbles at the bottom of the tubes and the liquid levels look even. Place the Gel Bead strip back in the holder and secure the holder lid.
- If the required volume of beads cannot be recovered, place the pipette tips against the sidewalls and slowly dispense the Gel Beads back into the tubes. DO NOT introduce bubbles into the tubes and verify that the pipette tips contain no leftover Gel Beads. Withdraw the full volume of beads again by pipetting slowly.

10x Gasket Attachment

- After reagents are loaded, attach the gasket by holding the tongue (curved end, to the right) and hook it on the left-hand tabs of the holder. Gently pull the gasket toward the right and hook it on the two right-hand tabs.
- DO NOT touch the smooth side of the gasket. DO NOT press down on the top of the gasket after attachment.
- Keep the assembly horizontal to avoid wetting the gasket with Partitioning Oil.

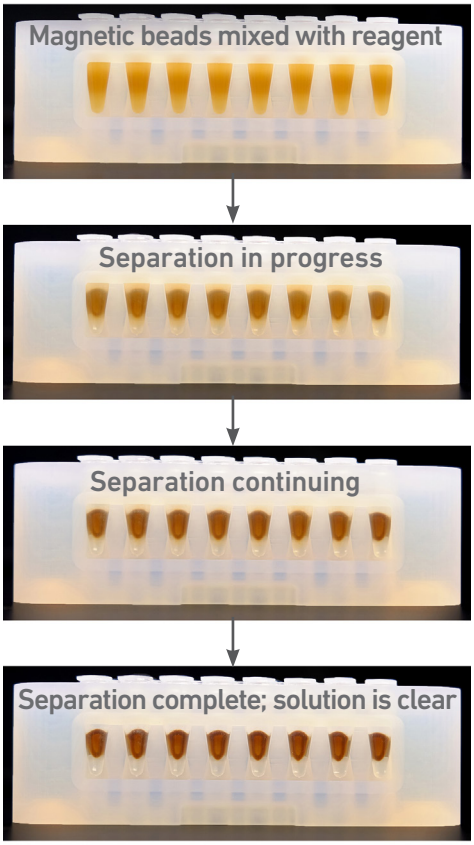
10x Magnetic Separator

- Offers two positions of the magnets (high and low) relative to a tube, depending on its orientation. Flip the magnetic separator over to switch between high (magnet•**High)** or low (magnet•**Low)** positions.
- If using MicroAmp 8-Tube Strips, use the high position (magnet•**High)** only throughout the protocol.



Magnetic Bead Cleanup Steps

- During magnetic bead based cleanup steps that specify waiting "until the solution clears", visually confirm clearing of solution before proceeding to the next step. See adjacent panel for an example.
- The time needed for the solution to clear may vary based on specific step, reagents, volume of reagents etc.



SPRIselect Cleanup & Size Selection

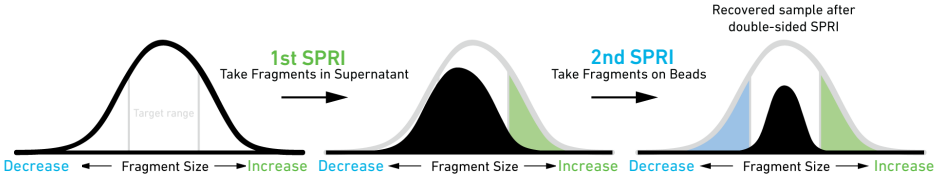
- After aspirating the desired volume of SPRIselect reagent, examine the pipette tips before dispensing to ensure the correct volume is transferred.
- Pipette mix thoroughly as insufficient mixing of sample and SPRIselect reagent will lead to inconsistent results.
- Use fresh preparations of 80% Ethanol.

Tutorial — SPRIselect Reagent:DNA Sample Ratios

SPRI beads selectively bind DNA according to the ratio of SPRIselect reagent (beads).

Example: Ratio = $\frac{\text{Volume of SPRIselect reagent added to the sample}}{\text{Volume of DNA sample}} = \frac{50\ \mu l}{100\ \mu l} = \mathbf{0.5X}$

Schematic of Double Sided Size Selection



After the first SPRI, supernatant is transferred for a second SPRI while larger fragments are discarded (green). After the second SPRI, fragments on beads are eluted and kept while smaller fragments are discarded (blue). Final sample has a tight fragment size distribution with reduced overall amount (black).

Tutorial — Double Sided Size Selection

Step a – First SPRIselect: Add **50 µl** SPRIselect reagent to **100 µl** sample (**0.5X**).

Ratio = $\frac{\text{Volume of SPRIselect reagent added to the sample}}{\text{Volume of DNA sample}} = \frac{50\ \mu l}{100\ \mu l} = \mathbf{0.5X}$

Step b – Second SPRIselect: Add **30 µl** SPRIselect reagent to supernatant from step a (**0.8X**).

Ratio = $\frac{\text{Total Volume of SPRIselect reagent added to the sample (step a + b)}}{\text{Original Volume of DNA sample}} = \frac{50\ \mu l + 30\ \mu l}{100\ \mu l} = \mathbf{0.8X}$

Enzymatic Fragmentation

- Ensure enzymatic fragmentation reactions are prepared on ice and then loaded into a thermal cycler pre-cooled to 4°C prior to initiating the Fragmentation, End Repair, and A-tailing incubation steps.

Sample Indices in Sample Index PCR

- Choose the appropriate sample index sets to ensure that no sample indices overlap in a multiplexed sequencing run.
- Each well in the i7 Sample Index plate contains a unique mix of 4 oligos.
- The sample indexes can therefore be used in any combination.
- Each sample index set is base-balanced to avoid monochromatic signal issues when it is the sole sample loaded on an Illumina sequencer.

Step 1

GEM Generation & Barcoding

1.1 Prepare Single Cell Master Mix

1.2 Load Chromium Next GEM Chip G

1.3 Run the Chromium Controller

1.4 Transfer GEMs

1.5 GEM-RT Incubation

1

1.0 GEM Generation & Barcoding

Next GEM

GET STARTED!					
Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature		**Single Cell 3' v3.1 Gel Beads**	2000164	Equilibrate to room temperature 30 min before loading the chip.	–80°C
	●	**RT Reagent B**	2000165	Vortex, verify no precipitate, centrifuge briefly.	–20°C
	●	**Template Switch Oligo**	3000228	Centrifuge briefly, resuspend in 80 µl Low TE Buffer. Vortex 15 sec at maximum speed, centrifuge briefly, leave at room temperature for ≥ 30 min. After resuspension, store at –80°C.	–20°C
	○	**Reducing Agent B**	2000087	Vortex, verify no precipitate, centrifuge briefly.	–20°C
Place on Ice	●	**RT Enzyme C**	2000085/ 2000102	Centrifuge briefly before adding to the mix.	–20°C
		Cell Suspension			
Obtain	●	**Partitioning Oil**	2000190	-	Ambient
		Chromium Next GEM Chip G	2000177	-	Ambient
		10x Gasket	370017/ 3000072	See Tips & Best Practices.	Ambient
		Chromium Next GEM Secondary Holder	3000332	See Tips & Best Practices.	Ambient
		10x Vortex Adapter	330002	See Tips & Best Practices.	Ambient
		50% glycerol solution If using <8 reactions	-	See Tips & Best Practices.	-

Firmware Version 4.0 or higher is required in the Chromium Controller or the Chromium Single Cell Controller used for this Single Cell 3' v3.1 protocol.

1.1 Prepare Master Mix

Next GEM

a. Prepare Master Mix on ice. Pipette mix 15x and centrifuge briefly.

Master Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
● **RT Reagent B**	2000165	18.8	82.2	165.0
● **Template Switch Oligo**	3000228	2.4	10.4	20.8
○ **Reducing Agent B**	2000087	2.0	8.6	17.3
● **RT Enzyme C**	2000085/ 2000102	8.7	38.4	76.8
Total	-	**31.8**	**139.9**	**279.8**

b. Add **31.8 µl** Master Mix into each tube of a PCR 8-tube strip on ice.

Assemble Chromium Next GEM Chip G

After removing the chip from the sealed bag, use the chip in ≤ 24 h.

TIPS See Tips & Best Practices for chip handling instructions.

- Align notch on the chip (upper left corner) and the holder.
- Insert the left-hand side of the chip under the guide. Depress the right-hand side of the chip until the spring-loaded clip engages.
- Close the lid before dispensing reagents into the wells.
- The assembled chip is ready for loading the indicated reagents. Refer to step 1.2 for reagent volumes and loading order.

For GEM generation, load the indicated reagents only in the specified rows, starting from row labeled 1, followed by rows labeled 2 and 3. DO NOT load reagents in the bottom row labeled NO FILL. See step 1.2 for details.

Cell Suspension Volume Calculator Table

(for step 1.2 of Chromium Next GEM Single Cell 3' v3.1 protocol)

Volume of Cell Suspension Stock per reaction (µl) | Volume of Nuclease-free Water per reaction (µl)

Cell Stock Concentration (Cells/µl)	Targeted Cell Recovery										
	500	1000	2000	3000	4000	5000	6000	7000	8000	9000	10000
100	8.3 35.0	16.5 26.7	33.0 10.2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
200	4.1 39.1	8.3 35.0	16.5 26.7	24.8 18.5	33.0 10.2	41.3 2.0	n/a	n/a	n/a	n/a	n/a
300	2.8 40.5	5.5 37.7	11.0 32.2	16.5 26.7	22.0 21.2	27.5 15.7	33.0 10.2	38.5 4.7	44.0 -0.8	n/a	n/a
400	2.1 41.1	4.1 39.1	8.3 35.0	12.4 30.8	16.5 26.7	20.6 22.6	24.8 18.5	28.9 14.3	33.0 10.2	37.1 6.1	41.3 2.0
500	1.7 41.6	3.3 39.9	6.6 36.6	9.9 33.3	13.2 30.0	16.5 26.7	19.8 23.4	23.1 20.1	26.4 16.8	29.7 13.5	33.0 10.2
600	1.4 41.8	2.8 40.5	5.5 37.7	8.3 35.0	11.0 32.2	13.8 29.5	16.5 26.7	19.3 24.0	22.0 21.2	24.8 18.5	27.5 15.7
700	1.2 42.0	2.4 40.8	4.7 38.5	7.1 36.1	9.4 33.8	11.8 31.4	14.1 29.1	16.5 26.7	18.9 24.3	21.2 22.0	23.6 19.6
800	1.0 42.2	2.1 41.1	4.1 39.1	6.2 37.0	8.3 35.0	10.3 32.9	12.4 30.8	14.4 28.8	16.5 26.7	18.6 24.6	20.6 22.6
900	0.9 42.3	1.8 41.4	3.7 39.5	5.5 37.7	7.3 35.9	9.2 34.0	11.0 32.2	12.8 30.4	14.7 28.5	16.5 26.7	18.3 24.9
1000	0.8 42.4	1.7 41.6	3.3 39.9	5.0 38.3	6.6 36.6	8.3 35.0	9.9 33.3	11.6 31.7	13.2 30.0	14.9 28.4	16.5 26.7
1100	0.8 42.5	1.5 41.7	3.0 40.2	4.5 38.7	6.0 37.2	7.5 35.7	9.0 34.2	10.5 32.7	12.0 31.2	13.5 29.7	15.0 28.2
1200	0.7 42.5	1.4 41.8	2.8 40.5	4.1 39.1	5.5 37.7	6.9 36.3	8.3 35.0	9.6 33.6	11.0 32.2	12.4 30.8	13.8 29.5
1300	0.6 42.6	1.3 41.9	2.5 40.7	3.8 39.4	5.1 38.1	6.3 36.9	7.6 35.6	8.9 34.3	10.2 33.0	11.4 31.8	12.7 30.5
1400	0.6 42.6	1.2 42.0	2.4 40.8	3.5 39.7	4.7 38.5	5.9 37.3	7.1 36.1	8.3 35.0	9.4 33.8	10.6 32.6	11.8 31.4
1500	0.6 42.7	1.1 42.1	2.2 41.0	3.3 39.9	4.4 38.8	5.5 37.7	6.6 36.6	7.7 35.5	8.8 34.4	9.9 33.3	11.0 32.2
1600	0.5 42.7	1.0 42.2	2.1 41.1	3.1 40.1	4.1 39.1	5.2 38.0	6.2 37.0	7.2 36.0	8.3 35.0	9.3 33.9	10.3 32.9
1700	0.5 42.7	1.0 42.2	1.9 41.3	2.9 40.3	3.9 39.3	4.9 38.3	5.8 37.4	6.8 36.4	7.8 35.4	8.7 34.5	9.7 33.5
1800	0.5 42.7	0.9 42.3	1.8 41.4	2.8 40.5	3.7 39.5	4.6 38.6	5.5 37.7	6.4 36.8	7.3 35.9	8.3 35.0	9.2 34.0
1900	0.4 42.8	0.9 42.3	1.7 41.5	2.6 40.6	3.5 39.7	4.3 38.9	5.2 38.0	6.1 37.1	6.9 36.3	7.8 35.4	8.7 34.5
2000	0.4 42.8	0.8 42.4	1.7 41.6	2.5 40.7	3.3 39.9	4.1 39.1	5.0 38.3	5.8 37.4	6.6 36.6	7.4 35.8	8.3 35.0

Grey boxes: Volumes that would exceed the allowable water volume in each reaction
Yellow boxes: Indicate a low transfer volume that may result in higher cell load variability
Blue boxes: Optimal range of cell stock concentration to maximize the likelihood of achieving the desired cell recovery target

1.2 Load Chromium Next GEM Chip G

Next GEM

After removing the chip from the sealed bag, use in ≤ 24 h. When **loading the chip,** raising and depressing the pipette plunger should each take ~5 sec. When dispensing, raise the pipette tips at the same rate as the liquid is rising, keeping the tips slightly submerged.

a. Dispense 50% Glycerol into Unused Chip Wells (if < 8 samples per chip)

i. **70 µl** to unused wells in **row labeled 1**.
ii. **50 µl** to unused wells in **row labeled 2**.
iii. **45 µl** to unused wells in **row labeled 3**.

DO NOT add 50% glycerol solution to the bottom row of NO FILL wells. DO NOT use any substitute for 50% glycerol solution.

b. Prepare Master Mix + Cell Suspension

Refer to the Cell Suspension Volume Calculator Table. Add the appropriate volume of nuclease-free water and corresponding volume of single cell suspension to Master Mix for a total of **75 µl** in each tube. Gently pipette mix the cells suspension before adding to the Master Mix.

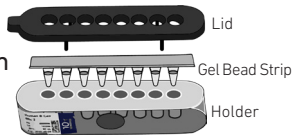
c. Load Row Labeled 1

Gently pipette mix the Master Mix + Cell Suspension and using the same pipette tip, dispense **70 µl** Master Mix + Cell Suspension into the bottom center of each well in **row labeled 1** without introducing bubbles.

The illustrated chip is being loaded for 8 samples.

d. Prepare Gel Beads

Snap the tube strip holder with the Gel Bead strip into a 10x Vortex Adapter. Vortex **30 sec.** Centrifuge the Gel Bead strip for ~**5 sec.** Confirm there are no bubbles at the bottom of the tubes and the liquid levels are even. Place the Gel Bead strip back in the holder. Secure the holder lid.

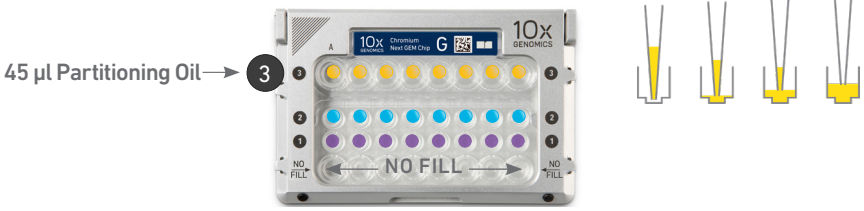


e. Load Row Labeled 2

Puncture the foil seal of the Gel Bead tubes. Slowly aspirate **50 µl** Gel Beads. Dispense into the wells in **row labeled 2** without introducing bubbles. Wait **30 sec**.

f. Load Row Labeled 3

Dispense **45 µl** Partitioning Oil into the wells in **row labeled 3** from a reagent reservoir. Failure to add Partitioning Oil to the top row labeled 3 will prevent GEM generation and can damage the Chromium Controller.



Attach the gasket and run the chip in the Chromium Controller **immediately** after loading the Partitioning Oil.

g. Attach 10x Gasket

Align the notch with the top left-hand corner. Ensure the gasket holes are aligned with the wells. Avoid touching the smooth surface.

Keep horizontal to avoid wetting the gasket. DO NOT press down on the gasket.

1.3 Run the Chromium Controller

Next GEM

a. Press the eject button on the Controller to eject the tray.

b. Place the assembled chip with the gasket in the tray, ensuring that the chip stays horizontal. Press the button to retract the tray.

c. Confirm the Chromium Chip G program on screen. Press the play button.

d. At completion of the run **(~18 min)**, the Controller will chime. **Immediately** proceed to the next step.

Firmware Version 4.0 or higher is required in the Chromium Controller or the Chromium Single Cell Controller used for the Single Cell 3' v3.1 protocol.

1.4 Transfer GEMs

Next GEM

a. Place a tube strip on ice.

b. Press the eject button of the Controller and remove the chip.

c. Discard the gasket. Open the chip holder. Fold the lid back until it clicks to expose the wells at 45 degrees.

d. Check the volume in rows labeled 1-2. Abnormally high volume in any well indicates a clog.

e. Slowly aspirate **100 µl** GEMs from the lowest points of the recovery wells in the top row labeled 3 without creating a seal between the tips and the bottom of the wells.

f. Withdraw pipette tips from the wells. GEMs should appear opaque and uniform across all channels. Excess Partitioning Oil (clear) in the pipette tips indicates a potential clog.

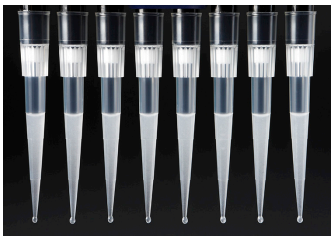
g. Over the course of **~20 sec**, dispense GEMs into the tube strip on ice with the pipette tips against the sidewalls of the tubes.

h. If multiple chips are run back-to-back, cap/cover the GEM-containing tube strip and place on ice for no more than **1 h**.

Expose Wells at 45 Degrees

Transfer GEMs

GEMs



1.5 GEM-RT Incubation

Use a thermal cycler that can accommodate at least 100 µl volume. A volume of 125 µl is the preferred setting on Bio-Rad C1000 Touch. In alternate thermal cyclers, use highest reaction volume setting.

a. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
53°C	125 µl	~55 min
Step	**Temperature**	**Time**
1	53°C	00:45:00
2	85°C	00:05:00
3	4°C	Hold

b. Store at **4°C** for up to **72 h** or at **–20°C** for up to a **week**, or proceed to the next step.

Step 2

Post GEM–RT Cleanup & cDNA Amplification

2.0 Post GEM-RT Cleanup & cDNA Amplification

GET STARTED!					
Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature		**Reducing Agent B**	2000087	Thaw, vortex, verify no precipitate, centrifuge.	–20°C
	●	**cDNA Primers**	2000089	Vortex, centrifuge briefly.	–20°C
		Dynabeads MyOne SILANE	2000048	Vortex thoroughly (≥30 sec) **immediately** before adding to the mix.	4°C
		Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations.	-
		Agilent Bioanalyzer High Sensitivity Kit If used for QC and quantification	-	Manufacturer's recommendations.	-
		Agilent TapeStation ScreenTape and Reagents If used for QC and quantification	-	Manufacturer's recommendations.	-
		Qubit dsDNA HS Assay Kit If used for QC and quantification	-	Manufacturer's recommendations.	-
Place on ice	○	**Amp Mix**	2000047/ 2000103	Vortex, centrifuge briefly.	–20°C
Thaw at 65°C	●	**Cleanup Buffer**	2000088	Thaw for 10 min at 65°C at max speed on a thermomixer. Verify no visible crystals. Cool to room temperature.	–20°C
Obtain	○	**Recovery Agent**	220016	-	Ambient
		Qiagen Buffer EB	-	Manufacturer's recommendations.	-
		Bio-Rad 10% Tween 20	-	Manufacturer's recommendations.	-
		10x Magnetic Separator	230003	-	Ambient
		Prepare 80% Ethanol Prepare 15 ml for 8 reactions.	-	-	-

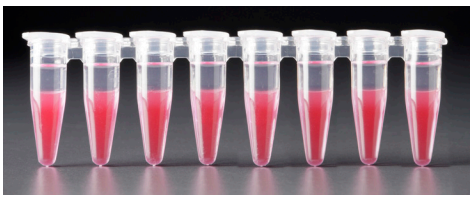
2.1 Post GEM-RT Cleanup – Dynabeads

Next GEM

a. Add **125 µl** Recovery Agent to each sample at room temperature. DO NOT pipette mix or vortex the biphasic mixture. Wait **2 min**.

The resulting biphasic mixture contains Recovery Agent/Partitioning Oil (pink) and aqueous phase (clear), with no persisting emulsion (opaque).

Biphasic Mixture

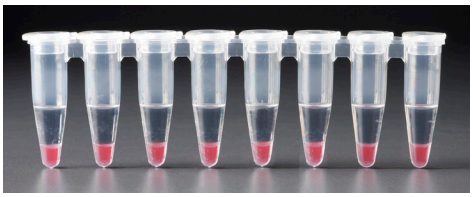


If biphasic separation is incomplete:
Firmly secure the cap on the tube strip, ensuring that no liquid is trapped between the cap and the tube rim. Mix by inverting the capped tube strip 5x, centrifuge briefly, and proceed to step b. DO NOT invert without firmly securing the caps.

A smaller aqueous phase volume indicates a clog during GEM generation.

b. Slowly remove and discard **125 µl** Recovery Agent/Partitioning Oil (pink) from the bottom of the tube. DO NOT aspirate any aqueous sample.

Remove Recovery Agent



c. Prepare Dynabeads Cleanup Mix.

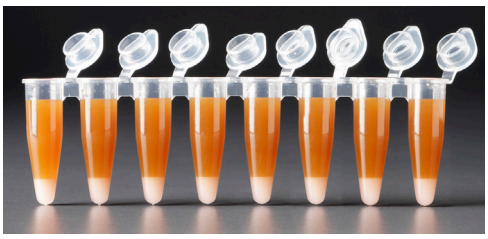
Dynabeads Cleanup Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Cleanup Buffer	2000088	182	801	1602
Dynabeads MyOne SILANE Vortex thoroughly (≥**30 sec**) **immediately** before adding to the mix. Aspirate the full liquid volume with a pipette tip to verify that the beads have not settled in the bottom of the tube. If clumps are present, pipette mix to resuspend completely. DO NOT centrifuge before use.	2000048	8	35	70
Reducing Agent B	2000087	5	22	44
Nuclease-free Water		5	22	44
Total	-	**200**	**880**	**1760**

Resuspend clump

d. Vortex and add **200 µl** to each sample. Pipette mix 10x (pipette set to 200 µl).

e. Incubate **10 min** at **room temperature**. Pipette mix again at **~5 min** after start of incubation to resuspend settled beads.

Add Dynabeads Cleanup Mix



f. Prepare Elution Solution I. Vortex and centrifuge briefly.

	Elution Solution I *Add reagents in the order listed*	PN	1X (µl)	10X (µl)
	Buffer EB	-	98	980
	10% Tween 20	-	1	10
◯	**Reducing Agent B**	2000087	1	10
	Total	-	**100**	**1000**

TIPS

g. At the end of **10 min** incubation, place on a 10x Magnetic Separator•**High** position (magnet•**High**) until the solution clears.

A white interface between the aqueous phase and Recovery Agent is normal.

h. Remove the supernatant.

i. Add **300 µl** 80% ethanol to the pellet while on the magnet. Wait **30 sec**.

j. Remove the ethanol.

k. Add **200 µl** 80% ethanol to pellet. Wait **30 sec**.

l. Remove the ethanol.

m. Centrifuge briefly. Place on the magnet•**Low**.

n. Remove remaining ethanol. Air dry for **1 min**.

o. Remove from the magnet. Immediately add **35.5 µl** Elution Solution I.

p. Pipette mix (pipette set to 30 µl) without introducing bubbles.

q. Incubate **2 min** at **room temperature**.

r. Place on the magnet•**Low** until the solution clears.

s. Transfer **35 µl** sample to a new tube strip.

2.2 cDNA Amplification

a. Prepare cDNA Amplification Mix on ice. Vortex and centrifuge briefly.

cDNA Amplification Reaction Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
○ **Amp Mix**	2000047/ 2000103	50	220	440
● **cDNA Primers**	2000089	15	66	132
Total	-	**65**	**286**	**572**

b. Add **65 µl** cDNA Amplification Reaction Mix to **35 µl** sample.

c. Pipette mix 15x (pipette set to 90 µl). Centrifuge briefly.

d. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~30-45 min
Step	**Temperature**	**Time**
1	98°C	00:03:00
2	98°C	00:00:15
3	63°C	00:00:20
4	72°C	00:01:00
5	Go to Step 2, see table below for total # of cycles	
6	72°C	00:01:00
7	4°C	Hold

The optimal number of cycles is a trade-off between generating sufficient final mass for library construction and minimizing PCR amplification artifacts. The number of cDNA cycles should also be reduced if large numbers of cells are sampled.

Recommended starting point for cycle number optimization.

Targeted Cell Recovery	Total Cycles
<500	13
500–6,000	12
>6,000	11

STOP

e. Store at **4°C** for up to **72 h** or or **–20°C** for **≤1 week**, or proceed to the next step.

2.3 cDNA Cleanup – SPRIselect

a. Vortex to resuspend the SPRIselect reagent. Add **60 µl** SPRIselect reagent **(0.6X)** to each sample and pipette mix 15x (pipette set to 150 µl).

b. Incubate **5 min** at **room temperature**.

c. Place on the magnet•**High** until the solution clears.

d. Remove the supernatant.

e. Add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

f. Remove the ethanol.

g. **Repeat** steps e and f for a total of 2 washes.

h. Centrifuge briefly and place on the magnet•**Low**.

i. Remove any remaining ethanol. Air dry for **2 min**. DO NOT exceed **2 min** as this will decrease elution efficiency.

j. Remove from the magnet. Add **40.5 µl** Buffer EB. Pipette mix 15x.

k. Incubate **2 min** at **room temperature**.

l. Place the tube strip on the magnet•**High** until the solution clears.

m. Transfer **40 µl** sample to a new tube strip.

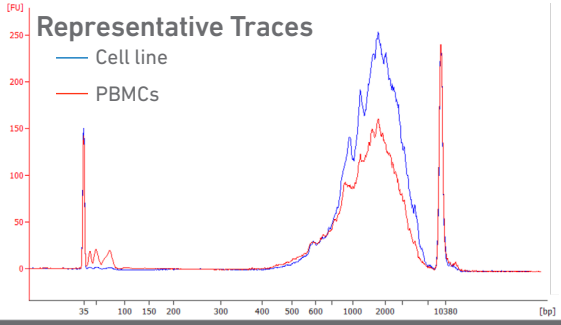
STOP

n. Store at **4°C** for up to **72 h** or at **–20°C** for up to **4 weeks**, or proceed to the next step.

2.4 cDNA QC & Quantification

a. Run **1 µl** of sample (Dilution Factor 1:10) on an Agilent Bioanalyzer High Sensitivity chip.

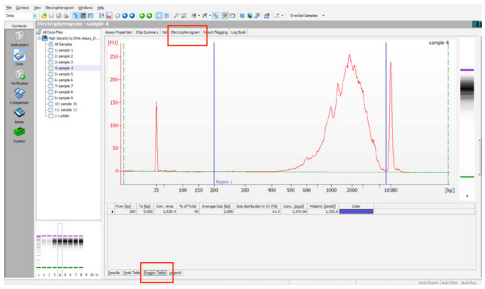
For input cells with low RNA content (<1pg total RNA/cell), 1 µl undiluted product may be run.
Lower molecular weight product (35 – 150 bp) may be present. This is normal and does not affect sequencing or application performance.



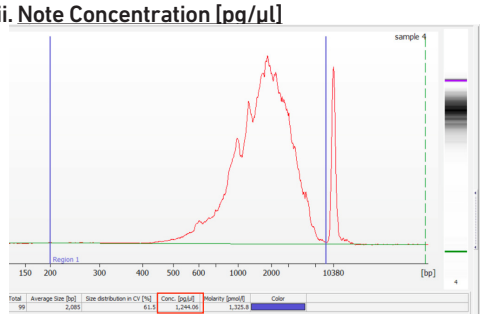
EXAMPLE CALCULATION

i. Select Region

Under the "Electropherogram" view choose the "Region Table". Manually select the region of ~200 – ~9000 bp



ii. Note Concentration [pg/µl]



iii. Calculate

Multiply the cDNA concentration [pg/µl] reported via the Agilent 2100 Expert Software by the elution volume (40 µl) of the Post cDNA Amplification Reaction Clean Up sample (taking any dilution factors into account) and then divide by 1000 to obtain the total cDNA yield in ng.

Example Calculation of cDNA Total Yield

Concentration: 1244.06 pg/µl
Elution Volume: 40
Dilution Factor: 10

Total cDNA Yield

$$= \frac{\text{Conc'n (pg/µl)} \times \text{Elution Volume (µl)} \times \text{Dilution Factor}}{1000 \text{ (pg/ng)}}$$

$$= \frac{1244.06 \text{ (pg/µl)} \times 40 \text{ (µl)} \times 10}{1000 \text{ (pg/ng)}} = 497.62 \text{ ng}$$

Carry forward **ONLY 25%** of total cDNA yield into 3' Gene Expression Library Construction (step 3)

= 0.25 x Total cDNA yield

= 0.25 x 497.62= 124.4ng

Refer to step 3.5 for appropriate number of Sample Index PCR cycles based on carry forward cDNA yield/input cDNA.

Alternate Quantification Methods:

- Agilent TapeStation. See Appendix for representative traces

Agilent Bioanalyzer or Agilent TapeStation are the recommended methods for accurate quantification.

(If using Qubit Fluorometer and Qubit dsDNA HS Assay Kit, see Appendix)

Step 3

3' Gene Expression Library Construction

3.0 3' Gene Expression Library Construction

GET STARTED!

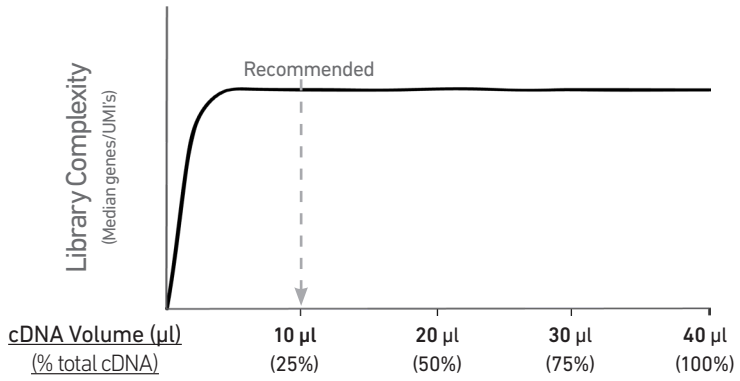
Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	●	**Fragmentation Buffer**	2000091	Vortex, verify no precipitate, centrifuge briefly.	–20°C
	●	**Adaptor Oligos**	2000094	Vortex, centrifuge briefly.	–20°C
	●	**Ligation Buffer**	2000092	Vortex, verify no precipitate, centrifuge briefly.	–20°C
	●	**SI Primer**	2000095	-	–20°C
		Chromium i7 Sample Index Plate	220103	-	–20°C
		Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations.	-
		Agilent TapeStation Screen Tape and Reagents If used for QC		Manufacturer's recommendations.	
		Agilent Bioanalyzer High Sensitivity kit	-	Manufacturer's recommendations.	-
Place on Ice	●	**Fragmentation Enzyme**	2000090/ 2000104	Centrifuge briefly.	–20°C
	●	**DNA Ligase**	220110/ 220131	Centrifuge briefly.	–20°C
	○	**Amp Mix**	2000047/ 2000103	Centrifuge briefly.	–20°C
		KAPA Library Quantification Kit for Illumina Platforms	-	Manufacturer's recommendations.	-
Obtain		**Qiagen Buffer EB**	-	-	Ambient
		10x Magnetic Separator	230003	See Tips & Best Practices.	Ambient
		Prepare 80% Ethanol Prepare 20 ml for 8 reactions	-	Prepare fresh.	Ambient

Step Overview (Step 3.1d)

Correlation between input & library complexity

A Single Cell 3' Gene Expression library is generated using a fixed proportion (10 µl, 25%) of the total cDNA (40 µl) obtained at step 2.3m. The complexity of this library will be comparable to one generated using a higher proportion (>25%) of the cDNA. The remaining proportion (30 µl, 75%) of the cDNA may be stored at **4°C** for up to **72 h** or at **–20°C** for longer-term storage (up to **4 weeks**).

Correlation: cDNA input & Library Complexity



Note that irrespective of the total cDNA yield (ng), which may vary based on cell type, targeted cell recovery etc., this protocol has been optimized for a broad range of input mass (ng), as shown in the example below. The total number of SI PCR cycles (step 3.5e) should be optimized based on carrying forward a fixed proportion (10 µl, 25%) of the total cDNA yield calculated during Post cDNA Amplification QC & Quantification (step 2.4).

Example: Library Construction Input Mass & SI PCR Cycles

Cell Type	Targeted Cell Recovery	Total cDNA Yield (ng)	cDNA Input into Fragmentation		SI PCR Cycle Number
			Volume (µl)	Mass (ng)	
High RNA Content	Low	250 ng	10 µl	62.5 ng	13
	High	1900 ng	10 µl	475 ng	10
Low RNA Content	Low	1 ng	10 µl	0.25 ng	16
	High	200 ng	10 µl	50 ng	12

3.1 Fragmentation, End Repair & A-tailing

a. Prepare a thermal cycler with the following incubation protocol.

Lid Temperature	Reaction Volume	Run Time
65°C	50 µl	~35 min
Step	**Temperature**	**Time**
Pre-cool block Pre-cool block prior to preparing the Fragmentation Mix	4°C	Hold
Fragmentation	32°C	00:05:00
End Repair & A-tailing	65°C	00:30:00
Hold	4°C	Hold

b. Vortex Fragmentation Buffer. Verify there is no precipitate.

c. Prepare Fragmentation Mix on ice. Pipette mix and centrifuge briefly.

Fragmentation Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Fragmentation Buffer	2000091	5	22	44
Fragmentation Enzyme	2000090/ 2000104	10	44	88
Total	-	**15**	**66**	**132**

d. Transfer **ONLY 10 µl** purified cDNA sample from cDNA Cleanup (step 2.3n) to a tube strip.
Note that only **10 µl** (25%) cDNA sample is sufficient for generating 3' Gene Expression library. The remaining **30 µl** (75%) cDNA sample can be stored at **4°C** for up to **72 h** or at **–20°C** for up to **4 weeks** for generating additional 3' Gene Expression libraries.

e. Add **25 µl** Buffer EB to each sample.

f. Add **15 µl** Fragmentation Mix to each sample.

g. Pipette mix 15x (pipette set to 35 µl) on ice. Centrifuge briefly.

h. Transfer into the pre-cooled thermal cycler **(4°C)** and press "SKIP" to initiate the protocol.

3.2 Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect

a. Vortex to resuspend SPRIselect reagent. Add **30 µl** SPRIselect **(0.6X)** reagent to each sample. Pipette mix 15x (pipette set to 75 µl).

b. Incubate **5 min** at **room temperature**.

c. Place on the magnet•**High** until the solution clears. DO NOT discard supernatant.

d. Transfer 75 **µl** supernatant to a new tube strip.

e. Vortex to resuspend SPRIselect reagent. Add **10 µl** SPRIselect reagent **(0.8X)** to each sample. Pipette mix 15x (pipette set to 80 µl).

f. Incubate **5 min** at **room temperature**.

g. Place on the magnet•**High** until the solution clears.

h. Remove **80 µl** supernatant. DO NOT discard any beads.

i. Add **125 µl** 80% ethanol to the pellet. Wait **30 sec.**

j. Remove the ethanol.

k. **Repeat** steps i and j for a total of 2 washes.

l. Centrifuge briefly. Place on the magnet•**Low** until the solution clears. Remove remaining ethanol. DO NOT over dry to ensure maximum elution efficiency.

m. Remove from the magnet. Add **50.5 µl** Buffer EB to each sample. Pipette mix 15x.

n. Incubate **2 min** at **room temperature**.

o. Place on the magnet•**High** until the solution clears.

p. Transfer **50 µl** sample to a new tube strip.

3.3 Adaptor Ligation

a. Prepare Adaptor Ligation Mix. Pipette mix and centrifuge briefly.

Adaptor Ligation Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Ligation Buffer	2000092	20	88	176
DNA Ligase	220110/ 220131	10	44	88
Adaptor Oligos	2000094	20	88	176
Total	-	**50**	**220**	**440**

b. Add **50 µl** Adaptor Ligation Mix to **50 µl** sample. Pipette mix 15x (pipette set to 90 µl). Centrifuge briefly.

c. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
30°C	100 µl	15 min
Step	**Temperature**	**Time**
1	20°C	00:15:00
2	4°C	Hold

3.4 Post Ligation Cleanup – SPRIselect

a. Vortex to resuspend SPRIselect Reagent. Add **80 µl** SPRIselect Reagent (**0.8X**) to each sample. Pipette mix 15x (pipette set to 150 µl).

b. Incubate **5 min** at **room temperature**.

c. Place on the magnet•**High** until the solution clears.

d. Remove the supernatant.

e. Add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

f. Remove the ethanol.

g. **Repeat** steps e and f for a total of 2 washes.

h. Centrifuge briefly. Place on the magnet•**Low**.

i. Remove any remaining ethanol. Air dry for **2 min**. DO NOT exceed **2 min** as this will decrease elution efficiency.

j. Remove from the magnet. Add **30.5 µl** Buffer EB. Pipette mix 15x.

k. Incubate **2 min** at **room temperature**.

l. Place on the magnet•**Low** until the solution clears.

m. Transfer **30 µl** sample to a new tube strip.

3.5 Sample Index PCR

a. Choose the appropriate sample index sets to ensure that no sample indices overlap in a multiplexed sequencing run. Record the 10x Sample Index name (PN-220103 Chromium i7 Sample Index Plate well ID) used.

b. Prepare Sample Index PCR Mix.

Sample Index PCR Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
○ **Amp Mix**	2000047/ 2000103	50	220	440
● **SI Primer**	2000095	10	44	88
Total	-	**60**	**264**	**528**

c. Add **60 µl** Sample Index PCR Mix to **30 µl** sample.

d. Add **10 µl** of an individual Chromium i7 Sample Index to each well and record the well ID used. Pipette mix 5x (pipette set to 90 µl). Centrifuge briefly.

e. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~25-40 min
Step	**Temperature**	**Time**
1	98°C	00:00:45
2	98°C	00:00:20
3	54°C	00:00:30
4	72°C	00:00:20
5	Go to step 2, see below for # of cycles	
6	72°C	00:01:00
7	4°C	Hold

The total cycles should be optimized based on 25% carry forward cDNA yield/input calculated during Post cDNA Amplification QC & Quantification (step 2.4)

Recommended cycle numbers

cDNA Input	Total Cycles
0.25-25 ng	14-16
25-150 ng	12-14
150-500 ng	10-12
500-1,000 ng	8-10
1,000-1,500 ng	6-8
>1500 ng	5

STOP

f. Store at **4°C** for up to **72 h** or proceed to the next step.

3.6 Post Sample Index PCR Double Sided Size Selection – SPRIselect

a. Vortex to resuspend the SPRIselect reagent. Add **60 µl** SPRIselect Reagent **(0.6X)** to each sample. Pipette mix 15x (pipette set to 150 µl).

b. Incubate **5 min** at **room temperature**.

c. Place the magnet•**High** until the solution clears. DO NOT discard supernatant.

d. Transfer **150 µl** supernatant to a new tube strip.

e. Vortex to resuspend the SPRIselect reagent. Add **20 µl** SPRIselect Reagent **(0.8X)** to each sample. Pipette mix 15x (pipette set to 150 µl).

f. Incubate **5 min** at **room temperature**.

g. Place the magnet•**High** until the solution clears.

h. Remove **165 µl** supernatant. DO NOT discard any beads.

i. With the tube still in the magnet, add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

j. Remove the ethanol.

k. **Repeat** steps i and j for a total of 2 washes.

l. Centrifuge briefly. Place on the magnet•**Low**. Remove remaining ethanol.

m. Remove from the magnet. Add **35.5 µl** Buffer EB. Pipette mix 15x.

n. Incubate **2 min** at **room temperature**.

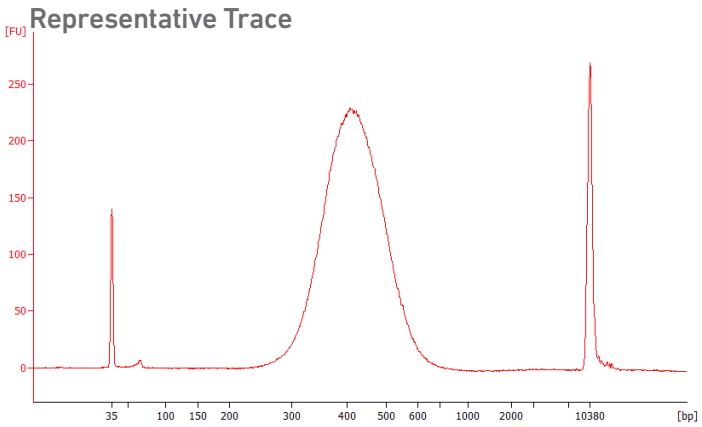
o. Place on the magnet•**Low** until the solution clears.

p. Transfer **35 µl** to a new tube strip.

q. Store at **4°C** for up to **72 h** or at **–20°C** for **long-term** storage.

3.7 Post Library Construction QC

Run **1 µl s**ample at **1:10 dilution** on an Agilent Bioanalyzer High Sensitivity chip.



Determine the average fragment size from the Bioanalyzer trace. This will be used as the insert size for library quantification.

Alternate QC Method:

- Agilent TapeStation. See Appendix for representative traces

See Appendix for Post Library Construction Quantification

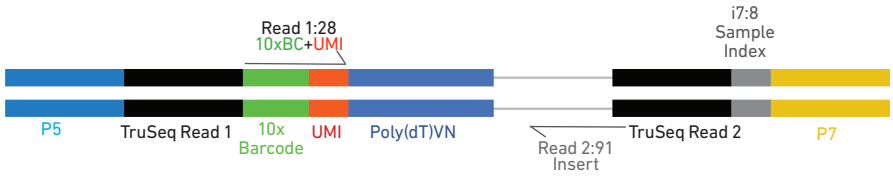
Sequencing

4

Sequencing Libraries

Single Cell 3' Gene Expression libraries comprise standard Illumina paired-end constructs which begin with P5 and end with P7. 16 bp 10x Barcodes are encoded at the start of TruSeq Read 1, while 8 bp sample index sequences are incorporated as the i7 index read. TruSeq Read 1 and Read 2 are standard Illumina sequencing primer sites used in paired-end sequencing. TruSeq Read 1 is used to sequence 16 bp 10x Barcodes and 12 bp UMI. Sequencing these libraries produce a standard Illumina BCL data output folder.

Chromium Single Cell 3' Gene Expression Library



Illumina Sequencer Compatibility

The compatibility of the listed sequencers has been verified by 10x Genomics. Some variation in assay performance is expected based on sequencer choice. For more information about performance variation, visit the 10x Genomics Support website.

- MiSeq
- NextSeq 500/550*
- HiSeq 2500 (Rapid Run)
- HiSeq 3000/4000
- NovaSeq

*Sequencing Chromium Single Cell libraries on the NextSeq 500/550 platform may yield reduced sequence quality and sensitivity relative to the MiSeq, HiSeq, and NovaSeq platforms. Refer to the 10x Genomics Support website for more information.

Sample Indices

Each sample index in the Chromium i7 Sample Index Kit (PN-120262) is a mix of 4 different sequences to balance across all 4 nucleotides. If multiple samples are pooled in a sequence lane, the sample index name (i.e. the Chromium i7 Sample Index plate well ID, SI-GA-) is needed in the sample sheet used for generating FASTQs with "cellranger mkfastq". Samples utilizing the same sample index should not be pooled together, or run on the same flow cell lane, as this would not enable correct sample demultiplexing.

3' Gene Expression Library Sequencing Depth & Run Parameters

Sequencing Depth	Minimum 20,000 read pairs per cell
Sequencing Type	Paired-end, single indexing
Sequencing Read	Recommended Number of Cycles
Read 1	28 cycles
i7 Index	8 cycles
i5 Index	0 cycles
Read 2	91 cycles

Library Loading

Once quantified and normalized, the 3' Gene Expression libraries should be denatured and diluted as recommended for Illumina sequencing platforms. Refer to Illumina documentation for denaturing and diluting libraries. Refer to the 10x Genomics Support website, for more information.

Instrument	Loading Concentration (pM)	PhiX (%)
MiSeq	11	1
NextSeq 500/550	1.8	1
HiSeq 2500 (RR)	11	1
HiSeq 4000	240	1
NovaSeq	150*/300	1

* Use 150 pM loading concentration for Illumina XP workflow.

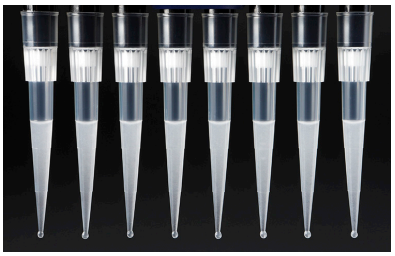
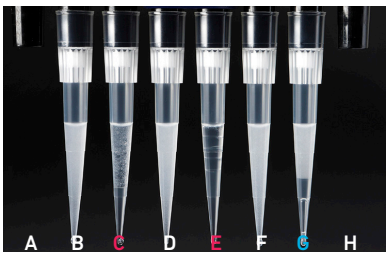
Library Pooling

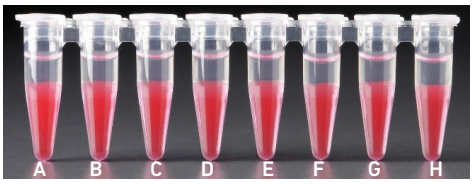
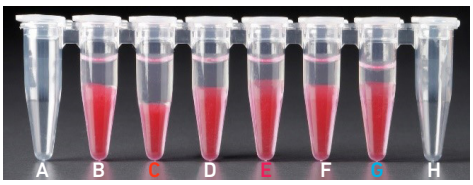
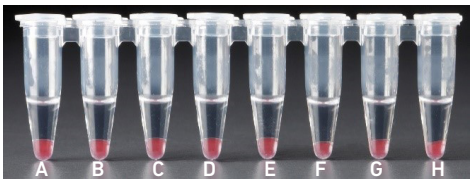
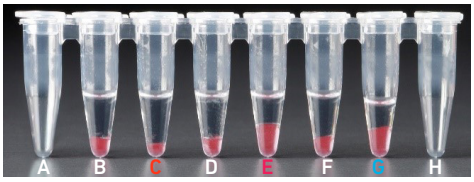
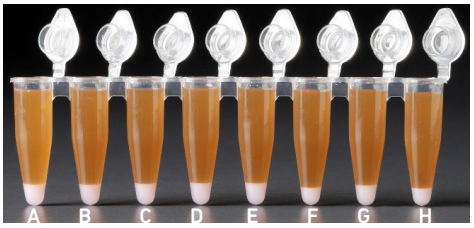
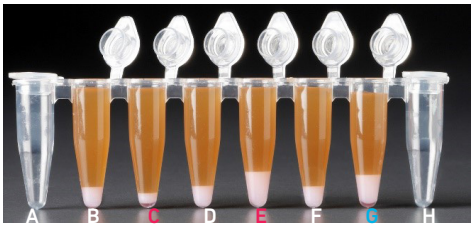
The 3' Gene Expression libraries maybe pooled for sequencing, taking into account the differences in cell number and per-cell read depth requirements between each library. Samples utilizing the same sample index should not be pooled together, or run on the same flow cell lane, as this would not enable correct sample demultiplexing.

Troubleshooting

5

GEMs

STEP	NORMAL	REAGENT CLOGS & WETTING FAILURES
1.4 d After Chip G is removed from the Controller and the wells are exposed	 All 8 recovery wells are similar in volume and opacity.	 Recovery well G indicates a reagent clog. Recovery well C and E indicate a wetting failure. Recovery wells B, D, and F are normal. Wells A and H contain 50% Glycerol Solution.
1.4 f Transfer GEMs from Chip G Row Labeled 3	 All liquid levels are similar in volume and opacity without air trapped in the pipette tips.	 Pipette tips C and E indicate a wetting failure. Pipette tip C contains partially emulsified GEMs. Emulsion is absent in pipette tip E. Pipette tip G indicates a reagent clog.

STEP	NORMAL	REAGENT CLOGS & WETTING FAILURES
2.1 a After transfer of the GEMs + Recovery Agent	 All liquid levels are similar in the aqueous sample volume (clear) and Recovery Agent/Partitioning Oil (pink).	 Tube G indicates a reagent clog has occurred. There is a decreased volume of aqueous layer (clear). Tube C and E indicate a wetting failure has occurred. There is an abnormal volume of Recovery Agent/Partitioning Oil (pink).
2.1 b After aspiration of Recovery Agent/ Partitioning Oil	 All liquid volumes are similar in the aqueous sample volume (clear) and residual Recovery Agent/Partitioning Oil (pink).	 Tube G indicates a reagent clog has occurred. There is a decreased volume of aqueous layer (clear). There is also a greater residual volume of Recovery Agent/Partitioning Oil (pink). Tube C and E indicate a wetting failure has occurred. There is an abnormal residual volume of Recovery Agent/Partitioning Oil (pink).
2.1 d After addition of Dynabeads Cleanup Mix	 All liquid volumes are similar after addition of the Dynabeads Cleanup Mix.	 Tube G indicates a reagent clog has occurred. There is an abnormal ratio of Dynabeads Cleanup Mix (brown) to Recovery Agent/Partitioning Oil (appears white). Tube C and E indicate a wetting failure has occurred. There is an abnormal ratio of Dynabeads Cleanup Mix (brown) to Recovery Agent/Partitioning Oil (appears white).

If a channel clogs or wetting failure occurs during GEM generation, it is recommended that the sample be remade. If any of the listed issues occur, take a picture and send it to support@10xgenomics.com for further assistance.

Chromium Controller Errors

If the Chromium Controller or the Chromium Single Cell Controller fails to start, an error tone will sound and one of the following error messages will be displayed:

a. **Chip not read – Try again:** Eject the tray, remove and/or reposition the Chromium Next GEM Secondary Holder assembly and try again. If the error message is still received after trying this more than twice, contact support@10xgenomics.com for further assistance.

b. **Check gasket:** Eject the tray by pressing the eject button to check that the 10x Gasket is correctly installed on the Chromium Next GEM Chip. If the error message persists, contact support@10xgenomics.com for further assistance.

c. **Pressure not at Setpoint:**

   i. If this message is received within a few seconds of starting a run, eject the tray by pressing the eject button and check for dirt or deposits on the 10x Gasket. If dirt is observed, replace with a new 10x Gasket and try again. If the error message is still received after trying this more than twice, contact support@10xgenomics.com for further assistance.

   ii. If this message is received after a few minutes into the run, the Chromium Next GEM Chip must be discarded. **Do not try running this Chromium Next GEM Chip again as this may damage the Chromium Controller.**

d. **Invalid Chip CRC Value:** This indicates that a Chromium Next GEM Chip has been used with an older firmware version. The chip must be discarded. Contact support@10xgenomics.com for further assistance.

Appendix

Post Library Construction Quantification
Agilent TapeStation Traces
Oligonucleotide Sequences

Post Library Construction Quantification

a. Thaw KAPA Library Quantification Kit for Illumina Platforms.

b. Dilute **2 µl** sample with deionized water to appropriate dilutions that fall within the linear detection range of the KAPA Library Quantification Kit for Illumina Platforms. (For more accurate quantification, make the dilution(s) in duplicate).

c. Make enough Quantification Master Mix for the DNA dilutions per sample and the DNA Standards (plus 10% excess) using the guidance for 1 reaction volume below.

Quantification Master Mix	1X (µl)
SYBR Fast Master Mix + Primer	12
Water	4
Total	**16**

d. Dispense **16 µl** Quantification Master Mix for sample dilutions and DNA Standards into a 96 well PCR plate.

e. Add **4 µl** sample dilutions and **4 µl** DNA Standards to appropriate wells. Centrifuge briefly.

f. Incubate in a thermal cycler with the following protocol.

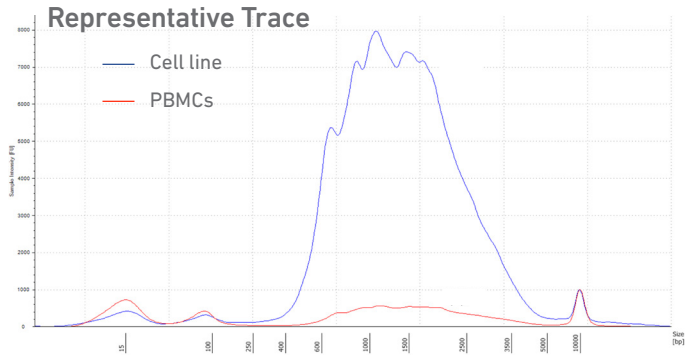
Step	Temperature	Run Time
1	95°C	00:03:00
2	95°C	00:00:05
3	67°C	00:00:30
4	Go to Step 2, 29X (Total 30 cycles)	

g. Follow the manufacturer's recommendations for qPCR-based quantification. For library quantification for sequencer clustering, determine the concentration based on insert size derived from the Bioanalyzer/TapeStation trace.

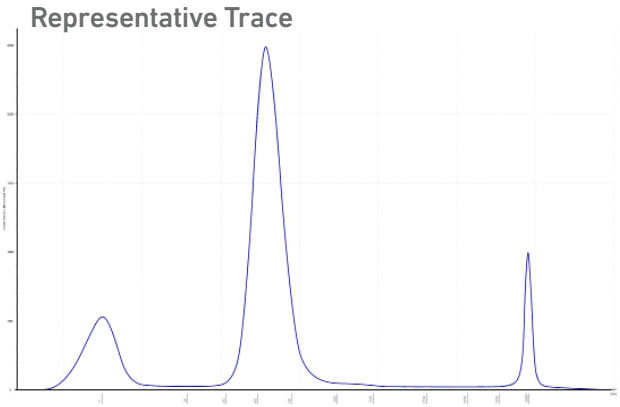
Agilent TapeStation Traces

Agilent TapeStation Traces
Agilent TapeStation High Sensitivity D5000 ScreenTape was used.
Protocol steps correspond to the Chromium Next GEM Single Cell 3' Reagent Kits v3.1 User Guide (CG000204).

Protocol Step 2.4 – cDNA QC & Quantification



Protocol Step 3.7 – Post Library Construction QC



Alternate QC Method:
Qubit Fluorometer and Qubit dsDNA HS Assay Kit

Multiply the cDNA concentration reported via the Qubit Fluorometer by the elution volume (40 µl) to obtain the total cDNA yield in ng. To determine the equivalent range using the Agilent 2100 Expert Software, select the region encompassing 35-10,000 bp.

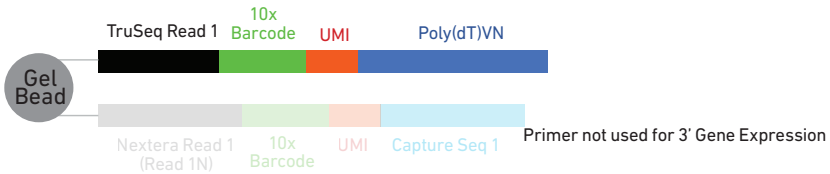
Oligonucleotide Sequences

Protocol steps correspond to the Chromium Next GEM Single Cell 3' Reagent Kits v3.1 User Guide (CG000204)

Protocol Step 1.5 – GEM-RT Incubation

Gel Bead Primers

5'-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNNNNN-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-3'



5'-GTCAGATGTGTATAAGAGACAG-NNNNNNNNNNNNNNNN-NNNNNNNNNNNN-TTGCTAGGACCGGCCTTAAAGC-3'

Template Switch Oligonucleotide PN-3000228

5'-AAGCAGTGGTATCAACGCAGAGTACATrGrGrG-3'

PCR Product

5'-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNNNNN-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-cDNA_Insert-CCCATGTACTCTGCGTTGATACCACTGCTT-3'

Protocol Step 2.2 – cDNA Amplification

cDNA Primers PN-2000089

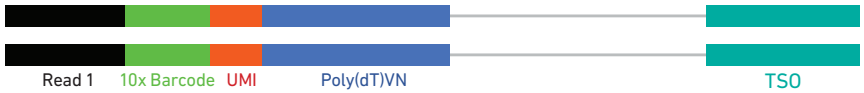
Forward Primer: Partial Read 1

5'-CTACACGACGCTCTTCCGATCT-3'

Reverse Primer: Partial TSO

5'-AAGCAGTGGTATCAACGCAGAG-3'

Amplification Products



5'-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNNNNN-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-cDNA_Insert-CCCATGTACTCTGCGTTGATACCACTGCTT-3'

3'-GATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNNNNN-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAABN-cDNA_Insert-GGGTACATGAGACGCAACTATGGTGACGAA-5'

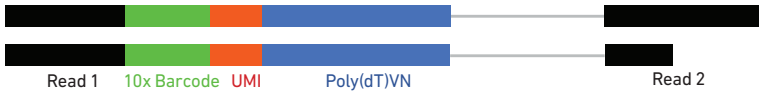
Protocol Step 3.3 – Adaptor Ligation

Adaptor Oligos PN -2000094

5'- GATCGGAAGAGCACACGTCTGAACTCCAGTCA-3'

3'-TCTAGCCTTCTCG-5'

Ligation Product



5'-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNNNNN-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-cDNA_Insert-AGATCGGAAGAGC ACACGTCTGAACTCCAGTCAC-3'

3'-GATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNNNNN-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAABN-cDNA_Insert-TCTAGCCTTCTCG-5'

Oligonucleotide Sequences

Protocol Step 3.5 – Sample Index PCR

Sample Index PCR Primer PN-2000095

Chromium i7Sample Index PN-220103

5'-AATGATACGGCGACCACCGAGATCT-ACACTCTTTCCCTACACGACGCTC-3'

5'-CAAGCAGAAGACGGCATACGAGAT-NNNNNNNN GTGACTGGAGTTCAGACGTGT-3'

Sample Index PCR Product

5'-AATGATACGGCGACCACCGAGATCT-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNNNN-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-cDNA_Insert-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-NNNNNNNN-ATCTCGTATGCCGTCTTCTGCTTG-3'

3'-TTACTATGCCGCTGGTGGCTCTAGA-TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNNNN-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAABN-cDNA_Insert-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG-NNNNNNNN-TAGAGCATACGGCAGAAGACGAAC-5'

EXHIBIT 8

CG000207 Rev D

USER GUIDE

Chromium Next GEM Single Cell V(D)J Reagent Kits v1.1

FOR USE WITH

Chromium Next GEM Single Cell 5' Library & Gel Bead Kit v1.1, 16 rxns PN-1000165
Chromium Next GEM Single Cell 5' Library & Gel Bead Kit v1.1, 4 rxns PN-1000167
Chromium Single Cell 5' Library Construction Kit, 16 rxns PN-1000020
Chromium Single Cell V(D)J Enrichment Kit, Human T Cell, 96 rxns PN-1000005
Chromium Single Cell V(D)J Enrichment Kit, Human B Cell, 96 rxns PN-1000016
Chromium Single Cell V(D)J Enrichment Kit, Mouse T Cell, 96 rxns PN-1000071
Chromium Single Cell V(D)J Enrichment Kit, Mouse B Cell, 96 rxns PN-1000072
Chromium Next GEM Chip G Single Cell Kit, 48 rxns PN-1000120
Chromium Next GEM Chip G Single Cell Kit, 16 rxns PN-1000127
Chromium i7 Multiplex Kit, 96 rxns PN-120262

Next GEM reagents are specific to Next GEM products and should not be used interchangeably with non-Next GEM reagents.

10x GENOMICS

Notices

Document Number

CG000207 • Rev D

Legal Notices

Instrument & Licensed Software Updates Warranties

Updates to existing Instruments and Licensed Software may be required to enable customers to use new or existing products. In the event of an Instrument failure resulting from an update, such failed Instrument will be replaced or repaired in accordance with the 10x Limited Warranty, Assurance Plan or service agreement, only if such Instrument is covered by any of the foregoing at the time of such failure. Instruments not covered under a current 10x Limited Warranty, Assurance Plan or service agreement will not be replaced or repaired.

Support

Email: support@10xgenomics.com

10x Genomics

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Pleasanton, CA 94588 USA

Document Revision Summary

Document Number	CG000207
Title	Chromium Next GEM Single Cell V(D)J Reagent Kits v1.1 User Guide
Revision	Rev C to Rev D
Revision Date	August 2019

Specific Changes:

- Updated to state that Next GEM reagents are specific to Next GEM products.

General Changes:

- Updates for general minor consistency of language and terms throughout.

Table of Contents

Introduction

Chromium Next GEM Single Cell V(D)J Reagent Kits v1.1
Chromium Accessories
Recommended Thermal Cyclers
Additional Kits, Reagents & Equipments
Protocol Steps & Timing
Stepwise Objectives

Chromium Next GEM Single Cell V(D)J Reagent Kits v1.1

Chromium Next GEM Single Cell 5' Library and Gel Bead Kit v1.1, 16 rxns PN-1000165

Chromium Next GEM Single Cell 5' Library Kit v1.1, 16 rxns PN-1000166 (store at –20°C)

Chromium Next GEM Single Cell 5' Reagents Module 1 v1.1

	#	PN
RT Reagent B	1	2000165
RT Enzyme Mix B	1	2000010
Additive A	1	220074
Poly-dT RT Primer	1	2000007
Buffer Sample Clean Up 1	2	220020
Amplification Master Mix	2	220125
cDNA Primer Mix	1	220106
cDNA Additive	1	220067

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Chromium Next GEM Single Cell 5' Reagents Module 2 v1.1

	#	PN
Fragmentation Enzyme Blend	1	220107
Fragmentation Buffer	1	220108
Ligation Buffer	1	220109
DNA Ligase	1	220110
Adaptor Mix	1	220026
SI-PCR Primer	1	220111
Amplification Master Mix	1	220125

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Chromium Next GEM Single Cell 5' Gel Bead Kit v1.1, 16 rxns PN-1000169 (store at –80°C)

Chromium Next GEM Single Cell 5' Gel Beads v1.1

	#	PN
Single Cell VDJ 5' Gel Beads v1.1	2	2000209

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Dynabeads™ MyOne™ SILANE PN-2000048 (store at 4°C)

	#	PN
Dynabeads MyOne SILANE	1	2000048

Chromium Next GEM Single Cell 5' Library and Gel Bead Kit v1.1, 4 rxns PN-1000167

Chromium Next GEM Single Cell 5' Library Kit v1.1, 4 rxns PN-1000168 (store at –20°C)

Chromium Next GEM Single Cell 5' Reagents Module 1 v1.1

	#	PN
RT Reagent B	1	2000165
RT Enzyme Mix B	1	2000021
Additive A	1	220074
Poly-dT RT Primer	1	2000007
Buffer Sample Clean Up 1	1	220020
Amplification Master Mix	1	220125
cDNA Primer Mix	1	220106
cDNA Additive	1	220067

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Chromium Next GEM Single Cell 5' Reagents Module 2 v1.1

	#	PN
Fragmentation Enzyme Blend	1	220130
Fragmentation Buffer	1	220108
Ligation Buffer	1	220109
DNA Ligase	1	220131
Adaptor Mix	1	220026
SI-PCR Primer	1	220111

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Chromium Next GEM Single Cell 5' Gel Bead Kit v1.1, 4 rxns PN-1000170 (store at –80°C)

Chromium Next GEM Single Cell 5' Gel Beads v1.1

	#	PN
Single Cell VDJ 5' Gel Beads v1.1	1	2000209

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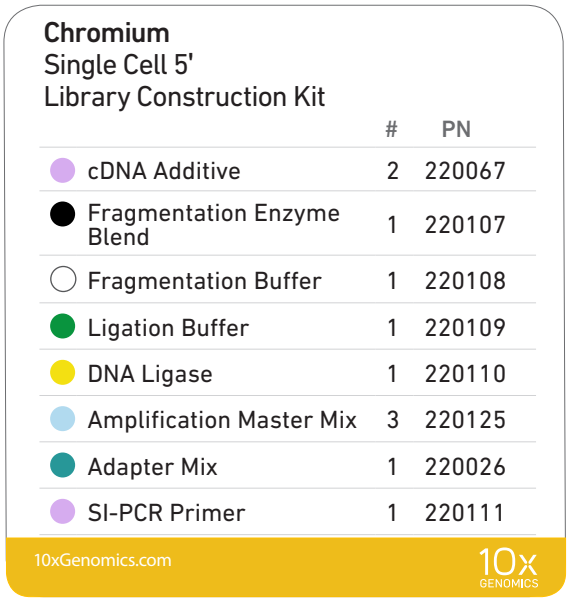
Dynabeads™ MyOne™ SILANE PN-2000048 (store at 4°C)

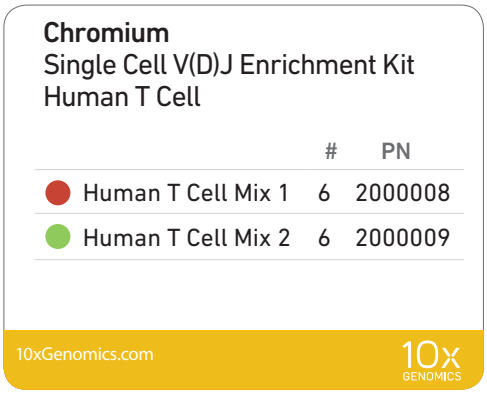
	#	PN
Dynabeads MyOne SILANE	1	2000048

Chromium Single Cell 5' Library Construction Kit, 16 rxns PN-1000020 (store at -20°C)

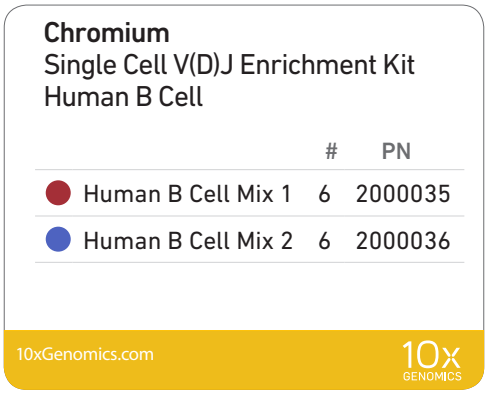


Chromium Single Cell V(D)J Enrichment Kits, Human (store at –20°C)

Human T Cell, 96 rxns
PN-1000005

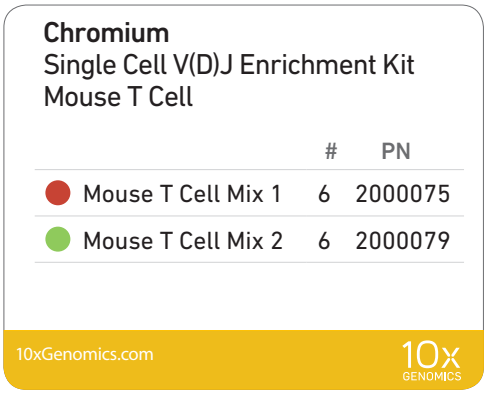


Human B Cell, 96 rxns
PN-1000016

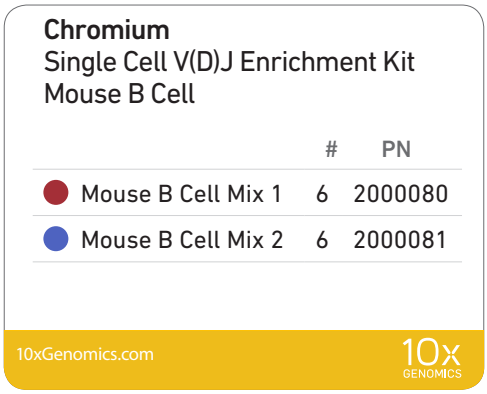


Chromium Single Cell V(D)J Enrichment Kits, Mouse (store at –20°C)

Mouse T Cell, 96 rxns
PN-1000071



Mouse B Cell, 96 rxns
PN-1000072



Chromium Next GEM Chip G Single Cell Kit, 48 rxns PN-1000120 (store at ambient temperature)

Chromium
Partitioning Oil

	#	PN
Partitioning Oil	6	2000190

Chromium
Recovery Agent

	#	PN
Recovery Agent	6	220016

Chromium
Next GEM
Chip G & Gaskets

	#	PN
Chromium Next GEM Chip G	6	2000177
Gasket, 6-pack	1	370017

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Chromium Next GEM Chip G Single Cell Kit, 16 rxns PN-1000127 (store at ambient temperature)

Chromium
Partitioning Oil

	#	PN
Partitioning Oil	2	2000190

Chromium
Recovery Agent

	#	PN
Recovery Agent	2	220016

Chromium
Next GEM
Chip G & Gaskets

	#	PN
Chromium Next GEM Chip G	2	2000177
Gasket, 2-pack	1	3000072

10xGenomics.com 10x GENOMICS

Chromium i7 Multiplex Kit, 96 rxns PN-120262 (store at –20°C)

Chromium
i7 Multiplex Kit

	#	PN
Chromium i7 Sample Index Plate	1	220103

Chromium Accessories

Product	PN (Kit)	PN (Item)
10x Vortex Adapter	120251	330002
Chromium Next GEM Secondary Holder	1000195	3000332
10x Magnetic Separator	120250	230003

Recommended Thermal Cyclers

Thermal cyclers used must support uniform heating of 100 µl emulsion volumes.

Supplier	Description	Part Number
BioRad	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module	1851197
Eppendorf	MasterCycler Pro	North America 950030010 International 6321 000.019
Thermo Fisher Scientific	Veriti 96-Well Thermal Cycler	4375786

Additional Kits, Reagents & Equipment

The items in the table below have been validated by 10x Genomics and are highly recommended for the Single Cell V(D)J protocol. Substituting materials may adversely affect system performance.

Supplier	Description	Part Number (US)
Plastics		
Eppendorf	PCR Tubes 0.2 ml 8-tube strips DNA LoBind Tubes, 1.5 ml DNA LoBind Tubes, 2.0 ml	951010022 022431021 022431048
USA Scientific	TempAssure PCR 8-tube strip	1402-4700
Thermo Fisher Scientific	MicroAmp 8-Tube Strip, 0.2 ml MicroAmp 8-Cap Strip, clear	N8010580 N8010535
Rainin	Tips LTS 200UL Filter RT-L200FLR Tips LTS 1ML Filter RT-L1000FLR Tips LTS 20UL Filter RT-L10FLR	30389240 30389213 30389226
Kits & Reagents		
Thermo Fisher Scientific	Nuclease-free Water	AM9937
Millipore Sigma	Ethanol, Pure (200 Proof, anhydrous)	E7023-500ML
Beckman Coulter	SPRIselect Reagent Kit	B23318
Bio-Rad	10% Tween 20	1662404
Ricca Chemical Company	Glycerin (glycerol), 50% (v/v) Aqueous Solution	3290-32
Qiagen	Qiagen Buffer EB	19086
Equipment		
VWR	Vortex Mixer Divided Polystyrene Reservoirs	10153-838 41428-958
Eppendorf	Eppendorf ThermoMixer C Eppendorf SmartBlock 1.5 ml, Thermoblock for 24 reaction vessel (alternatively, use a temperature-controlled Heat Block)	5382000023 5360000038
Rainin	Pipet-Lite Multi Pipette L8-50XLS+ Pipet-Lite Multi Pipette L8-200XLS+ Pipet-Lite Multi Pipette L8-10XLS+ Pipet-Lite Multi Pipette L8-20XLS+ Pipet-Lite LTS Pipette L-2XLS+ Pipet-Lite LTS Pipette L-10XLS+ Pipet-Lite LTS Pipette L-20XLS+ Pipet-Lite LTS Pipette L-100XLS+ Pipet-Lite LTS Pipette L-200XLS+ Pipet-Lite LTS Pipette L-1000XLS+	17013804 17013805 17013802 17013803 17014393 17014388 17014392 17014384 17014391 17014382

Choose either Eppendorf, USA Scientific or Thermo Fisher Scientific PCR 8-tube strips.

Additional Kits, Reagents & Equipment

The items in the table below have been validated by 10x Genomics and are highly recommended for the Single Cell V(D)J protocol. Substituting materials may adversely affect system performance.

Supplier	Description		Part Number (US)
Quantification & Quality Control			
Agilent	2100 Bioanalyzer Laptop Bundle High Sensitivity DNA Kit 4200 TapeStation High Sensitivity D5000 ScreenTape High Sensitivity D5000 Reagents	Choose Bioanalyzer, TapeStation or Qubit based on availability & preference.	G2943CA 5067-4626 G2991AA 5067-5592 5067-5593
Thermo Fisher Scientific	Qubit 4.0 Fluorometer Qubit dsDNA HS Assay Kit		Q33226 Q32854
KAPA Biosystems	KAPA Library Quantification Kit for Illumina Platforms		KK4824

Protocol Steps & Timing

Day	Steps		Timing	Stop & Store
3 h	**Cell Preparation**			
	Dependent on Cell Type		~1-1.5 h	
	Step 1 – GEM Generation & Barcoding			
	1.1	Prepare Reaction Mix	20 min	
	1.2	Load Chromium Next GEM Chip G	10 min	
	1.3	Run the Chromium Controller	18 min	
	1.4	Transfer GEMs	3 min	
	1.5	GEM-RT Incubation	55 min	STOP 4°C ≤72 h or –20°C ≤1 week
6 h	**Step 2 – Post GEM RT Cleanup**			
	2.1	Post GEM-RT Cleanup – Dynabead	45 min	
	Step 3 – cDNA Amplification & QC*			
	3.1	cDNA Amplification	50 min	
	3.2	cDNA Cleanup	15 min	STOP 4°C ≤72 h or –20°C ≤1 week
	3.3	cDNA Quantification & QC	50 min	
	*After cDNA Amplification & QC, for Target Enrichment & Enriched Library Construction proceed to steps 4-5. For 5' Gene Expression Library Construction proceed directly to step 6.			
8 h plus* *Time dependent on Stop options used and protocol steps executed (steps 4-5 only/ steps 4,5 & 6)	**Step 4 – Target Enrichment from cDNA**			
	4.1	Target Enrichment 1	40 min	STOP 4°C ≤72 h
	4.2	Post Target Enrichment 1 Cleanup – SPRIselect	20 min	STOP 4°C ≤72 h or –20°C ≤1 week
	4.3	Target Enrichment 2	40 min	STOP 4°C ≤72 h
	4.4	Post Target Enrichment 2 Double Sided Size Selection – SPRIselect	30 min	STOP 4°C ≤72 h or –20°C ≤1 week
	4.5	Post Target Enrichment QC & Quantification	50 min	
	Step 5 – Enriched Library Construction			
	5.1	Fragmentation, End Repair & A – tailing	45 min	
	5.2	Adaptor Ligation	25 min	
	5.3	Post Ligation Cleanup – SPRIselect	20 min	
	5.4	Sample Index PCR	40 min	STOP 4°C ≤72 h
	5.5	Post Sample Index PCR Cleanup – SPRIselect	20 min	STOP 4°C ≤72 h or –20°C long-term
	5.6	Post Library Construction QC	50 min	
	Step 6 – 5' Gene Expression (GEX) Library Construction			
	6.1	GEX Fragmentation, End Repair & A-tailing	45 min	
	6.2	GEX Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect	30 min	
	6.3	GEX Adaptor Ligation	25 min	
	6.4	GEX Post Ligation Cleanup – SPRIselect	20 min	
	6.5	GEX Sample Index PCR	40 min	STOP 4°C ≤72 h
	6.6	GEX Post Sample Index PCR Double Sided Cleanup – SPRIselect	30 min	STOP 4°C ≤72 h or –20°C long-term
	6.7	GEX Post Library Construction QC	50 min	

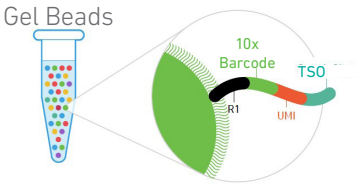
Stepwise Objectives

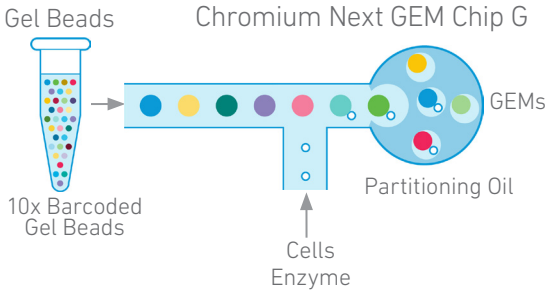
The Single Cell V(D)J protocols offer comprehensive, scalable solutions for measuring immune repertoire information and gene expression from the same cell. Profile full-length (5' UTR to constant region), paired T-cell receptor (TCR), or B-cell immunoglobulin (Ig) transcripts from 100-10,000 individual cells per sample. A pool of ~750,000 barcodes are sampled separately to index each cell's transcriptome. It does so by partitioning thousands of cells into nanoliter-scale Gel Beads-in-emulsion (GEMs), where all generated cDNA share a common 10x Barcode. Libraries are generated and sequenced and 10x Barcodes are used to associate individual reads back to the individual partitions. This document outlines the protocol to generate an enriched T-cell library and/or an enriched B-cell library, and/or a 5' Gene Expression library from amplified cDNA from the same cells.

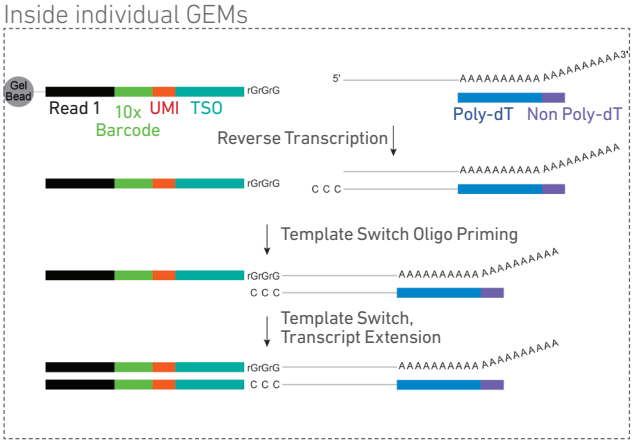
Step 1 GEM Generation & Barcoding

GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads v1.1, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell.

Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.







Step 2 Post GEM-RT Cleanup & QC

GEMs are broken and pooled after GEM-RT reaction mixtures are recovered. Silane magnetic beads are used to purify the 10x Barcoded first-strand cDNA from the post GEM-RT reaction mixture, which includes leftover biochemical reagents and primers. After cleanup a user may decide to pursue target enrichment directly from first-strand cDNA, in which case, consult Demonstrated Protocol - Chromium Single Cell V(D)J Reagent Kits-Direct Target Enrichment (Document CG000166). Otherwise, users should proceed to cDNA amplification in this protocol.

Step 3 cDNA Amplification & QC

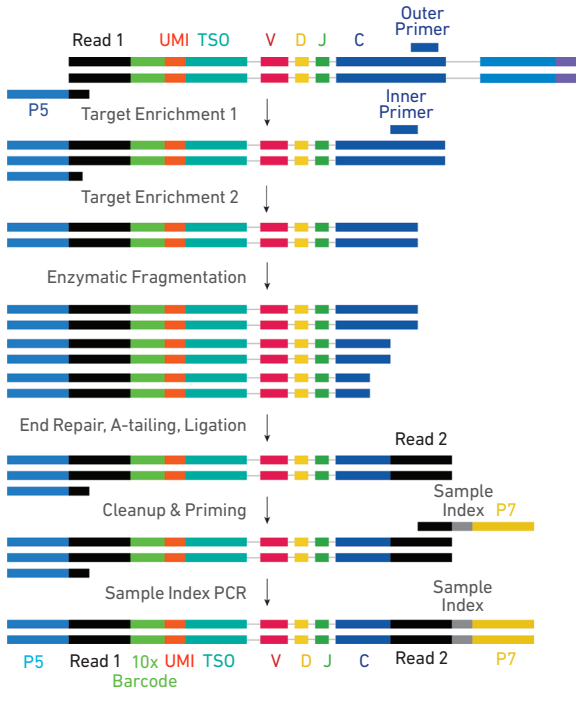
10x Barcoded, full-length cDNA is amplified via PCR with primers against common 5' and 3' ends added during GEM-RT. Amplification generates sufficient material to construct multiple libraries from the same cells, e.g. both T cell and/or B cell enriched libraries (steps 4 and 5) and 5' Gene Expression libraries (step 6).

Pooled cDNA amplification

Step 4 Target Enrichment from cDNA

Full-length V(D)J segments (10x Barcoded) are enriched from amplified cDNA via PCR amplification with primers specific to either the TCR or Ig constant regions. If both T and B cells are expected to be present in the partitioned cell population, TCR and Ig transcripts can be enriched in separate reactions from the same amplified cDNA material. P5 is added during enrichment.

Pooled amplified cDNA processed in bulk

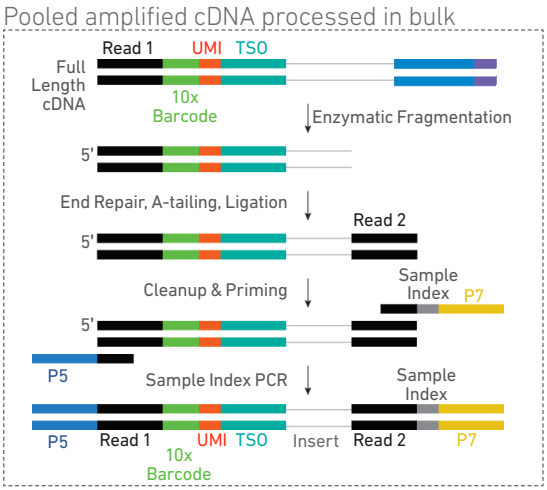


Step 5 Enriched Library Construction

Enzymatic fragmentation and size selection are used to generate variable length fragments that collectively span the V(D)J segments of the enriched TCR or Ig transcripts prior to library construction. P7, a sample index, and an Illumina R2 sequence (read 2 primer sequence) are added via End Repair, A-tailing, Adaptor Ligation, and Sample Index PCR. The final libraries contain the P5 and P7 priming sites used in Illumina sequencing.

Step 6
5' Gene Expression (GEX) Library Construction

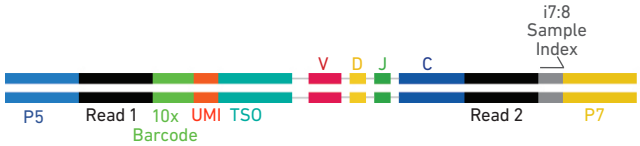
Enzymatic fragmentation and size selection are used to optimize the cDNA amplicon size prior to 5' Gene Expression library construction. P5, P7, a sample index, and Illumina R2 sequence (read 2 primer sequence) are added via End Repair, A-tailing, Adaptor Ligation, and Sample Index PCR. The final libraries contain the P5 and P7 priming sites used in Illumina sequencers.



Step 7
Sequencing

Illumina-ready sequencing libraries can be sequenced at the recommended depth & run parameters. Illumina sequencer compatibility, sample indices, library loading and pooling for sequencing are summarized in step 7.

Chromium Single Cell V(D)J Enriched Library



Chromium Single Cell 5' Gene Expression Library

See Appendix for Oligonucleotide Sequences

Tips & Best Practices

Icons

			
Tips & Best Practices section includes additional guidance	Signifies critical step requiring accurate execution	Troubleshooting section includes additional guidance	Next GEM specific protocol step updates

Emulsion-safe Plastics

- Use validated emulsion-safe plastic consumables when handling GEMs as some plastics can destabilize GEMs.

Cell Concentration

- Recommended starting point is to load ~1700 cells per reaction, resulting in recovery of ~1000 cells, and a multiplet rate of ~0.8%. The optimal input cell concentration is 700-1,200 cells/µl.
- The presence of dead cells in the suspension may also reduce the recovery rate. Consult the 10x Genomics Single Cell Protocols Cell Preparation Guide and the Guidelines for Optimal Sample Preparation flowchart (Documents CG00053 and CG000126 respectively) for more information on preparing cells.
- Refer to the 10x Genomics Support website for more information regarding cell type specific sample preparation, for example, the Demonstrated Protocol for Enrichment of CD3+ T Cells from Dissociated Tissues for Single Cell RNA Sequencing and Immune Repertoire Profiling (Document CG000123).

Multiplet Rate (%)	# of Cells Loaded	# of Cells Recovered
~0.4%	~870	~500
~0.8%	~1,700	~1,000
~1.6%	~3,500	~2,000
~2.3%	~5,300	~3,000
~3.1%	~7,000	~4,000
~3.9%	~8,700	~5,000
~4.6%	~10,500	~6,000
~5.4%	~12,200	~7,000
~6.1%	~14,000	~8,000
~6.9%	~15,700	~9,000
~7.6%	~17,400	~10,000

General Reagent Handling

- Fully thaw and thoroughly mix reagents before use.
- Keep all enzymes and Master Mixes on ice during setup and use. Promptly move reagents back to the recommended storage after use.
- Calculate reagent volumes with 10% excess of 1 reaction values.
- Cover Partitioning Oil tubes and reservoirs to minimize evaporation.
- If using multiple chips, use separate reagent reservoirs for each chip during loading.
- Thoroughly mix samples with the beads during bead-based cleanup steps.

50% Glycerol Solution

- Purchase 50% glycerol solution from Ricca Chemical Company, Glycerin (glycerol), 50% (v/v) Aqueous Solution, PN-3290-32.
- Prepare 50% glycerol solution:
  i. Mix an equal volume of water and 99% Glycerol, Molecular Biology Grade.
  ii. Filter through a 0.2-µm filter.
  iii. Store at –20°C in 1-ml LoBind tubes. 50% glycerol solution should be equilibrated to room temperature before use.

Pipette Calibration

- Follow manufacturer's calibration and maintenance schedules.
- Pipette accuracy is particularly important when using SPRIselect reagents.

Chromium Next GEM Chip Handling

Next GEM

- Minimize exposure of reagents, chips, and gaskets to sources of particles and fibers, laboratory wipes, frequently opened flip-cap tubes, clothing that sheds fibers, and dusty surfaces.
- After removing the chip from the sealed bag, use in ≤ 24 h.
- Execute steps without pause or delay, unless indicated. When multiple chips are to be used, load, run, and collect the content from one chip before loading the next.
- Fill all unused input wells in rows labeled 1, 2, and 3 on a chip with an appropriate volume of 50% glycerol solution before loading the used wells. DO NOT add glycerol to the wells in the bottom NO FILL row.
- Avoid contacting the bottom surface of the chip with gloved hands and other surfaces. Frictional charging can lead to inadequate priming of the channels, potentially leading to either clogs or wetting failures.
- Minimize the distance that a loaded chip is moved to reach the Chromium Controller.
- Keep the chip horizontal to prevent wetting the gasket with oil, which depletes the input volume and may adversely affect the quality of the resulting emulsion.

Chromium Next GEM Secondary Holders

Next GEM

- Chromium Next GEM Secondary Holders encase Chromium Next GEM Chips.
- The holder lid flips over to become a stand, holding the chip at 45 degrees for optimal recovery well content removal.
- Squeeze the black sliders on the back side of the holder together to unlock the lid and return the holder to a flat position.

Chromium Next GEM Chip & Holder Assembly

Next GEM

- Align notch on the chip (upper left corner) and the holder.
- Insert the left-hand side of the chip under the guide. Depress the right-hand side of the chip until the spring-loaded clip engages.
- Close the lid before dispensing reagents into the wells.

Chromium Next GEM Chip Loading

Next GEM

- Place the assembled chip and holder flat on the bench with the lid closed.
- Dispense at the bottom of the wells without introducing bubbles.
- When dispensing Gel Beads into the chip, wait for the remainder to drain into the bottom of the pipette tips and dispense again to ensure complete transfer.
- Refer to Load Chromium Next GEM Chip G for specific instructions.

Gel Bead Handling

Next GEM

- Use one tube of Gel Beads per sample. DO NOT puncture the foil seals of tubes not used at the time.
- Equilibrate the Gel Beads strip to room temperature before use.
- Store unused Gel Beads at –80°C and avoid more than 10 freeze-thaw cycles. DO NOT store Gel Beads at –20°C.

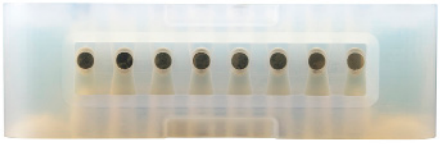
- Snap the tube strip holder with the Gel Bead strip into a 10x Vortex Adapter. Vortex **30 sec**.
- Centrifuge the Gel Bead strip for ~**5 sec** after removing from the holder. Confirm there are no bubbles at the bottom of the tubes and the liquid levels look even. Place the Gel Bead strip back in the holder and secure the holder lid.
- If the required volume of beads cannot be recovered, place the pipette tips against the sidewalls and slowly dispense the Gel Beads back into the tubes. DO NOT introduce bubbles into the tubes and verify that the pipette tips contain no leftover Gel Beads. Withdraw the full volume of beads again by pipetting slowly.

10x Gasket Attachment

- After reagents are loaded, attach the gasket by holding the tongue (curved end, to the right) and hook it on the left-hand tabs of the holder. Gently pull the gasket toward the right and hook it on the two right-hand tabs.
- DO NOT touch the smooth side of the gasket. DO NOT press down on the top of the gasket after attachment.
- Keep the assembly horizontal to avoid wetting the gasket with Partitioning Oil.

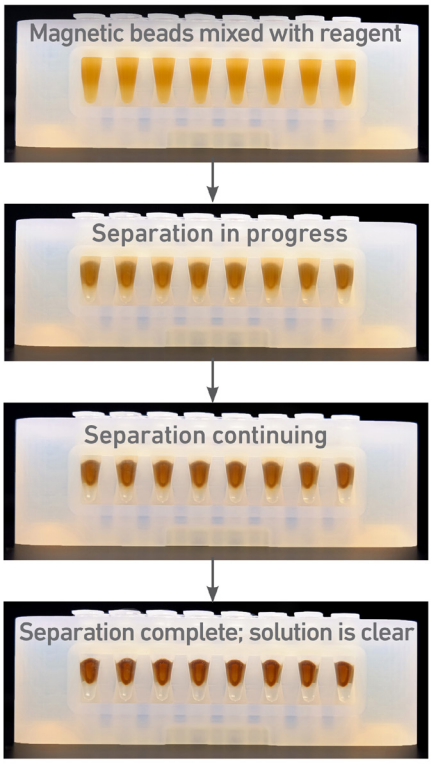
10x Magnetic Separator

- Offers two positions of the magnets (high and low) relative to a tube, depending on its orientation. Flip the magnetic separator over to switch between high (magnet•**High)** or low (magnet•**Low)** positions.
- If using MicroAmp 8-Tube Strips, use the high position (magnet•**High)** only throughout the protocol.



Magnetic Bead Cleanup Steps

- During magnetic bead based cleanup steps that specify waiting "until the solution clears", visually confirm clearing of solution before proceeding to the next step. See adjacent panel for an example.
- The time need for the solution to clear may vary based on specific step, reagents, volume of reagents used etc.



cDNA Amplification PCR Cycle Numbers

- Follow cycle number recommendations for high and low RNA content cells based on Targeted Cell Recovery and cell sample.
- Cycle numbers in the table below have been optimized assuming that the sample has >80% T and/or B cells. Samples with lower fraction of T and/or B cells may require additional cycle number optimization and/or may be enriched to increase the fraction of T or B cells. Refer to the Demonstrated Protocol for Enrichment of CD3+ T Cells from Dissociated Tissues for Single Cell RNA Sequencing and Immune Repertoire Profiling (Document CG000123).
- If the fraction of T and/or B cells in a cell sample is known, adjust PCR cycle number based on that fraction to ensure sufficient product generation.

Tutorial – Optimizing cDNA Amplification PCR Cycle Numbers

Examples

Sample A:
Primary cells with 15% T cell fraction.
Targeted Cell Recovery is 10,000 cells.
Only 1,500 (15%) cells are T cells.
Total PCR cycles – 16.

Sample B:
Cell line with high RNA content.
Targeted Cell Recovery is 10,000 cells.
Total PCR cycles – 11.

Sample C:
Cell mix with 90% low RNA content and 10% high RNA content B cells.
Targeted Cell Recovery is 10,000 cells.
90% B cells are low RNA content.
Total PCR cycles – 13.

Recommended starting point for cycle number optimization.

Targeted Cell Recovery	Low RNA Content Cells Total Cycles	High RNA Content Cells Total Cycles
100–500	18	16
501–2,000	16	14
2,001–6,000	14	12
6,001–10,000	13	11

Enzymatic Fragmentation

- Ensure enzymatic fragmentation reactions are prepared on ice and then loaded into a thermal cycler pre-cooled to 4°C prior to initiating the Fragmentation, End Repair, and A-tailing incubation steps.

SPRIselect Cleanup & Size Selection

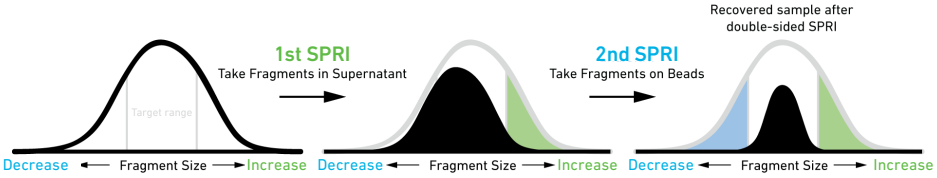
- After aspirating the desired volume of SPRIselect reagent, examine the pipette tips before dispensing to ensure the correct volume is transferred.
- Pipette mix thoroughly as insufficient mixing of sample and SPRIselect reagent will lead to inconsistent results.
- Use fresh preparations of 80% Ethanol.

Tutorial — SPRIselect Reagent:DNA Sample Ratios

SPRI beads selectively bind DNA according to the ratio of SPRIselect reagent (beads).

Example: Ratio = $\frac{\text{Volume of SPRIselect reagent added to the sample}}{\text{Volume of DNA sample}} = \frac{50\ \mu l}{100\ \mu l} = \mathbf{0.5X}$

Schematic of Double Sided Size Selection



After the first SPRI, supernatant is transferred for a second SPRI while larger fragments are discarded (green). After the second SPRI, fragments on beads are eluted and kept while smaller fragments are discarded (blue). Final sample has a tight fragment size distribution with reduced overall amount (black).

Tutorial — Double Sided Size Selection

Step a – First SPRIselect: Add **50 µl** SPRIselect reagent to **100 µl** sample (**0.5X**).

Ratio = $\frac{\text{Volume of SPRIselect reagent added to the sample}}{\text{Volume of DNA sample}} = \frac{50\ \mu l}{100\ \mu l} = \mathbf{0.5X}$

Step b – Second SPRIselect: Add **30 µl** SPRIselect reagent to supernatant from step a (**0.8X**).

Ratio = $\frac{\text{Total Volume of SPRIselect reagent added to the sample (step a + b)}}{\text{Original Volume of DNA sample}} = \frac{50\ \mu l + 30\ \mu l}{100\ \mu l} = \mathbf{0.8X}$

Sample Indices in Sample Index PCR

- Choose the appropriate sample index sets to ensure that no sample indices overlap in a multiplexed sequencing run.
- Each well in the i7 Sample Index plate contains a unique mix of 4 oligos.
- The sample indices can therefore be used in any combination.
- Each sample index set is base-balanced to avoid monochromatic signal issues when it is the sole sample loaded on an Illumina sequencer.

Step 1

GEM Generation & Barcoding

1.1 Prepare Master Mix

1.2 Load Chromium Next GEM Chip G

1.3 Run the Chromium Controller

1.4 Transfer GEMs

1.5 GEM-RT Incubation

1

1.0 GEM Generation & Barcoding

Next GEM

GET STARTED!

Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature		**Chromium Next GEM Single Cell 5' Gel Bead v1.1**	2000209	Equilibrate to room temperature 30 min before loading the chip.	–80°C
	●	**RT Reagent B**	2000165	Vortex, verify no precipitate, centrifuge briefly.	–20°C
	●	**Poly-dT RT Primer**	2000007	Vortex, verify no precipitate, centrifuge briefly.	–20°C
	●	**Additive A**	220074	Vortex, verify no precipitate, centrifuge briefly.	–20°C
Place on Ice	○	**RT Enzyme Mix B**	2000010/ 2000021	Centrifuge briefly before adding to the mix.	–20°C
Obtain		**Partitioning Oil**	2000190	-	Ambient
		Chromium Next GEM Chip G	2000177	-	Ambient
		10x Gasket	370017/ 3000072	See Tips & Best Practices.	Ambient
		Chromium Next GEM Secondary Holder	3000332	See Tips & Best Practices.	Ambient
		10x Vortex Adapter	330002	See Tips & Best Practices.	Ambient
		50% glycerol solution If using <8 reactions	-	See Tips & Best Practices.	-

Firmware Version 4.0 or higher is required in the Chromium Controller or the Chromium Single Cell Controller used for the Single Cell V(D)J v1.1 protocols.

1.1 Prepare Reaction Mix

Next GEM

a. Prepare Master Mix on ice. Pipette mix 15x and centrifuge briefly.

Master Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
● **RT Reagent B**	2000165	18.8	82.5	165.0
● **Poly-dT RT Primer**	2000007	6.4	28.1	56.2
● **Additive A**	220074	2.0	8.8	17.6
○ **RT Enzyme Mix B**	2000010/ 2000021	10.0	44.0	88.0
Total	-	**37.2**	**163.4**	**326.8**

b. Add **37.2 µl** Master Mix into each tube of a PCR 8-tube strip on ice.

Assemble Chromium Next GEM Chip G

! After removing the chip from the sealed bag, use the chip in ≤ 24 h.

TIPS See Tips & Best Practices for chip handling instructions.

- Align notch on the chip (upper left corner) and the holder.
- Insert the left-hand side of the chip under the guide. Depress the right-hand side of the chip until the spring-loaded clip engages.
- Close the lid before dispensing reagents into the wells.
- The assembled chip is ready for loading the indicated reagents. Refer to step 1.2 for reagent volumes and loading order.

! For GEM generation, load the indicated reagents only in the specified rows, starting from row labeled 1, followed by rows labeled 2 and 3. DO NOT load reagents in the bottom row labeled NO FILL. See step 1.2 for details.

Next GEM

Cell Suspension Volume Calculator Table

(for step 1.2 of Chromium Next GEM Single Cell V(D)J v1.1 protocol)

Volume of Cell Suspension Stock per reaction (µl) | Volume of Nuclease-free Water per reaction (µl)

Cell Stock Concentration (Cells/µl)	Targeted Cell Recovery										
	500	1000	2000	3000	4000	5000	6000	7000	8000	9000	10000
100	8.3 29.5	16.5 21.3	33.0 4.8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
200	4.1 33.7	8.3 29.5	16.5 21.3	24.8 13.0	33.0 4.8	n/a	n/a	n/a	n/a	n/a	n/a
300	2.8 35.0	5.5 32.3	11.0 26.8	16.5 21.3	22.0 15.8	n/a	n/a	n/a	n/a	n/a	n/a
400	2.1 35.7	4.1 33.7	8.3 29.5	12.4 25.4	16.5 21.3	20.6 17.2	24.8 13.0	28.9 8.9	33.0 4.8	n/a	n/a
500	1.7 36.1	3.3 34.5	6.6 31.2	9.9 27.9	13.2 24.6	16.5 21.3	19.8 18.0	23.1 14.7	26.4 11.4	29.7 8.1	33.0 4.8
600	1.5 36.3	2.8 35.0	5.5 32.3	8.3 29.5	11.0 26.8	13.8 24.0	16.5 21.3	19.3 18.5	22.0 15.8	24.8 13.0	27.5 10.3
700	1.2 36.6	2.4 35.4	4.7 33.1	7.1 30.7	9.4 28.4	11.8 26.0	14.1 23.7	16.5 21.3	18.9 18.9	21.2 16.6	23.6 14.2
800	1.0 36.8	2.1 35.7	4.1 33.7	6.2 31.6	8.3 29.5	10.3 27.5	12.4 25.4	14.4 23.4	16.5 21.3	18.6 19.2	20.6 17.2
900	0.9 36.9	1.8 36.0	3.7 34.1	5.5 32.3	7.3 30.5	9.2 28.6	11.0 26.8	12.8 25.0	14.7 23.1	16.5 21.3	18.3 19.5
1000	0.8 37.0	1.7 36.1	3.3 34.5	5.0 32.8	6.6 31.2	8.3 29.5	9.9 27.9	11.6 26.2	13.2 24.6	14.8 23.0	16.5 21.3
1100	0.8 37.0	1.5 36.3	3.0 34.8	4.5 33.3	6.0 31.8	7.5 30.3	9.0 28.8	10.5 27.3	12.0 25.8	13.5 24.3	15.0 22.8
1200	0.7 37.1	1.4 36.4	2.8 35.0	4.1 33.7	5.5 32.3	6.9 30.9	8.3 29.5	9.6 28.2	11.0 26.8	12.4 25.4	13.8 24.0
1300	0.6 37.2	1.3 36.5	2.5 35.3	3.8 34.0	5.1 32.7	6.3 31.5	7.6 30.2	8.9 28.9	10.2 27.6	11.4 26.4	12.7 25.1
1400	0.6 37.2	1.2 36.6	2.4 35.4	3.5 34.3	4.7 33.1	5.9 31.9	7.1 30.7	8.3 29.5	9.4 28.4	10.6 27.2	11.8 26.0
1500	0.5 37.3	1.1 36.7	2.2 35.6	3.3 34.5	4.4 33.4	5.5 32.3	6.6 31.2	7.7 30.1	8.8 29.0	9.9 27.9	11.0 26.8
1600	0.5 37.3	1.0 36.8	2.1 35.7	3.1 34.7	4.1 33.7	5.2 32.6	6.2 31.6	7.2 30.6	8.3 29.5	9.3 28.5	10.3 27.5
1700	0.5 37.3	1.0 36.8	1.9 35.9	2.9 34.9	3.9 33.9	4.9 32.9	5.8 32.0	6.8 31.0	7.8 30.0	8.7 29.1	9.7 28.1
1800	0.5 37.3	0.9 36.9	1.8 36.0	2.8 35.0	3.7 34.1	4.6 33.2	5.5 32.3	6.4 31.4	7.3 30.5	8.3 29.5	9.2 28.6
1900	0.4 37.4	0.9 36.9	1.7 36.1	2.6 35.2	3.5 34.3	4.3 33.5	5.2 32.6	6.1 31.7	6.9 30.9	7.8 30.0	8.7 29.1
2000	0.4 37.4	0.8 37.0	1.7 36.1	2.5 35.3	3.3 34.5	4.1 33.7	5.0 32.8	5.8 32.0	6.6 31.2	7.4 30.4	8.3 29.5

Grey boxes: Volumes that would exceed the allowable water volume in each reaction
Yellow boxes: Indicate a low transfer volume that may result in higher cell load variability
Blue boxes: Optimal range of cell stock concentration to maximize the likelihood of achieving the desired cell recovery target

1.2 Load Chromium Next GEM Chip G

Next GEM

After removing the chip from the sealed bag, use in ≤ 24 h. For all **chip loading steps**, raising and depressing the pipette plunger should each take **~5 sec**. When dispensing, raise the pipette tips at the same rate as the liquid is rising, keeping the tips slightly submerged.

a. Dispense 50% Glycerol into Unused Chip Wells (if < 8 samples per chip)

i. **70 µl** to unused wells in **row labeled 1**.
ii. **50 µl** to unused wells in **row labeled 2**.
iii. **45 µl** to unused wells in **row labeled 3**.

DO NOT add 50% glycerol solution to the bottom row of NO FILL wells. DO NOT use any substitute for 50% glycerol solution.

b. Prepare Master Mix + Cell Suspension

Refer to the Cell Suspension Volume Calculator Table. Add the appropriate volume of nuclease-free water and corresponding volume of single cell suspension to Master Mix for a total of **75 µl** in each tube. Gently pipette mix the cells suspension before adding to the Master Mix.

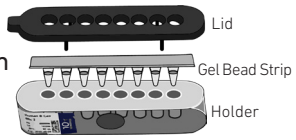
c. Load Row Labeled 1

Gently pipette mix the Master Mix + Cell Suspension and using the same pipette tip, dispense **70 µl** Master Mix + Cell Suspension into the bottom center of each well in **row labeled 1** without introducing bubbles.

The illustrated chip is being loaded for 8 samples.

d. Prepare Gel Beads

Snap the tube strip holder with the Gel Bead strip into a 10x Vortex Adapter. Vortex **30 sec.** Centrifuge the Gel Bead strip for **~5 sec.** Confirm there are no bubbles at the bottom of the tubes and the liquid levels are even. Place the Gel Bead strip back in the holder. Secure the holder lid.



e. Load Row Labeled 2

Puncture the foil seal of the Gel Bead tubes. Slowly aspirate **50 µl** Gel Beads. Dispense into the wells in **row labeled 2** without introducing bubbles. Wait **30 sec.**

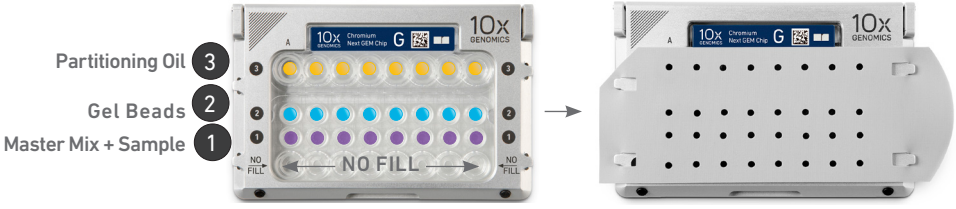
f. Load Row Labeled 3

Dispense **45 µl** Partitioning Oil into the wells in **row labeled 3** from a reagent reservoir. Failure to add Partitioning Oil to the top row labeled 3 will prevent GEM generation and can damage the Chromium Controller.

Attach the gasket and run the chip in the Chromium Controller **immediately** after loading the Partitioning Oil.

g. Attach 10x Gasket

Align the notch with the top left-hand corner. Ensure the gasket holes are aligned with the wells. Avoid touching the smooth surface.



Keep horizontal to avoid wetting the gasket. DO NOT press down on the gasket.

1.3 Run the Chromium Controller

Next GEM

a. Press the eject button on the Controller to eject the tray.

b. Place the assembled chip with the gasket in the tray, ensuring that the chip stays horizontal. Press the button to retract the tray.

c. Confirm the Chromium Chip G program on screen. Press the play button.

d. At completion of the run **(~18 min)**, the Controller will chime. **Immediately** proceed to the next step.

Firmware Version 4.0 or higher is required in the Chromium Controller or the Chromium Single Cell Controller used for the Single Cell V(D)J v1.1 protocol.

1.4 Transfer GEMs

Next GEM

a. Place a tube strip on ice.

b. Press the eject button of the Controller and remove the chip.

c. Discard the gasket. Open the chip holder. Fold the lid back until it clicks to expose the wells at 45 degrees.

d. Check the volume in rows labeled 1-2. Abnormally high volume in any well indicates a clog.

e. Slowly aspirate **100 µl** GEMs from the lowest points of the recovery wells in the top row labeled 3 without creating a seal between the pipette tips and the bottom of the wells.

f. Withdraw pipette tips from the wells. GEMs should appear opaque and uniform across all channels. Excess Partitioning Oil (clear) in the pipette tips indicates a potential clog.

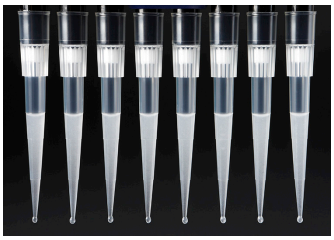
g. Over the course of **~20 sec**, dispense GEMs into the tube strip on ice with the pipette tips against the sidewalls of the tubes.

h. If multiple chips are run back-to-back, cap/cover the GEM-containing tube strip and place on ice for no more than **1 h**.

Expose Wells at 45 Degrees

Transfer GEMs

GEMs



1.5 GEM-RT Incubation

Use a thermal cycler that can accommodate at least 100 µl volume. A volume of 125 µl is the preferred setting on Bio-Rad C1000 Touch. In alternate thermal cyclers, use highest reaction volume setting.

a. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
53°C	125 µl	~55 min
Step	**Temperature**	**Time**
1	53°C	00:45:00
2	85°C	00:05:00
3	4°C	Hold

b. Store at **4°C** for up to **72 h** or at **–20°C** for up to a **week**, or proceed to the next step.

Step 2

Post GEM-RT Cleanup

2.1 Post GEM-RT Cleanup – Dynabeads

2.0 Post GEM-RT Cleanup

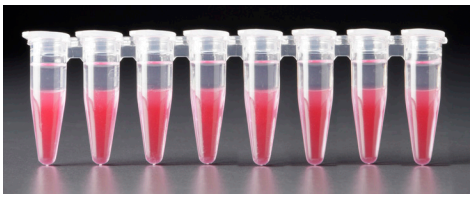
GET STARTED!

Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	●	**Additive A**	220074	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
		Dynabeads MyOne SILANE	2000048	Vortex thoroughly (≥30 sec) **immediately** before adding to the mix. If still clumpy, pipette mix to resuspend completely. DO NOT centrifuge before use.	4°C
Thaw at 65°C	●	**Buffer Sample Clean Up 1**	220020	Thaw for 10 min at 65°C at max speed on a thermomixer. Verify there are no visible crystals. Cool to room temperature.	–20°C
Obtain	○	**Recovery Agent**	220016	-	Ambient
		Qiagen Buffer EB	-	Manufacturer's recommendations.	-
		Bio-Rad 10% Tween 20	-	Manufacturer's recommendations.	-
		10x Magnetic Separator	230003	-	Ambient
		Prepare 80% Ethanol Prepare 15 ml for 8 reactions.	-	Prepare fresh.	-

2.1 Post GEM-RT Cleanup – Dynabeads

a. Add **125 µl** Recovery Agent to each sample (post GEM-RT incubation) at room temperature. DO NOT pipette mix or vortex the biphasic mixture. Wait **2 min**.

Biphasic Mixture

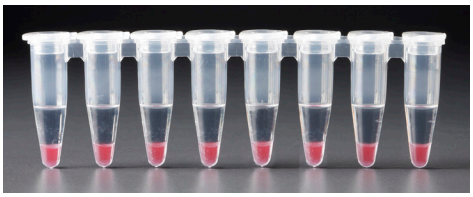


The resulting biphasic mixture contains Recovery Agent/Partitioning Oil (pink) and aqueous phase (clear), with no persisting emulsion (opaque).

If biphasic separation is incomplete:
Firmly secure the cap on the tube strip, ensuring that no liquid is trapped between the cap and the tube rim. Mix by inverting the capped tube strip 5x, centrifuge briefly, and proceed to step b. DO NOT invert without firmly securing the caps.

A smaller aqueous phase volume indicates a clog during GEM generation.

Remove Recovery Agent



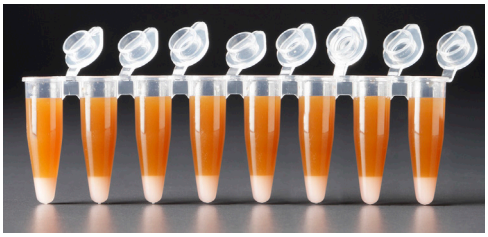
b. Slowly remove and discard **125 µl** Recovery Agent/Partitioning Oil (pink) from the bottom of the tube. DO NOT aspirate any aqueous sample.

c. Prepare Dynabeads Cleanup Mix.

Dynabeads Cleanup Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water		5	22	44
Buffer Sample Clean Up 1	220020	182	801	1602
Dynabeads MyOne SILANE Vortex thoroughly (≥30 sec) **immediately** before adding to the mix. Aspirate the full liquid volume with a pipette tip to verify that the beads have not settled in the bottom of the tube. If clumps are present, pipette mix to resuspend completely. DO NOT centrifuge before use.	2000048	8	35	70
Additive A	220074	5	22	44
Total	-	**200**	**880**	**1760**

Resuspend clump

Add Dynabeads Cleanup Mix



d. Vortex and add **200 µl** to each sample. Pipette mix 5x (pipette set to 200 µl).

e. Incubate **10 min** at **room temperature**.

f. Prepare Elution Solution I. Vortex and centrifuge briefly.

	Elution Solution I *Add reagents in the order listed*	PN	1X (µl)	10X (µl)
	Buffer EB	-	98	980
	10% Tween 20	-	1	10
●	**Additive A**	220074	1	10
	Total	**-**	**100**	**1000**

g. At the end of **10 min** incubation, place on a 10x Magnetic Separator•**High position** (magnet•**High)** until the solution clears.

A white interface between the aqueous phase and Recovery Agent is normal.

h. Remove the supernatant.

i. Add **300 µl** 80% ethanol to the pellet while on the magnet. Wait **30 sec**.

j. Remove the ethanol.

k. Add **200 µl** 80% ethanol to pellet. Wait **30 sec**.

l. Remove the ethanol.

m. Centrifuge briefly. Place on the magnet•**Low**.

n. Remove remaining ethanol. Air dry for **2 min**.

o. Remove from the magnet. Immediately add **35.5 µl** Elution Solution I.

p. Pipette mix (pipette set to 30 µl) without introducing bubbles. Pipette mix 15x. If beads still appear clumpy, continue pipette mixing until fully resuspended.

q. Incubate **1 min** at **room temperature**.

r. Place on the magnet•**Low** until the solution clears.

s. Transfer **35 µl** sample to a new tube strip.

Step 3

cDNA Amplification & QC

3.1 cDNA Amplification

3.2 cDNA Cleanup – SPRIselect

3.3 cDNA QC & Quantification

3.0 cDNA Amplification & QC

GET STARTED!

Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	**cDNA Additive**	220067	Vortex, centrifuge briefly.	–20°C
	cDNA Primer Mix	220106	Vortex, centrifuge briefly.	–20°C
	Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations.	-
	Agilent Bioanalyzer High Sensitivity Kit If used for QC and quantification	-	Manufacturer's recommendations.	-
	Agilent TapeStation ScreenTape and Reagents If used for QC and quantification	-	Manufacturer's recommendations.	-
	Qubit dsDNA HS Assay Kit If used for quantification	-	Manufacturer's recommendations.	-
Place on ice	**Amplification Master Mix**	220125	Vortex, centrifuge briefly.	–20°C
Obtain	**Qiagen Buffer EB**	-	Manufacturer's recommendations.	-
	10x Magnetic Separator	230003	-	Ambient
	Prepare 80% Ethanol Prepare 15 ml for 8 samples	-	Prepare fresh.	-

3.1 cDNA Amplification

a. Prepare cDNA Amplification Mix on ice. Vortex and centrifuge briefly.

cDNA Amplification Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water	-	8	35	70
Amplification Master Mix	220125	50	220	440
cDNA Additive	220067	5	22	44
cDNA Primer Mix	220106	2	9	18
Total	-	**65**	**286**	**572**

b. Add **65 µl** cDNA Amplification Mix to **35 µl** sample (Post GEM-RT Cleanup).

c. Pipette mix 5x (pipette set to 90 µl). Centrifuge briefly.

d. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~25-50 min
Step	**Temperature**	**Time**
1	98°C	00:00:45
2	98°C	00:00:20
3	67°C	00:00:30
4	72°C	00:01:00
5	Go to Step 2, see table below for total # of cycles	
6	72°C	00:01:00
7	4°C	Hold

TIPS

The optimal number of cycles is a trade-off between generating sufficient final mass for library construction and minimizing PCR amplification artifacts. Cycle numbers were optimized assuming that sample includes >80% T or B cells. If testing cells types with a known fraction of T and/or B cells, adjust cycle number based on that fraction to generate sufficient product. See Tips and Best Practices for examples.

Recommended starting point for cycle number optimization.

Targeted Cell Recovery	Primary Cells Total Cycles	Cell Lines Total Cycles
100 – 500	18	16
501 – 2,000	16	14
2,001 – 6,000	14	12
6,001 – 10,000	13	11

STOP

e. Store at **4°C** for up to **72 h** or proceed to the next step.

3.2 cDNA Cleanup – SPRIselect

a. Vortex to resuspend the SPRIselect reagent. Add **60 µl** SPRIselect reagent **(0.6X)** to each sample and pipette mix 15x (pipette set to 150 µl).

b. Incubate **5 min** at **room temperature**.

c. Place on the magnet•**High** until the solution clears.

d. Remove the supernatant.

e. Add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

f. Remove the ethanol.

g. **Repeat** steps e and f for a total of 2 washes.

h. Centrifuge briefly and place on the magnet•**Low**.

i. Remove any remaining ethanol. Air dry for **2 min**.

j. Remove from the magnet. Add **45.5 µl** Buffer EB. Pipette mix 15x.

k. Incubate **2 min** at **room temperature**.

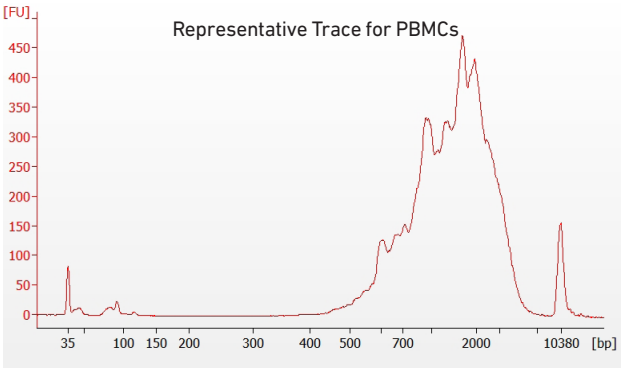
l. Place the tube strip on the magnet•**High** until the solution clears.

m. Transfer **45 µl** sample to a new tube strip.

n. Store at **4°C** for up to **72 h** or at **–20°C** for up to **a week,** or proceed to the next step.

3.3 cDNA QC & Quantification

a. Run **1 µl** undiluted sample (Dilution Factor 1) on an Agilent Bioanalyzer High Sensitivity chip.



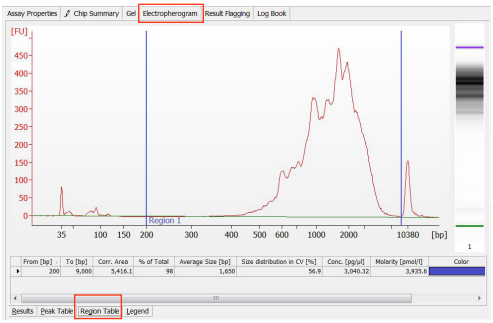
For 5' Gene Expression Library Construction proceed directly to step 6 after step 3.3.

b. If proceeding to 5' GEX Library Construction (step 6), determine cDNA yield for each sample. Example calculation below.

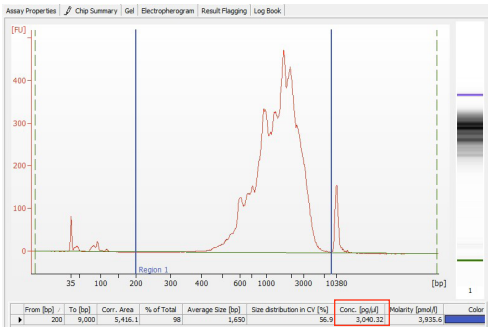
EXAMPLE CALCULATION

i. Select Region

Under the "Electropherogram" view choose the "Region Table". Manually select the region of ~200 – ~9000 bp



ii. Note Concentration [pg/µl]



iii. Calculate

Concentration: 3040.32 pg/µl
Dilution Factor: 1

cDNA Conc. =

$$\frac{\text{Conc. (pg/µl)} \times \text{Dilution Factor}}{\text{1000 (pg/ng)}} = \frac{3040.32 \times 1}{1000} = \textbf{3 ng/µl}$$

Example Calculation for Carrying Forward 50 ng Sample for 5' GEX Library Construction

Volume for 50 ng = $\frac{\text{50 ng}}{\text{3 (ng/µl)}}$ = **16.7 µl**

5' GEX Library Construction Sample
=16.7 µl + 3.3 µl nuclease-free water
= 20 µl total

If <50 ng available, carry forward 20 µl sample (2-50 ng) into 5' GEX Library Construction.

DO NOT exceed a mass of 50 ng in the 20 µl carry forward volume.

Alternate Quantification Methods:

- Agilent TapeStation. See Appendix for representative traces
- Qubit Fluorometer and Qubit dsDNA HS Assay Kit.

Step 4

Target Enrichment from cDNA

4.0 Target Enrichment from cDNA

GET STARTED!

Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	**For Human Samples** (Choose B or T-cell primers based on desired enrichment products)			
	Human T Cell Mix 1	2000008	Vortex, centrifuge briefly.	–20°C
	Human T Cell Mix 2	2000009	Vortex, centrifuge briefly.	–20°C
	Human B Cell Mix 1	2000035	Vortex, centrifuge briefly.	–20°C
	Human B Cell Mix 2	2000036	Vortex, centrifuge briefly.	–20°C
	For Mouse Samples (Choose B or T-cell primers based on desired enrichment products)			
	Mouse T Cell Mix 1	2000075	Vortex, centrifuge briefly.	–20°C
	Mouse T Cell Mix 2	2000079	Vortex, centrifuge briefly.	–20°C
	Mouse B Cell Mix 1	2000080	Vortex, centrifuge briefly.	–20°C
	Mouse B Cell Mix 2	2000081	Vortex, centrifuge briefly.	–20°C
	For all Samples			
	cDNA Additive	220067	Vortex, centrifuge briefly.	–20°C
	Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations.	-
	Agilent Bioanalyzer High Sensitivity Kit If used for QC and quantification	-	Manufacturer's recommendations.	-
	Agilent TapeStation ScreenTape and Reagents If used for QC and quantification	-	Manufacturer's recommendations.	-
	Qubit dsDNA HS Assay Kit If used for quantification	-	Manufacturer's recommendations.	-
Place on Ice	**Amplification Master Mix**	220125	Vortex, centrifuge briefly.	–20°C
Obtain	**Qiagen Buffer EB**	-	-	Ambient
	10x Magnetic Separator	230003	See Tips & Best Practices.	Ambient
	Prepare 80% Ethanol Prepare 15 ml for 8 reactions	-	See Tips & Best Practices.	Ambient

4.1 Target Enrichment 1

a. Add **33 µl** nuclease-free water into a tube strip on ice and then transfer **2 µl** sample (post cDNA Amplification & QC, step 3.3) to the same tube for a total of **35 µl.**

b. Prepare Target Enrichment 1 Reaction Mix on ice. Vortex and centrifuge briefly.

Target Enrichment 1 Reaction Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water	-	5	22	44
Amplification Master Mix	220125	50	220	440
cDNA Additive	220067	5	22	44
T Cell Mix 1 **or** **B Cell Mix 1**	Human 2000008/ Mouse 2000075 or Human 2000035/ Mouse 2000080	5	22	44
Total	-	**65**	**286**	**572**

c. Add **65 µl** Target Enrichment 1 Reaction Mix to each tube containing **35 µl** sample.

d. Pipette mix 5x (pipette set to 90 µl). Centrifuge briefly.

e. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~20-30 min
Step	**Temperature**	**Time**
1	98°C	00:00:45
2	98°C	00:00:20
3	67°C	00:00:30
4	72°C	00:01:00
5 Different cycle numbers for T & B cells	T Cell: Go to Step 2, 9x (total 10 cycles) B Cell: Go to Step 2, 5x (total 6 cycles)	
6	72°C	00:01:00
7	4°C	Hold

STOP

f. Store at **4°C** for up to **72 h** or proceed to the next step.

4.2 Post Target Enrichment 1 Cleanup – SPRIselect

a. Vortex to resuspend the SPRIselect reagent. Add **80 µl** SPRIselect reagent **(0.8X)** to each sample. Pipette mix 15x (pipette set to 150 µl).

b. Incubate **5 min** at **room temperature**.

c. Place tube strip on the magnet•**High** until the solution clears.

d. Remove the supernatant.

e. Add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

f. Remove the ethanol.

g. **Repeat** steps e and f for a total of 2 washes.

h. Centrifuge briefly. Place on the magnet•**Low**.

i. Remove remaining ethanol. Air dry for **2 min**.

j. Remove from the magnet. Add **35.5 µl** Buffer EB. Pipette mix 15x.

k. Incubate **2 min** at **room temperature**.

l. Place on the magnet•**Low** until the solution clears.

m. Transfer **35 µl** sample to a new tube strip.

n. Store at **4°C** in for up to **72 h** or at **–20°C** for up to **a week**, or proceed to the next step.

4.3 Target Enrichment 2

a. Prepare Target Enrichment 2 Reaction Mix on ice. Vortex and centrifuge briefly.

Target Enrichment 2 Reaction Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water	-	5	22	44
Amplification Master Mix	220125	50	220	440
cDNA Additive	220067	5	22	44
T Cell Mix 2 **or** **B Cell Mix 2**	Human 2000009/ Mouse 2000079 or Human 2000036/ Mouse 2000081	5	22	44
Total	-	**65**	**286**	**572**

c. Add **65 µl** Target Enrichment 2 Reaction Mix to each tube containing **35 µl** sample.

d. Pipette mix 5x (pipette set to 90 µl). Centrifuge briefly.

e. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~25-30 min
Step	**Temperature**	**Time**
1	98°C	00:00:45
2	98°C	00:00:20
3	67°C	00:00:30
4	72°C	00:01:00
5 Different cycle numbers for T & B cells	T Cell: Go to Step 2, 9x (total 10 cycles) B Cell: Go to Step 2, 7x (total 8 cycles)	
6	72°C	00:01:00
7	4°C	Hold

STOP

f. Store at **4°C** for up to **72 h** or proceed to the next step.

4.4 Post Target Enrichment 2 Double Sided Size Selection – SPRIselect

a. Vortex to resuspend SPRIselect reagent. Add **50 µl** SPRIselect reagent **(0.5X)** to each sample. Pipette mix 15x (pipette set to 145 µl).

b. Incubate **5 min** at **room temperature**.

c. Place on the magnet•**High** until the solution clears. DO NOT discard supernatant.

d. Transfer **145 µl** supernatant to a new tube strip.

e. Vortex to resuspend SPRIselect reagent. Add **30 µl** SPRIselect reagent **(0.8X)** to each sample. Pipette mix 15x (pipette set to 150 µl).

f. Incubate **5 min** at **room temperature**.

g. Place on the magnet•**High** until the solution clears.

h. Remove **170 µl** supernatant. DO NOT discard any beads.

i. Add **200 µl** 80% ethanol. Wait **30 sec**.

j. Remove the ethanol.

k. **Repeat** steps i and j for a total of 2 washes.

l. Centrifuge briefly. Place on the magnet•**Low**.

m. Remove remaining ethanol wash. DO NOT over-dry beads to ensure maximum elution efficiency.

n. Remove from the magnet. Add **45.5 µl** Buffer EB. Pipette mix 15x.

o. Incubate **2 min** at **room temperature**.

p. Place on the magnet•**Low** until the solution clears.

q. Transfer **45 µl** sample to a new tube strip.

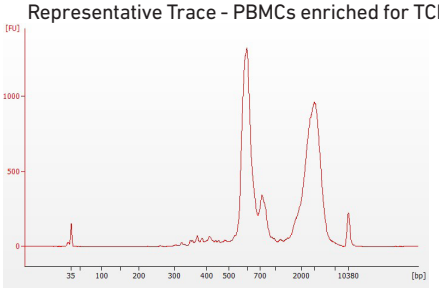
r. Store at **4°C** for up to **72 h** or at **–20°C** for up to **1 week**, or proceed to the next step.

4.5 Post Target Enrichment QC & Quantification

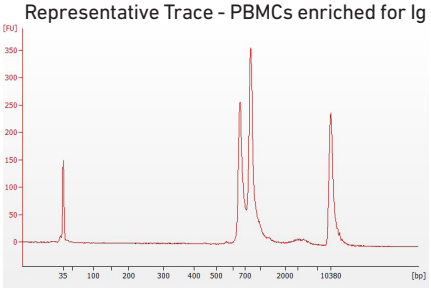
a. Run **1 µl** sample at **1:5 dilution** (Dilution Factor 5) on an Agilent Bioanalyzer High Sensitivity chip.

Samples of RNA-rich cells may require additional dilution in nuclease-free water. The number of distinct peaks may vary. Higher molecular weight product (2,000- 9,000 bp) may be present. This does not affect sequencing.

Representative Trace - PBMCs enriched for TCR



Representative Trace - PBMCs enriched for Ig

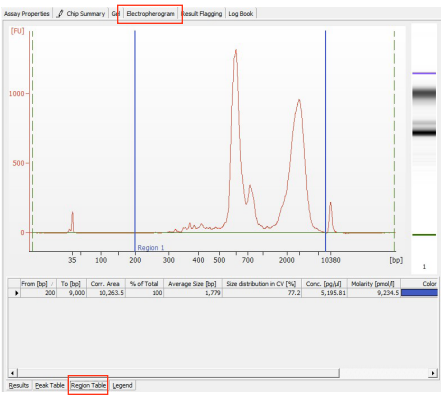


b. Determine yield for each sample. Example calculation below.

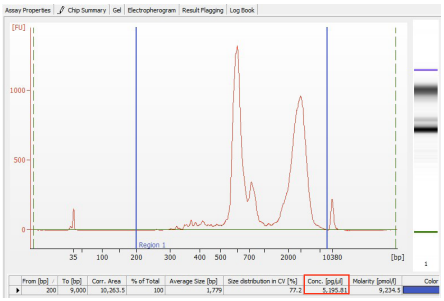
EXAMPLE CALCULATION

i. Select Region

Under the "Electropherogram" view choose the "Region Table". Manually select the region of ~200 – ~9000 bp.



ii. Note Concentration [pg/µl]



iii. Calculate

Concentration: 5195.81 pg/µl
Dilution Factor: 5

Enriched Product Conc.

$$\frac{\text{Conc. (pg/µl)} \times \text{Dilution Factor}}{\text{1000 (pg/ng)}} = \frac{5195.81 \times 5}{1000} = \textbf{26 ng/µl}$$

Example Calculation for Carrying Forward 50 ng Sample for Enriched Library Construction

$$\text{Volume for 50 ng} = \frac{\text{50 ng}}{\text{26 (ng/µl)}} = \textbf{1.9 µl}$$

Enriched Library Construction Sample
=1.9 µl + 18.1 µl nuclease-free water
=20 µl total

If <50 ng available, carry forward 20 µl sample (2-50 ng) into Enriched Library Construction.

DO NOT exceed a mass of 50 ng in the 20 µl carry forward volume.

Alternate Quantification Methods:

- Qubit Fluorometer and Qubit dsDNA HS Assay Kit.

Step 5

Enriched Library Construction

5.0 Enriched Library Construction

GET STARTED!

Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	**Fragmentation Buffer**	220108	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
	Adaptor Mix	220026	Vortex, centrifuge briefly.	–20°C
	Ligation Buffer	220109	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
	SI-PCR Primer	220111	Vortex, centrifuge briefly.	–20°C
	Chromium i7 Sample Index Plate	220103	-	–20°C
	Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations.	-
	Agilent Bioanalyzer High Sensitivity Kit If used for QC	-	Manufacturer's recommendations.	-
	Agilent TapeStation ScreenTape and Reagents If used for QC	-	Manufacturer's recommendations.	-
Place on Ice	**Fragmentation Enzyme Blend**	220107/ 220130	Centrifuge briefly.	–20°C
	DNA Ligase	220110/ 220131	Centrifuge briefly.	–20°C
	Amplification Master Mix	220125	Vortex, centrifuge briefly.	–20°C
Obtain	**Qiagen Buffer EB**	-	-	Ambient
	10x Magnetic Separator	230003	See Tips & Best Practices.	Ambient
	Prepare 80% Ethanol Prepare 15 ml for 8 reactions	-	Prepare fresh.	Ambient

5.1 Fragmentation, End Repair & A-tailing

a. Determine the volume for **50 ng** mass of sample (see example calculation at step 4.5). Dispense the sample volume in a tube strip **on ice**. If the volume required for **50 ng** is less than **20 µl**, adjust the total volume of each sample to **20 µl** with nuclease-free water. If the volume for **50 ng** exceeds **20 µl**, carry only **20 µl** sample into library construction.

b. Prepare a thermal cycler with the following incubation protocol.

Lid Temperature	Reaction Volume	Run Time
65°C	50 µl	~35 min
Step	**Temperature**	**Time**
Pre-cool block Pre-cool block prior to preparing the Fragmentation Mix	4°C	Hold
Fragmentation	32°C	00:02:00
End Repair & A-tailing	65°C	00:30:00
Hold	4°C	Hold

c. Vortex Fragmentation Buffer. Verify there is no precipitate.

d. Prepare Fragmentation Mix on ice. Pipette mix and centrifuge briefly.

Fragmentation Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water	-	15	66	132
○ **Fragmentation Buffer**	220108	5	22	44
● **Fragmentation Enzyme Blend**	220107/ 220130	10	44	88
Total	-	**30**	**132**	**264**

e. Add **30 µl** Fragmentation Mix into each tube containing **20 µl** sample.

f. Pipette mix 15x (pipette set to 30 µl) on ice. Centrifuge briefly.

g. Transfer into the pre-cooled thermal cycler **(4°C)** and press "SKIP" to initiate the protocol.

5.2 Adaptor Ligation

a. Prepare Adaptor Ligation Mix. Pipette mix and centrifuge briefly.

Adaptor Ligation Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water	-	17.5	77	154
Ligation Buffer	220109	20	88	176
DNA Ligase	220110/ 220131	10	44	88
Adaptor Mix	220026	2.5	11	22
Total	-	**50**	**220**	**440**

b. Remove the sample from the thermal cycler.

c. Add **50 µl** Adaptor Ligation Mix to **50 µl** sample. Pipette mix 15x (pipette set to 90 µl). Centrifuge briefly.

d. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
30°C	100 µl	15 min
Step	**Temperature**	**Time**
1	20°C	00:15:00
2	4°C	Hold

5.3 Post Ligation Cleanup – SPRIselect

a. Vortex to resuspend SPRIselect Reagent. Add **80 µl** SPRIselect Reagent (**0.8X**) to each sample. Pipette mix 15x (pipette set to 150 µl).

b. Incubate **5 min** at **room temperature**.

c. Place on the magnet•**High** until the solution clears.

d. Remove the supernatant.

e. Add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

f. Remove the ethanol.

g. **Repeat** steps e and f for a total of 2 washes.

h. Centrifuge briefly. Place on the magnet•**Low**.

i. Remove any remaining ethanol. Air dry for **2 min**.

j. Remove from the magnet. Add **30.5 µl** Buffer EB. Pipette mix 15x. If beads still appear clumpy, continue pipette mixing until fully resuspended.

k. Incubate **2 min** at **room temperature**.

l. Place on the magnet•**Low** until the solution clears.

m. Transfer **30 µl** sample to a new tube strip.

5.4 Sample Index PCR

a. Choose the appropriate sample index sets to ensure that no sample indices overlap in a multiplexed sequencing run.
Record the 10x Sample Index name (PN-220103 Chromium i7 Sample Index Plate well ID) used.

b. Prepare Sample Index PCR Mix.

Sample Index PCR Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water	-	8	35	70
Amplification Master Mix	220125	50	220	440
SI-PCR Primer	220111	2	9	18
Total	-	**60**	**264**	**528**

c. Add **60 µl** Sample Index PCR Mix to **30 µl** sample.

d. Add **10 µl** of an individual Chromium i7 Sample Index to each well and record the well ID. Pipette mix 5x (pipette set to 90 µl). Centrifuge briefly.

e. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~30 min
Step	**Temperature**	**Time**
1	98°C	00:00:45
2	98°C	00:00:20
3	54°C	00:00:30
4	72°C	00:00:20
5	Go to step 2, 8x (total 9 cycles)	
6	72°C	00:01:00
7	4°C	Hold

STOP

f. Store at **4°C** for up to **72 h** or proceed to the next step.

5.5 Post Sample Index PCR Cleanup – SPRIselect

a. Vortex to resuspend the SPRIselect reagent. Add **80 µl** SPRIselect Reagent **(0.8X)** to each sample. Pipette mix 15x (pipette set to 150 µl).

b. Incubate **5 min** at **room temperature**.

c. Place the magnet•**High** until the solution clears.

d. Remove the supernatant.

e. Add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

f. Remove the ethanol.

g. **Repeat** steps e and f for a total of 2 washes.

h. Centrifuge briefly. Place on the magnet•**Low**.

i. Remove remaining ethanol. Air dry for **2 min**.

j. Remove from the magnet. Add **35.5 µl** Buffer EB. Pipette mix 15x.

k. Incubate **2 min** at **room temperature**.

l. Place on the magnet•**Low** until the solution clears.

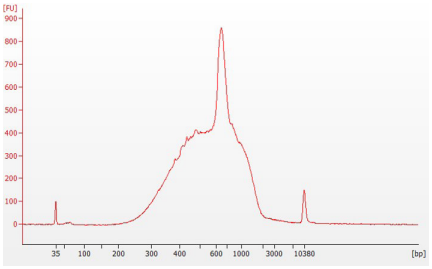
m. Transfer **35 µl** to a new tube strip.

n. Store at **4°C** for up to **72 h** or at **–20°C** for long-term storage.

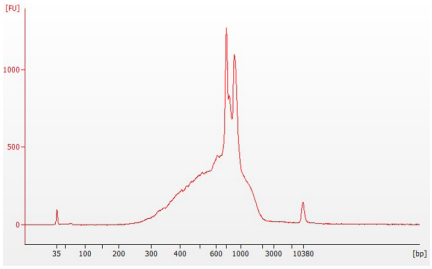
5.6 Post Library Construction QC

a. Run **1 µl s**ample at **1:10 dilution** on an Agilent Bioanalyzer High Sensitivity chip.

Representative Trace - PBMCs enriched for TCR



Representative Trace - PBMCs enriched for Ig



b. Determine the average fragment size from the trace. This will be used as the insert size for library quantification.

Alternate QC Method:

- Agilent TapeStation. See Appendix for representative traces

See Appendix for Post Library Construction Quantification

Step 6

5' Gene Expression (GEX) Library Construction

6.0 5' Gene Expression (GEX) Library Construction

GET STARTED!

Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	**Fragmentation Buffer**	220108	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
	Adaptor Mix	220026	Vortex, centrifuge briefly.	–20°C
	Ligation Buffer	220109	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
	SI-PCR Primer	220111	Vortex, centrifuge briefly.	–20°C
	Chromium i7 Sample Index Plate	220103	-	–20°C
	Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations.	-
	Agilent Bioanalyzer DNA 1000 kit If used for QC	-	Manufacturer's recommendations.	-
	Agilent TapeStation ScreenTape and Reagents If used for QC	-	Manufacturer's recommendations.	-
Place on Ice	**Fragmentation Enzyme Blend**	220107/ 220130	Centrifuge briefly.	–20°C
	DNA Ligase	220110/ 220131	Centrifuge briefly.	–20°C
	Amplification Master Mix	220125	Vortex, centrifuge briefly.	–20°C
Obtain	**Qiagen Buffer EB**	-	-	Ambient
	10x Magnetic Separator	230003	See Tips & Best Practices.	Ambient
	Prepare 80% Ethanol Prepare 15 ml for 8 reactions	-	Prepare fresh.	Ambient

6.1 GEX Fragmentation, End Repair & A-tailing

a. Determine the volume for **50 ng** mass of sample (see example calculation at step 3.3). Dispense the sample volume in a tube strip **on ice**. If the volume required for **50 ng** is less than **20 µl**, adjust the total volume of each sample to **20 µl** with nuclease-free water. If the volume for **50 ng** exceeds **20 µl**, carry only **20 µl** sample into library construction.

b. Prepare a thermal cycler with the following incubation protocol.

Lid Temperature	Reaction Volume	Run Time
65°C	50 µl	~35 min
Step	**Temperature**	**Time**
Pre-cool block Pre-cool block prior to preparing the Fragmentation Mix	4°C	Hold
Fragmentation	32°C	00:05:00
End Repair & A-tailing	65°C	00:30:00
Hold	4°C	Hold

c. Vortex Fragmentation Buffer. Verify there is no precipitate.

d. Prepare Fragmentation Mix on ice. Pipette mix and centrifuge briefly.

Fragmentation Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water	-	15	66	132
○ **Fragmentation Buffer**	220108	5	22	44
● **Fragmentation Enzyme Blend**	220107/ 220130	10	44	88
Total	-	**30**	**132**	**264**

e. Add **30 µl** Fragmentation Mix into each tube containing **20 µl** sample.

f. Pipette mix 15x (pipette set to 30 µl) on ice. Centrifuge briefly.

g. Transfer into the pre-cooled thermal cycler **(4°C)** and press "SKIP" to initiate the protocol.

6.2 GEX Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect

a. Vortex to resuspend SPRIselect Reagent. Add 3**0 µl** SPRIselect Reagent **(0.6X)** to each sample. Pipette mix 15x (pipette set to 75 µl).

b. Incubate **5 min** at **room temperature**.

c. Place on the magnet•**High** until the solution clears. DO NOT discard supernatent.

d. Transfer **75 µl** supernatant to a new tube strip.

e. Add **10 µl** SPRIselect reagent **(0.8X)** to each sample. Pipette mix 15x (pipette set to 75 µl).

f. Incubate **5 min** at **room temperature**.

g. Place on the magnet•**High** until the solution clears.

h. Remove **80 µl** supernatant. DO NOT discard any beads.

i. With the tube strip still on the magnet, add **125 µl** 80% ethanol to the pellet. Wait **30 sec**.

j. Remove the ethanol.

k. **Repeat** steps i and j for a total of 2 washes.

l. Centrifuge briefly. Place on the magnet •**Low**.

m. Remove the ethanol. DO NOT over-dry beads to ensure maximum elution efficiency.

n. Remove from the magnet. Add **50.5 µl** Buffer EB. Pipette mix 15x.

o. Incubate **2 min** at **room temperature**.

p. Place on the magnet•**High** until the solution clears.

q. Transfer **50 µl** sample to a new tube strip.

6.3 GEX Adaptor Ligation

a. Prepare Adaptor Ligation Mix. Pipette mix and centrifuge briefly.

Adaptor Ligation Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water	-	17.5	77	154
Ligation Buffer	220109	20	88	176
DNA Ligase	220110/ 220131	10	44	88
Adaptor Mix	220026	2.5	11	22
Total	-	**50**	**220**	**440**

b. Add **50 µl** Adaptor Ligation Mix to **50 µl** sample. Pipette mix 15x (pipette set to 90 µl). Centrifuge briefly.

c. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
30°C	100 µl	15 min
Step	**Temperature**	**Time**
1	20°C	00:15:00
2	4°C	Hold

6.4 GEX Post Ligation Cleanup – SPRIselect

a. Vortex to resuspend SPRIselect Reagent. Add **80 µl** SPRIselect Reagent **(0.8X)** to each sample. Pipette mix 15x (pipette set to 150 µl).

b. Incubate **5 min** at **room temperature**.

c. Place on the magnet•**High** until the solution clears.

d. Remove the supernatant.

e. Add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

f. Remove the ethanol.

g. **Repeat** steps e and f for a total of 2 washes.

h. Centrifuge briefly. Place on the magnet•**Low**.

i. Remove any remaining ethanol. Air dry for **2 min**.

j. Remove from the magnet. Add **30.5 µl** Buffer EB. Pipette mix 15x.

k. Incubate **2 min** at **room temperature**.

l. Place on the magnet•**Low** until the solution clears.

m. Transfer **30 µl** sample to a new tube strip.

6.5 GEX Sample Index PCR

a. Choose the appropriate sample index sets to ensure that no sample indices overlap in a multiplexed sequencing run.

b. Record the 10x Sample Index name (PN-220103 Chromium i7 Sample Index Plate well ID) used, especially if running more than one sample.

c. Prepare Sample Index PCR Mix. Pipette mix and centrifuge briefly.

Sample Index PCR Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water	-	8	35	70
Amplification Master Mix	220125	50	220	440
SI-PCR Primer	220111	2	9	18
Total	-	**60**	**264**	**528**

d. Add **60 µl** Sample Index PCR Mix to **30 µl** sample.

e. Add **10 µl** of an individual Chromium i7 Sample Index to each well and record their assignment. Pipette mix 5x (pipette set to 90 µl). Centrifuge briefly.

f. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~40 min
Step	**Temperature**	**Time**
1	98°C	00:00:45
2	98°C	00:00:20
3	54°C	00:00:30
4	72°C	00:00:20
5	Go to step 2, see table below for # cycles	
6	72°C	00:01:00
7	4°C	Hold

The table recommends starting point for optimization. If less than 50 ng was carried into 5' Gene Expression Library Construction, refer to the product yield calculation example in step 3.3 to determine the mass input into Library Construction.

Input into Library Construction	Total Sample Index Cycles
1 - 25 ng	16
26 - 50 ng	14

STOP

g. Store at **4°C** for up to **72 h** or proceed to the next step.

6.6 GEX Post Sample Index PCR Double Sided Size Selection – SPRIselect

a. Vortex to resuspend SPRIselect reagent. Add **60 µl** SPRIselect reagent **(0.6X)** to each sample. Pipette mix 15x (pipette set to 150 µl).

b. Incubate **5 min** at **room temperature**.

c. Place on the magnet•**High** until the solution clears. DO NOT discard supernatant.

d. Transfer **150 µl** supernatant to a new tube strip.

e. Vortex to resuspend SPRIselect reagent. Add **20 µl** SPRIselect reagent **(0.8X)** to each sample. Pipette mix 15x (pipette set to 150 µl).

f. Incubate **5 min** at **room temperature.**

g. Place on the magnet•**High** until the solution clears.

h. Remove **165 µl** supernatant. DO NOT discard any beads.

i. With the tube strip still on the magnet, add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

j. Remove the ethanol.

k. **Repeat** steps i and j for a total of 2 washes.

l. Centrifuge briefly. Place on the magnet•**Low**.

m. Remove the remaining ethanol. DO NOT over-dry beads to ensure maximum elution efficiency.

n. Remove the tube strip from the magnet. Add **35.5 µl** Buffer EB. Pipette mix 15x.

o. Incubate **2 min** at **room temperature**.

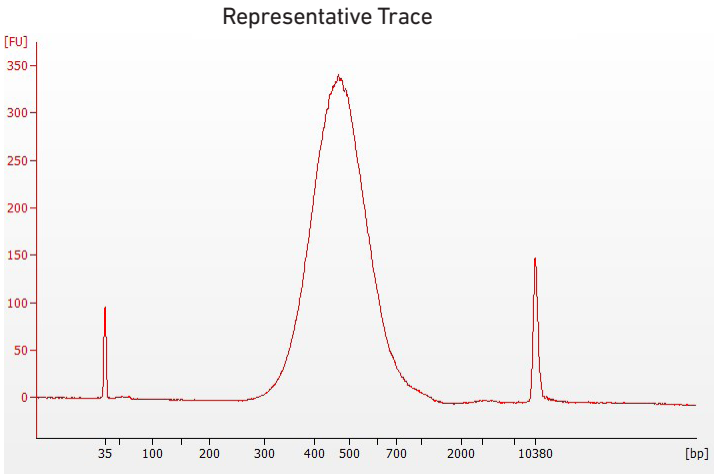
p. Place on the magnet•**Low** until the solution clears.

q. Transfer **35 µl** sample to a new tube strip.

r. Store at **4°C** for up to **72 h** or at **–20°C** for long-term storage.

6.7 GEX Post Library Construction QC

a. Run **1 µl s**ample at **1:10 dilution** on an Agilent Bioanalyzer High Sensitivity chip.



b. Determine the average fragment size from the trace. This will be used as the insert size for library quantification.

Alternate QC Method:

- Agilent TapeStation. See Appendix for representative traces

See Appendix for GEX Post Library Construction Quantification

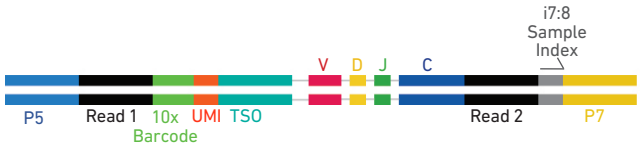
Sequencing

7

Sequencing Libraries

Chromium Single Cell V(D)J enriched libraries and 5' Gene Expression libraries comprise standard Illumina paired-end constructs which begin with P5 and end with P7. 16 bp 10x Barcodes are encoded at the start of Read 1, while sample index sequences are incorporated as the i7 index read. Read 1 and Read 2 are standard Illumina sequencing primer sites used in paired-end sequencing. Read 1 is used to sequence 16 bp 10x Barcodes and 10 bp UMI. Sequencing these libraries produce a standard Illumina BCL data output folder.

Chromium Single Cell V(D)J Enriched Library



Chromium Single Cell 5' Gene Expression Library

Illumina Sequencer Compatibility

The compatibility of the listed sequencers has been verified by 10x Genomics. Some variation in assay performance is expected based on sequencer choice. For more information about performance variation, visit the 10x Genomics Support website.

- MiSeq
- NexSeq 500/550*
- HiSeq 2500 (Rapid Run)
- HiSeq 3000/4000
- NovaSeq

*Sequencing Chromium Single Cell libraries on the NextSeq 500/550 platform may yield reduced sequence quality and sensitivity relative to the MiSeq, HiSeq, and NovaSeq platforms. Refer to the 10x Genomics Support website for more information.

Sample Indices

Each sample index in the Chromium i7 Sample Index Kit (PN-120262) is a mix of 4 different sequences to balance across all 4 nucleotides. If multiple samples are pooled in a sequence lane, the sample index name (i.e. the Chromium i7 Sample Index plate well ID) is needed in the sample sheet used for generating FASTQs with "cellranger mkfastq".

Library Sequencing Depth & Run Parameters

Sequencing Depth	Minimum 5,000 read pairs per cell for V(D)J Enriched library Minimum 20,000 read pairs per cell for 5' Gene Expression library
Sequencing Type	Paired-end, single indexing
Sequencing Read	Recommended cycles for all library types – 26 x 91 bp Recommended cycles for all library type combinations – 26 x 91 bp

V(D)J Enriched libraries (alone or in combination with 5' Gene Expression libraries) may be sequenced at 150 x 150 bp.

Library Loading

Once quantified and normalized, V(D)J Enriched libraries and 5' Gene Expression libraries should be denatured and diluted as recommended for Illumina sequencing platforms. Refer to Illumina documentation for denaturing and diluting libraries. Refer to the 10x Genomics Support website, for more information.

Instrument	Loading Concentration (pM)	PhiX (%)
MiSeq	10	1
NextSeq 500	1.5	1
HiSeq 2500 (RR)	10	1
HiSeq 4000	180	1
NovaSeq	200	1

Library Pooling

V(D)J Enriched libraries and the 5' Gene Expression libraries maybe pooled for sequencing, taking into account the differences in depth requirements between the pooled libraries. 5' Gene Expression libraries may be sequenced using enriched library parameters, however the cost of sequencing using enriched library parameters is higher.

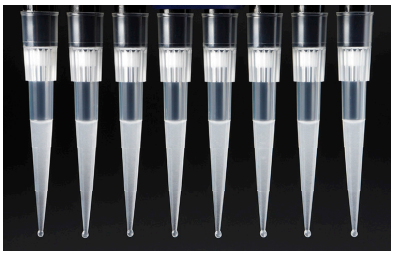
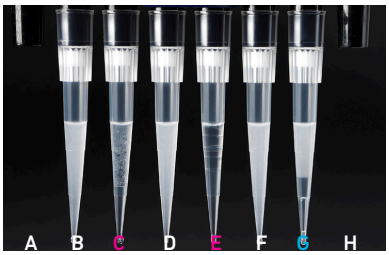
Library Pooling Examples:

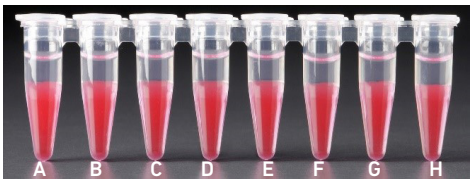
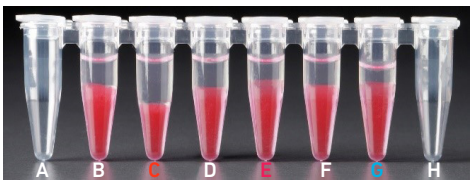
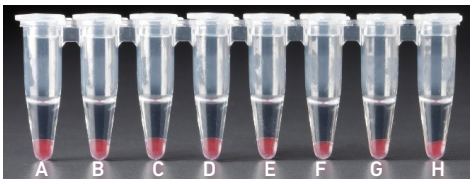
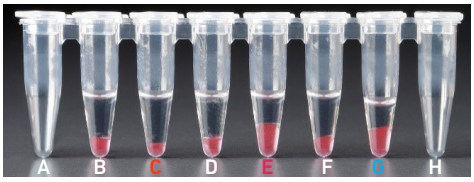
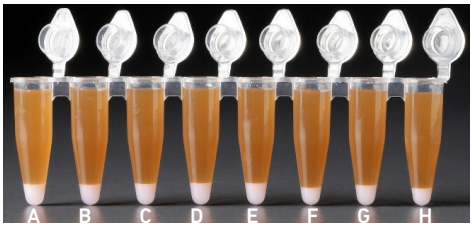
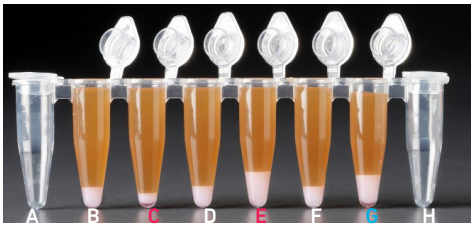
Libraries	Sequencing Depth (read pairs per cell)	Library Pooling Ratio
Example 1		
V(D)J Enriched library 5' Gene Expression library	5,000 20,000	1 4
Example 2		
V(D)J Enriched library 5' Gene Expression library	5,000 50,000	1 10

Troubleshooting

8

GEMs

STEP	NORMAL	REAGENT CLOGS & WETTING FAILURES
1.4 d After Chip G is removed from the Controller and the wells are exposed	 All 8 recovery wells are similar in volume and opacity.	 Recovery well G indicates a reagent clog. Recovery well C and E indicate a wetting failure. Recovery wells B, D, and F are normal. Wells A and H contain 50% Glycerol Solution.
1.4 e Transfer GEMs from Chip G Recovery Wells	 All liquid levels are similar in volume and opacity without air trapped in the pipette tips.	 Pipette tips C and E indicate a wetting failure. Pipette tip C contains partially emulsified GEMs. Emulsion is absent in pipette tip E. Pipette tip G indicates a reagent clog.

STEP	NORMAL	REAGENT CLOGS & WETTING FAILURES
2.1 a After transfer of the GEMs + Recovery Agent	 All liquid levels are similar in the aqueous sample volume (clear) and Recovery Agent/Partitioning Oil (pink).	 Tube G indicates a reagent clog has occurred. There is a decreased volume of aqueous layer (clear). Tube C and E indicate a wetting failure has occurred. There is an abnormal volume of Recovery Agent/Partitioning Oil (pink).
2.1 b After aspiration of Recovery Agent/ Partitioning Oil	 All liquid volumes are similar in the aqueous sample volume (clear) and residual Recovery Agent/Partitioning Oil (pink).	 Tube G indicates a reagent clog has occurred. There is a decreased volume of aqueous layer (clear). There is also a greater residual volume of Recovery Agent/Partitioning Oil (pink). Tube C and E indicate a wetting failure has occurred. There is an abnormal residual volume of Recovery Agent/Partitioning Oil (pink).
2.1 c After addition of Dynabeads Cleanup Mix	 All liquid volumes are similar after addition of the Dynabeads Cleanup Mix.	 Tube G indicates a reagent clog has occurred. There is an abnormal ratio of Dynabeads Cleanup Mix (brown) to Recovery Agent/Partitioning Oil (appears white). Tube C and E indicate a wetting failure has occurred. There is an abnormal ratio of Dynabeads Cleanup Mix (brown) to Recovery Agent/Partitioning Oil (appears white).

If a channel clogs or wetting failure occurs during GEM generation, it is recommended that the sample be remade. If any of the listed issues occur, take a picture and send it to support@10xgenomics.com for further assistance.

Chromium Controller Errors

If the Chromium Controller or the Chromium Single Cell Controller fails to start, an error tone will sound and one of the following error messages will be displayed:

a. **Chip not read – Try again:** Eject the tray, remove and/or reposition the Chromium Next GEM Secondary Holder assembly and try again. If the error message is still received after trying this more than twice, contact support@10xgenomics.com for further assistance.

b. **Check gasket:** Eject the tray by pressing the eject button to check that the 10x Gasket is correctly installed on the Chromium Next GEM Chip. If the error message persists, contact support@10xgenomics.com for further assistance.

c. **Error Detected: Row _ Pressure**

   i. If this message is received within a few seconds of starting a run, eject the tray by pressing the eject button and check for dirt or deposits on the 10x Gasket. If dirt is observed, replace with a new 10x Gasket and try again. If the error message is still received after trying this more than twice, contact support@10xgenomics.com for further assistance.

   ii. If this message is received after a few minutes into the run, the Chromium Next GEM Chip must be discarded. **Do not try running this Chromium Next GEM Chip again as this may damage the Chromium Controller.**

d. **Invalid Chip CRC Value:** This indicates that a Chromium Next GEM Chip has been used with an older firmware version. The chip must be discarded. Contact support@10xgenomics.com for further assistance.

Appendix

Post Library Construction Quantification

Agilent TapeStation Traces

Oligonucleotide Sequences

Post Library Construction Quantification

a. Thaw KAPA Library Quantification Kit for Illumina Platforms.

b. Dilute **1 µl** sample with deionized water to appropriate dilutions that fall within the linear detection range of the KAPA Library Quantification Kit for Illumina Platforms. (For more accurate quantification, make the dilution(s) in duplicate).

c. Make enough Quantification Master Mix for the DNA dilutions per sample and the DNA Standards (plus 10% excess) using the guidance for 1 reaction volume below.

Quantification Master Mix	1X (µl)
SYBR Fast Master Mix + Primer	12
Water	4
Total	**16**

d. Dispense **16 µl** Quantification Master Mix for sample dilutions and DNA Standards into a 96 well PCR plate.

e. Add **4 µl** sample dilutions and **4 µl** DNA Standards to appropriate wells. Centrifuge briefly.

f. Incubate in a thermal cycler with the following protocol.

Step	Temperature	Run Time
1	95°C	00:03:00
2	95°C	00:00:05
3	67°C	00:00:30
4	Go to Step 2, 29X (Total 30 cycles)	

g. Follow the manufacturer's recommendations for qPCR-based quantification. For library quantification for sequencer clustering, determine the concentration based on insert size derived from the Bioanalyzer/TapeStation trace.

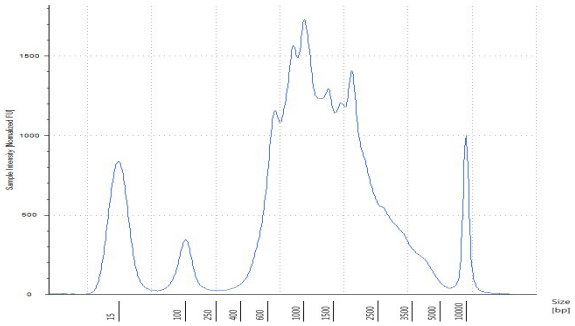
Agilent TapeStation Traces

Agilent TapeStation Traces

Agilent TapeStation High Sensitivity D5000 ScreenTape was used. Protocol steps correspond to the Chromium Next GEM Single Cell V(D)J v1.1 Reagent Kits User Guide (CG000207).

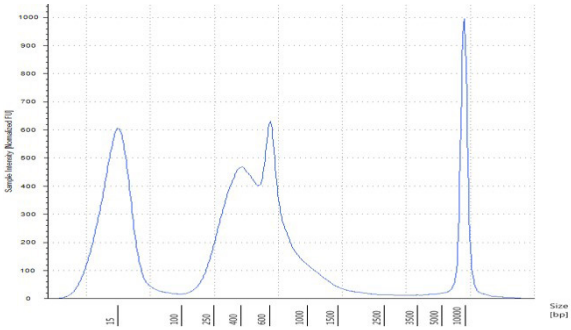
Protocol Step 3.3 – cDNA QC & Quantification

Representative Trace

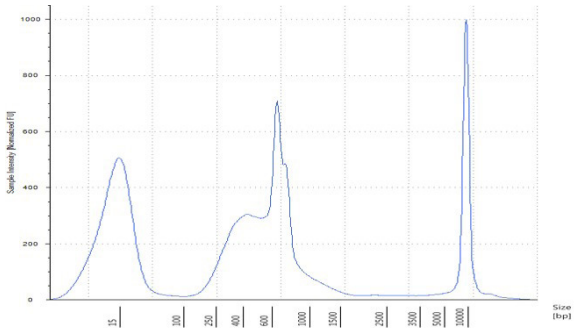


Protocol Step 5.6 – Post Library Construction QC

Representative Trace - PBMCs enriched for TCR

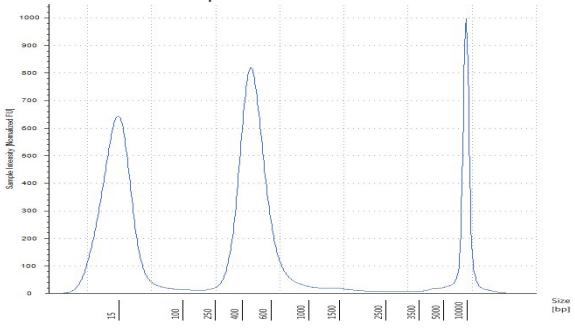


Representative Trace - PBMCs enriched for Ig



Protocol Step 6.7 – GEX Post Library Construction QC

Representative Trace



Oligonucleotide Sequences

Protocol steps correspond to the Chromium Next GEM Single Cell V(D)J v1.1 Reagent Kits User Guide (CG000207).

Protocol Step 1.5 – GEM-RT Incubation

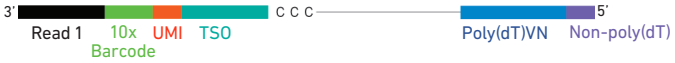
Gel Bead Oligo Primer (TSO)

5'-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNN-TTTCTTATATrGrGrG-3'

Poly-dT RT Primer

5'-AAGCAGTGGTATCAACGCAGAGTAC-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-3'

Reverse Transcript Product



3'-GATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNN-AAAGAATATACCC-cDNA_Insert-NVTTTTTTTTTTTTTTTTTTTTTTTTTTTT-CATGAGACGCAACTATGGTGACGAA-5'

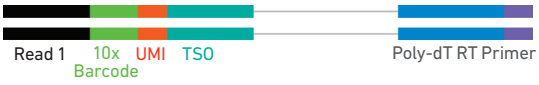
Protocol Step 3.1 – cDNA Amplification

cDNA Primer Mix PN-220106

Forward Primer: Partial Read 1
5'-CTACACGACGCTCTTCCGATCT-3'

Reverse Primer: Non-poly(dT)
5'-AAGCAGTGGTATCAACGCAGAG-3'

cDNA Amplified Product



5'-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNN-TTTCTTATATGGG-cDNA_Insert-GTACTCTGCGTTGATACCACTGCTT-3'
3'-GATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNN-AAAGAATATACCC-cDNA_Insert-CATGAGACGCAACTATGGTGACGAA-5'

Protocol Step 4.1 – Target Enrichment 1

Human T Cell Mix 1 PN-2000008

Forward Primer: (final conc. 2 µM) PCR Primer
5'-AATGATACGGCGACCACCGA-GATCTACACTCTTTCCCTACACGACGCTC-3'

Reverse Outer Primers: (final conc. 1 µM each) Enrichment Outer Primer
5'-TGAAGGCGTTTGCACATGCA-3'
5'-TCAGGCAGTATCTGGAGTCATTGAG-3'

Human B Cell Mix 1 PN-2000035

Forward Primer: (final conc. 1 µM) PCR Primer
5'-AATGATACGGCGACCACCGA-GATCTACACTCTTTCCCTACACGACGCTC-3'

Reverse Outer Primers: (final conc. 0.5 µM each) Enrichment Outer Primer
5'-CAGGGCACAGTCACATCCT-3'
5'-TGCTGGACCACGCATTTGTA-3'
5'-GGTTTTGTTGTCGACCCAGTCT-3'
5'-TTGTCCACCTTGGTGTTGCT-3'
5'-CATGACGTCCTTGGAAGGCA-3'
5'-TGTGGGACTTCCACTG-3'
5'-TTCTCGTAGTCTGCTTTGCTCAG-3'

Mouse T Cell Mix 1 PN-2000075

Forward Primer: (final conc. 2 µM) PCR Primer
5'-AATGATACGGCGACCACCGA-GATCTACACTCTTTCCCTACACGACGCTC-3'

Reverse Outer Primers: (final conc. 0.5 µM each) Enrichment Outer Primer
5'-CTGGTTGCTCCAGGCAATGG-3'
5'-TGTAGGCCTGAGGGTCCGT-3'

Mouse B Cell Mix 1 PN-2000080

Forward Primer: (final conc. 1 µM) PCR Primer
5'-AATGATACGGCGACCACCGA-GATCTACACTCTTTCCCTACACGACGCTC-3'

Reverse Outer Primers: Enrichment Outer Primer
5'-TCAGCACGGGACAAACTCTTCT-3' (final conc. 0.375 µM)
5'-GCAGGAGACAGACTCTTCTCCA-3' (final conc. 0.175 µM)
5'-AACTGGCTGCTCATGGTGT-3' (final conc. 0.1 µM)
5'-TGGTGCAAGTGTGGTTGAGGT-3' (final conc. 0.3 µM)
5'-TGGTCACTTGGCTGGTGGTG-3' (final conc. 0.25 µM)
5'-CACTTGGCAGGTGAACTGTTTTCT-3' (final conc. 0.25 µM)
5'-AACCTTCAAGGATGCTCTTGGGA-3' (final conc. 0.3 µM)
5'-GGACAGGGATCCAGAGTTCCA-3' (final conc. 0.5 µM)
5'-AGGTGACGGTCTGACTTGGC-3' (final conc. 0.125 µM)
5'-GCTGGACAGGGCTCCATAGTT-3' (final conc. 0.125 µM)
5'-GGCACCTTGTCCAATCATGTTCC-3' (final conc. 0.250 µM)
5'-ATGTCGTTCATACTCGTCCTTGGT-3' (final conc. 0.1 µM)

Protocol Step 4.3 – Target Enrichment 2

Human T Cell Mix 2
PN-2000009

Forward Primer: (final conc. 2 µM) PCR Primer
5'-AATGATACGGCGACCACCGA-GATCT-3'

Reverse Inner Primers: (final conc. 1 µM each) Enrichment Inner Primer
5'-AGTCTCTCAGCTGGTACACG-3'
5'-TCTGATGGCTCAAACACAGC-3'

Human B Cell Mix 2
PN-2000036

Forward Primer: (final conc. 1 µM) PCR Primer
5'-AATGATACGGCGACCACCGA-GATCT-3'

Reverse Inner Primers: (final conc. 0.5 µM each) Enrichment Inner Primer
5'-GGGAAGTTTCTGGCGGTCA-3'
5'-GGTGGTACCCAGTTATCAAGCAT-3'
5'-GTGTCCCAGGTCACCATCAC-3'
5'-TCCTGAGGACTGTAGGACAGC-3'
5'-CACGCTGCTCGTATCCGA-3'
5'-TAGCTGCTGGCCGC-3'
5'-GCGTTATCCACCTTCCACTGT-3'

Mouse T Cell Mix 2
PN-2000079

Forward Primer: (final conc. 0.5 µM) PCR Primer
5'-AATGATACGGCGACCACCGA-GATCT-3'

Reverse Inner Primers: (final conc. 0.5 µM each) Enrichment Inner Primer
5'-AGTCAAAGTCGGTGAACAGGCA-3'
5'-GGCCAAGCACACGAGGGTA-3'

Mouse B Cell Mix 2
PN-2000081

Forward Primer: (final conc. 1 µM) PCR Primer
5'-AATGATACGGCGACCACCGA-GATCT-3'

Reverse Inner Primers: Enrichment Inner Primer
5'-TACACACCAGTGTGGCCTT-3' (final conc. 0.375 µM)
5'-CAGGCCACTGTCACACCACT-3' (final conc. 0.175 µM)
5'-CAGGTCACATTCATCGTGCCG-3' (final conc. 0.1 µM)
5'-GAGGCCAGCACAGTGACCT-3' (final conc. 0.3 µM)
5'-GCAGGGAAGTTCACAGTGCT-3' (final conc. 0.25 µM)
5'-CTGTTTGAGATCAGTTTGCCATCCT-3' (final conc. 0.25 µM)
5'-TGCGAGGTGGCTAGGTACTTG-3' (final conc. 0.3 µM)
5'-CCCTTGACCAGGCATCC-3' (final conc. 0.5 µM)
5'-AGGTCACGGAGGAACCAGTTG-3' (final conc. 0.125 µM)
5'-GGCATCCCAGTGTCACCGA-3' (final conc. 0.125 µM)
5'-AGAAGATCCACTTCACCTTGAAC-3' (final conc. 0.250 µM)
5'-GAAGCACACGACTGAGGCAC-3' (final conc. 0.1 µM)

Target Enrichment Product

5'-AATGATACGGCGACCACCGA-GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNN-TTTCTTATATGGG-cDNA_Insert-Inner_Primer-3'
3'-TTACTATGCCGCTGGTGGCT-CTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNN-AAAGAATATACCC-cDNA_Insert-Inner_Primer-5'

Protocol Step 5.2 – Adaptor Ligation

Adaptor (Read 2)
PN-220026

Read 2
5'-GATCGGAAGAGCACACGTCTGAACTCCAGTCAC-3'
3'-TCTAGCCTTCTCG-5'

Ligation Product

5'-AATGATACGGCGACCACCGA-GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNN-TTTCTTATATGGG-cDNA_Insert-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-3'
3'-TTACTATGCCGCTGGTGGCT-CTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNN-AAAGAATATACCC-cDNA_Insert-TCTAGCCTTCTCG-5'

Protocol Step 5.4 – Sample Index PCR

Sample Index PCR Primer
PN-220111
PN-220103

Forward Primer: SI-PCR Primer

P5 Partial Read 1

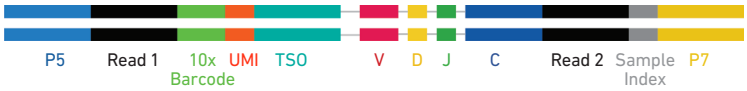
5'-AATGATACGGCGACCACCGA-GATCTACACTCTTTCCCTACACGACGCTC-3'

Reverse Primer: Chromium i7 Sample Index

P7 Sample Index Partial Read 2

5'-CAAGCAGAAGACGGCATACGAGAT-NNNNNNNN-GTGACTGGAGTTCAGACGTGT-3'

Sample Index PCR Product



5'-AATGATACGGCGACCACCGA-GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNN-TTTCTTATATGGG-cDNA _ Insert-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-NNNNNNNN-ATCTCGTATGCCGTCTTCTGCTTG-3'

3'-TTACTATGCCGCTGGTGGCT-CTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNN-AAAGAATATACCC-cDNA _ Insert-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG-NNNNNNNN-TAGAGCATACGGCAGAAGACGAAC-5'

Protocol Step 6.3 –GEX Adaptor Ligation

Adaptor (Read 2)
PN-220026

Read 2

5'–GATCGGAAGAGCACACGTCTGAACTCCAGTCAC–3'

3'–TCTAGCCTTCTCG–5'

Ligation Product

5'–CTACACGACGCTCTTCCGATCT–NNNNNNNNNNNNNNNN–NNNNNNNNN–TTTCTTATATGGG–cDNA _ Insert–AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC–3'

3'–GATGTGCTGCGAGAAGGCTAGA–NNNNNNNNNNNNNNNN–NNNNNNNNN–AAAGAATATACCC–cDNA _ Insert–TCTAGCCTTCTCG–5'

Protocol Step 6.5 – Sample Index PCR

Sample Index PCR Primer
PN-220111
PN-220103

Forward Primer: SI-PCR Primer

P5 Partial Read 1

5'-AATGATACGGCGACCACCGA-GATCTACACTCTTTCCCTACACGACGCTC-3'

Reverse Primer: Chromium i7 Sample Index

P7 Sample Index Partial Read 2

5'-CAAGCAGAAGACGGCATACGAGAT-NNNNNNNN-GTGACTGGAGTTCAGACGTGT-3'

Sample Index PCR Product

5'-AATGATACGGCGACCACCGA-GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNN-NNNNNNNNN-TTTCTTATATGGG-cDNA _ Insert-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-NNNNNNNN-ATCTCGTATGCCGTCTTCTGCTTG-3'

3'-TTACTATGCCGCTGGTGGCT-CTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNN-AAAGAATATACCC-cDNA _ Insert-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG-NNNNNNNN-TAGAGCATACGGCAGAAGACGAAC-5'

EXHIBIT 9

CG000209 Rev C

USER GUIDE

Chromium Next GEM Single Cell ATAC Reagent Kits v1.1

FOR USE WITH

Chromium Next GEM Single Cell ATAC Library & Gel Bead Kit, 16 rxns PN-1000175
Chromium Next GEM Single Cell ATAC Library & Gel Bead Kit, 4 rxns PN-1000176
Chromium Next GEM Chip H Single Cell Kit, 48 rxns PN-1000161
Chromium Next GEM Chip H Single Cell Kit, 16 rxns PN-1000162
Chromium i7 Multiplex Kit N, Set A, 96 rxns PN-1000084

Next GEM reagents are specific to Next GEM products and should not be used interchangeably with non-Next GEM reagents.

10x GENOMICS

Notices

Document Number

CG000209 • Rev C

Legal Notices

Instrument & Licensed Software Updates Warranties

Updates to existing Instruments and Licensed Software may be required to enable customers to use new or existing products. In the event of an Instrument failure resulting from an update, such failed Instrument will be replaced or repaired in accordance with the 10x Limited Warranty, Assurance Plan or service agreement, only if such Instrument is covered by any of the foregoing at the time of such failure. Instruments not covered under a current 10x Limited Warranty, Assurance Plan or service agreement will not be replaced or repaired.

Support

Email: support@10xgenomics.com

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Pleasanton, CA 94588 USA

Document Revision Summary

Document Number	CG000209
Title	Chromium Next GEM Single Cell ATAC Reagent Kits v1.1 User Guide
Revision	Rev B to Rev C
Revision Date	August 2019

Specific Changes:

- Updated to state that Next GEM reagents are specific to Next GEM products.

General Changes:

- Updates for general minor consistency of language and terms throughout.

Table of Contents

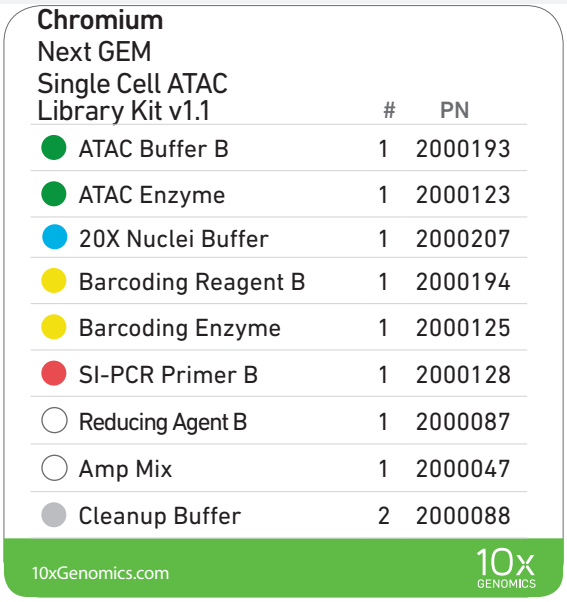
Introduction

Chromium Next GEM Single Cell ATAC Reagent Kits v1.1
Chromium Accessories
Recommended Thermal Cyclers
Additional Kits, Reagents & Equipment
Protocol Steps & Timing
Stepwise Objectives

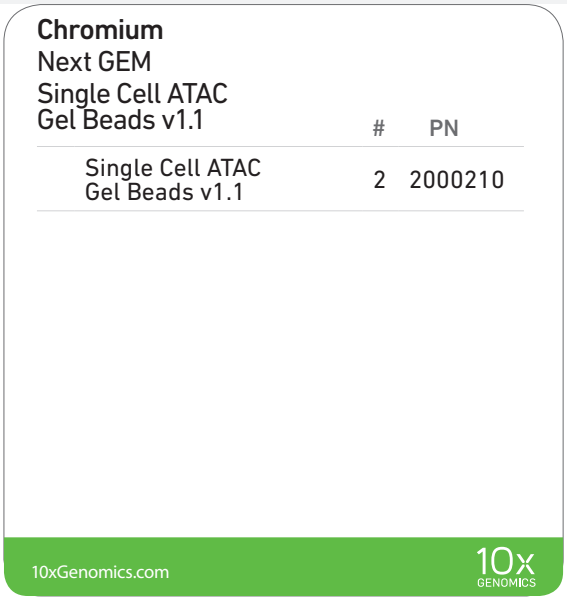
Chromium Next GEM Single Cell ATAC Reagent Kits v1.1

Chromium Next GEM Single Cell ATAC Library & Gel Bead Kit v1.1, 16 rxns PN-1000175

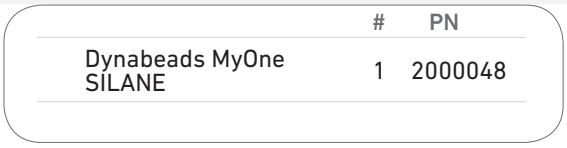
Chromium Next GEM Single Cell ATAC Library Kit v1.1, 16 rxns PN-1000163 (store at –20°C)



Chromium Next GEM Single Cell ATAC Gel Bead Kit v1.1, 16 rxns PN-1000159 (store at –80°C)



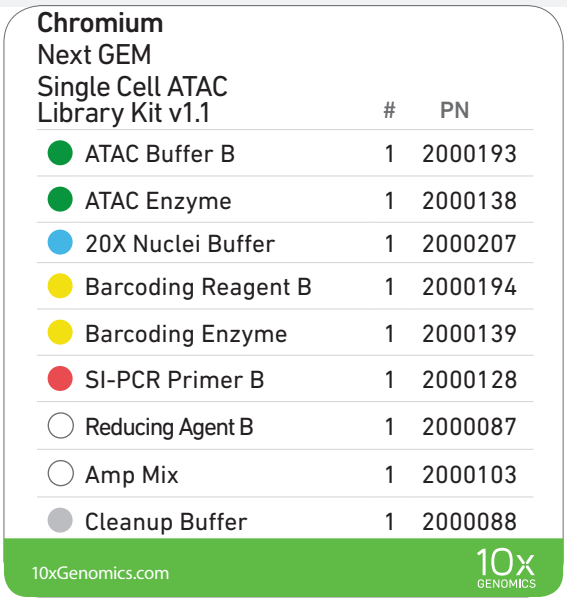
Dynabeads™ MyOne™ SILANE, PN-2000048 (store at 4°C)



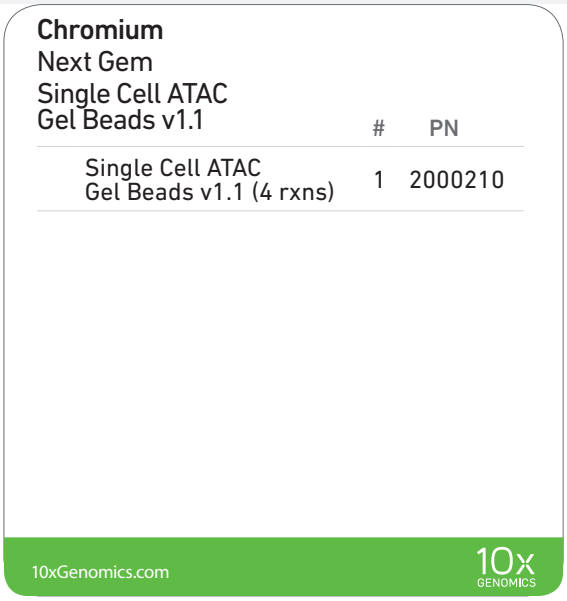
Chromium Next GEM Single Cell ATAC Reagent Kits v1.1

Chromium Next GEM Single Cell ATAC Library & Gel Bead Kit v1.1, 4 rxns PN-1000176

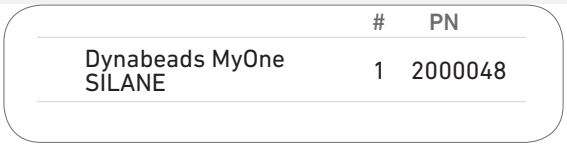
Chromium Next GEM Single Cell ATAC Library Kit v1.1, 4 rxns PN-1000164 (store at –20°C)



Chromium Next GEM Single Cell ATAC Gel Bead Kit v1.1, 4 rxns PN-1000160 (store at –80°C)



Dynabeads™ MyOne™ SILANE, PN-2000048 (store at 4°C)



Chromium Next GEM Chip H Single Cell Kit v1.1, 48 rxns PN-1000161 (store at ambient temperature)

Chromium
Partitioning Oil

	#	PN
Partitioning Oil	6	2000190

Chromium
Recovery Agent

	#	PN
Recovery Agent	6	220016

Chromium
Next GEM
Chip H & Gaskets

	#	PN
Chromium Next GEM Chip H	6	2000180
Gasket, 6-pack	1	370017

10xGenomics.com

10x GENOMICS

Chromium Next GEM Chip H Single Cell Kit v1.1, 16 rxns PN-1000162 (store at ambient temperature)

Chromium
Partitioning Oil

	#	PN
Partitioning Oil	2	2000190

Chromium
Recovery Agent

	#	PN
Recovery Agent	2	220016

Chromium
Next GEM
Chip H & Gaskets

	#	PN
Chromium Next GEM Chip H	2	2000180
Gasket, 2-pack	1	3000072

10xGenomics.com

10x GENOMICS

Chromium i7 Multiplex Kit N, Set A, 96 rxns PN-1000084 (store at –20°C)

Chromium
i7 Multiplex Kit N
Set A

	#	PN
Chromium i7 Sample Index Plate N, Set A	1	3000262

Chromium Accessories

Product	PN (Orderable)	PN (Item)
10x Vortex Adapter	120251	330002
Chromium Next GEM Secondary Holder	1000195	3000332
10x Magnetic Separator	120250	230003

Recommended Thermal Cyclers

Thermal cyclers used must support uniform heating of 100 µl emulsion volumes.

Supplier	Description	Part Number
BioRad	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module	1851197
Eppendorf	MasterCycler Pro	North America 950030010 International 6321 000.019
Thermo Fisher Scientific	Veriti 96-Well Thermal Cycler	4375786

Additional Kits, Reagents & Equipment

The items in the table below have been validated by 10x Genomics and are highly recommended for the Single Cell ATAC protocols. Substituting materials may adversely affect system performance.

Supplier	Description	Part Number (US)
Plastics		
Eppendorf	PCR Tubes 0.2 ml 8-tube strips DNA LoBind Tubes, 1.5 ml DNA LoBind Tubes, 2.0 ml	951010022 022431021 022431048
USA Scientific	TempAssure PCR 8-tube strip	1402-4700
Thermo Fisher Scientific	MicroAmp 8-Tube Strip, 0.2 ml MicroAmp 8-Cap Strip, clear	N8010580 N8010535
Rainin	Tips LTS 200UL Filter RT-L200FLR Tips LTS 1ML Filter RT-L1000FLR Tips LTS 20UL Filter RT-L10FLR	30389240 30389213 30389226
Kits & Reagents		
Thermo Fisher Scientific	Nuclease-free Water	AM9937
Millipore Sigma	Ethanol, Pure (200 Proof, anhydrous)	E7023-500ML
Beckman Coulter	SPRIselect Reagent Kit	B23318
Bio-Rad	10% Tween 20	1662404
Ricca Chemical Company	Glycerin (glycerol), 50% (v/v) Aqueous Solution	3290-32
Qiagen	Qiagen Buffer EB	19086
Equipment		
VWR	Vortex Mixer Divided Polystyrene Reservoirs	10153-838 41428-958
Eppendorf	Eppendorf ThermoMixer C Eppendorf ThermoMixer C Bundle, includes SmartBlock 1.5 ml, Thermoblock for 24 reaction vessel (alternatively, use a temperature-controlled Heat Block)	5382000023 2231000574
Rainin	Pipet-Lite Multi Pipette L8-50XLS+ Pipet-Lite Multi Pipette L8-200XLS+ Pipet-Lite Multi Pipette L8-10XLS+ Pipet-Lite Multi Pipette L8-20XLS+ Pipet-Lite LTS Pipette L-2XLS+ Pipet-Lite LTS Pipette L-10XLS+ Pipet-Lite LTS Pipette L-20XLS+ Pipet-Lite LTS Pipette L-100XLS+ Pipet-Lite LTS Pipette L-200XLS+ Pipet-Lite LTS Pipette L-1000XLS+	17013804 17013805 17013802 17013803 17014393 17014388 17014392 17014384 17014391 17014382

Choose either Eppendorf, USA Scientific or Thermo Fisher Scientific PCR 8-tube strips.

Additional Kits, Reagents & Equipment

The items in the table below have been validated by 10x Genomics and are highly recommended for the Single Cell ATAC protocols. Substituting materials may adversely affect system performance.

Supplier	Description		Part Number (US)
Quantification & Quality Control			
Agilent	2100 Bioanalyzer Laptop Bundle High Sensitivity DNA Kit 4200 TapeStation High Sensitivity D1000 ScreenTape High Sensitivity D1000 Reagents	Choose Bioanalyzer, or TapeStation based on availability & preference.	G2943CA 5067-4626 G2991AA 5067-5584 5067-5585
KAPA Biosystems	KAPA Library Quantification Kit for Illumina Platforms		KK4824

Protocol Steps & Timing

	Steps		Timing	Stop & Store
2 h	**Nuclei Isolation**			
	Dependent on Cell Type		~1-2 h	
	Step 1 – Transposition			
	1.1	Prepare Transposition Mix	10 min	
	1.2	Isothermal Incubation	60 min	
4 h	**Step 2 – GEM Generation & Barcoding**			
	2.1	Prepare Master Mix	10 min	
	2.2	Load Chromium Next GEM Chip H	10 min	
	2.3	Run the Chromium Controller	18 min	
	2.4	Transfer GEMs	3 min	
	2.5	GEM Incubation	45 min	STOP 15°C ≤18 h or –20°C ≤ 1 week
6 h	**Step 3 – Post GEM Incubation Cleanup**			
	3.1	Post GEM Incubation Cleanup – Dynabeads	35 min	
	3.2	Post GEM Incubation Cleanup – SPRIselect	15 min	STOP 4°C ≤ 72 h or –20°C ≤ 2 weeks
	Step 4 – Library Construction			
	4.1	Sample Index PCR	45 min	
	4.2	Post Sample Index Double Sided Size Selection – SPRIselect	20 min	STOP 4°C ≤72 h or –20°C long-term
	4.3	Post Library Construction QC	60 min	

Stepwise Objectives

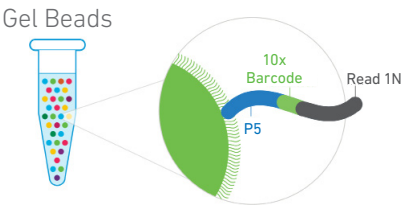
The Chromium Single Cell ATAC Solution provides a comprehensive, scalable approach to determine the regulatory landscape of chromatin in hundreds to thousands of cells in a single sample. This is achieved by transposing nuclei in a bulk solution; then using a microfluidic chip, the nuclei are partitioned into nanoliter-scale Gel Beads-in-emulsion (GEMs). A pool of ~750,000 10x Barcodes is sampled to separately and uniquely index the transposed DNA of each individual nucleus. Libraries are generated and sequenced, and 10x Barcodes are used to associate individual reads back to the individual partitions, and thereby, to each individual nucleus.

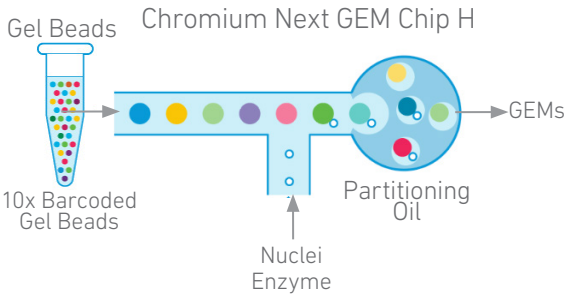
Step 1 Transposition

Nuclei suspensions are incubated in a Transposition Mix that includes a Transposase. The Transposase enters the nuclei and preferentially fragments the DNA in open regions of the chromatin. Simultaneously, adapter sequences are added to the ends of the DNA fragments.

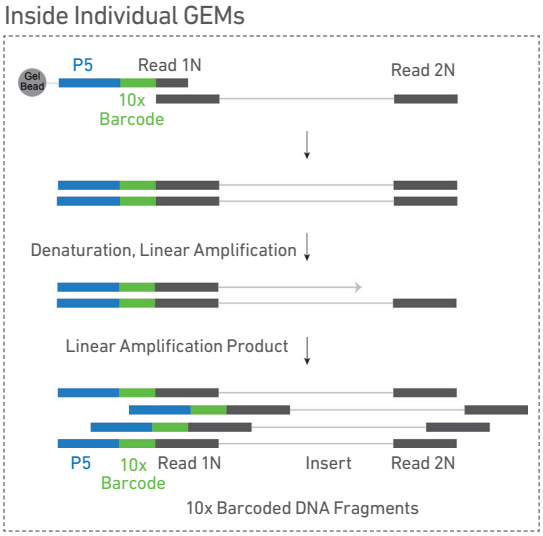
Step 2 GEM Generation & Barcoding

GEMs are generated by combining barcoded Gel Beads, transposed nuclei, a Master Mix, and Partitioning Oil on a Chromium Next GEM Chip H. To achieve single nuclei resolution, the nuclei are delivered at a limiting dilution, such that the majority (~90-99%) of generated GEMs contains no nuclei, while the remainder largely contain a single nucleus.





Upon GEM generation, the Gel Bead is dissolved. Oligonucleotides containing (i) an Illumina® P5 sequence, (ii) a 16 nt 10x Barcode and (iii) a Read 1 (Read 1N) sequence are released and mixed with DNA fragments and Master Mix. Thermal cycling of the GEMs produces 10x barcoded single-stranded DNA. After incubation, the GEMs are broken and pooled fractions are recovered.

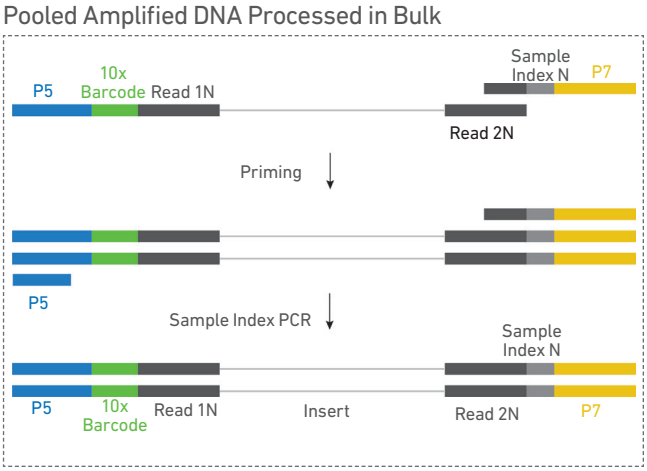


Step 3 Post GEM Incubation Cleanup

Silane magnetic beads are used to remove leftover biochemical reagents from the post GEM reaction mixture. Solid Phase Reversible Immobilization (SPRI) beads are used to eliminate unused barcodes from the sample.

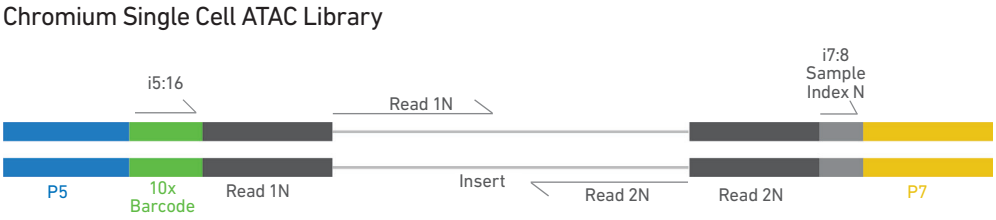
Step 4 Library Construction

P7 and a sample index are added during library construction via PCR. The final libraries contain the P5 and P7 sequences used in Illumina® bridge amplification.



Step 5 Sequencing

The Chromium Next GEM Single Cell ATAC Reagent Kits v1.1 protocol produces Illumina®-ready sequencing libraries. Illumina® sequencer compatibility, sample indices, sequencing depth & run parameters, library loading and pooling are summarized.



See Appendix for Oligonucleotide Sequences

Tips & Best Practices

Icons

Tips & Best Practices section includes additional guidance

Signifies critical step requiring accurate execution

Troubleshooting section includes additional guidance

Next GEM specific protocol step updates

Emulsion-safe Plastics

- Use 10x Genomics validated emulsion-safe plastic consumables when handling GEMs as some plastics can destabilize GEMs.

Multiplet Rate

Multiplet Rate (%)	# of Nuclei Loaded	# of Nuclei Recovered
0.4%	~775	~500
0.8%	~1,550	~1,000
1.6%	~3,075	~2,000
2.3%	~4,625	~3,000
3.1%	~6,150	~4,000
3.9%	~7,700	~5,000
4.6%	~9,250	~6,000
5.4%	~10,750	~7,000
6.2%	~12,300	~8,000
6.9%	~13,850	~9,000
7.7%	~15,400	~10,000

General Reagent Handling

- Fully thaw and thoroughly mix reagents before use.
- Keep all enzymes and Master Mixes on ice during setup and use. Promptly move reagents back to the recommended storage.
- Calculate reagent volumes with 10% excess of 1 reaction values.
- Cover Partitioning Oil tubes and reservoirs to minimize evaporation.
- Thoroughly mix samples with the beads during bead-based cleanup steps.

50% Glycerol Solution

- Purchase 50% glycerol solution from Ricca Chemical Company, Glycerin (glycerol), 50% (v/v) Aqueous Solution, PN-3290-32.
- Prepare 50% glycerol solution:
  i. Mix an equal volume of water and 99% Glycerol, Molecular Biology Grade.
  ii. Filter through a 0.2-µm filter.
  iii. Store at –20°C in 1-ml LoBind tubes. 50% glycerol solution should be equilibrated to room temperature before use.

Pipette Calibration

- Follow manufacturer's calibration and maintenance schedules.
- Pipette accuracy is particularly important when using SPRIselect reagents.

Chromium Next GEM Chip Handling

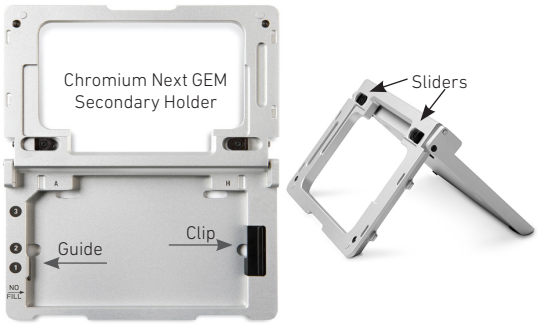
Next GEM

- Minimize exposure of reagents, chips, and gaskets to sources of particles and fibers, laboratory wipes, frequently opened flip-cap tubes, clothing that sheds fibers, and dusty surfaces.
- After removing the chip from the sealed bag, use within 24 h.
- Execute steps without pause or delay, unless indicated. When multiple chips are to be used, load, run, and collect the content from one chip before loading the next.
- Fill all unused input wells in rows labeled 1, 2, and 3 on a chip with an appropriate volume of 50% glycerol solution before loading the used wells. DO NOT add glycerol to the wells in the bottom NO FILL row.
- Avoid contacting the bottom surface of the chip with gloved hands and other surfaces. Frictional charging can lead to inadequate priming of the channels, potentially leading to either clogs or wetting failures.
- Minimize the distance that a loaded chip is moved to reach the Chromium Controller.
- Keep the chip horizontal to prevent wetting the gasket with oil, which depletes the input volume and may adversely affect the quality of the assay.

Chromium Next GEM Secondary Holders

Next GEM

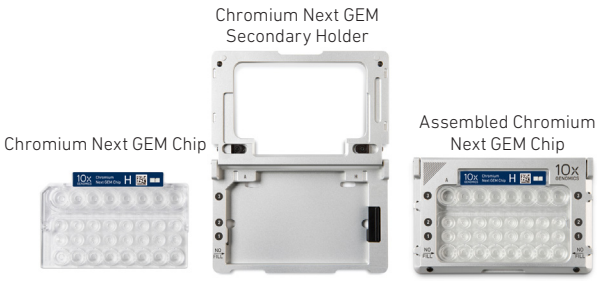
- Chromium Next GEM Secondary Holders encase Chromium Next GEM Chips.
- The holder lid flips over to become a stand, holding the chip at 45 degrees for optimal Recovery Well content removal.
- Squeeze the black sliders on the back side of the holder together to unlock the lid and return the holder to a flat position.



Chromium Next GEM Chip & Holder Assembly

Next GEM

- Align notch on the chip (upper left corner) and the holder.
- Insert the left-hand side of the chip under the guide. Depress the right-hand side of the chip until the spring-loaded clip engages.
- Close the lid before dispensing reagents into the wells.



Chromium Next GEM Chip Loading

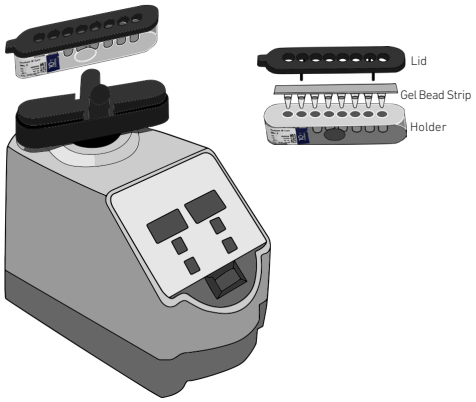
Next GEM

- Place the assembled chip and holder flat on the bench with the lid closed.
- Dispense at the bottom of the wells without introducing bubbles.
- When dispensing Gel Beads into the chip, wait for the remainder to drain into the bottom of the pipette tips and dispense again to ensure complete transfer.
- Refer to Load Chromium Next GEM Chip H for specific instructions.

Gel Bead Handling

Next GEM

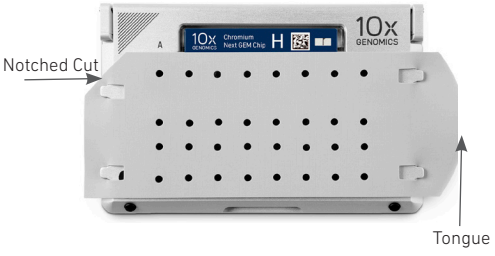
- Use one tube of Gel Beads per sample. DO NOT puncture the foil seals of tubes not used at the time.
- Equilibrate the Gel Beads strip to room temperature before use.
- Store unused Gel Beads at –80°C and avoid more than 12 freeze-thaw cycles. DO NOT store Gel Beads at –20°C.



- Snap the tube strip holder with the Gel Bead strip into a 10x Vortex Adapter. Vortex **30 sec**.
- Centrifuge the Gel Bead strip for ~**5 sec**. Confirm there are no bubbles at the bottom of the tubes and the liquid levels look even. Place the Gel Bead strip back in the holder and secure the holder lid.
- If the required volume of beads cannot be recovered, place the pipette tips against the sidewalls and slowly dispense the Gel Beads back into the tubes. DO NOT introduce bubbles into the tubes and verify that the pipette tips contain no leftover Gel Beads. Withdraw the full volume of beads again by pipetting slowly.

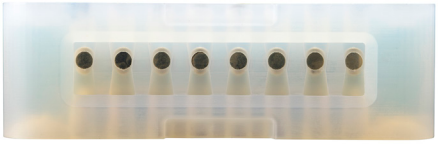
10x Gasket Attachment

- After reagents are loaded, attach the gasket by holding the tongue (curved end, to the right) and hook it on the left-hand tabs of the holder. Gently pull the gasket toward the right and hook it on the two right-hand tabs.
- DO NOT touch the smooth side of the gasket. DO NOT press down on the top of the gasket after attachment.
- Keep the assembly horizontal to avoid wetting the gasket with Partitioning Oil.



10x Magnetic Separator

- Offers two positions of the magnets (high and low) relative to a tube, depending on its orientation. Flip the magnetic separator over to switch between high (magnet•**High**) or low (magnet•**Low**) positions.



SPRIselect Cleanup & Size Selection

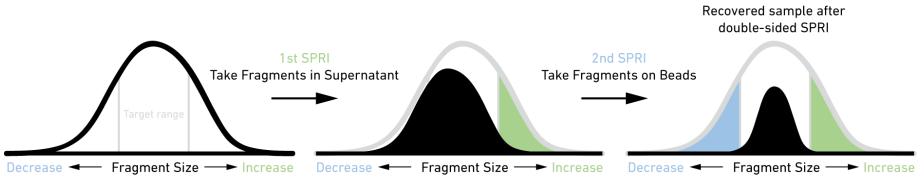
- After aspirating the desired volume of SPRIselect reagent, examine the pipette tips before dispensing to ensure the correct volume is transferred.
- Pipette mix thoroughly as insufficient mixing of sample and SPRIselect reagent will lead to inconsistent results.
- Use fresh preparations of 80% Ethanol.

Tutorial — SPRIselect Reagent:DNA Sample Ratios

SPRI beads selectively bind DNA according to the ratio of SPRIselect reagent (beads).

Example: Ratio = $\frac{\text{Volume of SPRIselect reagent added to the sample}}{\text{Volume of DNA sample}} = \frac{50\ \mu l}{100\ \mu l} = \mathbf{0.5X}$

Schematic of Double Sided Size Selection



After the first SPRI, supernatant is transferred for a second SPRI while larger fragments are discarded (green). After the second SPRI, fragments on beads are eluted and kept while smaller fragments are discarded (blue). Final sample has a tight fragment size distribution with reduced overall amount (black).

Tutorial — Double Sided Size Selection

Step a – First SPRIselect: Add **50 µl** SPRIselect reagent to **100 µl** sample (**0.5X**).

Ratio = $\frac{\text{Volume of SPRIselect reagent added to the sample}}{\text{Volume of DNA sample}} = \frac{50\ \mu l}{100\ \mu l} = \mathbf{0.5X}$

Step b – Second SPRIselect: Add **30 µl** SPRIselect reagent to supernatant from step a (**0.8X**).

Ratio = $\frac{\text{Total Volume of SPRIselect reagent added to the sample (step a + b)}}{\text{Original Volume of DNA sample}} = \frac{50\ \mu l + 30\ \mu l}{100\ \mu l} = \mathbf{0.8X}$

Sample Indices in Sample Index PCR

- Choose the appropriate sample index sets to ensure that no sample indices overlap in a multiplexed sequencing run.
- Each well in the i7 Sample Index plate N, Set A contains a unique mix of 4 oligos.
- The sample indexes can therefore be used in any combination.
- Each sample index set is base-balanced to avoid monochromatic signal issues when it is the sole sample loaded on an Illumina® sequencer.

Step 1

Transposition

1.1 Prepare Transposition Mix

1.2 Isothermal Incubation

1.0 Transposition

Next GEM

GET STARTED!

Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	🟢	**ATAC Buffer B**	2000193	Vortex, centrifuge briefly.	–20°C
	🔵	**20X Nuclei Buffer*** *Concentrated 20X stock; dilute 1:20 in nuclease-free water before use. (See below to Prepare Diluted Nuclei Buffer)	2000207	Thaw. Vortex, centrifuge briefly.	–20°C
Place on Ice	🟢	**ATAC Enzyme**	2000123/ 2000138	Centrifuge briefly.	–20°C
		Nuclei** in Diluted Nuclei Buffer (See below to Prepare Diluted Nuclei Buffer)			

**Refer to Demonstrated Protocols for isolating nuclei for ATAC Sequencing (Documents CG000169; CG000212). Adhering to this protocol is critical for optimal assay performance. If following a different nuclei isolation protocol, use the Diluted Nuclei Buffer for final nuclei pellet suspension.

The use of the Tris-based Diluted Nuclei Buffer for nuclei suspension is critical for optimal assay performance. The composition of the Diluted Nuclei Buffer, including Magnesium concentration, has been optimized for the Transposition and Barcoding steps. Suspension of nuclei in a different buffer may not be compatible with the downstream protocol steps.

Prepare — **Diluted Nuclei Buffer**

Diluted Nuclei Buffer Maintain at 4°C	Stock	Final	1 ml
🔵 20X Nuclei Buffer (PN-2000207)	20X	1X	50 µl
Nuclease-free Water	-	-	950 µl

Nuclei Concentration Guidelines

Based on the Targeted Nuclei Recovery, resuspend the nuclei in Diluted Nuclei Buffer to get corresponding Nuclei Stock Concentrations (see Table). This enables pipetting volumes of the Nuclei Stock for Transposition (step 1.1) to be 2-5 µl. Higher Nuclei Stock Concentrations will result in lower pipetting volumes that may increase nuclei input variability.

Targeted Nuclei Recovery	Nuclei Stock Concentration (nuclei/µl)
500	155-390
1,000	310-780
2,000	610-1,540
3,000	925-2,300
4,000	1,230-3,075
5,000	1,540-3,850
6,000	1,850-4,600
7,000	2,150-5,400
8,000	2,460-6,150
9,000	2,770-6,900
10,000	3,080-7,700

Calculate volume of Nuclei Stock and Diluted Nuclei Buffer for a total volume of 5 µl

$$\text{Volume of Nuclei Stock (µl)} = \frac{\text{Targeted Nuclei Recovery x 1.53 (Recovery efficiency factor)}}{\text{Nuclei Stock Concentration (nuclei/ µl)}}$$

Volume of Diluted Nuclei Buffer* (µl) = **5 µl** - volume of Nuclei Stock (µl)

*Use ONLY Diluted Nuclei Buffer (Dilute 20X Nuclei Buffer (PN-2000207) 1:20 in nuclease-free water)

Example Calculation

Targeted Nuclei Recovery = 4000 nuclei

Nuclei Stock Concentration = 2500 nuclei/ µl

Recovery efficiency factor 1.53

Volume of Nuclei Stock (µl) =

$$\frac{\text{Targeted Nuclei Recovery x 1.53 (Recovery efficiency factor)}}{\text{Nuclei Stock Concentration (nuclei/µl)}} = \frac{4000 \times 1.53}{2500} = \mathbf{2.45\ µl}$$

Volume of Diluted Nuclei Buffer = 5 µl - 2.45 µl = **2.55 µl**

Add calculated volumes of Diluted Nuclei Buffer and Nuclei Stock to the Transposition Mix in step 1.1

1.1 Prepare Transposition Mix

Next GEM

a. Prepare Transposition Mix on ice. Pipette mix 10x and centrifuge briefly.

Transposition Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
ATAC Buffer B	2000193	7.0	30.8	61.6
ATAC Enzyme	2000123/ 2000138	3.0	13.2	26.4
Total	-	**10.0**	**44.0**	**88.0**

b. Add **10 µl** Transposition Mix to a tube of a PCR 8-tube strip for each sample. Centrifuge briefly and maintain on ice.

c. Refer to Nuclei Concentration Guidelines to calculate the volume of Nuclei Stock and Diluted Nuclei Buffer for a total volume of **5 µl**.

d. Add the calculated volume of Diluted Nuclei Buffer to the Transposition Mix. Pipette mix. Centrifuge briefly.

e. Gently pipette mix the Nuclei Stock. Add the calculated volume of the Nuclei Stock to the tube containing the Transposition Mix. Gently pipette mix 6x (pipette set to 10 µl). DO NOT centrifuge.

1.2 Isothermal Incubation

a. Incubate in a thermal cycler using the following protocol.

Lid Temperature	Reaction Volume	Run Time
50°C	15 µl	60 min
Step	**Temperature**	**Time**
Incubate	37°C	00:60:00
Hold	4°C	Hold

Step 2

GEM Generation & Barcoding

2.0 GEM Generation & Barcoding

Next GEM

GET STARTED!

Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	**Single Cell ATAC Gel Beads v1.1**	2000210	Equilibrate to room temperature 30 min before loading the chip.	–80°C
	Nuclease-free Water	-	-	-
	Reducing Agent B	2000087	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
	Barcoding Reagent B	2000194	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
Place on Ice	**Barcoding Enzyme**	2000125/ 2000139	Maintain on ice. Store at –20°C immediately after use.	–20°C
Obtain	**Partitioning Oil**	2000190	-	Ambient
	Chromium Next GEM Chip H	2000180	See Tips & Best Practices.	Ambient
	10x Gasket	370017/ 3000072	See Tips & Best Practices.	Ambient
	10x Vortex Adapter	330002	See Tips & Best Practices.	Ambient
	Chromium Next GEM Secondary Holder	3000332	See Tips & Best Practices	Ambient
	50% glycerol solution If using <8 reactions	-	See Tips & Best Practices.	-

Firmware Version 4.00 or higher is required in the Chromium Controller or the Chromium Single Cell Controller used for the Single Cell ATAC v1.1 protocol.

2.1 Prepare Master Mix

Next GEM

a. Prepare Master Mix on ice. Pipette mix 10x and centrifuge briefly.

	Master Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
●	**Barcoding Reagent B**	2000194	56.5	248.6	497.2
○	**Reducing Agent B**	2000087	1.5	6.6	13.2
●	**Barcoding Enzyme**	2000125/ 2000139	2.0	8.8	17.6
	Total	-	**60.0**	**264.0**	**528.0**

Assemble Chromium Next GEM Chip H

After removing the chip from the sealed bag, use the chip in ≤ 24 h.

TIPS See Tips & Best Practices for chip handling instructions.

- Align notch on the chip (upper left corner) and the holder.
- Insert the left-hand side of the chip under the guide. Depress the right-hand side of the chip until the spring-loaded clip engages.
- Close the lid before dispensing reagents into the wells.
- The assembled chip is ready for loading the indicated reagents. Refer to step 2.2 for reagent volumes and loading order.

For GEM generation, load the indicated reagents only in the specified rows, starting from row labeled 1, followed by rows labeled 2 and 3. DO NOT load reagents in the bottom row labeled NO FILL. See step 2.2 for details.

2.2 Load Chromium Next GEM Chip H

Next GEM

After removing the chip from the sealed bag, use in ≤ 24 h. **For all chip loading steps, raising and depressing the pipette plunger should each take ~5 sec**. When dispensing, raise the pipette tips at the same rate as the liquid is rising, keeping the tips slightly submerged.

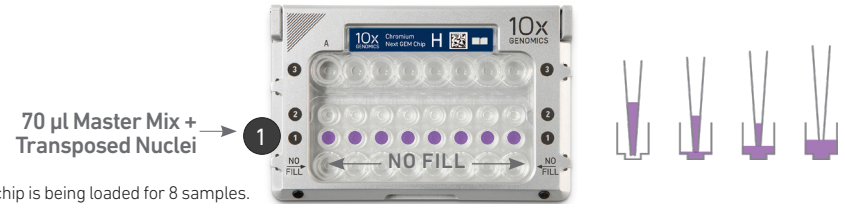
a. Dispense 50% Glycerol into Unused Chip Wells (if < 8 samples per chip)

i. **70 µl** to unused wells in **row labeled 1**.
ii. **50 µl** to unused wells in **row labeled 2**.
iii. **40 µl** to unused wells in **row labeled 3**.

DO NOT add 50% glycerol solution to the bottom row of NO FILL wells. DO NOT use any substitute for 50% glycerol solution.

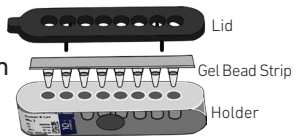
b. Prepare Master Mix + Transposed Nuclei
Add **60 µl** Master Mix to each tube containing Transposed Nuclei for a total of **75 µl** in each tube.

c. Load Row Labeled 1
Gently pipette mix the Master Mix + Transposed Nuclei 5x. Using the same pipette tip, dispense **70 µl** Master Mix + Transposed Nuclei into the bottom center of each well in **row labeled 1** without introducing bubbles.



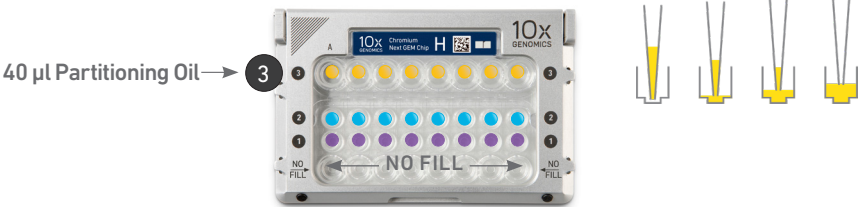
The illustrated chip is being loaded for 8 samples.

d. Prepare Gel Beads
Snap the tube strip holder with the Gel Bead strip into a 10x Vortex Adapter. Vortex **30 sec.** Centrifuge the Gel Bead strip for **~5 sec**. Confirm there are no bubbles at the bottom of the tubes and the liquid levels are even. Place the Gel Bead strip back in the holder. Secure the holder lid.



e. Load Row Labeled 2
Puncture the foil seal of the Gel Bead tubes. Slowly aspirate **50 µl** Gel Beads. Dispense into the wells in **row labeled 2** without introducing bubbles. Wait **30 sec**.

f. Load Row Labeled 3
Dispense **40 µl** Partitioning Oil into the wells in **row labeled 3** from a reagent reservoir. Failure to add Partitioning Oil to the top row labeled 3 will prevent GEM generation and can damage the Chromium Controller.



Attach the gasket and run the chip in the Chromium Controller **immediately** after loading the Partitioning Oil.

g. Attach 10x Gasket
Align the notch with the top left-hand corner. Ensure the gasket holes are aligned with the wells. Avoid touching the smooth surface.

Keep horizontal to avoid wetting the gasket. DO NOT press down on the gasket.

2.3 Run the Chromium Controller

Next GEM

a. Press the eject button on the Controller to eject the tray.

b. Place the assembled chip with the gasket in the tray, ensuring that the chip stays horizontal. Press the button to retract the tray.

c. Confirm the program on screen. Press the play button.

d. At completion of the run **(~18 min)**, the Controller will chime. **Immediately** proceed to the next step.

Firmware Version 4.00 or higher is required in the Chromium Controller or the Chromium Single Cell Controller used for the Single Cell ATAC v1.1 protocol.

2.4 Transfer GEMs

Next GEM

a. Place a PCR 8-tube strip on ice.

b. Press the eject button of the Controller to remove the chip.

c. Discard the gasket. Open the chip holder. Fold the lid back until it clicks to expose the wells at 45 degrees.

d. Check the volume in row labeled 1-2. Abnormally high volume in any well indicates a clog.

e. Slowly aspirate **100 µl** GEMs from the lowest points of the recovery wells in the top row labeled 3 without creating a seal between the pipette tips and the wells.

f. Withdraw pipette tips from the wells. GEMs should appear opaque and uniform across all channels. Excess Partitioning Oil (clear) in the pipette tips indicates a potential clog.

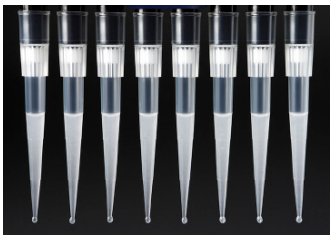
g. Over the course of **~20 sec**, dispense GEMs into the tube strip on ice with the pipette tips against the sidewalls of the wells.

h. If multiple chips are run back-to-back, cap/cover the GEM-containing tube strip or plate and place on ice for no more than **1 h**.

Expose Wells at 45 Degrees

Transfer GEMs

GEMs



2.5 GEM Incubation

Use a thermal cycler that can accommodate at least 100 µl volume. A volume of 125 µl is the preferred setting on Bio-Rad C1000 Touch. In alternate thermal cyclers, use highest reaction volume setting.

a. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	30 min
Step	**Temperature**	**Time**
1	72°C	00:05:00
2	98°C	00:00:30
3	98°C	00:00:10
4	59°C	00:00:30
5	72°C	00:01:00 Go to step 3, repeat 11X (Total 12 cycles)
6	15°C	Hold

b. Store at **15°C** for up to **18 h** or at **–20°C** for up to **1 week,** or proceed to the next step.

Step 3

Post GEM Incubation Cleanup

3.0 Post GEM Incubation Cleanup

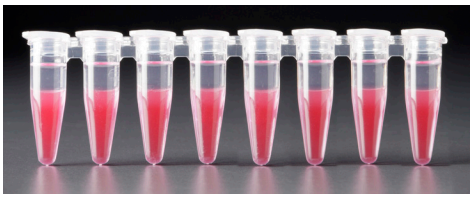
GET STARTED!					
Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	○	**Reducing Agent B**	2000087	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
		Nuclease-free Water	-	-	-
		Dynabeads MyOne SILANE	2000048	Vortex thoroughly (≥**30 sec**) to resuspend beads immediately before use.	4°C
		Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations.	-
Thaw at 65°C	●	**Cleanup Buffer**	2000088	Thaw for **10 min** at **65°C** at max speed on a thermomixer. Verify there are no visible crystals. Cool to room temperature.	–20°C
Obtain		**Recovery Agent**	220016	-	Ambient
		Qiagen Buffer EB	-	Manufacturer's recommendations.	-
		Bio-Rad 10% Tween 20	-	Manufacturer's recommendations.	-
		10x Magnetic Separator	230003	-	Ambient
		Prepare 80% Ethanol Prepare 10 ml for 8 reactions	-	Prepare fresh.	-

3.1 Post GEM Incubation Cleanup – Dynabeads

a. Add **125 µl** Recovery Agent to each sample at room temperature. DO NOT pipette mix or vortex the biphasic mixture. Gently invert tube 10x to mix. Centrifuge briefly.

The resulting biphasic mixture contains Recovery Agent/Partitioning Oil (pink) and aqueous phase (clear), with no persisting emulsion (opaque).

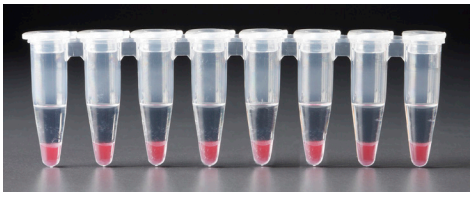
Biphasic Mixture



A smaller aqueous phase volume indicates a clog during GEM generation.

b. Slowly remove and discard **125 µl** Recovery Agent/Partitioning Oil (pink) from the bottom of the tube. DO NOT aspirate any aqueous sample.

Remove Recovery Agent



c. Prepare Dynabeads Cleanup Mix.

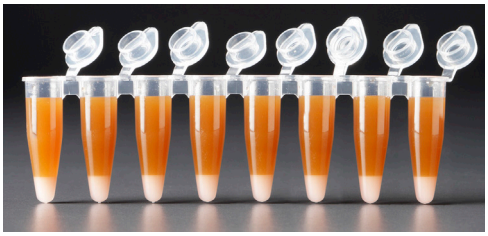
Dynabeads Cleanup Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Cleanup Buffer	2000088	182	800.8	1601.6
Dynabeads MyOne SILANE Vortex thoroughly (≥**30 sec**) **immediately** before adding to the mix. Aspirate the full liquid volume with a pipette tip to verify that the beads have not settled in the bottom of the tube. If clumps are present, pipette mix to resuspend completely. DO NOT centrifuge before use.	2000048	8	35.2	70.4
Reducing Agent B	2000087	5	22	44
Nuclease-free Water	-	5	22	44
Total	-	**200**	**880**	**1760**

Resuspend clump

d. Vortex and add **200 µl** to each sample. Pipette mix 5x (pipette set to 200 µl).

e. Incubate **10 min** at **room temperature**.

Add Dynabeads Cleanup Mix



f. Prepare Elution Solution I. Vortex and centrifuge briefly.

Elution Solution I* *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Buffer EB	-	98.0	431.2	862.4
10% Tween 20	-	1.0	4.4	8.8
○ **Reducing Agent B**	200087	1.0	4.4	8.8
Total	-	**100.0**	**440.0**	**880.0**

*Elution Solution I will be used in steps 3.1o and 3.2j

TIPS

g. At the end of **10 min** incubation, place on the 10x Magnetic Separator, high position (magnet•**High**) until the solution clears.

h. Remove the supernatant.

i. Add **300 µl** freshly prepared 80% ethanol to the pellet while on the magnet•**High**. Wait **30 sec**.

j. Remove the ethanol.

k. Add **200 µl** 80% ethanol to pellet. Wait **30 sec**.

l. Remove the ethanol.

m. Centrifuge briefly. Place on the magnet•**Low**.

n. Remove remaining ethanol.

o. Remove from the magnet. **Immediately** add **40.5 µl** Elution Solution I to avoid clumping.

p. Pipette mix (pipette set to 40 µl) without introducing bubbles.

q. Incubate **1 min** at **room temperature**.

r. Centrifuge briefly. Place on the magnet•**Low** until the solution clears.

s. Transfer **40 µl** sample to a new tube strip.

3.2 Post GEM Incubation Cleanup – SPRIselect

a. Vortex the SPRIselect reagent until fully resuspended. Add **48 µl** SPRIselect reagent to each sample. Pipette mix thoroughly.

b. Incubate **5 min** at **room temperature**.

c. Centrifuge briefly. Place on the magnet•**High** until the solution clears.

d. Remove the supernatant.

e. Add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

f. Remove the ethanol.

g. **Repeat** steps e and f for a total of 2 washes.

h. Centrifuge briefly. Place on the magnet•**Low.**

i. Remove any remaining ethanol.

j. Remove the tube strip from the magnet. **Immediately** add **40.5 µl** Elution Solution I.

k. Pipette mix (pipette set to 30 µl) without introducing bubbles.

l. Incubate **2 min** at **room temperature**.

m. Centrifuge briefly. Place on the magnet•**Low** until the solution clears.

n. Transfer **40 µl** sample to a new tube strip.

o. Store at **4°C** for up to **72 h** or at **–20°C** for up to **2 weeks**, or proceed to the next step.

Step 4

Library Construction

4.0 Library Construction

GET STARTED!				
Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	**Chromium i7 Sample Index Plate N, Set A**	3000262	-	–20°C
	Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations.	-
	Agilent Bioanalyzer DNA kit (if used for QC)	-	Manufacturer's recommendations.	-
Place on Ice	**SI-PCR Primer B**	2000128	Vortex, centrifuge briefly.	–20°C
	Amp Mix	2000047/ 2000103	Gently pipette mix, centrifuge briefly.	–20°C
	KAPA Library Quantification Kit for Illumina® Platforms	-	Manufacturer's recommendations.	-
Obtain	**Qiagen Buffer EB**	-	-	Ambient
	10x Magnetic Separator	230003	See Tips & Best Practices.	Ambient
	Prepare 80% Ethanol Prepare 10 ml for 8 reactions	-	Prepare fresh.	Ambient

4.1 Sample Index PCR

Choose the appropriate sample index sets to ensure that no sample indices overlap in a multiplexed sequencing run.

a. Prepare Sample Index PCR Mix.

Sample Index PCR Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
○ **Amp Mix**	2000047/ 2000103	50	220	440
● **SI- PCR Primer B**	2000128	7.5	33	66
Total	-	**57.5**	**253**	**506**

b. Add **57.5 µl** Sample Index PCR Mix to **40 µl** sample. Pipette mix and centrifuge briefly.

c. Add **2.5 µl** of an individual Chromium i7 Sample Index N, Set A to each well. Record assignment. Pipette mix and centrifuge briefly.

d. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~30 min
Step	**Temperature**	**Time**
1	98°C	00:00:45
2	98°C	00:00:20
3	67°C	00:00:30
4	72°C	00:00:20 Go to step 2, see table below for # cycles
5	72°C	00:01:00
6	4°C	Hold

The table recommends a starting point for cycle number optimization based on Targeted Nuclei Recovery.

Cycle Number Optimization Table

Targeted Nuclei Recovery	Total Cycles
500-2,000	11
2,001-6,000	10
6,001-10,000	9

STOP

e. Store at **4°C** for up to **72 h** or proceed to the next step.

4.2 Post Sample Index Double Sided Size Selection – SPRIselect

a. Vortex to resuspend SPRIselect reagent. Add **40 µl** SPRIselect reagent to each sample. Pipette mix.

b. Incubate **5 min** at **room temperature**.

c. Place on the magnet•**High** until the solution clears.

d. Transfer **130 µl** supernatant to a new strip tube. DO NOT discard the supernatant.

e. Vortex to resuspend SPRIselect reagent. Add **74 µl** SPRIselect reagent to each sample. Pipette mix.

f. Incubate **5 min** at **room temperature**.

g. Place on the magnet•**High** until the solution clears.

h. Remove the supernatant.

i. Add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

j. Remove the ethanol.

k. **Repeat** steps i and j for a total of 2 washes.

l. Centrifuge briefly. Place on the magnet•**Low**.

m. Remove remaining ethanol.

n. Remove from the magnet. **Immediately** add **20.5 µl** Buffer EB. Pipette mix.

o. Incubate **2 min** at **room temperature**.

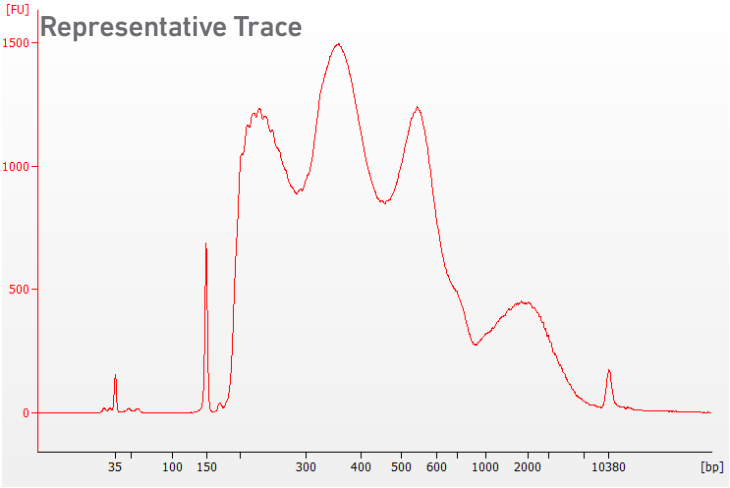
p. Centrifuge briefly. Place on the magnet•**Low** until the solution clears.

q. Transfer **20 µl** sample to a new tube strip.

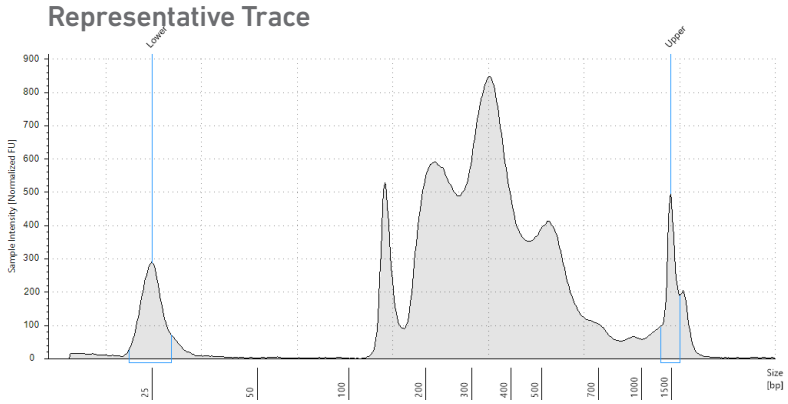
r. Store at **4°C** for up to **72 h** or at **–20°C** for **long-term** storage.

4.3 Post Library Construction QC

a. EITHER Run **1 µl** sample on the Agilent Bioanalyzer High Sensitivity DNA chip to determine fragment size. Lower molecular weight product (≤ 150 bp) may be present. This does not affect sequencing.



b. OR Run **2 µl** sample on the Agilent TapeStation High Sensitivity D1000 ScreenTape to determine fragment size.



4.4 Post Library Construction Quantification

a. Thaw KAPA Library Quantification Kit for Illumina® Platforms.

b. Dilute **1 µl** sample with deionized water to appropriate dilutions that fall within the linear detection range of the KAPA Library Quantification Kit for Illumina® Platforms. (For more accurate quantification, make the dilution(s) in duplicate).

c. Make enough Quantification Master Mix for the DNA dilutions per sample and the DNA Standards (plus 10% excess) using the guidance for 1 reaction volume below.

Quantification Master Mix	1X (µl)
SYBR Fast Master Mix + Primer	12
Water	4
Total	**16**

d. Dispense **16 µl** Quantification Master Mix for sample dilutions and DNA Standards into a 96 well PCR plate.

e. Add **4 µl** sample dilutions and **4 µl** DNA Standards to appropriate wells. Centrifuge briefly.

f. Incubate in a thermal cycler with the following protocol.

Step	Temperature	Run Time
1	95°C	00:03:00
2	95°C	00:00:05
3	67°C	00:00:30
4	Go to Step 2, 29X (Total 30 cycles)	

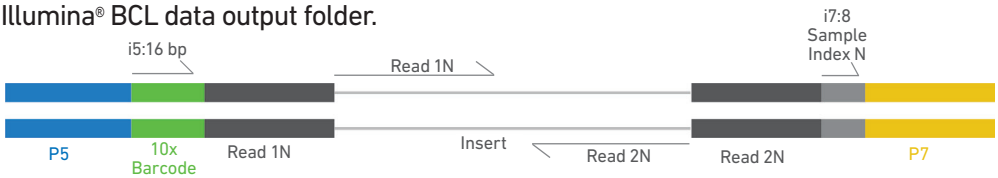
g. Follow the manufacturer's recommendations for qPCR-based quantification. For library quantification for sequencer clustering, determine the concentration using the average size in the region of 175-1,000 bp.

Sequencing

5

Sequencing Libraries

Chromium Single Cell ATAC libraries comprise double stranded DNA fragments which begin with P5 and end with P7. Sequencing these libraries produces a standard Illumina® BCL data output folder.



The BCL data for Single Cell ATAC libraries include:

- Paired-end Read 1N containing insert sequence only
- Read 2N containing insert sequence, starting from the opposite end of fragment
- 8 bp sample index in the i7 read
- 16 bp 10X barcode sequence in the i5 read

The Cell Ranger scATAC pipeline performs demultiplexing and leverages the 10x Barcodes to group read-pairs and associate them to individual cells for secondary analysis and visualization. In addition to performing standard analysis steps such as alignment, Cell Ranger scATAC leverages the 10x Barcodes to generate chromatin accessibility data with single cell resolution. This enables applications including cell clustering, cell type classification, and differential accessibility at a scale of hundreds to thousands of cells.

Illumina® Sequencer Compatibility

The compatibility of the listed sequencers has been verified by 10x Genomics. Some variation in assay performance is expected based on sequencer choice. For more information about performance variation, visit the 10x Genomics Support website.

- MiSeq™
- NextSeq™ 500/550 (High Output)
- HiSeq 2500™ (Rapid Run)
- HiSeq™ 3000/4000
- NovaSeq™

Sample Indices

Each sample index in the Chromium i7 Sample Index Plate Kit N, Set A (PN-3000262) is a mix of 4 different sequences to balance across all 4 nucleotides. If multiple samples are pooled in a sequence lane, the sample index name (i.e. the Chromium i7 Sample Index Plate N, Set A well ID) is needed in the sample sheet used for generating FASTQs with “cellranger-scATAC mkfastq”.

Sequencing Depth & Run Parameters

Sequencing Depth	25,000 read pairs per nucleus (25,000 reads for Read 1N; 25,000 reads for Read 2N)
Sequencing Type	Paired-end, dual indexing
Sequencing Read	Recommended Number of Cycles
Read 1N	50 cycles
i7 Index	8 cycles
i5 Index	16 cycles
Read 2N	50 cycles

Library Loading

Once quantified and normalized, Single Cell ATAC libraries should be denatured and diluted according to the table below. Consult the Technical Note on Sequencing Metrics and Base Composition of Chromium Single Cell ATAC Libraries (Document CG000181), available at the 10x Genomics Support website, for more information.

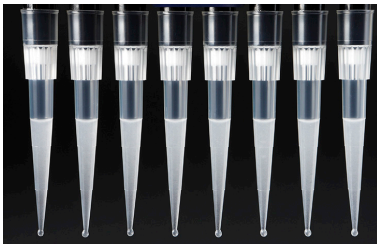
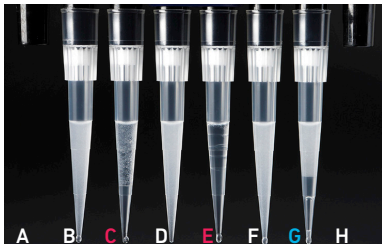
Instrument	Loading Concentration (pM)	PhiX (%)
MiSeq™	11	1
NextSeq™ 500/550	1.7	1
HiSeq™ 2500 (RR)	11	1
HiSeq™ 4000	180	1
NovaSeq™	300	1

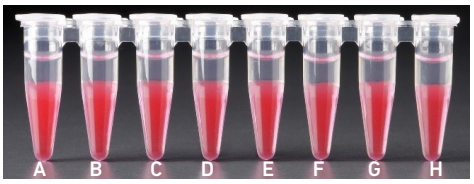
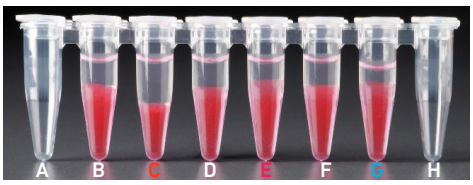
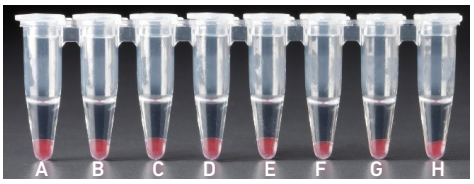
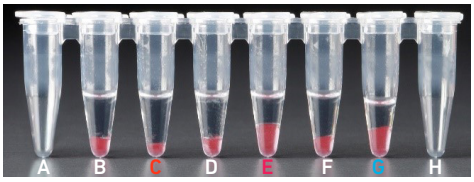
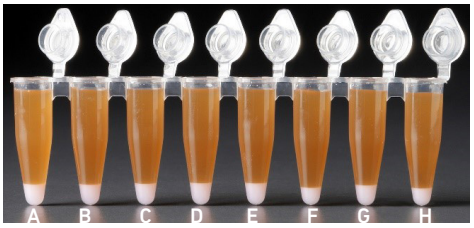
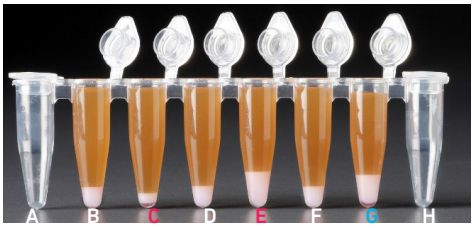
Library Pooling

Pooling dissimilar libraries may compromise the ability to pool effectively due to differences in insert sizes. DO NOT pool Single Cell ATAC libraries with other 10x Genomics libraries.

Troubleshooting

GEMs

STEP	NORMAL	REAGENT CLOGS & WETTING FAILURES
2.4 d After Chip H is removed from the Controller and the wells are exposed	 All 8 recovery wells (row labeled 3) are similar in volume and opacity.	 Recovery well G indicates a reagent clog. Recovery well C and E indicate a wetting failure. Recovery wells B, D, and F are normal. Wells A and H contain 50% Glycerol Solution.
2.4 f Transfer GEMs from Chip H Row Labeled 3	All liquid levels are similar in volume and opacity without air trapped in the pipette tips. 	Pipette tips C and E indicate a wetting failure. Pipette tip C contains partially emulsified GEMs. Emulsion is absent in pipette tip E. Pipette tip G indicates a reagent clog. 

STEP	NORMAL	REAGENT CLOGS & WETTING FAILURES
3.1 a After transfer of the GEMs + Recovery Agent	 All liquid levels are similar in the aqueous sample volume (clear) and Recovery Agent/Partitioning Oil (pink).	 Tube G indicates a reagent clog has occurred. There is a decreased volume of aqueous layer (clear). Tube C and E indicate a wetting failure has occurred. There is an abnormal volume of Recovery Agent/Partitioning Oil (pink).
3.1 b After aspiration of Recovery Agent/ Partitioning Oil	 All liquid volumes are similar in the aqueous sample volume (clear) and residual Recovery Agent/Partitioning Oil (pink).	 Tube G indicates a reagent clog has occurred. There is a decreased volume of aqueous layer (clear). There is also a greater residual volume of Recovery Agent/Partitioning Oil (pink). Tube C and E indicate a wetting failure has occurred. There is an abnormal residual volume of Recovery Agent/Partitioning Oil (pink).
3.1 d After addition of Dynabeads Cleanup Mix	 All liquid volumes are similar after addition of the Dynabeads Cleanup Mix.	 Tube G indicates a reagent clog has occurred. There is an abnormal ratio of Dynabeads Cleanup Mix (brown) to Recovery Agent/Partitioning Oil (appears white). Tube C and E indicate a wetting failure has occurred. There is an abnormal ratio of Dynabeads Cleanup Mix (brown) to Recovery Agent/Partitioning Oil (appears white).

If a channel clogs or wetting failure occurs during GEM generation, it is recommended that the sample be remade. If any of the listed issues occur, take a picture and send it to support@10xgenomics.com for further assistance.

Chromium Controller Errors

If the Chromium Controller or the Chromium Single Cell Controller fails to start, an error tone will sound and one of the following error messages will be displayed:

a. **Chip not read – Try again:** Eject the tray, remove and/or reposition the Chromium Next GEM Secondary Holder assembly and try again. If the error message is still received after trying this more than twice, contact support@10xgenomics.com for further assistance.

b. **Check gasket:** Eject the tray by pressing the eject button to check that the 10x Gasket is correctly installed on the Chromium Next GEM Chip. If the error message persists, contact support@10xgenomics.com for further assistance.

c. **Error Detected: Row _ Pressure:**

   i. If this message is received within a few seconds of starting a run, eject the tray by pressing the eject button and check for dirt or deposits on the 10x Gasket. If dirt is observed, replace with a new 10x Gasket and try again. If the error message is still received after trying this more than twice, contact support@10xgenomics.com for further assistance.

   ii. If this message is received after a few minutes into the run, the Chromium Next GEM Chip must be discarded. **Do not try running this Chromium Next GEM Chip again as this may damage the Chromium Controller.**

d. **Invalid Chip CRC Value:** This indicates that a Chromium Next GEM Chip has been used with an older firmware version. The chip must be discarded. Contact support@10xgenomics.com for further assistance.

Appendix

Oligonucleotide Sequences

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Oligonucleotide Sequences

Protocol steps correspond to the Chromium Next GEM Single Cell ATAC Reagent Kits v1.1 User Guide (CG000209)

Protocol Step 1 – Transposition

Transposition Mix

Read 1N primer sequence:
5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'

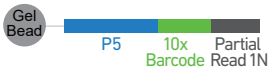
Read 2N primer sequence:
5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'

Transposed DNA Product

5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-----insert-- CTGTCTCTTATACACATCTCCGAGCCCACGAGAC-3'
3'-AGCAGCCGTCGCAGTCTACACATATTCTCTGTC --insert-----GACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG-5'

Protocol Step 2.5 – GEM Incubation

Gel Bead Oligo Primer PN-2000210



5'-AATGATACGGCGACCACCGAGATCTACAC-NNNNNNNNNNNNNNNN-TCGTCGGCAGCGTC-3'

Linear Amplification DNA Product

5'-AATGATACGGCGACCACCGAGATCTACAC-NNNNNNNNNNNNNNNN-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG----insert----CTGTCTCTTATACACATCTCCGAGCCCACGAGAC-3'

Protocol Step 4.1 – Sample Index PCR

SI-PCR Primer B PN-2000128

Forward Primer:

5'-AATGATACGGCGACCACCGAGA-3'

Reverse Primer:

5'- CAAGCAGAAGACGGCATACGAGAT-NNNNNNNN-GTCTCGTGGGCTCGG-3'

i7 Sample Index Plate N, Set A PN-3000262

Sample Index PCR Product

5'-AATGATACGGCGACCACCGAGATCTACAC-NNNNNNNNNNNNNNNN-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG---insert---CTGTCTCTTATACACATCTCCGAGCCCACGAGAC-NNNNNNNN-ATCTCGTATGCCGTCTTCTGCTTG-3'
3'-TTACTATGCCGCTGGTGGCTCTAGATGTG-NNNNNNNNNNNNNNNN-AGCAGCCGTCGCAGTCTACACATATTCTCTGTC---insert---GACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG-NNNNNNNN-TAGAGCATACGGCAGAAGACGAAC-5'

EXHIBIT 10

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				Art Unit	1639
				Examiner Name	Unassigned
Sheet	1	of	17	Attorney Docket Number	43487-703.201

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	1.	US 2001/0020588 A1	09/13/2001	Adourian et al.	
	2.	US 2001/0044109 A1	11/22/2001	Mandecki	
	3.	US 2002/0034737 A1	03/21/2002	Drmanac	
	4.	US 2002/0051992 A1	05/02/2002	Bridgham et al.	
	5.	US 2002/0092767 A1	07/18/2002	Bjornson et al.	
	6.	US 2002/0179849 A1	12/05/2002	Maher et al.	
	7.	US 2003/0008285 A1	01/09/2003	Fischer	
	8.	US 2003/0008323 A1	01/09/2003	Ravkin et al.	
	9.	US 2003/0028981 A1	02/13/2003	Chandler et al.	
	10.	US 2003/0039978 A1	02/27/2003	Hannah	
	11.	US 2003/0044777 A1	03/06/2003	Beattie	
	12.	US 2003/0044836 A1	03/06/2003	Levine et al.	
	13.	US 2003/0104466 A1	06/05/2003	Knapp et al.	
	14.	US 2003/0108897 A1	06/12/2003	Drmanac	
	15.	US 2003/0170698 A1	09/11/2003	Gascoyne et al.	
	16.	US 2003/0182068 A1	09/25/2003	Battersby et al.	
	17.	US 2003/0207260 A1	11/06/2003	Trnovsky et al.	
	18.	US 2003/0215862 A1	11/20/2003	Parce et al.	
	19.	US 2004/0063138 A1	04/01/2004	McGinnis et al.	
	20.	US 2004/0132122 A1	07/08/2004	Banerjee et al.	
	21.	US 2005/0019839 A1	01/27/2005	Jesperson et al.	
	22.	US 2005/0042625 A1	02/24/2005	Schmidt et al.	
	23.	US 2005/0130188 A1	06/16/2005	Walt et al.	
	24.	US 2005/0172476 A1	08/11/2005	Stone et al.	
	25.	US 2005/0181379 A1	08/18/2005	Su et al.	
	26.	US 2005/0221339 A1	10/06/2005	Griffiths et al.	
	27.	US 2005/0244850 A1	11/03/2005	Huang et al.	
	28.	US 2005/0287572 A1	12/29/2005	Mathies et al.	
	29.	US 2006/0020371 A1	01/26/2006	Ham et al.	

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Sheet	2	of	17	Attorney Docket Number	43487-703.201

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	31.	US 2006/0078888 A1	04/13/2006	Griffiths et al.	
	32.	US 2006/0153924 A1	07/13/2006	Griffiths et al.	
	33.	US 2006/0163385 A1	07/27/2006	Link et al.	
	34.	US 2006/0199193 A1	09/07/2006	Koo et al.	
	35.	US 2006/0240506 A1	10/26/2006	Kushmaro et al.	
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	53.	US 2009/0012187 A1	01/08/2009	Chu et al.	
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	58.	US 2009/0118488 A1	05/07/2009	Drmanac et al.	

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	61.	US 2009/0143244 A1	06/04/2009	Bridgham et al.	
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	68.	US 2010/0105112 A1	04/29/2010	Holtz et al.	
	69.	US 2010/0136544 A1	06/03/2010	Agresti et al.	
	70.	US 2010/0210479 A1	08/19/2010	Griffiths et al.	
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	73.	US 2011/0086780 A1	04/14/2011	Colston Jr et al.	
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	75.	US 2011/0160078 A1	06/30/2011	Fodor et al.	
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	87.	US 2012/0121481 A1	05/17/2012	Romanowsky et al.	

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	89.	US 2012/0135893 A1	05/31/2012	Drmanac et al.	
	90.	US 2012/0190032 A1	07/26/2012	Ness et al.	
	91.	US 2012/0211084 A1	08/23/2012	Weitz et al.	
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	93.	US 2012/0220497 A1	08/30/2012	Jacobson et al.	
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	98.	US 2013/0157899 A1	06/20/2013	Adler Jr et al.	
	99.	US 2013/0210639 A1	08/15/2013	Link et al.	
	100.	US 2013/0274117 A1	10/17/2013	Church et al.	
	101.	US 2014/0037514 A1	02/06/2014	Stone et al.	
	102.	US 2014/0065234 A1	03/06/2014	Shum et al.	
	103.	US 5,202,231	04/13/1993	Drmanac et al.	
	104.	US 5,436,130	07/25/1995	Mathies et al.	
	105.	US 5,512,131	04/30/1996	Kumar et al.	
	106.	US 5,587,128	12/24/1996	Wilding et al.	
	107.	US 5,618,711	04/08/1997	Gelfand et al.	
	108.	US 5,695,940	12/09/1997	Drmanac et al.	
	109.	US 5,736,330	04/07/1998	Fulton	
	110.	US 5,834,197	11/10/1998	Parton	
	111.	US 5,856,174	01/05/1999	Lipshutz et al.	
	112.	US 5,958,703	09/28/1999	Dower et al.	
	113.	US 5,994,056	11/30/1999	Higuchi et al.	
	114.	US 6,046,003	04/04/2000	Mandecki	
	115.	US 6,051,377	04/18/2000	Mandecki	
	116.	US 6,057,107	05/02/2000	Fulton	

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	118.	US 6,143,496	11/07/2000	Brown et al.	
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				Application Number	14/104,650
				Filing Date	December 12, 2013
				First Named Inventor	Benjamin Hindson
				Art Unit	1639
				Examiner Name	Unassigned
Sheet	6	of	17	Attorney Docket Number	43487-703.201

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	146.	US 7,425,431	09/16/2008	Church et al.	
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				First Named Inventor	Benjamin Hindson
				Art Unit	1639
				Examiner Name	Unassigned
Sheet	7	of	17	Attorney Docket Number	43487-703.201

UNPUBLISHED PATENT APPLICATIONS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	174.	US 13/966,150	Appln filed 08/13/2013	Hindson et al.	
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Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] – Number[4] – Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
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	220.	WO 2007/138178 A2	12/06/2007	Baroud et al.		
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NON PATENT LITERATURE DOCUMENTS			
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				Application Number	14/104,650
				Filing Date	December 12, 2013
				First Named Inventor	Benjamin Hindson
				Art Unit	1639
				Examiner Name	Unassigned
Sheet	12	of	17	Attorney Docket Number	43487-703.201

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
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				Filing Date	February 7, 2014
				First Named Inventor	Benjamin Hindson
				Art Unit	1621
				Examiner Name	Unassigned
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				Filing Date	February 7, 2014
				First Named Inventor	Benjamin Hindson
				Art Unit	1621
				Examiner Name	Unassigned
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U.S. PATENT DOCUMENTS

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				First Named Inventor	Benjamin Hindson
				Art Unit	1621
				Examiner Name	Unassigned
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				Art Unit	1621
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				Application Number	14/175,935
				Filing Date	February 7, 2014
				First Named Inventor	Benjamin Hindson
				Art Unit	1621
				Examiner Name	Unassigned
Sheet	8	of	17	Attorney Docket Number	43487-705.201

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] – Number[4] – Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
		abstract)				
	192.	JPS 5949832 A (in Japanese with English abstract)	03/22/1984	Manfureeto et al.		
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NON PATENT LITERATURE DOCUMENTS			
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				First Named Inventor	Benjamin Hindson
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Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	14/175,935
				Filing Date	February 7, 2014
				First Named Inventor	Benjamin Hindson
				Art Unit	1621
				Examiner Name	Unassigned
Sheet	16	of	17	Attorney Docket Number	43487-705.201

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
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				First Named Inventor	Benjamin Hindson
				Art Unit	1621
				Examiner Name	Unassigned
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NON PATENT LITERATURE DOCUMENTS			
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				Application Number	14/175,973
				Filing Date	February 7, 2014
				First Named Inventor	Benjamin Hindson
				Art Unit	1652
				Examiner Name	Unassigned
Sheet	1	of	17	Attorney Docket Number	43487-706.201

U.S. PATENT DOCUMENTS

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	29.	US 2006/0020371 A1	01/26/2006	Ham et al.	

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				Filing Date	February 7, 2014
				First Named Inventor	Benjamin Hindson
				Art Unit	1652
				Examiner Name	Unassigned
Sheet	2	of	17	Attorney Docket Number	43487-706.201

U.S. PATENT DOCUMENTS

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				Art Unit	1652
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	59.	US 2009/0137404 A1	05/28/2009	Drmanac et al.	
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	88.	US 2012/0132288 A1	05/31/2012	Weitz et al.	
	89.	US 2012/0135893 A1	05/31/2012	Drmanac et al.	
	90.	US 2012/0190032 A1	07/26/2012	Ness et al.	
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	95.	US 2013/0046030 A1	02/21/2013	Rotem et al.	
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	98.	US 2013/0157899 A1	06/20/2013	Adler Jr et al.	
	99.	US 2013/0210639 A1	08/15/2013	Link et al.	
	100.	US 2013/0274117 A1	10/17/2013	Church et al.	
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				Application Number	14/175,973
				Filing Date	February 7, 2014
				First Named Inventor	Benjamin Hindson
				Art Unit	1652
				Examiner Name	Unassigned
Sheet	5	of	17	Attorney Docket Number	43487-706.201

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	117.	US 6,103,537	08/15/2000	Ullman et al.	
	118.	US 6,143,496	11/07/2000	Brown et al.	
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	120.	US 6,297,006	10/02/2001	Drmanac et al.	
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				First Named Inventor	Benjamin Hindson
				Art Unit	1652
				Examiner Name	Unassigned
Sheet	6	of	17	Attorney Docket Number	43487-706.201

U.S. PATENT DOCUMENTS

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	146.	US 7,425,431	09/16/2008	Church et al.	
	147.	US 7,536,928	05/26/2009	Kazuno	
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	150.	US 7,638,276	12/29/2009	Griffiths et al.	
	151.	US 7,645,596	01/12/2010	Williams et al.	
	152.	US 7,666,664	02/23/2010	Sarofim et al.	
	153.	US 7,708,949	05/04/2010	Stone et al.	
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	155.	US 7,709,197	05/04/2010	Drmanac	
	156.	US 7,745,178	06/29/2010	Dong et al.	
	157.	US 7,776,927	08/17/2010	Chu et al.	
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	159.	US 7,842,457	11/30/2010	Berka et al.	
	160.	US 7,901,891	03/08/2011	Drmanac et al.	
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				First Named Inventor	Benjamin Hindson
				Art Unit	1652
				Examiner Name	Unassigned
Sheet	7	of	17	Attorney Docket Number	43487-706.201

UNPUBLISHED PATENT APPLICATIONS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	174.	US 13/966,150	Appln filed 08/13/2013	Hindson et al.	
	175.	US 14/104,650	Appln filed 12/12/2013	Hindson et al.	
	176.	US 14/175,935	Appln filed 02/07/2014	Hindson et al.	

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Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] – Number[4] – Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	177.	EP 0249007 A2	12/16/1987	Matsueda et al.		
	178.	EP 0637996 B1	07/23/1997	Wilding et al.		
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	181.	EP 1905828 B1	08/08/2012	Griffiths et al.		
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		abstract)				
	192.	JPS 5949832 A (in Japanese with English abstract)	03/22/1984	Manfureeto et al.		
	193.	WO 00/08212 A1	02/17/2000	Trnovsky et al.		
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Continued on the next page with more references.

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NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	244.	ABATE et al., Valve-based flow focusing for drog formation. Appl Phys Lett. 2009;94. 3 pages.	
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				First Named Inventor	Benjamin Hindson
				Art Unit	1652
				Examiner Name	Unassigned
Sheet	11	of	17	Attorney Docket Number	43487-706.201

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				Filing Date	August 13, 2013
				First Named Inventor	Benjamin Hindson
				Art Unit	1639
				Examiner Name	Unassigned
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Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
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				Application Number	13/966,150
				Filing Date	August 13, 2013
				First Named Inventor	Benjamin Hindson
				Art Unit	1639
				Examiner Name	Unassigned
Sheet	2	of	17	Attorney Docket Number	43487-702.201

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	30.	US 2006/0073487 A1	04/06/2006	Oliver et al.	
	31.	US 2006/0078888 A1	04/13/2006	Griffiths et al.	
	32.	US 2006/0153924 A1	07/13/2006	Griffiths et al.	
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				Art Unit	1639
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				First Named Inventor	Benjamin Hindson
				Art Unit	1639
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Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1]Applicant's unique citation designation number (optional). [2]Applicant is to place a checkmark here if English language Translation is attached.

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Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	13/966,150
				Filing Date	August 13, 2013
				First Named Inventor	Benjamin Hindson
				Art Unit	1639
				Examiner Name	Unassigned
Sheet	17	of	17	Attorney Docket Number	43487-702.201

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	314.	YAMAMOTO, et al. Chemical modification of Ce(IV)/EDTA-base artificial restriction DNa cutter for versatile manipulation of doulbe-stranded DNa. Nucleic Acids Research. 2007; 35(7):e53	
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Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1]Applicant's unique citation designation number (optional). [2]Applicant is to place a checkmark here if English language Translation is attached.

EXHIBIT 14

US009919277B2

(12) **United States Patent**
Griffiths et al.

(10) **Patent No.: US 9,919,277 B2**
(45) **Date of Patent: *Mar. 20, 2018**

(54) **IN VITRO EVOLUTION IN MICROFLUIDIC SYSTEMS**

(71) Applicants: **Medical Research Council**, London (GB); **President and Fellows of Harvard College**, Cambridge, MA (US)

(72) Inventors: **Andrew David Griffiths**, Strasbourg (FR); **David A. Weitz**, Cambridge, MA (US); **Darren Roy Link**, Lexington, MA (US); **Keunho Ahn**, Boston, MA (US); **Jerome Bibette**, Paris (FR)

(73) Assignees: **Medical Research Council**, London (GB); **President and Fellows of Harvard College**, Cambridge, MA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **15/588,210**

(22) Filed: **May 5, 2017**

(65) **Prior Publication Data**

US 2017/0282133 A1 Oct. 5, 2017

Related U.S. Application Data

(63) Continuation of application No. 15/587,026, filed on May 4, 2017, now abandoned, which is a continuation of application No. 15/012,209, filed on Feb. 1, 2016, which is a continuation of application No. 13/705,833, filed on Dec. 5, 2012, now abandoned, which is a continuation of application No. 11/665,030, filed as application No. PCT/GB2005/003889 on Oct. 10, 2005, now Pat. No. 9,029,083, which is a continuation of application No. 10/961,695, filed on Oct. 8, 2004, now Pat. No. 7,968,287.

(51) **Int. Cl.**

B01F 3/08	(2006.01)
B01F 5/06	(2006.01)
B01F 13/00	(2006.01)
B01L 3/00	(2006.01)
C12N 15/10	(2006.01)
C12P 21/00	(2006.01)
B01J 19/00	(2006.01)
C12Q 1/68	(2018.01)

(52) **U.S. Cl.**

CPC ***B01F 3/0807*** (2013.01); ***B01F 5/0646*** (2013.01); ***B01F 5/0647*** (2013.01); ***B01F 5/0655*** (2013.01); ***B01F 13/0062*** (2013.01); ***B01F 13/0071*** (2013.01); ***B01F 13/0076*** (2013.01); ***B01J 19/0046*** (2013.01); ***B01L 3/502746*** (2013.01); ***B01L 3/502753*** (2013.01); ***B01L 3/502776*** (2013.01); ***B01L 3/502784*** (2013.01); ***C12N 15/1058*** (2013.01); ***C12N 15/1075*** (2013.01); ***C12P 21/00*** (2013.01); ***C12Q 1/6874*** (2013.01); *B01J 2219/005* (2013.01); *B01J 2219/0052* (2013.01); *B01J 2219/00466* (2013.01); *B01J 2219/00468* (2013.01); *B01J 2219/00545* (2013.01); *B01J 2219/00576* (2013.01); *B01J 2219/00657* (2013.01); *B01J 2219/00722* (2013.01); *B01L 2200/0636* (2013.01); *B01L 2200/0673* (2013.01); *B01L 2300/0654* (2013.01); *B01L 2300/0816* (2013.01); *B01L 2300/0864* (2013.01); *B01L 2300/0867* (2013.01); *B01L 2400/0415* (2013.01); *B01L 2400/0487* (2013.01)

(58) **Field of Classification Search**

CPC B01L 3/50; C12N 15/1075; C12P 21/00; C12Q 1/68; G01N 15/06

See application file for complete search history.

Primary Examiner — Narayan Bhat

(74) *Attorney, Agent, or Firm* — Thomas C. Meyers; Brown Rudnick LLP

(57) **ABSTRACT**

The invention describes a method for isolating one or more genetic elements encoding a gene product having a desired activity, comprising the steps of: (a) compartmentalising genetic elements into microcapsules; and (b) sorting the genetic elements which express the gene product having the desired activity; wherein at least one step is under microfluidic control. The invention enables the in vitro evolution of nucleic acids and proteins by repeated mutagenesis and iterative applications of the method of the invention.

14 Claims, 25 Drawing Sheets

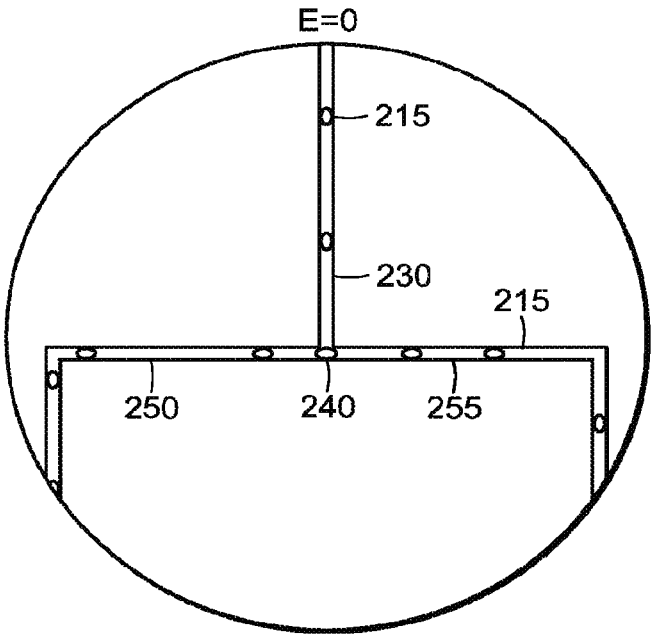

FIG. 1A

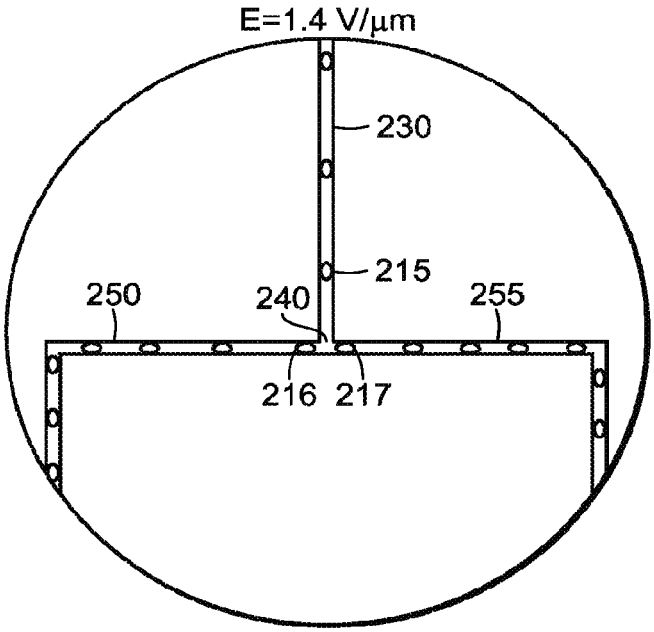

FIG. 1B

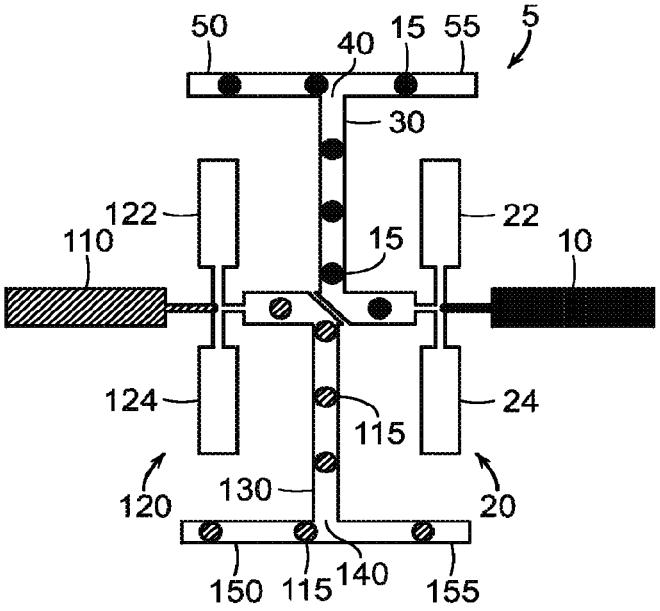
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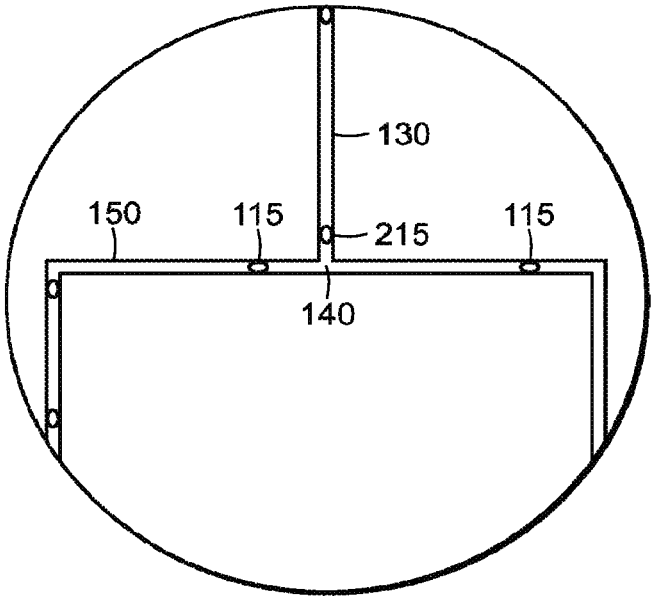
FIG. 2A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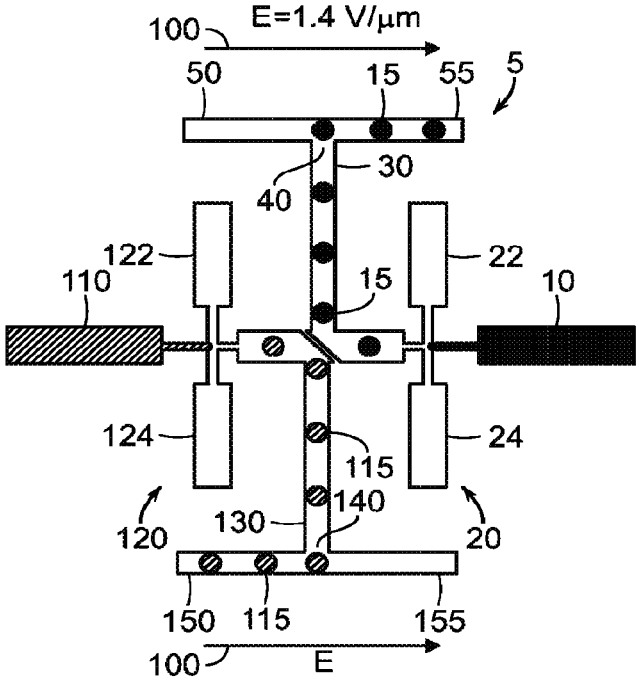
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FIG. 3A

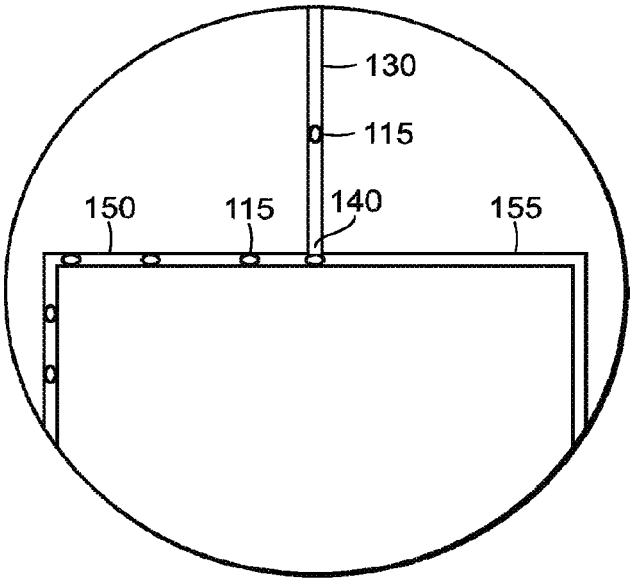
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FIG. 3B

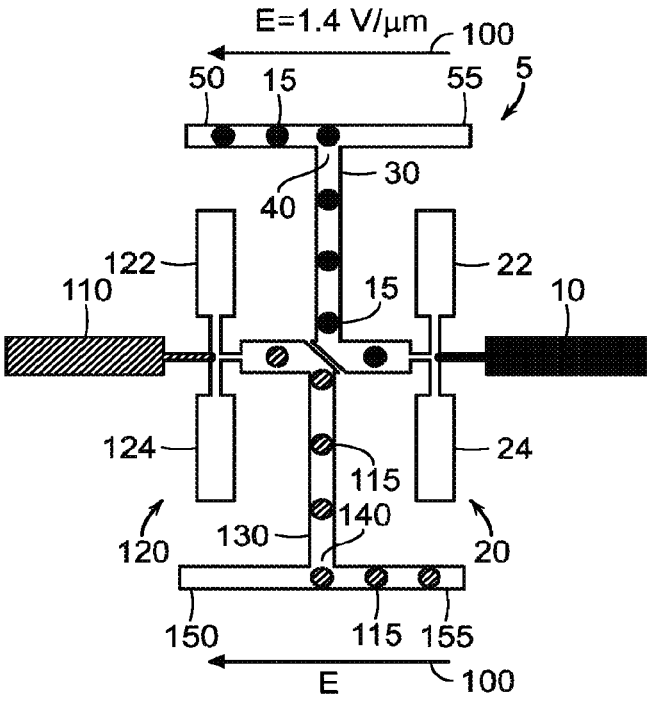
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FIG. 4A

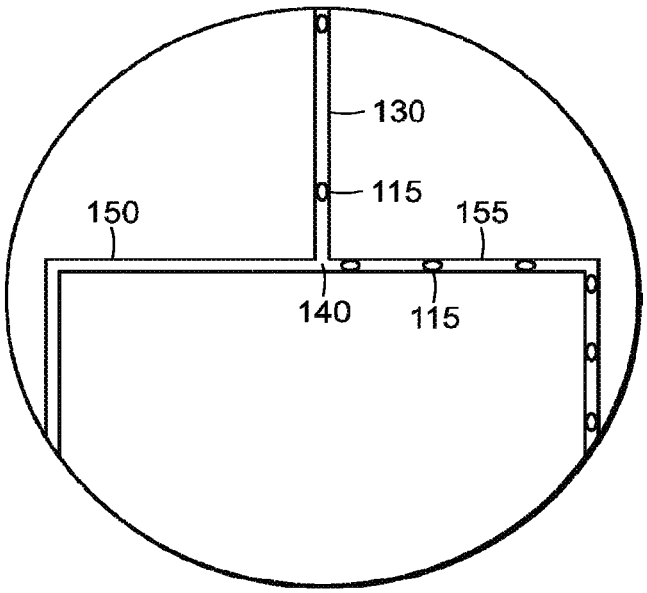
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FIG. 4B

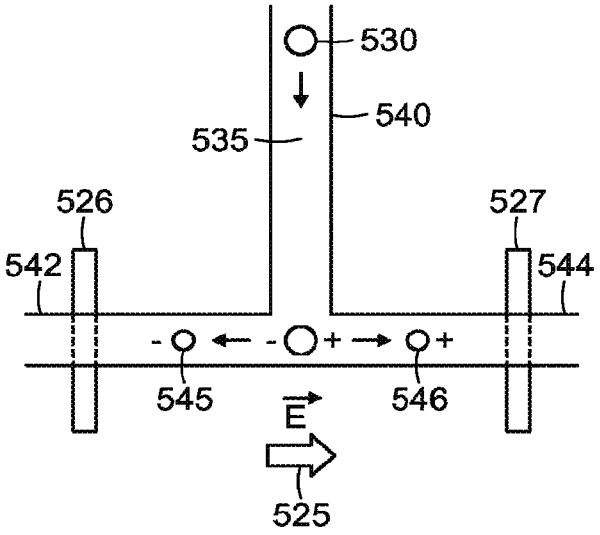

FIG. 5

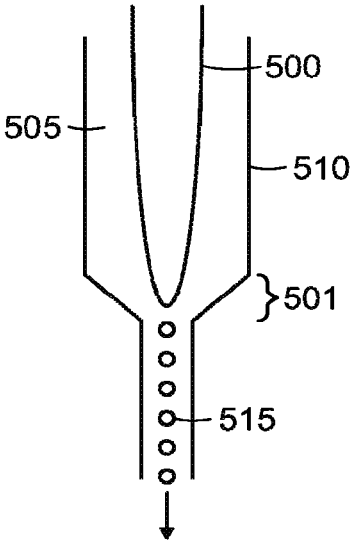

FIG. 7A

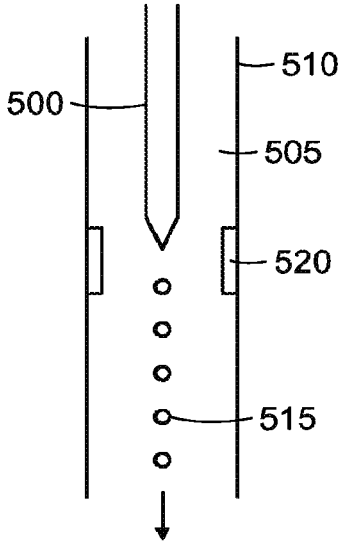

FIG. 7B

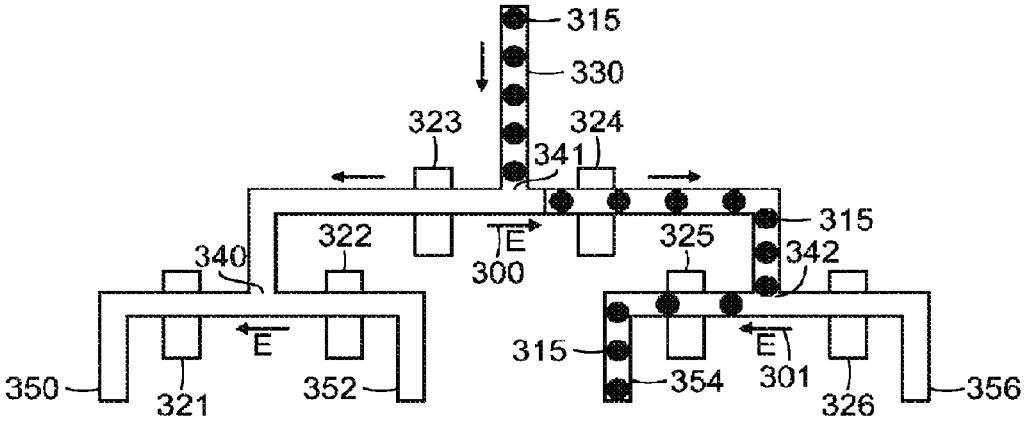
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FIG. 6A

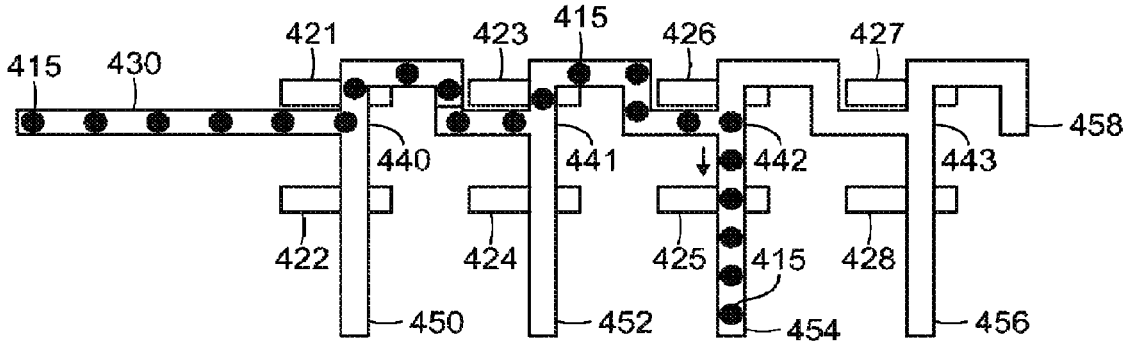
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FIG. 6B

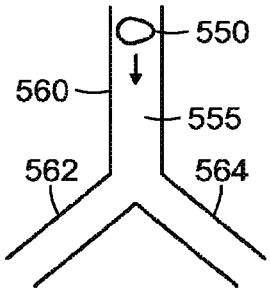


FIG. 8A

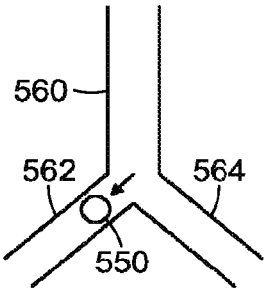


FIG. 8B

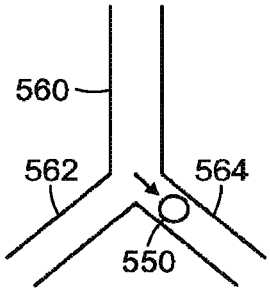


FIG. 8C

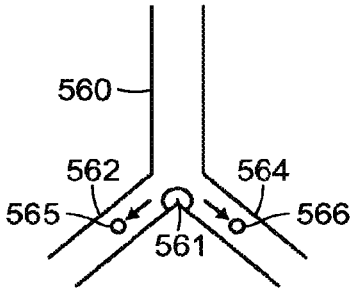


FIG. 8D

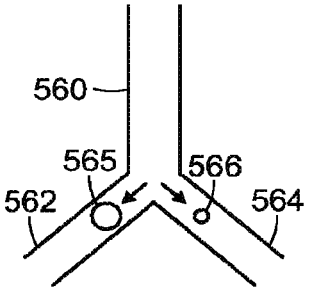


FIG. 8E

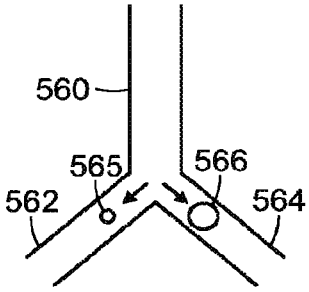


FIG. 8F

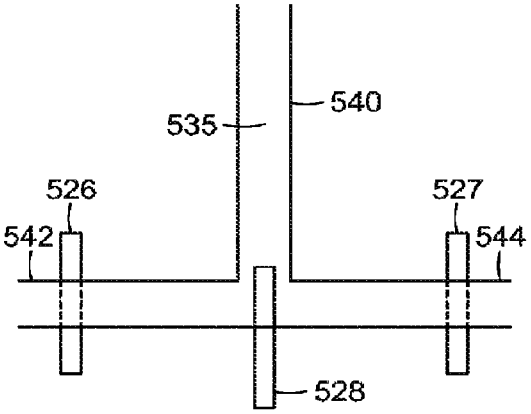


FIG. 9A

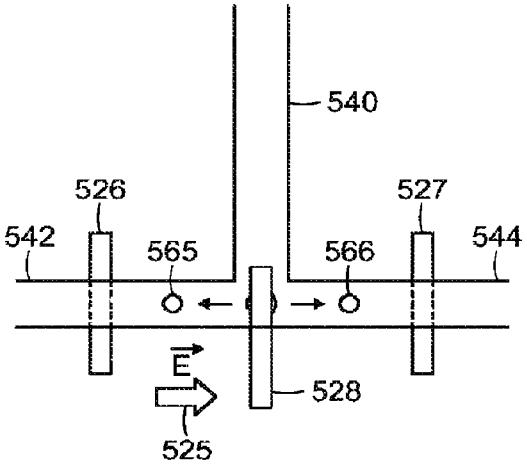


FIG. 9B

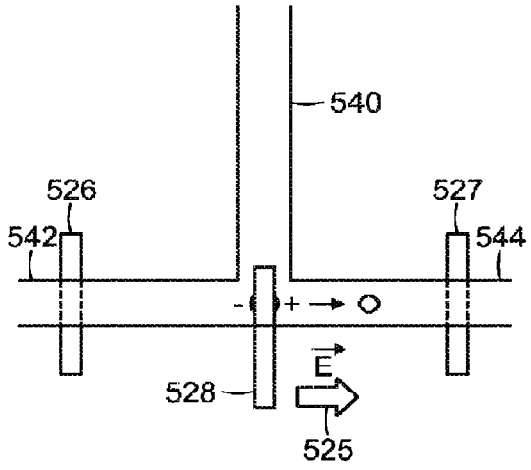


FIG. 9C

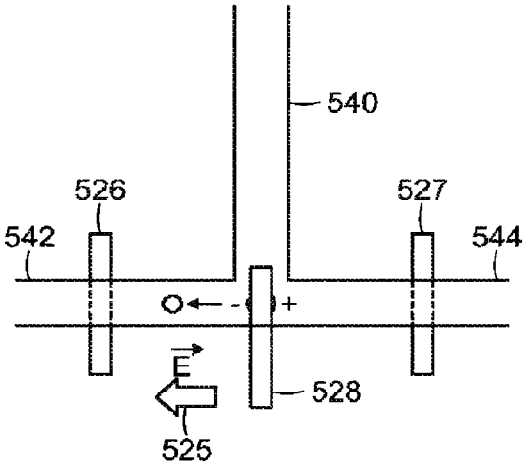


FIG. 9D

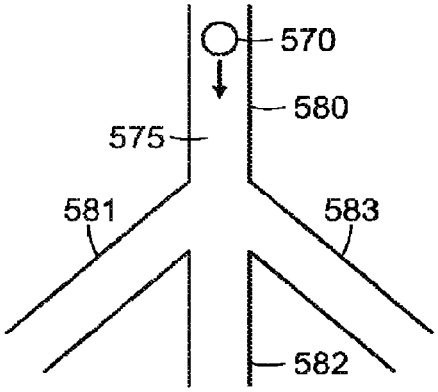
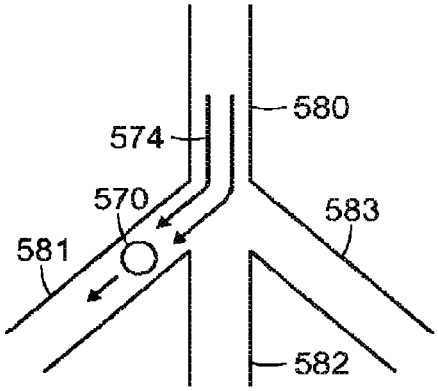


FIG. 10A



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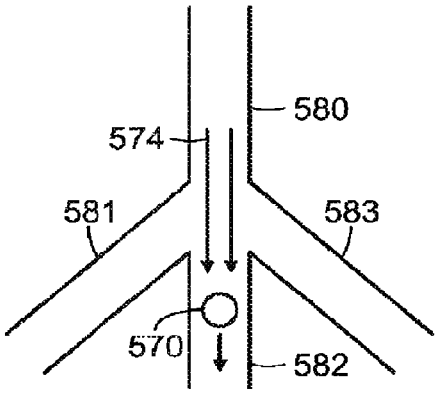

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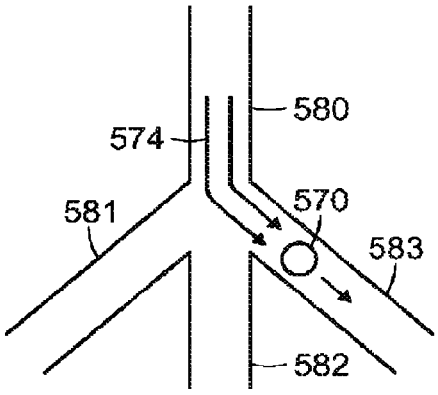


FIG. 10D

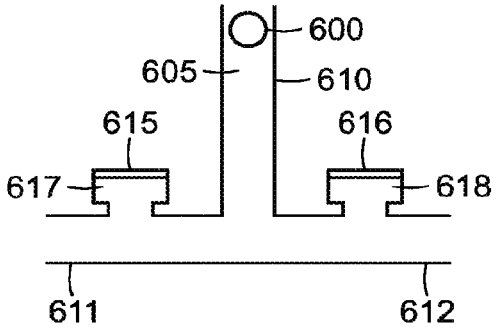


FIG. 11A

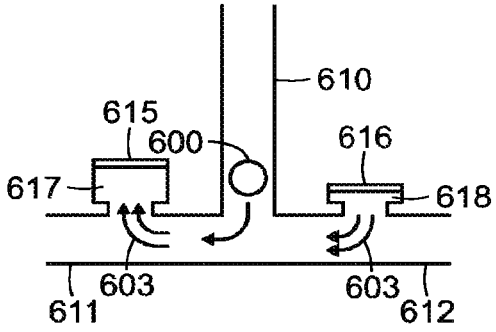


FIG. 11B

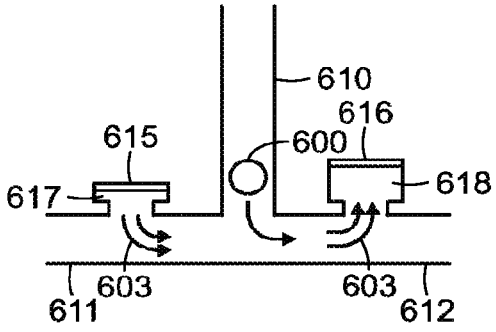
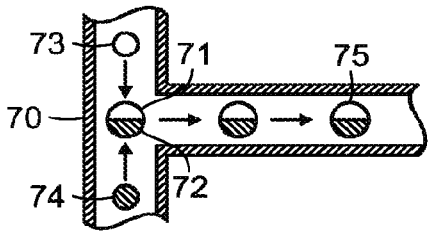


FIG. 11C



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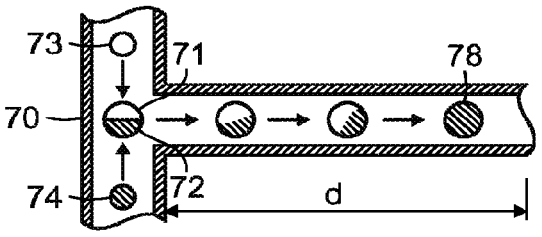


FIG. 12B

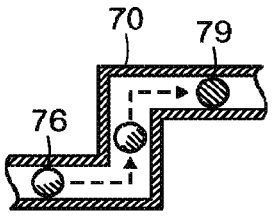


FIG. 12C

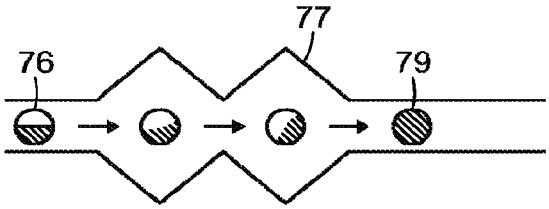


FIG. 12D

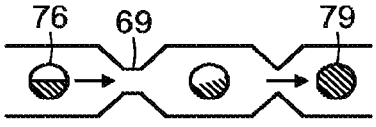


FIG. 12E

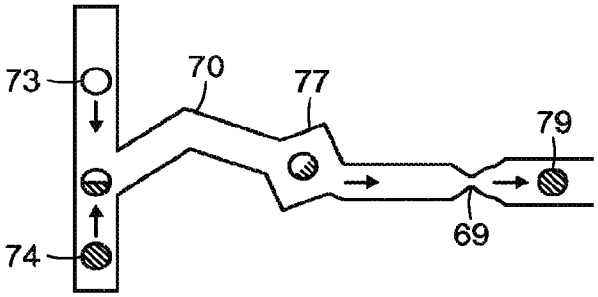


FIG. 12F

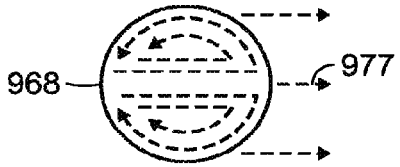


FIG. 12G

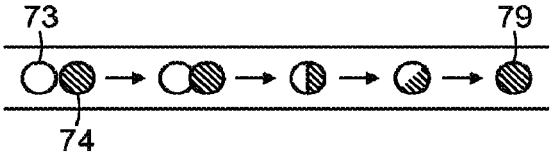


FIG. 12H

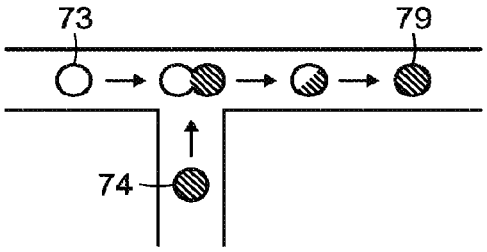


FIG. 12I

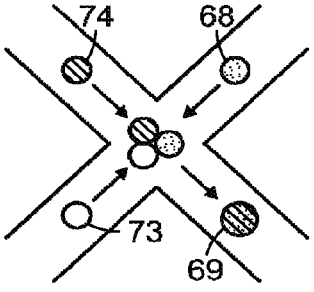


FIG. 12J

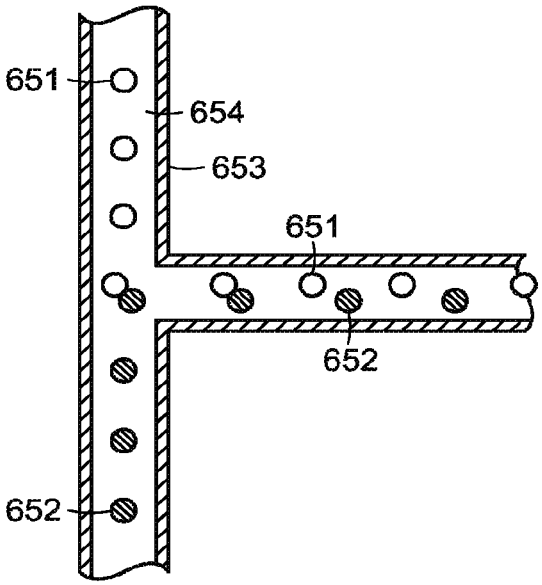


FIG. 13A

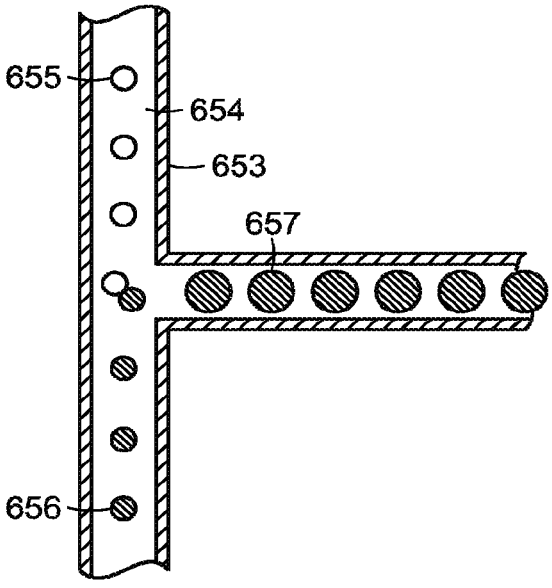


FIG. 13B

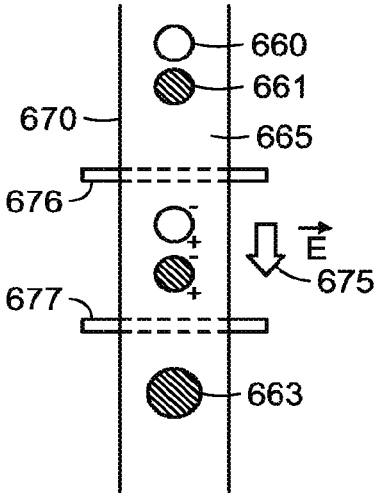


FIG. 13C

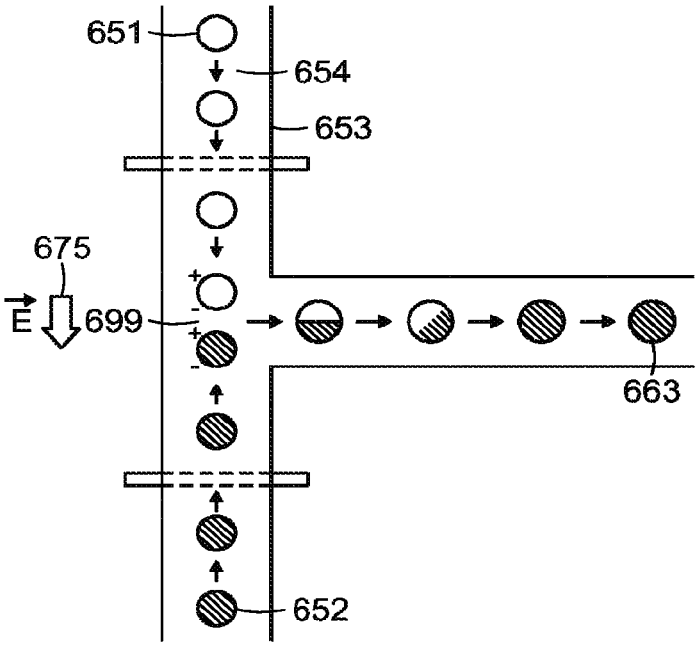


FIG. 13D

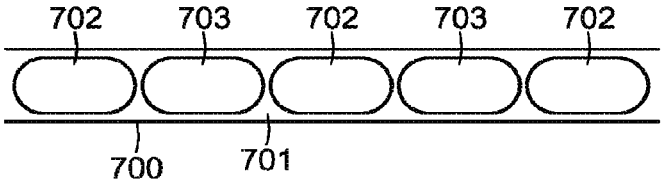


FIG. 14A

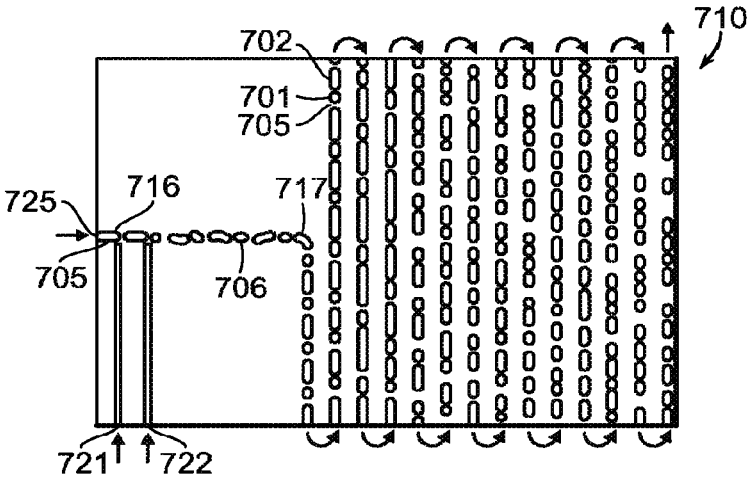


FIG. 14B

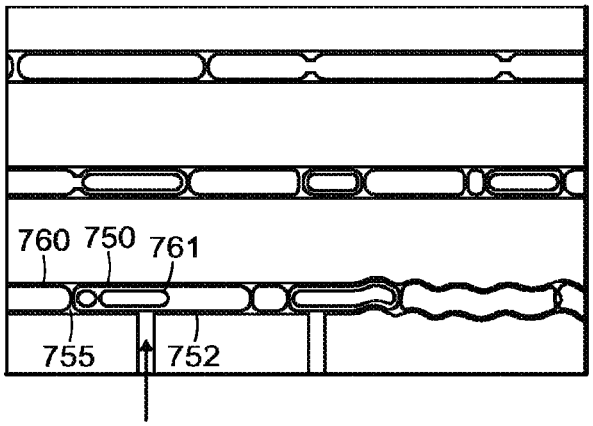


FIG. 14C

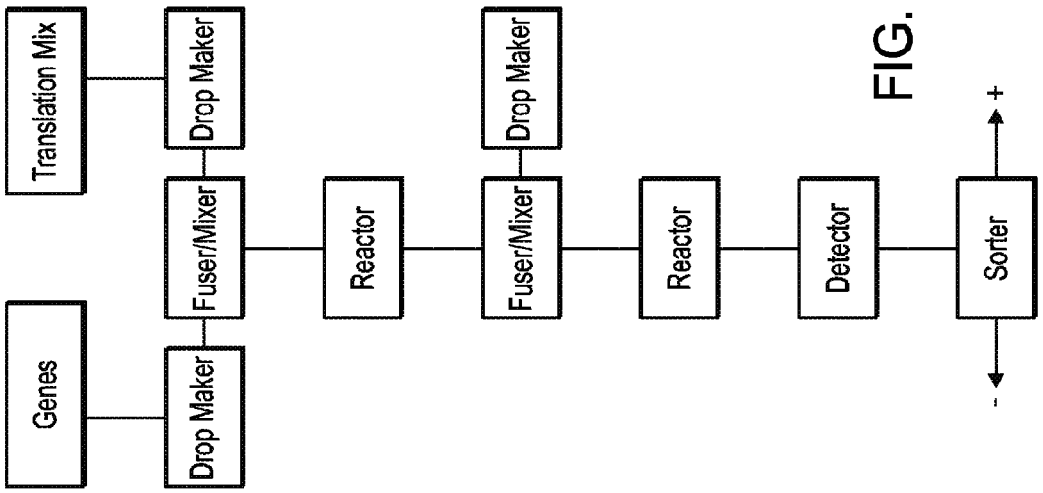


FIG. 15B

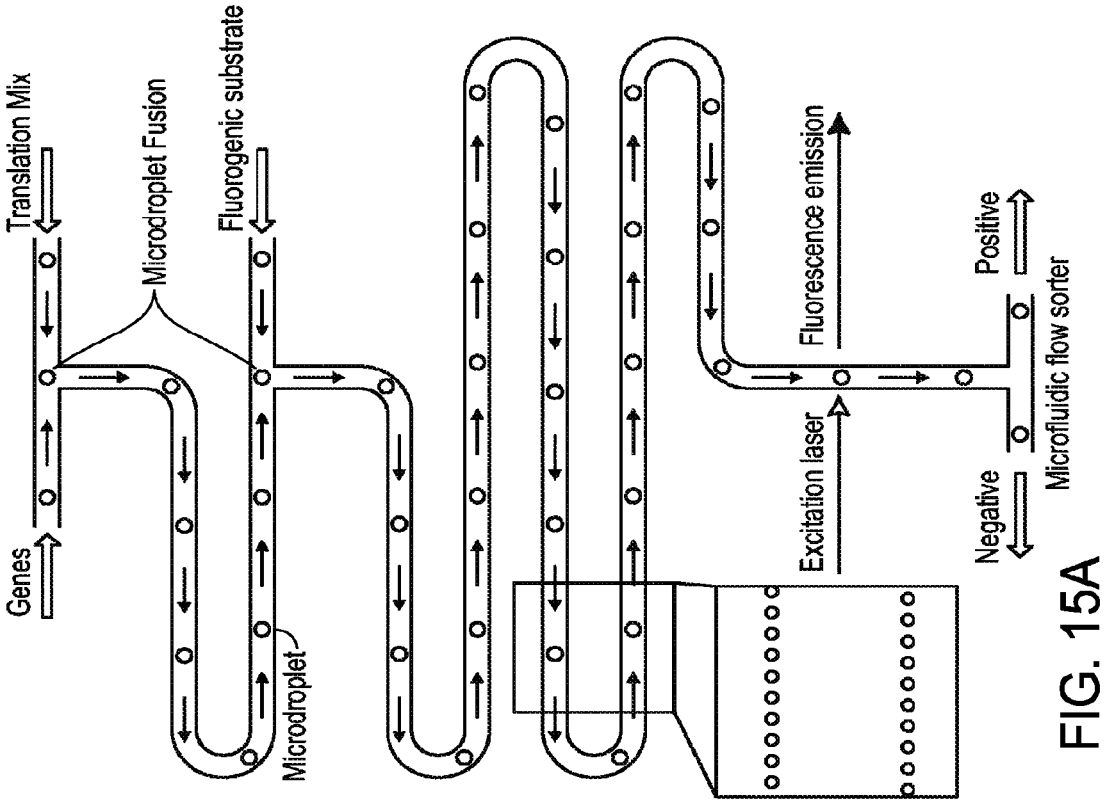


FIG. 15A

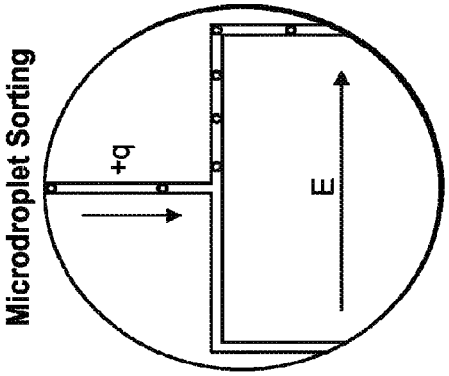


FIG. 16D

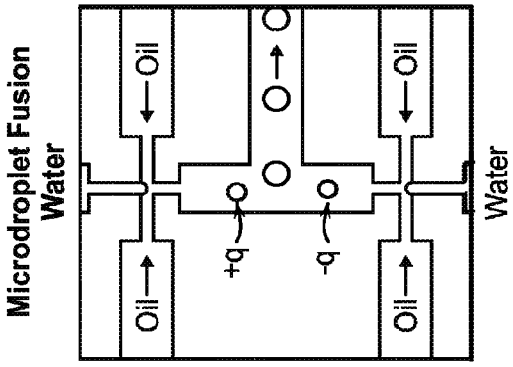


FIG. 16C

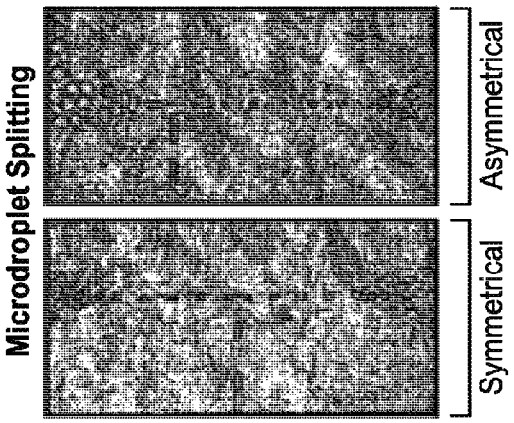


FIG. 16B

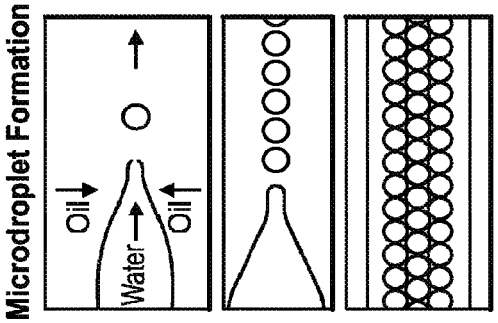


FIG. 16A

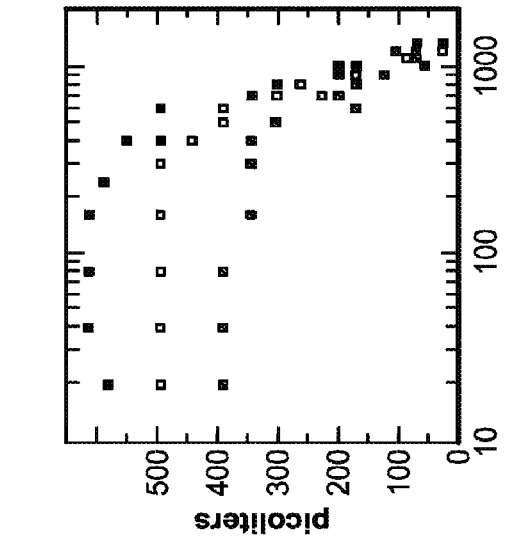


FIG. 17F

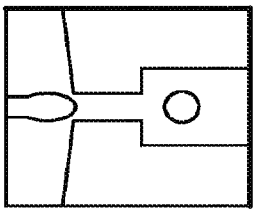


FIG. 17C

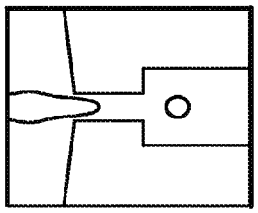


FIG. 17E

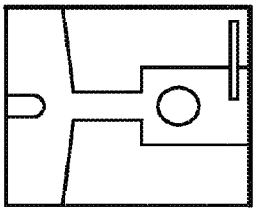


FIG. 17B

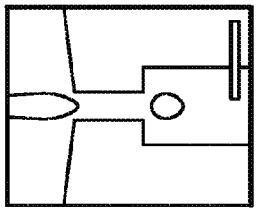


FIG. 17D

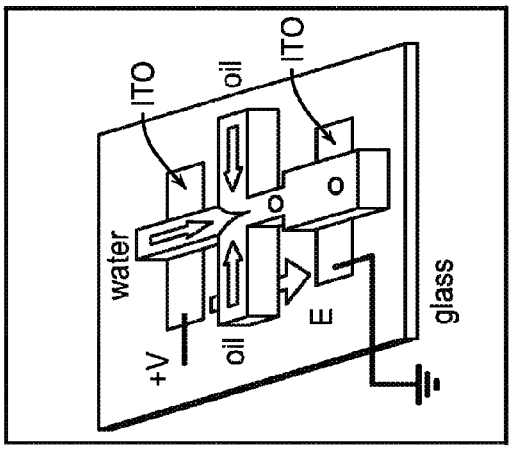


FIG. 17A

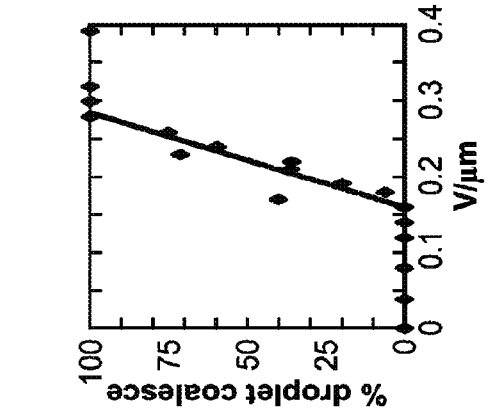


FIG. 18D

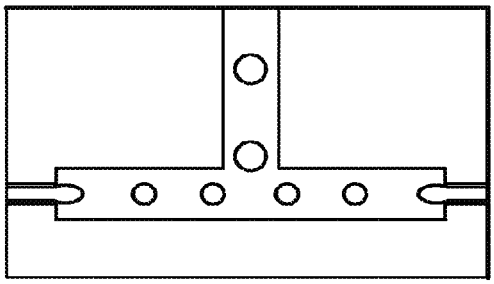


FIG. 18C

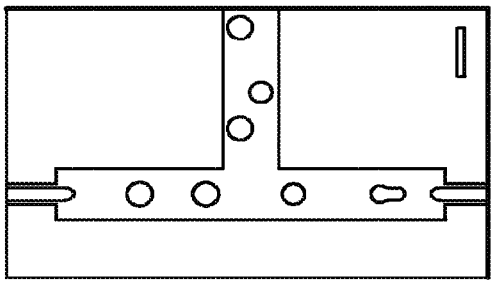


FIG. 18B

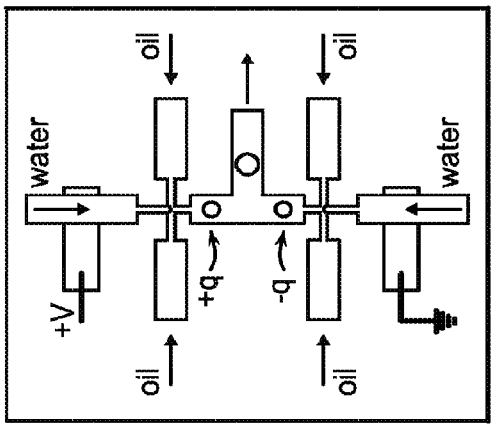


FIG. 18A

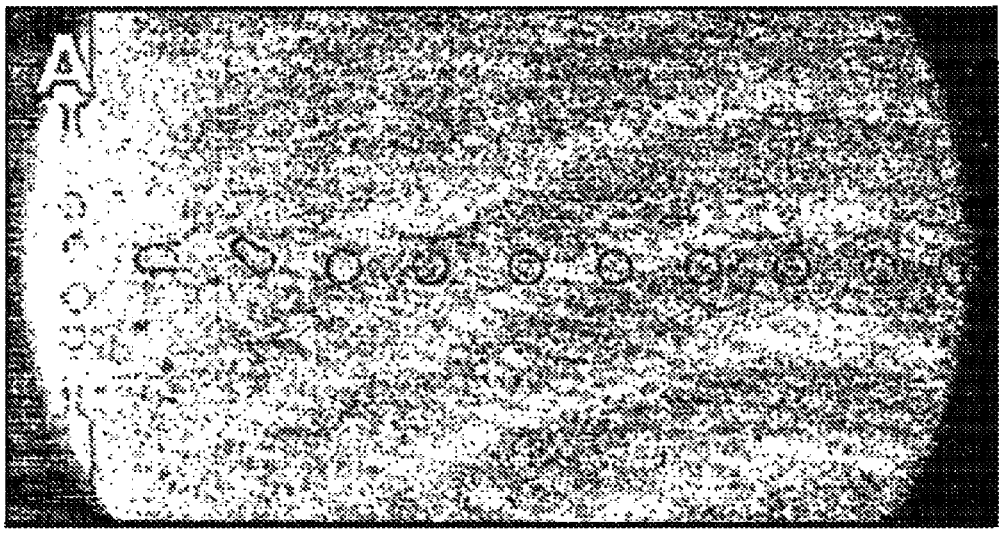


FIG. 19A

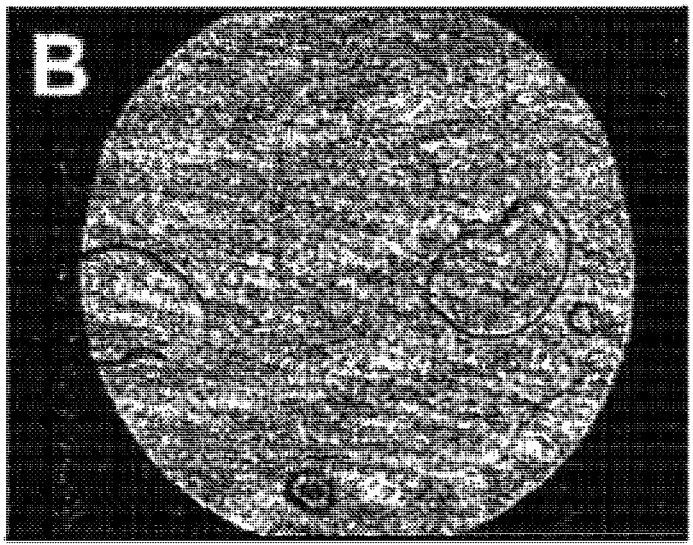


FIG. 19B

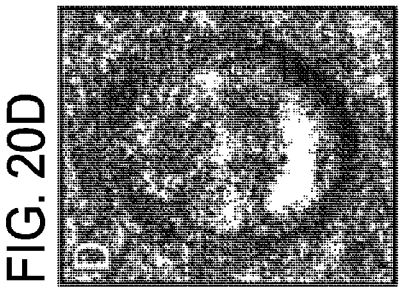


FIG. 20D

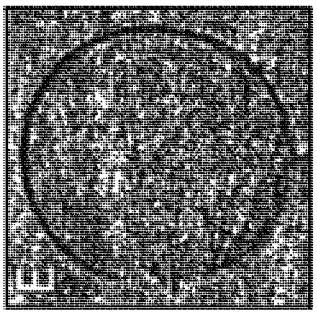


FIG. 20E

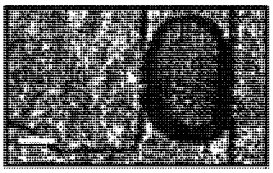


FIG. 20I

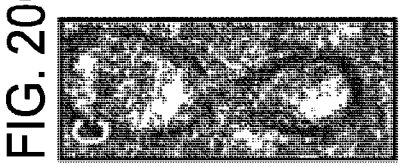


FIG. 20C

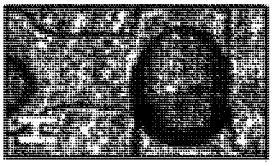


FIG. 20H

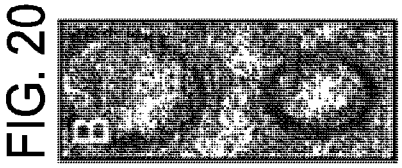


FIG. 20B

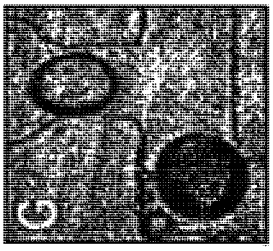


FIG. 20G

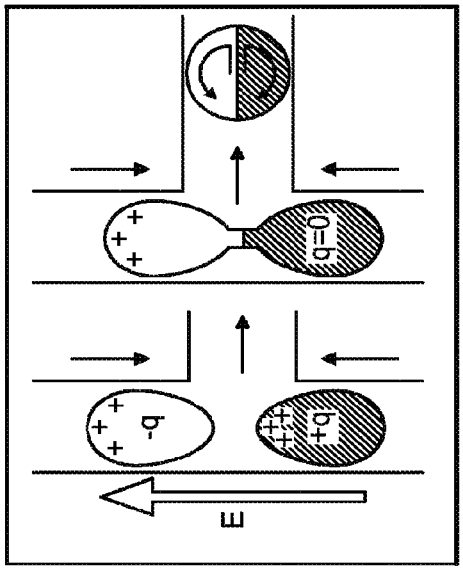


FIG. 20A

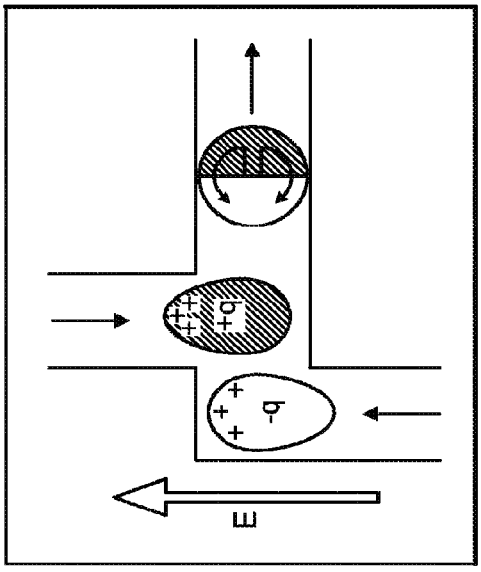


FIG. 20F

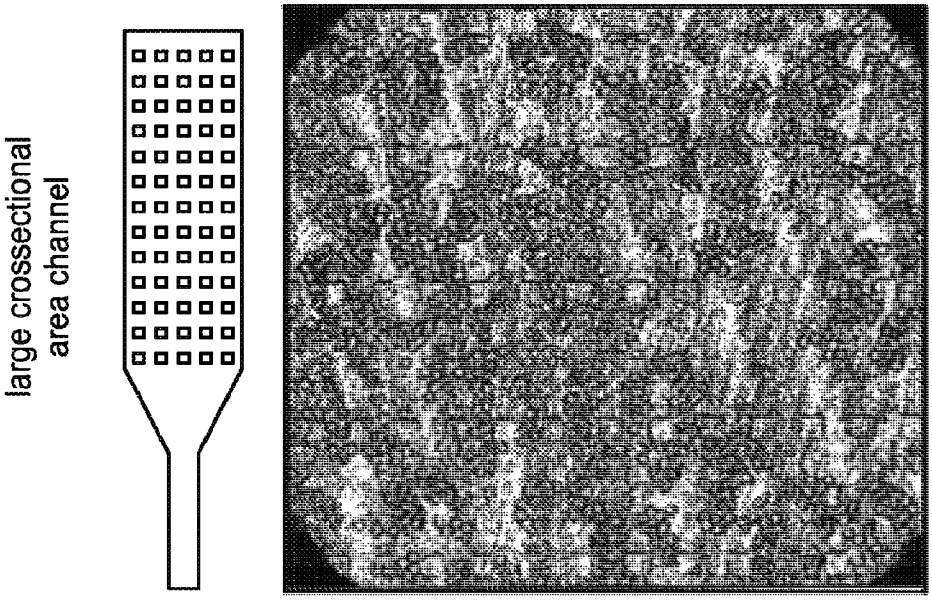


FIG. 21B

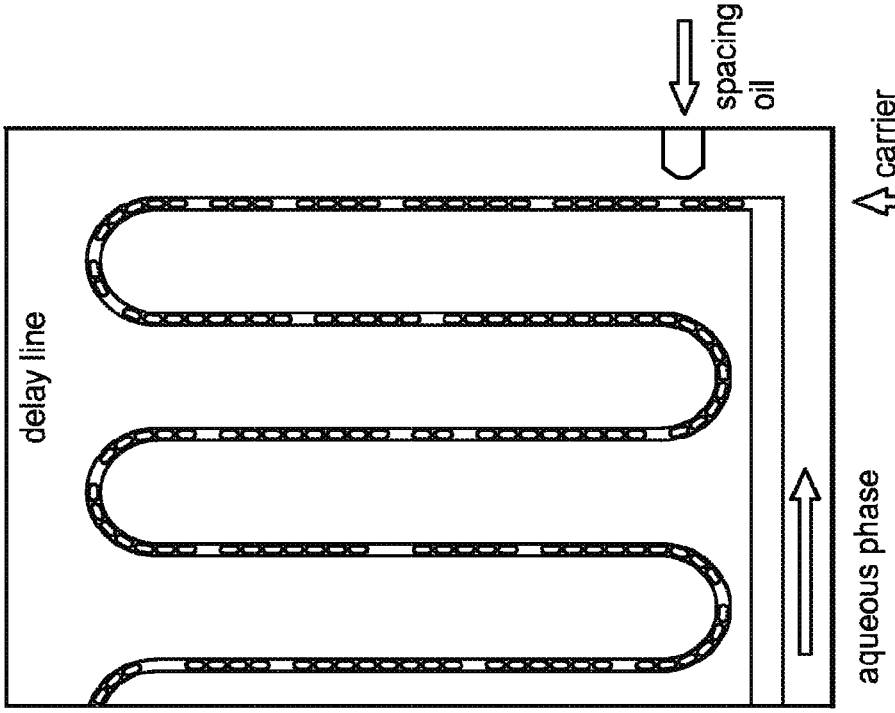


FIG. 21A

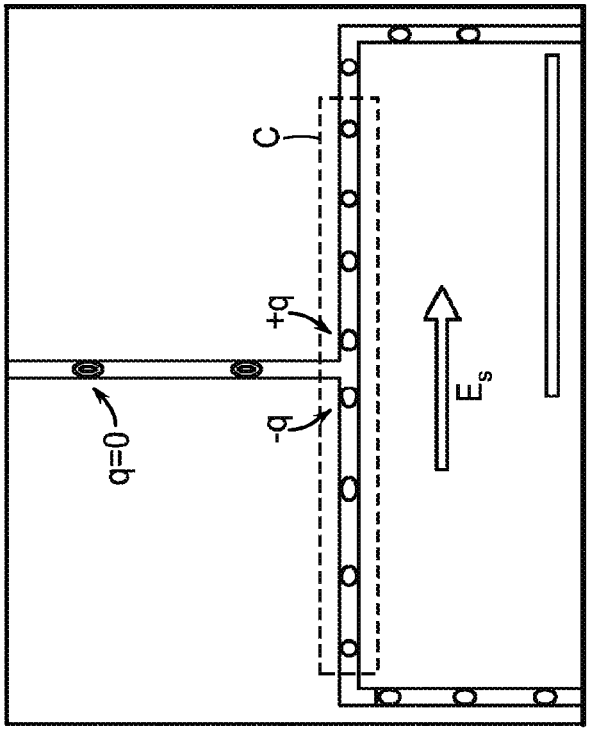


FIG. 22B

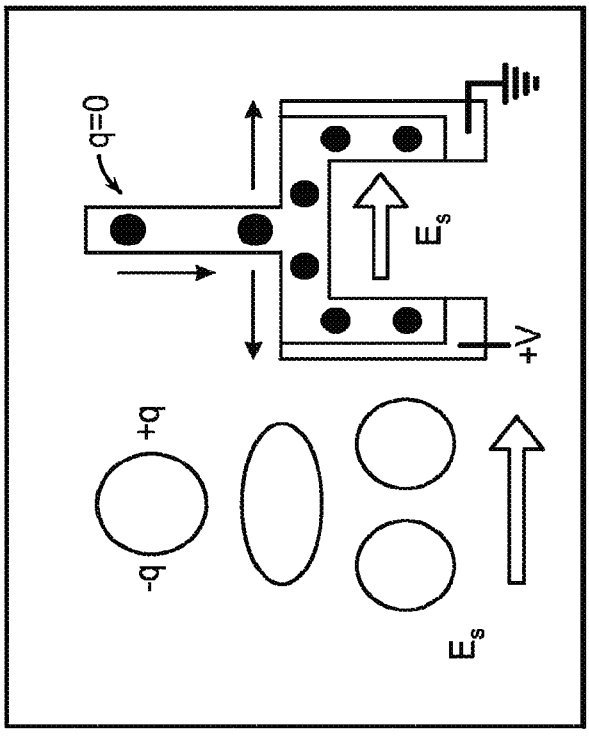


FIG. 22A

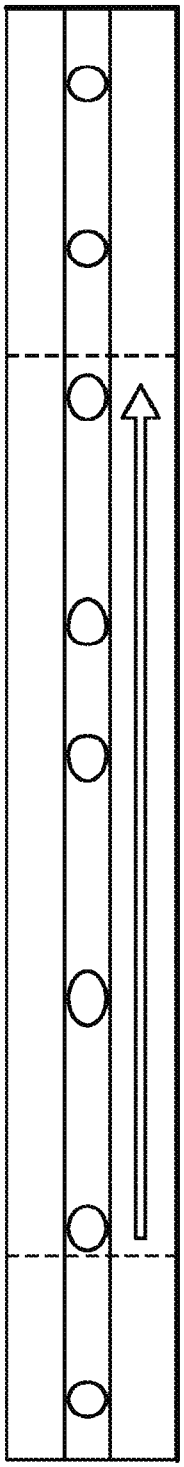


FIG. 22C

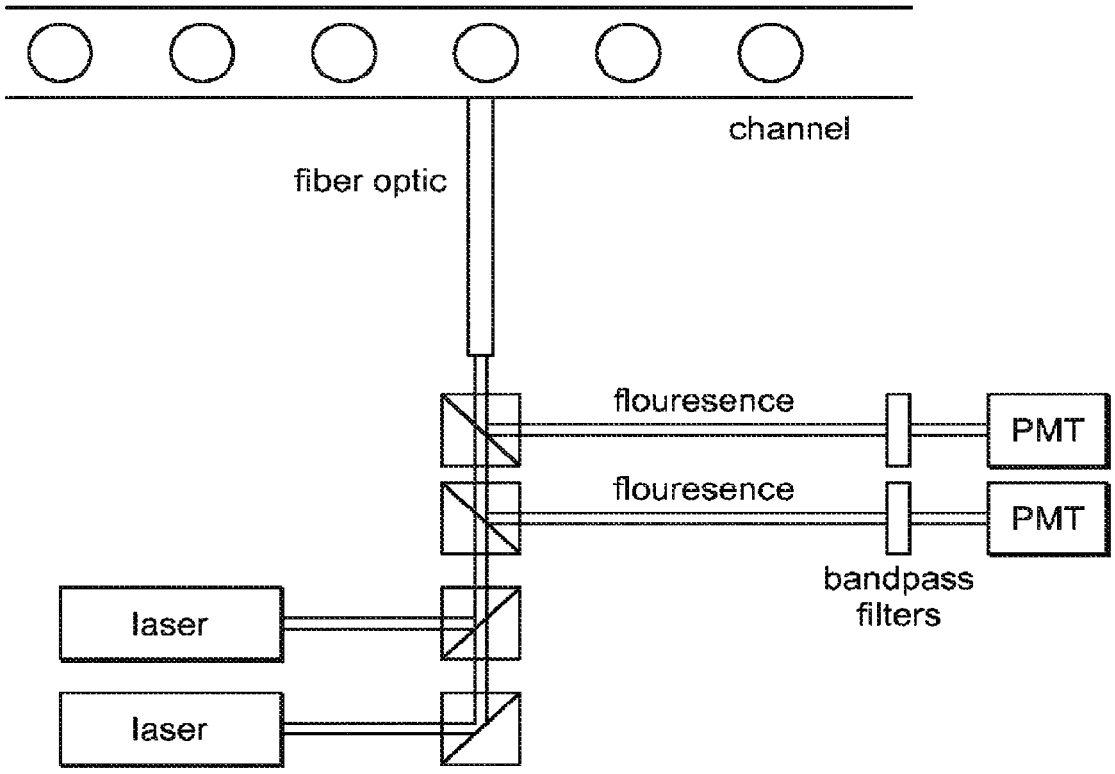

channel
fiber optic
flouresence
PMT
flouresence
PMT
bandpass
filters
laser
laser

FIG. 23

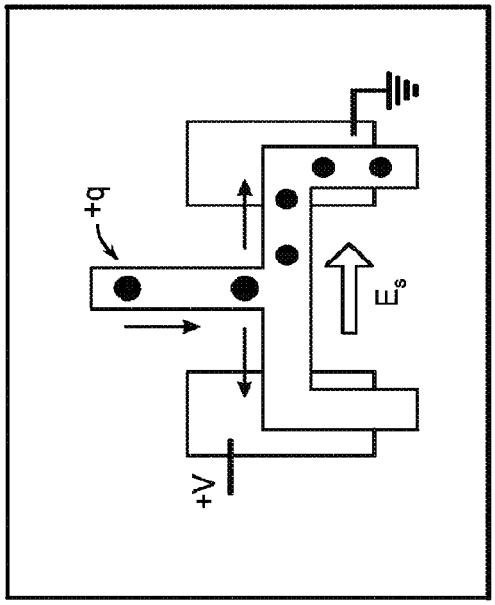


FIG. 24B

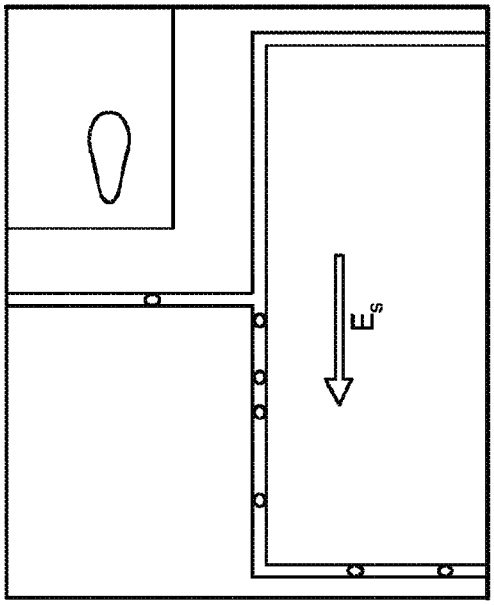


FIG. 24D

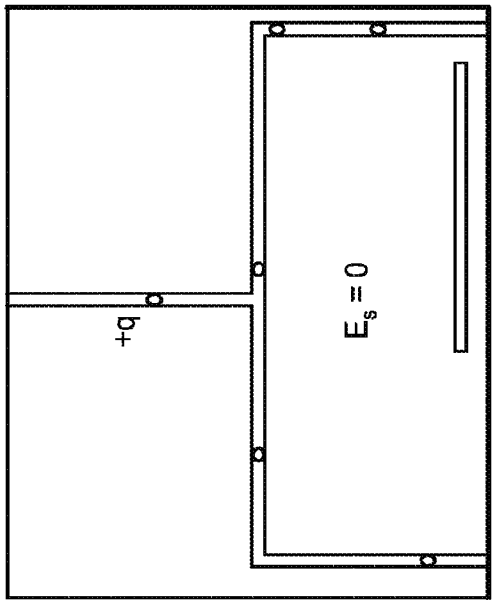


FIG. 24A

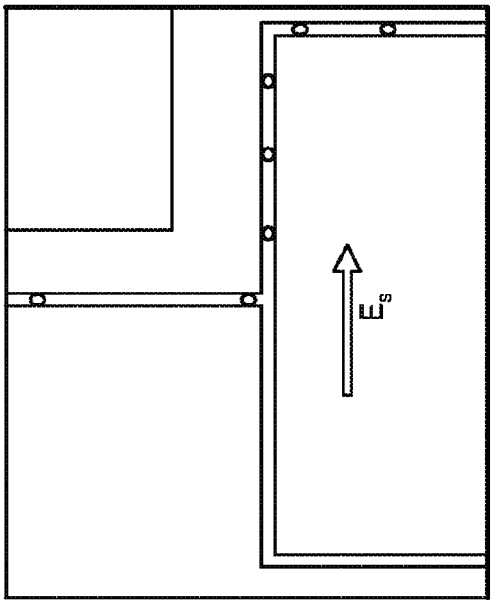


FIG. 24C

IN VITRO EVOLUTION IN MICROFLUIDIC SYSTEMS

RELATED APPLICATIONS

The present application is a continuation of U.S. Ser. No. 15/012,209, filed Feb. 1. 2016, which is a continuation of U.S. Ser. No. 13/705,833, filed Dec. 5, 2012, which is a continuation of U.S. Ser. No. 11/665,030, filed Apr. 14, 2009, now U.S. Pat. No. 9,029,083, which application is a 35 U.S.C. § 371 National Phase Application of PCT/GB2005/003889, filed Oct. 10, 2005; which claims priority from U.S. Ser. No. 10/961,695, filed Oct. 8, 2004, now U.S. Pat. No. 7,938,287, each of which is incorporated by reference in its entirety.

SEQUENCE LISTING

The application contains a sequence listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. The ASCII-formatted sequence listing, created on May 4, 2017, is named RDT-301-US06-Sequence Listing, and is 3,600 bytes in size.

The present invention relates to methods for use in in vitro evolution of molecular libraries. In particular, the present invention relates to methods of selecting nucleic acids encoding gene products in which the nucleic acid and the activity of the encoded gene product are linked by compartmentation, using microfluidic systems to create and/or handle the compartments.

Evolution requires the generation of genetic diversity (diversity in nucleic acid) followed by the selection of those nucleic acids which result in beneficial characteristics. Because the nucleic acid and the activity of the encoded gene product of an organism are physically linked (the nucleic acids being confined within the cells which they encode) multiple rounds of mutation and selection can result in the progressive survival of organisms with increasing fitness. Systems for rapid evolution of nucleic acids or proteins in vitro advantageously mimic this process at the molecular level in that the nucleic acid and the activity of the encoded gene product are linked and the activity of the gene product is selectable.

Recent advances in molecular biology have allowed some molecules to be co-selected according to their properties along with the nucleic acids that encode them. The selected nucleic acids can subsequently be cloned for further analysis or use, or subjected to additional rounds of mutation and selection.

Common to these methods is the establishment of large libraries of nucleic acids. Molecules having the desired characteristics (activity) can be isolated through selection regimes that select for the desired activity of the encoded gene product, such as a desired biochemical or biological activity, for example binding activity.

Phage display technology has been highly successful as providing a vehicle that allows for the selection of a displayed protein by providing the essential link between nucleic acid and the activity of the encoded gene product (Smith, 1985; Bass et al., 1990; McCafferty et al., 1990; for review see Clackson and Wells, 1994). Filamentous phage particles act as genetic display packages with proteins on the outside and the genetic elements which encode them on the inside. The tight linkage between nucleic acid and the activity of the encoded gene product is a result of the assembly of the phage within bacteria. As individual bacteria are rarely multiply infected, in most cases all the phage produced from an individual bacterium will carry the same genetic element and display the same protein.

However, phage display relies upon the creation of nucleic acid libraries in vivo in bacteria. Thus, the practical limitation on library size allowed by phage display technology is of the order of 10^7 to 10^{11}, even taking advantage of λ phage vectors with excisable filamentous phage replicons. The technique has mainly been applied to selection of molecules with binding activity. A small number of proteins with catalytic activity have also been isolated using this technique, however, selection was not directly for the desired catalytic activity, but either for binding to a transition-state analogue (Widersten and Mannervik, 1995) or reaction with a suicide inhibitor (Soumillion et al., 1994; Janda et al., 1997). More recently there have been some examples of enzymes selected using phage-display by product formation (Atwell & Wells, 1999; Demartis et al., 1999; Jestin et al., 1999; Pederson, et al., 1998), but in all these cases selection was not for multiple turnover.

Specific peptide ligands have been selected for binding to receptors by affinity selection using large libraries of peptides linked to the C terminus of the lac repressor LacI (Cull et al, 1992). When expressed in *E. coli* the repressor protein physically links the ligand to the encoding plasmid by binding to a lac operator sequence on the plasmid.

An entirely in vitro polysome display system has also been reported (Mattheakis et al., 1994; Hanes and Plückthun, 1997) in which nascent peptides are physically attached via the ribosome to the RNA which encodes them. An alternative, entirely in vitro system for linking genotype to phenotype by making RNA-peptide fusions (Roberts and Szostak, 1997; Nemoto et al., 1997) has also been described.

However, the scope of the above systems is limited to the selection of proteins and furthermore does not allow direct selection for activities other than binding, for example catalytic or regulatory activity.

In vitro RNA selection and evolution (Ellington and Szostak, 1990), sometimes referred to as SELEX (systematic evolution of ligands by exponential enrichment) (Tuerk and Gold, 1990) allows for selection for both binding and chemical activity, but only for nucleic acids. When selection is for binding, a pool of nucleic acids is incubated with immobilised substrate. Non-binders are washed away, then the binders are released, amplified and the whole process is repeated in iterative steps to enrich for better binding sequences. This method can also be adapted to allow isolation of catalytic RNA and DNA (Green and Szostak, 1992; for reviews see Chapman and Szostak, 1994; Joyce, 1994; Gold et al., 1995; Moore, 1995).

However, selection for "catalytic" or binding activity using SELEX is only possible because the same molecule performs the dual role of carrying the genetic information and being the catalyst or binding molecule (aptamer). When selection is for "auto-catalysis" the same molecule must also perform the third role of being a substrate. Since the genetic element must play the role of both the substrate and the catalyst, selection is only possible for single turnover events. Because the "catalyst" is in this process itself modified, it is by definition not a true catalyst. Additionally, proteins may not be selected using the SELEX procedure. The range of catalysts, substrates and reactions which can be selected is therefore severely limited.

Those of the above methods that allow for iterative rounds of mutation and selection are mimicking in vitro mechanisms usually ascribed to the process of evolution: iterative variation, progressive selection for a desired the activity and

replication. However, none of the methods so far developed have provided molecules of comparable diversity and functional efficacy to those that are found naturally. Additionally, there are no man-made "evolution" systems which can evolve both nucleic acids and proteins to effect the full range of biochemical and biological activities (for example, binding, catalytic and regulatory activities) and that can combine several processes leading to a desired product or activity.

There is thus a great need for an in vitro system that overcomes the limitations discussed above.

In Tawfik and Griffiths (1998), and in International patent application PCT/GB98/01889, we describe a system for in vitro evolution that overcomes many of the limitations described above by using compartmentalisation in microcapsules to link genotype and phenotype at the molecular level.

In Tawfik and Griffiths (1998), and in several embodiments of International patent application WO9902671, the desired activity of a gene product results in a modification of the genetic element which encoded it (and is present in the same microcapsule). The modified genetic element can then be selected in a subsequent step.

Our subsequent international patent application WO0040712 describes a variation of this technology in which the modification of the genetic element causes a change in the optical properties of the element itself, and which has many advantages over the methods described previously.

The manipulation of fluids to form fluid streams of desired configuration, discontinuous fluid streams, droplets, particles, dispersions, etc., for purposes of fluid delivery, product manufacture, analysis, and the like, is a relatively well-studied art. For example, highly monodisperse gas bubbles, less than 100 microns in diameter, have been produced using a technique referred to as capillary flow focusing. In this technique, gas is forced out of a capillary tube into a bath of liquid, the tube is positioned above a small orifice, and the contraction flow of the external liquid through this orifice focuses the gas into a thin jet which subsequently breaks into equal-sized bubbles via a capillary instability. In a related technique, a similar arrangement was used to produce liquid droplets in air.

An article entitled "Generation of Steady Liquid Microthreads and Micron-Sized Monodisperse Sprays and Gas Streams," Phys. Rev. Lett., 80:2, Jan. 12, 1998, 285-288 (Ganan-Calvo) describes formation of a microscopic liquid thread by a laminar accelerating gas stream, giving rise to a fine spray.

An articled entitled "Dynamic Pattern Formation in a Vesicle-Generating Microfluidic Device," Phys. Rev. Lett., 86:18, Apr. 30, 2001 (Thorsen, et al.) describes formation of a discontinuous water phase in a continuous oil phase via microfluidic cross-flow, specifically, by introducing water, at a "T" junction between two microfluidic channels, into flowing oil.

U.S. Pat. No. 6,120,666, issued Sep. 19, 2000, describes a micofabricated device having a fluid focusing chamber for spatially confining first and second sample fluid streams for analysing microscopic particles in a fluid medium, for example in biological fluid analysis.

U.S. Pat. No. 6,116,516, issued Sep. 12, 2000, describes formation of a capillary microjet, and formation of a monodisperse aerosol via disassociation of the microjet.

U.S. Pat. No. 6,187,214, issued Feb. 13, 2001, describes atomised particles in a size range of from about 1 to about 5 microns, produced by the interaction of two immiscible fluids.

U.S. Pat. No. 6,248,378, issued Jun. 19, 2001, describes production of particles for introduction into food using a microjet and a monodisperse aerosol formed when the microjet dissociates.

Microfluidic systems have been described in a variety of contexts, typically in the context of miniaturised laboratory (e.g., clinical) analysis. Other uses have been described as well. For example, International Patent Publication No. WO 01/89789, published Nov. 29, 2001 by Anderson, et al., describes multi-level microfluidic systems that can be used to provide patterns of materials, such as biological materials and cells, on surfaces. Other publications describe microfluidic systems including valves, switches, and other components.

BRIEF DESCRIPTION OF THE INVENTION

According to a first aspect of the present invention, there is provided a method for isolating one or more genetic elements encoding a gene product having a desired activity, comprising the steps of:

(a) compartmentalising the genetic elements into microcapsules;

(b) sorting the genetic elements which express gene product (s) having the desired activity;

wherein at least one step is under microfluidic control.

In the method of the invention, a genetic element may be expressed to form its gene product before or after compartmentalisation; where the gene product is expressed before compartmentalisation, it is linked to the genetic element such that they are compartmentalised together.

Preferably, at least one step is performed using electronic control of fluidic species.

Advantageously at least one step involves fusion or splitting of microcapsules.

Methods for electronic control of fluidic species, as well as splitting (and fusing) of microcapsules under microfluidic control, are described herein.

Preferably, the method of the invention comprises the steps of:

(a) compartmentalising the genetic elements into microcapsules;

(c) expressing the genetic elements to produce their respective gene products within the microcapsules; and

(d) sorting the genetic elements which encode gene product (s) having the desired activity.

Alternatively, the method of the invention comprises the steps of:

(a) expressing the genetic elements to produce their respective gene products such that the gene products are linked to the genes encoding them;

(b) compartmentalising the genetic elements into microcapsules; and

(c) sorting the genetic elements which encode gene product(s) having the desired activity.

The microcapsules according to the present invention compartmentalise genetic elements and gene products such that they remain physically linked together.

As used herein, a genetic element is a molecule or molecular construct comprising a nucleic acid. The genetic elements of the present invention may comprise any nucleic acid (for example, DNA, RNA or any analogue, natural or artificial, thereof). The nucleic acid component of the genetic element may moreover be linked, covalently or non-covalently, to one or more molecules or structures, including proteins, chemical entities and groups, and solid-phase supports such as beads (including nonmagnetic, mag-

netic and paramagnetic beads), and the like. In the method of the invention, these structures or molecules can be designed to assist in the sorting and/or isolation of the genetic element encoding a gene product with the desired activity.

Expression, as used herein, is used in its broadest meaning, to signify that a nucleic acid contained in the genetic element is converted into its gene product. Thus, where the nucleic acid is DNA, expression refers to the transcription of the DNA into RNA; where this RNA codes for protein, expression may also refer to the translation of the RNA into protein. Where the nucleic acid is RNA, expression may refer to the replication of this RNA into further RNA copies, the reverse transcription of the RNA into DNA and optionally the transcription of this DNA into further RNA molecule(s), as well as optionally the translation of any of the RNA species produced into protein. Preferably, therefore, expression is performed by one or more processes selected from the group consisting of transcription, reverse transcription, replication and translation.

Expression of the genetic element may thus be directed into either DNA, RNA or protein, or a nucleic acid or protein containing unnatural bases or amino acids (the gene product) within the microcapsule of the invention, so that the gene product is confined within the same microcapsule as the genetic element.

The genetic element and the gene product thereby encoded are linked by confining each genetic element and the respective gene product encoded by the genetic element within the same microcapsule. In this way the gene product in one microcapsule cannot cause a change in any other microcapsules. In addition, further linking means may be employed to link gene products to the genetic elements encoding them, as set forth below.

The term "microcapsule" is used herein in accordance with the meaning normally assigned thereto in the art and further described hereinbelow. In essence, however, a microcapsule is an artificial compartment whose delimiting borders restrict the exchange of the components of the molecular mechanisms described herein which allow the sorting of the genetic elements according to the function of the gene products which they encode.

Preferably, the microcapsules used in the method of the present invention will be capable of being produced in very large numbers, and thereby to compartmentalise a library of genetic elements which encodes a repertoire of gene products.

As used herein, a change in optical properties refers to any change in absorption or emission of electromagnetic radiation, including changes in absorbance, luminescence, phosphorescence or fluorescence. All such properties are included in the term "optical". Microcapsules and/or genetic elements can be sorted, for example, by luminescence, fluorescence or phosphorescence activated sorting. In a preferred embodiment, flow cytometry is employed to sort microcapsules and/or genetic elements, for example, light scattering (Kerker, 1983) and fluorescence polarisation (Rolland et aL, 1985) can be used to trigger flow sorting. In a highly preferred embodiment genetic elements are sorted using a fluorescence activated cell sorter (FACS) sorter (Norman, 1980; Mackenzie and Pinder, 1986). Such a sorting device can be integrated directly on the microfluidic device, and can use electronic means to sort the microcapsules and/or genetic elements. Optical detection, also integrated directly on the microfluidic device, can be used to screen the microcapsules and/or genetic elements to trigger the sorting. Other means of control of the microcapsules and/or genetic elements, in addition to charge, can also be incorporated onto the microfluidic device.

Changes in optical properties may be direct or indirect. Thus, the change may result in the alteration of an optical property in the microcapsule or genetic element itself, or may lead indirectly to such a change. For example, modification of a genetic element may alter its ability to bind an optically active ligand, thus indirectly altering its optical properties.

Alternatively, imaging techniques can be used to screen thin films of genetic elements to allow enrichment for a genetic element with desirable properties, for example by physical isolation of the region where a genetic element with desirable properties is situated, or ablation of non-desired genetic elements. The genetic elements can be detected by luminescence, phosphorescence or fluorescence.

The sorting of genetic elements may be performed in one of essentially seven techniques.

(I) In a first embodiment, the microcapsules are sorted according to an activity of the gene product or derivative thereof which makes the microcapsule detectable as a whole. Accordingly, a gene product with the desired activity induces a change in the microcapsule, or a modification of one or more molecules within the microcapsule, which enables the microcapsule containing the gene product and the genetic element encoding it to be sorted. In this embodiment the microcapsules are physically sorted from each other according to the activity of the gene product(s) expressed from the genetic element(s) contained therein, which makes it possible selectively to enrich for microcapsules containing gene products of the desired activity.

(II) In a second embodiment, the genetic elements are sorted following pooling of the microcapsules into one or more common compartments. In this embodiment, a gene product having the desired activity modifies the genetic element which encoded it (and which resides in the same microcapsule) in such a way as to make it selectable in a subsequent step. The reactions are stopped and the microcapsules are then broken so that all the contents of the individual microcapsules are pooled. Selection for the modified genetic elements enables enrichment of the genetic elements encoding the gene product(s) having the desired activity. Accordingly, the gene product having the desired activity modifies the genetic element encoding it to enable the isolation of the genetic element. It is to be understood, of course, that modification may be direct, in that it is caused by the direct action of the gene product on the genetic element, or indirect, in which a series of reactions, one or more of which involve the gene product having the desired activity, leads to modification of the genetic element.

(III) In a third embodiment, the genetic elements are sorted following pooling of the microcapsules into one or more common compartments. In this embodiment, a gene with a desired activity induces a change in the microcapsule containing the gene product and the genetic element encoding it. This change, when detected, triggers the modification of the gene within the compartment. 'The reactions are stopped and the microcapsules are then broken so that all the contents of the individual microcapsules are pooled. Selection for the modified genetic elements enables enrichment of the genetic elements encoding the gene product(s) having the desired activity. Accordingly the gene product having the desired activity induces a change in the compartment which is detected and triggers the modification of the genetic element

within the compartment so as to allow its isolation. It is to be understood that the detected change in the compartment may be caused by the direct action of the gene product, or indirect action, in which a series of reactions, one or more of which involve the gene product having the desired activity leads to the detected change.

(IV) In a fourth embodiment, the genetic elements may be sorted by a multi-step procedure, which involves at least two steps, for example, in order to allow the exposure of the genetic elements to conditions which permit at least two separate reactions to occur. As will be apparent to a persons skilled in the art, the first microencapsulation step of the invention must result in conditions which permit the expression of the genetic elements—be it transcription, transcription and/or translation, replication or the like. Under these conditions, it may not be possible to select for a particular gene product activity, for example because the gene product may not be active under these conditions, or because the expression system contains an interfering activity. The method therefore comprises expressing the genetic elements to produce their respective gene products within the microcapsules, linking the gene products to the genetic elements encoding them and isolating the complexes thereby formed. This allows for the genetic elements and their associated gene products to be isolated from the capsules before sorting according to gene product activity takes place. In a preferred embodiment, the complexes are subjected to a further compartmentalisation step prior to isolating the genetic elements encoding a gene product having the desired activity. This further compartmentalisation step, which advantageously takes place in microcapsules, permits the performance of further reactions, under different conditions, in an environment where the genetic elements and their respective gene products are physically linked. Eventual sorting of genetic elements may be performed according to embodiment (I), (II) or (III) above.

Where the selection is for optical changes in the genetic elements, the selection may be performed as follows:

(V) In a fifth embodiment, the genetic elements are sorted following pooling of the microcapsules into one or more common compartments. In this embodiment, a gene product having the desired activity modifies the genetic element which encoded it (and which resides in the same microcapsule) so as to make it selectable as a result of its modified optical properties in a subsequent step. The reactions are stopped and the microcapsules are then broken so that all the contents of the individual microcapsules are pooled. The modification of the genetic element in the microcapsule may result directly in the modification of the optical properties of the genetic element. Alternatively, the modification may allow the genetic elements to be further modified outside the microcapsules so as to induce a change in their optical properties. Selection for the genetic elements with modified optical properties enables enrichment of the genetic elements encoding the gene product(s) having the desired activity. Accordingly, the gene product having the desired activity modifies the genetic element encoding it to enable the isolation of the genetic element as a result in a change in the optical properties of the genetic element. It is to be understood, of course, that modification may be direct, in that it is caused by the direct action of the gene product on the genetic element, or indirect, in which a series of reactions, one or more of which involve the gene product having the desired activity, leads to modification of the genetic element.

(VI) In a sixth embodiment, the genetic elements may be sorted by a multi-step procedure, which involves at least two steps, for example, in order to allow the exposure of the genetic elements to conditions which permit at least two separate reactions to occur. As will be apparent to persons skilled in the art, the first microencapsulation step of the invention advantageously results in conditions which permit the expression of the genetic elements—be it transcription, transcription and/or translation, replication or the like. Under these conditions, it may not be possible to select for a particular gene product activity, for example because the gene product may not be active under these conditions, or because the expression system contains an interfering activity. The method therefore comprises expressing the genetic elements to produce their respective gene products within the microcapsules, linking the gene products to the genetic elements encoding them and isolating the complexes thereby formed. This allows for the genetic elements and their associated gene products to be isolated from the capsules before sorting according to gene product activity takes place. In a preferred embodiment, the complexes are subjected to a further compartmentalisation step prior to isolating the genetic elements encoding a gene product having the desired activity. This further compartmentalisation step, which advantageously takes place in microcapsules, permits the performance of further reactions, under different conditions, in an environment where the genetic elements and their respective gene products are physically linked. Eventual sorting of genetic elements may be performed according to embodiment (V) above.

The "secondary encapsulation" may also be performed with genetic elements linked to gene products by other means, such as by phage display, polysome display, RNA-peptide fusion or lac repressor peptide fusion, optionally where expression takes place prior to encapsulation; or even by the encapsulation of whole cells containing the desired genetic element.

The selected genetic element(s) may also be subjected to subsequent, possibly more stringent rounds of sorting in iteratively repeated steps, reapplying the method described above either in its entirety or in selected steps only. By tailoring the conditions appropriately, genetic elements encoding gene products having a better optimised activity may be isolated after each round of selection.

Additionally, the genetic elements isolated after a first round of sorting may be subjected to mutagenesis before repeating the sorting by iterative repetition of the steps of the method of the invention as set out above. After each round of mutagenesis, some genetic elements will have been modified in such a way that the activity of the gene products is enhanced.

Moreover, the selected genetic elements can be cloned into an expression vector to allow further characterisation of the genetic elements and their products.

(VII) In a seventh embodiment, the microcapsules may be sorted using microfluidic approaches. The microcapsules may be produced using microfluidic droplet formation techniques, such as those described herein, or by other techniques, for example conventional emulsification by forcing together two fluid phases. Sorting using microfluidics is applicable to embodiments I to VI above, and provides enhanced processing of microcapsules leading to improved sorting. Microcapsules may be split or fused according to methods described herein, or the contents thereof mixed. Moreover, the contents of the microcap-

sules may be analysed and the microcapsules sorted using detectors in microfluidic systems.

In a second aspect, the invention provides a product when selected according to the first aspect of the invention. As used in this context, a "product" may refer to a gene product, selectable according to the invention, or the genetic element (or genetic information comprised therein).

In a third aspect, the invention provides a method for preparing a gene product, the expression of which may result, directly or indirectly, in the modification the optical properties of a genetic element encoding it, comprising the steps of:

(a) preparing a genetic element encoding the gene product;

(b) compartmentalising genetic elements into microcapsules;

(c) expressing the genetic elements to produce their respective gene products within the microcapsules;

(d) sorting the genetic elements which produce the gene product(s) having the desired activity using the changed optical properties of the genetic elements; and

(e) expressing the gene product having the desired activity;

wherein one or both of steps (b) and (d) is performed under microfluidic control.

In accordance with the third aspect, step (a) preferably comprises preparing a repertoire of genetic elements, wherein each genetic element encodes a potentially differing gene product. Repertoires may be generated by conventional techniques, such as those employed for the generation of libraries intended for selection by methods such as phage display. Gene products having the desired activity may be selected from the repertoire, according to the present invention, according to their ability to modify the optical properties of the genetic elements in a manner which differs from that of other gene products. For example, desired gene products may modify the optical properties to a greater extent than other gene products, or to a lesser extent, including not at all.

In a fourth aspect, the invention provides a method for screening a compound or compounds capable of modulation the activity of a gene product, the expression of which may result, directly or indirectly, in the modification of the optical properties of a genetic element encoding it, comprising the steps of:

(a) preparing a repertoire of genetic elements encoding gene product;

(b) compartmentalising genetic elements into microcapsules;

(c) expressing the genetic elements to produce their respective gene products within the microcapsules;

(d) sorting the genetic elements which produce the gene product(s) having the desired activity using the changed optical properties of the genetic elements; and

(e) contacting a gene product having the desired activity with the compound or compounds and monitoring the modulation of an activity of the gene product by the compound or compounds; wherein one or both of steps (b) and (d) is performed under microfluidic control.

Advantageously, the method further comprises the step of:

(f) identifying the compound or compounds capable of modulating the activity of the gene product and synthesising said compound or compounds.

This selection system can be configured to select for RNA, DNA or protein molecules with catalytic, regulatory or binding activity.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. **1**A and **1**B illustrate the splitting of droplets in accordance with one embodiment of the invention;

FIGS. **2**A and **2**B illustrate an apparatus in accordance with an embodiment of the invention, before the application of an electric field thereto;

FIGS. **3**A and **3**B illustrate the apparatus of FIGS. **2**A and **2**B after the application of an electric field thereto;

FIGS. **4**A and **4**B illustrate the apparatus of FIGS. **2**A and **2**B after the application of a reversed electric field thereto;

FIG. **5** is a schematic diagram of droplet splitting, in accordance with one embodiment of the invention;

FIGS. **6**A and **6**B are schematic diagrams of additional embodiments of the invention;

FIGS. **7**A and **7**B are schematic diagrams of the-formation of microfluidic droplets in accordance with the present invention;

FIGS. **8**A-F illustrate the splitting of droplets in accordance with the invention;

FIGS. **9**A-D illustrate the induction of dipoles in droplets in accordance with the invention;

FIGS. **10**A-D illustrate the sorting of microcapsules by altering the flow of carrier fluid in a microfluidic system;

FIGS. **11**A-C illustrate the use of pressure changes in the microfluidic system to control **10** the direction of flow of droplets;

FIGS. **12**A-J illustrate flow patterns for droplets in microfluidic systems in accordance with the invention;

FIGS. **13**A-D illustrate the use of oppositely charged droplets in the invention;

FIGS. **14**A-C are illustrations of the formation and maintenance of microfluidic droplets using three immiscible liquids;

FIGS. **15**A-B: Directed evolution of enzymes using microdroplets in a microfluidic system. FIG. **15**A: schematic of the core system. FIG. **15**B: process block diagram showing the modules in the core system. Libraries of mutated enzyme genes are encapsulated in aqueous microdroplets (FIG. **16**A) such that, statistically, the majority of droplets contain no more than one gene per droplet. Each of these microdroplets is fused with a second microdroplet (FIG. **16**C) containing an in vitro translation system. After allowing time for the genes to be translated into protein each microdroplet is fused with another microdroplet containing an inhibitor of translation (puromycin) and a fluorogenic enzyme substrate. The rate of the enzymatic reaction is determined by measuring the fluorescence of each microdroplet, ideally at multiple points (corresponding to different times). Microdroplets with catalytic rates over a desired threshold value (e.g. the fastest 1%) will be sorted (FIG. **16**D) and collected and the genes contained therein amplified using the polymerase chain reaction (PCR). The selected genes will then either be characterised, re-selected directly, or first re-mutated randomly, or recombined before re-selection.

FIGS. **16**A-D: Examples of microdroplet formation and manipulation using microfluidics. FIG. **16**A: microdroplets can be created at up to 10^4 sec^1 by hydrodynamic-focussing (top two panels) and show <1.5% polydispersity (bottom panel). FIG. **16**B: microdroplets can be split symmetrically or asymmetrically. FIG. **16**C: microdroplets carrying positive (+q) and negative (−q) electrical charges fuse spontaneously. FIG. **16**D: charged microdroplets can also be steered using an applied electrical field (E).

FIGS. **17**A-F: Charged droplet generation. (FIG. **17**A), Oil and water streams converge at a 30 micron orifice. A

voltage V applied to indium-tin-oxide (ITO) electrodes on the glass produces an electric field E to capacitively charges the aqueous-oil interface. Drop size is independent of charge at low field strengths but decreases at higher fields, as shown in the photomicrographs, [(FIG. **17**B) V=0, (FIG. **17**C) V=400, (FIG. **17**D) V=600 and (FIG. **17**E) V=800] at higher fields. (FIG. **17**F) Droplet size as a function of voltage showing the crossover between flow-dominated and field-dominated snap-off for three different flow rates of the continuous phase oil (Q_c,=80 nL/s, 110 nL/s, and 140 nL/s). The infusion rate of the water is constant Q_d=20 nL/s.}

FIGS. **18**A-D: Coalescing drops. (FIG. **18**A) Drops having opposite sign of electrostatic charge can be generated by applying a voltage across the two aqueous streams. (FIG. **18**B) In the absence of the field the frequency and timing of drop formation at the two nozzles are independent and each nozzle produces a different size drop at a different frequency; infusion rates are the same at both nozzles. After the confluence of the two streams, drops from the upper and lower nozzles stay in their respective halves of the stream and due to surfactant there are no coalescence events even in the case of large slugs that fill the channel width. (FIG. **18**C) With an applied voltage of 200V across the 500 micron separation of the nozzles, the drops simultaneously break-off from the two nozzles and are identical; simultaneous drop formation can be achieved for unequal infusion rates of the aqueous streams even up to a factor of two difference in volumes. (FIG. **18**D) The fraction of the drops that encounter each other and coalesce increases linearly above a critical field when a surfactant, sorbiton-monooleate 3% is present.

FIGS. **19**A-B: Droplets carrying a pH sensitive dye coalesce with droplets of a different pH fluid. Chaotic advection rapidly mixes the two fluids through a combination of translation and rotation as the droplets pass around corners.

FIGS. **20**A-I: Diffusion limited and rapid mixing strategies. (FIG. **20**A) Drops meet and coalesce along the direction of E and then move off in a perpendicular direction, as sketched the counter rotating vortices after coalescence do not mix the two fluid parts as each vortex contains a single material. (FIG. **20**B) As the drops approach each other the increasing field causes there interfaces to deform and (FIG. **20**C) a bridge to jump out connecting the drops, to create (FIG. **20**D) in the case of 20 nm silica particles and MgCl_2 a sharp interface where the particles begin to gel. (FIG. **20**E) A typical unmixed droplet with particles in one hemisphere. (FIG. **20**F) To achieve fast mixing, droplets are brought together in the direction perpendicular to the electric field and move off in the direction parallel to the direction they merged along. Counter rotating vortexes are then created where each vortex is composed of half of the contents from each of the premerger-droplets. (FIG. **20**G) Shows a pH sensitive dye in the lower drop and a different pH fluid in the upper droplet. (FIG. **20**H) After merger the droplets are split by a sharp line. (FIG. **20**I) A uniform intensity indicating that mixing has been occurred is achieved in the droplet after it translates one diameter, typically this takes 1 to 2 ms.

FIGS. **21**A-B: Time delay reaction module. (FIG. **21**A) Droplets of perfluorodecaline alternate with aqueous droplets in a hexadecane carrier fluid. The 'single-file' ordering of the droplets provides for long delays with essentially no deviation in the precise spacing of aqueous droplets or droplet order. (FIG. **21**B) Increasing the width and height of the channel to create a 'large cross-sectional area' channel provides for extremely long time delays from minutes to hours. The exact ordering and spacing between the droplets is not maintained in this type of delay line.

FIGS. **22**A-C: Recharging neutral drops. (FIG. **22**A) Schematic to recharge neutral drops by breaking them in the presence of an electric field. Uncharged drops (q=0) are polarized in an electric field ($E_s \neq 0$), and provided E_s is sufficiently large, as shown in the photomicrograph of (FIG. **22**B), they break into two oppositely charged daughter drops in the extensional flow at a bifurcation. The enlargement of the dashed rectangle, shown in (FIG. **22**C), reveals that the charged drops are stretched in the electric field E_s but return to spherical on contacting the electrodes indicated by dashed vertical lines.

FIG. **23**: Detection module. One or more lasers are coupled to an optical fibre that is used to excite the fluorescence in each droplet as it passes over the fibre. The fluorescence is collected by the same fibre and dichroic beam splitters separate off specific wavelengths of the fluorescent light and the intensity of the fluorescence is measured with a photomultiplier tube (PMT) after the light passes through a band-pass filter.

FIGS. **24**A-D: Manipulating charged drops. In (FIG. **24**A) charged drops alternately enter the right and left channels when there is no field applied (E_s=0). The sketch in (FIG. **24**B) shows the layout for using an electric field E_s to select the channel charged drops will enter at a bifurcation. When an electric field is applied to the right (FIG. **24**C), the drops enter the right branch at the bifurcation; they enter the left branch when the field is reversed (FIG. **24**D). After the bifurcation, the distance between drops is reduced to half what it was before indicating the oil stream is evenly divided. The inset of (FIG. **24**D) shows the deformation in the shape of a highly charged drop in an electric field.

DEFINITIONS

As used herein, "or" is understood to mean "inclusively or," i.e., the inclusion of at least one, but including more than one, of a number or list of elements. In contrast, the term "exclusively or" refers to the inclusion of exactly one element of a number or list of elements.

The indefinite articles "a" and "an," as used herein in the specification and in the claims, should be understood to mean "at least one."

The term "about," as used herein in reference to a numerical parameter (for example, a physical, chemical, electrical, or biological property), will be understood by those of ordinary skill in the art to be an approximation of a numerical value, the exact value of which may be subject to errors such as those resulting from measurement errors of the numerical parameter, uncertainties resulting from the variability and/or reproducibility of the numerical parameter (for example, in separate experiments), and the like.

The term "microcapsule" is used herein in accordance with the meaning normally assigned thereto in the art and further described hereinbelow. In essence, however, a microcapsule is an artificial compartment whose delimiting borders restrict the exchange of the components of the molecular mechanisms described herein which allow the identification of the molecule with the desired activity. The delimiting borders preferably completely enclose the contents of the microcapsule. Preferably, the microcapsules used in the method of the present invention will be capable of being produced in very large numbers, and thereby to compartmentalise a library of genetic elements. Optionally, the genetic elements can comprise genes attached to microbeads. The microcapsules used herein allow mixing and sorting to be performed thereon, in order to facilitate the high throughput potential of the methods of the invention. A

microcapsule can be a droplet of one fluid in a different fluid, where the confined components are soluble in the droplet but not in the carrier fluid. In another embodiment there is a third material defining a wall, such as a membrane.

Arrays of liquid droplets on solid surfaces, multiwell plates and "plugs" in microfluidic systems, that is fluid droplets that are not completely surrounded by a second fluid as defined herein, are not microcapsules as defined herein.

The term "microbead" is used herein in accordance with the meaning normally assigned thereto in the art and further described hereinbelow. Microbeads, are also known by those skilled in the art as microspheres, latex particles, beads, or minibeads, are available in diameters from 20 nm to 1 mm and can be made from a variety of materials including silica and a variety of polymers, copolymers and terpolymers. Highly uniform derivatised and non-derivatised nonmagnetic and paramagnetic microparticles (beads) are commercially available from many sources (e.g. Sigma, Bangs Laboratories, Luminex and Molecular Probes) (Fornusek and Vetvicka, 1986).

Microbeads can be "compartmentalised" in accordance with the present invention by distribution into microcapsules. For example, in a preferred aspect the microbeads can be placed in a water/oil mixture and emulsified to form a water-in-oil emulsion comprising microcapsules according to the invention. The concentration of the microbeads can be adjusted such that a single microbead, on average, appears in each microcapsule.

As used herein, the "target" is any compound, molecule, or supramolecular complex. Typical targets include targets of medical significance, including drug targets such as receptors, for example G protein coupled receptors and hormone receptors; transcription factors, protein kinases and phosphatases involved in signalling pathways; gene products specific to microorganisms, such as components of cell walls, replicases and other enzymes; industrially relevant targets, such as enzymes used in the food industry, reagents intended for research or production purposes, and the like.

A "desired activity", as referred to herein, is the modulation of any activity of a target, or an activity of a molecule which is influenced by the target, which is modulatable directly or indirectly by a genetic element or genetic elements as assayed herein. The activity of the target may be any measurable biological or chemical activity, including binding activity, an enzymatic activity, an activating or inhibitory activity on a third enzyme or other molecule, the ability to cause disease or influence metabolism or other functions, and the like. Activation and inhibition, as referred to herein, denote the increase or decrease of a desired activity 1.5 fold, 2 fold, 3 fold, 4 fold, 5 fold, 10 fold, 100 fold or more. Where the modulation is inactivation, the inactivation can be substantially complete inactivation. The desired activity may moreover be purely a binding activity, which may or may not involve the modulation of the activity of the target bound to.

A compound defined herein as "low molecular weight" or a "small molecule" is a molecule commonly referred to in the pharmaceutical arts as a "small molecule". Such compounds are smaller than polypeptides and other, large molecular complexes and can be easily administered to and assimilated by patients and other subjects. Small molecule drugs can advantageously be formulated for oral administration or intramuscular injection. For example, a small molecule may have a molecular weight of up to 2000 Dalton; preferably up to 1000 Dalton; advantageously between 250 and 750 Dalton; and more preferably less than 500 Dalton.

A "selectable change" is any change which can be measured and acted upon to identify or isolate the genetic element which causes it. The selection may take place at the level of the micro capsule, the microbead, or the genetic element itself, optionally when complexed with another reagent. A particularly advantageous embodiment is optical detection, in which the selectable change is a change in optical properties, which can be detected and acted upon for instance in a flow sorting device to separate microcapsules or microbeads displaying the desired change.

As used herein, a change in optical properties refers to any change in absorption or emission of electromagnetic radiation, including changes in absorbance, luminescence, phosphorescence or fluorescence. All such properties are included in the term "optical". Microcapsules or microbeads can be identified and, optionally, sorted, for example, by luminescence, fluorescence or phosphorescence activated sorting. In a preferred embodiment, flow sorting is employed to identify and, optionally, sort microcapsules or microbeads. A variety of optical properties can be used for analysis and to trigger sorting, including light scattering (Kerker, 1983) and fluorescence polarisation (Rolland et al., 1985).

The genetic elements in microcapsules or on beads can be identified using a variety of techniques familiar to those skilled in the art, including mass spectroscopy, chemical tagging or optical tagging.

As used herein, "microfluidic control" refers to the use of a microfluidic system comprising microfluidic channels as defined herein to direct or otherwise control the formation and/or movement of microcapsules (or "droplets") in order to carry out the methods of the present invention. For example, "microfluidic control" of microcapsule formation refers to the creation of microcapsules using a microfluidic device to form "droplets" of fluid within a second fluid, thus creating a microcapsule. Microcapsules sorted under microfluidic control are sorted, as described herein, using a microfluidic device to perform one or more of the functions associated with the sorting procedure. "Microfluidic control of fluidic species", therefore, refers to the handling of fluids in a microfluidic system as defined in order to carry out the methods of the present invention.

As used herein, a "cell" is given its ordinary meaning as used in biology. The cell may be any cell or cell type. For example, the cell may be a bacterium or other single-cell organism, a plant cell, or an animal cell. If the cell is a single-cell organism, then the cell may be, for example, a protozoan, a trypanosome, an amoeba, a yeast cell, algae, etc. If the cell is an animal cell, the cell may be, for example, an invertebrate cell (e.g., a cell from a fruit fly), a fish cell (e.g., a zebrafish cell), an amphibian cell (e.g., a frog cell), a reptile cell, a bird cell, or a mammalian cell such as a primate cell, a bovine cell, a horse cell, a porcine cell, a goat cell, a dog cell, a cat cell, or a cell from a rodent such as a rat or a mouse. If the cell is from a multicellular organism, the cell may be from any part of the organism. For instance, if the cell is from an animal, the cell may be a cardiac cell, a fibroblast, a keratinocyte, a heptaocyte, a chondrocyte, a neural cell, a osteocyte, a muscle cell, a blood cell, an endothelial cell, an immune cell (e.g., a T-cell, a B-cell, a macrophage, a neutrophil, a basophil, a mast cell, an eosinophil), a stem cell, etc. In some cases, the cell may be a

genetically engineered cell. In certain embodiments, the cell may be a Chinese hamster ovarian ("CHO") cell or a 3T3 cell.

"Microfluidic," as used herein, refers to a device, apparatus or system including at least one fluid channel having a cross-sectional dimension of less than 1 mm, and a ratio of length to largest cross-sectional dimension of at least 3:1. A "microfluidic channel," as used herein, is a channel meeting these criteria.

The "cross-sectional dimension" of the channel is measured perpendicular to the direction of fluid flow. Most fluid channels in components of the invention have maximum cross-sectional dimensions less than 2 mm, and in some cases, less than 1 mm. In one set of embodiments, all fluid channels containing embodiments of the invention are microfluidic or have a largest cross sectional dimension of no more than 2 mm or 1 mm. In another embodiment, the fluid channels may be formed in part by a single component (e.g. an etched substrate or moulded unit). Of course, larger channels, tubes, chambers, reservoirs, etc. can be used to store fluids in bulk and to deliver fluids to components of the invention. In one set of embodiments, the maximum cross-sectional dimension of the channel(s) containing embodiments of the invention are less than 500 microns, less than 200 microns, less than 100 microns, less than 50 microns, or less than 25 microns.

A "channel," as used herein, means a feature on or in an article (substrate) that at least partially directs the flow of a fluid. The channel can have any cross-sectional shape (circular, oval, triangular, irregular, square or rectangular, or the like) and can be covered or uncovered. In embodiments where it is completely covered, at least one portion of the channel can have a cross-section that is completely enclosed, or the entire channel may be completely enclosed along its entire length with the exception of its inlet(s) and outlet(s). A channel may also have an aspect ratio (length to average cross sectional dimension) of at least 2:1, more typically at least 3:1, 5:1, or 10:1 or more. An open channel generally will include characteristics that facilitate control over fluid transport, e.g., structural characteristics (an elongated indentation) and/or physical or chemical characteristics (hydrophobicity vs. hydrophilicity) or other characteristics that can exert a force (e.g., a containing force) on a fluid. The fluid within the channel may partially or completely fill the channel. In some cases where an open channel is used, the fluid may be held within the channel, for example, using surface tension (i.e., a concave or convex meniscus).

The channel may be of any size, for example, having a largest dimension perpendicular to fluid flow of less than about 5 mm or 2 mm, or less than about 1 mm, or less than about 500 microns, less than about 200 microns, less than about 100 microns, less than about 60 microns, less than about 50 microns, less than about 40 microns, less than about 30 microns, less than about 25 microns, less than about 10 microns, less than about 3 microns, less than about 1 micron, less than about 300 nm, less than about 100 nm, less than about 30 nrn, or less than about 10 nrn. In some cases the dimensions of the channel may be chosen such that fluid is able to freely flow through the article or substrate. The dimensions of the channel may also be chosen, for example, to allow a certain volumetric or linear flowrate of fluid in the channel. Of course, the number of channels and the shape of the channels can be varied by any method known to those of ordinary skill in the art. In some cases, more than one channel or capillary may be used. For example, two or more channels may be used, where they are positioned inside each other, positioned adjacent to each other, positioned to intersect with each other, etc.

As used herein, "integral" means that portions of components are joined in such a way that they cannot be separated from each other without cutting or breaking the components from each other.

A "droplet," as used herein is an isolated portion of a first fluid that is completely surrounded by a second fluid. It is to be noted that a droplet is not necessarily spherical, but may assume other shapes as well, for example, depending on the external environment. In one embodiment, the droplet has a minimum cross-sectional dimension that is substantially equal to the largest dimension of the channel perpendicular to fluid flow in which the droplet is located.

The "average diameter" of a population of droplets is the arithmetic average of the diameters of the droplets. Those of ordinary skill in the art will be able to determine the average diameter of a population of droplets, for example, using laser light scattering or other known techniques. The diameter of a droplet, in a non-spherical droplet, is the mathematically-defined average diameter of the droplet, integrated across the entire surface. As non-limiting examples, the average diameter of a droplet may be less than about 1 mm, less than about 500 micrometers, less than about 200 micrometers, less than about 100 micrometers, less than about 75 micrometers, less than about 50 micrometers, less than about 25 micrometers, less than about 10 micrometers, or less than about 5 micrometers. The average diameter of the droplet may also be at least about 1 micrometer, at least about 2 micrometers, at least about 3 micrometers, at least about 5 micrometers, at least about 10 micrometers, at least about 15 micrometers, or at least about 20 micrometers in certain cases.

As used herein, a "fluid" is given its ordinary meaning, i.e., a liquid or a gas. Preferably, a fluid is a liquid. The fluid may have any suitable viscosity that permits flow. If two or more fluids are present, each fluid may be independently selected among essentially any fluids (liquids, gases, and the like) by those of ordinary skill in the art, by considering the relationship between the fluids. The fluids may each be miscible or immiscible. For example, two fluids can be selected to be immiscible within the time frame of formation of a stream of fluids, or within the time frame of reaction or interaction. Where the portions remain liquid for a significant period of time then the fluids should be significantly immiscible. Where, after contact and/or formation, the dispersed portions are quickly hardened by polymerisation or the like, the fluids need not be as immiscible. Those of ordinary skill in the art can select suitable miscible or immiscible fluids, using contact angle measurements or the like, to carry out the techniques of the invention.

As used herein, a first entity is "surrounded" by a second entity if a closed loop can be drawn around the first entity through only the second entity. A first entity is "completely surrounded" if closed loops going through only the second entity can be drawn around the first entity regardless of direction. In one aspect, the first entity may be a cell, for example, a cell suspended in media is surrounded by the media. In another aspect, the first entity is a particle. In yet another aspect of the invention, the entities can both be fluids. For example, a hydrophilic liquid may be suspended in a hydrophobic liquid, a hydrophobic liquid may be suspended in a hydrophilic liquid, a gas bubble may be suspended in a liquid, etc. Typically, a hydrophobic liquid and a hydrophilic liquid are substantially immiscible with respect to each other, where the hydrophilic liquid has a greater affinity to water than does the hydrophobic liquid.

Examples of hydrophilic liquids include, but are not limited to, water and other aqueous solutions comprising water, such as cell or biological media, ethanol, salt solutions, etc. Examples of hydrophobic liquids include, but are not limited to, oils such as hydrocarbons, silicon oils, fluorocarbon oils, organic solvents etc.

The term "determining," as used herein, generally refers to the analysis or measurement of a species, for example, quantitatively or qualitatively, or the detection of the presence or absence of the species. "Determining" may also refer to the analysis or measurement of an interaction between two or more species, for example, quantitatively or qualitatively, or by detecting the presence or absence of the interaction. Example techniques include, but are not limited to, spectroscopy such as infrared, absorption, fluorescence, UV/visible, FTIR ("Fourier Transform Infrared Spectroscopy"), or Raman; gravimetric techniques; ellipsometry; piezoelectric measurements; immunoassays; electrochemical measurements; optical measurements such as optical density measurements; circular dichroism; light scattering measurements such as quasielectric light scattering; polarimetry; refractometry; or turbidity measurements.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art (e.g., in cell culture, molecular genetics, nucleic acid chemistry, hybridisation techniques and biochemistry). Standard techniques are used for molecular, genetic and biochemical methods (see generally, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. and Ausubel et al., Short Protocols in Molecular Biology (1999) 4th Ed, John Wiley & Sons, Inc. which are incorporated herein by reference) and chemical methods. In addition Harlow & Lane, A Laboratory Manual Cold Spring Harbor, N.Y. is referred to for standard Immunological Techniques.

(A) General Description

The microcapsules of the present invention require appropriate physical properties to allow the working of the invention.

First, to ensure that the genetic elements and gene products may not diffuse between microcapsules, the contents of each microcapsule are preferably isolated from the contents of the surrounding microcapsules, so that there is no or little exchange of the genetic elements and gene products between the microcapsules over the timescale of the experiment. However, the permeability of the microcapsules may be adjusted such that reagents may be allowed to diffuse into and/or out of the microcapsules if desired.

Second, the method of the present invention requires that there are only a limited number of genetic elements per microcapsule. This ensures that the gene product of an individual genetic element will be isolated from other genetic elements. Thus, coupling between genetic element and gene product will be highly specific. The enrichment factor is greatest with on average one or fewer genetic elements per microcapsule, the linkage between nucleic acid and the activity of the encoded gene product being as tight as is possible, since the gene product of an individual genetic element will be isolated from the products of all other genetic elements. However, even if the theoretically optimal situation of, on average, a single genetic element or less per microcapsule is not used, a ratio of 5, 10, 50, 100 or 1000 or more genetic elements per microcapsule may prove beneficial in sorting a large library. Subsequent rounds of sorting, including renewed encapsulation with differing genetic element distribution, will permit more stringent sorting of the genetic elements. Preferably, there is a single genetic element, or fewer, per microcapsule.

Third, the formation and the composition of the microcapsules advantageously does not abolish the function of the machinery the expression of the genetic elements and the activity of the gene products.

Consequently, any microencapsulation system used preferably fulfils these three requirements. The appropriate system(s) may vary depending on the precise nature of the requirements in each application of the invention, as will be apparent to the skilled person.

A wide variety of microencapsulation procedures are available (see Benita, 1996) and may be used to create the microcapsules used in accordance with the present invention.

Indeed, more than 200 microencapsulation methods have been identified in the literature (Finch, 1993).

Enzyme-catalysed biochemical reactions have also been demonstrated in microcapsules generated by a variety of other methods. Many enzymes are active in reverse micellar solutions (Bru & Walde, 1991; Bru & Walde, 1993; Creagh et al., 1993; Haber et al., 1993; Kumar et al., 1989; Luisi & B., 1987; Mao & Walde, 1991; Mao et al., 1992; Perez et al., 1992; Walde et al., 1994; Walde et al., 1993; Walde et al., 1988) such as the AOT-isooctane-water system (Menger & Yamada, 1979).

Microcapsules can also be generated by interfacial polymerisation and interfacial complexation (Whateley, 1996). Microcapsules of this sort can have rigid, nonpermeable membranes, or semipermeable membranes. Semipermeable microcapsules bordered by cellulose nitrate membranes, polyamide membranes and lipid-polyamide membranes can all support biochemical reactions, including multienzyme systems (Chang, 1987; Chang, 1992; Lim, 1984). Alginate/polylysine microcapsules (Lim & Sun, 1980), which can be formed under very mild conditions, have also proven to be very biocompatible, providing, for example, an effective method of encapsulating living cells and tissues (Chang, 1992; Sun et al., 1992).

Non-membranous microencapsulation systems based on phase partitioning of an aqueous environment in a colloidal system, such as an emulsion, may also be used.

Preferably, the microcapsules of the present invention are formed from emulsions; heterogeneous systems of two immiscible liquid phases with one of the phases dispersed in the other as droplets of microscopic or colloidal size (Becher, 1957; Sherman, 1968; Lissant, 1974; Lissant, 1984).

Emulsions may be produced from any suitable combination of immiscible liquids. Preferably the emulsion of the present invention has "water" (an aqueous liquid containing the biochemical components) as the phase present in the form of finely divided droplets (the disperse, internal or discontinuous phase) and a hydrophobic, immiscible liquid (an 'oil') as the matrix in which these droplets are suspended (the nondisperse, continuous or external phase). Such emulsions are termed 'water-in-oil' (W/O). This has the advantage that the entire aqueous phase containing the biochemical components is compartmentalised in discreet droplets (the internal phase). The external phase, being a hydrophobic liquid, generally contains none of the biochemical components and hence is inert.

The emulsion may be stabilised by addition of one or more surface-active agents (surfactants). These surfactants are termed emulsifying agents and act at the water/oil interface to prevent (or at least delay) separation of the phases. Many oils and many emulsifiers can be used for the

generation of water-in-oil emulsions; a recent compilation listed over 16,000 surfactants, many of which are used as emulsifying agents (Ash and Ash, 1993). Suitable oils include light white mineral oil and decane. Suitable surfactants include: non-ionic surfactants (Schick, 1966) such as sorbitan monooleate (Span™80; ICI), sorbitan monosteatate (Span™60; ICI), polyoxyethylenesorbitan monooleate (Tween™80; ICI), and octylphenoxyethoxyethanol (Triton X-100); ionic surfactants such as sodium cholate and sodium taurocholate and sodium deoxycholate; chemically inert silicone-based surfactants such as polysiloxane-polycetyl-polyethylene glycol copolymer (Cetyl Dimethicone Copolyol) (e.g. Abil™EM90; Goldschmidt); and cholesterol.

Emulsions with a fluorocarbon (or perfluorocarbon) continuous phase (Krafft et al., 2003; Riess, 2002) may be particularly advantageous. For example, stable water-in-perfluorooctyl bromide and water-in-perfluorooctylethane emulsions can be formed using F-alkyl dimorpholinophosphates as surfactants (Sadtler et al., 1996). Non-fluorinated compounds are essentially insoluble in fluorocarbons and perfluorocarbons (Curran, 1998; Hildebrand and Cochran, 1949; Hudlicky, 1992; Scott, 1948; Studer et al., 1997) and small drug-like molecules (typically <500 Da and Log P<5) (Lipinski et al., 2001) are compartmentalised very effectively in the aqueous microcapsules of water-in-fluorocarbon and water-in-perfluorocarbon emulsions—with little or no exchange between microcapsules.

Creation of an emulsion generally requires the application of mechanical energy to force the phases together. There are a variety of ways of doing this which utilise a variety of mechanical devices, including stirrers (such as magnetic stir-bars, propeller and turbine stirrers, paddle devices and whisks), homogenisers (including rotor-stator homogenisers, high-pressure valve homogenisers and jet homogenisers), colloid mills, ultrasound and 'membrane emulsification' devices (Becher, 1957; Dickinson, 1994), and microfluidic devices (Umbanhowar et al., 2000).

Complicated biochemical processes, notably gene transcription and translation are also active in aqueous microcapsules formed in water-in-oil emulsions. This has enabled compartmentalisation in water-in-oil emulsions to be used for the selection of genes, which are transcribed and translated in emulsion microcapsules and selected by the binding or catalytic activities of the proteins they encode (Doi and Yanagawa, 1999; Griffiths and Tawfik, 2003; Lee et al., 2002; Sepp et al., 2002; Tawfik and Griffiths, 1998). This was possible because the aqueous microcapsules formed in the emulsion were generally stable with little if any exchange of nucleic acids, proteins, or the products of enzyme catalysed reactions between microcapsules.

The technology exists to create emulsions with volumes all the way up to industrial scales of thousands of liters (Becher, 1957; Sherman, 1968; Lissant, 1974; Lissant, 1984).

The preferred microcapsule size will vary depending upon the precise requirements of any individual selection process that is to be performed according to the present invention. In all cases, there will be an optimal balance between gene library size, the required enrichment and the required concentration of components in the individual microcapsules to achieve efficient expression and reactivity of the gene products.

The processes of expression occurs within each individual microcapsule provided by the present invention. Both in vitro transcription and coupled transcription-translation become less efficient at sub-nanomolar DNA concentrations. Because of the requirement for only a limited number of DNA molecules to be present in each microcapsule, this therefore sets a practical upper limit on the possible microcapsule size. Preferably, the mean volume of the microcapsules is less that 5.2×10^{-16} m^3, (corresponding to a spherical microcapsule of diameter less than 10 μm, more preferably less than 6.5×10^{-17} m^3 (5 μm diameter), more preferably about 4.2×10^{-18} m^3 (2 μm diameter) and ideally about 9×10^{-18} m^3 (2.6 μm diameter).

The effective DNA or RNA concentration in the microcapsules may be artificially increased by various methods that will be well-known to those versed in the art. These include, for example, the addition of volume excluding chemicals such as polyethylene glycols (PEG) and a variety of gene amplification techniques, including transcription using RNA polymerases including those from bacteria such as *E. coli* (Roberts, 1969; Blattner and Dahlberg, 1972; Roberts et al., 1975; Rosenberg et al., 1975), eukaryotes e.g. (Weil et al., 1979; Manley et al, 1983) and bacteriophage such as T7, T3 and SP6 (Melton et al., 1984); the polymerase chain reaction (PCR) (Saiki et al., 1988); Qb replicase amplification (Miele et al., 1983; Cahill et al., 1991; Chetverin and Spirin, 1995; Katanaev et al., 1995); the ligase chain reaction (LCR) (Landegren et al, 1988; Barany, 1991); and self-sustained sequence replication system (Fahy et al., 1991) and strand displacement amplification (Walker et al, 1992). Gene amplification techniques requiring thermal cycling such as PCR and LCR may be used if the emulsions and the in vitro transcription or coupled transcription-translation systems are thermostable (for example, the coupled transcription-translation systems can be made from a thermostable organism such as *Thermus aquaticus*).

Increasing the effective local nucleic acid concentration enables larger microcapsules to be used effectively. This allows a preferred practical upper limit to the microcapsule volume of about 5.2×10^{-16} m^3 (corresponding to a sphere of diameter 10 μm).

The microcapsule size is preferably sufficiently large to accommodate all of the required components of the biochemical reactions that are needed to occur within the microcapsule. For example, in vitro, both transcription reactions and coupled transcription-translation reactions require a total nucleoside triphosphate concentration of about 2 mM.

For example, in order to transcribe a gene to a single short RNA molecule of 500 bases in length, this would require a minimum of 500 molecules of nucleoside triphosphate per microcapsule (8.33×10^{-22} moles). In order to constitute a 2 mM solution, this number of molecules is contained within a microcapsule of volume 4.17×10^{-19} liters (4.17×10^{-22} m^3 which if spherical would have a diameter of 93 nm.

Furthermore, particularly in the case of reactions involving translation, it is to be noted that the ribosomes necessary for the translation to occur are themselves approximately 20 nm in diameter. Hence, the preferred lower limit for microcapsules is a diameter of approximately 0.1 μm (100 nm).

Therefore, the microcapsule volume is preferably of the order of between 5.2×10^{-22} m^3 and 5.2×10^{-16} m^3 corresponding to a sphere of diameter between 0.1 μm and 10 μm, more preferably of between about 5.2×10^{-19} m^3 and 6.5×10^{-17} m^3 (1 μm and 5 μm). Sphere diameters of about 2.6 μm are most advantageous.

It is no coincidence that the preferred dimensions of the compartments (droplets of 2.6 μm mean diameter) closely resemble those of bacteria, for example, *Escherichia* are 1.1-1.5×2.0-6.0 μm rods and *Azotobacter* are 1.5-2.0 1. μm diameter ovoid cells. In its simplest form, Darwinian evolution is based on a 'one genotype one phenotype' mechanism. The concentration of a single compartmentalised gene,

or genome, drops from 0.4 nM in a compartment of 2 μm diameter, to 25 pM in a compartment of 5 μm diameter. The prokaryotic transcription/translation machinery has evolved to operate in compartments of ~1-2 μm diameter, where single genes are at approximately nanomolar concentrations. A single gene, in a compartment of 2.6 μm diameter is at a concentration of 0.2 nM. This gene concentration is high enough for efficient translation. Compartmentalisation in such a volume also ensures that even if only a single molecule of the gene product is formed it is present at about 0.2 nM, which is important if the gene product is to have a modifying activity of the genetic element itself. The volume of the microcapsule is thus selected bearing in mind not only the requirements for transcription and translation of the genetic element, but also the modifying activity required of the gene product in the method of the invention.

The size of emulsion microcapsules may be varied simply by tailoring the emulsion conditions used to form the emulsion according to requirements of the selection system. The larger the microcapsule size, the larger is the volume that will be required to encapsulate a given genetic element library, since the ultimately limiting factor will be the size of the microcapsule and thus the number of microcapsules possible per unit volume.

The size of the microcapsules is selected not only having regard to the requirements of the transcription/translation system, but also those of the selection system employed for the genetic element. Thus, the components of the selection system, such as a chemical modification system, may require reaction volumes and/or reagent concentrations which are not optimal for transcription/translation. As set forth herein, such requirements may be accommodated by a secondary re-encapsulation step; moreover, they may be accommodated by selecting the microcapsule size in order to maximise transcription/translation and selection as a whole. Empirical determination of optimal microcapsule volume and reagent concentration, for example as set forth herein, is preferred.

A "genetic element" in accordance with the present invention is as described above. Preferably, a genetic element is a molecule or construct selected from the group consisting of a DNA molecule, an RNA molecule, a partially or wholly artificial nucleic acid molecule consisting of exclusively synthetic or a mixture of naturally-occurring and synthetic bases, any one of the foregoing linked to a polypeptide, and any one of the foregoing linked to any other molecular group or construct. Advantageously, the other molecular group or construct may be selected from the group consisting of nucleic acids, polymeric substances, particularly beads, for example polystyrene beads, and magnetic or paramagnetic substances such as magnetic or paramagnetic beads.

The nucleic acid portion of the genetic element may comprise suitable regulatory sequences, such as those required for efficient expression of the gene product, for example promoters, enhancers, translational initiation sequences, polyadenylation sequences, splice sites and the like.

As will be apparent from the following, in many cases the polypeptide or other molecular group or construct is a ligand or a substrate which directly or indirectly binds to or reacts with the gene product in order to alter the optical properties of the genetic element. This allows the sorting of the genetic element on the basis of the activity of the gene product. The ligand or substrate can be connected to the nucleic acid by a variety of means that will be apparent to those skilled in the art (see, for example, Hermanson, 1996).

One way in which the nucleic acid molecule may be linked to a ligand or substrate is through biotinylation. This can be done by PCR amplification with a 5'-biotinylation primer such that the biotin and nucleic acid are covalently linked.

The ligand or substrate can be attached to the modified nucleic acid by a variety of means that will be apparent to those of skill in the art (see, for example, Hermanson, 1996). A biotinylated nucleic acid may be coupled to a polystyrene or paramagnetic microbead (0.02 to approx. 5.0 μm in diameter) that is coated with avidin or streptavidin, that will therefore bind the nucleic acid with very high affinity. This bead can be derivatised with substrate or ligand by any suitable method such as by adding biotinylated substrate or by covalent coupling.

Alternatively, a biotinylated nucleic acid may be coupled to avidin or streptavidin complexed to a large protein molecule such as thyroglobulin (669 Kd) or ferritin (440 Kd). This complex can be derivatised with substrate or ligand, for example by covalent coupling to the E-amino group of lysines or through a non-covalent interaction such as biotin-avidin.

The substrate may be present in a form unlinked to the genetic element but containing an inactive "tag" that requires a further step to activate it such as photoactivation (e.g. of a "caged" biotin analogue, (Sundberg et al., 1995; Pirrung and Huang, 1996)). The catalyst to be selected then converts the substrate to product. The "tag" is then activated and the "tagged" substrate and/or product bound by a tag-binding molecule (e.g. avidin or streptavidin) complexed with the nucleic acid. The ratio of substrate to product attached to the nucleic acid via the "tag" will therefore reflect the ratio of the substrate and product in solution.

An alternative is to couple the nucleic acid to a product-specific antibody (or other product-specific molecule). In this scenario, the substrate (or one of the substrates) is present in each microcapsule unlinked to the genetic element, but has a molecular "tag" (for example biotin, DIG or DNP or a fluorescent group). When the catalyst to be selected converts the substrate to product, the product retains the "tag" and is then captured in the microcapsule by the product-specific antibody. In this way the genetic element only becomes associated with the "tag" when it encodes or produces an enzyme capable of converting substrate to product.

The terms "isolating", "sorting" and "selecting", as well as variations thereof; are used herein. Isolation, according to the present invention, refers to the process of separating an entity from a heterogeneous population, for example a mixture, such that it is free of at least one substance with which it was associated before the isolation process. In a preferred embodiment, isolation refers to purification of an entity essentially to homogeneity. Sorting of an entity refers to the process of preferentially isolating desired entities over undesired entities. In as far as this relates to isolation of the desired entities, the terms "isolating" and "sorting" are equivalent. The method of the present invention permits the sorting of desired genetic elements from pools (libraries or repertoires) of genetic elements which contain the desired genetic element. Selecting is used to refer to the process (including the sorting process) of isolating an entity according to a particular property thereof.

In a highly preferred application, the method of the present invention is useful for sorting libraries of genetic elements. The invention accordingly provides a method according to preceding aspects of the invention, wherein the

genetic elements are isolated from a library of genetic elements encoding a repertoire of gene products. Herein, the terms "library", "repertoire" and "pool" are used according to their ordinary signification in the art, such that a library of genetic elements encodes a repertoire of gene products. In general, libraries are constructed from pools of genetic elements and have properties which facilitate sorting.

Initial selection of a genetic element from a genetic element library using the present invention will in most cases require the screening of a large number of variant genetic elements. Libraries of genetic elements can be created in a variety of different ways, including the following.

Pools of naturally occurring genetic elements can be cloned from genomic DNA or cDNA (Sambrook et al., 1989); for example, phage antibody libraries, made by PCR amplification repertoires of antibody genes from immunised or unimmunised donors have proved very effective sources of functional antibody fragments (Winter et al., 1994; Hoogenboom, 1997). Libraries of genes can also be made by encoding all (see for example Smith, 1985; Parmley and Smith: 1988) or part of genes (see for example Lowman et al., 1991) or pools of genes (see for example Nissim et al., 1994) by a randomised or doped synthetic oligonucleotide. Libraries can also be made by introducing mutations into a genetic element or pool of genetic elements 'randomly by a variety of techniques in vivo, including; using mutator strains of bacteria such as *E. coli* mutD5 (Liao et al., 1986; Yamagishi et al., 1990; Low et at, 1996); using the antibody hypermutation system of B-lymphocytes (Yelamos et al., 1995). Random mutations can also be introduced both in vivo and in vitro by chemical mutagens, and ionising or UV irradiation (see Friedberg et al., 1995), or incorporation of mutagenic base analogues (Frees; 1959; Zaccolo et at, 1996). Random' mutations can also be introduced into genes in vitro during polymerisation for example by using error-prone polymerases (Leung et al., 1989).

Further diversification can be introduced by using homologous recombination either in vivo (see Kowalczykowski et al, 1994) or in vitro (Stemmer, 1994a; Stemmer, 1994b).

According to a further aspect of the present invention, therefore, there is provided a method of in vitro evolution comprising the steps of:

(a) selecting one or more genetic elements from a genetic element library according to the present invention;

(b) mutating the selected genetic element(s) in order to generate a further library of genetic elements encoding a repertoire to gene products; and

(c) iteratively repeating steps (a) and (b) in order to obtain a gene product with enhanced activity.

Mutations may be introduced into the genetic elements(s) as set forth above.

The genetic elements according to the invention advantageously encode enzymes, preferably of pharmacological or industrial interest, activators or inhibitors, especially of biological systems, such as cellular signal transduction mechanisms, antibodies and fragments thereof, and other binding agents (e.g. transcription factors) suitable for diagnostic and therapeutic applications. In a preferred aspect, therefore, the invention permits the identification and isolation of clinically or industrially useful products. In a further aspect of the invention, there is provided a product when isolated by the method of the invention.

The selection of suitable encapsulation conditions is desirable. Depending on the complexity and size of the library to be screened, it may be beneficial to set up the encapsulation procedure such that 1 or less than 1 genetic element is encapsulated per microcapsule. This will provide the greatest power of resolution. Where the library is larger and/or more complex, however, this may be impracticable; it may be preferable to encapsulate several genetic elements together and rely on repeated application of the method of the invention to achieve sorting of the desired activity. A combination of encapsulation procedures may be used to obtain the desired enrichment.

Theoretical studies indicate that the larger the number of genetic element variants created the more likely it is that a molecule will be created with the properties desired (see Perelson and Oster, 1979 for a description of how this applies to repertoires of antibodies). Recently it has also been confirmed practically that larger phage-antibody repertoires do indeed give rise to more antibodies with better binding affinities than smaller repertoires (Griffiths et al., 1994). To ensure that rare variants are generated and thus are capable of being selected, a large library size is desirable. Thus, the use of optimally small microcapsules is beneficial.

The largest repertoire created to date using methods that require an in vivo step (phage-display and LacI systems) has been a 1.6×10^{11} clone phage-peptide library which required the fermentation of 15 liters of bacteria (Fisch et al., 1996). SELEX experiments are often carried out on very large numbers of variants (up to 10^{15}).

Using the present invention, at a preferred microcapsule diameter of 2.6 μm, a repertoire size of at least 10^{11} can be selected using 1 ml aqueous phase in a 20 ml emulsion.

In addition to the genetic elements described above, the microcapsules according to the invention will comprise further components required for the sorting process to take place. Other components of the system will for example comprise those necessary for transcription and/or translation of the genetic element. These are selected for the requirements of a specific system from the following; a suitable buffer, an in vitro transcription/replication system and/or an in vitro translation system containing all the necessary ingredients, enzymes and cofactors, RNA polymerase, nucleotides, nucleic acids (natural or synthetic), transfer RNAs, ribosomes and amino acids, and the substrates of the reaction of interest in order to allow selection of the modified gene product.

A suitable buffer will be one in which all of the desired components of the biological system are active and will therefore depend upon the requirements of each specific reaction system. Buffers suitable for biological and/or chemical reactions are known in the art and recipes provided in various laboratory texts, such as Sambrook et al., 1989.

The in vitro translation system will usually comprise a cell extract, typically from bacteria (Zubay, 1973; Zubay, 1980; Lesley et al., 1991; Lesley, 1995), rabbit reticulocytes (Pelham and Jackson, 1976), or wheat germ (Anderson et al., 1983). Many suitable systems are commercially available (for example from Promega) including some which will allow coupled transcription/translation (all the bacterial systems and the reticulocyte and wheat germ TNT™ extract systems from Promega). The mixture of amino acids used may include synthetic amino acids if desired, to increase the possible number or variety of proteins produced in the library. This can be accomplished by charging tRNAs with artificial amino acids and using these tRNAs for the in vitro translation of the proteins to be selected (Ellman et al., 1991; Benner, 1994; Mendel et al., 1995).

After each round of selection the enrichment of the pool of genetic elements for those encoding the molecules of interest can be assayed by non-compartmentalised in vitro

transcription/replication or coupled transcription-translation reactions. The selected pool is cloned into a suitable plasmid vector and RNA or recombinant protein is produced from the individual clones for further purification and assay.

In a preferred aspect, the internal environment of a microcapsule may be altered by addition of reagents to the oil phase of the emulsion. The reagents diffuse through the oil phase to the aqueous microcapsule environment. Preferably, the reagents are at least partly water-soluble; such that a proportion thereof is distributed from the oil phase to the aqueous microcapsule environment. Advantageously, the reagents are substantially insoluble in the oil phase. Reagents are preferably mixed into the oil phase by mechanical mixing, for example vortexing.

The reagents which may be added via the oil phase include substrates, buffering components, factors and the like. In particular, the internal pH of microcapsules may be altered in situ by adding acidic or basic components to the oil phase.

The invention moreover relates to a method for producing a gene product, once a genetic element encoding the gene product has been sorted by the method of the invention. Clearly, the genetic element itself may be directly expressed by conventional means to produce the gene product. However, alternative techniques may be employed, as will be apparent to those skilled in the art. For example, the genetic information incorporated in the gene product may be incorporated into a suitable expression vector, and expressed therefrom.

The invention also describes the use of conventional screening techniques to identify compounds which are capable of interacting with the gene products identified by the first aspect of the invention. In preferred embodiments, gene product encoding nucleic acid is incorporated into a vector, and introduced into suitable host cells to produce transformed cell lines that express the gene product. The resulting cell lines can then be produced for reproducible qualitative and/or quantitative analysis of the effect(s) of potential drugs affecting gene product function. Thus gene product expressing cells may be employed for the identification of compounds, particularly small molecular weight compounds, which modulate the function of gene product. Thus host cells expressing gene product are useful for drug screening and it is a further object of the present invention to provide a method for identifying compounds which modulate the activity of the gene product, said method comprising exposing cells containing heterologous DNA encoding gene product, wherein said cells produce functional gene product, to at least one compound or mixture of compounds or signal whose ability to modulate the activity of said gene product is sought to be determined, and thereafter monitoring said cells for changes caused by said modulation. Such an assay enables the identification of modulators, such as agonists, antagonists and allosteric modulators, of the gene product. As used herein, a compound or signal that modulates the activity of gene product refers to a compound that alters the activity of gene product in such a way that the activity of the gene product is different in the presence of the compound or signal (as compared to the absence of said compound or signal).

Cell-based screening assays can be designed by constructing cell lines in which the expression of a reporter protein, i.e. an easily assayable protein, such as □-galactosidase, chloramphenicol acetyltransferase (CAT), green fluorescent protein (GFP) or luciferase, is dependent on gene product. Such an assay enables the detection of compounds that directly modulate gene product function, such as compounds that antagonise gene product, or compounds that inhibit or potentiate other cellular functions required for the activity of gene product.

The present invention also provides a method to exogenously affect gene product dependent processes occurring in cells. Recombinant gene product producing host cells, e.g. mammalian cells, can be contacted with a test compound, and the modulating effect(s) thereof can then be evaluated by comparing the gene product-mediated response in the presence and absence of test compound, or relating the gene product-mediated response of test cells, or control cells (i.e., cells that do not express gene product), to the presence of the compound.

In a further aspect, the invention relates to a method for optimising a production process which involves at least one step which is facilitated by a polypeptide. For example, the step may be a catalytic step, which is facilitated by an enzyme. Thus, the invention provides a method for preparing a compound or compounds comprising the steps of:

(a) providing a synthesis protocol wherein at least one step is facilitated by a polypeptide;
(b) preparing genetic elements encoding variants of the polypeptide which facilitates this step, the expression of which may result, directly or indirectly, in the modification of the optical properties of the genetic elements;
(c) compartmentalising genetic elements into microcapsules;
(d) expressing the genetic elements to produce their respective gene products within the microcapsules;
(e) sorting the genetic elements which produce polypeptide gene product(s) having the desired activity using the changed optical properties of the genetic elements; and
(f) preparing the compound or compounds using the polypeptide gene product identified in (g) to facilitate the relevant step of the synthesis.

By means of the invention, enzymes involved in the preparation of a compound may be optimised by selection for optimal activity. The procedure involves the preparation of variants of the polypeptide to be screened, which equate to a library of polypeptides as refereed to herein. The variants may be prepared in the same manner as the libraries discussed elsewhere herein.

The size of emulsion microcapsules may be varied simply by tailoring the emulsion conditions used to form the emulsion according to requirements of the screening system. The larger the microcapsule size, the larger is the volume that will be required to encapsulate a given library, since the ultimately limiting factor will be the size of the microcapsule and thus the number of microcapsules possible per unit volume.

Water-in-oil emulsions can be re-emulsified to create water-in-oil-in water double emulsions with an external (continuous) aqueous phase. These double emulsions can be analysed and, optionally, sorted using a flow cytometer (Bernath et al., 2004).

Highly monodisperse microcapsules can be produced using microfluidic techniques. For example, water-in-oil emulsions with less than 1.5% polydispersity can be generated by droplet break off in a co-flowing steam of oil (Umbanhowar et al., 2000). Microfluidic systems can also be used for laminar-flow of aqueous microdroplets dispersed in a stream of oil in microfluidic channels (Thorsen et al., 2001). This allows the construction of microfluidic devices for flow analysis and, optionally, flow sorting of microdroplets (Fu et al., 2002).

Advantageously, highly monodisperse microcapsules can be formed using systems and methods for the electronic control of fluidic species. One aspect of the invention relates to systems and methods for producing droplets of fluid surrounded by a liquid. The fluid and the liquid may be essentially immiscible in many cases, i.e., immiscible on a time scale of interest (e.g., the time it takes a fluidic droplet to be transported through a particular system or device). In certain cases, the droplets may each be substantially the same shape or size, as further described below. The fluid may also contain other species, for example, certain molecular species (e.g., as further discussed below), cells, particles, etc.

In one set of embodiments, electric charge may be created on a fluid surrounded by a liquid, which may cause the fluid to separate into individual droplets within the liquid. In some embodiments, the fluid and the liquid may be present in a channel, e.g., a microfluidic channel, or other constricted space that facilitates application of an electric field to the fluid (which may be "AC" or alternating current, "DC" or direct current etc.), for example, by limiting movement of the fluid with respect to the liquid. Thus, the fluid can be present as a series of individual charged and/or electrically inducible droplets within the liquid. In one embodiment, the electric force exerted on the fluidic droplet may be large enough to cause the droplet to move within the liquid. In some cases, the electric force exerted on the fluidic droplet may be used to direct a desired motion of the droplet within the liquid, for example, to or within a channel or a microfluidic channel (e.g., as further described herein), etc. As one example, in apparatus **5** in FIG. **3**A, droplets **15** created by fluid source **10** can be electrically charged using an electric filed created by electric field generator **20**.

Electric charge may be created in the fluid within the liquid using any suitable technique, for example, by placing the fluid within an electric field (which may be AC, DC, etc.), and/or causing a reaction to occur that causes the fluid to have an electric charge, for example, a chemical reaction, an ionic reaction, a photocatalyzed reaction, etc. In one embodiment, the fluid is an electrical conductor. As used herein, a "conductor" is a material having a conductivity of at least about the conductivity of 18 megohm (MOhm or MΩ) water. The liquid surrounding the fluid may have a conductivity less than that of the fluid. For instance, the liquid may be an insulator, relative to the fluid, or at least a "leaky insulator," i.e., the liquid is able to at least partially electrically insulate the fluid for at least a short period of time. Those of ordinary skill in the art will be able to identify the conductivity of fluids. In one non-limiting embodiment, the fluid may be substantially hydrophilic, and the liquid surrounding the fluid may be substantially hydrophobic.

In some embodiments, the charge created on the fluid (for example, on a series of fluidic droplets) may be at least about 10^{-22} C/micrometer^3. In certain cases, the charge may be at least about 10^{-21} C/micrometer^3, and in other cases, the charge may be at least about 10^{-20} C/micrometer^3, at least about 10^{-19} C/micrometer^3, at least about 10^{-18} C/micrometer^3, at least about 10^{-17} C/micrometer^3, at least about 10^{-16} C/micrometer^3, at least about 10^{-15} C/micrometer^3, at least about 10^{-14} C/micrometer^3, at least about 10^{-13} C/micrometer^3, at least about 10^{-12} C/micrometer^3, at least about 10^{-11} C/micrometer^3, at least about 10^{-10} C/micrometer^3, or at least about 10^{-9} C/micrometer^3 or more. In certain embodiments, the charge created on the fluid may be at least about 10^{-21} C/micrometer^2, and in some cases, the charge may be at least about 10^{-20} C/micrometer^2, at least about 10^{-19} C/micrometer^2, at least about 10^{-18} C/micrometer^2, at least about 10^{-17} C/micrometer^2, at least about 10^{-16} C/micrometer^2, at least about 10^{-15} C/micrometer^2, at least about 10^{-14} C/micrometer^2, or at least about 10^{-13} C/micrometer^2 or more. In other embodiments, the charge may be at least about 10^{-14} C/droplet, and, in some cases, at least about 10^{-13} C/droplet, in other cases at least about 10^{-12} C/droplet, in other cases at least about 10^{-11} C/droplet, in other cases at least about 10^{-10} C/droplet, or in still other cases at least about 10^{-9} C/droplet.

The electric field, in some embodiments, is generated from an electric field generator, i.e., a device or system able to create an electric field that can be applied to the fluid. The electric field generator may produce an AC field (i.e., one that varies periodically with respect to time, for example, sinusoidally, sawtooth, square, etc.), a DC field (i.e., one that is constant with respect to time), a pulsed field, etc. The electric field generator may be constructed and arranged to create an electric field within a fluid contained within a channel or a microfluidic channel. The electric field generator may be integral to or separate from the fluidic system containing the channel or microfluidic channel, according to some embodiments. As used herein, "integral" means that portions of the components integral to each other are joined in such a way that the components cannot be manually separated from each other without cutting or breaking at least one of the components.

Techniques for producing a suitable electric field (which may be AC, DC, etc.) are known to those of ordinary skill in the art. For example, in one embodiment, an electric field is produced by applying voltage across a pair of electrodes, which may be positioned on or embedded within the fluidic system (for example, within a substrate defining the channel or microfluidic channel), and/or positioned proximate the fluid such that at least a portion of the electric field interacts with the fluid. The electrodes can be fashioned from any suitable electrode material or materials known to those of ordinary skill in the art, including, but not limited to, silver, gold, copper, carbon, platinum, copper, tungsten, tin, cadmium, nickel, indium tin oxide ("ITO"), etc., as well as combinations thereof. In some cases, transparent or substantially transparent electrodes can be used. In certain embodiments, the electric field generator can be constructed and arranged (e.g., positioned) to create an electric field applicable to the fluid of at least about 0.01 V/micrometer, and, in some cases, at least about 0.03 V/micrometer, at least about 0.05 V/micrometer, at least about 0.08 V/micrometer, at least about 0.1 V/micrometer, at least about 0.3 V/micrometer, at least about 0.5 V/micrometer, at least about 0.7 V/micrometer, at least about 1 V/micrometer, at least about 1.2 V/micrometer, at least about 1.4 V/micrometer, at least about 1.6 V/micrometer, or at least about 2 V/micrometer. In some embodiments, even higher electric field intensities may be used, for example, at least about 2 V/micrometer, at least about 3 V/micrometer, at least about 5 V/micrometer, at least about 7 V/micrometer, or at least about 10 V/micrometer or more.

In some embodiments, an electric field may be applied to fluidic droplets to cause the droplets to experience an electric force. The electric force exerted on the fluidic droplets may be, in some cases, at least about 10^{-16} N/micrometer^3. In certain cases, the electric force exerted on the fluidic droplets may be greater, e.g., at least about 10^{-15} N/micrometer^3, at least about 10^{-14} N/micrometer^3, at least about 10^{-13} N/micrometer^3, at least about 10^{-12} N/micrometer^3, at least about 10^{-11} N/micrometer^3, at least about 10^{-10} N/micrometer^3, at least about 10^{-9} N/micrometer^3, at least about 10^{-8} N/micrometer^3, or at least about 10^{-7}

N/micrometer^3 or more. In other embodiments, the electric force exerted on the fluidic droplets, relative to the surface area of the fluid, may be at least about 10^{-15} N/micrometer^2, and in some cases, at least about 10^{-14} N/micrometer^2, at least about 10^{-13} N/micrometer^2, at least about 10^{-12} N/micrometer^2, at least about 10^{-11} N/micrometer^2, at least about 10^{-10} N/micrometer^2, at least about 10^{-9} N/micrometer^2, at least about 10^{-8} N/micrometer^2, at least about 10^{-7} N/micrometer^2, or at least about 10^{-6} N/micrometer^2 or more. In yet other embodiments, the electric force exerted on the fluidic droplets may be at least about 10^{-9} N, at least about 10^{-8} N, at least about 10^{-7} N, at least about 10^{-6} N, at least about 10^{-5} N, or at least about 10^4 N or more in some cases.

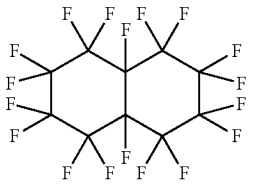
In some embodiments of the invention, systems and methods are provided for at least partially neutralizing an electric charge present on a fluidic droplet, for example, a fluidic droplet having an electric charge, as described above. For example, to at least partially neutralize the electric charge, the fluidic droplet may be passed through an electric field and/or brought near an electrode, e.g., using techniques such as those described herein. Upon exiting of the fluidic droplet from the electric field (i.e., such that the electric field no longer has a strength able to substantially affect the fluidic droplet), and/or other elimination of the electric field, the fluidic droplet may become electrically neutralized, and/or have a reduced electric charge.

In another set of embodiments, droplets of fluid can be created from a fluid surrounded by a liquid within a channel by altering the channel dimensions in a manner that is able to induce the fluid to form individual droplets. The channel may, for example, be a channel that expands relative to the direction of flow, e.g., such that the fluid does not adhere to the channel walls and forms individual droplets instead, or a channel that narrows relative to the direction of flow, e.g., such that the fluid is forced to coalesce into individual droplets. One example is shown in FIG. **7**A, where channel **510** includes a flowing fluid **500** (flowing downwards), surrounded by liquid **505**. Channel **510** narrows at location **501**, causing fluid **500** to form a series of individual fluidic droplets **515**. In other embodiments, internal obstructions may also be used to cause droplet formation to occur. For instance, baffles, ridges, posts, or the like may be used to disrupt liquid flow in a manner that causes the fluid to coalesce into fluidic droplets.

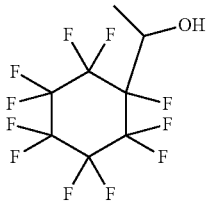
In some cases, the channel dimensions may be altered with respect to time (for example, mechanically or electromechanically, pneumatically, etc.) in such a manner as to cause the formation of individual fluidic droplets to occur. For example, the channel may be mechanically contracted ("squeezed") to cause droplet formation, or a fluid stream may be mechanically disrupted to cause droplet formation, for example, through the use of moving baffles, rotating blades, or the like. As a non-limiting example, in FIG. **7**B, fluid **500** flows through channel **510** in a downward direction. Fluid **500** is surrounded by liquid **505**. Piezoelectric devices **520** positioned near or integral to channel **510** may then mechanically constrict or "squeeze" channel **510**, causing fluid **500** to break up into individual fluidic droplets **515**.

In yet another set of embodiments, individual fluidic droplets can be created and maintained in a system comprising three essentially mutually immiscible fluids (i.e., immiscible on a time scale of interest), where one fluid is a liquid carrier, and the second fluid and the third fluid alternate as individual fluidic droplets within the liquid carrier. In such a system, surfactants are not necessarily required to ensure separation of the fluidic droplets of the second and third fluids. As an example, with reference to FIG. **14**A, within channel **700**, a first fluid **701** and a second fluid **702** are each carried within liquid carrier **705**. First fluid **701** and second fluid **702** alternate as a series of alternating, individual droplets, each carried by liquid carrier **705** within channel **700**. As the first fluid, the second fluid, and the liquid carrier are all essentially mutually immiscible, any two of the fluids (or all three fluids) can come into contact without causing droplet coalescence to occur. A photomicrograph of an example of such a system is shown in FIG. **14**B, illustrating first fluid **701** and second fluid **702**, present as individual, alternating droplets, each contained within liquid carrier **705**.

One example of a system involving three essentially mutually immiscible fluids is a silicone oil, a mineral oil, and an aqueous solution (i.e., water, or water containing one or more other species that are dissolved and/or suspended therein, for example, a salt solution, a saline solution, a suspension of water containing particles or cells, or the like). Another example of a system is a silicone oil, a fluorocarbon oil, and an aqueous solution. Yet another example of a system is a hydrocarbon oil (e.g., hexadecane), a fluorocarbon oil, and an aqueous solution. In these examples, any of these fluids may be used as the liquid carrier. Non-limiting examples of suitable fluorocarbon oils include octadecafluorodecahydro naphthalene:



or 1-(1,2,2,3,3,4,4,5,5,6,6-undecafluorocyclohexyl)ethanol:



A non-limiting example of such a system is illustrated in FIG. **14**B. In this figure, fluidic network **710** includes a channel containing liquid carrier **705**, and first fluid **701** and second fluid **702**. Liquid carrier **705** is introduced into fluidic network **710** through inlet **725**, while first fluid **701** is introduced through inlet **721**, and second fluid **702** is introduced through inlet **722**. Channel **716** within fluidic network **710** contains liquid carrier **715** introduced from inlet **725**. Initially, first fluid **701** is introduced into liquid **10** carrier **705**, forming fluidic droplets therein. Next, second fluid **702** is introduced into liquid **705**, forming fluidic droplets therein that are interspersed with the fluidic droplets containing first fluid **701**. Thus, upon reaching channel **717**, liquid carrier **705** contains a first set of fluidic droplets containing first fluid **701**, interspersed with a second set of fluidic droplets containing second fluid **702**. In the embodiment illustrated, channel **706** optionally comprises a series of bends, which may allow mixing to occur within each of the fluidic droplets, as further discussed below. However, it should be noted that in this embodiment, since first fluid **701** and

second fluid **702** are essentially immiscible, significant fusion and/or mixing of the droplets containing first fluid **701** with the droplets containing second fluid **702** is not generally expected.

Other examples of the production of droplets of fluid surrounded by a liquid are described in International Patent Application Serial No. PCT/US2004/010903, filed Apr. 9, 2004 by Link, et al. and International Patent Application Serial No. PCT/US03/20542, filed Jun. 30, 2003 by Stone, et al., published as WO 2004/002627 on Jan. 8, 2004, each incorporated herein by reference.

In some embodiments, the fluidic droplets may each be substantially the same shape and/or size. The shape and/or size can be determined, for example, by measuring the average diameter or other characteristic dimension of the droplets. The term "determining," as used herein, generally refers to the analysis or measurement of a species, for example, quantitatively or qualitatively, and/or the detection of the presence or absence of the species. "Determining" may also refer to the analysis or measurement of an interaction between two or more species, for example, quantitatively or qualitatively, or by detecting the presence or absence of the interaction. Examples of suitable techniques include, but are not limited to, spectroscopy such as infrared, absorption, fluorescence, UV/visible, FTIR ("Fourier Transform Infrared Spectroscopy"), or Raman; gravimetric techniques; ellipsometry; piezoelectric measurements; immunoassays; electrochemical measurements; optical measurements such as optical density measurements; circular dichroism; light scattering measurements such as quasi-electric light scattering; polarimetry; refractometry; or turbidity measurements.

The "average diameter" of a plurality or series of droplets is the arithmetic average of the average diameters of each of the droplets. Those of ordinary skill in the art will be able to determine the average diameter (or other characteristic dimension) of a plurality or series of droplets, for example, using laser light scattering, microscopic examination, or other known techniques. The diameter of a droplet, in a non-spherical droplet, is the mathematically-defined average diameter of the droplet, integrated across the entire surface. The average diameter of a droplet (and/or of a plurality or series of droplets) may be, for example, less than about 1 mm, less than about 500 micrometers, less than about 200 micrometers, less than about 100 micrometers, less than about 75 micrometers, less than about 50 micrometers, less than about 25 micrometers, less than about 10 micrometers, or less than about 5 micrometers in some cases. The average diameter may also be at least about 1 micrometer, at least about 2 micrometers, at least about 3 micrometers, at least about 5 micrometers, at least about 10 micrometers, at least about 15 micrometers, or at least about 20 micrometers in certain cases.

In certain instances, the invention provides for the production of droplets consisting essentially of a substantially uniform number of entities of a species therein (i.e., molecules, compounds, cells, genetic elements, particles, etc.). For example, about 90%, about 93%, about 95%, about 97%, about 98%, or about 99%, or more of a plurality or series of droplets may each contain the same number of entities of a particular species.

For instance, a substantial number of fluidic droplets produced, e.g., as described above, may each contain 1 entity, 2 entities, 3 entities, 4 entities, 5 entities, 7 entities, 10 entities, 15 entities, 20 entities, 25 entities, 30 entities, 40 entities, 50 entities, 60 entities, 70 entities, 80 entities, 90 entities, 100 entities, etc., where the entities are molecules or macromolecules, cells, particles, etc. In some cases, the droplets may each independently contain a range of entities, for example, less than 20 entities, less than 15 entities, less than 10 entities, less than 7 entities, less than 5 entities, or less than 3 entities in some cases. In one set of embodiments, in a liquid containing droplets of fluid, some of which contain a species of interest and some of which do not contain the species of interest, the droplets of fluid may be screened or sorted for those droplets of fluid containing the species as further described below (e.g., using fluorescence or other techniques such as those described above), and in some cases, the droplets may be screened or sorted for those droplets of fluid containing a particular number or range of entities of the species of interest, e.g., as previously described. Thus, in some cases, a plurality or series of fluidic droplets, some of which contain the species and some of which do not, may be enriched (or depleted) in the ratio of droplets that do contain the species, for example, by a factor of at least about 2, at least about 3, at least about 5, at least about 10, at least about 15, at least about 20, at least about 50, at least about 100, at least about 125, at least about 150, at least about 200, at least about 250, at least about 500, at least about 750, at least about 1000, at least about 2000, or at least about 5000 or more in some cases. In other cases, the enrichment (or depletion) may be in a ratio of at least about 10^4, at least about 10^5, at least about 10^6, at least about 10^7, at least about 10^8, at least about 10^9, at least about 10^{10}, at least about 10^{11}, at least about 10^{12}, at least about 10^{13}, at least about 10^{14}, at least about 10^{15}, or more. For example, a fluidic droplet containing a particular species may be selected from a library of fluidic droplets containing various species, where the library may have about 10^5, about 10^6, about 10^7, about 10^8, about 10^9, about 10^{10}, about 10^{11}, about 10^{12}, about 10^{13}, about 10^{14}, about 10^{15}, or more items, for example, a DNA library, an RNA library, a protein library, a combinatorial chemistry library, a library of genetic elements, etc. In certain embodiments, the droplets carrying the species may then be fused, reacted, or otherwise used or processed, etc., as further described below, for example, to initiate or determine a reaction.

The use of microfluidic handling to create microcapsules according to the invention has a number of advantages:

(a) They allow the formation of highly monodisperse microcapsules (<1.5% polydispersity), each of which functions as an almost identical, very small microreactor;

(b) The microcapsules can have volumes ranging from about 1 femtoliter to about 1 nanoliter;

(c) Compartmentalisation in microcapsules prevents diffusion and dispersion due to parabolic flow;

(d) By using a perfluorocarbon carrier fluid it is possible to prevent exchange of molecules between microcapsules;

(e) Reagents in microcapsules cannot react or interact with the fabric of the microchannels as they are separated by a layer of inert perfluorocarbon carrier fluid.

(f) Microcapsules can be created at up to 10,000 per second and screened using optical methods at the same rate. This is a throughput of ~10^9 per day.

Microcapsules (or droplets; the terms may be used interchangeably for the purposes envisaged herein) can, advantageously, be fused or split. For example, aqueous microdroplets can be merged and split using microfluidics systems (Link et al., 2004; Song et al., 2003). Microcapsule fusion allows the mixing of reagents. Fusion, for example, of a microcapsule containing the genetic element with a microcapsule containing a transcription factor could initiate tran-

scription of the genetic information. Microcapsule splitting allows single microcapsules to be split into two or more smaller microcapsules. For example a single microcapsule containing a reagent can be split into multiple microcapsules which can then each be fused with a different microcapsule containing a different reagent or genetic element. A single microcapsule containing a reagent can also be split into multiple microcapsules which can then each be fused with a different microcapsule containing a different genetic element, or other reagents, for example at different concentrations.

In one aspect, the invention relates to microfluidic systems and methods for splitting a fluidic droplet into two or more droplets. The fluidic droplet may be surrounded by a liquid, e.g., as previously described, and the fluid and the liquid are essentially immiscible in some cases. The two or more droplets created by splitting the original fluidic droplet may each be substantially the same shape and/or size, or the two or more droplets may have different shapes and/or sizes, depending on the conditions used to split the original fluidic droplet. In many cases, the conditions used to split the original fluidic droplet can be controlled in some fashion, for example, manually or automatically (e.g., with a processor, as discussed below). In some cases, each droplet in a plurality or series of fluidic droplets may be independently controlled. For example, some droplets may be split into equal parts or unequal parts, while other droplets are not split.

According to one set of embodiments, a fluidic droplet can be split using an applied electric field. The electric field may be an AC field, a DC field, etc. The fluidic droplet, in this embodiment, may have a greater electrical conductivity than the surrounding liquid, and, in some cases, the fluidic droplet may be neutrally charged. In some embodiments, the droplets produced from the original fluidic droplet are of approximately equal shape and/or size. In certain embodiments, in an applied electric field, electric charge may be urged to migrate from the interior of the fluidic droplet to the surface to be distributed thereon, which may thereby cancel the electric field experienced in the interior of the droplet. In some embodiments, the electric charge on the surface of the fluidic droplet may also experience a force due to the applied electric field, which causes charges having opposite polarities to migrate in opposite directions. The charge migration may, in some cases, cause the drop to be pulled apart into two separate fluidic droplets. The electric field applied to the fluidic droplets may be created, for example, using the techniques described above, such as with a reaction an electric field generator, etc.

As a non-limiting example, in FIG. **1**A, where no electric field is applied, fluidic droplets **215** contained in channel **230** are carried by a surrounding liquid, which flows towards intersection **240**, leading to channels **250** and **255**. In this example, the surrounding liquid flows through channels **250** and **255** at equal flowrates. Thus, at intersection **240**, fluidic droplets **215** do not have a preferred orientation or direction, and move into exit channels **250** and **255** with equal probability due to the surrounding liquid flow. In contrast, in FIG. **1**B, while the surrounding liquid flows in the same fashion as FIG. **1**A, under the influence of an applied electric field of 1.4 V/micrometers, fluidic droplets **215** are split into two droplets at intersection **240**, forming new droplets **216** and **217**. Droplet **216** moves to the left in channel **250**, while droplet **217** moves to the right in channel **255**.

A schematic of this process can be seen in FIG. **5**, where a neutral fluidic droplet **530**, surrounded by a liquid **535** in channel **540**, is subjected to applied electric field **525**, created by electrodes **526** and **527**. Electrode **526** is positioned near channel **542**, while electrode **527** is positioned near channel **544**. Under the influence of electric field **525**, charge separation is induced within fluidic droplet **530**, i.e., such that a positive charge is induced at one end of the droplet, while a negative charge is induced at the other end of the droplet. The droplet may then split into a negatively charged droplet **545** and a positively charged droplet **546**, which then may travel in channels **542** and **544**, respectively. In some cases, one or both of the electric charges on the resulting charged droplets may also be neutralized, as previously described.

Other examples of splitting a fluidic droplet into two droplets are described in International Patent Application Serial No. PCT/US2004/010903, filed Apr. 9, 2004 by Link, et al.; U.S. Provisional Patent Application Ser. No. 60/498,091, filed Aug. 27, 2003, by Link, et. al.; and International Patent Application Serial No. PCT/US03/20542, filed Jun. 30, 2003 by Stone, et al., published as WO 2004/002627 on Jan. 8, 2004, each incorporated herein by reference.

The invention, in yet another aspect, relates to systems and methods for fusing or coalescing two or more fluidic droplets into one droplet. For example, in one set of embodiments, systems and methods are provided that are able to cause two or more droplets (e.g., arising from discontinuous streams of fluid) to fuse or coalesce into one droplet in cases where the two or more droplets ordinarily are unable to fuse or coalesce, for example, due to composition, surface tension, droplet size, the presence or absence of surfactants, etc. In certain microfluidic systems, the surface tension of the droplets, relative to the size of the droplets, may also prevent fusion or coalescence of the droplets from occurring in some cases.

In one embodiment, two fluidic droplets may be given opposite electric charges (i.e., positive and negative charges, not necessarily of the same magnitude), which may increase the electrical interaction of the two droplets such that fusion or coalescence of the droplets can occur due to their opposite electric charges, e.g., using the techniques described herein. For instance, an electric field may be applied to the droplets, the droplets may be passed through a capacitor, a chemical reaction may cause the droplets to become charged, etc. As an example, as is shown schematically in FIG. **13**A, uncharged droplets **651** and **652**, carried by a liquid **654** contained within a microfluidic channel **653**, are brought into contact with each other, but the droplets are not able to fuse or coalesce, for instance, due to their size and/or surface tension. The droplets, in some cases, may not be able to fuse even if a surfactant is applied to lower the surface tension of the droplets. However, if the fluidic droplets are electrically charged with opposite charges (which can be, but are not necessarily of, the same magnitude), the droplets may be able to fuse or coalesce. For instance, in FIG. **13**B, positively charged droplets **655** and negatively charged droplets **656** are directed generally towards each other such that the electrical interaction of the oppositely charged droplets causes the droplets to fuse into fused droplets **657**.

In another embodiment, the fluidic droplets may not necessarily be given opposite electric charges (and, in some cases, may not be given any electric charge), and are fused through the use of dipoles induced in the fluidic droplets that causes the fluidic droplets to coalesce. In the example illustrated in FIG. **13**C, droplets **660** and **661** (which may each independently be electrically charged or neutral), surrounded by liquid **665** in channel **670**, move through the channel such that they are the affected by an applied electric field **675**. Electric field **675** may be an AC field, a DC field,

etc., and may be created, for instance, using electrodes **676** and **677**, as shown here. The induced dipoles in each of the fluidic droplets, as shown in FIG. **13**C, may cause the fluidic droplets to become electrically attracted towards each other due to their local opposite charges, thus causing droplets **660** and **661** to fuse to produce droplet **663**. In FIG. **13**D, droplets **651** and **652** flow together to fuse to form droplet **653**, which flows in a third channel.

It should be noted that, in various embodiments, the two or more droplets allowed to coalesce are not necessarily required to meet "head-on". Any angle of contact, so long as at least some fusion of the droplets initially occurs, is sufficient. As an example, in FIG. **12**H, droplets **73** and **74** each are traveling in substantially the same direction (e.g., at different velocities), and are able to meet and fuse. As another example, in FIG. **12**I, droplets **73** and **74** meet at an angle and fuse. In FIG. **12**J, three fluidic droplets **73**, **74** and **68** meet and fuse to produce droplet **79**.

Other examples of fusing or coalescing fluidic droplets are described in International Patent Application Serial No. PCT/US2004/010903, filed Apr. 9, 2004 by Link, et al., incorporated herein by reference.

Fluidic handling of microcapsules therefore results in further advantages:

(a) Microcapsules can be split into two or more smaller microdroplets allowing the reagents contained therein to be reacted with a series of different molecules in parallel or assayed in multiplicate.

(b) Microcapsules can be fused. This allows molecules to be: (a) diluted, (b) mixed with other molecules, and (c) reactions initiated, terminated or modulated at precisely defined times.

(c) Reagents can be mixed very rapidly (in <2 ms) in microcapsules using chaotic advection, allowing fast kinetic measurements and very high throughput.

(d) Reagents can be mixed in a combinatorial manner. For example, allowing the effect of all possible pairwise combinations of compounds in a library to be tested.

Creating and manipulating microcapsules in microfluidic systems means that:

(a) Stable streams of microcapsules can be formed in microchannels and identified by their relative positions.

(b) If the reactions are accompanied by an optical signal (e.g. a change in fluorescence) a spatially-resolved optical image of the microfluidic network allows time resolved measurements of the reactions in each microcapsules.

(c) Microcapsules can be separated using a microfluidic flow sorter to allow recovery and further analysis or manipulation of the molecules they contain.

Screening/Sorting of Microcapsules

In still another aspect, the invention provides systems and methods for screening or sorting fluidic droplets in a liquid, and in some cases, at relatively high rates. For example, a characteristic of a droplet may be sensed and/or determined in some fashion (e.g., as further described below), then the droplet may be directed towards a particular region of the device, for example, for sorting or screening purposes.

In some embodiments, a characteristic of a fluidic droplet may be sensed and/or determined in some fashion, for example, as described herein (e.g., fluorescence of the fluidic droplet may be determined), and, in response, an electric field may be applied or removed from the fluidic droplet to direct the fluidic droplet to a particular region (e.g. a channel). In some cases, high sorting speeds may be achievable using certain systems and methods of the invention. For instance, at least about 10 droplets per second may be determined and/or sorted in some cases, and in other cases, at least about 20 droplets per second, at least about 30 droplets per second, at least about 100 droplets per second, at least about 200 droplets per second, at least about 300 droplets per second, at least about 500 droplets per second, at least about 750 droplets per second, at least about 1000 droplets per second, at least about 1500 droplets per second, at least about 2000 droplets per second, at least about 3000 droplets per second, at least about 5000 droplets per second, at least about 7500 droplets per second, at least about 10,000 droplets per second, at least about 15,000 droplets per second, at least about 20,000 droplets per second, at least about 30,000 droplets per second, at least about 50,000 droplets per second, at least about 75,000 droplets per second, at least about 100,000 droplets per second, at least about 150,000 droplets per second, at least about 200,000 droplets per second, at least about 300,000 droplets per second, at least about 500,000 droplets per second, at least about 750,000 droplets per second, at least about 1,000,000 droplets per second, at least about 1,500,000 droplets per second, at least about 2,000,000 or more droplets per second, or at least about 3,000,000 or more droplets per second may be determined and/or sorted in such a fashion.

In one set of embodiments, a fluidic droplet may be directed by creating an electric charge (e.g., as previously described) on the droplet, and steering the droplet using an applied electric field, which may be an AC field, a DC field, etc. As an example, in reference to FIGS. **2-4**, an electric field may be selectively applied and removed (or a different electric field may be applied, e.g., a reversed electric field as shown in FIG. **4**A) as needed to direct the fluidic droplet to a particular region. The electric field may be selectively applied and removed as needed, in some embodiments, without substantially altering the flow of the liquid containing the fluidic droplet. For example, a liquid may flow on a substantially steady-state basis (i.e., the average flowrate of the liquid containing the fluidic droplet deviates by less than 20% or less than 15% of the steady-state flow or the expected value of the flow of liquid with respect to time, and in some cases, the average flowrate may deviate less than 10% or less than 5%) or other predetermined basis through a fluidic system of the invention (e.g., through a channel or a microchannel), and fluidic droplets contained within the liquid may be directed to various regions, e.g., using an electric field, without substantially altering the flow of the liquid through the fluidic system. As a particular example, in FIGS. **2**A, **3**A and **4**A, a liquid containing fluidic droplets **15** flows from fluid source **10**, through channel **30** to intersection **40**, and exits through channels **50** and **55**. In FIG. **2**A, fluidic droplets **15** are directed through both channels **50** and **55**, while in FIG. **3**A, fluidic droplets **15** are directed to only channel **55** and, in FIG. **4**A, fluidic droplets **15** are directed to only channel **50**.

In another set of embodiments, a fluidic droplet may be sorted or steered by inducing a dipole in the fluidic droplet (which may be initially charged or uncharged), and sorting or steering the droplet using an applied electric field. The electric field may be an AC field, a DC field, etc. For example, with reference to FIG. **9**A, a channel **540**, containing fluidic droplet **530** and liquid **535**, divides into channel **542** and **544**. Fluidic droplet **530** may have an electric charge, or it may be uncharged. Electrode **526** is positioned near channel **542**, while electrode **527** is positioned near channel **544**. Electrode **528** is positioned near the junction of channels **540**, **542**, and **544**. In FIGS. **9**C and **9**D, a dipole is induced in the fluidic droplet using electrodes **526**, **527**, and/or **528**. In FIG. **9**C, a dipole is induced in

droplet **530** by applying an electric field **525** to the droplet using electrodes **527** and **528**. Due to the strength of the electric field, the droplet is strongly attracted to the right, into channel **544**. Similarly, in FIG. **9**D, a dipole is induced in droplet **530** by applying an electric field **525** to the droplet using electrodes **526** and **528**, causing the droplet to be attracted into channel **542**. Thus, by applying the proper electric field, droplet **530** can be directed to either channel **542** or **544** as desired.

In other embodiments, however, the fluidic droplets may be screened or sorted within a fluidic system of the invention by altering the flow of the liquid containing the droplets. For instance, in one set of embodiments, a fluidic droplet may be steered or sorted by directing the liquid surrounding the fluidic droplet into a first channel, a second channel, etc. As a non-limiting example, with reference to FIG. **10**A, fluidic droplet **570** is surrounded by a liquid **575** in channel **580**. Channel **580** divides into three channels **581**, **582**, and **583**. The flow of liquid **575** can be directed into any of channels **581**, **582**, and **583** as desired, for example, using flow-controlling devices known to those of ordinary skill in the art, for example, valves, pumps, pistons, etc. Thus, in FIG. **10**B, fluidic droplet **570** is directed into channel **581** by directing liquid **575** to flow into channel **581** (indicated by arrows **574**); in FIG. **10**C, fluidic droplet **570** is directed into channel **582** by directing liquid **575** to flow into channel **582** (indicated by arrows **574**); and in FIG. **10**D, fluidic droplet **570** is directed into channel **583** by directing liquid **575** to flow into channel **583** (indicated by arrows **574**).

However, it is preferred that control of the flow of liquids in microfluidic systems is not used to direct the flow of fluidic droplets therein, but that an alternative method is used. Advantageously, therefore, the microcapsules are not sorted by altering the direction of the flow of a carrier fluid in a microfluidic system.

In another set of embodiments, pressure within a fluidic system, for example, within different channels or within different portions of a channel, can be controlled to direct the flow of fluidic droplets. For example, a droplet can be directed toward a channel junction including multiple options for further direction of flow (e.g., directed toward a branch, or fork, in a channel defining optional downstream flow channels). Pressure within one or more of the optional downstream flow channels can be controlled to direct the droplet selectively into one of the channels, and changes in pressure can be effected on the order of the time required for successive droplets to reach the junction, such that the downstream flow path of each successive droplet can be independently controlled. In one arrangement, the expansion and/or contraction of liquid reservoirs may be used to steer or sort a fluidic droplet into a channel, e.g., by causing directed movement of the liquid containing the fluidic droplet. The liquid reservoirs may be positioned such that, when activated, the movement of liquid caused by the activated reservoirs causes the liquid to flow in a preferred direction, carrying the fluidic droplet in that preferred direction. For instance, the expansion of a liquid reservoir may cause a flow of liquid towards the reservoir, while the contraction of a liquid reservoir may cause a flow of liquid away from the reservoir. In some cases, the expansion and/or contraction of the liquid reservoir may be combined with other flow-controlling devices and methods, e.g., as described herein. Non-limiting examples of devices able to cause the expansion and/or contraction of a liquid reservoir include pistons and piezoelectric components. In some cases, piezoelectric components may be particularly useful due to their relatively rapid response times, e.g., in response to an electrical signal.

As a non-limiting example, in FIG. **11**A, fluidic droplet **600** is surrounded by a liquid **605** in channel **610**. Channel **610** divides into channels **611**, **612**. Positioned in fluidic communication with channels **611** and **612** are liquid reservoirs **617** and **618**, which may be expanded and/or contracted, for instance, by piezoelectric components **615** and **616**, by a piston (not shown), etc. In FIG. **11**B, liquid reservoir **617** has been expanded, while liquid reservoir **618** has been contracted. The effect of the expansion/contractions of the reservoirs is to cause a net flow of liquid towards channel **611**, as indicated by arrows **603**. Thus, fluidic droplet **600**, upon reaching the junction between the channels, is directed to channel **611** by the movement of liquid **605**. The reverse situation is shown in FIG. **11**C, where liquid reservoir **617** has contracted while liquid reservoir **618** has been expanded. A net flow of liquid occurs towards channel **612** (indicated by arrows **603**), causing fluidic droplet **600** to move into channel **612**. It should be noted, however, that reservoirs **617** and **618** do not both need to be activated to direct fluidic droplet **600** into channels **611** or **612**. For example, in one embodiment, fluidic droplet **600** may be directed to channel **611** by the expansion of liquid reservoir **617** (without any alteration of reservoir **618**), while in another embodiment, fluidic droplet **600** may be directed to channel **611** by the contraction of liquid reservoir **618** (without any alteration of reservoir **617**). In some cases, more than two liquid reservoirs may be used.

In some embodiments, the fluidic droplets may be sorted into more than two channels. Non-limiting examples of embodiments of the invention having multiple regions within a fluidic system for the delivery of droplets are shown in FIGS. **6**A and **6**B. Other arrangements are shown in FIGS. **10**A-**10**D. In FIG. **6**A, charged droplets **315** in channel **330** may be directed as desired to any one of exit channels **350**, **352**, **354**, or **356**, by applying electric fields to control the movement of the droplets at intersections **340**, **341**, and **342**, using electrodes **321**/**322**, **323**/**324**, and **325**/**326**, respectively. In FIG. **6**A, droplets **315** are directed to channel **354** using applied electric fields **300** and **301**, using 5 principles similar to those discussed above. Similarly, in FIG. **6**B, charged droplets **415** in channel **430** can be directed to any one of exit channels **450**, **452**, **454**, **456**, or **458**, by applying electric fields to control the movement of the droplets at intersections **440**, **441**, **442**, and **443**, using electrodes **421**/**422**, **423**/**424**, **425**/**426**, and **427**/**428**, respectively. In this figure, droplets **415** are directed to channel **454**; of course, the charged droplets may be directed to any other exit channel as desired.

In another example, in apparatus **5**, as schematically illustrated in FIG. **2**A, fluidic droplets **15** created by fluid source **10** are positively charged due to an applied electric field created using electric field generator **20**, which comprises two electrodes **22**, **24**. Fluidic droplets **15** are directed through channel **30** by a liquid containing the droplets, and are directed towards intersection **40**. At intersection **40**, the fluidic droplets do not have a preferred orientation or direction, and move into exit channels **50** and **55** with equal probability (in this embodiment, liquid drains through both exit channels **50** and **55** at substantially equal rates). Similarly, fluidic droplets **115** created by fluid source **110** are negatively charged due to an applied electric field created using electric field generator **120**, which comprises electrodes **122** and **124**. After traveling through channel **130** towards intersection **140**, the fluidic droplets do not have a

preferred orientation or direction, and move into exit channels **150** and **155** with equal probability, as the liquid exits through exit channels **150** and **155** at substantially equal rates. A representative photomicrograph of intersection **140** is shown in FIG. **2**B.

In the schematic diagram of FIG. **3**A, an electric field **100** of 1.4 V/micrometer has been applied to apparatus **5** of FIG. **2**A, in a direction towards the right of apparatus **5**. Positively-charged fluidic droplets **15** in channel **30**, upon reaching intersection **40**, are directed to the right in channel **55** due to the applied electric field **100**, while the liquid containing the droplets continues to exit through exit channels **50** and **55** at substantially equal rates. Similarly, negatively-charged fluidic droplets **115** in channel **130**, upon reaching intersection **140**, are directed to the left in channel **150** due to the applied electric field **100**, while the liquid fluid continues to exit the device through exit channels **150** and **155** at substantially equal rates. Thus, electric field **100** can be used to direct fluidic droplets into particular channels as desired. A representative photomicrograph of intersection **140** is shown in FIG. **3**B.

FIG. **4**A is a schematic diagram of apparatus **5** of FIG. **2**A, also with an applied electric field **100** of 1.4 V/micrometer, but in the opposite direction (i.e., −1.4 V/micrometer). In this figure, positively-charged fluidic droplets **15** in channel **30**, upon reaching intersection **40**, are directed to the left into channel **50** due to the applied electric field **100**, while negatively-charged fluidic droplets **115** in channel **130**, upon reaching intersection **140**, are directed to the right into channel **155** due to applied electric field **100**. The liquid containing the droplets exits through exit channels **50** and **55**, and **150** and **155**, at substantially equal rates. A representative photomicrograph of intersection **140** is shown in FIG. **4**B.

In some embodiments of the invention, a fluidic droplet may be sorted and/or split into two or more separate droplets, for example, depending on the particular application. Any of the above-described techniques may be used to spilt and/or sort droplets. As a non-limiting example, by applying (or removing) a first electric field to a device (or a portion thereof), a fluidic droplet may be directed to a first region or channel; by applying (or removing) a second electric field to the device (or a portion thereof), the droplet may be directed to a second region or channel; by applying a third electric field to the device (or a portion thereof), the droplet may be directed to a third region or channel; etc., where the electric fields may differ in some way, for example, in intensity, direction, frequency, duration, etc. In a series of droplets, each droplet may be independently sorted and/or split; for example, some droplets may be directed to one location or another, while other droplets may be split into multiple droplets directed to two or more locations.

As one particular example, in FIG. **8**A, fluidic droplet **550**, surrounding liquid **555** in channel **560** may be directed to channel **556**, channel **557**, or be split in some fashion between channels **562** and **564**. In FIG. **8**B, by directing surrounding liquid **555** towards channel **562**, fluidic droplet **550** may be directed towards the left into channel **562**; in FIG. **8**C, by directing surrounding liquid **555** towards channel **564**, fluidic droplet **550** may be directed towards the right into channel **564**, In FIG. **8**D, an electric field may be applied, in combination with control of the flow of liquid **555** surrounding fluidic droplet **550**, that causes the droplet to impact junction **561**, which may cause the droplet to split into two separate fluidic droplets **565**, **566**. Fluidic droplet **565** is directed to channel **562**, while fluidic droplet **566** is directed to channel **566**. A high degree of control of the applied electric field may be achieved to control droplet formation; thus, for example, after fluidic droplet **565** has been split into droplets **565** and **566**, droplets **565** and **566** may be of substantially equal size, or either of droplets **565** and **566** may be larger, e.g., as is shown in FIGS. **8**E and **8**F, respectively.

As another, example, in FIG. **9**A, channel **540**, carrying fluidic droplet **530** and liquid **535**, divides into channel **542** and **544**. Fluidic droplet **530** may be electrically charged, or it may uncharged. Electrode **526** is positioned near channel **542**, while electrode **527** is positioned near channel **544**. Electrode **528** is positioned near the junction of channels **540**, **542**, and **544**. When fluidic droplet **530** reaches the junction, it may be subjected to an electric field, and/or directed to a channel or other region, for example, by directing the surrounding liquid into the channel. As shown in FIG. **9**B, fluidic droplet **530** may be split into two separate droplets **565** and **566** by applying an electric field **525** to the droplet using electrodes **526** and **527**. In FIG. **9**C, a dipole can be induced in droplet **530** by applying an electric field **525** to the droplet using electrodes **527** and **528**. Due to the strength of the applied electric field, the droplet may be strongly attracted to the right, into channel **544**. Similarly, in FIG. **9**D, a dipole may be induced in droplet **530** by applying an electric field **525** to the droplet using electrodes **526** and **528**, causing the droplet to be attracted into channel **542**. By controlling which electrodes are used to induce an electric field across droplet **530**, and/or the strength of the applied electric field, one or more fluidic droplets within channel **540** may be sorted and/or split into two droplets, and each droplet may independently be sorted and/or split.

Microcapsules can be optically tagged by, for example, incorporating fluorochromes. In a preferred configuration, the microcapsules are optically tagged by incorporating quantum dots: quantum dots of 6 colours at 10 concentrations would allow the encoding of 10^6 microcapsules (Han et al., 2001). Microcapsules flowing in an ordered sequence in a microfluidic channel can be encoded (wholly or partially) by their sequence in the stream of microcapsules (positional encoding).

By means of the invention, enzymes involved in the preparation of a compound may be optimised by selection for optimal activity. The procedure involves the preparation of variants of the polypeptide to be screened, which equate to a library of polypeptides as refereed to herein. The variants may be prepared in the same manner as the libraries discussed elsewhere herein.

(B) Selection Procedures

The system can be configured to select for RNA, DNA or protein gene product molecules with catalytic, regulatory or binding activity.

(i) Selection for Binding

In the case of selection for a gene product with affinity for a specific ligand the genetic element may be linked to the gene product in the microcapsule via the ligand. Only gene products with affinity for the ligand will therefore bind to the genetic element and only those genetic elements with gene product bound via the ligand will acquire the changed optical properties which enable them to be retained in the selection step. In this embodiment, the genetic element will thus comprise a nucleic acid encoding the gene product linked to a ligand for the gene product.

The change in optical properties of the genetic element after binding of the gene product to the ligand may be induced in a variety of ways, including:

(1) the gene product itself may have distinctive optical properties, for example, it is fluorescent (e.g. green fluorescent protein, (Lorenz et al., 1991)).

(2) the optical properties of the gene product may be modified on binding to the ligand, for example, the fluorescence of the gene product is quenched or enhanced on binding (Guixe et al., 1998; Qi and Grabowski, 1998)

(3) the optical properties of the ligand may be modified on binding of the gene product, for example, the fluorescence of the ligand is quenched or enhanced on binding (Voss, 1993; Masui and Kuramitsu, 1998).

(4) the optical properties of both ligand and gene product are modified on binding, for example, there can be a fluorescence resonance energy transfer (FRET) from ligand to gene product (or vice versa) resulting in emmission at the "acceptor" emmission wavelength when excitation is at the "donor" absorption wavelength (Heim & Tsien, 1996; Mahaj an et al., 1998; Miyawaki et al., 1997).

In this embodiment, it is not necessary for binding of the gene product to the genetic element via the ligand to directly induce a change in optical properties. All the gene products to be selected can contain a putative binding domain, which is to be selected for, and a common feature—a tag. The genetic element in each microcapsule is physically linked to the ligand. If the gene product produced from the genetic element has affinity for the ligand, it will bind to it and become physically linked to the same genetic element that encoded it, resulting in the genetic element being 'tagged'. At the end of the reaction, all of the microcapsules are combined, and all genetic elements and gene products pooled together in one environment. Genetic elements encoding gene products exhibiting the desired binding can be selected by adding reagents which specifically bind to, or react specifically with, the "tag" and thereby induce a change in the optical properties of the genetic element allowing there sorting. For example, a fluorescently-labelled anti-"tag" antibody can be used, or an anti-"tag" antibody followed by a second fluorescently labelled antibody which binds the first.

In an alternative embodiment, genetic elements may be sorted on the basis that the gene product, which binds to the ligand, merely hides the ligand from, for example, further binding partners which would otherwise modify the optical properties of the genetic element. In this case genetic elements with unmodified optical properties would be selected.

In an alternative embodiment, the invention provides a method according to the first aspect of the invention, wherein in step (b) the gene products bind to genetic elements encoding them. The gene products together with the attached genetic elements are then sorted as a result of binding of a ligand to gene products having the desired binding activity. For example, all gene products can contain an invariant region which binds covalently or non-covalently to the genetic element, and a second region which is diversified so as to generate the desired binding activity.

In an alternative embodiment, the ligand for the gene product is itself encoded by the genetic element and binds to the genetic element. Stated otherwise, the genetic element encodes two (or indeed more) gene products, at least one of which binds to the genetic element, and which can potentially bind each other. Only when the gene products interact in a microcapsule is the genetic element modified in a way that ultimately results in a change in a change in its optical properties that enables it to be sorted. This embodiment, for example, is used to search gene libraries for pairs of genes encoding pairs of proteins which bind each other.

Fluorescence may be enhanced by the use of Tyramide Signal Amplification (TSA™) amplification to make the genetic elements fluorescent. This involves peroxidase (linked to another protein) binding to the genetic elements and catalysing the conversion of fluorescein-tyramine in to a free radical form which then reacts (locally) with the genetic elements. Methods for performing TSA are known in the art, and kits are available commercially from NEN.

TSA may be configured such that it results in a direct increase in the fluorescence of the genetic element, or such that a ligand is attached to the genetic element which is bound by a second fluorescent molecule, or a sequence of molecules, one or more of which is fluorescent.

(ii) Selection for Catalysis

When selection is for catalysis, the genetic element in each microcapsule may comprise the substrate of the reaction. If the genetic element encodes a gene product capable of acting as a catalyst, the gene product will catalyse the conversion of the substrate into the product. Therefore, at the end of the reaction the genetic element is physically linked to the product of the catalysed reaction.

It may also be desirable, in some cases, for the substrate not to be a component of the genetic element. In this case the substrate would contain an inactive "tag" that requires a further step to activate it such as photoactivation (e.g. of a "caged" biotin analogue, (Sundberg et al., 1995; Pirrung and Huang, 1996)). The catalyst to be selected then converts the substrate to product. The "tag" is then activated and the "tagged" substrate and/or product bound by a tag-binding molecule (e.g. avidin or streptavidin) complexed with the nucleic acid. The ratio of substrate to product attached to the nucleic acid via the "tag" will therefore reflect the ratio of the substrate and product in solution.

The optical properties of genetic elements with product attached and which encode gene products with the desired catalytic activity can be modified by either:

(1) the product-genetic element complex having characteristic optical properties not found in the substrate-genetic element complex, due to, for example;

(a) the substrate and product having different optical properties (many fluorogenic enzyme substrates are available commercially (see for example Haugland, 1996) including substrates for glycosidases, phosphatases, peptidases and proteases (Craig et al., 1995; Huang et al., 1992; Brynes et al., 1982; Jones et al., 1997; Matayoshi et al., 1990; Wang et al., 1990)), or

(b) the substrate and product having similar optical properties, but only the product, and not the substrate binds to, or reacts with, the genetic element;

(2) adding reagents which specifically bind to, or react with, the product and which thereby induce a change in the optical properties of the genetic elements allowing their sorting (these reagents can be added before or after breaking the microcapsules and pooling the genetic elements). The reagents;

(a) bind specifically to, or react specifically with, the product, and not the substrate, if both substrate and product are attached to the genetic element, or

(b) optionally bind both substrate and product if only the product, and not the substrate binds to, or reacts with, the genetic element.

The pooled genetic elements encoding catalytic molecules can then be enriched by selecting for the genetic elements with modified optical properties.

An alternative is to couple the nucleic acid to a product-specific antibody (or other product-specific molecule). In this scenario, the substrate (or one of the substrates) is present in each microcapsule unlinked to the genetic element, but has a molecular "tag" (for example biotin, DIG or DNP or a fluorescent group). When the catalyst to be selected converts the substrate to product, the product retains the "tag" and is then captured in the microcapsule by the product-specific antibody. In this way the genetic element only becomes associated with the "tag" when it encodes or produces an enzyme capable of converting substrate to product. When all reactions are stopped and the microcapsules are combined, the genetic elements encoding active enzymes will be "tagged" and may already have changed optical properties, for example, if the "tag" was a fluorescent group. Alternatively, a change in optical properties of "tagged" genes can be induced by adding a fluorescently labelled ligand which binds the "tag" (for example fluorescently-labelled avidin/streptavidin, an anti-"tag" antibody which is fluorescent, or a non-fluorescent anti-"tag" antibody which can be detected by a second fluorescently-labelled antibody).

Alternatively, selection may be performed indirectly by coupling a first reaction to subsequent reactions that takes place in the same microcapsule. There are two general ways in which this may be performed. In a first embodiment, the product of the first reaction is reacted with, or bound by, a molecule which does not react with the substrate of the first reaction. A second, coupled reaction will only proceed in the presence of the product of the first reaction. A genetic element encoding a gene product with a desired activity can then be purified by using the properties of the product of the second reaction to induce a change in the optical properties of the genetic element as above.

Alternatively, the product of the reaction being selected may be the substrate or cofactor for a second enzyme-catalysed reaction. The enzyme to catalyse the second reaction can either be translated in situ in the microcapsules or incorporated in the reaction mixture prior to microencapsulation. Only when the first reaction proceeds will the coupled enzyme generate a product which can be used to induce a change in the optical properties of the genetic element as above.

This concept of coupling can be elaborated to incorporate multiple enzymes, each using as a substrate the product of the previous reaction. This allows for selection of enzymes that will not react with an immobilised substrate. It can also be designed to give increased sensitivity by signal amplification if a product of one reaction is a catalyst or a cofactor for a second reaction or series of reactions leading to a selectable product (for example, see Johannsson and Bates, 1988; Johannsson, 1991). Furthermore an enzyme cascade system can be based on the production of an activator for an enzyme or the destruction of an enzyme inhibitor (see Mize et al., 1989). Coupling also has the advantage that a common selection system can be used for a whole group of enzymes which generate the same product and allows for the selection of complicated chemical transformations that cannot be performed in a single step.

Such a method of coupling thus enables the evolution of novel "metabolic pathways" in vitro in a stepwise fashion, selecting and improving first one step and then the next. The selection strategy is based on the final product of the pathway, so that all earlier steps can be evolved independently or sequentially without setting up a new selection system for each step of the reaction.

Expressed in an alternative manner, there is provided a method of isolating one or more genetic elements encoding a gene product having a desired catalytic activity, comprising the steps of:

(1) expressing genetic elements to give their respective gene products;

(2) allowing the gene products to catalyse conversion of a substrate to a product, which may or may not be directly selectable, in accordance with the desired activity;

(3) optionally coupling the first reaction to one or more subsequent reactions, each reaction being modulated by the product of the previous reactions, and leading to the creation of a final, selectable product;

(4) linking the selectable product of catalysis to the genetic elements by either:

a) coupling a substrate to the genetic elements in such a way that the product remains associated with the genetic elements, or

b) reacting or binding the selectable product to the genetic elements by way of a suitable molecular "tag" attached to the substrate which remains on the product,

or

c) coupling the selectable product (but not the substrate) to the genetic elements by means of a product-specific reaction or interaction with the product; and

(5) selecting the product of catalysis, together with the genetic element to which it is bound, either by means of its characteristic optical properties, or by adding reagents which specifically bind to, or react specifically with, the product and which thereby induce a change in the optical properties of the genetic elements wherein steps (1) to

(6) each genetic element and respective gene product is contained within a microcapsule.

(iii) Selecting for Enzyme Substrate Specificity/Selectivity

Genetic elements encoding enzymes with substrate specificity or selectivity can be specifically enriched by carrying out a positive selection for reaction with one substrate and a negative selection for reaction with another substrate. Such combined positive and negative selection pressure should be of great importance in isolating regio-selective and stereo-selective enzymes (for example, enzymes that can distinguish between two enantiomers of the same substrate). For example, two substrates (e.g. two different enantiomers) are each labelled with different tags (e.g. two different fluorophores) such that the tags become attached to the genetic element by the enzyme-catalysed reaction. If the two tags confer different optical properties on the genetic element the substrate specificity of the enzyme can be determined from the optical properties of the genetic element and those genetic elements encoding gene products with the wrong (or no) specificity rejected. Tags conferring no change in optical activity can also be used if tag-specific ligands with different optical properties are added (e.g. tag-specific antibodies labelled with different fluorophores).

(iv) Selection for Regulation

A similar system can be used to select for regulatory properties of enzymes.

In the case of selection for a regulator molecule which acts as an activator or inhibitor of a biochemical process, the components of the biochemical process can either be translated in situ in each microcapsule or can be incorporated in the reaction mixture prior to microencapsulation. If the genetic element being selected is to encode an activator, selection can be performed for the product of the regulated

reaction, as described above in connection with catalysis. If an inhibitor is desired, selection can be for a chemical property specific to the substrate of the regulated reaction.

There is therefore provided a method of sorting one or more genetic elements coding for a gene product exhibiting a desired regulatory activity, comprising the steps of:

(1) expressing genetic elements to give their respective gene products;

(2) allowing the gene products to activate or inhibit a biochemical reaction, or sequence of coupled reactions, in accordance with the desired activity, in such a way as to allow the generation or survival of a selectable molecule;

(3) linking the selectable molecule to the genetic elements either by

a) having the selectable molecule, or the substrate from which it derives, attached to the genetic elements, or

b) reacting or binding the selectable product to the genetic elements, by way of a suitable molecular "tag" attached to the substrate which remains on the product,

or

c) coupling the product of catalysis (but not the substrate) to the genetic elements, by means of a product-specific reaction or interaction with the product;

(4) selecting the selectable product, together with the genetic element to which it is bound, either by means of its characteristic optical properties, or by adding reagents which specifically bind to, or react specifically with, the product and which thereby induce a change in the optical properties of the genetic elements wherein steps (1) to (3) each genetic element and respective gene product is contained within a microcapsule.

(v) Selection for Optical Properties of the Gene Product

It is possible to select for inherent optical properties of gene products if, in the microcapsules, the gene product binds back to the genetic element, for example through a common element of the gene product which binds to a ligand which is part of the genetic element. After pooling the genetic elements they can then be sorted using the optical properties of the bound gene products. This embodiment can be used, for example, to select variants of green fluorescent protein (GFP) (Cormack et al., 1996; Delagrave et al., 1995; Ehrig et al., 1995), with improved fluorescence and/or novel absorption and emission spectra.

(vi) Screening Using Cells

In the current drug discovery paradigm, validated recombinant targets form the basis of in vitro high-throughput screening (HTS) assays. Isolated genetic constructs or polypeptides cannot, however, be regarded as representative of complex biological systems; hence, cell-based systems can provide greater confidence in compound activity in an intact biological system. A wide range of cell-based assays for drug leads are known to those skilled in the art. Cells can be compartmentalised in microcapsules, such as the aqueous microdroplets of a water-in-oil emulsion (Ghadessy, 2001). The effect of a compound(s) on a target can be determined by compartmentalising a cell (or cells) in a microcapsule together with a genetic element(s) and using an appropriate cell-based assay to identify those compartments containing genetic elements with the desired effect on the cell(s). The use of water-in-fluorocarbon emulsions may be particularly advantageous: the high gas dissolving capacity of fluorocarbons can support the exchange of respiratory gases and has been reported to be beneficial to cell culture systems (Lowe, 2002).

(vii) Flow Analysis and Sorting

In a preferred embodiment of the invention the microcapsules will be analysed and, optionally, sorted by flow cytometry. Many formats of microcapsule can be analysed and, optionally, sorted directly using flow cytometry.

In a highly preferred embodiment, microfluidic devices for flow analysis and, optionally, flow sorting (Fu, 2002) of microcapsules will be used. Such a sorting device can be integrated directly on the microfluidic device, and can use electronic means to sort the microcapsules and/or genetic elements. Optical detection, also integrated directly on the microfluidic device, can be used to screen the microcapsules to trigger the sorting. Other means of control of the microcapsules, in addition to charge, can also be incorporated onto the microfluidic device.

A variety of optical properties can be used for analysis and to trigger sorting, including light scattering (Kerker, 1983) and fluorescence polarisation (Rolland et al., 1985). In a highly preferred embodiment the difference in optical properties of the microcapsules or microbeads will be a difference in fluorescence and, if required, the microcapsules or microbeads will be sorted using a microfluidic or conventional fluorescence activated cell sorter (Norman, 1980; Mackenzie and Pinder, 1986), or similar device. Flow cytometry has a series of advantages:

(1) fluorescence activated cell sorting equipment from established manufacturers (e.g. Becton-Dickinson, Coulter, Cytomation) allows the analysis and sorting at up to 100,000 microcapsules or microbeads per second.

(2) the fluorescence signal from each microcapsule or microbead corresponds tightly to the number of fluorescent molecules present. As little as few hundred fluorescent molecules per microcapsules or microbeads can be quantitatively detected;

(3) the wide dynamic range of the fluorescence detectors (typically 4 log units) allows easy setting of the stringency of the sorting procedure, thus allowing the recovery of the optimal number microcapsules or microbeads from the starting pool (the gates can be set to separate microcapsules or microbeads with small differences in fluorescence or to only separate out microcapsules or microbeads with large differences in fluorescence, dependant on the selection being performed);

(4) fluorescence-activated cell sorting equipment can perform simultaneous excitation and detection at multiple wavelengths (Shapiro, 1995) allowing positive and negative selections to be performed simultaneously by monitoring the labelling of the microcapsules or microbeads with two to thirteen (or more) fluorescent markers, for example, if substrates for two alternative targets are labelled with different fluorescent tags the microcapsules or microbeads can labelled with different fluorophores dependent on the target regulated.

If the microcapsules or microbeads are optically tagged, flow cytometry can also be used to identify the genetic element or genetic elements in the microcapsule or coated on the microbeads (see below). Optical tagging can also be used to identify the concentration of reagents in the microcapsule (if more than one concentration is used in a single experiment) or the number of compound molecules coated on a microbead (if more than one coating density is used in a single experiment). Furthermore, optical tagging can be used to identify the target in a microcapsule (if more than one target is used in a single experiment). This analysis can be performed simultaneously with measuring activity, after sorting of microcapsules containing microbeads, or after sorting of the microbeads.

(viii) Microcapsule Identification and Sorting

The invention provides for the identification and, optionally, the sorting of intact microcapsules where this is enabled by the sorting techniques being employed. Microcapsules may be identified and, optionally, sorted as such when the change induced by the desired genetic element either occurs or manifests itself at the surface of the microcapsule or is detectable from outside the microcapsule. The change may be caused by the direct action of the gene product, or indirect, in which a series of reactions, one or more of which involve the gene product having the desired activity leads to the change. For example, where the microcapsule is a membranous microcapsule, the microcapsule may be so configured that a component or components of the biochemical system comprising the target are displayed at its surface and thus accessible to reagents which can detect changes in the biochemical system regulated by the gene product within the microcapsule.

In a preferred aspect of the invention, however, microcapsule identification and, optionally, sorting relies on a change in the optical properties of the microcapsule, for example absorption or emission characteristics thereof, for example alteration in the optical properties of the microcapsule resulting from a reaction leading to changes in absorbance, luminescence, phosphorescence or fluorescence associated with the microcapsule. All such properties are included in the term "optical". In such a case, microcapsules can be identified and, optionally, sorted by luminescence, fluorescence or phosphorescence activated sorting. In a highly preferred embodiment, flow cytometry is employed to analyse and, optionally, sort microcapsules containing gene products having a desired activity which result in the production of a fluorescent molecule in the microcapsule.

The methods of the current invention allow reagents to be mixed rapidly (in <2 ms), hence a spatially-resolved optical image of microcapsules in microfluidic network allows time resolved measurements of the reactions in each microcapsule. Microcapsules can, optionally, be separated using a microfluidic flow sorter to allow recovery and further analysis or manipulation of the molecules they contain. Advantageously, the flow sorter would be an electronic flow sorting device. Such a sorting device can be integrated directly on the microfluidic device, and can use electronic means to sort the microcapsules. Optical detection, also integrated directly on the microfluidic device, can be used to screen the microcapsules to trigger the sorting. Other means of control of the microcapsules, in addition to charge, can also be incorporated onto the microfluidic device.

In an alternative embodiment, a change in microcapsule fluorescence, when identified, is used to trigger the modification of the microbead within the compartment. In a preferred aspect of the invention, microcapsule identification relies on a change in the optical properties of the microcapsule resulting from a reaction leading to luminescence, phosphorescence or fluorescence within the microcapsule. Modification of the microbead within the microcapsules would be triggered by identification of luminescence, phosphorescence or fluorescence. For example, identification of luminescence, phosphorescence or fluorescence can trigger bombardment of the compartment with photons (or other particles or waves) which leads to modification of the microbead or molecules attached to it. A similar procedure has been described previously for the rapid sorting of cells (Keij et al., 1994). Modification of the microbead may result, for example, from coupling a molecular "tag", caged by a photolabile protecting group to the microbeads: bombardment with photons of an appropriate wavelength leads to the removal of the cage. Afterwards, all microcapsules are combined and the microbeads pooled together in one environment. Genetic elements exhibiting the desired activity can be selected by affinity purification using a molecule that specifically binds to, or reacts specifically with, the "tag".

(ix) Flow Sorting of Genetic Elements

In a preferred embodiment of the invention the genetic elements will be sorted by flow cytometry. A variety of optical properties can be used to trigger sorting, including light scattering (Kerker, 1983) and fluorescence polarisation (Rolland et al., 1985). In a highly preferred embodiment the difference in optical properties of the genetic elements will be a difference in fluorescence and the genetic elements will be sorted using a fluorescence activated cell sorter (Norman, 1980; Mackenzie and Pinder, 1986), or similar device. Such a sorting device can be integrated directly on the microfluidic device, and can use electronic means to sort the genetic elements. Optical detection, also integrated directly on the microfluidic device, can be used to screen the genetic elements to trigger the sorting. Other means of control of the genetic elements, in addition to charge, can also be incorporated onto the microfluidic device. In an especially preferred embodiment the genetic element comprises of a nonfluorescent nonmagnetic (e.g. polystyrene) or paramagnetic microbead (see Fornusek and Vetvicka, 1986), optimally 0.6 to 1.0 μm diameter, to which are attached both the gene and the groups involved in generating a fluorescent signal:

(1) commercially available fluorescence activated cell sorting equipment from established manufacturers (e.g. Becton-Dickinson, Coulter) allows the sorting of up to 10^8 genetic elements (events) per hour;

(2) the fluorescence signal from each bead corresponds tightly to the number of fluorescent molecules attached to the bead. At present as little as few hundred fluorescent molecules per particle can be quantitatively detected;

(3) the wide dynamic range of the fluorescence detectors (typically 4 log units) allows easy setting of the stringency of the sorting procedure, thus allowing the recovery of the optimal number of genetic elements from the starting pool (the gates can be set to separate beads with small differences in fluorescence or to only separate out beads with large differences in fluorescence, dependant on the selection being performed;

(4) commercially available fluorescence-activated cell sorting equipment can perform simultaneous excitation at up to two different wavelengths and detect fluorescence at up to four different wavelengths (Shapiro, 1983) allowing positive and negative selections to be performed simultaneously by monitoring the labelling of the genetic element with two (or more) different fluorescent markers, for example, if two alternative substrates for an enzyme (e.g. two different enantiomers) are labelled with different fluorescent tags the genetic element can labelled with different fluorophores dependent on the substrate used and only genes encoding enzymes with enantioselectivity selected.

(5) highly uniform derivatised and non-derivatised nonmagnetic and paramagnetic microparticles (beads) are commercially available from many sources (e.g. Sigma, and Molecular Probes) (Fornusek and Vetvicka, 1986).

(x) Multi-Step Procedure

It will be also be appreciated that according to the present invention, it is not necessary for all the processes of tran-

scription/replication and/or translation, and selection to proceed in one single step, with all reactions taking place in one microcapsule. The selection procedure may comprise two or more steps. First, transcription/replication and/or translation of each genetic element of a genetic element library may take place in a first microcapsule. Each gene product is then linked to the genetic element which encoded it (which resides in the same microcapsule), for example via a gene product-specific ligand such as an antibody. The microcapsules are then broken, and the genetic elements attached to their respective gene products optionally purified. Alternatively, genetic elements can be attached to their respective gene products using methods which do not rely on encapsulation. For example phage display (Smith, G. P., 1985), polysome display (Mattheakkis et al., 1994), RNA-peptide fusion (Roberts and Szostak, 1997) or lac repressor peptide fusion (Cull, et al., 1992).

In the second step of the procedure, each purified genetic element attached to its gene product is put into a second microcapsule containing components of the reaction to be selected. This reaction is then initiated. After completion of the reactions, the microcapsules are again broken and the modified genetic elements are selected. In the case of complicated multistep reactions in which many individual components and reaction steps are involved, one or more intervening steps may be performed between the initial step of creation and linking of gene product to genetic element, and the final step of generating the selectable change in the genetic element.

If necessary, release of the gene product from the genetic element within a secondary microcapsule can be achieved in a variety of ways, including by specific competition by a low-molecular weight product for the binding site or cleavage of a linker region joining the binding domain of the gene product from the catalytic domain either enzymatically (using specific proteases) or autocatalytically (using an integrin domain).

(xi) Selection by Activation of Reporter Gene Expression In Situ

The system can be configured such that the desired binding, catalytic or regulatory activity encoded by a genetic element leads, directly or indirectly to the activation of expression of a "reporter gene" that is present in all microcapsules. Only gene products with the desired activity activate expression of the reporter gene. The activity resulting from reporter gene expression allows the selection of the genetic element (or of the compartment containing it) by any of the methods described herein.

For example, activation of the reporter gene may be the result of a binding activity of the gene product in a manner analogous to the "two hybrid system" (Fields and Song; 1989). Activation can also result from the product of a reaction catalysed by a desirable gene product. For example, the reaction product can be a transcriptional inducer of the reporter gene. For example arabinose may be used to induce transcription from the araBAD promoter. The activity of the desirable gene product can also result in the modification of a transcription factor, resulting in expression of the reporter gene. For example, if the desired gene product is a kinase or phosphatase the phosphorylation or dephosphorylation of a transcription factor may lead to activation of reporter gene expression.

(xii) Amplification

According to a further aspect of the present invention the method comprises the further step of amplifying the genetic elements. Selective amplification may be used as a means to enrich for genetic elements encoding the desired gene product.

In all the above configurations, genetic material comprised in the genetic elements may be amplified and the process repeated in iterative steps. Amplification may be by the polymerase chain reaction (Saiki et al., 1988) or by using one of a variety of other gene amplification techniques including; Qb replicase amplification (Cahill, Foster and Mahan, 1991; Chetverin and Spirin, 1995; Katanaev, Kurnasov and Spirin, 1995); the ligase chain reaction (LCR) (Landegren et al., 1988; Barany, 1991); the self-sustained sequence replication system (Fahy, Kwoh and Gingeras, 1991) and strand displacement amplification (Walker et al., 1992). Advantageously, the amplification procedure can be performed in a microfluidic device.

(C) Rapid Mixing of Reagents in Microcapsules

Advantageously, after fusion of microcpasules, the reagents contained in the fused microcapsule can be mixed rapidly using chaotic advection by passing the droplets through channels that disrupt the laminar flow lines of the fluid within the droplets, their contents can be rapidly mixed, fully initiating any chemical reactions.

(D) Sensing Microcapsule Characteristics

In certain aspects of the invention, sensors are provided that can sense and/or determine one or more characteristics of the fluidic droplets, and/or a characteristic of a portion of the fluidic system containing the fluidic droplet (e.g., the liquid surrounding the fluidic droplet) in such a manner as to allow the determination of one or more characteristics of the fluidic droplets. Characteristics determinable with respect to the droplet and usable in the invention can be identified by those of ordinary skill in the art. Non-limiting examples of such characteristics include fluorescence, spectroscopy (e.g., optical, infrared, ultraviolet, etc.), radioactivity, mass, volume, density, temperature, viscosity, pH, concentration of a substance, such as a biological substance (e.g., a protein, a nucleic acid, etc.), or the like.

In some cases, the sensor may be connected to a processor, which in turn, causes an operation to be performed on the fluidic droplet, for example, by sorting the droplet, adding or removing electric charge from the droplet, fusing the droplet with another droplet, splitting the droplet, causing mixing to occur within the droplet, etc., for example, as previously described. For instance, in response to a sensor measurement of a fluidic droplet, a processor may cause the fluidic droplet to be split, merged with a second fluidic droplet, sorted etc.

One or more sensors and/or processors may be positioned to be in sensing communication with the fluidic droplet. "Sensing communication," as used herein, means that the sensor may be positioned anywhere such that the fluidic droplet within the fluidic system (e.g., within a channel), and/or a portion of the fluidic system containing the fluidic droplet may be sensed and/or determined in some fashion. For example, the sensor may be in sensing communication with the fluidic droplet and/or the portion of the fluidic system containing the fluidic droplet fluidly, optically or visually, thermally, pneumatically, electronically, or the like. The sensor can be positioned proximate the fluidic system, for example, embedded within or integrally connected to a wall of a channel, or positioned separately from the fluidic system but with physical, electrical, and/or optical communication with the fluidic system so as to be able to sense and/or determine the fluidic droplet and/or a portion of the fluidic system containing the fluidic droplet (e.g., a channel or a microchannel, a liquid containing the fluidic droplet,

etc.). For example, a sensor may be free of any physical connection with a channel containing a droplet, but may be positioned so as to detect electromagnetic radiation arising from the droplet or the fluidic system, such as infrared, ultraviolet, or visible light. The electromagnetic radiation may be produced by the droplet, and/or may arise from other portions of the fluidic system (or externally of the fluidic system) and interact with the fluidic droplet and/or the portion of the fluidic system containing the fluidic droplet in such as a manner as to indicate one or more characteristics of the fluidic droplet, for example, through absorption, reflection, diffraction, refraction, fluorescence, phosphorescence, changes in polarity, phase changes, changes with respect to time, etc. As an example, a laser may be directed towards the fluidic droplet and/or the liquid surrounding the fluidic droplet, and the fluorescence of the fluidic droplet and/or the surrounding liquid may be determined. "Sensing communication," as used herein may also be direct or indirect. As an example, light from the fluidic droplet may be directed to a sensor, or directed first through a fiber optic system, a waveguide, etc., before being directed to a sensor.

Non-limiting examples of sensors useful in the invention include optical or electromagnetically-based systems. For example, the sensor may be a fluorescence sensor (e.g., stimulated by a laser), a microscopy system (which may include a camera or other recording device), or the like. As another example, the sensor may be an electronic sensor, e.g., a sensor able to determine an electric field or other electrical characteristic. For example, the sensor may detect capacitance, inductance, etc., of a fluidic droplet and/or the portion of the fluidic system containing the fluidic droplet.

As used herein, a "processor" or a "microprocessor" is any component or device able to receive a signal from one or more sensors, store the signal, and/or direct one or more responses (e.g., as described above), for example, by using a mathematical formula or an electronic or computational circuit. The signal may be any suitable signal indicative of the environmental factor determined by the sensor, for example a pneumatic signal, an electronic signal, an optical signal, a mechanical signal, etc.

As a particular non-limiting example, a device of the invention may contain fluidic droplets containing one or more cells. The desired activity of one or more gene products may result in the expression (or inhibition of expression) of a 'marker' gene, for example a gene for green fluorescent protein (GFP). The cells may be exposed to a fluorescent signal marker that binds if a certain condition is present, for example, the marker may bind to a first cell type but not a second cell type, the marker may bind to an expressed protein, the marker may indicate viability of the cell (i.e., if the cell is alive or dead), the marker may be indicative of the state of development or differentiation of the cell, etc., and the cells may be directed through a fluidic system of the invention based on the presence/absence, and/or magnitude of the fluorescent signal marker. For instance, determination of the fluorescent signal marker may cause the cells to be directed to one region of the device (e.g., a collection chamber), while the absence of the fluorescent signal marker may cause the cells to be directed to another region of the device (e.g., a waste chamber). Thus, in this example, a population of cells may be screened and/or sorted on the basis of one or more determinable or targetable characteristics of the cells, for example, to select live cells, cells expressing a certain protein, a certain cell type, etc.

(E) Materials

A variety of materials and methods, according to certain aspects of the invention, can be used to form any of the above-described components of the microfluidic systems and devices of the invention. In some cases, the various materials selected lend themselves to various methods. For example, various components of the invention can be formed from solid materials, in which the channels can be formed via micromachining, film deposition processes such as spin coating and chemical vapor deposition, laser fabrication, photolithographic techniques, etching methods including wet chemical or plasma processes, and the like. See, for example, *Scientific American,* 248:44-55, 1983 (Angell, et al). In one embodiment, at least a portion of the fluidic system is formed of silicon by etching features in a silicon chip. Technologies for precise and efficient fabrication of various fluidic systems and devices of the invention from silicon are known. In another embodiment, various components of the systems and devices of the invention can be formed of a polymer, for example, an elastomeric polymer such as polydimethylsiloxane ("PDMS"), polytetrafluoroethylene ("PTFE" or Teflon®), or the like.

Different components can be fabricated of different materials. For example, a base portion including a bottom wall and side walls can be fabricated from an opaque material such as silicon or PDMS, and a top portion can be fabricated from a transparent or at least partially transparent material, such as glass or a transparent polymer, for observation and/or control of the fluidic process. Components can be coated so as to expose a desired chemical functionality to fluids that contact interior channel walls, where the base supporting material does not have a precise, desired functionality. For example, components can be fabricated as illustrated, with interior channel walls coated with another material. Material used to fabricate various components of the systems and devices of the invention, e.g., materials used to coat interior walls of fluid channels, may desirably be selected from among those materials that will not adversely affect or be affected by fluid flowing through the fluidic system, e.g., material(s) that is chemically inert in the presence of fluids to be used within the device.

In one embodiment, various components of the invention are fabricated from polymeric and/or flexible and/or elastomeric materials, and can be conveniently formed of a hardenable fluid, facilitating fabrication via molding (e.g. replica molding, injection molding, cast molding, etc.). The hardenable fluid can be essentially any fluid that can be induced to solidify, or that spontaneously solidifies, into a solid capable of containing and/or transporting fluids contemplated for use in and with the fluidic network. In one embodiment, the hardenable fluid comprises a polymeric liquid or a liquid polymeric precursor (i.e. a "prepolymer"). Suitable polymeric liquids can include, for example, thermoplastic polymers, thermoset polymers, or mixture of such polymers heated above their melting point. As another example, a suitable polymeric liquid may include a solution of one or more polymers in a suitable solvent, which solution forms a solid polymeric material upon removal of the solvent, for example, by evaporation. Such polymeric materials, which can be solidified from, for example, a melt state or by solvent evaporation, are well known to those of ordinary skill in the art. A variety of polymeric materials, many of which are elastomeric, are suitable, and are also suitable for forming molds or mold masters, for embodiments where one or both of the mold masters is composed of an elastomeric material. A non-limiting list of examples of such polymers includes polymers of the general classes of silicone polymers, epoxy polymers, and acrylate polymers. Epoxy polymers are characterized by the presence of a three-membered cyclic ether group commonly referred to as

an epoxy group, 1,2-epoxide, or oxirane. For example, diglycidyl ethers of bisphenol A can be used, in addition to compounds based on aromatic amine, triazine, and cycloaliphatic backbones. Another example includes the well-known Novolac polymers. Non-limiting examples of silicone elastomers suitable for use according to the invention include those formed from precursors including the chlorosilanes such as methylchlorosilanes, ethylchlorosilanes, phenylchlorosilanes, etc.

Silicone polymers are preferred in one set of embodiments, for example, the silicone elastomer polydimethylsiloxane. Non-limiting examples of PDMS polymers include those sold under the trademark Sylgard by Dow Chemical Co., Midland, Mich., and particularly Sylgard 182, Sylgard 184, and Sylgard 186. Silicone polymers including PDMS have several beneficial properties simplifying fabrication of the microfluidic structures of the invention. For instance, such materials are inexpensive, readily available, and can be solidified from a prepolymeric liquid via curing with heat. For example, PDMSs are typically curable by exposure of the prepolymeric liquid to temperatures of about, for example, about 65° C. to about 75° C. for exposure times of, for example, about an hour. Also, silicone polymers, such as PDMS, can be elastomeric and thus may be useful for forming very small features with relatively high aspect ratios, necessary in certain embodiments of the invention. Flexible (e.g., elastomeric) molds or masters can be advantageous in this regard.

One advantage of forming structures such as microfluidic structures of the invention from silicone polymers, such as PDMS, is the ability of such polymers to be oxidized, for example by exposure to an oxygen-containing plasma such as an air plasma, so that the oxidized structures contain, at their surface, chemical groups capable of cross-linking to other oxidized silicone polymer surfaces or to the oxidized surfaces of a variety of other polymeric and non-polymeric materials. Thus, components can be fabricated and then oxidized and essentially irreversibly sealed to other silicone polymer surfaces, or to the surfaces of other substrates reactive with the oxidized silicone polymer surfaces, without the need for separate adhesives or other sealing means. In most cases, sealing can be completed simply by contacting an oxidized silicone surface to another surface without the need to apply auxiliary pressure to form the seal. That is, the pre-oxidized silicone surface acts as a contact adhesive against suitable mating surfaces. Specifically, in addition to being irreversibly sealable to itself, oxidized silicone such as oxidized PDMS can also be sealed irreversibly to a range of oxidized materials other than itself including, for example, glass, silicon, silicon oxide, quartz, silicon nitride, polyethylene, polystyrene, glassy carbon, and epoxy polymers, which have been oxidized in a similar fashion to the PDMS surface (for example, via exposure to an oxygen-containing plasma). Oxidation and sealing methods useful in the context of the present invention, as well as overall molding techniques, are described in the art, for example, in an article entitled "Rapid Prototyping of Microfluidic Systems and Polydimethylsiloxane," *Anal. Chem.*, 70:474-480, 1998 (Duffy et al.), incorporated herein by reference.

Another advantage to forming microfluidic structures of the invention (or interior, fluid contacting surfaces) from oxidized silicone polymers is that these surfaces can be much more hydrophilic than the surfaces of typical elastomeric polymers (where a hydrophilic interior surface is desired). Such hydrophilic channel surfaces can thus be more easily filled and wetted with aqueous solutions than can structures comprised of typical, unoxidized elastomeric polymers or other hydrophobic materials.

In one embodiment, a bottom wall is formed of a material different from one or more side walls or a top wall, or other components. For example, the interior surface of a bottom wall can comprise the surface of a silicon wafer or microchip, or other substrate. Other components can, as described above, be sealed to such alternative substrates. Where it is desired to seal a component comprising a silicone polymer (e.g. PDMS) to a substrate (bottom wall) of different material, the substrate may be selected from the group of materials to which oxidized silicone polymer is able to irreversibly seal (e.g., glass, silicon, silicon oxide, quartz, silicon nitride, polyethylene, polystyrene, epoxy polymers, and glassy carbon surfaces which have been oxidized). Alternatively, other sealing techniques can be used, as would be apparent to those of ordinary skill in the art, including, but not limited to, the use of separate adhesives, thermal bonding, solvent bonding, ultrasonic welding, etc.

Various aspects and embodiments of the present invention are illustrated in the following examples. It will be appreciated that modification of detail may be made without departing from the scope of the invention.

All documents mentioned in the text are incorporated by reference.

EXAMPLES

Example 1

Microfluidic Device for Selection of Genes Using In Vitro Compartmentalisation

A schematic representation of the microfluidic device is shown in FIG. **15**. Microchannels are fabricated with rectangular cross-sections using rapid prototyping in poly(dimethylsiloxane) (PDMS) (McDonald and Whitesides, 2002) and rendered hydrophobic as (Song and Ismagilov, 2003). Syringe pumps were used to drive flows (Harvard Apparatus PHD 2000 Infusion pumps). For aqueous solutions, 250 μi Hamilton Gastight syringes (1700 series, TLL) with removeable needles of 27-gaugeare used with 30-gauge Teflon tubing (WeiCo Wire and Cable). For the carrier fluid, 1 ml Hamilton Gastight syringes (1700 series, TLL) are used with 30-gauge Teflon needles with one hub from Hamilton (Song and Ismagilov, 2003). The carrier fluid is 9% (v/v) $C_6F_{11}C_2H_4OH$ in perfluorodecaline (PFD) (Song et al., 2003). The microfluidic device consists of a series of interconnected modules. Each module has a specific function. These include modules that will produce droplets, fuse droplets, mix droplets, react droplets, detect droplets, and 20 sort droplets (see FIG. **16**). In one example, droplets are made, consisting of different molecules or different concentrations of molecules. Droplets are made at rates of up to 10^4 sec^{-1}, and are made with a polydispersity of less than 1.5% and with sizes ranging from 1 μm to 100 μm. Each droplet is fused with a second droplet containing a second set of reactants, and is rapidly mixed to initiate the chemical reaction. This chemical reaction is allowed to proceed in each droplet by passing it through a delay channel. Each droplet is then fused with another droplet containing a second set of reactants, and is subsequently rapidly mixed to initiate the second set of chemical reactions. After the second reaction has proceeded in a delay module, the results of the reaction is determined using an optical sensor or other form of detection module. Finally, the desired droplets are sorted into two populations based on signal form the optical

detection module, one population is kept for further processing and the other discarded. These and other modules can be used in this combination, or in other combinations.

Droplet Generation Module: We use a flow-focusing geometry to form the drops. A water stream is infused from one channel through a narrow constriction; counter propagating oil streams hydrodynamically focus the water stream reducing its size as it passes through the constriction as shown in FIG. **17**A. This droplet generator can be operated in a flow regime that produces a steady stream of uniform droplets of water in oil. The size of the water droplets is controlled by the relative flow rates of the oil and the water; the viscous forces overcome surface tension to create uniform droplets. If the flow rate of the water is too high a longer jet of fluid passes through the orifice and breaks up into droplets further down stream; these droplets are less uniform in size. If the flow rate of the water is too low, the droplet breakup in the orifice becomes irregular again, producing a wider range of droplet sizes. While this emulsification technology is robust, it is limited to producing droplets of one size at any given flow rate; this droplet size is largely determined by the channel dimensions. Moreover, the timing of the droplet production cannot be controlled.

We overcome these limitations by incorporating electric fields to create an electrically addressable emulsification system. To achieve this, we apply high voltage to the aqueous stream and charge the oil water interface, as shown schematically in FIG. **17**A. The water stream behaves as a conductor while the oil is an insulator; electrochemical reactions charge the fluid interface like a capacitor. At snap-off, charge on the interface remains on the droplet. In addition, the droplet volume, V_d, and frequency, f, can be tailored over nearly three orders of magnitude without changing the infusion rate of the oil or water. Droplet size and frequency are not independent; instead their product is determined by the infusion rate of the dispersed phase $Q_d=fV_d$. The droplet size decreases with increasing field strength, as shown in FIGS. **17**, B to E. The dependence of the droplet size on applied voltage for three different flow rates is summarized in FIG. **17**F. At low applied voltages the electric field has a negligible effect, and droplet formation is driven exclusively by the competition between surface tension and viscous flow. By contrast, at high electric field strengths, there is a significant additional force on the growing drop, F=qE, where q is the charge on the droplet. Since the droplet interface behaves as a capacitor, q is proportional to the applied voltage, V. This leads to a V^2 dependence of the force, which accounts for the decrease in droplet size with increasing applied field shown in FIG. **17**F. If the electric field becomes too large, the charged interface of the water stream is repelled by the highly charged drops; this destabilizes the production and increases the variation in droplet size.

The electronic control afforded by the field-induced droplet formation provides an additional valuable benefit: it allows the phase of the droplet break-off to be adjusted within the production cycle. This is accomplished by increasing the field above the critical break-off field only at the instant the droplet is required. This provides a convenient means to precisely synchronize the production and arrival of individual droplets at specific locations.

Droplet Coalesces Module: An essential component in any droplet-based reaction confinement system is a droplet coalescing module which combines two or more reagents to initiate a chemical reaction. This is particularly difficult to achieve in a microfluidic device because surface tension, surfactant stabilization, and drainage forces all hinder droplet coalescence; moreover, the droplets must cross the stream lines that define their respective flows and must be perfectly synchronized to arrive at a precise location for coalescence.

Use of electrostatic charge overcomes these difficulties; placing charges of opposite sign on each droplet and applying an electric field forces them to coalesce. As an example we show a device consisting of two separate nozzles that generate droplets with different compositions and opposite charges, sketched in FIG. **18**A. The droplets are brought together at the confluence of the two streams. The electrodes used to charge the droplets upon formation also provide the electric field to force the droplets across the stream lines, leading to coalesce. Slight variations in the structure of the two nozzles result in slight differences in the frequency and phase of their droplet generation in the absence of a field. Thus the droplets differ in size even though the infusion rates are identical. Moreover, the droplets do not arrive at the point of confluence at exactly the same time. As a result the droplets do not coalesce as shown in FIG. **18**B. By contrast, upon application of an electric field, droplet formation becomes exactly synchronized, ensuring that pairs of identically sized droplets each reach the point of confluence simultaneously. Moreover, the droplets are oppositely charged, forcing them to traverse the stream lines and contact each other, thereby causing them to coalesce, as shown in FIG. **18**C. The remarkable synchronization of the droplet formation results from coupling of the break-off of each of the pair of droplets as mediated by the electric field; the magnitude of the electric field varies as the separation between the leading edges of the two droplets changes and the frequency of droplet break-off is mode-locked to the electric field. A minimum charge is required to cause droplets to coalesce, presumably because of the stabilizing effects of the surfactant coating; this is clear from FIG. **18**D which shows the voltage dependence of the percentage of drops that contact each other that actually coalesce.

Droplet Mixer Module: Rapid mixing is achieved through either successive iterations of translation and rotation, FIG. **19**, or by coalescing drops along the direction parallel to the flow direction, FIG. **20**.

Droplet Reactor/Time Delay Module: A delay line is used to provide a fixed time for a reaction. Two non-limiting examples of how this can be achieved are 'single file' and 'large cross-section' channels. The 'single file' delay line uses length to achieve a fixed reaction time. As this often results in exceptionally long channels, it is desirable to place spacer droplets of a third fluid, immicible with both the carrier oil and the aqueous droplets inbetween aqueous droplet pairs. There is then an alternation between aqueous and non-aqueous droplets in a carrier oil. This is shown in FIG. **21**A. A second possibility for achieving a long time delay is to use wide and deep channel having a 'large cross-sectional area' to slow the average velocity of the droplets. An example of this is shown in FIG. **21**B.

Recharging Module: The use of oppositely charged droplets and an electric field to combine and mix reagents is extremely robust, and 100% of the droplets coalesce with their partner from the opposite stream. However, after they coalesce the resultant drops carry no electrostatic charge. While it is convenient to charge droplets during formation, other methods must be employed in any robust droplet-based micro fluidic system to recharge the mixed droplets if necessary for further processing. This is readily accomplished through the use of extensional flow to split neutral droplets in the presence of an electric field which polarizes them, resulting in two oppositely charged daughter droplets;

this is sketched in FIG. **22**A. The photomicrograph in FIG. **22**B shows neutral droplets entering a bifurcation and splitting into charged daughter droplets. The dashed region in FIG. **22**B is enlarged in FIG. **22**C to illustrate the asymmetric stretching of the charged droplets in the electric field. The vertical dashed lines indicate the edges of the electrodes where the droplets return to their symmetric spherical shape. The electric field also allows precision control of the droplet splitting providing the basis for a robust droplet division module which allows the splitting of the contents into two or more aliquots of identical reagent, facilitating multiple assays on the contents of the same microreactor.

Detection Module: The detection module consists of an optical fiber, one or more laser, one or more dichroic beam splitter, bandpass filters, and one or more photo multiplying tube (PMT) as sketched in FIG. **23**.

Sorting Module: The Contents of individual droplets must be probed, and selected droplets sorted into discreet streams. The use of electrostatic charging of droplets provides a means for sorting that can be precisely controlled, can be switched at high frequencies, and requires no moving parts. Electrostatic charge on the droplets enables drop-by-drop sorting based on the linear coupling of charge to an external electric field. As an example, a T-junction bifurcation that splits the flow of carrier fluid equally will also randomly split the droplet population equally into the two streams, as shown in FIG. **24**A. However, a small electric field applied at the bifurcation precisely dictates which channel the drops enter; a schematic of the electrode configuration is shown in FIG. **24**B. Varying the direction of the field varies the direction of the sorted droplets as shown in FIGS. **24**C and **24**D. The large forces that can be imparted on the droplets and the high switching frequency make this a fast and robust sorting engine with no moving parts; thus the processing rate is limited only by the rate of droplet generation.

Example 2

Enrichment of lacZ Genes from a Pool of Mutant lacZ Genes Based on β-galactosidase Activity Inside Aqueous Droplets in a Microfluidic Device

An example is given how single genes encoding enzymes with a desired activity can be selected from a pool of genes by making and manipulating aqueous droplets using the microfluidic device described in Example 1. It is demonstrated that lacZ genes encoding for active β-galactosidase enzyme can be selected from a pool of mutant lacZ genes by:

(1) forming droplets containing (a) a coupled in vitro transcription/translation system and (b) genes; (2) fusing droplets (a) and (b) to initiate translation with the concentration of genes such that the majority of combined droplets (c) contain no more than one gene per droplet; (3) passing the combined droplets (c) down a microfluidic channel to allow translation; (4) fusing each droplet (c) with a droplet (d) which contains an inhibitor of translation (puromycin) and the fluorogenic substrate, fluorescein digalactoside (FDG); (5) passing the combined droplets (e) down a microfluidic channel to allow catalysis and; (6) monitoring the fluorescence of the droplets. When the gene present in the aqueous droplet encodes for an active β-galactosidase enzyme, FDG inside the compartment will be converted into the fluorescent product fluorescein (excitation 488 nm, emission 514 nm). After a single round of selection, lacZ genes can be enriched from a mixture of genes by over 100-fold.

DNA Preparation

The lacZ gene encoding for β-galactosidase is amplified from genomic DNA isolated from strain BL21 of *Escherichia coli* using primers GALBA and GALFO (GALBA: 5'-CAGACTGCACCATGGCCATGATTACGGAT-TCACTGGCCGTCGTTTTAC-3' (SEQ ID NO: 1); GALFO: 5'-ACGATGTCAGGATCCTTATTATTTTT-GACACCAGACCAACTG GTAA TGGTAG-3' (SEQ ID NO: 2)) The PCR product is digested with restriction endonueleases NcoI and BamHI (New England Biolabs Inc., Beverly, Mass., USA). Digested DNA is gel purified and ligated into vector pIVEX2.2b (Roche Biochemicals GmbH, Mannheim, Germany) that is digested with the same enzymes. The ligation product is transformed into XL-10 gold cells (Stratagene). Minicultures are grown from 5 single colonies in 3 ml LB medium supplemented with 100 μg/ml ampicillin at 37° C. over night. From these overnight cultures, plasmid DNA (pDNA) is isolated and sequenced for the presence of the right insert. Linear DNA constructs are generated by PCR using pDNA from a sequenced clone (containing the correct lacZ sequence) as template and primers LMB2-10E (5'-GATGGCGCCCAACAGTCC-3' (SEQ ID NO: 3)) and PIVB-4 (5'-TTTGGCCGCCGC-CCAGT-3' (SEQ ID NO: 4)).

Full-length mutant lacZ (lacZmut), which has an internal frameshift and hence does not encode an active β-galactosidase, is obtained by cutting pIVEX2.2b-LacZ with restriction enzyme SacI (NEB). Digested DNA is blunted by incubation for 15 min at 12° C. with T4 DNA polymerase (2 U) and dNTPs (500 μM final concentration). The reaction is quenched by adding EDTA to a final concentration of 10 mM and heating to 75° C. for 20 minutes. Blunted DNA is purified and self-ligated with T4 DNA ligase (1 Weiss unit) in the presence of 5% PEG 4,000 by incubating for 2 hrs at 22° C. pDNA is directly transformed into XL-10 Gold cells. Minicultures are grown from 5 single colonies in 3 ml LB medium supplemented with 100 μg/ml ampicillin at 37° C. over night and plasmid DNA is isolated. pDNA is digested with SacI and one of the clones lacking the internal SacI site is used to generate linear DNA constructs as described above.

In Vitro Transcription and Translation Inside Aqueous Droplets in a Microfluidic System

LacZ and lacZmut linear DNA constructs are mixed at a molar ratio of 1:5, 1:100 and 1:1000, respectively in nuclease-free water.

A commercial in vitro translation system (EcoProT7, Novagen/EMD biosciences Ltd, Madison, Wi, USA) is used according to the manufacturer's protocol. Using the device described in Example 1, EcoProT7 extract is compartmentalised into droplets (a) of mean μm diameter (520 fl volume). Droplets (b), of mean 7.4 μm diameter (220 fl volume) are formed containing 0.67 mM_L-methionine and 0.25 mM 7-hydroxycoumarin-3-carboxylic acid (Sigma Aldrich) (excitation 386 nm, emission 448 nm), and 0.75 pM DNA (mixes of LacZ and lacZmut linear DNA at the ratios described above) in nuclease-free water. The droplets are formed in a carrier fluid consisting of perfluorinated oil; the perfluorinated oil can either consist of the mixture described in example 1 or alternatively one of the 3M™ Fluorinert™ liquids. Each droplet (a) is fused with a droplet (b). The concentration of DNA is such that the majority of combined droplets (c) contain no more than one gene per droplet (the mean number of genes per droplet=0.1). According to the Poisson Distribution, $P(a)=e^{-m}[m^a/a!]$, where m=0.1=the mean number of genes per droplet, and P(a)=the probability of finding a genes per droplet, 90.5% of droplets contain no genes, 9.05% contain 1 gene, and 0.45% contain 2 genes and

0.016% contain more than two genes). The combined droplets (c) are passed down the microfluidic channel held at 30° C. for 30 minutes to allow in vitro transcription and translation.

Screening and Selection for β-galactosidase Activity

After the translation step, a series of droplets (d) of 11.2 μm diameter (740 fl volume, equal in volume to droplets (c)) and which contain 4 mM puromycin (to stop translation) and 1 mM FDG (Molecular Probes) in water. Each droplet (c) is fused with a droplet (d) to stop translation and start the catalytic reaction. The combined droplets (e) are passed down the microfluidic channel held at 30° C. for 10 minutes to allow catalysis. The fluorescence of the droplets is monitored. All droplets contain 7-hydroxycoumarin-3-carboxylic acid allowing their identification. Monitoring of the fluorescence signal from individual droplets is achieved by coupling both excitation and fluorescent signals to the droplets through an optical fiber. The continuous wave emission from two diode lasers (363 nm and 488 nm) is used for excitation dichroic beam splitters and band pass filters (450±20 nm and 530±20 nm) are used to isolate the fluorescent emission to detect the 7-hydroxycoumarin-3-carboxylic acid fluorescence and the fluorescein fluorescence as measured with photomultiplying tubes. Droplets with the highest fluorescein fluorescence (with a sorting gate set such that less than 0.05% of the population of droplets from a negative control without DNA) are sorted. For each sort, 10,000 droplets are collected.

DNA Recovery from Sorted Droplets

DNA from the sorted droplets is precipitated by adding 100 μl 0.3M sodium acetate pH 5.2 and 70 μl isopropanol in the presence of 20 μg glycogen as carrier (Roche 20 Biochemicals GmbH, Mannheim, Germany). DNA is pelleted by centrifugation at 20,000×g for 15 rain at 4° C. Precipitated DNA is ished twice with 100 μl 70% ethanol and the DNA pellet is dried using a Speedvac (Eppendorf). DNA is resuspended into 10 μl nuclease-free water.

PCR Amplification of—Recovered DNA

PCR reactions are set up at 50 μl total volume, using Expand Long Template PCR mix with buffer 1 according to the manufacturer's protocol (Roche). Primers LMB2-11E (5'-GCCCGATCTTCCCCATCGG-3' (SEQ ID NO: 5)) and PIVB-8 (5'-CACACCCGTCCTGTGGA-3' (SEQ ID NO: 6)) are used at a concentration of 300 μM each. Reactions are incubated for 2 min at 94° C. and subsequently subjected to 10 cycles at 94° C., 15 s; 55° C., 30 s; 68° C., 2 min, another 22 cycles with an increment in elongation time of 10 s/cycle and a final incubation step for 7 min at 68° C. PCR products are purified using a Wizard PCR prep kit (Promega).

SacI, Digestion of PCR Products

To be able to distinguish between lacZ DNA and lacZmut DNA, purified PCR products are digested with 20 U of SacI enzyme. SacI cuts the lacZ gene but not lacZmut. SacI enzyme is heat-inactivated (15 min at 65° C.) and 5 μl of digested DNA is loaded onto a 1% agarose gel in TAE. DNA is electrophoresed at 5V/cm. DNA is visualized by staining with ethidium bromide and quantified using ImageQuant TL gel analysis software (Amersham Biosciences).

Genes encoding an active β-galactosidase (lacZ genes) are significantly enriched from a pool of mutant genes (lacZmut genes) encoding an inactive β-galactosidase with all ratios of lacZ:lacZmut tested. With an initial gene concentration of 0.1% lacZ genes, the lacZ genes could be enriched over 100-fold in a single round of selection.

Example 3

Mutants with Improved β-galactosidase Activity can be Selected from a Random Mutagenesis Library of Evolved β-galactosidase (Ebg) Using Compartmentalisation of Genes in Aqueous Droplets in a Microfluidic Device

The gene encoding for evolved β-galactosidase (Ebg) is often used as a model to study the evolution of novel enzyme functions within an organism. The wild type ebgA gene of *Escherichia coli* encodes an enzyme—with feeble β-galactosidase activity, but ebgA has the potential to evolve sufficient activity to replace the lacZ gene for growth on the sugars lactose and lactulose. Genetic analysis of these mutants has revealed that only two amino acid replacements account for the drastic increase in β-galactosidase activity.

Here we show that similar mutants can be obtained in vitro by creating a random mutagenesis library of the ebg gene and subjecting them to selection for β-galactosidase activity by making and manipulating aqueous droplets using the microfluidic device described in Example 1.

Error Prone Mutagenesis of EbgAC Using Base Analogues

A gene segment encoding for the A domain and the C domain of evolved β-galactosidase enzyme is amplified from genomic DNA of *E. coli* strain BL21 using primers EbgACFw (5'-CAGACTGCACCGCGGGATGAATCGCTGGGAAAACATTCAGC-3' (SEQ ID NO: 7)) and EbgACBw (5'-GCGAGGAGCTCTTATTGTTATGGAAATAACCATCTTCG-3' (SEQ ID NO: 8)). The PCR product is cloned into vector pIVEX2.2b using restriction endonucleases SacIi and SacI (NEB). DNA is transfected into XLIO-gold cells and single colonies are screened for the presence of the EbgAC gene construct with the right nucleotide sequence. pDNA from a single clone with the right EbgAC gene sequence is used as template to generate a random mutagenesis library using nucleoside analogues essentially as described by Zaccolo et al. (*J Mol Biol* 255(4): 589-603, 1996). A mixture of the 5'-triphosphates of 6-(2-deoxy-b-D-ribofuranosyl)-3,4-dihydro-8H-pyrimido-[4,5-C][1,2]oxazin-7-one (dPTP) and of 8-oxo-2'deoxyguanosine (8-oxodG) is prepared in PCR grade water at 2 mM and 10 mM concentrations, respectively. This base analogue mix is diluted 167× and 333× in expand long template PCR buffer 1 (Roche); containing $MgCl_2$ (2 mM), dNTPs (500 μM), expand long template PCR polymerase enzyme mix (Roche), primer LMB2-9E (5'-GCATTTATCAGGGTTATTGTC-3 (SEQ ID NO: 9); 500 nM') and triple biotinylated primer PIVB-1 (5'-3Bi-GCGTTGATGCAATTTCT-3' (SEQ ID NO: 10); 500 nM) in a total reaction volume of 50 μl. Five nanograms of pIVEX2.2b-EbgAC DNA is added and samples are subjected to 1 cycle of 2 minutes at 94° C., followed by 3 cycles at 94° C., 1 min; at 50° C., 1 min; at 68° C., 4 min), followed by a final extension of 7 min at 68° C. Ten micrograms of molecular biology-grade glycogen is added to the DNA prior to purification using a Qiaquick PCR purification kit. After purification DNA is recovered in 50 μl PCR-grade water. Ten micrograms of Streptavidin-coated magnetic beads (Dynabeads M-280 streptavidin, Dynal Biotech, Oslo, Norway) are rinsed in 2× binding buffer provided with the beads, resuspended into 50 μl 2× binding buffer and added to the purified DNA. Beads and DNA are incubated for 2.5 hrs at room temperature in a rotating device. Beads are collected with a magnet and rinsed twice with ish buffer that is provided with the beads and twice with PCR-grade water. Finally, beads are resuspended into 25 μl water. 5 ml of bead-bound DNA is used as template in a second PCR

reaction (25 cycles of 15 s at 94° C., 30 s at 55° C. and 2 min at 68° C.). PCR product is purified using a Qiaquick PCR purification kit and recovered in 50 μl of PCR-grade water.

Iterative Rounds of In Vitro Selection Using a Microfluidic System

The generated random mutagenesis library of ebgAC is subjected to 2 successive rounds of selection. Each selection round consisted of 7 separate steps: (1) forming droplets containing (a) a coupled in vitro transcription/translation system and (b) genes; (2) fusing droplets (a) and (b) to initiate translation with the concentration of genes such that the majority of combined droplets (c) contain no more than one gene per droplet; (3) passing the combined droplets (c) down a microfluidic channel to allow translation; (4) fusing each droplet (c) with a droplet (d) which contains an inhibitor of translation (puromycin) and the fluorogenic substrate, fluorescein digalactoside (FDG); (5) passing the combined droplets (e) down a microfluidic channel to allow catalysis; (6) monitoring the fluorescence of the droplets. When the gene present in the aqueous droplet encodes for an active β-galactosidase enzyme, FDG inside the compartment will be converted into the fluorescent product fluorescein (excitation 488 nm, emission 514 nm) and; (7) recovery and amplification of genes from the selected double emulsion droplets. The entire procedure is described in detail above (Example 2). Sets of nested primers are used for subsequent selection rounds (Table 1).

TABLE 1

list of primers used to amplify recovered DNA from successive rounds of selection

Selection round	Forward primer	Backward primer
0	LMB2-9E 5'-GCATTTATCAGG GTTATTGTC-3' (SEQ ID NO: 11)	PIVB-1 5'-GCGTTGATGCAA TTTCT-3' (SEQ ID NO: 12)
1	LMB2-10E 5'-GATGGCGCCCAA CAGTCC-3' (SEQ ID NO: 13)	PIVB-4 5'-TTTGGCCGCCGC CCAGT-3' (SEQ ID NO: 14)
2	LMB2-11 5'-ATGCGTCCGGCG TAGAGG-3' (SEQ ID NO: 15)	PIVB-11 5'-AGCAGCCAACTC AGCTTCC-3' (SEQ ID NO: 16)

After each selection round, the number of positive droplets within the Ebg library increased by at least 10-fold.

Characterisation of the β-galactosidase Activity of Single Members of the Ebg Library

After the 2nd selection round, DNA is recovered from the double emulsions by standard isopropanol precipitation and PCR amplified using primers LMB2-11 and PIVB-11. Amplified DNA is digested with restriction endonucleases SacI and SacII and cloned into pIVEX2.2b that is digested with the same enzymes. The ligation product is transformed into ElectroBlue electrocompetent cells (Strategene) by electroporation (at 17 kV/cm, 600 Ω, 25 μF) and plated onto LB agar plates with ampicillin. Ebg gene constructs are amplified from single colonies by colony PCR using primers LMB2-10E and PIVB-4. One microliter of PCR product is added for 14 μl of IVT mix (Novagen's EcoProT7 extract, supplemented with 200 μM L-methionine) and incubated for 90 min at 30° C. Forty microliters substrate solution (250 μM FDG, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT and 100 μg/ml BSA in 10 mM Tris-HCl, pH 7.9) is added and the conversion of FDG into fluorescein is monitored every 45 s for 90 min at 37° C.

The screened clones show a broad variety of β-galactosidase activities. ~50% of colonies have β-galactosidase activities that are comparable to or lower than wild type Ebg. ~12.5% of clones show β-galactosidase activity that is comparable to the Class I and Class II mutants (single point mutations) described by Hall et al. (*FEMS Microbiol Lett* 174(1): 1-8, 1999; *Genetica* 118(2-3): 143-56, 2003). In conclusion, the system described here can be used for the selection of ebg variants with improved β-galactosidase activity from a large gene library.

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All publications mentioned in the above specification, and references cited in said publications, are herein incorporated, by reference. Various modifications and variations of the described methods and system of the invention will be

apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be-unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claim.

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The invention claimed is:

1. A method for conducting an enzymatic reaction, comprising the steps of:

providing a droplet generator to produce, under microfluidic control, a plurality of aqueous microcapsules surrounded by an immiscible continuous phase that comprises a fluorinated oil that comprises a fluorinated polymer surfactant, each of the plurality of microcapsules comprising an enzyme, a genetic element linked covalently or non-covalently to a bead, and reagents for the enzymatic reaction;

pooling the microcapsules into one or more common compartments such that a portion of the plurality of microcapsules contact each other but do not fuse with each other due to the presence of the surfactant; and

conducting the enzymatic reaction on the genetic element of at least one of the plurality of microcapsules within the one or more common compartments.

2. The method of claim **1**, wherein the genetic element comprises nucleic acids.

3. The method of claim **2**, wherein the nucleic acids further comprise primers for a polymerase chain reaction (PCR).

4. The method of claim **2**, wherein the genetic element comprises RNA.

5. The method of claim **1**, wherein the genetic element is labeled.

6. The method of claim **1**, wherein the microcapsules are monodisperse with respect to each other.

7. The method of claim **1**, wherein the droplet generator further comprises an aqueous fluid channel and two immiscible continuous phase channels and producing the plurality of microcapsules surrounded by the immiscible continuous phase under the microfluidic control comprises partitioning an aqueous fluid that is flowing through the aqueous fluid channel with two counter propagating streams of the immiscible continuous phase that are flowing through the immiscible continuous phase channels.

8. The method of claim **1**, wherein the concentration of the beads is adjusted such that a single bead appears in each microcapsule.

9. The method of claim **1**, wherein the genetic element is identified with a tag.

10. The method of claim **1**, wherein the droplet generator comprises a channel that narrows relative to the direction of flow.

11. The method of claim **1**, wherein the enzymatic reaction is an amplification reaction.

12. The method of claim **11**, wherein the amplification reaction is a strand displacement amplification.

13. The method of claim **1**, wherein the microcapsules are produced by infusing an aqueous fluid through a narrow constriction into a counter-propagating oil stream.

14. The method of claim **1**, wherein the enzymatic reaction is a reverse transcription reaction.

* * * * *

EXHIBIT 15

US010190115B2

(12) **United States Patent**
Saxonov

(10) **Patent No.: US 10,190,115 B2**
(45) **Date of Patent: *Jan. 29, 2019**

(54) **METHODS AND COMPOSITIONS FOR NUCLEIC ACID ANALYSIS**

(71) Applicant: **Bio-Rad Laboratories, Inc.**, Hercules, CA (US)

(72) Inventor: **Serge Saxonov**, Oakland, CA (US)

(73) Assignee: **Bio-Rad Laboratories, Inc.**, Hercules, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 331 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **14/493,272**

(22) Filed: **Sep. 22, 2014**

(65) **Prior Publication Data**

US 2015/0011430 A1 Jan. 8, 2015

Related U.S. Application Data

(63) Continuation of application No. 13/456,121, filed on Apr. 25, 2012, now Pat. No. 9,347,059.

(60) Provisional application No. 61/478,777, filed on Apr. 25, 2011.

(51) **Int. Cl.**

C12N 15/00	(2006.01)
C12Q 1/68	(2018.01)
C12N 15/10	(2006.01)
C12Q 1/6876	(2018.01)

(52) **U.S. Cl.**

CPC ***C12N 15/1065*** (2013.01); ***C12N 15/10*** (2013.01); ***C12N 15/1075*** (2013.01); ***C12Q 1/6876*** (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

Provided herein are methods, compositions, and kits for assays, many of which involve amplification reactions such as digital PCR or droplet digital PCR. The assays may be used for such applications as sequencing, copy number variation analysis, and others. In some cases, the assays involve subdividing a sample into multiple partitions (e.g., droplets) and merging the partitions with other partitions that comprise adaptors with barcodes.

26 Claims, 3 Drawing Sheets

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FIG. 1A

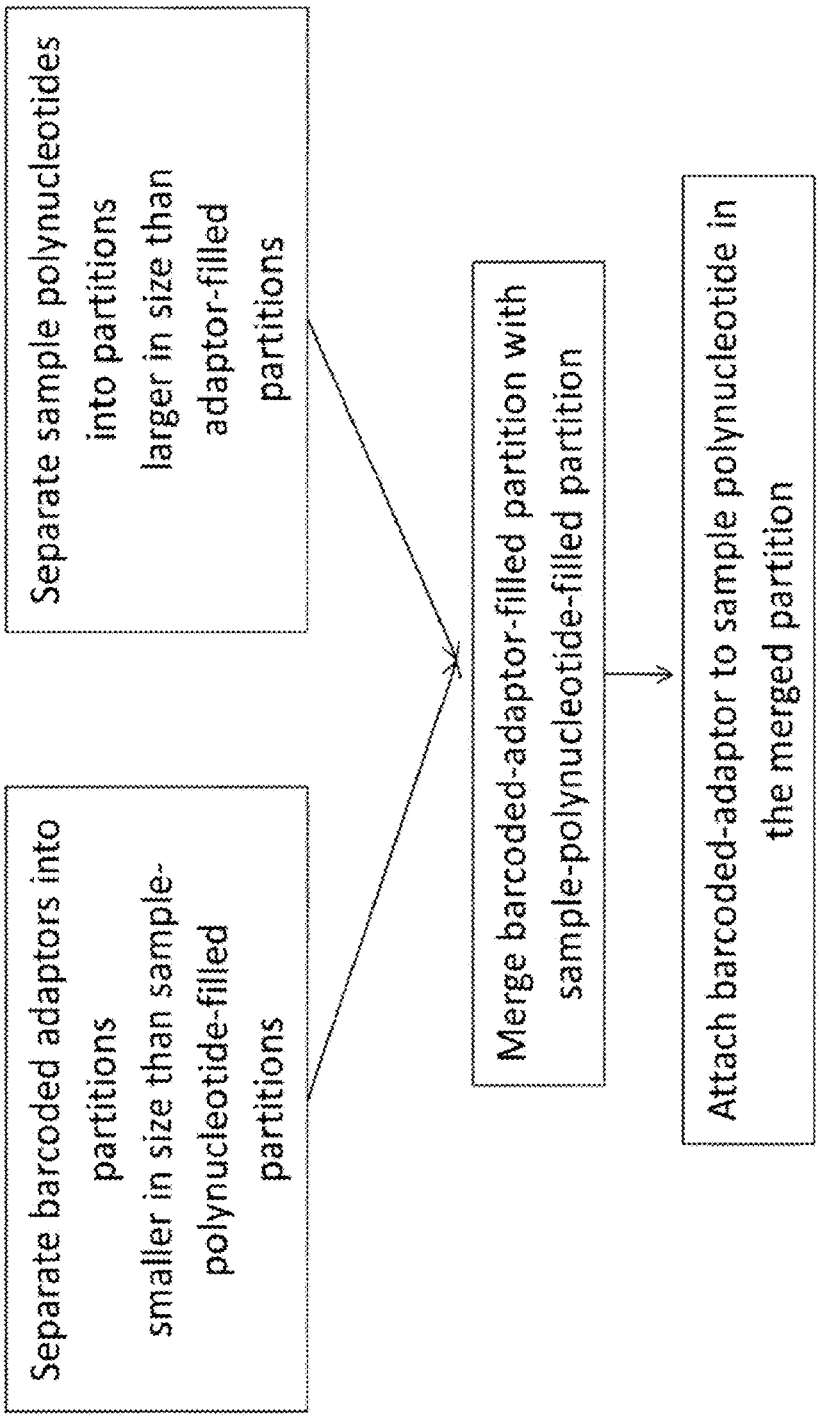

Separate sample polynucleotides into partitions larger in size than adaptor-filled partitions
Separate barcoded adaptors into partitions smaller in size than sample-polynucleotide-filled partitions
Merge barcoded-adaptor-filled partition with sample-polynucleotide-filled partition
Attach barcoded-adaptor to sample polynucleotide in the merged partition

FIG. 1B

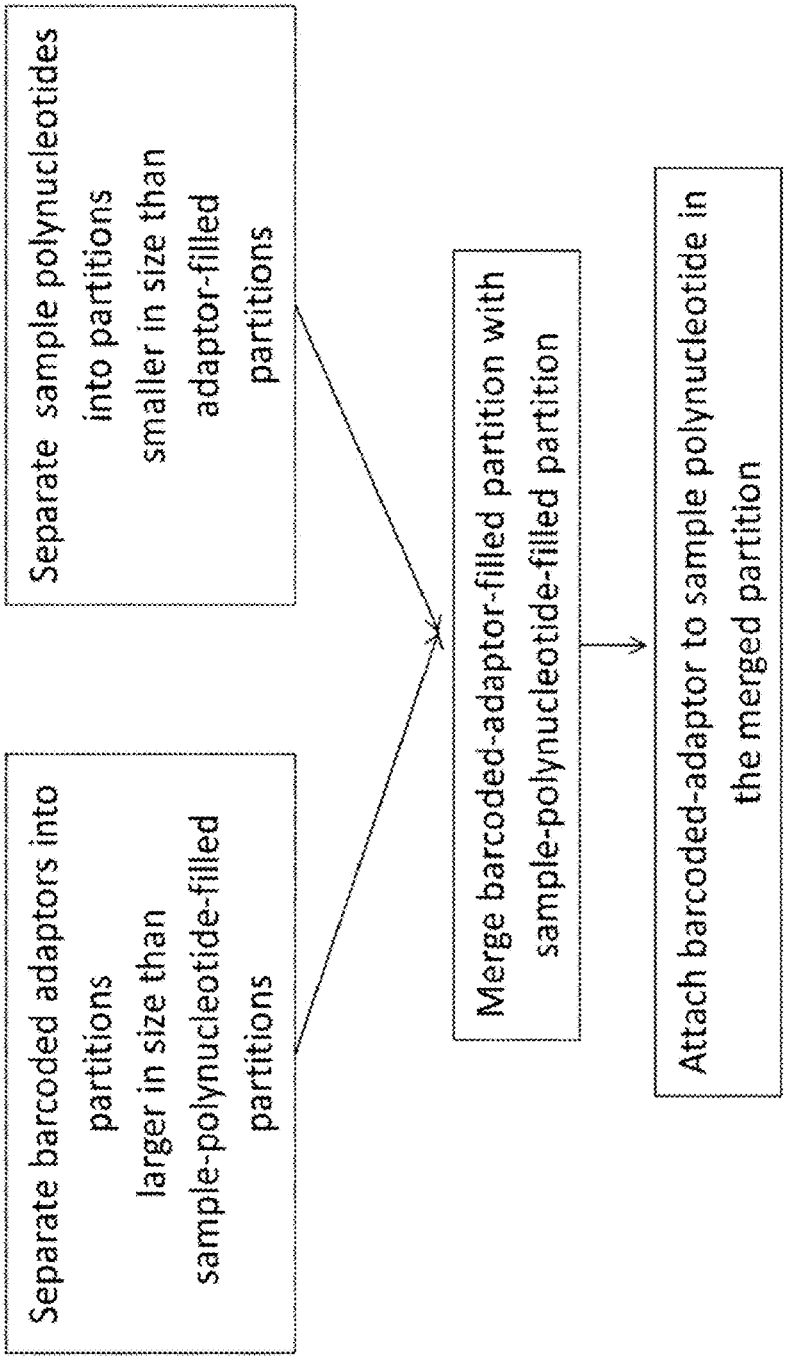
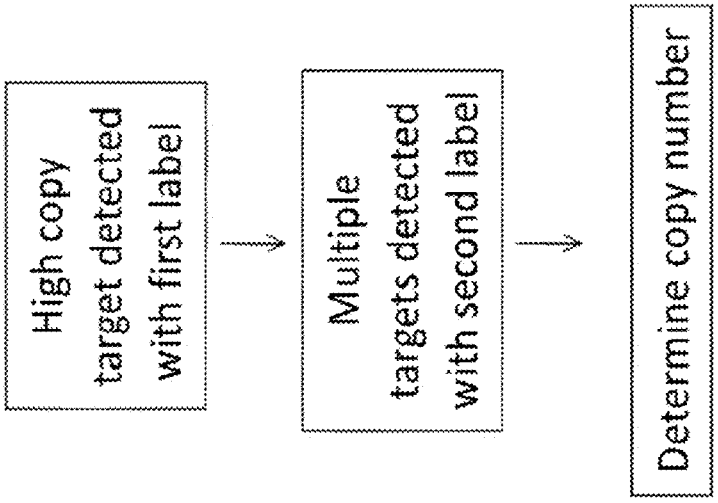

Separate sample polynucleotides into partitions smaller in size than adaptor-filled partitions
Separate barcoded adaptors into partitions larger in size than sample-polynucleotide-filled partitions
Merge barcoded-adaptor-filled partition with sample-polynucleotide-filled partition
Attach barcoded-adaptor to sample polynucleotide in the merged partition

FIG. 2


High copy target detected with first label
Multiple targets detected with second label
Determine copy number

METHODS AND COMPOSITIONS FOR NUCLEIC ACID ANALYSIS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 13/456,121, filed on Apr. 25, 2012, which claims the benefit of priority under 35 U.S.C. .§ 119(e) of U.S. Provisional Patent Application No. 61/478,777, filed Apr. 25, 2011, each of which is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

Next generation sequencing has many useful applications and can be used to analyze multiple samples. There is a need for improved methods of multiplexing samples for applications of next generation sequencing. There is also a need for improved methods of barcode tagging partitioned polynucleotides and analyzing the barcode tagged polynucleotides.

Determining the copy number of a target sequence can have many useful applications. There is a need for improved methods of determining the copy number of a target sequence.

BRIEF SUMMARY OF THE INVENTION

This disclosure provides methods that can be used in sequencing and other applications. In some instances, this disclosure provides a method comprising: a. subdividing a plurality of adaptors into a plurality of first partitions, wherein each of the first partitions has on average a first volume and wherein the adaptors comprise unique barcodes; b. subdividing a sample comprising multiple polynucleotides into a plurality of second partitions, wherein each of the second partitions has on average a second volume, wherein the second volume is greater than the first volume; c. merging at least one of the first partitions with at least one of the second partitions to form a merged partition; and d. tagging one of the multiple polynucleotides, or fragment thereof, with at least one of the adaptors.

The method may comprise: a. subdividing a plurality of adaptors into a plurality of first partitions, wherein each of the first partitions has on average a first volume and wherein the adaptors comprise unique barcodes; b. subdividing a sample comprising multiple polynucleotides into a plurality of second partitions, wherein each of the second partitions has on average a second volume, wherein said second volume is less than said first volume; c. merging at least one of said first partitions with at least one of said second partitions to form a merged partition; and d. tagging one of said multiple polynucleotides, or fragment thereof, with at least one of said adaptors.

Often, in a method disclosed herein, the first partitions are droplets. In some instances, said second partitions are droplets. In some cases, said droplets are within an immiscible fluid.

In some cases, the polynucleotides are genomic DNA. For example, the genomic DNA may be high molecular weight DNA. In some cases, the sample of genomic DNA is partitioned so that it is unlikely that a given partition comprises two or more polynucleotides, or fragments thereof, from the same locus but from different chromosomes.

In some cases, the first partitions are first droplets and the second partitions are second droplets; and prior to the merging, the at least one second droplet comprises the at least one first droplet. In other cases, the first partitions are first droplets and the second partitions are second droplets; and prior to the merging, the at least one second droplet does not comprise the at least one first droplet. In some cases, the first partitions are first droplets and the second partitions are second droplets; and prior to the merging, the at least one first droplet comprises the at least one second droplet. In some cases, the first partitions are first droplets and the second partitions are second droplets; and prior to the merging, the at least one first droplet does not comprise the at least one second droplet.

The volumes of the partitions containing the sample may be different than the volumes of the partitions containing the adaptors. For example, the second volume is at least two times the volume of the first volume. In other cases, the first volume is at least two times the volume of the second volume. The methods disclosed herein may further comprise modifying the temperature of the droplets.

In some cases, the method further comprises merging droplets by a method comprising use of a controller such that each of the first droplets merges with each of the second droplets. In some cases, merging comprises randomly merging droplets comprising polynucleotides with droplets comprising adaptors. The methods may further comprise pooling the adaptor-tagged polynucleotides, or fragments thereof.

Often, the method further comprises analyzing the adaptor-tagged polynucleotides, or fragments thereof. The analyzing may involve sequencing the adaptor-tagged polynucleotides, or fragments thereof. The analyzing may comprise determining whether the adaptor-tagged polynucleotides, or fragments thereof, were located in the same partition; or, in some cases, estimating the likelihood that any two sequence reads generated by the sequencing came from the same or different partitions.

In some cases, the method further comprises fragmenting the polynucleotides within the second partitions to form polynucleotide fragments. The polynucleotides fragments may be generated by fragmenting the polynucleotides with an endonuclease.

In some cases, the polynucleotides are tagged by ligating the adaptors to the polynucleotides within a plurality of the merged partitions. The tagging may be accomplished by multiple means; for example, tagging can be accomplished using transposons.

Often, the methods herein include an amplification reaction. Often, the amplification comprises a polymerase chain reaction; or, the amplification can be a different type of reaction such as multiple-displacement amplification. Often, tagged polynucleotides are amplified; and, in some cases, they are amplified before tagging.

In some cases, each of the first partitions comprises, on average, less than five adaptors. Often, each of said second partitions comprises, on average, less than five of the multiple polynucleotides. In some cases, the subdividing of the sample comprises emulsifying or mixing the sample with the second partitions. Often, the subdividing of the plurality of adaptors comprises emulsifying or mixing the plurality of adaptors with the second partitions.

In some aspects, this disclosure provides a method comprising: a. partitioning organelles into a plurality of partitions, wherein each partition comprises on average less than five organelles per partition; b. lysing the extracellular organelles in the plurality of partitions, wherein the lysing releases RNA from the organelles; c. generating tagged

cDNA from the released RNA in the plurality of partitions with adaptors comprising a barcode, wherein each partition in the plurality of partitions comprises adaptors with a unique barcode. In some cases, the organelles are extracellular organelles such as exosomes. In some cases, the generating tagged cDNA comprises reverse transcription of the released RNA with partition-specific barcoded primers. The method may further comprise sequencing the tagged cDNA and/or determining if the tagged cDNA is from the same organelle.

This disclosure also provides a method comprising: a. partitioning microorganisms into a plurality of partitions, b. obtaining polynucleotides from the microorganisms in the plurality of partitions; and c. tagging the polynucleotides in the plurality of partitions with adaptors comprising a barcode, wherein each partition in the plurality of partitions comprises adaptors with a unique barcode. In some cases, each of said partitions comprises, on average, less than five microorganisms. The method may further comprise sequencing the tagged polynucleotides and/or determining if the tagged polynucleotide fragments are from the same partition.

INCORPORATION BY REFERENCE

All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

Novel features are set forth with particularity in the appended claims. A better understanding of the features and advantages will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which principles are utilized, and the accompanying drawings of which:

FIGS. **1**A and **1**B illustrate methods of merging droplets comprising a sample with droplets comprising adaptors with barcodes.

FIG. **2** illustrates a method of determining copy number of a high copy number target using references detected with a common label.

DETAILED DESCRIPTION OF THE INVENTION

In general, described herein are methods, compositions, and kits for library preparation for sequencing polynucleotides. The methods, compositions, and kits can be used to separate a sample of polynucleotides into a plurality of partitions, and each of the plurality of partitions can be provided with a unique set of adaptors comprising a barcode. Library preparation can be performed in each of the plurality of partitions (e.g., droplets). The contents of the partitions can be pooled and sequenced to generate sequence reads, and the barcodes can be used to identify which sequence reads came from the same partition. A number of embodiments of methods, compositions, systems, and kits are provided herein.

Overview

In general, barcoding (or "tagging") can enable one to pool samples of nucleic acids in order to reduce the cost of sequencing per sample, yet retain the ability to determine from which sample a sequence read is derived. Separate library preparations can be prepared for each sample, and each sample can have its own unique barcode. The separately prepared libraries with unique barcodes can then be pooled and sequenced. Each sequence read of the resulting dataset can be traced back to an original sample via the barcode in the sequence read.

In methods provided herein, polynucleotides in a sample can be separated into a plurality of partitions, e.g., droplets. Adaptors with a unique barcode (or "tag") can be supplied to each of a plurality of partitions comprising polynucleotides. Polynucleotides with barcode adaptors can be sequenced, and the barcodes can be used determine if two or more sequence reads were generated from one or more polynucleotides in the same partition.

Barcode adaptors can be bundled within a partition, e.g., an aqueous phase of an emulsion, e.g., a droplet. Barcode tagging may be accomplished by merging adaptor-filled partitions (e.g., droplets) with sample-polynucleotide-containing partitions (e.g., droplets). In some cases, adaptor-filled partitions are smaller than sample-polynucleotide-containing partitions (see e.g., FIG. **1**A). Barcoded-adaptors can be separated into a plurality of partitions smaller in size than sample-polynucleotide-containing partitions. Larger sample-polynucleotide-containing partitions can be formed. A barcoded-adaptor-filled partition can be merged with a sample-polynucleotide-containing partition, and an adaptor can be attached to a polynucleotide. For example, the partitions containing sample polynucleotide may be, on average, greater than 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5 fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 50-fold, 75-fold, 100-fold, 500-fold, 1000-fold, 10,000-fold, 50,000-fold, or 100,000-fold the average size the of the partitions containing the adaptors. The partitions containing sample polynucleotide may be, on average, greater than 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5 fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 50-fold, 75-fold, 100-fold, 500-fold, 1000-fold, 10,000-fold, 50,000-fold, or 100,000-fold the average volume of the partitions containing the adaptors. In some cases, sample-polynucleotide-containing partitions are formed so that they contain adaptor-filled partitions. For example, adaptor-filled partitions (e.g., droplets) can be emulsified with a polynucleotide sample so that sample-polynucleotide-containing partitions (e.g., droplets) end up containing adaptor-filled partitions. The adaptor-filled droplets can be burst (e.g., through a temperature adjustment) to release reaction components (e.g., PCR or ligation components) that can be used for library preparation. In some embodiments, the temperature adjustment comprises raising the temperature to about, more than about, or at least about 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100° C. for about, more than about, or at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 min. In some embodiments, the temperature adjustment can last for about, more than about, or at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hrs. In some cases, the adaptor-filled droplets are not contained within the sample-containing droplets. In such cases, separate droplets may be merged together.

In some cases, an adaptor-filled partition is larger than a sample-polynucleotide-containing partition (see e.g., FIG. **1**B). Barcoded-adaptors can be separated into a plurality of

partitions larger in size than sample-polynucleotide-containing partitions. Smaller sample-polynucleotide-containing partitions can be formed. A barcoded-adaptor-filled partition can be merged with a sample-polynucleotide-containing partition, and an adaptor can be attached to a polynucleotide. For example, partitions containing adaptors may be, on average, greater than 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5 fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 50-fold, 75-fold, 100-fold, 500-fold, 1000-fold, 10,000-fold, 50,000-fold, or 100,000-fold the average size of the partitions containing the samples. The partitions containing adaptors can be, on average, greater than 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5 fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 50-fold, 75-fold, 100-fold, 500-fold, 1000-fold, 10,000-fold, 50,000-fold, or 100,000-fold the average volume of the partitions containing the samples. In some cases, adaptor-containing partitions are formed so that they contain sample-containing partitions. For example, in some embodiments, sample-polynucleotide-filled partitions (e.g., droplets) can be emulsified with adaptors so that adaptor-containing partitions (e.g., droplets) end up enveloping sample-containing partitions. In such cases, the sample-containing droplets can be burst (e.g., through a temperature adjustment) so that the contents of the different types of droplets can merge. In some embodiments, the temperature adjustment comprises raising the temperature to about, more than about, or at least about 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100° C. for about, more than about, or at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 min. In some embodiments, the temperature adjustment can last for about, more than about, or at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hrs. In some cases, the sample-polynucleotide-filled droplets are not contained within the adaptor-containing droplets. In such cases, separate droplets may be merged together.

A microfluidic device can be used to merge pre-made adaptor reagents with a plurality of sample-polynucleotide partitions such that every sample-polynucleotide partition comprises adaptor reagents. For example, a square-shaped device can be used with 1000×1000 (one million) partitions, and each polynucleotide can be tagged with two barcodes. One million unique identifiers can be constructed with 2,000 different barcodes. Reagents with 1,000 different barcodes can be loaded in horizontal channels of the device and reagents for another set of 1,000 different barcodes can be loaded in vertical channels of the device. Every one of the million partitions can have its own unique combination of barcodes.

In some cases, sample-polynucleotide-containing partitions (e.g., droplets) and adaptor-filled partitions (e.g., droplets) are merged in a controlled manner, e.g., one droplet of sample polynucleotides with one droplet of unique adaptors. In some cases, adaptor-filled partitions are randomly merged with sample-polynucleotide-containing partitions.

The following example illustrates an embodiment of a method. A large set of droplets of a number (N) types can be made. Each type of droplet can be loaded with its own barcode. The value N can be determined in part by the length of the barcode (L). For example, N can be as large as 4 L. Thus, if L=10, around 1 million different droplet types can be generated. Standard sequencing library preparation within each partition can be performed. Once the libraries are prepared, the contents of all the partitions can be merged (e.g., by breaking droplets) and loaded onto a nucleic acid sequencer. The sequencer can generate sequence reads for many of the library polynucleotides. Polynucleotides prepared within the same droplet can contain the same barcode. If the number of barcodes is sufficiently large, it can be surmised that molecules containing the same barcode came from the same partition. If N is sufficiently large (e.g., larger than the number of adaptor-filled partitions actually used in the experiment), it can be expected that any two sample-polynucleotide-containing partitions can be tagged by different adaptor-filled partitions. However, if N is not very large, sample-polynucleotide partitions can be tagged with the same adaptors. In that case, the likelihood that any two reads came from the same or different sample containing partitions can be estimated probabilistically. For many applications, a probabilistic assessment can be sufficient.

In some embodiments, the number of different samples that can be multiplexed, e.g., in a sequencing reaction, can be about, more than about, less than about, or at least about 1000, 5000, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000, 100,000, 200,000, 300,000, 400,000, 500,000, 600,000, 700,000, 800,000, 900,000, 1,000,000, 2,000,000, 3,000,000, 4,000,000, 5,000,000, 6,000,000, 7,000,000, 8,000,000, 9,000,000, or 10,000,000 samples. In some embodiments, about 1000 to about 10,000, about 10,000 to about 100,000, about 10,000 to about 500,000, about 100,000 to about 500,000, about 100,000 to about 1,000,000, about 500,000 to about 1,000,000, about 1,000,000 to about 5,000,000, or about 1,000,000 to about 10,000,000 samples are multiplexed in the methods described herein.

Some methods of barcode tagging are described, for example, in U.S. Patent Application Publication No. 20110033854.

Fusing Smaller Droplets (E.g., Inner Droplets) with Larger Droplets (E.g., Outer Droplets)

Some methods for fusing smaller droplets (e.g., inner droplets) with larger droplets (e.g., outer droplets) are disclosed, e.g., in U.S. Patent Application Publication No. 20110053798. In some cases, an inner droplet (or partition) can be fused with an outer droplet (or partition) by heating/cooling to change temperature, applying pressure, altering composition (e.g., via a chemical additive), applying acoustic energy (e.g., via sonication), exposure to light (e.g., to stimulate a photochemical reaction), applying an electric field, or any combination thereof. In some cases, the inner droplet may fuse to the outer droplet spontaneously. The treatment may be continuous or may vary temporally (e.g., pulsatile, shock, and/or repetitive treatment). The treatment may provide a gradual or rapid change in an emulsion parameter, to effect steady state or transient initiation of droplet fusion. The stability of the partitions, and their responsiveness to a treatment to induce droplet fusion, may be determined during their formation by selection of an appropriate surfactant type, surfactant concentration, critical micelle concentration, ionic strength, etc., for one or more phases of the inner/outer partition.

The fusing can result in a fused emulsion. Fusion may occur spontaneously, such that no treatment, other than a sufficient time delay (or no delay), is necessary before processing fused droplets. Alternatively, the inner/outer droplet may be treated to controllably induce fusion of droplets to form assay mixtures.

The fused emulsion may be processed. Processing may include subjecting the fused emulsion to any condition or set of conditions under which at least one reaction of interest can occur (and/or is stopped), and for any suitable time period. Accordingly, processing may include maintaining the temperature of the fused emulsion near a predefined set point, varying the temperature of the fused emulsion between two or more predefined set points (such as thermally cycling the fused emulsion), exposing the fused emulsion to light, changing a pressure exerted on the fused emulsion, adding at least one chemical substance to the fused emulsion, applying an electric field to the fused emulsion, or any combination thereof, among others.

Signals may be detected from the fused emulsion after and/or during processing. Detection is described further in other sections herein. The signals may be detected optically, electrically, chemically, or a combination thereof, among others. The detected signals may include test signals that correspond to at least one reaction of interest performed in the fused emulsion. Alternatively, or in addition, the detected signals may include code signals that correspond to codes present in the fused emulsion. Test signals and code signals generally are distinguishable and may be detected using the same or distinct detectors. For example, the test signals and code signals each may be detected as fluorescence signals, which may be distinguishable based on excitation wavelength (or spectrum), emission wavelength (or spectrum), and/or distinct positions in a fused droplet (e.g., code signals may be detectable as more localized than test signals with respect to fused droplets), among others. As another example, the test signals and code signals may be detected as distinct optical characteristics, such as test signals detected as fluorescence and code signals detected as optical reflectance. As a further example, the test signals may be detected optically and the code signals electrically, or vice versa.

Adaptors

Barcodes can be present on adaptors, and an adaptor with a barcode can be attached to a polynucleotide by ligation. A variety of types of adaptors can be used in the methods, compositions, systems, and kits described herein. For example, an adaptor can comprise double stranded sequence. An adaptor with double stranded sequence can comprise one blunt end. In some cases, an adaptor with double stranded sequence comprises two blunt ends. An adaptor with double stranded sequence can comprise one 3' overhang. An adaptor with double stranded sequence can comprise two 3' overhangs. An adaptor with double stranded sequence can comprise one 5' overhang. In some cases, an adaptor with double stranded sequence can comprise two 5' overhangs. An adaptor with double stranded sequence can comprise a 5' overhang and a 3' overhang. In some cases, an adaptor comprises only single stranded nucleic acid.

When an adaptor has one or more overhangs, the overhang can be about, more than about, less than about, or at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 bases. For example, a 3' overhang can be about, more than about, less than about, or at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 bases. A 5' overhang can be about, more than about, at least about, or less than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 bases. If an adaptor comprises two overhangs, the overhangs can comprise the same or different number of bases.

The longest strand of an adaptor can be about, more than about, less than about, or at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 bases. If an adaptor comprises a double-stranded portion, the double stranded portion can be about, more than about, at least about, or less than about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 base-pairs.

An adaptor can comprise DNA and/or RNA. In some cases, an adaptor comprises DNA. In some cases, an adaptor comprises RNA. In some cases, an adaptor comprises DNA and RNA.

An adaptor can comprise double stranded nucleic acid. In some cases, an adaptor comprises double stranded DNA. In some cases, an adaptor comprises double stranded RNA. In some cases, an adaptor comprises a DNA/RNA hybrid duplex.

An adaptor can comprise single stranded nucleic acid. In some embodiments, an adaptor comprises single stranded RNA. In some cases, an adaptor comprises single stranded DNA. In some cases, an adaptor comprises single stranded RNA and DNA.

When an adaptor comprises double stranded sequence, one strand of the adaptor can comprise only DNA and one strand of the adaptor can comprise only RNA. A first strand can comprise DNA and RNA and a second strand can comprise DNA only. A first stand can comprise DNA and RNA, and a second strand can comprise RNA only. If a strand of an adaptor comprises both DNA and RNA, the DNA can be 5' of the RNA or the DNA and be 3' or the RNA. In some embodiments, an adaptor is single stranded and comprises DNA and RNA, and the DNA is 5' of the RNA or 3' of the RNA.

An adaptor can comprise a hairpin (or hairpin loop). A hairpin can comprise DNA and/or RNA. The number of nonbase-paired bases in a loop of a hairpin can be about, more than about, or at least about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 bases. The number of nonbase-paired bases in a loop of a hairpin can be about 4 to about 8, about 4 to about 10, about 4 to about 14, about 4 to about 16, about 4 to about 20, about 4 to about 24, about 4 to about 26, or about 4 to about 30 bases. The length of the stem (base-paired portion) of the adaptor can be about, more than about, or at least about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 base-pairs.

In some cases, a hairpin adaptor is ligated to only one end of a polynucleotide. In some cases, a first hairpin adaptor is ligated to one end of a polynucleotide and a second hairpin adaptor is ligated to the other end of the polynucleotide. The hairpin adaptors that are ligated to each end of a polynucleotide can comprise the same nucleic acid sequence or different nucleic acid sequence. The hairpin adaptors that ligate to each end of a polynucleotide can have barcodes, and the barcodes can be the same or different. A hairpin adaptor that ligates to one end of a polynucleotide can have a barcode, and a hairpin adaptor that ligates to the other end of a polynucleotide can lack a barcode.

In some cases, adaptors are ligated to polynucleotides such that multiple adaptors and polynucleotides are interspersed.

Barcode

An adaptor can comprise one or more barcode (tag) sequences. A barcode sequence can be a unique identifier. In some embodiments, a barcode is about, more than about, less than about, or at least about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 bases or bases pairs. In some embodiments, a barcode is about 4 to about 6 bases or bp, about 4 to about 7 bases or bp, about 4 to about 8 bases or bp, about 4 to about 9 bases or bp, about 4 to about 10 bases or bp, about 4 to about 12 bases or bp, about 4 to about 14 bases or bp, about 4 to about 16 bases or bp, about 4 to about 18 bases or bp, about 4 to about 20 bases or bp, about 5 to about 10 bases or bp, about 5 to about 15 bases or bp, about 5 to about 20 bases or bp, about 5 to about 25 bases or bp, about 5 to about 30 bases or bp, about 5 to about 35 bases or bp, about 5 to about 40 bases or bp, or about 5 to about 50 bases or bp. In some embodiments, bases in a barcode are contiguous. In some embodiments, bases in a barcode are noncontiguous. In some embodiments, an adaptor comprises no barcodes.

A barcode can be double stranded in an adaptor. In some cases, a barcode is single stranded in an adaptor. A barcode can comprise double stranded and single stranded sequence in an adaptor. An adaptor can comprise about, more than about, at least about, or less than about 1, 2, 3, 4, 5, 6, 7, 8, 9, or different barcodes. If an adaptor comprises more than one barcode, the barcodes can be separated from each other by about, more than about, or at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 bases or base pairs on the adaptor.

Commercially available kits comprising adaptors with barcodes can be used in the methods described herein. For example, a kit comprising adaptors with barcodes can include the ENCORE™ 384 Multiplex System (NUGEN®) which can comprise 384 molecularly barcoded library adaptors. The ENCORE™ NGS Multiplex Library Systems for ION TORRENT™ can comprise adaptors with barcodes that can be ligated to fragments. The ENCORE™ Complete RNA-Seq IL Multiplex System 1-8 (NUGEN®) and ENCORE™ Complete RNA-Seq IL Multiplex System 9-16 (NUGEN®) can provide barcoded adaptors for multiplex sequencing. The ENCORE™ Complete RNA-Seq DR Multiplex system 1-8 (NUGEN®) and ENCORE™ Complete RNA-Seq DR Multiplex system 9-16 (NUGEN®) can provide a dedicated read (DR) barcode design. Examples of kits with adaptors with barcodes from LIFE TECHNOLOGIES™ include 5500 SOLiD™ Fragment Library Barcode Adaptors 1-16, 5500 SOLiD™ Fragment Library Barcode Adaptors 1-96, 5500 SOLiD™ Fragment Library Barcode Adaptors 17-32, 5500 SOLiD™ Fragment Library Barcode Adaptors 33-48, 5500 SOLiD™ Fragment Library Barcode Adaptors 49-64, 5500 SOLiD™ Fragment Library Barcode Adaptors 65-80, 5500 SOLiD™ Fragment Library Barcode Adaptors 81-96, 5500 SOLiD™ Fragment Library Core Kit, 5500 SOLiD™ Fragment Library Standard Adaptors, LIBRARY BUILDER™ Fragment Core Kit for 5500 Genetic Analysis Systems, SOLiD™ Fragment Library Barcoding Kit 1-96, SOLiD™ Fragment Library Barcoding Kit Module 17-32, SOLiD™ Fragment Library Barcoding Kit Module 33-48, SOLiD™ Fragment Library Barcoding Kit Module 49-64, SOLiD™ Fragment Library Barcoding Kit Module 65-80, SOLiD™ Fragment Library Barcoding Kit Module 81-96, SOLiD™ RNA Barcoding Kit, Module 1-16, SOLiD™ RNA Barcoding Kit, Module 1-48, SOLiD™ RNA Barcoding Kit, Module 1-96, SOLiD™ RNA Barcoding Kit, Module 17-32, SOLiD™ RNA Barcoding Kit, Module 33-48, SOLiD™ RNA Barcoding Kit, Module 49-64, SOLiD™ RNA Barcoding Kit, Module 49-96, SOLiD™ RNA Barcoding Kit, Module 65-80, or SOLiD™ RNA Barcoding Kit, Module 81-96.

Other commercially available kits with adaptors with barcodes include SureSelect AB Barcode Adaptor Kit (AGILENT TECHONOLOGIES), Bioo Scientific's AIR™ Barcoded Adapters, NEXTFLEX™ DNA Barcodes, ILLUMINA® TRUSEQ™ RNA and DNA Sample Preparation Kits, RAINDANCE® Technologies DEEPSEQ™ FFPE solution, NEBNEXT® Multiplex Oligos for ILLUMNIA® (Index Primers 1-12), or NEBNEXT® Multiplex Small RNA Library Prep set for ILLUMNIA® (Index Primers 1-12).

A polynucleotide can receive a barcode by being ligated to an adaptor comprising a barcode. The ligation can involve use of one or more ligases. A barcode can be attached to a polynucleotide by amplification with a primer comprising a barcode.

A barcode can be adjacent to a primer binding site. A barcode can be 0 or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 bases 3' of a primer binding (annealing, hybridization) site.

Primer/Probe Binding Site

An adaptor can comprise one or more primer, probe, or oligonucleotide hybridization sites. The one or more primer, probe, or oligonucleotide hybridization sites can be about, more than about, less than about, or at least about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 bases. A primer, probe, or oligonucleotide hybridization site can be used to anneal an oligonucleotide primer to the adaptor for amplification or to anneal a primer to the adaptor for a sequencing reaction. An adaptor can comprise sequence for annealing of more than one oligonucleotide primer or probes, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10 or more oligonucleotide primers or probes. An adaptor can have a site for annealing a sequencing primer and an amplification primer. A primer or probe that anneals to an adaptor can be about, more than about, less than about, or at least about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 bases in length.

Restriction Enzyme Site

An adaptor can comprise one or more restriction enzyme binding sites and or cleavage sites. A restriction enzyme that can bind or cleave an adaptor can be, e.g., AatII, Acc65I, AccI, AciI, AclI, AcuI, AfeI, AflII, AflIII, AgeI, AhdI, AleI, AluI, AlwI, AlwNI, ApaI, ApaLI, ApeKI, ApoI, AscI, AseI, AsiSI, AvaI, AvaII, AvrII, BaeGI, BaeI, BamHI, BanI, BanII, BbsI, BbvCI, BbvI, BccI, BceAI, BcgI, BciVI, BclI, BfaI, BfuAI, BfuCI, BglI, BglII, BlpI, BmgBI, BmrI, BmtI, BpmI, Bpu10I, BpuEI, BsaAI, BsaBI, BsaHI, BsaI, BsaJI, BsaWI, BsaXI, BseRI, BseYI, BsgI, BsiEI, BsiHKAI, BsiWI, BslI, BsmAI, BsmBI, BsmFI, BsmI, BsoBI, Bsp1286I, BspCNI, BspDI, BspEI, BspHI, BspMI, BspQI, BsrBI, BsrDI, BsrFI, BsrGI, BsrI, BssHII, BssKI, BssSI, BstAPI, BstBI, BstEII, BstNI, BstUI, BstXI, BstYI, BstZ17I, Bsu36I, BtgI, BtgZI, BtsCI, BtsI, Cac8I, ClaI, CspCI, CviAII, CviKI-1, CviQI, DdeI, DpnI, DpnII, DraI, DraIII, DrdI, EaeI, EagI, EarI, EciI, Eco53kI, EcoNI, Eco0109I, EcoP15I, EcoRI, EcoRV, FatI, FauI, Fnu4HI, FokI, FseI, FspI, HaeII, HaeIII, HgaI, HhaI, HincII, HindIII, HinfI, HinP1I, HpaI, HpaII, HphI, Hpy166I, Hpy188I, Hpy188 III, Hpy99I, HpyAV, HpyCH4III, HpyCH41V, HpyCH4V, KasI, KpnI, MboI, MboII, MfeI, MluI, MlyI, MmeU, MnlI, MscI, MseI, MslI, MspAlI, MspI, MwoI, NaeI, NarI, Nb.BbvCI, Nb.BsmI,

Nb.BsrDI, Nb.BtsI, NciI, NcoI, NdeI, NgoMIV, NheI, NlaIII, NlaIV, NmeAIII, NotI, NruI, NsiI, NspI, Nt.AlwI, Nt.BbvCI, Nt.BsmAI, Nt.BspQI, Nt.BstNBI, Nt.CviPII, PacI, PaeR7I, PciI, PflFI, PflMI, PhoI, PleI, PmeI, PmlI, PpuMI, PshAI, PsiI, PspGI, PspOMI, PspXI, PstI, PvuI, PvuII, RsaI, RsrII, SacI, SacII, SalI, SapI, Sau3AI, Sau96I, SbfI, ScaI, ScrFI, SexAI, SfaNI, SfcI, SfiI, SfoI, SgrAI, SmaI, SmlI, SnaBI, SpeI, SphI, SspI, StuI, StyD4I, StyI, SwaI, T, Taq.alpha.I, TfiI, TliI, TseI, Tsp45I, Tsp509I, TspMI, TspRI, Tth111I, XbaI, XcmI, XhoI, XmaI, XmnI, or ZraI.

An adaptor can comprise a Type IIS restriction enzyme binding site. A Type IIS restriction enzyme can cleave DNA at a defined distance from a non-palindromic asymmetric recognition site. Examples of Type IIS restriction enzymes include AarI, Acc36I, AccBSI, AciI, AclWI, AcuI, AloI, Alw26I, AlwI, AsuHPI, BaeI, BbsI, BbvCI, BbvI, BccI, BceAI, BcgI, BciVI, BfiI, BfuAI, BfuI, BmgBI, BmrI, BpiI, BpmI, Bpu10I, Bpu10I, BpuAI, BpuEI, BsaI, BsaMI, BsaXI, BseII, Bse3DI, BseGI, BseMI, BseMII, BseNI, BseRI, BseXI, BseYI, BsgI, BsmAI, BsmBI, BsmFI, BsmI, Bso31I, BspCNI, BspMI, BspQI, BspTNI, BsrBI, BsrDI, BsrI, BsrSI, BssSI, Bst2BI, Bst6I, BstF5I, BstMAI, BstV1I, BstV2I, BtgZI, BtrI, BtsCI, BtsI, CspCI, Eam1104I, EarI, EciI, Eco31I, Eco57I, Eco57MI, Esp3I, FauI, FauI, FokI, GsuI, HgaI, Hin4I, HphI, HpyAV, Ksp632I, LweI, MbiI, MboII, MlyI, MmeI, MnlI, Mva1269I, NmeAIII, PctI, PleI, PpiI, PpsI, PsrI, SapI, SchI, SfaNI, SmuI, TspDTI, TspGWI, or Taq II. A restriction enzyme can bind recognition sequence within an adaptor and cleave sequence outside the adaptor (e.g., in a polynucleotide).

The restriction enzyme can be a methylation sensitive restriction enzyme. The methylation sensitive restriction enzyme can specifically cleave methylated DNA. The methylation sensitive restriction enzyme can specifically cleave unmethylated DNA. A methylation sensitive enzyme can include, e.g., DpnI, Acc65I, KpnI, ApaI, Bsp120I, Bsp143I, MboI, BspOI, NheI, Cfr9I, SmaI, Csp6I, RsaI, Ecl136II, SacI, EcoRII, MvaI, HpaII, MSpJI, LpnPI, FsnEI, DpnII, McrBc, or MspI.

An adaptor can comprise one or more recognition sites for one or more nicking endonucleases, Type I endonucleases, or Type III endonucleases. A nicking endonuclease can hydrolyze only one strand of a duplex to produce DNA molecules that are "nicked" rather than cleaved. The nicking can result in a 3-hydroxyl and a 5'-phosphate. Examples of nicking enzymes include Nt.CviPII, Nb.BsmI, Nb.BbvCI, Nb.BsrDI, Nb.BtsI, Nt.BsmAI, Nt.BspQI, Nt.AlwI, Nt.BbvCI, or Nt.BstNBI. A Type I endonuclease can cleave at a site that differs and is at a random distance away from the recognition site. A Type III endonuclease can recognize two separate non-palindromic sequences that are inversely oriented. Examples of Type III restriction enzymes include EcoP15 and EcoP1.

One or more restriction enzymes used in the methods, compositions and/or kits described herein can be a component of a hybrid or chimeric protein. For example, a domain of a restriction enzyme comprising an enzymatic activity (e.g., endonuclease activity) can be fused to another protein, e.g., a DNA binding protein. The DNA binding protein can target the hybrid to a specific sequence on a DNA. The nucleic acid cleavage activity of the domain with enzymatic activity can be sequence specific or sequence non-specific. For example, the non-specific cleavage domain from the Type IIs restriction endonuclease FokI can be used as the enzymatic (cleavage) domain of the hybrid nuclease. The sequence the domain with the enzymatic activity can cleave can be limited by the physical tethering of the hybrid to DNA by the DNA binding domain. The DNA binding domain can be from a eukaryotic or prokaryotic transcription factor. The DNA binding domain can recognize about, or at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 bases or base pairs of continuous nucleic acid sequence. The DNA binding domain can recognize about 9 to about 18 bases or base pairs of sequence. The DNA binding domain can be, e.g., a zinc finger DNA binding domain. The DNA binding domain can be from a naturally occurring protein. The DNA binding domain can engineered to specifically bind any desired nucleotide sequence. The hybrid can be a zinc finger nuclease (e.g., zinc finger nuclease). The hybrid protein can function as a multimer (e.g., dimer, trimer, tetramer, pentamer, hexamer, etc.).

Modifications

An adaptor can comprise one or more end modifications. An adaptor can comprise one 5' phosphate. An adaptor can comprise two 5' phosphates. An adaptor can comprise one 3' hydroxyl. An adaptor can comprise two 3' hydroxyls. An adaptor can lack a 3' hydroxyl.

An adaptor can comprise one or more 3' end modifications. The 3' end modification can be, e.g., 3'-amino, 3'-black hole quencher (e.g., BHQ-0, BHQ-1, BHQ-2), 3'-biotin, 3'-chloesterol, 3'-dabcyl CPG, 3' dabsyl CPG, 3'-dye (e.g., fluorescein-CGP, Tamra-CPG, Rox-CPG, Cal Fluor 560-CPG, Quasar 570 (Cy3 substitute)-CGP, Quasar 670 (Cy5 substitute)-CPG, Quasar 705 (Cy5.5 substitute)-CPG, Pulsar 650-CPG, Epoch Richmond Red-CPG, Epoch Yakima Yellow-CPG, Acridine-CPG, 3'-inverted linkage (with 5'-OH attached to support and 3'-OH available for chain extension), 3'-phosphate. An adaptor can comprise any fluorescent dye described herein.

An adaptor can comprise one or more 5' end modifications. The 5' end modification can be, e.g., a 5'-amino group, 5'-biotin, 5'-digoxigenin (DIG), 5' phosphate group, or 5'-thiol. An adaptor can comprise a 5' aldehyde, 5' alkaline phosphatase, 5' amine, 5' horse radish peroxidase (HRP), 5' fluorescein, 5' HEX, 5' ROX, 5' TET, or 5' TAMRA. The 5' modification can be a molecular probe dye, e.g., Alexa Fluor 488, Alexa Fluoro 532, Alexa Fluor 546, Alexa Fluor 555, Alexa Fluor 594, Alexa Fluor 647, Alexa Fluor 660, Alexa Fluor 750, BODIPY® FL, BODIPY® 530/550, BODIPY® 493/503, BODIPY® 558/569, BODIPY® 564/570, BODIPY® 576/589, BODIPY® 581/591, BODIPY® FL-X, BODIPY® TR-X, BODIPY® TMR, BODIPY® R6G, BODIPY® R6G-X, BODIPY® 630/650, BODIPY® 650/665, CASCADE BLUE™ Dye, MARINA BLUE™, OREGON GREEN® 514, OREGON GREEN® 488, OREGON GREEN® 488-X, PACIFIC BLUE™ Dye, RHODAMINE GREEN™ Dye, RHODOL GREEN™ Dye, RHODAMINE GREEN™-X, RHODAMINE RED™-X, TEXAS RED®, or TEXAS RED™-X.

A modification can be attached to a nucleic acid strand through a linker, e.g., C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, C11, C12, C13, C14, C15, C16, C17, C18, C19, or C20. A linker can be, e.g., PC (photo cleavable) spacer, hexanediol, spacer 9 (a triethylene glycol spacer), spacer 18 (an 18-atom hexa-ethyleneglycol spacer), 1',2' dideoxyribose (dspacer), or I-linker (from Exiqon).

An adaptor can comprise one or more methyl groups.

An adaptor can be synthesized with canonical nucleotides (dATP, dCTP, dGTP, and dTTP). An adaptor can be made one or more noncanonical nucleotides. The noncanonical nucleotide can be dUTP. An adaptor can comprise deoxyuracil or deoxyinosine.

An adaptor can comprise one or more RNA-like nucleosides, e.g., ANA (arabino), LNA (locked), 2'-O methyl RNA, FANA (2'-fluoroarabino), or 2'-fluoro RNA. An adaptor can comprise a DNA-like nucleoside, e.g., β-D-DNA, β-L-DNA, or α-D-DNA. An adaptor can comprise one or more 5'-3 phosphorothioate linkages or inverted linkages (5'-5' or 3'-3'). An adaptor can comprise A-phosphorothioate, C-phosphorothioate, G-phosphorothioate, or T-phosphorothioate.

Modified bases in an adaptor can include, e.g., LNA (locked nucleic acid), 2-aminopurine, trimer-20, fluoro bases, 2,6-diaminopurine (2-amino-dA), 5-bromo dU, deoxyuridine, inverted dT, dideoxy C, 5-methyldC, deoxylnosine, 5-nitroindole, ribo A, ribo C, ribo G, ribo U, or −+2' 0-methyl RNA bases. An adaptor can have any type of nucleic acid modification described herein.

In some embodiments, an adaptor is chemically synthesized. In some embodiments, an adaptor is not chemically synthesized.

The modifications described herein can be found on sample polynucleotides.

Partitions

A partition can be formed by any mode of separating that can be used for digital PCR. A partition can be a microfluidic channel, a well on a nano- or microfluidic device or on a microtiter plate, or a reaction chamber in a microfluidic device. A partition can be an area on an array surface. A partition can be an aqueous phase of an emulsion (e.g., a droplet). Methods of generating droplets are described herein.

Droplet Generation

The present disclosure includes compositions, methods, and kits for manipulation of genetic material in droplets, e.g., using droplet digital PCR. The droplets described herein can include emulsion compositions (or mixtures of two or more immiscible fluids) described in U.S. Pat. No. 7,622,280, and droplets generated by devices described in International Application Publication No. WO/2010/036352, first inventor: Colston, each of which is hereby incorporated by reference in its entirety. The term emulsion, as used herein, can refer to a mixture of immiscible liquids (such as oil and water). Oil-phase and/or water-in-oil emulsions can allow for the compartmentalization of reaction mixtures within aqueous droplets. In some embodiments, the emulsions can comprise aqueous droplets within a continuous oil phase. In other embodiments, the emulsions provided herein are oil-in-water emulsions, wherein the droplets are oil droplets within a continuous aqueous phase. The droplets provided herein can be used to prevent mixing between compartments, and each compartment can protect its contents from evaporation and coalescing with the contents of other compartments. One or mote enzymatic reactions can occur in a droplet.

The mixtures or emulsions described herein can be stable or unstable. The emulsions can be relatively stable and have minimal coalescence. Coalescence can occur when small droplets combine to form progressively larger droplets. Less than about 0.00001%, 0.00005%, 0.00010%, 0.00050%, 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 2%. 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 6%, 7%, 8%, 9%, or 10% of droplets generated from a droplet generator can coalesce with other droplets. The emulsions can also have limited flocculation, a process by which the dispersed phase comes out of suspension in flakes.

Splitting a sample into small reaction volumes as described herein can enable the use of reduced amounts of reagents, thereby lowering the material cost of the analysis. Reducing sample complexity by partitioning can also improve the dynamic range of detection, since higher-abundance molecules can be separated from low-abundance molecules in different compartments, thereby allowing lower-abundance molecules greater proportional access to reaction reagents, which in turn can enhance the detection of lower-abundance molecules.

Droplets can be generated having an average diameter of about, more than about, less than about, or at least about 0.001, 0.01, 0.05, 0.1, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 100, 120, 130, 140, 150, 160, 180, 200, 300, 400, or 500 microns. The average diameter of the droplets can be about 0.001 microns to about 0.01 microns, about 0.001 microns to about 0.005 microns, about 0.001 microns to about 0.1 microns, about 0.001 microns to about 1 micron, about 0.001 microns to about 10 microns, about 0.001 microns to about 100 microns, about 0.001 microns to about 500 microns, about 0.01 microns to about 0.1 microns, about 0.01 microns to about 1 micron, about 0.01 microns to about 10 microns, about 0.01 microns to about 100 microns, about 0.01 microns to about 500 microns, about 0.1 microns to about 1 micron, about 0.1 microns to about 10 microns, about 0.1 microns to about 100 microns, about 0.1 microns to about 500 microns, about 1 micron to about 10 microns, about 1 micron to about 100 microns, 1 micron to about 500 microns, about 10 microns to about 100 microns, about 10 microns to about 500 microns, or about 100 microns to about 500 microns.

Droplet volume can be about, more than about, less than about, or at least about 0.001 nL, 0.01 nL, 0.1 nL, 1 nL (100 μm^3), 10 nL, or 100 nL. Droplets can be generated using, e.g., RAINSTORM™ (RAINDANCE™), microfluidics from ADVACED LIQUID LOGIC, or ddPCR™ (BIO-RAD).

Microfluidic methods of producing emulsion droplets using microchannel cross-flow focusing or physical agitation can produce either monodisperse or polydisperse emulsions. The droplets can be monodisperse droplets. The droplets can be generated such that the size of said droplets does not vary by more than plus or minus 5% of the average size of said droplets. The droplets can be generated such that the size of said droplets does not vary by more than plus or minus 2% of the average size of said droplets. A droplet generator can generate a population of droplets from a single sample, wherein none of the droplets can vary in size by more than plus or minus 0.1%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 5.5%, 6%, 6.5%, 7%, 7.5%, 8%, 8.5%, 9%, 9.5%, or 10% of the average size of the total population of droplets.

Both the flow rate in a droplet generator and the length of nucleic acids in a sample can have an impact on droplet generation. One way to decrease extension is to decrease flow rate; however, this can have the undesirable side effect of lower throughput and also increased droplet size. Long nucleic acids can disrupt droplet formation in extreme cases, resulting in a steady flow rather than discrete droplets. Reducing nucleic acid size in a sample can improve droplet formation when nucleic acid loads are high. Samples with high nucleic acid loads (e.g., high DNA loads, high RNA loads, etc.) can be used. Reducing the length of nucleic acids in a sample (e.g., by digestion, sonication, heat treatment, or shearing) can improve droplet formation.

Higher mechanical stability can be useful for microfluidic manipulations and higher-shear fluidic processing (e.g., in microfluidic capillaries or through 90 degree turns, such as valves, in a fluidic path). Pre- and post-thermally treated

droplets or capsules can be mechanically stable to standard pipette manipulations and centrifugation.

A droplet can be formed by flowing an oil phase through an aqueous sample. A partition, e.g., an aqueous phase of an emulsion, can comprise a buffered solution and reagents for performing an amplification reaction, e.g., a PCR reaction, including nucleotides, primers, probe(s) for fluorescent detection, template nucleic acids, DNA polymerase enzyme, and/or reverse transcriptase enzyme.

A partition, e.g., an aqueous phase of an emulsion, can comprise a buffered solution and reagents for performing an enzymatic reaction (e.g., a PCR) without solid-state beads, such as magnetic-beads. The buffered solution can comprise about, more than about, at least about, or less than about 1, 5, 10, 15, 20, 30, 50, 100, or 200 mM Tris. A partition, e.g., an aqueous phase of an emulsion, can comprise one or more buffers including, e.g., TAPS, bicine, Tris, Tricine, TAPSO, HEPES, TES, MOPS, PIPES, cacodylate, SSC, ADA, ACES, cholamine chloride, acetamidoglycine, glycinamide, maleate, phosphate, CABS, piperidine, glycine, citrate, glycylglycine, malate, formate, succinate, acetate, propionate, pyridine, piperazine, histidine, bis-tris, ethanolamine, carbonate, MOPSO, imidazole, BIS-TRIS propane, BES, MOBS, triethanolamine (TEA), HEPPSO, POPSO, hydrazine, Trizma (tris), EPPS, HEPPS, bicine, HEPBS, AMPSO, taurine (AES), borate, CHES, 2-amino-2-methyl-1-propanol (AMP), ammonium hydroxide, methylamine, or MES. The pH of the partition, e.g., an aqueous phase of an emulsion, can be about 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.5, 10, 10.5, 11, 11.5, 12, or 12.5. The pH of the partition, e.g., an aqueous phase of an emulsion, can be about 5 to about 9, about 5 to about 8, about 5 to about 7, about 6.5 to about 8, about 6.5 to about 7.5, about 6 to about 7, about 6 to about 9, or about 6 to about 8.

A partition, e.g., an aqueous phase of an emulsion, can comprise a salt, e.g., potassium acetate, potassium chloride, magnesium acetate, magnesium chloride, sodium acetate, or sodium chloride. The concentration of potassium chloride can be about, more than about, at least about, or less than about 10, 20, 30, 40, 50, 60, 80, 100, 200 mM. The buffered solution can comprise about 15 mM Tris and about 50 mM KCl.

A partition, e.g., an aqueous phase of an emulsion, can comprise nucleotides. The nucleotides can comprise deoxyribonucleotide triphosphate molecules, including dATP, dCTP, dGTP, dTTP, in concentrations of about, more than about, less than about, or at least about 50, 100, 200, 300, 400, 500, 600, or 700 μM each. dUTP can be added within a partition, e.g., an aqueous phase of an emulsion, to a concentration of about, less than about, more than about, or at least about 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 μM. The ratio of dUTP to dTTP in a partition, e.g., an aqueous phase of an emulsion, can be about 1:1000, 1:500, 1:250, 1:100, 1:75, 1:50, 1:40, 1:30, 1:20, 1:10, 1:5, 1:4, 1:3, 1:2, or 1:1.

A partition, e.g., an aqueous phase of an emulsion, can comprise one or more divalent cations. The one or more divalent cations can be, e.g., Mg^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, or Zn^{2+} Magnesium chloride ($MgCl_2$) can be added to a partition, e.g., an aqueous phase of an emulsion, at a concentration of about, more than about, at least about, or less than about 1.0, 2.0, 3.0, 4.0, or 5.0 mM. The concentration of $MgCl_2$ can be about 3.2 mM. Magnesium sulfate can be substituted for magnesium chloride, at similar concentrations. A partition, e.g., an aqueous phase of an emulsion, can comprise both magnesium chloride and magnesium sulfate. A wide range of common, commercial PCR buffers from varied vendors can be substituted for the buffered solution.

A non-ionic Ethylene Oxide/Propylene Oxide block copolymer can be added to a partition, e.g., an aqueous phase of an emulsion, in a concentration of about, more than about, less than about, or at least about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, or 1.0%. A partition, or aqueous phase, can comprise a biosurfactant. Common biosurfactants include non-ionic surfactants such as Pluronic F-68, Tetronics, Zonyl FSN. Pluronic F-68 can be present at a concentration of about 0.5% w/v.

Additives

A partition, e.g., an aqueous phase of an emulsion, can comprise one or more additives including, but not limited to, non-specific background/blocking nucleic acids (e.g., salmon sperm DNA), biopreservatives (e.g., sodium azide), PCR enhancers (e.g., betaine (N,N,N-trimethylglycine; [carboxymethyl]trimethylammonium), trehalose, etc.), and/or inhibitors (e.g., RNAse inhibitors). A GC-rich additive comprising, e.g., betaine and DMSO, can be added to samples assayed in the methods provided herein.

The one or more additives can include a non-specific blocking agent such as BSA or gelatin from bovine skin. The gelatin or BSA can be present in a concentration range of approximately 0.1 to about 0.9% w/v. Other blocking agents can include betalactoglobulin, casein, dry milk, or other common blocking agents. In some cases, the concentration of BSA and gelatin are about 0.1% w/v.

The one or more additives can include 2-pyrrolidone, acetamide, N-methylpyrolidone (NMP), B-hydroxyethylpyrrolidone (HEP), propionamide, NN-dimethylacetamide (DMA), N-methylformamide (MMP), NN-dimethylformamide (DMF), formamide, N-methylacetamide (MMA), polyethylene glycol, tetramethylammonium chloride (TMAC), 7-deaza-2'-deoxyguanosine, T4 gene 32 protein, glycerol, or nonionic detergent (Triton X-100, Tween 20, Nonidet P-40 (NP-40), Tween 40, SDS (e.g., about 0.1% SDS)), salmon sperm DNA, sodium azide, formamide, dithiothreitol (DTT), betamercaptoethanol (BME), 2-mercaptoethylamine-HCl, tris(2-carboxythyl)phosphine (TCEP), cysteine-HCl, or a plant polysaccharide. The one or more additives can be ethanol, ethylene glycol, dimethylacetamide, dimethylformamide, or suphalane.

Primers

A partition, e.g., an aqueous phase of an emulsion, can comprise oligonucleotide primers. The oligonucleotide primers can be used for amplification. Primers for amplification within a partition, e.g., an aqueous phase of an emulsion, can have a concentration of about, more than about, less than about, or at least about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, or 1.0 μM. The concentration of each primer can be about 0.5 μM. Primers can be designed according to known parameters for avoiding secondary structures and self-hybridization. Different primer pairs can anneal and melt at about the same temperatures, for example, within about 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10° C. of another primer pair. In some cases, greater than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 100, 200, 500, 1000, 5000, 10,000 or more primers are initially used. Such primers may be able to hybridize to the genetic targets described herein. About 2 to about 10,000, about 2 to about 5,000, about 2 to about 2,500, about 2 to about 1,000, about 2 to about 500, about 2 to about 100, about 2 to about 50, about 2 to about 20, about 2 to about 10, or about 2 to about 6 primers can be used.

Primers can be prepared by a variety of methods including but not limited to cloning of appropriate sequences and direct chemical synthesis using methods well known in the art (Narang et al., *Methods Enzymol.* 68:90 (1979); Brown et al., *Methods Enzymol.* 68:109 (1979)). Primers can also be obtained from commercial sources such as Integrated DNA Technologies, Operon Technologies, Amersham Pharmacia Biotech, Sigma, or Life Technologies. The primers can have an identical melting temperature. The melting temperature of a primer can be about 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 81, 82, 83, 84, or 85° C. The melting temperature of a primer can be about 30 to about 85° C., about 30 to about 80° C., about 30 to about 75° C., about 30 to about 70° C., about 30 to about 65° C., about 30 to about 60° C., about 30 to about 55° C., about 30 to about 50° C., about 40 to about 85° C., about 40 to about 80° C., about 40 to about 75° C., about 40 to about 70° C., about 40 to about 65° C., about 40 to about 60° C., about 40 to about 55° C., about 40 to about 50° C., about 50 to about 85° C., about 50 to about 80° C., about 50 to about 75° C., about 50 to about 70° C., about 50 to about 65° C., about 50 to about 60° C., about 50 to about 55° C., about 52 to about 60° C., about 52 to about 58° C., about 52 to about 56° C., or about 52 to about 54° C.

The lengths of the primers can be extended or shortened at the 5' end or the 3' end to produce primers with desired melting temperatures. One of the primers of a primer pair can be longer than the other primer. The 3' annealing lengths of the primers, within a primer pair, can differ. Also, the annealing position of each primer pair can be designed such that the sequence and length of the primer pairs yield the desired melting temperature. An equation for determining the melting temperature of primers smaller than 25 base pairs is the Wallace Rule (Td=2(A+T)+4(G+C)). Computer programs can also be used to design primers, including but not limited to Array Designer Software (Arrayit Inc.), Oligonucleotide Probe Sequence Design Software for Genetic Analysis (Olympus Optical Co.), NetPrimer, and DNAsis from Hitachi Software Engineering. The TM (melting or annealing temperature) of each primer can be calculated using software programs such as Net Primer (free web based program at http://www.premierbiosoft.com/netprimer/index.html). The annealing temperature of the primers can be recalculated and increased after any cycle of amplification, including but not limited to about cycle 1, 2, 3, 4, 5, about cycle 6 to about cycle 10, about cycle 10 to about cycle 15, about cycle 15 to about cycle 20, about cycle 20 to about cycle 25, about cycle 25 to about cycle 30, about cycle 30 to about cycle 35, or about cycle 35 to about cycle 40. After the initial cycles of amplification, the 5' half of the primers can be incorporated into the products from each loci of interest; thus the TM can be recalculated based on both the sequences of the 5' half and the 3' half of each primer.

The annealing temperature of the primers can be recalculated and increased after any cycle of amplification, including but not limited to about cycle 1, 2, 3, 4, 5, about cycle 6 to about cycle 10, about cycle 10 to about cycle 15, about cycle 15 to about cycle 20, about cycle 20 to about cycle 25, about cycle 25 to about cycle 30, about cycle 30 to about 35, or about cycle 35 to about cycle 40. After the initial cycles of amplification, the 5' half of the primers can be incorporated into the products from each loci of interest, thus the TM can be recalculated based on both the sequences of the 5' half and the 3' half of each primer.

Probes

A partition, e.g., an aqueous phase of an emulsion, can comprise one or more probes for fluorescent detection. The concentration of each of the one or more probes can be about, more than about, at least about, or less than about 0.1, 0.2, 0.3, 0.4, or 0.5 μM. The concentration of the one or more probes for fluorescent detection can be about 0.25 μM. Amenable ranges for target nucleic acid concentrations in PCR can be between about 1 pg and about 500 ng. A probe can be about, more than about, less than about, or at least about, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 bases long. A probe can be about 8 to about 40, about 10 to about 40, about 10 to about 35, about 10 to about 30, about 10 to about 25, about 10 to about 20, about 15 to about 40, about 15 to about 35, about 15 to about 30, about 15 to about 25, about 15 to about 20, about 18 to about 40, about 18 to about 35, about 18 to about 30, about 18 to about 25, or about 18 to 22 bases. A label (fluorophore, dye) used on a probe (e.g., a Taqman probe) can be, e.g., 6-carboxyfluorescein (FAM), tetrachlorofluorescin (TET), 4,7,2'-trichloro-7'-phenyl-6-carboxyfluorescein (VIC), HEX, Cy3, Cy 3.5, Cy 5, Cy 5.5, Cy 7, tetramethylrhodamine, ROX, and JOE, Alexa Fluor dye, e.g., Alexa Fluor 350, 405, 430, 488, 532, 546, 555, 568, 594, 633, 647, 660, 680, 700, and 750; Cascade Blue, Marina Blue, Oregon Green 500, Oregon Green 514, Oregon Green 488, Oregon Green 488-X, Pacific Blue, Rhodamine Green, Rhodol Green, Rhodamine Green-X, Rhodamine Red-X, and Texas Red-X. The label can be at the 5' end of a probe, 3' end of the probe, at both the 5' and 3' end of a probe, or internal to the probe. A unique label can be used to detect each different locus in an experiment. A probe, e.g., a Taqman probe, can comprise a quencher, e.g., a 3' quencher. The 3' quencher can be, e.g., TAMARA, DABCYL, BHQ-1, BHQ-2, or BHQ-3. In some cases, a quencher used in the methods provided herein is a black hole quencher (BHQ). In some cases, the quencher is a minor groove binder (MGB). In some cases, the quencher is a fluorescent quencher. In other cases, the quencher is a non-fluorescent quencher (NFQ).

Polymerases

A partition, e.g., an aqueous phase of an emulsion, can comprise a polymerase. The polymerase can be a DNA polymerase. The DNA polymerase can be, e.g., T4 DNA polymerase, DEEP VENT™ DNA polymerase, LONGAMP® Tag, PHUSION® High Fidelity DNA polymerase, LONGAMP® Hot Start Taq, Crimson LONGAMP® Taq, Taq DNA polymerase, Crimson Taq DNA polymerase, ONETAQ® DNA polymerase, QUICK-LOAD® DNA polymerase, VENTR® DNA polymerase, Hemo KLENTAQ®, Bsu DNA polymerase, DNA polymerase I, DNA Polymerase I, Large (Klenow), Klenow Fragment, Phi29 DNA polymerase, Pfu DNA polymerase, Pfx DNA polymerase, Tth DNA polymerase, Vent DNA polymerase, bacteriophage 29, REDTAQ™, or T7 DNA polymerase. The DNA polymerase can comprise 3' to 5' exonuclease activity. The DNA polymerase can comprise 5' to 3' exonuclease activity. The DNA polymerase can comprise both 3' to 5' exonuclease activity and 5' to 3' exonuclease activity. The DNA polymerase can comprise neither 3' to 5' exonuclease activity nor 5' to 3' exonuclease activity. The DNA polymerase can comprise strand displacement activity. In some cases, the DNA polymerase does not comprise strand displacement activity. The error rate of the DNA polymerase can be less than 1×10^{-6} bases.

A partition, e.g., an aqueous phase of an emulsion, can comprise a reverse transcriptase. The reverse transcriptase

can be AMV reverse transcriptase or M-MuLV reverse transcriptase. The RNA polymerase can comprise 5' to 3' exonuclease activity. The reverse transcriptase can comprise both 3' to 5' exonuclease activity and 5' to 3' exonuclease activity. The reverse transcriptase can comprise neither 3' to 5' exonuclease activity nor 5' to 3' exonuclease activity. The reverse transcriptase can comprise strand displacement activity. In some embodiments, the reverse transcriptase does not comprise strand displacement activity.

A partition, e.g., an aqueous phase of an emulsion, can comprise an RNA polymerase. The RNA polymerase can be, e.g., phi6 RNA polymerase, SP6 RNA polymerase, or T7 RNA polymerase.

In some embodiments, a partition, e.g., an aqueous phase of an emulsion, comprises Poly(U) polymerase or Poly(A) polymerase.

Oil Phase

The oil phase can comprise a fluorinated base oil which can be additionally stabilized by combination with a fluorinated surfactant such as a perfluorinated polyether. The base oil can be one or more of HFE 7500, FC-40, FC-43, FC-70, or another common fluorinated oil. The anionic surfactant can be Ammonium Krytox (Krytox-AM), the ammonium salt of Krytox FSH, or morpholino derivative of Krytox-FSH. Krytox-AS can be present at a concentration of about, more than about, less than about, or at least about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 2.0%, 3.0%, or 4.0% w/w. The concentration of Krytox-AS can be about 1.8%. The concentration of Krytox-AS can be about 1.62%. Morpholino derivative of Krytox-FSH can be present at a concentration of about, more than about, less than about, or at least about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 2.0%, 3.0%, or 4.0% w/w. The concentration of morpholino derivative of Krytox-FSH can be about 1.8%. In some embodiments, the concentration of morpholino derivative of Krytox-FSH is about 1.62%.

The oil phase can comprise an additive for tuning the oil properties, such as vapor pressure or viscosity or surface tension. Nonlimiting examples include perfluoro-octanol and 1H, 1H,2H,2H-Perfluorodecanol. In some embodiments, 1H, 1H,2H,2H-Perfluorodecanol is added to a concentration of about 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 1.00%, 1.25%, 1.50%, 1.75%, 2.00%, 2.25%, 2.50%, 2.75%, or 3.00% w/w. In some embodiments, 1H,1H,2H, 2H-Perfluorodecanol is added to a concentration of 0.18% w/w.

Microcapsules

In some embodiments, the emulsion is formulated to produce highly monodisperse droplets having a liquid-like interfacial film that can be converted by heating into microcapsules having a solid-like interfacial film; such microcapsules can behave as bioreactors able to retain their contents through a reaction process such as PCR amplification. The conversion to microcapsule form can occur upon heating. For example, such conversion can occur at a temperature of greater than about, more than about, or at least about 50, 60, 70, 80, 90, or 95 degrees Celsius. In some embodiments this heating occurs using a thermocycler. During the heating process, a fluid or mineral oil overlay can be used to prevent evaporation. Excess continuous phase oil may or may not be removed prior to heating. The biocompatible capsules can be resistant to coalescence and/or flocculation across a wide range of thermal and mechanical processing.

Following conversion, the capsules can be stored at about, more than about, less than about, or at least about 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, or 40 degrees Celsius, with one embodiment comprising storage of capsules at less than about 25 degrees Celsius. In some embodiments, these capsules are useful in biomedical applications, such as stable, digitized encapsulation of macromolecules, particularly aqueous biological fluids containing a mix of nucleic acids or protein, or both together; drug and vaccine delivery; biomolecular libraries; clinical imaging applications, and others.

The microcapsules can contain one or more nucleic acid probes (e.g., molecular inversion probe, ligation probe, etc.) and can resist coalescence, particularly at high temperatures. Accordingly, PCR amplification reactions can occur at a very high density (e.g., number of reactions per unit volume). In some embodiments, greater than 100,000, 500,000, 1,000,000, 1,500,000, 2,000,000, 2,500,000, 5,000,000, or 10,000,000 separate reactions can occur per ml. In some embodiments, the reactions occur in a single well, e.g., a well of a microtiter plate, without inter-mixing between reaction volumes. The microcapsules can also contain other components necessary to enable an enzymatic reaction (e.g., a PCR reaction) to occur, e.g., nucleotides, primers, probes, dNTPs, DNA or RNA polymerases, reverse transcriptases, restriction enzymes, etc. These capsules exhibit resistance to coalescence and flocculation across a wide range of thermal and mechanical processing.

The compositions described herein can include compositions comprising mixtures of two or more immiscible fluids such as oil and water that contain a type of nucleic acid probe (e.g., TaqMan probe, molecular inversion probe, ligation probe, etc.). In some cases, the composition comprises a restriction enzyme described herein, e.g., a droplet comprising a restriction enzyme (e.g., methylation-sensitive enzyme). In other embodiments, the compositions described herein comprise microcapsules that contain a type of nucleic acid (e.g., TaqMan probe, molecular inversion probe, ligation probe, etc.). Such microcapsules can resist coalescence, particularly at high temperatures, and therefore enable amplification reactions to occur at a very high density (e.g., number of reactions per unit volume).

Fragmentation

Library preparation within partitions (e.g., droplets) can entail fragmentation of polynucleotides in a sample and ligation of adaptors. Generally, the fragmentation occurs within a partition (e.g., droplet); but, in some applications, the fragmentation may occur prior to the partitioning. Fragmentation can be accomplished enzymatically, e.g., using an endonuclease. The endonuclease can be, e.g., AatII, Acc65I, AccI, AciI, AclI, AcuI, AfeI, AflII, AflIII, AgeI, AhdI, AleI, AluI, AlwI, AlwNI, ApaI, ApaLI, ApeKI, ApoI, AscI, AseI, AsiSI, AvaI, AvaII, AvrUU, BaeGI, BaeI, BamHI, BanI, BanII, BbsI, BbvCI, BbvI, Bed, BceAI, BcgI, BciVI, BclI, BfaI, BfuAI, BfuCI, BglI, BglII, BlpI, BmgBI, BmrI, BmtI, BpmI, Bpu10I, BpuEI, BsaAI, BsaBI, BsaHI, BsaI, BsaJI, BsaWI, BsaXI, BseRI, BseYI, BsgI, BsiEI, BsiHKAI, BsiWI, BslI, BsmAI, BsmBI, BsmFI, BsmI, BsoBI, Bsp1286I, BspCNI, BspDI, BspEI, BspHI, BspMI, BspQI, BsrBI, BsrDI, BsrFI, BsrGI, BsrI, BssHII, BssKI, BssSI, BstAPI, BstBI, BstEII, BstNI, BstUI, BstXI, BstYI, BstZ17I, Bsu36I, BtgI, BtgZI, BtsCI, BtsI, Cac8I, ClaI, CspCI, CviAII, CviKI-1, CviQI, DdeI, DpnI, DpnII, DraI, DraIII, DrdI, EaeI, EagI, EarI, EciI, Eco53kI, EcoNU, EcoO109I, EcoP15I, EcoRI, EcoRV, FatI, FauI, Fnu4HI, FokI, FseI, FspI, HaeII, HaeIII, HgaI, HhaI, HincII, HincIII, HinfI, HinP1I, HpaI, HpaII, HphI, Hpy166I, Hpy188I, Hpy188III, Hpy99I, HpyAV, HpyCH4III, HpyCH4IV, HpyCH4V, KasI, KpnI, MboI, MboII, MfeI, MluI, MlyI, MmeI, MnlI, MscI, MseI, MslI, MspAlI, MspI, MwoI, NaeI, NatI, Nb.BbvCI,

Nb.BsmI, Nb.BsrDI, Nb.BtsI, NciI, NcoI, NdeI, NgoMIV, NheI, NlaIII, NlaIV, NmeAIII, NotI, NruI, NsiI, NspI, Nt.AiwI, Nt.BbvCI, Nt.BsmAI, Nt.BspQI, Nt.BstNBI, Nt.CviPII, PacI, PaeR7I, PciI, PflFI, PflMI, PhoI, PleI, PmeI, PmlI, PpuMI, PshAI, PsiI, PspGI, PspOMI, PspXI, PstI, PvuI, PvuII, RsaI, RsrII, SacI, SacII, SalI, SapI, Sau3AI, Sau96I, SbfI, ScaI, ScrFI, SexAI, SfaNI, SfcI, SfcI, SfoI, SgrAI, SmaI, SmlI, SnaBI, SpeI, SphI, SspI, StuI, StyD4I, StyO, SwaI, T, TaqαI, TfiI, TliI, TseI, Tsp45I, Tsp509I, TspMI, TspRI, Tth111I, XbaI, XcmI, XhoI, XmaI, XmnI, or ZraI

In some embodiments, the fragmentation is mechanical fragmentation. In some embodiments, shear forces created during lysis or extraction can mechanically fragment polynucleotides. Fragmentation can be accomplished by, e.g, sonication, heat treatment, or shearing. In some embodiments, mechanical fragmentation is by nebulization.

In some embodiments, the endonuclease is a methylation sensitive restriction enzyme. In some embodiments, the methylation sensitive restriction enzyme specifically cleaves methylated polynucleotides. In some embodiments, the methylation sensitive restriction enzyme specifically cleaves unmethylated polynucleotides. A methylation sensitive enzyme can include, e.g., DpnI, Acc65I, KpnI, ApaI, Bsp120I, Bsp143I, MboI, BspOI, NheI, Cfr9I, SmaI, Csp6I, RsaI, Ecl136II, SacI, EcoRII, MvaI, HpaII, MSpJI, LpnPI, FsnEI, DpnII, McrBc, or MspI.

In some embodiments, fragmentation of a polynucleotide is accomplished by introducing one or more noncanonical nucleotides (e.g., dUTP) into a polynucleotide, generating one or more abasic sites by cleaving the base of the non-canonical nucleotide (e.g., using, e.g., Uracil N-Glycosylase (UNG) or Uracil DNA glycosylase (UDG)), and fragmenting the polynucleotide at the one or more abasic sites. The fragmenting can be by an enzymatic agent or a chemical agent. The chemical agent can be, e.g., a polyamine, e.g., N,N'-dimethylethylenediamine (DMED). The enzymatic agent can be, e.g., apurinic/apyrimidinic endonuclease (APE 1). In some embodiments, fragmentation can be accomplished as described in U.S. Patent Application Publication Nos. 20110033854 or 20100022403.

Ligation

Fragmentation can be followed by a step of ligating adaptors to polynucleotides. In some embodiments, a ligation step does not following a fragmentation step. A partition, e.g., an aqueous phase of an emulsion, can comprise a ligase. The ligase can be, e.g., T4 DNA ligase, *E. coli* DNA ligase, Taq DNA ligase, 9° N™ DNA ligase, T4 RNA ligase 1 (ssRNA ligase), T4 RNA ligase 2 (dsRNA ligase), or T4 RNA Ligase 2, truncated (NEB).

A partition, e.g., an aqueous phase of an emulsion, can comprise reagents for a ligation reaction, e.g., buffer, salt, and/or reducing agent. Ligase and other reagents can be supplied in a partition, e.g., an aqueous phase of an emulsion, separate from a partition, e.g., an aqueous phase of an emulsion, comprising polynucleotides. A partition, e.g., an aqueous phase of an emulsion, comprising ligase can be merged with a partition, e.g., an aqueous phase of an emulsion, comprising polynucleotides.

The ligation reaction can occur at a temperature of about, more than about, less than about, or at least about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99° C. The ligation can occur at about 4° C. to about 16° C., about 16° C. to about 25° C., about 25° C. to about 30° C., about 25° C. to about 37° C., about 37° C. to about 45° C., about 37° C. to about 50° C., or about 50° C. to about 65° C.

The ligation reaction can occur for a time period of about, more than about, less than about, or at least about 5 min, 15 min, 30 min, 45 min, or 60 min. The ligation reaction can occur for a time period of about, more than about, less than about, or at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, or 48 hr. The ligation reaction can last for about 5 min to about 15 min, about 5 min to about 30 min, about 5 min to about 45 min, about 5 min to about 60 min, about 30 min to about 60 min, about 30 min to about 90 min, about 30 min to about 120 min, about 1 hr to about 2 hr, about 1 hr to about 6 hr, about 1 hr to about 12 hr, about 12 hr to about 24 hr, or about 12 hr to about 48 hr.

A transposon-based approach such as that provided by NEXTERA™ can be used in which DNA is fragmented and an adaptor can be ligated in a single step reaction (see e.g., http://www.epibio.com/newsletter/16-3_4-6.pdf). A TRANSPOSOME™ complex can comprise free transposon ends and a transposase. A TRANSPOSOME™ complex can be incubated with target double strand DNA, and the target can be fragmented. The transferred strand of a transposon end oligonucleotide can be covalently attached to the 5' end of a target fragment. Transposon integration and strand transfer can occur via a staggered, dsDNA break within a target polynucleotide. The resulting fragments can have single-stranded gaps. The concentration of TRANSPOSOME™ complexes can be varied to control the size distribution of the fragmented and tagged DNA library. The transposon ends can comprise barcodes. Adaptor ligation can be followed with PCR amplification of ligated products to increase their concentrations.

The NEXTERA™ technology can be used to generate di-tagged libraries. The libraries can be optionally barcoded. The libraries can be compatible, e.g., with Roche/454 or ILLUMINA®/SOLEXA® sequencing platforms. To generate platform-specific libraries, free transposon ends or appended transposon ends can be used. Platform specific tags, and optional barcoding, can be introduced by, e.g., PCR. Amplification can occur by, e.g., emulsion PCR (emPCR) or bridge PCR (bPCR).

In some embodiments, the methods of ligating adaptors to polynucleotides are those described in U.S. Pat. No. 5,789,206 or Arneson et al. (2008) Whole-Genome Amplification by Adaptor-Ligation PCR of Randomly Sheared Genomic DNA (PRSG) Cold Spring Harbor Protocols.

Sizes of fragments of polynucleotides that can be generated can be about, more than about, at least about, or less than about 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000, 100,000, 200,000, 300,000, 400,000, 500,000, 600,000, 700,000, 800,000, 900,000, 1,000,000, 2,000,000, 3,000,000, 4,000,000, 5,000,000, 6,000,000, 7,000,000, 8,000,000, 9,000,000, or 10,000,000 bases or base pairs. In some embodiments, the size of fragmented polynucleotides is about 50 to about 100, about 50 to about 150, about 50 to about 200, about 100 to about 150, about 100 to about 200, about 100 to about 300, about 150 to about 200, about 150 to about 250, about 200 to about 300, about 200 to about 400, about 300 to about 400, about 300 to about 500, about 400 to about 500, about 400 to about 600, about 500 to about 600, about 500 to about 700, about 600 to about

700, about 600 to about 800, about 700 to about 800, about 700 to about 900, about 800 to about 1000, about 50 to about 500, about 100 to about 500, about 100 to about 1000, about 50 to about 1500, about 50 to about 2000, about 1000 to about 2000, or about 1500 to about 2000 bases or base pairs. In some embodiments, the size of fragmented polynucleotides is about 1000 to about 5000, about 1000 to about 10,000, about 10,000 to about 20,000, about 10,000 to about 50,000, about 10,000 to about 100,000, about 50,000 to about 100,000, about 100,000 to about 200,000, about 100,000 to about 500,000, about 100,000 to about 1,000,000, about 200,000 to about 1,000,000, about 300,000 to about 1,000,000, about 400,000 to about 1,000,000, about 500,000 to about 1,000,000, or about 750,000 to about 1,000,000 bases or base pairs.

Amplification

Polynucleotides may be amplified before they are partitioned. In some embodiments, polynucleotides are amplified while in a partition (e.g., aqueous phase of an emulsion, e.g., droplet). In some embodiments, polynucleotides are amplified before fragmentation in a partition. In some embodiments, polynucleotides are amplified after fragmentation in a partition. In some embodiments, polynucleotides are amplified both before and after fragmentation in a partition. In some embodiments, polynucleotides are amplified in a partition before ligating an adaptor to a polynucleotide in a partition. In some embodiments, polynucleotides are amplified in a partition after ligating an adaptor to the polynucleotide in the partition. In some embodiments, polynucleotides are amplified after ligating an adaptor to the polynucleotides and pooling polynucleotides from different partitions.

In some embodiments, the amplification comprises polymerase chain reaction (PCR), digital PCR, reverse-transcription PCR, quantitative PCR, real-time PCR, isothermal amplification, linear amplification, or isothermal linear amplification, quantitative fluorescent PCR (QF-PCR), multiplex fluorescent PCR (MF-PCR), single cell PCR, restriction fragment length polymorphism PCR(PCR-RFLP), PCR-RFLP/RT-PCR-RFLP, hot start PCR, nested PCR, in situ polony PCR, in situ rolling circle amplification (RCA), bridge PCR (bPCR), picotiter PCR, digital PCR, droplet digital PCR, or emulsion PCR (emPCR). Other suitable amplification methods include ligase chain reaction (LCR (oligonucleotide ligase amplification (OLA)), transcription amplification, cycling probe technology (CPT), molecular inversion probe (MIP)PCR, self-sustained sequence replication, selective amplification of target polynucleotide sequences, consensus sequence primed polymerase chain reaction (CP-PCR), arbitrarily primed polymerase chain reaction (AP-PCR), transcription mediated amplification (TMA), degenerate oligonucleotide-primed PCR (DOP-PCR), multiple-displacement amplification (MDA), strand displacement amplification (SDA), and nucleic acid based sequence amplification (NABSA). Other amplification methods that can be used herein include those described in U.S. Pat. Nos. 5,242,794; 5,494,810; 4,988,617; and 6,582,938.

In some embodiments, a multiple-displacement amplification (MDA) step can be performed within a partition (e.g., droplet) prior to fragmentation of polynucleotides and adaptor ligation to amplify the amount of DNA in each droplet in order to cover more of the captured polynucleotides. MDA can be a non-PCR based amplification technique that can involve annealing multiple primers (e.g., hexamer primers) to a polynucleotide template, and initiating DNA synthesis (e.g., using Phi 29 polymerase). When DNA synthesis proceeds to the next synthesis starting site, the polymerase can displace the newly produced DNA strand and continues its strand elongation. Strand displacement can generate newly synthesized single stranded DNA template to which other primers can anneal. Further primer annealing and strand displacement on the newly synthesized template can result in a hyper-branched DNA network. The sequence debranching during amplification can result in a high yield of products. To separate the DNA branching network, one or more S1 nucleases can be used to cleave the fragments at displacement sites. The nicks on the resulting DNA fragments can be repaired by DNA polymerase I. The generated DNA fragments can be directly used for analysis or be ligated to generate genomic libraries for further sequencing analysis. MDA is described, e.g., in U.S. Pat. No. 7,074,600.

Amplification of polynucleotides can occur on a bead. In other embodiments, amplification does not occur on a bead. A hot start PCR can be performed wherein the reaction is heated to 95° C. for two minutes prior to addition of the polymerase or the polymerase can be kept inactive until the first heating step in cycle 1. Hot start PCR can be used to minimize nonspecific amplification. Other strategies for and aspects of amplification suitable for use in the methods described herein are described in U.S. Patent Application Publication No. 2010/0173394 A1, published Jul. 8, 2010, which is incorporated herein by reference.

Any number of PCR cycles can be used to amplify the DNA, e.g., about, more than about, at least about, or less than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44 or 45 cycles. The number of amplification cycles can be about 1 to about 45, about 10 to about 45, about 20 to about 45, about 30 to about 45, about 35 to about 45, about 10 to about 40, about 10 to about 30, about 10 to about 25, about 10 to about 20, about 10 to about 15, about 20 to about 35, about 25 to about 35, about 30 to about 35, or about 35 to about 40.

Thermocycling reactions can be performed on samples contained in droplets. The droplets can remain intact during thermocycling. Droplets can remain intact during thermocycling at densities of greater than about 10,000 droplets/mL, 100,000 droplets/mL, 200,000 droplets/mL, 300,000 droplets/mL, 400,000 droplets/mL, 500,000 droplets/mL, 600,000 droplets/mL, 700,000 droplets/mL, 800,000 droplets/mL, 900,000 droplets/mL or 1,000,000 droplets/mL. Droplets can remain intact during thermocycling at densities of greater than about 10,000 droplets/mL to about 100,000 droplets/mL, 10,000 droplets/mL to about 1,000,000 droplets/mL, or about 100,000 droplets/mL to about 1,000,000 droplets/mL. In other cases, two or more droplets may coalesce during thermocycling. In other cases, greater than about 100 or greater than about 1,000 droplets may coalesce during thermocycling.

Polynucleotides

The methods described herein can be used for manipulating and analyzing polynucleotides. The term polynucleotide, or grammatical equivalents, can refer to at least two nucleotides covalently linked together. A nucleic acid described herein can contain phosphodiester bonds, although in some cases, as outlined herein (for example in the construction of primers and probes such as label probes), nucleic acid analogs are included that can have alternate backbones, comprising, for example, phosphoramide (see e.g., Beaucage et al., *Tetrahedron* 49(10):1925 (1993) and references therein; Letsinger, *J. Org. Chem.* 35:3800 (1970); Sprinzl et al., *Eur. J. Biochem.* 81:579 (1977); Letsinger et al., *Nucl. Acids Res.* 14:3487 (1986); Sawai et al, *Chem. Lett.* 805 (1984), Letsinger et al., *J. Am. Chem. Soc.* 110:4470

(1988); and Pauwels et al., *Chemica Scripta* 26:141 91986)), phosphorothioate (Mag et al., *Nucleic Acids Res.* 19:1437 (1991); and U.S. Pat. No. 5,644,048), phosphorodithioate (Briu et al., *J. Chem. Soc.* 111:2321 (1989), O-methyl phosphoroamidate linkages (see e.g., Eckstein, Oligonucleotides and Analogues: A Practical Approach, Oxford University Press), and peptide nucleic acid (also referred to herein as "PNA") backbones and linkages (see e.g., Egholm, *J. Am. Chem. Soc.* 114:1895 (1992); Meier et al., *Chem. Int. Ed. Engl.* 31:1008 (1992); Nielsen, *Nature,* 365:566 (1993); Carlsson et al., *Nature* 380:207 (1996), all of which are incorporated by reference). Other analog nucleic acids include those with bicyclic structures including locked nucleic acids (LNAs are a class of nucleic acid analogues in which the ribose ring is "locked" by a methylene bridge connecting the 2'-O atom with the 4'-C atom), also referred to herein as "LNA" (see e.g., Koshkin et al., *J. Am. Chem. Soc.* 120.13252 3 (1998)); positive backbones (Denpcy et al., *Proc. Natl. Acad. Sci. USA* 92:6097 (1995); non-ionic backbones (see e.g., U.S. Pat. Nos. 5,386,023, 5,637,684, 5,602,240, 5,216,141 and 4,469,863; Kiedrowshi et al., Angew. Chem. Intl. Ed. English 30:423 (1991); Letsinger et al., *J. Am. Chem. Soc.* 110:4470 (1988); Letsinger et al., *Nucleoside & Nucleotide* 13:1597 (1994); Chapters 2 and 3, ASC Symposium Series 580, "Carbohydrate Modifications in Antisense Research", Ed. Y. S. Sanghui and P. Dan Cook; Mesmaeker et al., Bioorganic & Medicinal Chem. Lett. 4:395 (1994); Jeffs et al., J. Biomolecular NMR 34:17 (1994); Tetrahedron Lett. 37:743 (1996)), and non-ribose backbones, including those described in U.S. Pat. Nos. 5,235,033 and 5,034,506, and Chapters 6 and 7, ASC Symposium Series 580, "Carbohydrate Modifications in Antisense Research", Ed. Y. S. Sanghui and P. Dan Cook. Nucleic acids containing one or more carbocyclic sugars are also included within the definition of nucleic acids (see e.g., Jenkins et al., *Chem. Soc. Rev.* (1995) pp 169-176). Several nucleic acid analogs are described in Rawls, *C & E News* Jun. 2, 1997 page 35. All of these references are hereby expressly incorporated by reference. These modifications of the ribose-phosphate backbone can be done to increase the stability and half-life of such molecules in physiological environments. For example, PNA:DNA and LNA-DNA hybrids can exhibit higher stability and thus can be used in some embodiments. The target nucleic acids can be single stranded or double stranded, as specified, or contain portions of both double stranded or single stranded sequence. Depending on the application, the nucleic acids can be DNA (including, e.g., genomic DNA, mitochondrial DNA, and cDNA), RNA (including, e.g., mRNA and rRNA) or a hybrid, where the nucleic acid can contain any combination of deoxyribo- and ribo-nucleotides, and any combination of bases, including uracil, adenine, thymine, cytosine, guanine, inosine, xathanine hypoxathanine, isocytosine, isoguanine, etc.

The methods, compositions, and kits provided herein can be used to analyze polynucleotides (e.g., DNA, RNA, mitochondrial DNA, genomic DNA, mRNA, siRNA, miRNA, cRNA, single-stranded DNA, double-stranded DNA, single-stranded RNA, double-stranded RNA, tRNA, rRNA, cDNA, etc.). The methods, compositions and kits can be used to evaluate a quantity of a first polynucleotide compared to the quantity of a second polynucleotide. The methods can be used to analyze the quantity of synthetic plasmids in a solution; to detect a pathogenic organism (e.g., microbe, bacteria, virus, parasite, retrovirus, lentivirus, HIV-1, HIV-2, influenza virus, etc.) within a sample obtained from a subject or obtained from an environment. The methods also can be used in other applications wherein a rare population of polynucleotides exists within a larger population of polynucleotides. Polynucleotides can be obtained through cloning, e.g., cloning into plasmids, yeast, or bacterial artificial chromosomes. A polynucleotide can be obtained by reverse transcription of isolated mRNA.

In some embodiments, genomic DNA is analyzed. In some embodiments, the genomic DNA is from a mammal, e.g., a human. The genomic DNA can be obtained from normal somatic tissue, germinal tissue, or diseased tissue (e.g., tumor tissue). In some embodiments, about, more than about, at least about, or less than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 genome equivalents are used. A genome equivalent can be the amount of DNA in a single copy of a genome (e.g., a single diploid cell has 2 genome equivalents of DNA). In some embodiments, about 1 to about 10, about 1 to about 15, about 1 to about 20, about 1 to about 25, about 1 to about 30, about 1 to about 35, about 1 to about 40, about 1 to about 45, about 1 to about 50, about 1 to about 55, about 1 to about 60, about 5 to about 10, about 5 to about 15, about 5 to about 20, about 5 to about 25, about 5 to about 30, about 5 to about 35, about 5 to about 40, about 5 to about 45, about 5 to about 50, about 5 to about 55, about 5 to about 60, about 10 to about 20, about 10 to about 30, about 10 to about 40, about 10 to about 50, or about 10 to about 50 genome equivalents are used. In some embodiments, about, more than about, at least about, or less than about 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000, 100,000, 200,000, 300,000, 400,000, 500,000, 600,000, 700,000, 800,000, 900,000, 1,000,000, 2,000,000, 3,000,000, 4,000,000, 5,000,000, 6,000,000, 7,000,000, 8,000,000, 9,000,000, or 10,000,000 genome equivalents are used. In some embodiments, about 100 to about 1000, about 100 to about 10,000, about 100 to about 100,000, about 100 to about 1,000,000, about 100 to about 10,000,000, about 1000 to about 10,000, about 1000 to about 100,000, about 1000 to about 1,000,000, about 1000 to about 10,000,000, about 10,000 to about 100,000, about 10,000 to about 1,000,000, about 10,000 to about 10,000,000, about 100,000 to about 1,000,000, about 100,000 to about 10,000,000, or about 1,000,000 to about 10,000,000 genome equivalents are used.

In some embodiments, polynucleotides are protected from shearing. Additives that can protect polynucleotides from shearing include, e.g., spermidine, spermine, poly(N-vinylpyrrolidone) 40 (PVP40), or $Co(NH_3)_6Cl_3$. In some embodiments, wide pore pipettes are used to avoid shearing of polynucleotides, e.g., when polynucleotides are transferred from one receptacle to another. Methods and compositions for protecting polynucleotides from shearing are described, e.g., in Kovacic et al. (1995) *Nucleic Acids Research* 23: 3999-4000 and Gurrieri S and Bustamante C. (1997) *Biochem J.* 326: 131-138.

The length of polynucleotides, or fragments of polynucleotides, that can be partitioned (e.g., in droplets) as described herein can be about, more than about, at least about, or less than about 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000, 100,000, 200,000, 300,000, 400,000, 500,

000, 600,000, 700,000, 800,000, 900,000, 1,000,000, 2,000,000, 3,000,000, 4,000,000, 5,000,000, 6,000,000, 7,000,000, 8,000,000, 9,000,000, 10,000,000, 20,000,000, 30,000,000, 40,000,000, 50,000,000, 60,000,000, 70,000,000, 80,000,000, 90,000,000, 100,000,000, 110,000,000, 120,000,000, 130,000,000, 140,000,000, 150,000,000, 160,000,000, 170,000,000, 180,000,000, 190,000,000, 200,000,000, 210,000,000, 220,000,000, 230,000,000, 240,000,000, or 250,000,000 nucleotides or base pairs in length.

Individual chromosomes can be separated into individual partitions. Human chromosomes that can be partitioned as described herein can include chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, X or Y.

In some embodiments, gentle processing steps are used to obtain large polynucleotides from a sample. The gentle processing steps can include, e.g., low speed centrifugation, release of genomic DNA using proteinase K and/or RNase digestion, or dialysis. In some embodiments, steps such as vortexing, high speed centrifugation, or ethanol precipitation are not performed.

Next Generation Sequencing

The methods, compositions, and kits described herein can be used with next generation sequence platforms. For example, adaptors with barcodes can be ligated to polynucleotides, different samples of polynucleotides with different barcodes can be pooled, the pooled polynucleotides can be sequenced using next generation sequencing, and barcodes can be used to determine which sequence reads are generated from polynucleotides in the same partition (e.g., droplet).

In some embodiments, the next generation sequencing technique is 454 sequencing (Roche) (see e.g., Margulies, M et al. (2005) *Nature* 437: 376-380). 454 sequencing can involve two steps. In the first step, DNA can be sheared into fragments of approximately 300-800 base pairs, and the fragments can be blunt ended. Oligonucleotide adaptors can then ligated to the ends of the fragments. The adaptors can serve as sites for hybridizing primers for amplification and sequencing of the fragments. The fragments can be attached to DNA capture beads, e.g., streptavidin-coated beads using, e.g., Adaptor B, which can contain 5'-biotin tag. The fragments can be attached to DNA capture beads through hybridization. A single fragment can be captured per bead. The fragments attached to the beads can be PCR amplified within droplets of an oil-water emulsion. The result can be multiple copies of clonally amplified DNA fragments on each bead. The emulsion can be broken while the amplified fragments remain bound to their specific beads. In a second step, the beads can be captured in wells (pico-liter sized; PicoTiterPlate (PTP) device). The surface can be designed so that only one bead fits per well. The PTP device can be loaded into an instrument for sequencing. Pyrosequencing can be performed on each DNA fragment in parallel. Addition of one or more nucleotides can generate a light signal that can be recorded by a CCD camera in a sequencing instrument. The signal strength can be proportional to the number of nucleotides incorporated. Pyrosequencing can make use of pyrophosphate (PPi) which can be released upon nucleotide addition. PPi can be converted to ATP by ATP sulfurylase in the presence of adenosine 5' phosphosulfate. Luciferase can use ATP to convert luciferin to oxyluciferin, and this reaction can generate light that is detected and analyzed.

In some embodiments, the next generation sequencing technique is SOLiD technology (Applied Biosystems; Life Technologies). In SOLiD sequencing, genomic DNA can be sheared into fragments, and adaptors can be attached to the 5' and 3' ends of the fragments to generate a fragment library. Alternatively, internal adaptors can be introduced by ligating adaptors to the 5' and 3' ends of the fragments, circularizing the fragments, digesting the circularized fragment to generate an internal adaptor, and attaching adaptors to the 5' and 3' ends of the resulting fragments to generate a mate-paired library. Next, clonal bead populations can be prepared in microreactors containing beads, primers, template, and PCR components. Following PCR, the templates can be denatured and beads can be enriched to separate the beads with extended templates. Templates on the selected beads can be subjected to a 3' modification that permits bonding to a glass slide. A sequencing primer can bind to adaptor sequence. A set of four fluorescently labeled di-base probes can compete for ligation to the sequencing primer. Specificity of the di-base probe can be achieved by interrogating every first and second base in each ligation reaction. The sequence of a template can be determined by sequential hybridization and ligation of partially random oligonucleotides with a determined base (or pair of bases) that can be identified by a specific fluorophore. After a color is recorded, the ligated oligonucleotide can be cleaved and removed and the process can be then repeated. Following a series of ligation cycles, the extension product can be removed and the template can be reset with a primer complementary to the n−1 position for a second round of ligation cycles. Five rounds of primer reset can be completed for each sequence tag. Through the primer reset process, most of the bases can be interrogated in two independent ligation reactions by two different primers. Up to 99.99% accuracy can be achieved by sequencing with an additional primer using a multi-base encoding scheme.

In some embodiments, the next generation sequencing technique is SOLEXA sequencing (Illumina sequencing). SOLEXA sequencing can be based on the amplification of DNA on a solid surface using fold-back PCR and anchored primers. SOLEXA sequencing can involve a library preparation step. Genomic DNA can be fragmented, and sheared ends can be repaired and adenylated. Adaptors can be added to the 5' and 3' ends of the fragments. The fragments can be size selected and purified. SOLEXA sequence can comprise a cluster generation step. DNA fragments can be attached to the surface of flow cell channels by hybridizing to a lawn of oligonucleotides attached to the surface of the flow cell channel. The fragments can be extended and clonally amplified through bridge amplification to generate unique clusters. The fragments become double stranded, and the double stranded molecules can be denatured. Multiple cycles of the solid-phase amplification followed by denaturation can create several million clusters of approximately 1,000 copies of single-stranded DNA molecules of the same template in each channel of the flow cell. Reverse strands can be cleaved and washed away. Ends can be blocked, and primers can by hybridized to DNA templates. SOLEXA sequencing can comprise a sequencing step. Hundreds of millions of clusters can be sequenced simultaneously. Primers, DNA polymerase and four fluorophore-labeled, reversibly terminating nucleotides can be used to perform sequential sequencing. All four bases can compete with each other for the template. After nucleotide incorporation, a laser is used to excite the fluorophores, and an image is captured and the identity of the first base is recorded. The 3' terminators and fluorophores from each incorporated base are removed and the incorporation, detection and identification steps are repeated. A single base can be read each cycle.

In some embodiments, the next generation sequencing technique comprises real-time (SMRT™) technology by Pacific Biosciences. In SMRT, each of four DNA bases can be attached to one of four different fluorescent dyes. These dyes can be phospholinked. A single DNA polymerase can be immobilized with a single molecule of template single stranded DNA at the bottom of a zero-mode waveguide (ZMW). A ZMW can be a confinement structure which enables observation of incorporation of a single nucleotide by DNA polymerase against the background of fluorescent nucleotides that can rapidly diffuse in an out of the ZMW (in microseconds). It can take several milliseconds to incorporate a nucleotide into a growing strand. During this time, the fluorescent label can be excited and produce a fluorescent signal, and the fluorescent tag can be cleaved off. The ZMW can be illuminated from below. Attenuated light from an excitation beam can penetrate the lower 20-30 nm of each ZMW. A microscope with a detection limit of 20 zeptoliters (10^{-21} liters) can be created. The tiny detection volume can provide 1000-fold improvement in the reduction of background noise. Detection of the corresponding fluorescence of the dye can indicate which base was incorporated. The process can be repeated.

In some embodiments, the next generation sequencing is nanopore sequencing (See e.g., Soni G V and Meller A. (2007) *Clin Chem* 53: 1996-2001). A nanopore can be a small hole, of the order of about one nanometer in diameter. Immersion of a nanopore in a conducting fluid and application of a potential across it can result in a slight electrical current due to conduction of ions through the nanopore. The amount of current which flows can be sensitive to the size of the nanopore. As a DNA molecule passes through a nanopore, each nucleotide on the DNA molecule can obstruct the nanopore to a different degree. Thus, the change in the current passing through the nanopore as the DNA molecule passes through the nanopore can represent a reading of the DNA sequence. The nanopore sequencing technology can be from Oxford Nanopore Technologies; e.g., a GridION system. A single nanopore can be inserted in a polymer membrane across the top of a microwell. Each microwell can have an electrode for individual sensing. The microwells can be fabricated into an array chip, with 100,000 or more microwells per chip. An instrument (or node) can be used to analyze the chip. Data can be analyzed in real-time. One or more instruments can be operated at a time. The nanopore can be a protein nanopore, e.g., the protein alpha-hemolysin, a heptameric protein pore. The nanopore can be a solid-state nanopore made, e.g., a nanometer sized hole formed in a synthetic membrane (e.g., SiN_x, or SiO_2). The nanopore can be a hybrid pore (e.g., an integration of a protein pore into a solid-state membrane. The nanopore can be a nanopore with an integrated sensors (e.g., tunneling electrode detectors, capacitive detectors, or graphene based nano-gap or edge state detectors (see e.g., Garaj et al. (2010) *Nature* vol. 67, doi:10.1038/nature09379)). A nanopore can be functionalized for analyzing a specific type of molecule (e.g., DNA, RNA, or protein). Nanopore sequencing can comprise "strand sequencing" in which intact DNA polymers can be passed through a protein nanopore with sequencing in real time as the DNA translocates the pore. An enzyme can separate strands of a double stranded DNA and feed a strand through a nanopore. The DNA can have a hairpin at one end, and the system can read both strands. In some embodiments, nanopore sequencing is "exonuclease sequencing" in which individual nucleotides can be cleaved from a DNA strand by a processive exonuclease, and the nucleotides can be passed through a protein nanopore. The nucleotides can transiently bind to a molecule in the pore (e.g., cyclodextran). A characteristic disruption in current can be used to identify bases. Nanopore sequencing technology from GENIA or NABsys can be used. In GENIA's technology, an engineered protein pore can be embedded in a lipid bilayer membrane, and "Active Control" technology can enable efficient nanopore-membrane assembly and control of DNA movement through the channel. In some embodiments, the next generation sequencing comprises ion semiconductor sequencing (e.g., using technology from Life Technologies (Ion Torrent)). Ion semiconductor sequencing can take advantage of the fact that when a nucleotide is incorporated into a strand of DNA, an ion can be released. To perform ion semiconductor sequencing, a high density array of micro-machined wells can formed. Each well can hold a single DNA template. Beneath the well can be an ion sensitive layer, and beneath the ion sensitive layer can be an ion sensor. When a nucleotide is added to a DNA, H+ is released, when can be measured as a change in pH. The H+ ion can be converted to voltage and recorded by the semiconductor sensor. An array chip can be sequentially flooded with one nucleotide after another. No scanning, light, or cameras can be required.

In some embodiments, the next generation sequencing is DNA nanoball sequencing (as performed, e.g., by Complete Genomics; see e.g., Drmanac et al. (2010) *Science* 327: 78-81). DNA can be isolated, fragmented, and size selected. For example, DNA can be fragmented (e.g., by sonication) to a mean length of about 500 bp. Adaptors (Ad1) can be attached to the ends of the fragments. The adaptors can be used to hybridize to anchors for sequencing reactions. DNA with adaptors bound to each end can be PCR amplified. The adaptor sequences can be modified so that complementary single strand ends bind to each other forming circular DNA. The DNA can be methylated to protect it from cleavage by a Type IIS restriction enzyme used in a subsequent step. An adaptor (e.g., the right adaptor) can have a restriction recognition site, and the restriction recognition site can remain non-methylated. The non-methylated restriction recognition site in the adaptor can be recognized by a restriction enzyme (e.g., AcuI), and the DNA can be cleaved by AcuI 13 bp to the right of the right adaptor to form linear double stranded DNA. A second round of right and left adaptors (Ad2) can be ligated onto either end of the linear DNA, and all DNA with both adaptors bound can be PCR amplified (e.g., by PCR). Ad2 sequences can be modified to allow them to bind each other and form circular DNA. The DNA can be methylated, but a restriction enzyme recognition site can remain non-methylated on the left Ad1 adaptor. A restriction enzyme (e.g., AcuI) can be applied, and the DNA can be cleaved 13 bp to the left of the Ad1 to form a linear DNA fragment. A third round of right and left adaptor (Ad3) can be ligated to the right and left flank of the linear DNA, and the resulting fragment can be PCR amplified. The adaptors can be modified so that they can bind to each other and form circular DNA. A type III restriction enzyme (e.g., EcoP15) can be added; EcoP15 can cleave the DNA 26 bp to the left of Ad3 and 26 bp to the right of Ad2. This cleavage can remove a large segment of DNA and linearize the DNA once again. A fourth round of right and left adaptors (Ad4) can be ligated to the DNA, the DNA can be amplified (e.g., by PCR), and modified so that they bind each other and form the completed circular DNA template. Rolling circle replication (e.g., using Phi 29 DNA polymerase) can be used to amplify small fragments of DNA. The four adaptor sequences can contain palindromic sequences that can hybridize and a single strand can fold onto itself to form a

DNA nanoball (DNB™) which can be approximately 200-300 nanometers in diameter on average. A DNA nanoball can be attached (e.g., by adsorption) to a microarray (sequencing flowcell). The flow cell can be a silicon wafer coated with silicon dioxide, titanium and hexamethyldisilazane (HMDS) and a photoresist material. Sequencing can be performed by unchained sequencing by ligating fluorescent probes to the DNA. The color of the fluorescence of an interrogated position can be visualized by a high resolution camera. The identity of nucleotide sequences between adaptor sequences can be determined.

In some embodiments, the next generation sequencing technique is Helicos True Single Molecule Sequencing (tSMS) (see e.g., Harris T. D. et al. (2008) *Science* 320:106-109). In the tSMS technique, a DNA sample can be cleaved into strands of approximately 100 to 200 nucleotides, and a polyA sequence can be added to the 3' end of each DNA strand. Each strand can be labeled by the addition of a fluorescently labeled adenosine nucleotide. The DNA strands can then be hybridized to a flow cell, which can contain millions of oligo-T capture sites immobilized to the flow cell surface. The templates can be at a density of about 100 million templates/cm^2. The flow cell can then be loaded into an instrument, e.g., HELISCOPE™ sequencer, and a laser can illuminate the surface of the flow cell, revealing the position of each template. A CCD camera can map the position of the templates on the flow cell surface. The template fluorescent label can then be cleaved and washed away. The sequencing reaction can begin by introducing a DNA polymerase and a fluorescently labeled nucleotide. The oligo-T nucleic acid can serve as a primer. The DNA polymerase can incorporate the labeled nucleotides to the primer in a template directed manner. The DNA polymerase and unincorporated nucleotides can be removed. The templates that have directed incorporation of the fluorescently labeled nucleotide can be detected by imaging the flow cell surface. After imaging, a cleavage step can remove the fluorescent label, and the process can be repeated with other fluorescently labeled nucleotides until a desired read length is achieved. Sequence information can be collected with each nucleotide addition step. The sequencing can be asynchronous. The sequencing can comprise at least 1 billion bases per day or per hour.

In some embodiments, the sequencing technique can comprise paired-end sequencing in which both the forward and reverse template strand can be sequenced. In some embodiments, the sequencing technique can comprise mate pair library sequencing. In mate pair library sequencing, DNA can be fragments, and 2-5 kb fragments can be end-repaired (e.g., with biotin labeled dNTPs). The DNA fragments can be circularized, and non-circularized DNA can be removed by digestion. Circular DNA can be fragmented and purified (e.g., using the biotin labels). Purified fragments can be end-repaired and ligated to sequencing adaptors.

In some embodiments, a sequence read is about, more than about, less than about, or at least about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 525, 550, 575, 600, 625, 650, 675, 700, 725, 750, 775, 800, 825, 850, 875, 900, 925, 950, 975, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, or 3000 bases. In some embodiments, a sequence read is about 10 to about 50 bases, about 10 to about 100 bases, about 10 to about 200 bases, about 10 to about 300 bases, about 10 to about 400 bases, about 10 to about 500 bases, about 10 to about 600 bases, about 10 to about 700 bases, about 10 to about 800 bases, about 10 to about 900 bases, about 10 to about 1000 bases, about 10 to about 1500 bases, about 10 to about 2000 bases, about 50 to about 100 bases, about 50 to about 150 bases, about 50 to about 200 bases, about 50 to about 500 bases, about 50 to about 1000 bases, about 100 to about 200 bases, about 100 to about 300 bases, about 100 to about 400 bases, about 100 to about 500 bases, about 100 to about 600 bases, about 100 to about 700 bases, about 100 to about 800 bases, about 100 to about 900 bases, or about 100 to about 1000 bases.

In some embodiments, the sequencing depth is about, more than about, at least about, or less than about 1×, 2×, 3×, 4×, 5×, 6×, 7×, 8×, 9×, 10×, 11×, 12×, 13×, 14×, 15×, 16×, 17×, 18×, 19×, 20×, 21×, 22×, 23×, 24×, 25×, 26×, 27×, 28×, 29×, 30×, 31×, 32×, 33×, 34×, 35×, 36×, 37×, 38×, 39×, 40×, 41×, 42×, 43×, 44×, 45×, 46×, 47×, 48×, 49×, 50×, 51×, 52×, 53×, 54×, 55×, 56×, 57×, 58×, 59×, 60×, 61×60×, 61×, 63×, 64×, 65×, 66×, 67×, 68×, 69×, 70×, 71×, 72×, 73×, 74×, 75×, 76×, 77×, 78×, 79×, 80×, 81×, 82×, 83×, 84×, 85×, 86×, 87×, 88×, 89×, 90×, 91×, 92×, 93×, 94×, 95×, 96×, 97×, 98×, 99×, 100×, 110×, 120×, 130×, 140×, 150×, 160×, 170×, 180×, 190×, 200×, 210×, 220×, 230×, 240×, 250×, 260×, 270×, 280×, 290×, 300×, 350×, 400×, 450×, 500×, 550×, 600×, 650×, 700×, 750×, 800×, 850×, 900×, 950×, 1000×, 2000×, 3000×, 4000×, 5000×, 6000×, 7000×, 8000×, 9000×, 10,000×. In some embodiments, the sequencing depth is about 1× to about 4×, about 1× to about 5×, about 1× to about 8×, about 1× to about 10×, about 2× to about 4×, about 2× to about 8×, about 2× to about 10×, about 5× to about

10×, about 3× to about 6×, about 10× to about 15×, about 10× to about 20×, about 15× to about 20×, about 15× to about 25×, about 15× to about 30×, about 20× to about 30×, about 25× to about 30×, about 25× to about 50×, about 25× to about 75×, about 25× to about 100×, about 50× to about 100×, about 100× to about 200×, about 100× to about 500×, about 100× to about 1000×, about 200× to about 500×, about 500× to about 750×, about 500× to about 1000×, about 750× to about 1000×, about 1000× to about 2000×, about 1000× to about 5000×, about 1000× to about 10,000×, about 2000× to about 5000×, or about 5000× to about 10,000×. Depth of sequencing can be the number of times a sequence (e.g., a genome) is sequenced. In some embodiments, the Lander/Waterman equation is used for computing coverage. The general equation can be: C=LN/G, where C=coverage; G=haploid genome length; L=read length; and N=number of reads.

Applications

Long Reads, Phasing and De Novo Sequencing

In some embodiments, the methods, compositions, and kits described herein can be used for haplotype phasing. In some embodiments, short read sequencers, such as those made by Illumina and ABI, can be unable to provide phasing information. These sequencers can produce reads of 100-200 bases and as short as 30 bases. 454 sequencing (Roche) can produce sequence reads of about 400 bases. In some embodiments, 400 bases can be too short to yield sufficient phasing information. Sequencing using technology from Pacific Biosciences can produce sequence reads of about 1000 bases. In some embodiments, 1000 bases is too short to provide phasing information.

Short sequence reads can make it challenging to sequence a large genome de-novo. Short sequence reads can make it difficult to determine phasing information for all but a very small fraction of polymorphisms. The partitioning and barcoding schemes described herein can be used to reconstruct longer reads using long range assembly and supply phasing information while making use of existing sequencing approaches.

Next generation sequencing platforms can entail a library preparation step. Genomic DNA can be fragmented, optionally sized, and ligated to nucleic acid sequence (e.g., an adaptor) that can provide hybridization sites for a common set of primers. A common set of primers can be used for massive clonal amplification, e.g., in solution or on a solid support. In some embodiments, these clones can then be sequenced because the presence of a massive amount of identical sequence in a tightly confined space can allow for the amplification of a fluorescent (or other) signal emitted by the sequencing reaction.

Tag sequences can be appended to regions that serve as binding sites for primers so that a common barcode can be ligated to every sequence from a particular sample. Libraries from different samples can be mixed and sequenced in a single run. Because every read can contain a barcode, it can be determined which sample produced any given sequence read. This process can be known as sample multiplexing and can allow for much more cost effective pricing per sample for many sequencing applications. In some embodiments, part of every sequence read includes barcode sequence.

In some embodiments, a high molecular weight DNA sample can be partitioned so that a given partition is unlikely to contain two fragments from the same locus but different chromosomes. In some embodiments, high molecular weight DNA can comprise polynucleotides of greater than about 10,000, 100,000, 1,000,000, 10,000,000, 100,000,000, or 200,000,000 bases or base pairs. In some embodiments, polynucleotides are separated such that it is a rare event to have any given region of a genome of both a maternal and paternal polynucleotide in the same partition. In some embodiments, less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.1%, 0.01%, or 0.001% of partitions have two fragments from the same locus but from different chromosomes. In some embodiments, a sample is partitioned such that about, or less than about 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.1%, 0.01%, or 0.001% of a haploid genome is found per partition (e.g., droplet). In some embodiments, a sample is partitioned such that about 0.1% to about 1%, about 0.5% to about 1%, about 0.25% to about 0.75%, about 1% to about 5%, about 1% to about 2%, about 1% to about 10%, or about 5% to about 10% of a haploid genome is found per partition (e.g., droplet).

Library preparation can be performed within partitions (e.g., droplets) as described herein. Sequence reads that map somewhat close to each other in a genome and are determined to be from the same partition (e.g., in the same droplet) are likely linked to each other and thus reside on the same chromosome. In this fashion individual, short reads can be strung together into longer sequence fragments. See e.g., Example 1.

Single Cell Analysis

In some embodiments, the methods and compositions described herein can be used to analyze cells, e.g., individual cells. For example, individual cells can be separated into unique partitions, uniquely barcoded adaptors can be added to each partition, polynucleotides, or fragments of polynucleotides, within each partition can be barcoded by ligating adaptors to the polynucleotides or fragments of polynucleotides, barcoded polynucleotides from each partition can be pooled, the pooled polynucleotides can be sequenced, and barcodes can be used to determine if sequence reads were generated in the same or different partitions, and thus, in the same or different cells. In some embodiments, the methods and compositions described herein are used for single cell transcriptome sequencing, single cell genomic sequencing, or single cell methylome sequencing.

There are approximately 210 different types of cells in the human body. The individual cells that are partitioned can be any type of cell in the human body. A cell can be, e.g., a hormone secreting cell, an exocrine secretory epithelial cell, a keratinizing epithelial cell, a wet stratified barrier epithelial cell, a sensory transducer cell, an autonomic neuron cell, a sense organ or peripheral neuron supporting cell, a central nervous system neuron or glial cell, a lens cell, a metabolism or storage cell, a kidney cell, an extracellular matrix cell, a contractile cell, a blood or immune system cell, a pigment cell, a germ cell, a nurse cell, or an interstitial cell. The blood or immune system cell can be, e.g., erythrocyte (red blood cell), megakaryocyte (platelet precursor), monocyte, connective tissue macrophage, epidermal Langerhans cell, osteoclast, dendritic cell, microglial cell, neutrophil granulocyte, eosinophil granulocyte, basophil granulocyte, mast cell, Helper T cell, Suppressor T cell, Cytotoxic T cell, Natural Killer T cell, B cell, Natural killer cell, reticulocyte, or stem cell.

Individual cells can be from other types of samples described herein.

In some embodiments, the individual cell is from an environmental sample. In some embodiments, an environmental sample is separated into a plurality of partitions. The environmental sample can be, e.g., air, water, agricultural, or soil. The environmental sample can be, e.g., from a creek, river, pond, lake, lagoon, run, delta, marsh, salt marsh,

swamp, mangrove swamp, mill pond, moat, sea, barachois, basin, bayou, beck, boil, canal, cove, estuary, gulf, harbor, inlet, ocean, bay, sewage treatment facility, slough, sound, spring, stream, tide pool, wash, wetland, Superfund site, coal mine, farm, field, desert, glacier, mountain, or mere. In some embodiments, a sample is from a pool (e.g., swimming pool), gymnasium, school, workplace, office, lobby, elevator, restroom, hospital, medical office, ventilation shaft, or restaurant. In some embodiments, an environmental sample can be from a surface, e.g., floor, table, skin, keyboard, computer, laptop, crime screen evidence (e.g., a weapon, e.g., gun or knife), or doorknob. In some embodiments, the sample is from a bioterrorist attack. In some embodiments, the sample comprises bacteria and/or viruses. In some embodiments, the sample comprises about, at least about, more than about, or less than about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000, or 100,000 different species and/or types of viruses. In some embodiments, and environmental sample comprises about 10 to about 100, about 10 to about 1000, about 100 to about 1000, about 100 to about 10,000, about 1000 to 10,000, about 10,000 to about 50,000, or about 10,000 to about 100,000 different species and/or types of viruses.

Single Cell Transcriptome Sequencing

In one aspect, single cells can be captured within separate partitions (e.g., droplets), and the single cells can be lysed. Messenger RNA from the individual cells in each partition (e.g., droplet) can be reverse transcribed with partition-specific barcoded primers. In some embodiments, the appropriate reagents (e.g., reverse transcriptase, nucleotides) can be sequestered in a partition (e.g., droplet) that is inside a larger droplet. The inner droplet can be burst (e.g., by heating) when desired to allow the reverse transcriptase to contact the messenger RNA. A reverse transcription (RT) reaction can be followed by library prep, which can incorporate unique barcodes.

Calculations for the number of droplets and barcodes to be used in single cell transcriptome sequencing can be similar to those described in Example 1 for analyzing phasing. For example, for analyzing 2,000 cells, sufficient partitioning can be performed to capture each cell in a separate droplet. For example, the 2,000 cells can be partitioned among 20,000 partitions (e.g., droplets). Steps can be taken to ensure that each of the partitions (e.g., droplets) with cells receives a unique barcode (e.g., on adaptors). This goal can be accomplished, e.g., by using 10,000 different barcodes.

After partitioning, lysing, barcoding, and sequencing, sequence read data can be analyzed to determine which transcripts came from the same cell. In this way, the massive capacity of next generation sequencing can be applied to large collections of cells while preserving single cell resolution.

Single Cell Genomic Sequencing

In some instances, individual cells can be captured in separate partitions (e.g., droplets), and genomic DNA from a partition with a single cell can be uniquely barcoded (e.g., using adaptors). Barcoded genomic DNA from different partitions can be pooled and sequenced, and the barcodes can be used to determine which sequence reads are from the same cell. In some embodiments, genomic DNA is fragmented in a partition. In some embodiments, genomic DNA is amplified before and/or after adding adaptors with barcodes. In some embodiments, genomic DNA is not amplified before or after adaptors are ligated to the genomic DNA.

In some embodiments, the sequence coverage per cell can be shallow (e.g., few reads per locus). In some embodiments, single cell genomic DNA sequencing can be used to determine copy number variation (CNV).

In some embodiments, MDA is performed within a droplet on a cell's genome prior to fragmentation and adaptor ligation. In some embodiments, performing MDA can provide more genetic material from a cell to sequence. In some embodiments, MDA may introduce bias. In some embodiments, amplification may result in loss of some copy number variation (CNV) information. In some embodiments, MDA is not performed within a droplet on a cell's genome prior to fragmentation and adaptor ligation.

Single Cell Methylome Sequencing

In some embodiments, methods and compositions described herein can be used for analyzing genomic methylation. For example, methods described herein can be used for single cell methylome sequencing. In some embodiments, individual cells are partitioned, e.g., into droplets. The partitions can be comprised of methyl-sensitive enzymes (e.g., endonucleases). In some embodiments, the methyl-sensitive enzymes digest methylated sites. Each of the partitions can comprise uniquely barcoded adaptors. For example, partitions (e.g., droplets) comprising methyl-sensitive enzymes are merged with partitions comprising sample polynucleotides. The adaptors can be ligated to polynucleotides in the partition before or after digestion with the methyl-sensitive enzyme. Barcode tagged polynucleotides can be pooled, and the polynucleotides can be sequenced. Sequence reads from the same partition can be determined. Absence of sequence reads can indicate digestion of polynucleotides in a partition. In some embodiments, the methyl-sensitive enzymes do not digest methylated DNA, but digest unmethylated DNA.

Genomic Methylation

In some embodiments, methods and compositions described herein can be used for genomic methylation analysis. In some embodiments, polynucleotides can be treated with bisulfite. Bifsulfite can convert unmethylated cytosines to uracil. Bisulfite does not convert methylated cytosines to uracil. Treated and untreated polynucleotides can be partitioned into a plurality of partitions (e.g., droplets). Polynucleotides can be fragmented in the partitions. Uniquely barcoded adaptors can be provided to each partition and ligated to bisulfite treated polynucleotides. The tagged polynucleotides can be pooled and sequenced to determine the methylation status of nucleic acids from the same and different partitions.

Exosome Sequencing

Exosomes are generally organelles such as small extracellular vesicles that can contain RNA. Exosomes can contain mRNA and/or miRNA. In some embodiments, individual exosomes are partitioned into separate partitions (e.g., droplets). Exosomes can be partitioned such that on average, each partition comprises less than about five, four, three, two, or 1 exosomes. Reverse transcription can be used to convert RNA in the exosome into cDNA. Uniquely barcoded adaptors can be added to polynucleotides from a partitioned exosome. Polynucleotides from the partitions can be pooled, the pooled polynucleotides can be sequenced, and the barcodes can be used to determine which sequence

reads were derived from the same exosome. Other types of organelles that can be analyzed can include mitochondria (e.g., mitochondrial DNA can be analyzed).

Metagenomics Sequencing

In another aspect, the methods and compositions described herein can be used for metagenomic analysis. Metagenomics can be the study of genetic material in an environmental sample. In some embodiments, individual viruses and/or bacteria in a sample, e.g., an environmental sample, can be partitioned into a plurality of partitions, adaptors with unique barcodes can be added to each partition, and individual organisms or viruses can have their genomes and/or transcriptomes sequenced. Sequence reads with the same barcode can be assembled to determine the sequence of genomes or transcriptomes of the organisms and/or viruses.

Microfluidics

In another aspect, a microfluidics device can be devised that can partition a sample comprising cells so that every cell ends up in a unique partition (e.g., chamber) with its own set of barcodes. The contents of each chamber can then be processed separately to dilute and further partition (e.g., through an emulsion) in order to enable whole genome or transcriptome amplification separately for each cell. Whole genome amplification or other amplification schemes can benefit from partitioning because of a reduction in competition between different parts of the genome or transcriptome.

Slugs

In another aspect, slugs can be made to capture individual cells and supply them with their own barcodes (e.g., by ligating adaptors with unique barcodes). Slugs can be serial slugs of reagent that completely fill the diameter of a flow tube. Those slugs can be broken into many (e.g., thousands or more) smaller droplets in order to perform unbiased whole genome/transcriptome amplification in droplets. In some embodiments, a slug can be broken down into about, at least about, more than about, or less than about 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, or 10,000 droplets. In some embodiments, a slug can be broken down into about 100 to about 500, about 100 to about 1000, about 500 to about 1000, about 1000 to about 1500, about 1000 to about 2000, about 1000 to about 5000, about 1000 to about 10,000, or about 5,000 to about 10,000 droplets. In some embodiments, whole genome amplification can work better in droplets than in bulk. The droplets from all the slugs can be mixed together because they are already furnished with adaptors with unique barcodes. Sequencing information can be used to determine which reads came from which slug.

Protein Expression and Nucleic Acid Information

In another embodiment, methods described herein can be used to capture cells with specific cell surface markers and analyze polynucleotides (e.g., DNA or RNA) from the cells. In some embodiments, antibodies can be linked to beads coated with short DNA fragments with a unique barcode. Each antibody can be associated with its own unique sequence. The antibodies could also be linked to droplets containing DNA fragments—which can be burst as appropriate. Cells can be pre-coated with these antibodies, then captured in larger droplets along with droplet/cell-specific barcode adaptors. Library prep can ensue as described herein, contents of the droplets can be sequenced, and it can be inferred which reads came from which cell by the barcodes. Thus, this technique allows one to, in addition to sequencing a cell's genome or transcriptome, obtain information about their proteins. In some embodiments, some of the same information can be captured via FACS.

An antibody can include polyclonal and monoclonal antibodies, as well as antigen binding fragments of such antibodies. An antibody, or antigen binding fragment of such an antibody, can be characterized by having specific binding activity for a polypeptide or a peptide portion thereof of at least about 1×10^5 M^{-1}. Fab, $F(ab')_2$, Fd, Fv, single chain Fv (scFv) fragments of an antibody and the like, which retain specific binding activity for a polypeptide, can be used. Specific binding activity of an antibody for a polypeptide can be readily determined by one skilled in the art, for example, by comparing the binding activity of an antibody to a particular polypeptide versus a control polypeptide that is not the particular polypeptide. Methods of preparing polyclonal or monoclonal antibodies are well known to those skilled in the art (see, for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1988)).

An antibody can include naturally occurring antibodies as well as non-naturally occurring antibodies, including, for example, single chain antibodies, chimeric, bifunctional and humanized antibodies, as well as antigen-binding fragments thereof. Such non-naturally occurring antibodies can be constructed using solid phase peptide synthesis, can be produced recombinantly or can be obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and variable light chains as described by Huse et al. (Science 246:1275-1281 (1989)). These and other methods of making functional antibodies are well known to those skilled in the art (Winter and Harris, Immunol. Today 14:243-246 (1993); Ward et al., Nature 341:544-546 (1989); Harlow and Lane, supra, 1988); Hilyard et al., Protein Engineering: A practical approach (IRL Press 1992); Borrabeck, Antibody Engineering, 2d ed. (Oxford University Press 1995)).

Samples

Samples to be analyzed using the methods, compositions, and kits provided herein can be derived from a non-cellular entity comprising nucleic acid (e.g., a virus) or from a cell-based organism (e.g., member of archaea, bacteria, or eukarya domains). A sample can be obtained in some cases from a hospital, laboratory, clinical or medical laboratory. The sample can comprise nucleic acid, e.g., RNA or DNA. The sample can comprise cell-free nucleic acid. In some cases, the sample is obtained from a swab of a surface, such as a door or bench top.

The sample can from a subject, e.g., a plant, fungi, eubacteria, archaeabacteria, protest, or animal. The subject may be an organism, either a single-celled or multi-cellular organism. The subject may be cultured cells, which may be primary cells or cells from an established cell line, among others. The sample may be isolated initially from a multicellular organism in any suitable form. The animal can be a fish, e.g., a zebrafish. The animal can be a bird, e.g., a chicken. The animal can be a mammal. The mammal can be, e.g., a dog, cat, horse, cow, mouse, rat, or pig. The mammal can be a primate, e.g., a human, chimpanzee, orangutan, or gorilla. The human can be a male or female. The sample can be from a human embryo or human fetus. In some embodiments, the human can be an infant, child, teenager, adult, or elderly person. The female can be pregnant, can be suspected of being pregnant, or planning to become pregnant. In some embodiments, the sample is from a plant. In some embodiments, the samples comprises one or more viruses.

The sample can be from a subject (e.g., human subject) who is healthy. In some embodiments, the sample is taken

from a subject (e.g., an expectant mother) at at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26 weeks of gestation. In some embodiments, the subject is affected by a genetic disease, a carrier for a genetic disease or at risk for developing or passing down a genetic disease, where a genetic disease is any disease that can be linked to a genetic variation such as mutations, insertions, additions, deletions, translocation, point mutation, trinucleotide repeat disorders and/or single nucleotide polymorphisms (SNPs).

The sample can be from a subject who has a specific disease, disorder, or condition, or is suspected of having (or at risk of having) a specific disease, disorder or condition. For example, the sample can be from a cancer patient, a patient suspected of having cancer, or a patient at risk of having cancer. The cancer can be, e.g., acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), adrenocortical carcinoma, Kaposi Sarcoma, anal cancer, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, osteosarcoma, malignant fibrous histiocytoma, brain stem glioma, brain cancer, craniopharyngioma, ependymoblastoma, ependymoma, medulloblastoma, medulloepithelioma, pineal parenchymal tumor, breast cancer, bronchial tumor, Burkitt lymphoma, Non-Hodgkin lymphoma, carcinoid tumor, cervical cancer, chordoma, chronic lymphocytic leukemia (CLL), chromic myelogenous leukemia (CML), colon cancer, colorectal cancer, cutaneous T-cell lymphoma, ductal carcinoma in situ, endometrial cancer, esophageal cancer, Ewing Sarcoma, eye cancer, intraocular melanoma, retinoblastoma, fibrous histiocytoma, gallbladder cancer, gastric cancer, glioma, hairy cell leukemia, head and neck cancer, heart cancer, hepatocellular (liver) cancer, Hodgkin lymphoma, hypopharyngeal cancer, kidney cancer, laryngeal cancer, lip cancer, oral cavity cancer, lung cancer, non-small cell carcinoma, small cell carcinoma, melanoma, mouth cancer, myelodysplastic syndromes, multiple myeloma, medulloblastoma, nasal cavity cancer, paranasal sinus cancer, neuroblastoma, nasopharyngeal cancer, oral cancer, oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, papillomatosis, paraganglioma, parathyroid cancer, penile cancer, pharyngeal cancer, pituitary tumor, plasma cell neoplasm, prostate cancer, rectal cancer, renal cell cancer, rhabdomyosarcoma, salivary gland cancer, Sezary syndrome, skin cancer, nonmelanoma, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, testicular cancer, throat cancer, thymoma, thyroid cancer, urethral cancer, uterine cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenstrom Macroglobulinemia, or Wilms Tumor. The sample can be from the cancer and/or normal tissue from the cancer patient.

The sample can be from a subject who is known to have a genetic disease, disorder or condition. In some cases, the subject is known to be wild-type or mutant for a gene, or portion of a gene, e.g., CFTR, Factor VIII (F8 gene), beta globin, hemachromatosis, G6PD, neurofibromatosis, GAPDH, beta amyloid, or pyruvate kinase gene. In some cases, the status of the subject is either known or not known, and the subject is tested for the presence of a mutation or genetic variation of a gene, e.g., CFTR, Factor VIII (F8 gene), beta globin, hemachromatosis, G6PD, neurofibromatosis, GAPDH, beta amyloid, or pyruvate kinase gene.

In other embodiments, the sample is taken from a female patient of child-bearing age and, in some cases, the female patient is not pregnant or of unknown pregnancy status. In still other cases, the subject is a male patient, a male expectant father, or a male patient at risk of, diagnosed with, or having a specific genetic abnormality. In some cases, the female patient is known to be affected by, or is a carrier of, a genetic disease or genetic variation, or is at risk of, diagnosed with, or has a specific genetic abnormality. In some cases, the status of the female patient with respect to a genetic disease or genetic variation may not be known. In further embodiments, the sample is taken from any child or adult patient of known or unknown status with respect to copy number variation of a genetic sequence. In some cases, the child or adult patient is known to be affected by, or is a carrier of, a genetic disease or genetic variation.

The sample can be aqueous humour, vitreous humour, bile, whole blood, blood serum, blood plasma, breast milk, cerebrospinal fluid, cerumen, enolymph, perilymph, gastric juice, mucus, peritoneal fluid, saliva, sebum, semen, sweat, perspiration, tears, vaginal secretion, vomit, feces, or urine. The sample can be obtained from a hospital, laboratory, clinical or medical laboratory. The sample can taken from a subject. The sample can comprise nucleic acid. The nucleic acid can be, e.g., mitochondrial DNA, genomic DNA, mRNA, siRNA, miRNA, cRNA, single-stranded DNA, double-stranded DNA, single-stranded RNA, double-stranded RNA, tRNA, rRNA, or cDNA. The sample can comprise cell-free nucleic acid. The sample can be a cell line, genomic DNA, cell-free plasma, formalin fixed paraffin embedded (FFPE) sample, or flash frozen sample. A formalin fixed paraffin embedded sample can be deparaffinized before nucleic acid is extracted. The sample can be from an organ, e.g., heart, skin, liver, lung, breast, stomach, pancreas, bladder, colon, gall bladder, brain, etc.

In some embodiments, the sample is an environmental sample, e.g., air, water, agricultural, or soil.

When the nucleic acid is RNA, the source of the RNA can be any source described herein. For example, the RNA can a cell-free mRNA, can be from a tissue biopsy, core biopsy, fine needle aspirate, flash frozen, or formalin-fixed paraffin embedded (FFPE) sample. The FFPE sample can be deparaffinized before the RNA is extracted. The extracted RNA can be heated to about, more than about, less than about, or at least about 30, 31, 32, 33, 34, 35, 36, 37 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99° C. before analysis. The extracted RNA can be heated to any of these temperatures for about, or at least about, 15 min, 30 min, 45 min, 60 min, 1.5 hr, 2 hr, 2.5 hr, 3 hr, 3.5 hr, 4 hr, 4.5 hr, 5 hr, 5.5 hr, 6 hr, 6.5 hr, 7 hr, 7.5 hr, 8 hr, 8.5 hr, 9 hr, 9.5 hr, or 10 hr.

RNA can be used for a variety of downstream applications. For example, the RNA can be converted to cDNA with a reverse transcriptase and the cDNA can optionally be subject to PCR, e.g., real-time PCR. The RNA or cDNA can be used in an isothermal amplification reaction, e.g., an isothermal linear amplification reaction. The RNA, resulting cDNA, or molecules amplified therefrom can be used in a microarray experiment, gene expression experiment, Northern analysis, Southern analysis, sequencing reaction, next generation sequencing reaction, etc. Specific RNA sequences can be analyzed, or RNA sequences can be globally analyzed.

Nucleic acids can be extracted from a sample by means available to one of ordinary skill in the art.

The sample may be processed to render it competent for amplification. Exemplary sample processing can include lysing cells of the sample to release nucleic acid, purifying the sample (e.g., to isolate nucleic acid from other sample components, which may inhibit amplification), diluting/

concentrating the sample, and/or combining the sample with reagents for amplification, such as a DNA/RNA polymerase (e.g., a heat-stable DNA polymerase for PCR amplification), dNTPs (e.g., dATP, dCTP, dGTP, and dTTP (and/or dUTP)), a primer set for each allele sequence or polymorphic locus to be amplified, probes (e.g., fluorescent probes, such as TAQMAN probes or molecular beacon probes, among others) capable of hybridizing specifically to each allele sequence to be amplified, Mg^{2+}, DMSO, BSA, a buffer, or any combination thereof, among others. In some examples, the sample may be combined with a restriction enzyme, uracil-DNA glycosylase (UNG), reverse transcriptase, or any other enzyme of nucleic acid processing.

Computers

A computer can be used to store and process the data. A computer-executable logic can be employed to perform such functions grouping sequence reads by barcode sequence. A computer can be useful for displaying, storing, retrieving, or calculating diagnostic results from the molecular profiling; displaying, storing, retrieving, or calculating raw data from genomic or nucleic acid expression analysis; or displaying, storing, retrieving, or calculating any sample or patient information useful in the methods described herein. Provided herein are systems comprising computer readable instructions for performing methods described herein. Provided herein are computer readable medium comprising instructions which, when executed by a computer, cause the computer to perform methods described herein.

Kits

Provided herein are kits for performing methods described herein. The kits can comprise one or more restriction enzymes, endonucleases, exonucleases, ligases, polymerases, RNA polymerases, DNA polymerases, reverse transcriptases, topoisomerases, kinases, phosphatases, buffers, salts, metal ions, reducing agents, BSA, spermine, spermidine, glycerol, oligonucleotides, primers, probes, or labels (e.g., fluorescent labels). The kits can comprises one or more sets of instructions.

Multiplexing to Align the Dynamic Range of Targets Whose Concentrations are Different and to Smooth Out Biological Variation of Reference Genes

Also provided herein are methods for estimating copy number variation (CNV). Copy number variation of one or more target sequences can play a role in a number of diseases and disorders. One method to analyze copy number variation of a target sequence is through a digital analysis, such as digital PCR, or droplet digital PCR. However, digital analysis of copy number of a target sequence can underestimate the number of copies of a target nucleic acid sequence in a sample if multiple copies of the target nucleic acid sequence are on the same polynucleotide in a sample. For example, in a digital PCR assay that has multiple compartments (e.g., partitions, spatially isolated regions), nucleic acids in a sample can be partitioned such that each compartment receives on average about 0, 1, 2, or several target polynucleotides. Each partition can have, on average, less than 5, 4, 3, 2, or 1 copies of a target nucleic acid per partition (e.g., droplet). In some cases, at least 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, or 200 partitions (e.g., droplets) have zero copies of a target nucleic acid. The number of compartments that contain a polynucleotide can be enumerated. However, if two copies of a target nucleic acid sequence are on a single polynucleotide, a compartment containing that polynucleotide can be counted as having only one target sequence.

Methods of analyzing CNVs are disclosed, e.g., in U.S. patent application Ser. No. 13/385,277, filed Feb. 9, 2012. For example, methods can be used for physically separating target nucleic acids sequences. Often, the methods can avoid underestimating copy numbers of a target sequence due to the presence of multiple copies of the target sequence on a single polynucleotide. In some embodiments, a first sample of polynucleotides is obtained; the first sample can be, e.g., a genomic DNA sample. The target nucleic acid sequences in the first sample can be physically separated (e.g., by contacting the first sample with one or more restriction enzymes). The first sample can be separated into a plurality of partitions. The number of partitions with the target sequence can be enumerated. The copy number of the target can then be estimated.

The target nucleic acids can be identical; or, in other cases, the target nucleic acids can be different. In some cases, the target nucleic acids are located within the same gene. In some cases, the target nucleic acids are each located in a different copy (identical or near identical copy) of a gene. In still other cases, the target sequences are located within introns, or in a region between genes. Sometimes, one target sequence is located in a gene; and the second target sequence is located outside of the gene. In some cases, a target sequence is located within an exon.

Different targets within a sample may often be present at different copy numbers. In such cases, the target that is present at a lower copy number level, may be probed with multiple probes, each recognizing a different locus or region with the target sequence. For example, target A may be present at copy number 3, while target B is present at copy number 1. In such cases, target B may be probed with 3 primer/probe pairs to increase the number of B-positive droplets, or to increase the signal from droplets that comprise target B. The probes may be directed to different regions within target B. Often, the probes that target target B are labeled with the same label; but, in some cases, different labels may be used. Thus, such methods enable alignment of the dynamic range of targets with different copy numbers. Target B can be a different target or may be a reference sample, as described further herein. Therefore, such methods can also enable alignment of the dynamic range of targets with reference samples.

In some cases, a genome comprises one target sequence. In some cases, a genome comprises two or more target sequences. When a genome comprises two or more target sequences, the target sequences can be about, or more than about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% identical.

Separating two target sequences can comprise separating the target sequences by cleaving a specific site on the nucleic acid sequence. In some cases, the separating target nucleic acid sequences can comprise contacting the first sample with one or more restriction enzymes. Separating the target nucleic acid sequences can comprise digesting a polynucleotide at a site located between the target nucleic acid sequences. In some cases, the target nucleic acid sequences are each located within a gene. In some cases, the site that is targeted for digestion is located between the two genes. In some cases, the site selected for digestion is located in a gene; and, in some cases, the gene is the same gene as the gene which contains the target sequences. In other cases, the site selected for digestion is located in a different gene from that of the target sequence. In some cases, a target sequence and the site targeted for digestion are located in the same gene; and the target sequence is located upstream of the site targeted for digestion. In other cases, a target sequence and the site targeted for digestion are located in the same gene; but the target sequence is located downstream of the site

targeted for digestion. In some cases, target nucleic acids can be separated by treatment of a nucleic acid sample with one or more restriction enzymes. In some cases, target nucleic acids can be separated by shearing. In some cases, target nucleic acids can be separated by sonication.

Following the separation step (e.g., digesting with one or more restriction enzymes), the sample can be partitioned into multiple partitions. Each of the plurality of partitions can comprise about 0, 1, 2 or several target polynucleotides. In some cases, each partition can have, on average, less than 5, 4, 3, 2, or 1 copies of a target nucleic acid per partition (e.g., droplet). In some cases, at least 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, or 200 droplets have zero copies of a target nucleic acid.

Often, target nucleic acid is amplified in the partitions. In some cases, the amplification comprises use of one or more TaqMan probes.

In another embodiment, the method further comprises the step of enumerating the number of partitions comprising a reference nucleic acid sequence. A reference nucleic acid sequence can be known to be present in a certain number of copies per genome and can be used to estimate the number of genome copies of a target nucleic acid sequence in a sample. Estimating the copy number can comprise comparing the number of partitions comprising the target sequence to the number of partitions comprising the reference nucleic acid sequence. In another instance, a CNV estimate is determined by a ratio of the concentration of target nucleic acid sequence to a reference sequence.

In another embodiment, the method further comprises the step of analyzing a second sample, wherein the second sample and the first sample are derived from the same sample (e.g., a nucleic acid sample is split to the first sample and the second sample). The method can further comprise not contacting the second sample with one or more restriction enzymes. In some cases, the method further comprises separating the second sample into a plurality of partitions. The method can further comprise enumerating the number of partitions of the second sample that comprise the target sequence. In another embodiment, the method further comprises enumerating the number of partitions of the second sample that comprise a reference sequence. In another embodiment, the method comprises estimating the copy number of the target sequence in the second sample. In another embodiment, estimating the copy number of the target sequence in the second sample comprises comparing the number of partitions from the second sample with the target sequence and the number of partitions from the second sample with the reference sequence.

The copy number of the target sequence from the first sample and the copy number of the target sequence in the second sample can be compared to determine whether the copy number of the target sequence in the second sample was underestimated. The degree to which the copy number was underestimated may be indicative of whether interrogated copies were all on one chromosome or if at least one copy was on one homologous chromosome and at least one copy was on the other homologous chromosome. Values closer to one per diploid genome may indicate the first case, while values closer to two may indicate the second case.

Additional methods of determining copy number differences by amplification are described, e.g., in U.S. Patent Application Publication No. 20100203538. Methods for determining copy number variation are described in U.S. Pat. No. 6,180,349 and Taylor et al. (2008) *PLoS One* 3(9): e3179.

Copy number variations described herein can involve the loss or gain of nucleic acid sequence. Copy number variations can be inherited or can be caused by a de novo mutation. A CNV can be in one or more different classes. See, e.g. Redon et al. (2006) Global variation in copy number in the human genome. *Nature* 444 pp. 444-454. A CNV can result from a simple de novo deletion, from a simple de novo duplication, or from both a deletion and duplication. A CNV can result from combinations of multiallelic variants. A CNV can be a complex CNV with de novo gain. A CNV can include about, or more than about 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous genes. A CNV can include about 1 to about 10, about 1 to about 5, about 1 to about 4, about 1 to about 3, about 1 to about 2, about 0 to about 10, about 0 to about 5, or about 0 to about 2 contiguous genes. A copy number variation can involve a gain or a loss of about, or more than about, 100, 500, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000, 100,000, 200,000, 500,000, 750,000, 1 million, 5 million, or 10 million base pairs. In some cases, a copy number variation can involve the gain or loss of about 1,000 to about 10,000,000, about 10,000 to about 10,000,000, about 100,000 to about 10,000,000, about 1,000 to about 100,000, or about 1,000 to about 10,000 base-pairs of nucleic acid sequence. A copy number variation can be a deletion, insertion, or duplication of a nucleic acid sequence. In some cases, a copy number variation can be a tandem duplication.

In another embodiment, CNV haplotypes can be estimated from fluorescent signals generated by real-time PCR or ddPCR of partitioned samples. Before the late stages of a real-time PCR or ddPCR experiment, when reagents can become limiting, a partition with a higher copy number of a target sequence can have a higher signal than a partition with a lower copy number of the target sequence. In one embodiment, a sample (e.g., a subsample of a sample used in a linkage experiment) can be partitioned, and PCR can be performed on the partitions (e.g., droplets). The mean fluorescence intensity of partitions can be determined as they undergo exponential amplification for a target and/or reference nucleic acid sequence. The mean intensity can correspond to the number of starting copies of the target. If multiple targets are linked along a single polynucleotide strand, the intensity in the partition (e.g., droplet) that captures this strand may be higher than that of a partition (e.g., droplet) that captures a strand with only a single copy of the target. Excess presence of positive droplets with higher mean amplitudes can suggest the presence of a haplotype with multiple CNV copies. Conversely, presence of positive droplets with only low mean amplitudes can suggest that only haplotypes with single CNV copies are present in the sample. In another embodiment, the number of cycles used to estimate CNV can be optimized based on the size of the partitions and the amount of reagent in the partitions. For example, smaller partitions with lower amounts of reagent may require fewer amplification cycles than larger partitions that would be expected to have higher amounts of reagent.

The method can be useful because it can be used to analyze even target copies that are near each other on the polynucleotide, e.g., less than about 10, 9, 8, 7, 6, 5, 4, 5, 2, 1, 0.7 0.5, 0.3, 0.2, 0.1, 0.05, or 0.01 megabases apart; or that are very near each other on the polynucleotide, e.g., less than about 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 kilobase apart. In some cases, the method is useful for analyzing target copies that are very close to each other on the polynucleotide, e.g., within about 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200,

300, 400, 500, 600, 700, 800, 900, or 950 base pairs (bp's) apart. In some cases, the method is useful for analyzing target copies that are separated by zero (0) base pairs. In some cases, the method can be applied to identical, near identical, and completely different targets.

In some embodiments, the copy number of a target in a genome is about, more than about, less than about, or at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 500, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000, or 100,000 copies per haploid or diploid genome. In some embodiments, the copy number of a target is about 2 to about 5, about 2 to about 10, about 2 to about 20, about 2 to about 30, about 2 to about 40, about 2 to about 50, about 2 to about 100, about 5 to about 10, about 5 to about 25, about 5 to about 50, about 5 to about 100, about 10 to about 20, about 10 to about 50, about 10 to about 100, about 25 to about 50, about 25 to about 75, about 25 to about 100, about 100 to about 200, about 100 to about 500, about 100 to about 1000, about 500 to about 1000, about 1000 to about 5000, about 1000 to about 10,000, about 10,000 to about 20,000, about 10,000 to about 50,000, about 10,000 to about 100,000, or about 50,000 to about 100,000 per haploid or diploid genome.

In some embodiments, CNVs can be analyzed by measuring amounts of a target and a reference in a single reaction using probes with one fluorescence dye for the target and another for the reference. In some embodiments, e.g., when the target copy number is high, the concentration (or amount) of the target can be higher than the concentration (or amount) of the reference. In that case, it can be challenging to measure both the target and the reference in a single digital reaction (e.g., digital PCR), because the dynamic range of digital PCR can be limited. For example, a target may be present at 10,000 copies in a genome, but a reference may be present at only two copies per genome.

In some embodiments, several different targets for the reference can be multiplexed with each being detectable using probes with the same fluorescent dye. (See e.g., FIG. **2**) Often, these different reference targets represent different regions or loci within the same reference polynucleotide (e.g., gene); although, in some cases, different reference polynucleotides (e.g., genes) can be used. Use of multiple references can boost the counts of the reference and bring them closer to the counts of the target. In some embodiments, about, more than about, at least about, or less than about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 500, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000, or 100,000 different references are used. In some embodiments, about 2 to about 5, about 2 to about 10, about 2 to about 20, about 2 to about 30, about 2 to about 40, about 2 to about 50, about 2 to about 100, about 5 to about 10, about 5 to about 25, about 5 to about 50, about 5 to about 100, about 10 to about 20, about 10 to about 50, about 10 to about 100, about 25 to about 50, about 25 to about 75, about 25 to about 100, about 100 to about 200, about 100 to about 500, about 100 to about 1000, about 500 to about 1000, about 1000 to about 5000, about 1000 to about 10,000, about 10,000 to about 20,000, about 10,000 to about 50,000, about 10,000 to about 100,000, or about 50,000 to about 100,000 different references are used. The reference can be any reference sequence described herein. Generally, the reference may be present at a different copy number than the target sequence. For example, the target may have copy number that is about, more than about, less than about, or at least about 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5 fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 50-fold, 75-fold, 100-fold, 200-fold, 500-fold, 700-fold, the copy number of the reference number. In other cases the copy number of the target is equal to that of the reference. In still other cases, the reference has a copy number that is about, more than about, less than about, or at least about 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5 fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 50-fold, 75-fold, or 100-fold the copy number of the target sequence.

In some embodiments, probes that anneal to each of the references can comprise the same label, e.g., fluorescent dye. Depending on the number of targets to be multiplexed, one can use universal probes, LNA probes, or ligation approaches. Any type of probe described herein can be used to multiplex references.

The methods described herein can be used to measure several gene expression targets in a single reaction. Several assays can be designed to target the lowest expressed gene and bring the measured counts closer to those of the higher expressed gene(s).

If the abundance of expression of two or more different targets on the same gene is being investigated, e.g., by converting mRNA to cDNA, a restriction digest on the cDNA can be performed in order to ensure that the different targets on a given gene end up in different partitions (e.g., droplets). Other methods of fragmenting nucleic acids described herein can be used to separate the targets.

The methods described herein can also apply to measuring viral load levels in a single reaction. A viral load can be measured by estimating the amount of virus in a bodily fluid. In some embodiments, determination of viral load can comprise PCR, reverse transcription PCR, or Nucleic Acid Sequence Based Amplification (NASBA) (transcription-based amplification system (TAS)). For example, PCR can be used to quantify integrated DNA (e.g., integrated into a chromosome of a cell). Reverse transcription PCR can be used to quantify viral RNA by converting it to cDNA. In some embodiments, NASBA is used to convert viral RNA into DNA, and the DNA can be transcribed into RNA. NASBA can involve annealing a primer to the 3' end of an RNA template, reverse transcribing the RNA template, degrading the RNA template with RNAse H, annealing a primer to the 5' end of the DNA strand, and using T7 RNA polymerase to produce a complementary RNA strand. The complementary RNA strand can be reused in the reaction cycle. In some embodiments, multiple references are used to such that the amount of viral nucleic acid and reference nucleic acid in a sample are within the dynamic range of the method used to determine the viral load. In some embodiments, probes used to detect the different references use the same label. In some embodiments, probes used to detect the references comprise different labels.

In some embodiments, multiplexing can also be useful for evening out biological variation where a reference varies in copy number from individual to individual. By averaging across multiple targets and/or reference sequences, the

impact of the variation can be reduced. This method can be used, e.g., for diagnostic tests, including those used for measuring copy number alterations.

In some embodiments, a reference sequence that is present at two copies per diploid genome can be used, e.g., a housekeeping gene (e.g., a gene that is required for the maintenance of basic cellular function). Dividing the concentration or amount of the target by the concentration or amount of the reference can yield an estimate of the number of target copies per genome.

A housekeeping gene that can be used as reference in the methods described herein can include a gene that encodes a transcription factor, a transcription repressor, an RNA splicing gene, a translation factor, tRNA synthetase, RNA binding protein, ribosomal protein, RNA polymerase, protein processing protein, heat shock protein, histone, cell cycle regulator, apoptosis regulator, oncogene, DNA repair/replication gene, carbohydrate metabolism regulator, citric acid cycle regulator, lipid metabolism regulator, amino acid metabolism regulator, nucleotide synthesis regulator, NADH dehydrogenase, cytochrome C oxidase, ATPase, mitochondrial protein, lysosomal protein, proteosomal protein, ribonuclease, oxidase/reductase, cytoskeletal protein, cell adhesion protein, channel or transporter, receptor, kinase, growth factor, tissue necrosis factor, etc. Specific examples of housekeeping genes that can be used in the methods described include, e.g., HSP90, Beta-actin, tRNA, rRNA, ATF4, RPP30, and RPL3.

A single copy reference nucleic acid (e.g., gene) can be used to determine copy number variation. Multi-copy reference nucleic acids (e.g., genes) can be used to determine copy number to expand the dynamic range. For example, the multi-copy reference gene can comprise about, or more than about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 500, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000, or 100,000 copies in a genome.

Ranges can be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. The term "about" as used herein refers to a range that is 15% plus or minus from a stated numerical value within the context of the particular usage. For example, about 10 would include a range from 8.5 to 11.5.

EXAMPLES

Example 1

One thousand genome equivalents (about 6 ng of DNA) can be sequenced at 100× depth. From 1000 genome equivalents, there can be 2,000 copies of every (normal copy number) target. Steps can be taken such that for every locus, a large majority of fragments end up in separate partitions. Steps can also be taken to ensure that most of the 2,000 fragments are tagged with a unique barcode.

The first goal can be accomplished by increasing the number of partitions. For example, with 100,000 partitions, only about 0.5% of fragments at a particular locus from different chromosomes are expected to end up in the same partition. Note that many such cases will be readily identified by the appearance of distinct alleles from heterozygous SNPs with the same barcode as well as by increased coverage of the locus by a barcode.

In order to ensure that most fragments are tagged with distinct barcodes, a large number of different barcodes can be used, and an approach that distributes barcodes so that any given partition is furnished with a small number (preferably one) of barcode-containing droplets can be used. The distribution can be random so that some partitions receive zero barcodes, some one, some multiple. Thus, for 100,000 partitions 100,000 barcoding droplets can be supplied. In this case, it is anticipated that 37% of the partitions will receive no adaptors and will thus be unavailable for sequencing. The number of barcoding droplets can be increased if sample preservation is a goal. 37% of the partitions can be barcoded with a single barcode and up to 25% can be coded with potentially different barcodes. In the case above, 740 fragments will be unavailable for sequencing, 740 will be sequestered in with their own barcodes and 500 will be sequestered with multiple barcodes. Ideally all of the 740*1+360*2+ . . . =2,000 barcodes in the partitions associated with a particular fragment would be unique. If there are 10,000 different barcode types, then more than 80% of the fragments would be uniquely tagged.

If the number of genome equivalents is lower then fewer partitions and barcodes could be used.

Note that perfection is not necessary for this application, because only a small subset of SNPs from any given genomic location can be captured to yield phasing information. It can be acceptable if a substantial fraction of fragments is not informative.

One can attain greater efficiency of sample processing if each partition is supplied with a barcode in a controlled manner. For example, sample containing partitions and barcode containing partitions can be merged using droplet merging technology from RAINDANCE™ (RAINSTORM™). Droplet merging can be performed using a microfluidic circuit similar to FLUIDIGM's array designs. If it can be guaranteed that a given partition receives precisely one ADF, fewer ADFs and fewer ADF types can be used.

A microfluidic chip can be used in an analogous manner for partitioning. Sample partitions can be supplied with their own barcodes via a two-dimensional arrangement of channels as described above. A large number of unique barcodes can be readily supplied by combining vertical and horizontal barcodes.

While preferred embodiments of the methods, compositions, systems, and kits described herein have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the methods, compositions, systems, and kits described herein. It should be understood that various alternatives to the embodiments of the methods, compositions, systems, and kits described herein may be employed in practicing the methods, compositions, systems, and kits. It is intended that the following claims define the scope of the methods, compositions, systems, and kits within the scope of these claims and their equivalents be covered thereby.

What is claimed is:

1. A composition comprising a plurality of second partitions containing first partitions, wherein:

a. said first partitions are degradable upon the application of a stimulus to said first partitions such that contents of a first partition is mixed with contents of a second partition; and

b. said first partitions are contained within the second partitions;

c. said first partitions contain an oligonucleotide barcode; and

d. the first partitions have on average a first average volume and the second partitions have on average a second average volume, wherein the second average volume is at least twice as large as the first average volume.

2. The composition of claim 1, wherein said stimulus is selected from the group consisting of a biological, chemical, thermal, electrical, magnetic, or photo stimulus, and combination thereof.

3. The composition of claim 1, wherein said stimulus is a chemical stimulus.

4. The composition of claim 1, wherein said second partition is a droplet.

5. The composition of claim 1, wherein the first partition is a droplet.

6. The composition of claim 1, wherein the first partition and the second partition are droplets.

7. The composition of claim 1, wherein the second partition comprises a sample comprising polynucleotides.

8. The composition of claim 7, wherein the second partition further comprises a DNA polymerase enzyme or a reverse transcriptase enzyme.

9. The composition of claim 7, wherein the polynucleotides comprise DNA.

10. The composition of claim 9, wherein the DNA is genomic DNA.

11. The composition of claim 7, wherein the polynucleotides comprise RNA.

12. The composition of claim 7, wherein the polynucleotides comprise cDNA.

13. The composition of claim 7, wherein the partitions are less than 10 nL in volume.

14. A device comprising a plurality of second partitions, wherein:

a. at least one second partition of the plurality of second partitions contains a first partition comprising an oligonucleotide barcode, and the first partition has a first volume and the at least one second partition has a second volume, wherein the second volume is at least twice as large as the first volume; and

b. said first partition is degradable upon the application of a stimulus to said first partition such that contents of a first partition is mixed with contents of a second partition.

15. The device of claim 14, wherein said second partition is a droplet.

16. The device of claim 14, wherein said stimulus is selected from the group consisting of a biological, chemical, thermal, electrical, magnetic, or photo stimulus, and a combination thereof.

17. The device of claim 16, wherein said stimulus is a chemical stimulus.

18. The device of claim 14, wherein the first partition is a droplet.

19. The device of claim 14, wherein the first partition and the second partition are droplets.

20. The device of claim 19, wherein the droplets are less than 10 nL in volume.

21. The device of claim 14, wherein the second partition comprises a sample comprising polynucleotides.

22. The device of claim 21, wherein the second partition further comprises a DNA polymerase enzyme or a reverse transcriptase enzyme.

23. The device of claim 21, wherein the polynucleotides comprise DNA.

24. The device of claim 23, wherein the DNA is genomic DNA.

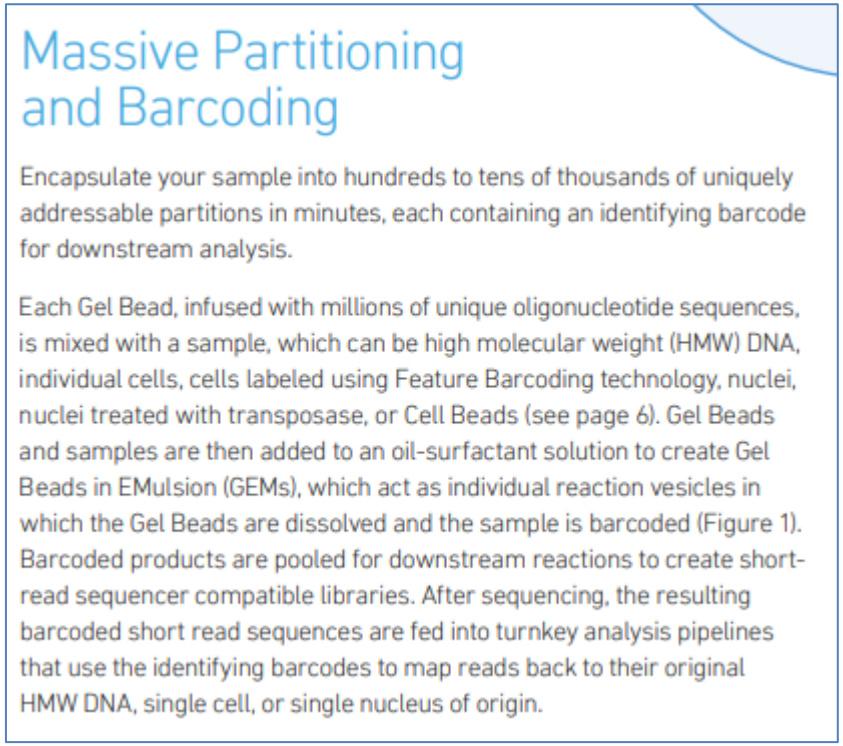
25. The device of claim 21, wherein the polynucleotides comprise RNA.

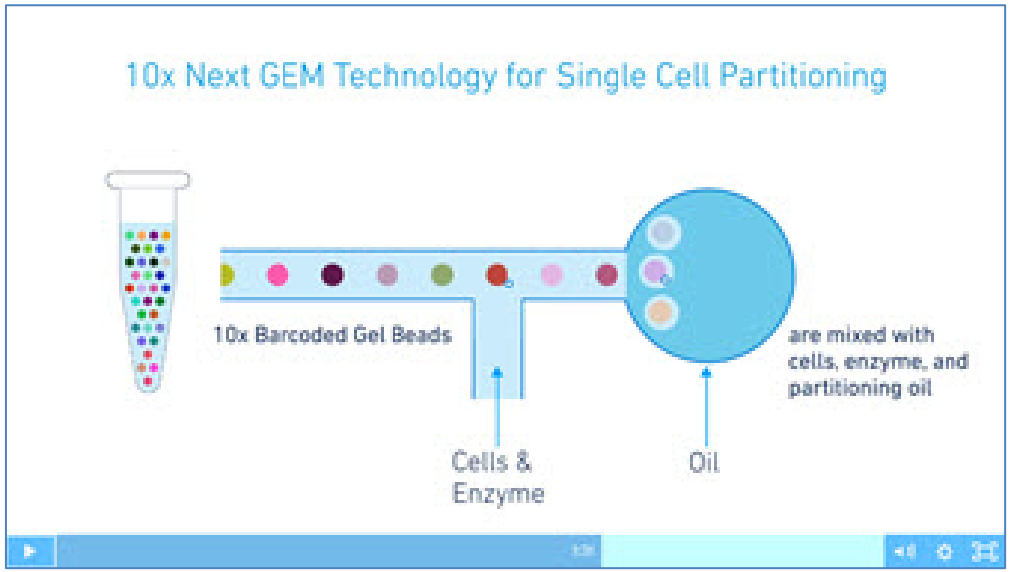
26. The device of claim 21, wherein the polynucleotides comprise cDNA.

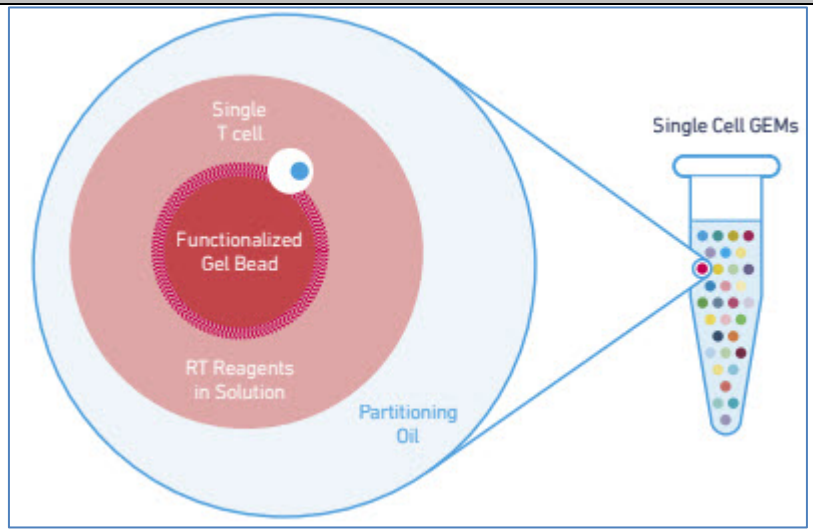
* * * * *

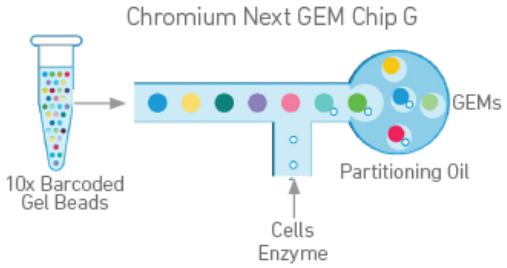
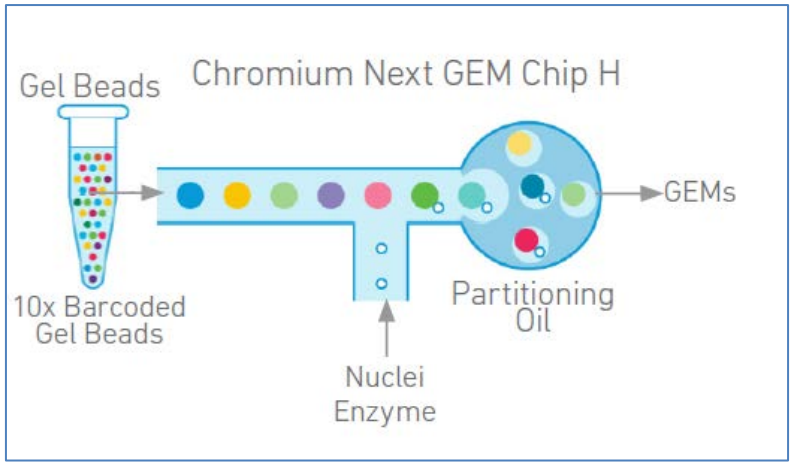
EXHIBIT 16

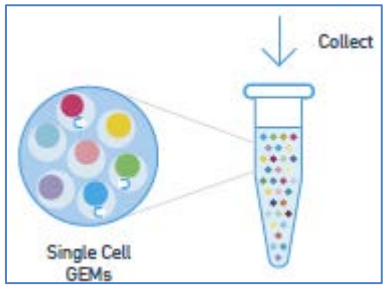
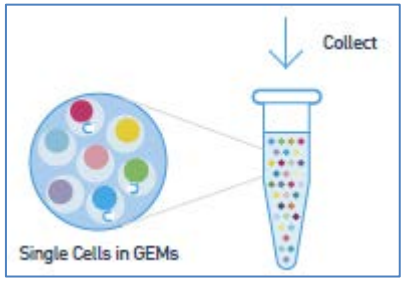
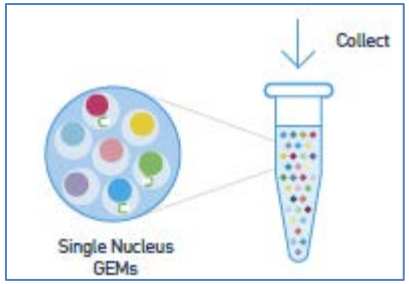
US 9,919,277
Infringement Analysis

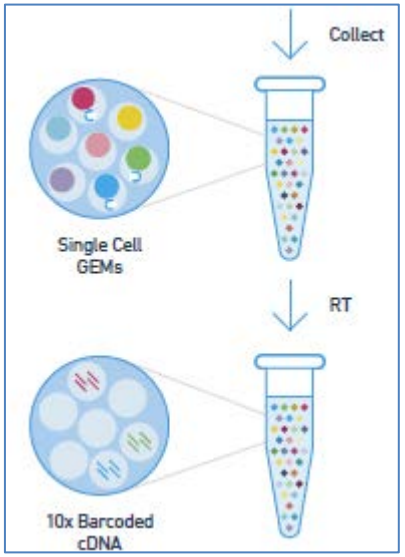
'277 Claim Language	**Infringement Support**
1. A method for conducting an enzymatic reaction, comprising the steps of:	10X's Next GEM platform conducts enzymatic barcoding reactions in microfluidic droplets:  Ex. 2 at 2.
providing a droplet generator to produce, under	10X's Next GEM platform includes a microfluidic droplet generator, which creates aqueous microcapsules:

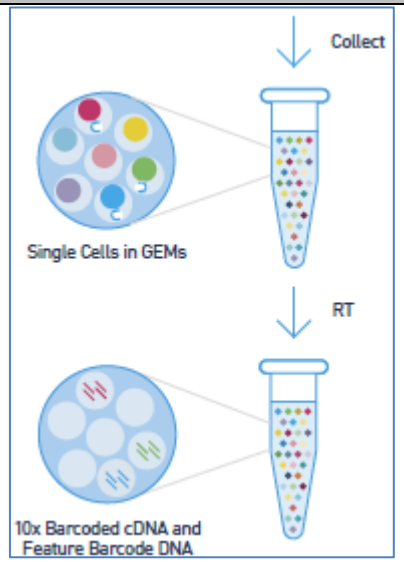
'277 Claim Language	**Infringement Support**
microfluidic control, a plurality of aqueous microcapsules surrounded by an immiscible continuous phase that comprises a fluorinated oil that comprises a fluorinated polymer surfactant, each of the plurality of microcapsules comprising an enzyme, a genetic element linked covalently or non-covalently to a bead, and reagents for the enzymatic reaction;	 Ex. 6. (Video, available at: https://www.10xgenomics.com/solutions/single-cell/). As shown in 10X's technology brochure, each of its reagent kits that utilize the Next GEM platform use the approach immediately above to generate aqueous microcapsules. *See* Ex. 2 at 3 (single cell partitioning for transcriptome analysis); *id.* at 4 (single cell partitioning for immunology applications); *id.* at 5 (single cell partition for epigenome analysis). Additionally, the 10X technology brochure shows that the aqueous microcapsules include an enzyme (included with the "RT Reagents in Solution"), a genetic element (the sample nucleic acids), and reagents for the enzymatic reaction (the "RT Reagents in Solution" and the barcodes included with the "Functionalized Gel Bead"):

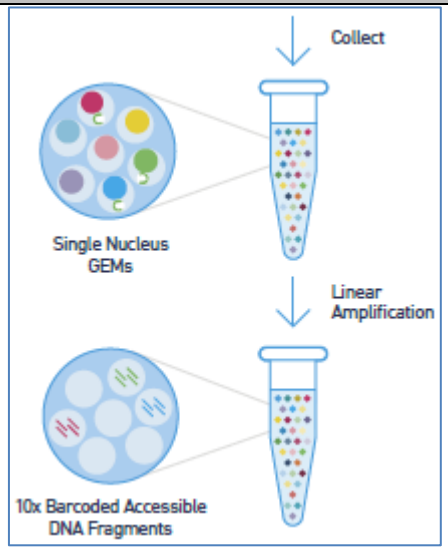
'277 Claim Language	Infringement Support
	 Figure 1 A GEM is a "Gel bead in EMulsion" droplet that encapsulates each micro-reaction within the Chromium System. Here we show a Single Cell GEM with a single T cell, reagents, and barcoded gel bead all partitioned within a single droplet. *Id.* at 1. As the image above shows, each aqueous microcapsule is surrounded by an immiscible continuous phase (the "Partitioning Oil"). As confirmed during 10X's November 2018 trial against Bio-Rad in this district, 10X's products all utilize fluorinated chemistry, including a fluorinated oil carrier fluid and a fluorinated polymer surfactant in the carrier fluid. 10X's product literature for its Next GEM platform depicts a cell or other genetic material (e.g., cell nuclei) being linked to a Gel Bead.

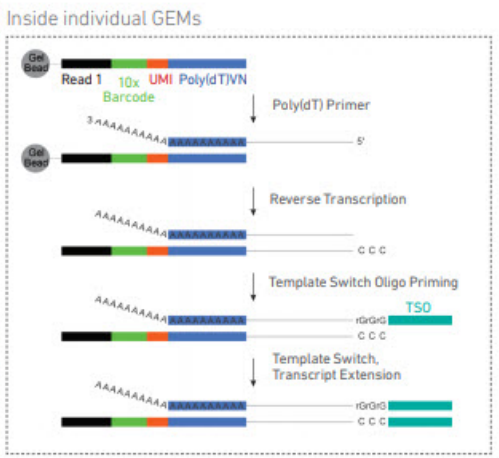
’277 Claim Language	Infringement Support
	Step 1 GEM Generation & Barcoding GEMs are generated by combining barcoded Single Cell 3' v3.1 Gel Beads, a Master Mix containing cells, and Partitioning Oil onto Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90-99%) of generated GEMs contain no cell, while the remainder largely contain a single cell.  Ex. 7 at 14.  Ex. 9 at 13.
pooling the microcapsules into one or more common compartments such that a portion of the plurality of	10X’s technology brochure confirms that “[b]arcoded products are pooled for downstream reactions to create short-read sequencer compatible libraries.” Ex. 2 at 2. The brochure shows that for each of 10X’s Next GEM applications the droplets are pooled in a compartment (e.g., a tube), and the droplets contact each other but do not fuse:

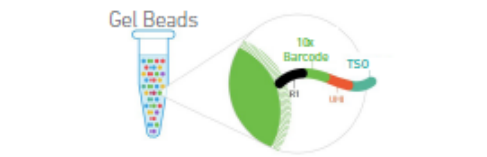
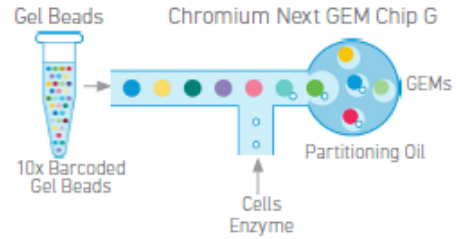
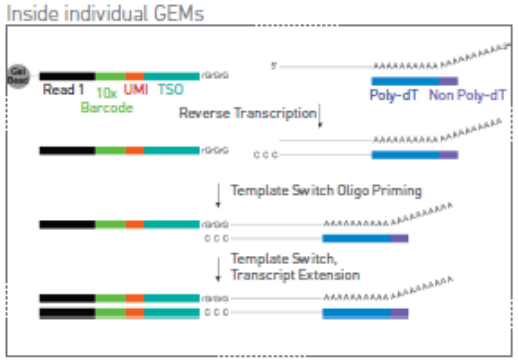
'277 Claim Language	**Infringement Support**
microcapsules contact each other but do not fuse with each other due to the presence of the surfactant;	 *Id.* at 3  *Id.* at 4.  *Id.* at 5.

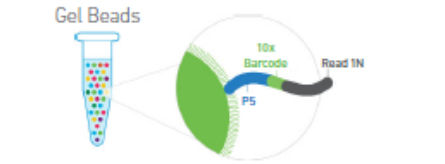
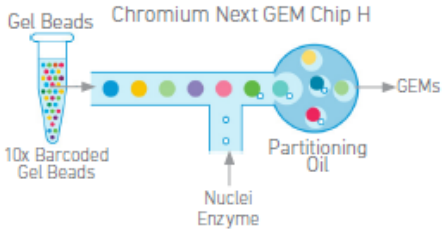
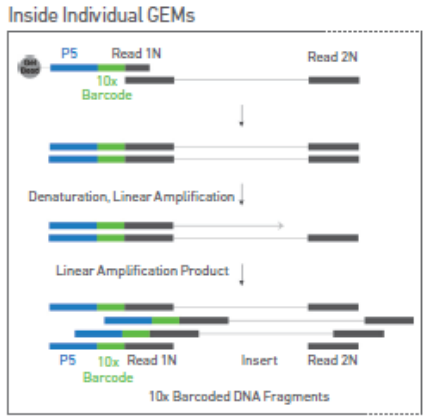
'277 Claim Language	**Infringement Support**
conducting the enzymatic reaction on the genetic element of at least one of the plurality of microcapsules within the one or more common compartments.	In 10X's Next GEM platform, an enzymatic reaction is conducted within the microcapsule that is within the compartment. This is shown in the product brochure, which shows an enzymatic reaction based on the use of reverse transcriptase ("RT") enzyme or another enzyme for "Linear Amplification" to yield barcoded DNA fragments:  *Id.* at 3.

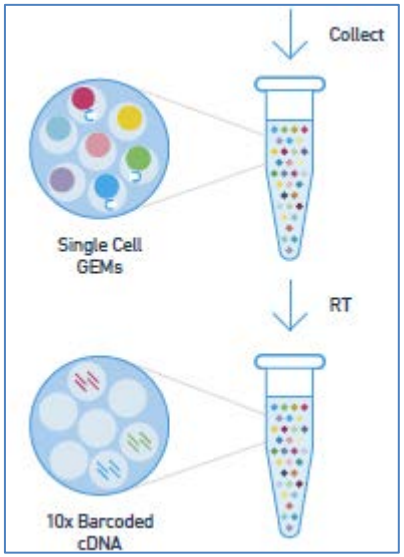
’277 Claim Language	Infringement Support
	 *Id.* at 4.

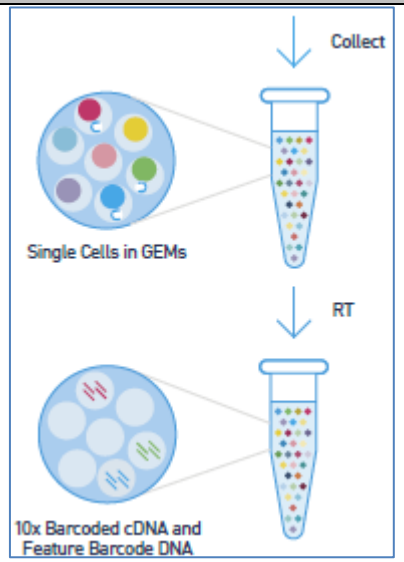
’277 Claim Language	Infringement Support
	 *Id.* at 5. The product manuals for each of 10X’s Next GEM products further depict the enzymatic reaction:

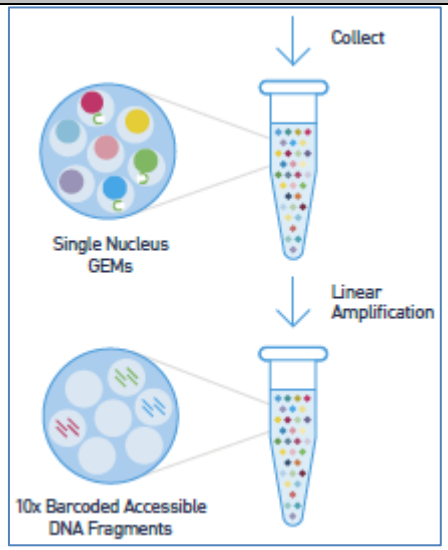
’277 Claim Language	Infringement Support
	Step 1 GEM Generation & Barcoding Immediately following GEM generation, the Gel Bead is dissolved, primers are released, and any co-partitioned cell is lysed. Primer containing: • an Illumina TruSeq Read 1 (read 1 sequencing primer) • 16 nt 10x Barcode • 12 nt unique molecular identifier (UMI) • 30 nt poly(dT) sequence are mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents. Incubation of the GEMs produces barcoded, full-length cDNA from poly-adenylated mRNA.  Ex. 7 at 15.

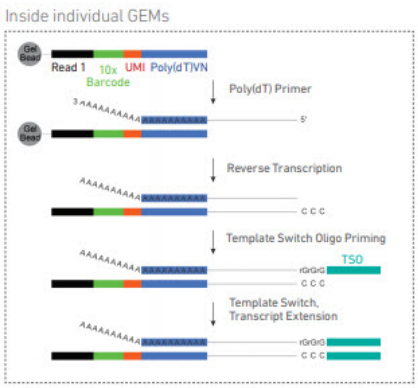
’277 Claim Language	Infringement Support
	Step 1 GEM Generation & Barcoding GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads v1.1, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell. Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.    Ex. 8 at 16.

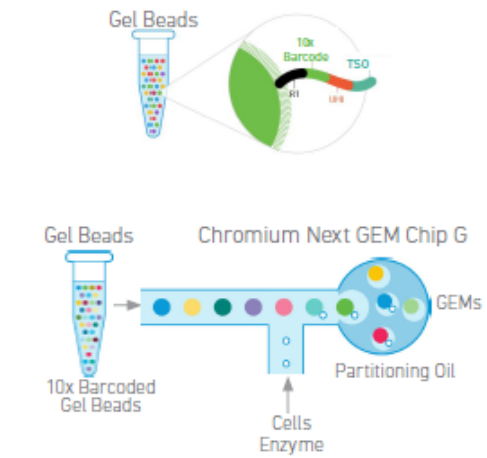
'277 Claim Language	Infringement Support
	Step 2 GEM Generation & Barcoding GEMs are generated by combining barcoded Gel Beads, transposed nuclei, a Master Mix, and Partitioning Oil on a Chromium Next GEM Chip H. To achieve single nuclei resolution, the nuclei are delivered at a limiting dilution, such that the majority (~90-99%) of generated GEMs contains no nuclei, while the remainder largely contain a single nucleus. Upon GEM generation, the Gel Bead is dissolved. Oligonucleotides containing (i) an Illumina® P5 sequence, (ii) a 16 nt 10x Barcode and (iii) a Read 1 (Read 1N) sequence are released and mixed with DNA fragments and Master Mix. Thermal cycling of the GEMs produces 10x barcoded single-stranded DNA. After incubation, the GEMs are broken and pooled fractions are recovered.    Ex. 9 at 13.
2. The method of claim 1, wherein the genetic elements comprises nucleic acids.	In 10X's Next GEM platform the genetic elements encompass at least nucleic acids. This is shown in the product brochure, which shows an enzymatic reaction based on the use of reverse

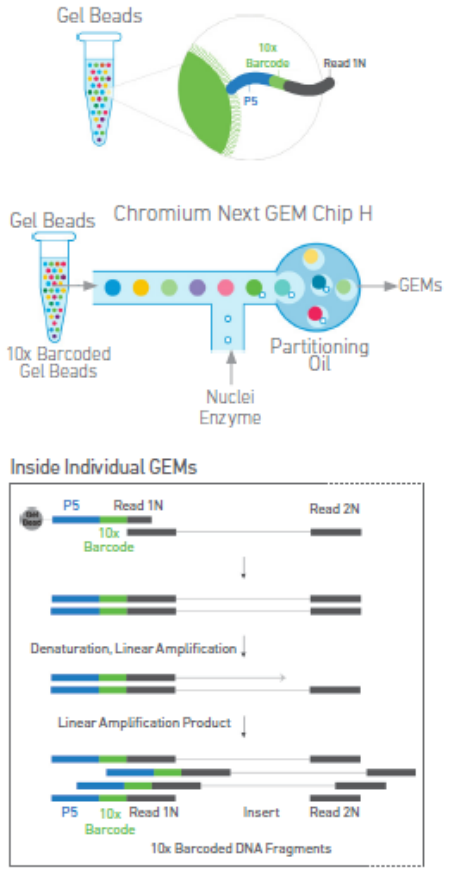
'277 Claim Language	**Infringement Support**
	transcriptase ("RT") enzyme or another enzyme for "Linear Amplification" to yield barcoded DNA fragments:  *Id.* at 3.

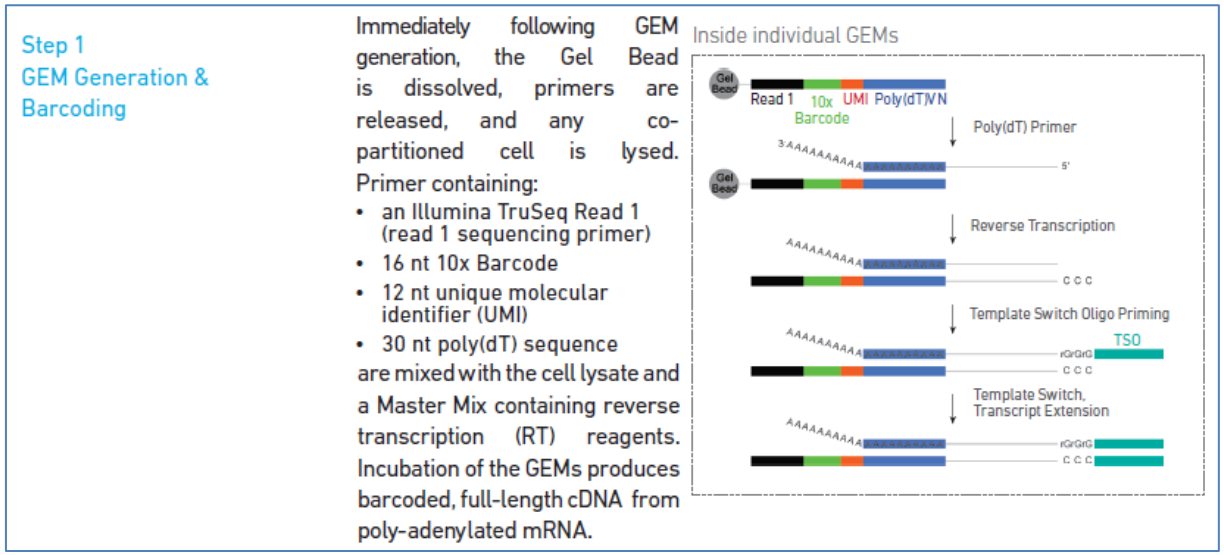
’277 Claim Language	Infringement Support
	 *Id.* at 4.

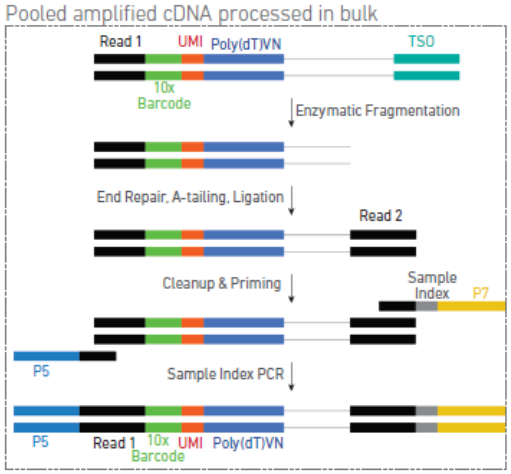
’277 Claim Language	Infringement Support
	 *Id.* at 5. The product manuals for each of 10X’s Next GEM products further depict the enzymatic reaction involving nucleic acids:

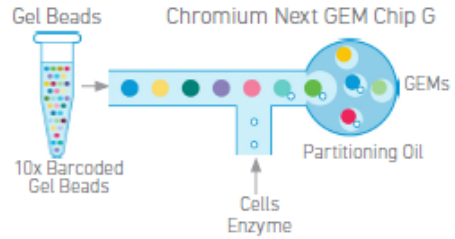
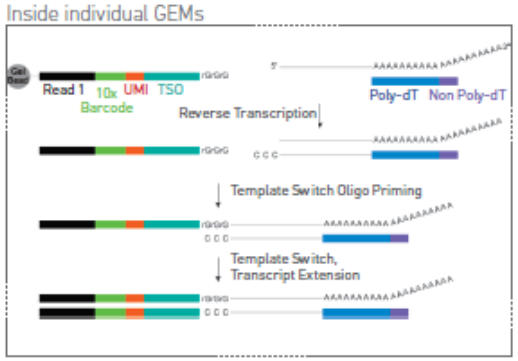
’277 Claim Language	Infringement Support
	Step 1 GEM Generation & Barcoding Immediately following GEM generation, the Gel Bead is dissolved, primers are released, and any co-partitioned cell is lysed. Primer containing: • an Illumina TruSeq Read 1 (read 1 sequencing primer) • 16 nt 10x Barcode • 12 nt unique molecular identifier (UMI) • 30 nt poly(dT) sequence are mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents. Incubation of the GEMs produces barcoded, full-length cDNA from poly-adenylated mRNA.  Ex. 7 at 15.

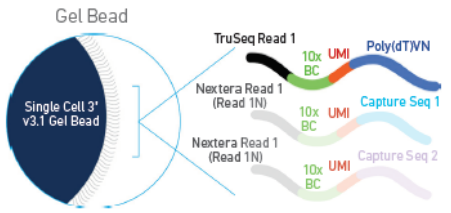
’277 Claim Language	Infringement Support
	Step 1 GEM Generation & Barcoding GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads v1.1, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell. Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.  Ex. 8 at 16.

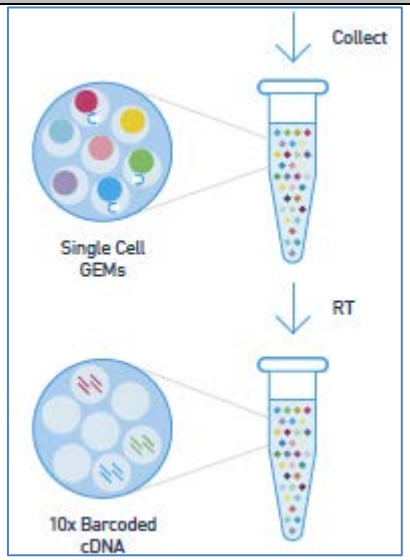
’277 Claim Language	Infringement Support
	Step 2 GEM Generation & Barcoding GEMs are generated by combining barcoded Gel Beads, transposed nuclei, a Master Mix, and Partitioning Oil on a Chromium Next GEM Chip H. To achieve single nuclei resolution, the nuclei are delivered at a limiting dilution, such that the majority (~90-99%) of generated GEMs contains no nuclei, while the remainder largely contain a single nucleus. Upon GEM generation, the Gel Bead is dissolved. Oligonucleotides containing (i) an Illumina® P5 sequence, (ii) a 16 nt 10x Barcode and (iii) a Read 1 (Read 1N) sequence are released and mixed with DNA fragments and Master Mix. Thermal cycling of the GEMs produces 10x barcoded single-stranded DNA. After incubation, the GEMs are broken and pooled fractions are recovered.  Ex. 9 at 13.
3. The method of claim 2, wherein the nucleic acids	In 10X’s Next GEM platform, primers are attached to cDNA during the GEM incubation step, which produces barcoded, full-length, cDNA within each droplet.

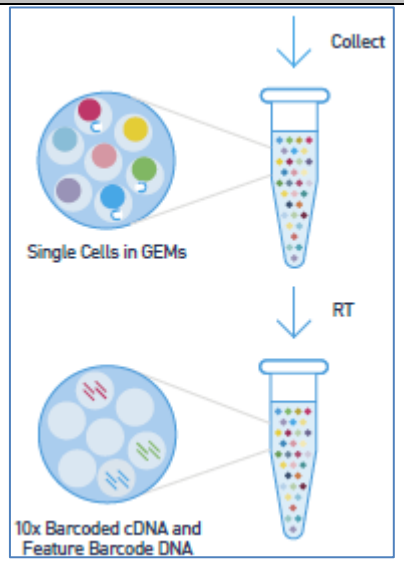
’277 Claim Language	Infringement Support
further comprise primers for a polymerase chain reaction (PCR).	 Ex. 7 at 15. In some Next GEM applications, TruSeq Read 1 primers are added to the cDNA molecules during incubation and are used for PCR.

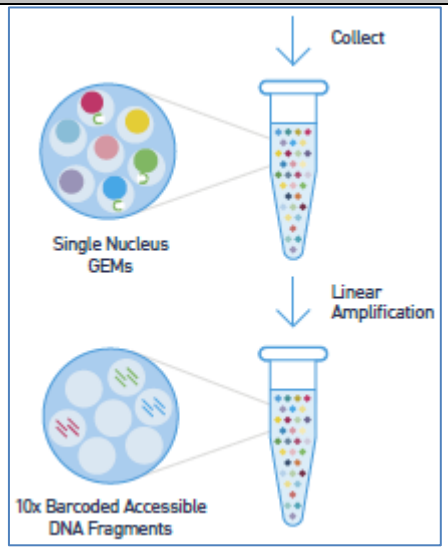
’277 Claim Language	Infringement Support
	Step 3 3' Gene Expression Library Construction Enzymatic fragmentation and size selection are used to optimize the cDNA amplicon size. TruSeq Read 1 (read 1 primer sequence) is added to the molecules during GEM incubation. P5, P7, a sample index, and TruSeq Read 2 (read 2 primer sequence) are added via End Repair, A-tailing, Adaptor Ligation, and PCR. The final libraries contain the P5 and P7 primers used in Illumina bridge amplification.  *Id.* In other examples, Illumina sequencing primers are added during the GEM incubation.

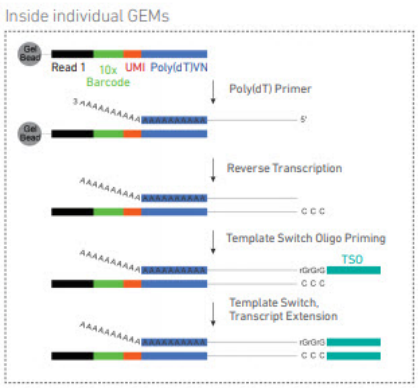
'277 Claim Language	Infringement Support
	Step 1 GEM Generation & Barcoding GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads v1.1, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell. Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.    Ex. 8 at 16
4. The method of claim 2, wherein the genetic element comprises RNA.	In the 10X Next GEM platform, barcoded cDNA molecules are produced from mRNA.

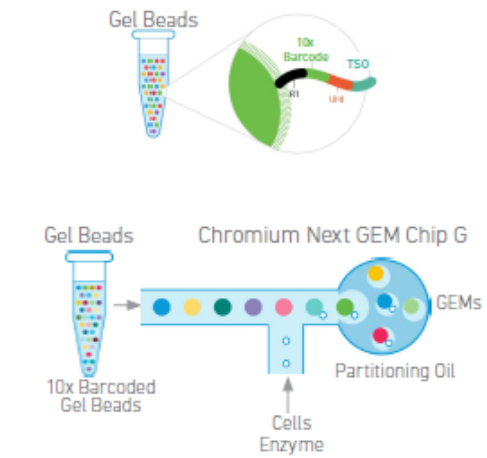
’277 Claim Language	Infringement Support
	Single Cell 3' v3.1 Gel Beads In addition to the poly(dT) primer that enables the production of barcoded, full-length cDNA from poly-adenylated mRNA, the Single Cell 3' v3.1 Gel Beads also include two additional primer sequences (Capture Sequence 1 and Capture Sequence 2), that enable capture and priming of Feature Barcoding technology compatible targets or analytes of interest. Only the poly(dT) primers are used in this protocol for generating Single Cell 3' Gene Expression libraries.  Ex. 7 at 14.
5. The method of claim 1, wherein the genetic element is labeled.	In 10X’s Next GEM platform the genetic elements encompass at least nucleic acids. This is shown in the product brochure, which shows an enzymatic reaction based on the use of reverse transcriptase (“RT”) enzyme or another enzyme for “Linear Amplification” to yield barcoded DNA fragments:

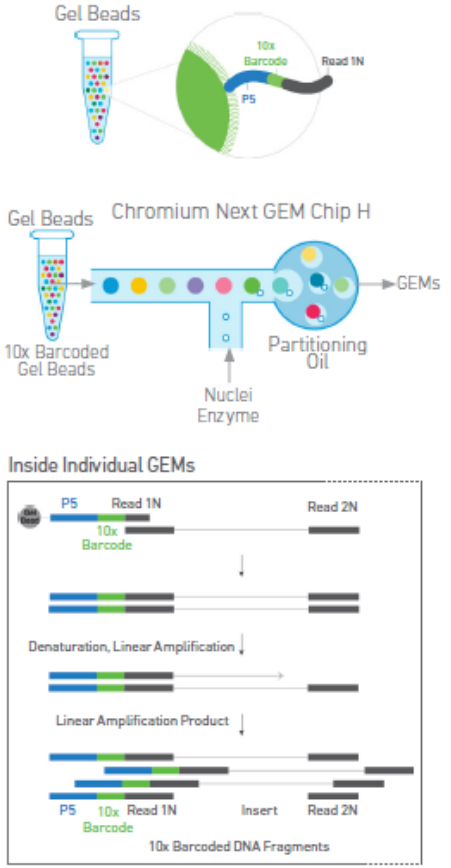
'277 Claim Language	**Infringement Support**
	 *Id.* at 3.

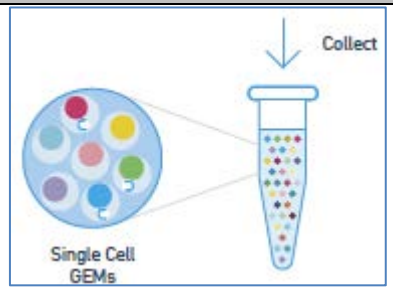
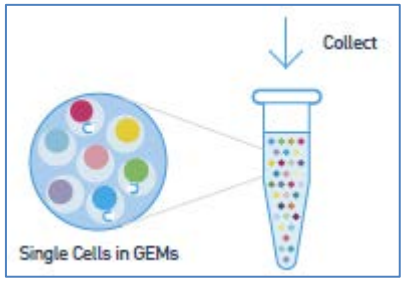
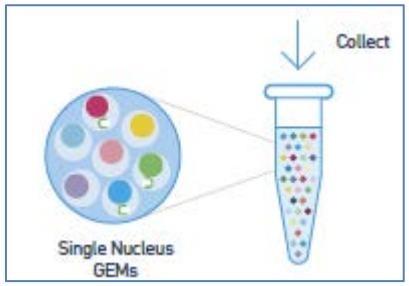
’277 Claim Language	Infringement Support
	 *Id.* at 4.

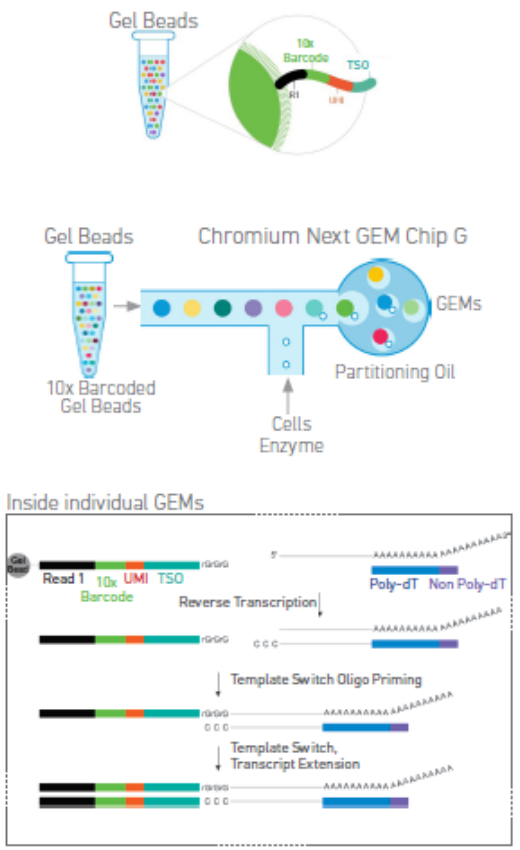
’277 Claim Language	Infringement Support
	 *Id.* at 5. The product manuals for each of 10X’s Next GEM products further depict the enzymatic reaction involving nucleic acids:

’277 Claim Language	Infringement Support
	Step 1 GEM Generation & Barcoding Immediately following GEM generation, the Gel Bead is dissolved, primers are released, and any co-partitioned cell is lysed. Primer containing: • an Illumina TruSeq Read 1 (read 1 sequencing primer) • 16 nt 10x Barcode • 12 nt unique molecular identifier (UMI) • 30 nt poly(dT) sequence are mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents. Incubation of the GEMs produces barcoded, full-length cDNA from poly-adenylated mRNA.  Ex. 7 at 15.

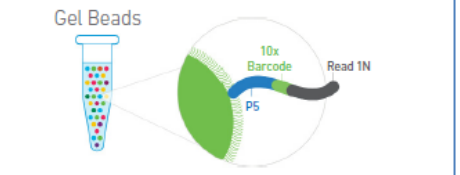
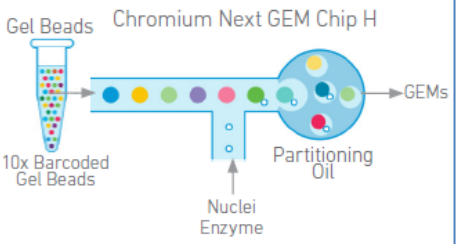
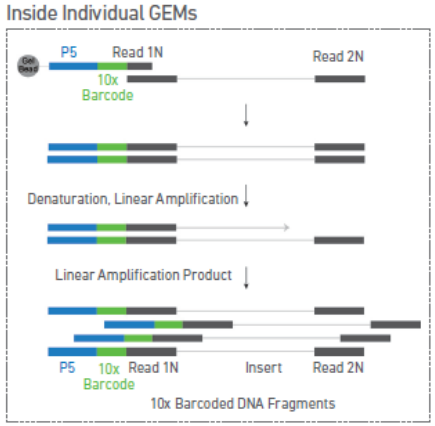
’277 Claim Language	Infringement Support
	Step 1 GEM Generation & Barcoding GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads v1.1, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell. Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.  Ex. 8 at 16.

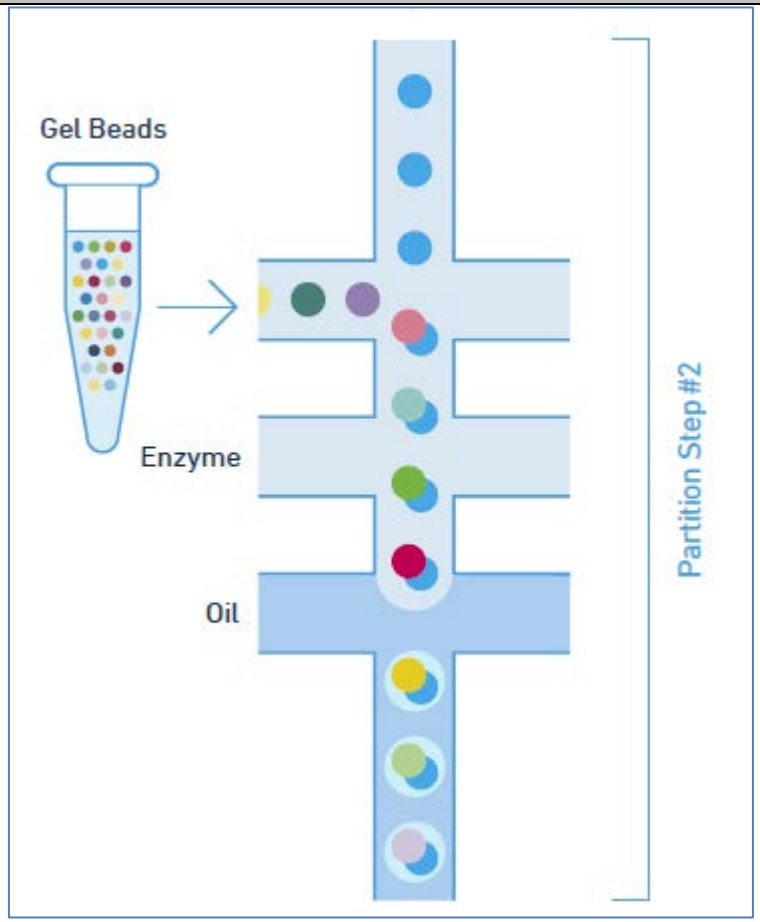
’277 Claim Language	Infringement Support
	Step 2 GEM Generation & Barcoding GEMs are generated by combining barcoded Gel Beads, transposed nuclei, a Master Mix, and Partitioning Oil on a Chromium Next GEM Chip H. To achieve single nuclei resolution, the nuclei are delivered at a limiting dilution, such that the majority (~90-99%) of generated GEMs contains no nuclei, while the remainder largely contain a single nucleus. Upon GEM generation, the Gel Bead is dissolved. Oligonucleotides containing (i) an Illumina® P5 sequence, (ii) a 16 nt 10x Barcode and (iii) a Read 1 (Read 1N) sequence are released and mixed with DNA fragments and Master Mix. Thermal cycling of the GEMs produces 10x barcoded single-stranded DNA. After incubation, the GEMs are broken and pooled fractions are recovered.  Ex. 9 at 13.
6. The method of claim 1, wherein the microcapsules are monodisperse with respect to each other.	10X’s product literature for its Next GEM platform depicts the microcapsules as being monodisperse with respect to each other:

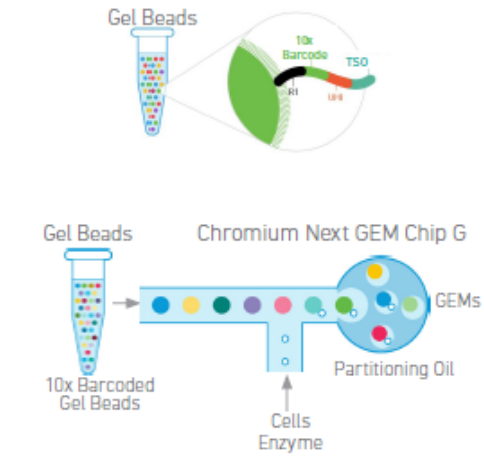
'277 Claim Language	**Infringement Support**
	 Ex. 2 at 3  *Id.* at 4.  *Id.* at 5.

'277 Claim Language	**Infringement Support**
8. The method of claim 1, wherein the concentration of the beads is adjusted such that a single bead appears in each microcapsule	10X's product literature for its Next GEM platform depicts the microcapsules as containing one bead per GEM. Step 1 GEM Generation & Barcoding GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads v1.1, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell. Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.  Ex. 8 at 16.

'277 Claim Language	**Infringement Support**
9. The method of claim 1, wherein the genetic element is identified with a tag.	10X's Next GEM sequencing platform attaches barcodes to cDNA molecules so that DNA from each droplet may be identified and thereby associated with a single cell. Encapsulate your sample into hundreds to tens of thousands of uniquely addressable partitions in minutes, each containing an identifying barcode for downstream analysis. Each Gel Bead, infused with millions of unique oligonucleotide sequences, is mixed with a sample, which can be high molecular weight (HMW) DNA, individual cells, cells labeled using Feature Barcoding technology, nuclei, nuclei treated with transposase, or Cell Beads (see page 6). Gel Beads and samples are then added to an oil-surfactant solution to create Gel Beads in EMulsion (GEMs), which act as individual reaction vesicles in which the Gel Beads are dissolved and the sample is barcoded (Figure 1). Barcoded products are pooled for downstream reactions to create short-read sequencer compatible libraries. After sequencing, the resulting barcoded short read sequences are fed into turnkey analysis pipelines that use the identifying barcodes to map reads back to their original HMW DNA, single cell, or single nucleus of origin. Ex. 2 at 2.
11. The method of claim 1, wherein the enzymatic reaction is an amplification reaction.	The enzymatic reaction wherein barcodes are attached to DNA includes denaturization and amplification of DNA molecules.

’277 Claim Language	Infringement Support
	Step 2 GEM Generation & Barcoding GEMs are generated by combining barcoded Gel Beads, transposed nuclei, a Master Mix, and Partitioning Oil on a Chromium Next GEM Chip H. To achieve single nuclei resolution, the nuclei are delivered at a limiting dilution, such that the majority (~90-99%) of generated GEMs contains no nuclei, while the remainder largely contain a single nucleus. Upon GEM generation, the Gel Bead is dissolved. Oligonucleotides containing (i) an Illumina® P5 sequence, (ii) a 16 nt 10x Barcode and (iii) a Read 1 (Read 1N) sequence are released and mixed with DNA fragments and Master Mix. Thermal cycling of the GEMs produces 10x barcoded single-stranded DNA. After incubation, the GEMs are broken and pooled fractions are recovered.    Ex. 9 at 13.
13. The method of claim 1, wherein the microcapsules are produced by infusing an aqueous fluid through a narrow constriction into a	In the Next GEM sequencing platform, GEMs are generated by infusing an aqueous fluid comprising Gel Beads and cells into partitioning oil. 10X’s technology brochure depicts a partitioning process wherein an aqueous fluid comprising Gel Beads and sample material is flowed through a channel to intersect with a channel of oil in order to create GEMs.

'277 Claim Language	**Infringement Support**
counter-propagating oil stream.	 Ex. 2 at 6. 10X's product manuals also depict this process of creating GEMs by infusing the aqueous fluid comprising Gel Beads and cells into the partitiong oil stream.

'277 Claim Language	Infringement Support
	Step 1 GEM Generation & Barcoding GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads v1.1, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell. Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.  Ex. 8 at 16
14. The method of claim 1, wherein the enzymatic	The product manuals for 10X's Next GEM products explain that the enzymatic reaction used to attach barcodes to genetic material includes a reverse transcription reaction.

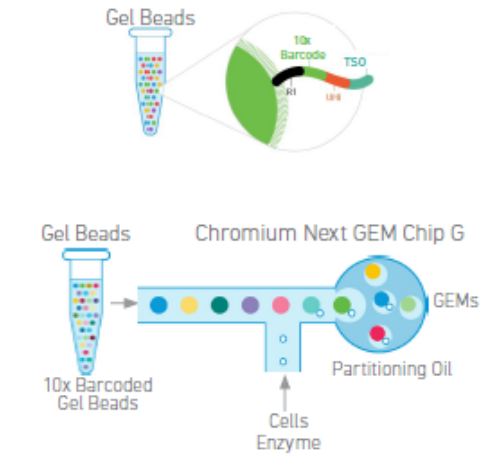
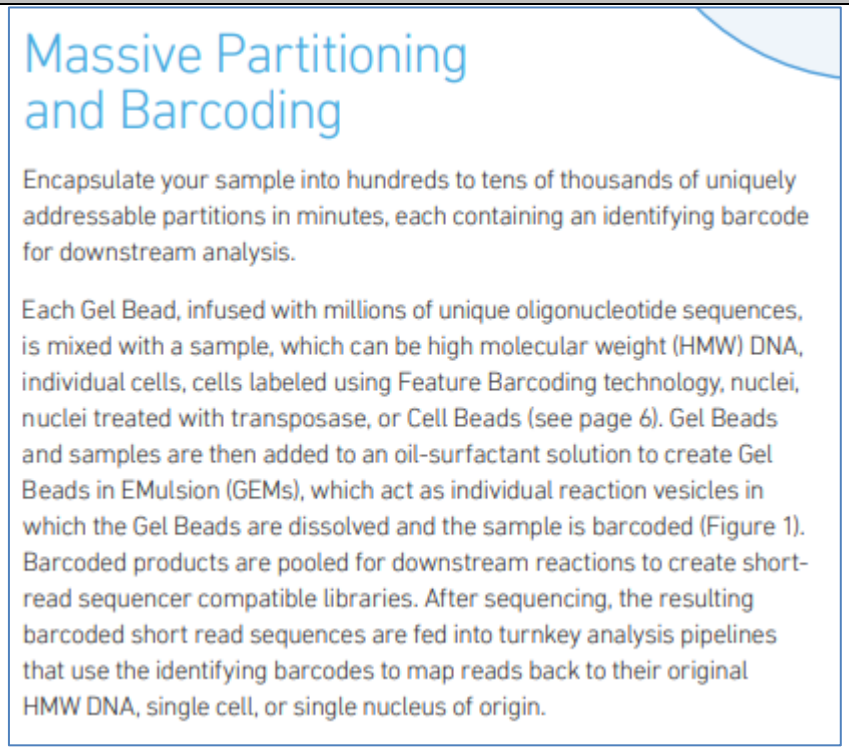
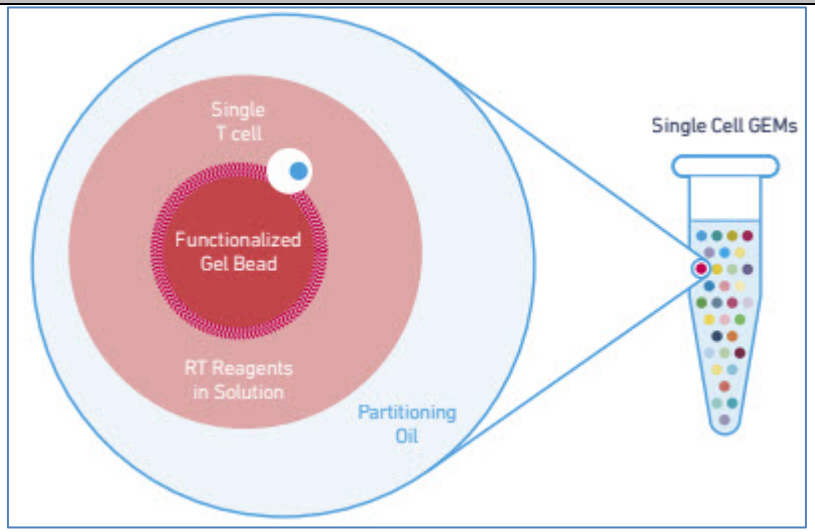
'277 Claim Language	Infringement Support
reaction is a reverse transcription reaction.	Step 1 GEM Generation & Barcoding GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads v1.1, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell. Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.  Ex. 8 at 16.

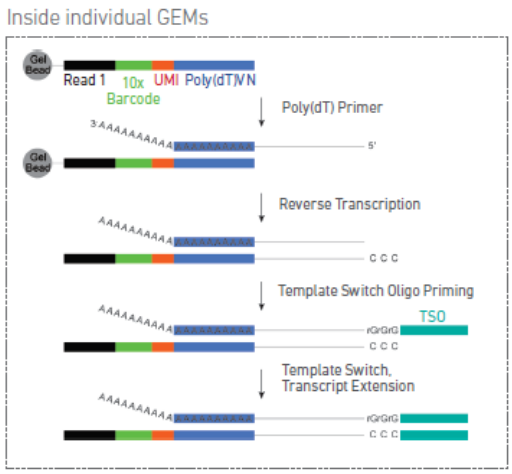
EXHIBIT 17

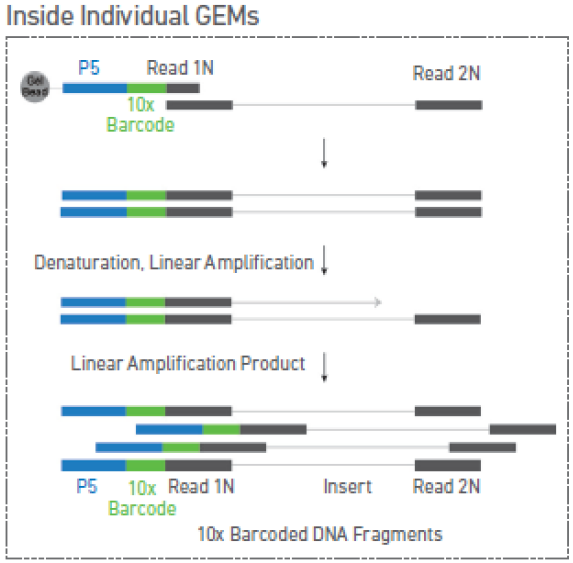
US 10,190,115
Infringement Analysis

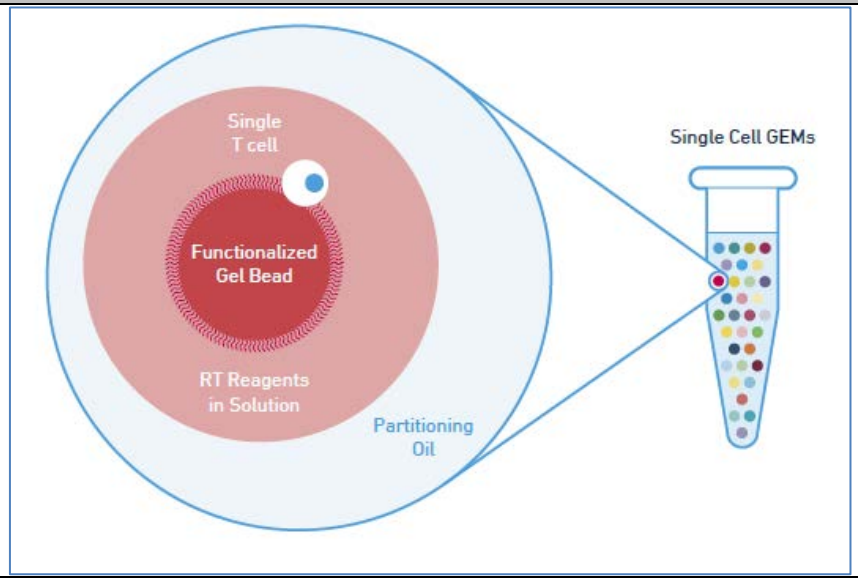
'115 Claim Language	**Infringement Support**
1. A composition comprising a plurality of second partitions containing first partitions, wherein:	10X's Next GEM platform includes a microfluidic droplet generator, which creates a plurality of aqueous microcapsules. The microcapsules are created by inserting a Gel Bead and genetic material (e.g. a cell, cell nucleus, DNA) into an aqueous solution, and then adding the aqueous solution comprising the Gel Bead and sample into an oil surfactant solution. Gel Beads are "infused with millions of unique oligonucleotide sequences." Ex. 2 at 2. This process creates "Gel Beads in Emulsion (GEMs), which act as individual reaction vesicles." *Id.*

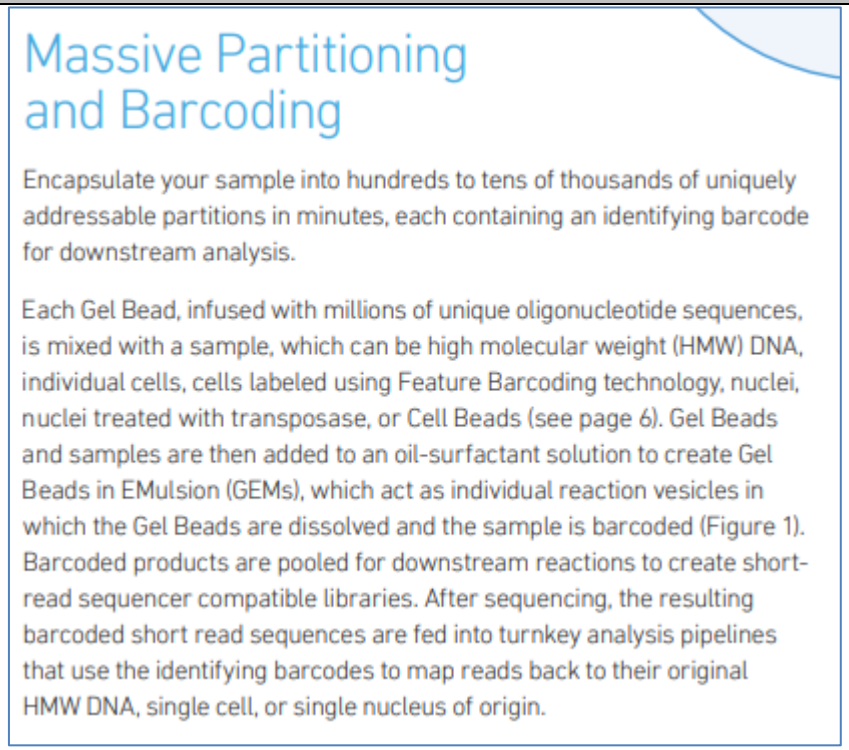
’115 Claim Language	Infringement Support
	 *Id.* 10X’s technology brochure depicts the creation of multiple GEMs.

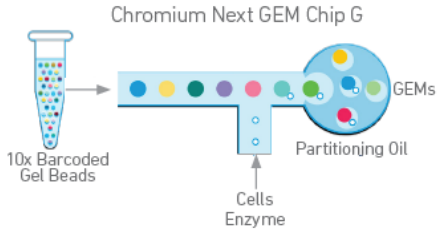
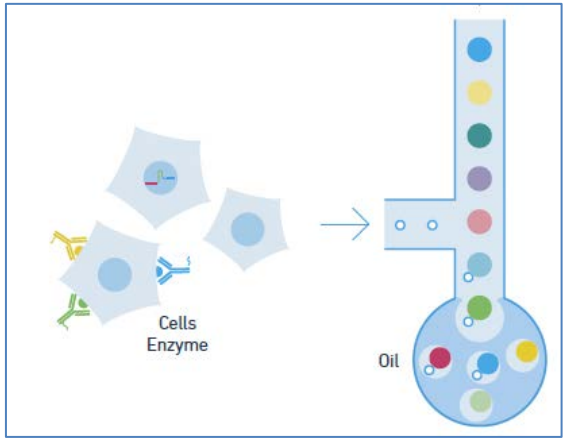
'115 Claim Language	**Infringement Support**
	 *Id.*
a. said first partitions are degradable upon the application of a stimulus to said first partitions such that contents of a first partition is mixed with contents of a second partition; and	10X's technology brochure explains that the Gel Beads (i.e. first partitions) are dissolved once the Bead and the genetic material are encapsulated in a GEM (i.e. a second partition). The Gel Beads are designed to dissolve "[i]mmediately following GEM generation." Ex. 7 at 15.

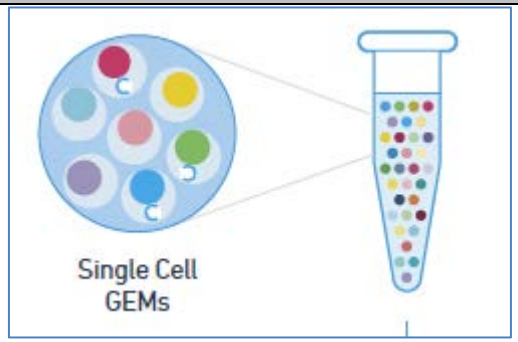
’115 Claim Language	Infringement Support
	Step 1 GEM Generation & Barcoding Immediately following GEM generation, the Gel Bead is dissolved, primers are released, and any co-partitioned cell is lysed. Primer containing: • an Illumina TruSeq Read 1 (read 1 sequencing primer) • 16 nt 10x Barcode • 12 nt unique molecular identifier (UMI) • 30 nt poly(dT) sequence are mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents. Incubation of the GEMs produces barcoded, full-length cDNA from poly-adenylated mRNA.  *Id.* Once the Gel Bead is dissolved, the contents of the Bead (primers, barcodes, unique molecular identifiers, for example) are released into the GEM so that they can mix with the genetic material in order to attach barcodes. *See* Ex. 9 at 13.

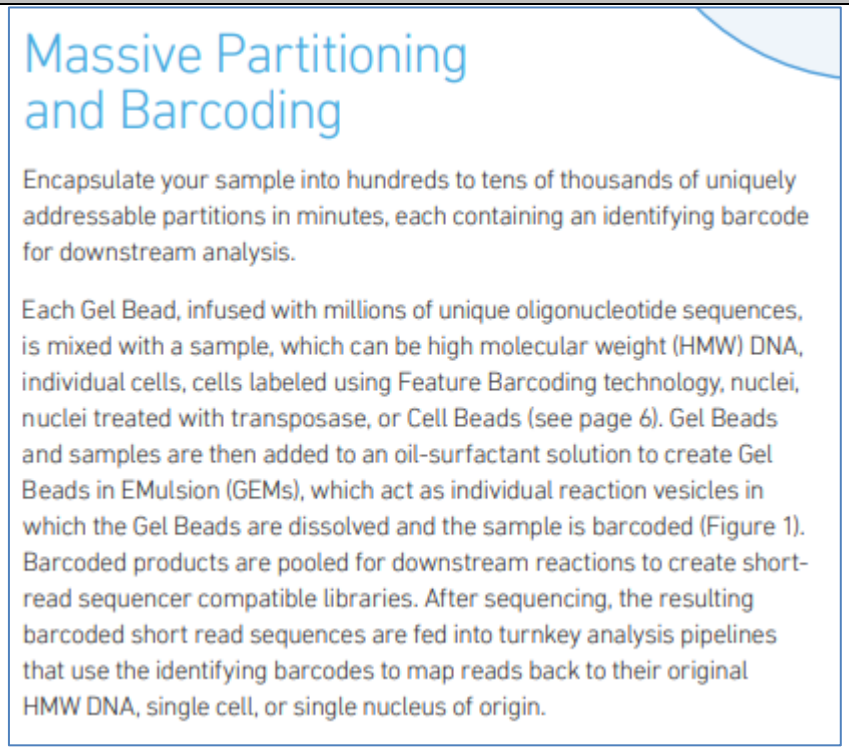
'115 Claim Language	**Infringement Support**
	Upon GEM generation, the Gel Bead is dissolved. Oligonucleotides containing (i) an Illumina® P5 sequence, (ii) a 16 nt 10x Barcode and (iii) a Read 1 (Read 1N) sequence are released and mixed with DNA fragments and Master Mix. Thermal cycling of the GEMs produces 10x barcoded single-stranded DNA. After incubation, the GEMs are broken and pooled fractions are recovered.  *Id.*
b. said first partitions are contained within the second partitions;	10X's technology brochure and product manuals for each of 10X's Next GEM Products repeatedly indicate that the Gel Beads are Contained within the GEMs. In 10X's Next GEM platform, the Gel Beads, along with genetic materials, are encapsulated by the GEMs.

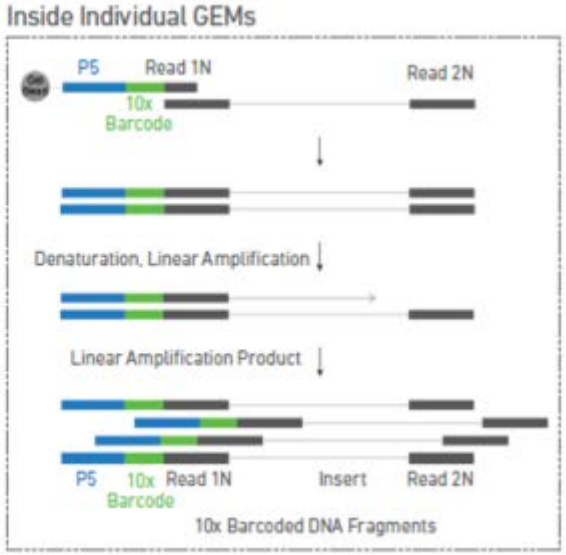
’115 Claim Language	Infringement Support
	

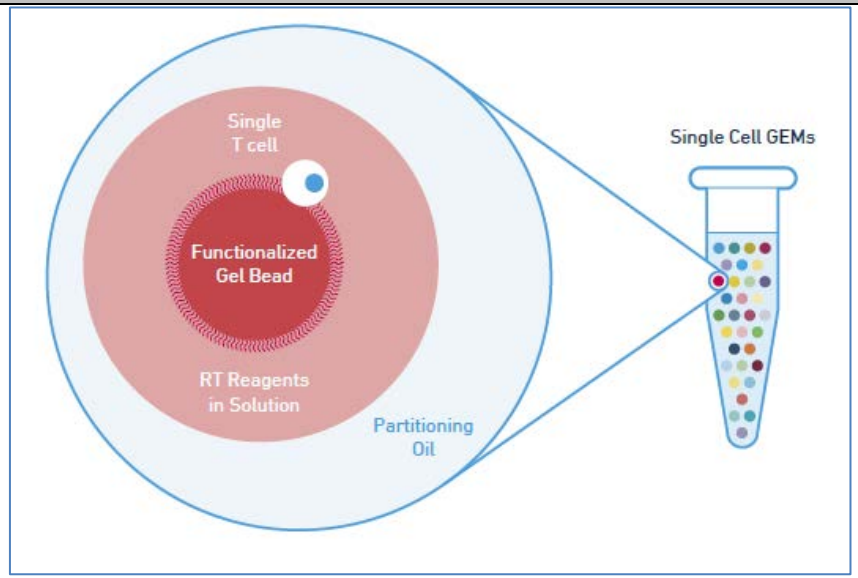
’115 Claim Language	Infringement Support
	 Ex. 2 at 2.

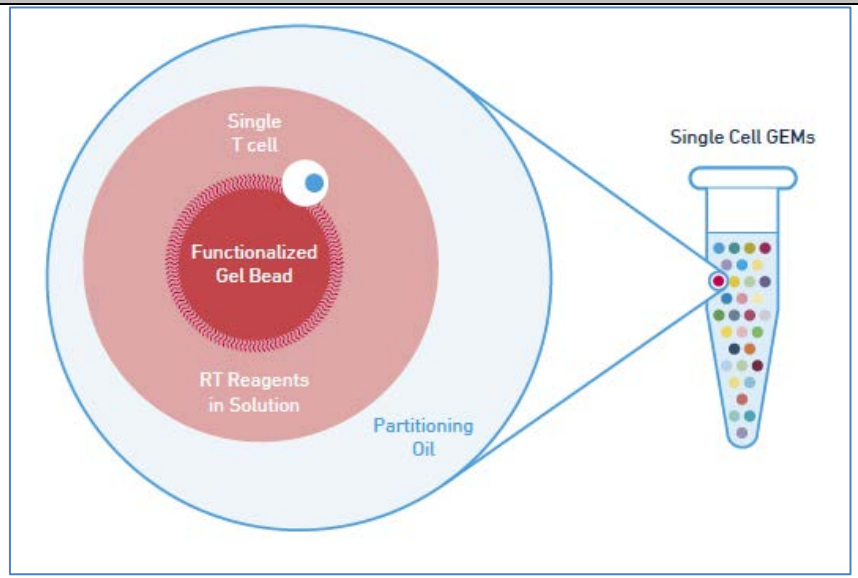
’115 Claim Language	Infringement Support
	Step 1 GEM Generation & Barcoding GEMs are generated by combining barcoded Single Cell 3' v3.1 Gel Beads, a Master Mix containing cells, and Partitioning Oil onto Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90-99%) of generated GEMs contain no cell, while the remainder largely contain a single cell.  Ex. 7 at 14.  Ex. 2 at 3.

'115 Claim Language	**Infringement Support**
	 *Id.*
c. said first partitions contain an oligonucleotide barcode; and	10X's technology brochures explain that each Gel Bead is "infused with millions of unique oligonucleotide sequences." These sequences include oligonucleotide barcodes. Ex. 2. at 2.

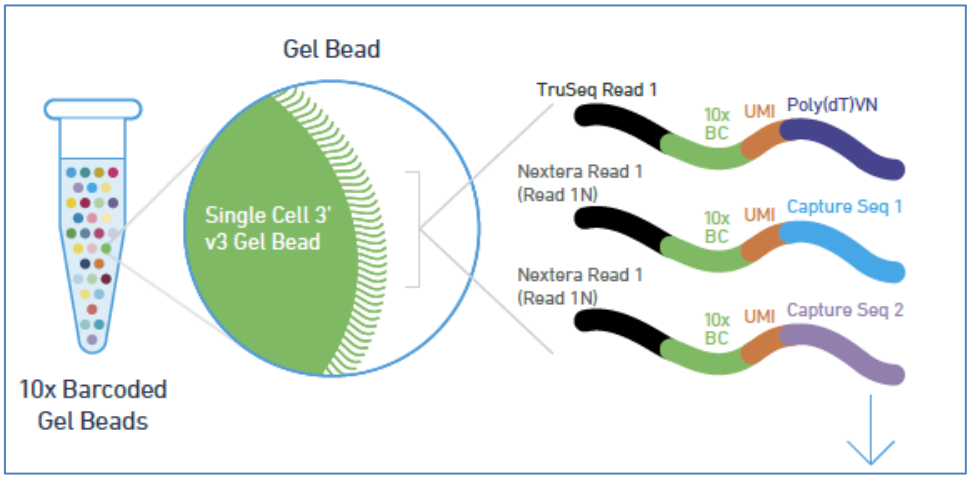
’115 Claim Language	Infringement Support
	 *Id.*

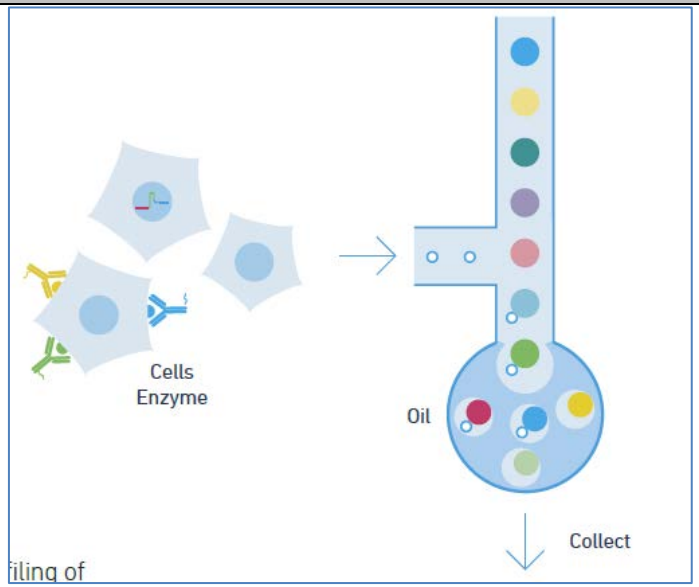
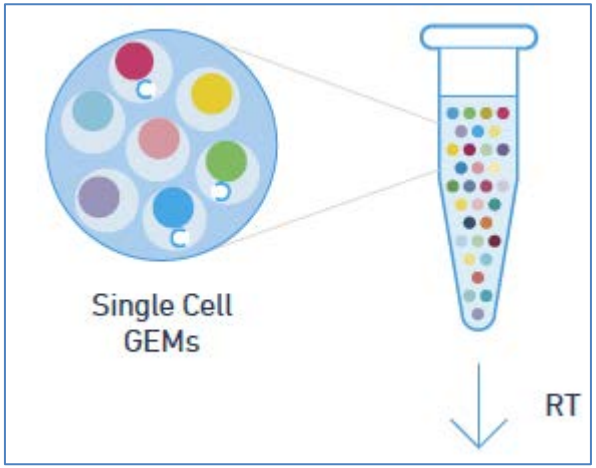
'115 Claim Language	**Infringement Support**
	Upon GEM generation, the Gel Bead is dissolved. Oligonucleotides containing (i) an Illumina® P5 sequence, (ii) a 16 nt 10x Barcode and (iii) a Read 1 (Read 1N) sequence are released and mixed with DNA fragments and Master Mix. Thermal cycling of the GEMs produces 10x barcoded single-stranded DNA. After incubation, the GEMs are broken and pooled fractions are recovered.  Ex. 9 at 13.
d. the first partitions have on average a first average volume and the second partitions have on average a second average volume, wherein the second average volume is at least twice as large as the first average volume.	The technology brochure for 10X's Next GEM system depicts GEMs with volumes that are at least twice as large of the volumes of the Gel Beads.

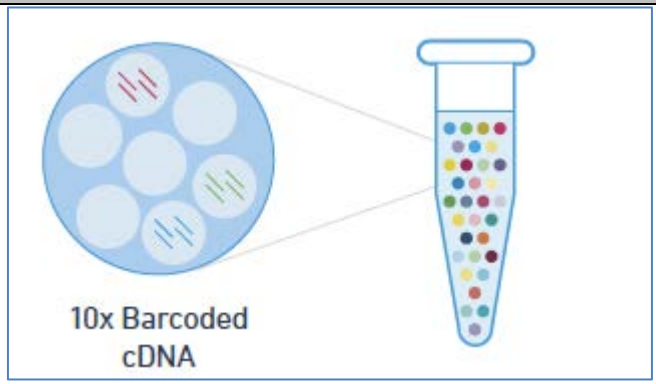
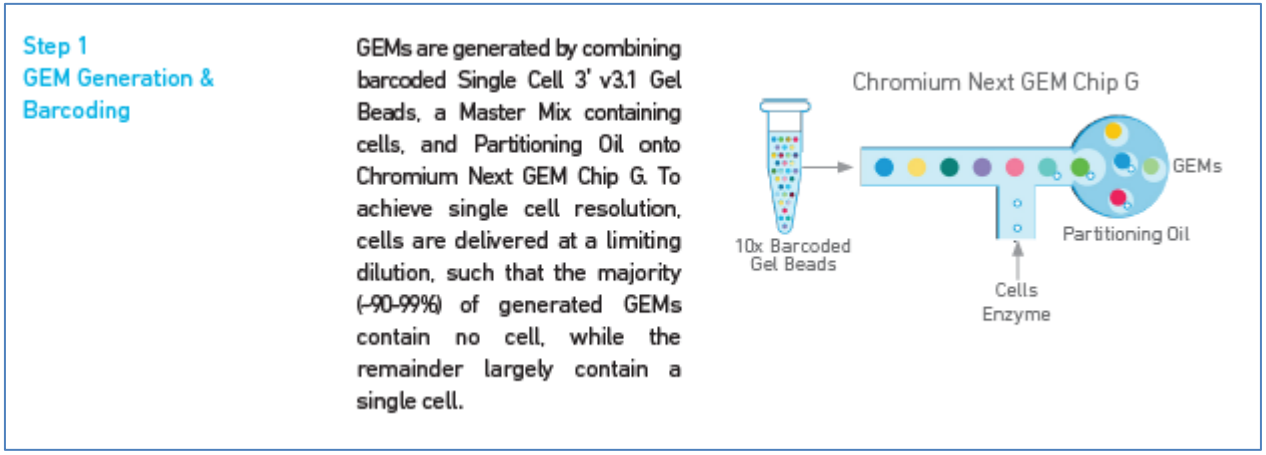
'115 Claim Language	**Infringement Support**
	 Ex. 2 at 2.
4. The composition of claim 1, wherein said second partition is a droplet.	10X's technology brochure confirms that "A GEM is a 'Gel bead in Emulsion' droplet that encapsulates each micro-reaction within the Chromium System. Ex. 2 at 2.

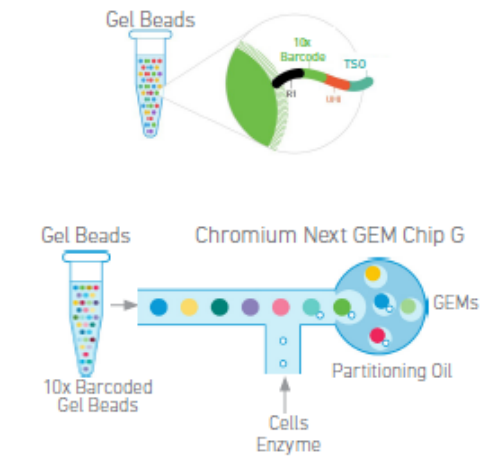
'115 Claim Language	**Infringement Support**
	 Figure 1 A GEM is a "Gel bead in EMulsion" droplet that encapsulates each micro-reaction within the Chromium System. Here we show a Single Cell GEM with a single T cell, reagents, and barcoded gel bead all partitioned within a single droplet. *Id.*
5. The composition of claim 1, wherein the first partition is a droplet.	10X's technology brochure indicates that barcodes are contained by a "gel bead." A gel bead is a type of droplet.
6. The composition of claim 1, wherein the first partition and	10X's technology brochure indicates that barcodes are contained by a "gel bead." A gel bead is a type of droplet.

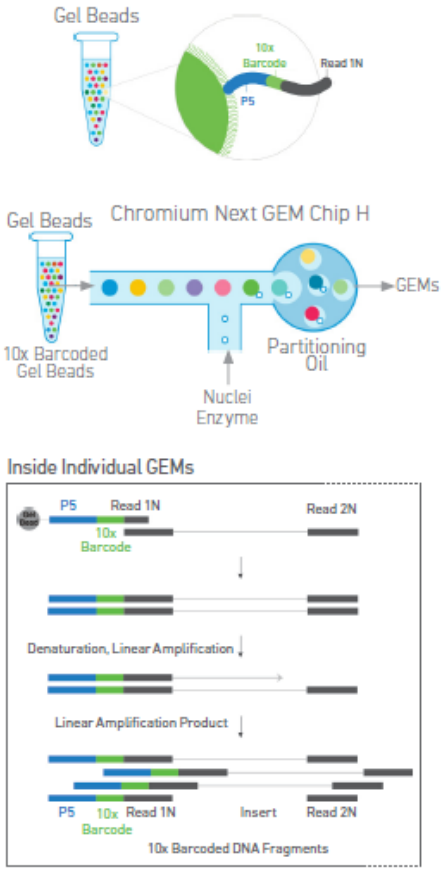
'115 Claim Language	**Infringement Support**
the second partition are droplets.	
7. The composition of claim 1, wherein the second partition comprises a sample comprising polynucleotides.	The 10X technology brochure explains that the goal of the Next GEM platform is to "encapsulate your sample into hundreds to tens of thousands of uniquely addressable partitions in minutes." Ex. 2 at 2. Samples "can be high molecular weight (HMW) DNA, individual cells, cells labeled using Feature Barcoding technology, nuclei, nuclei treated with transposase, or Cell Beads." *Id*. The end result of this process is to use the identifying barcodes that are attached to samples within the partitions (i.e. GEMs) "to map reads back to their original HMW DNA, single cell, or single nucleus of origin." *Id*. Massive Partitioning and Barcoding Encapsulate your sample into hundreds to tens of thousands of uniquely addressable partitions in minutes, each containing an identifying barcode for downstream analysis. Each Gel Bead, infused with millions of unique oligonucleotide sequences, is mixed with a sample, which can be high molecular weight (HMW) DNA, individual cells, cells labeled using Feature Barcoding technology, nuclei, nuclei treated with transposase, or Cell Beads (see page 6). Gel Beads and samples are then added to an oil-surfactant solution to create Gel Beads in EMulsion (GEMs), which act as individual reaction vesicles in which the Gel Beads are dissolved and the sample is barcoded (Figure 1). Barcoded products are pooled for downstream reactions to create short-read sequencer compatible libraries. After sequencing, the resulting barcoded short read sequences are fed into turnkey analysis pipelines that use the identifying barcodes to map reads back to their original HMW DNA, single cell, or single nucleus of origin.

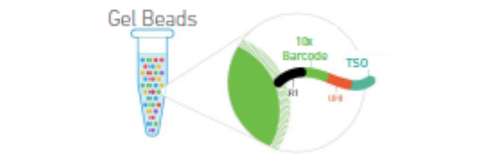
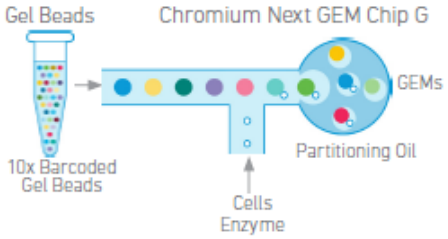
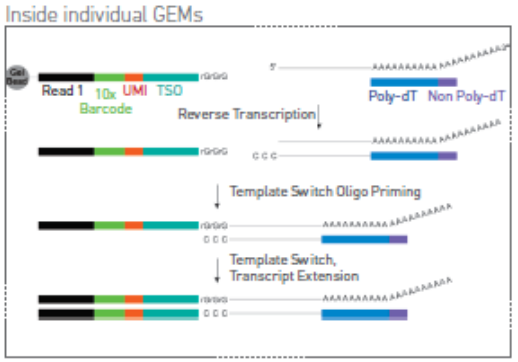
’115 Claim Language	Infringement Support
	Id. Samples comprising polynucleotides are encapsulated into GEMs for barcoding, as depicted in 10X’s technology brochure and product manuals. 

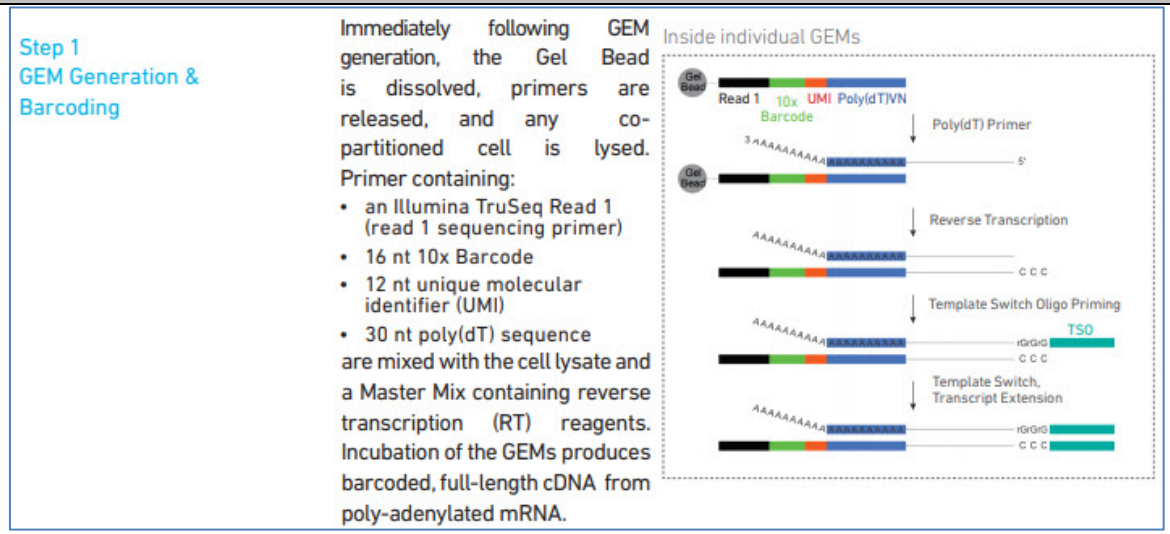
’115 Claim Language	Infringement Support
	 

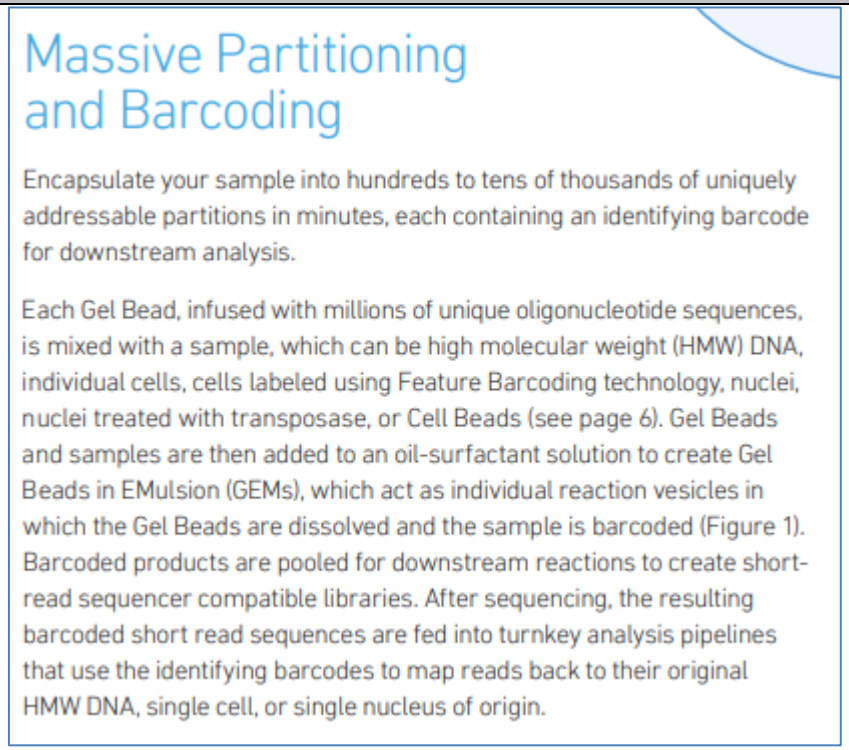
’115 Claim Language	Infringement Support
	 Ex. 2 at 3.  Ex. 7 at 15.

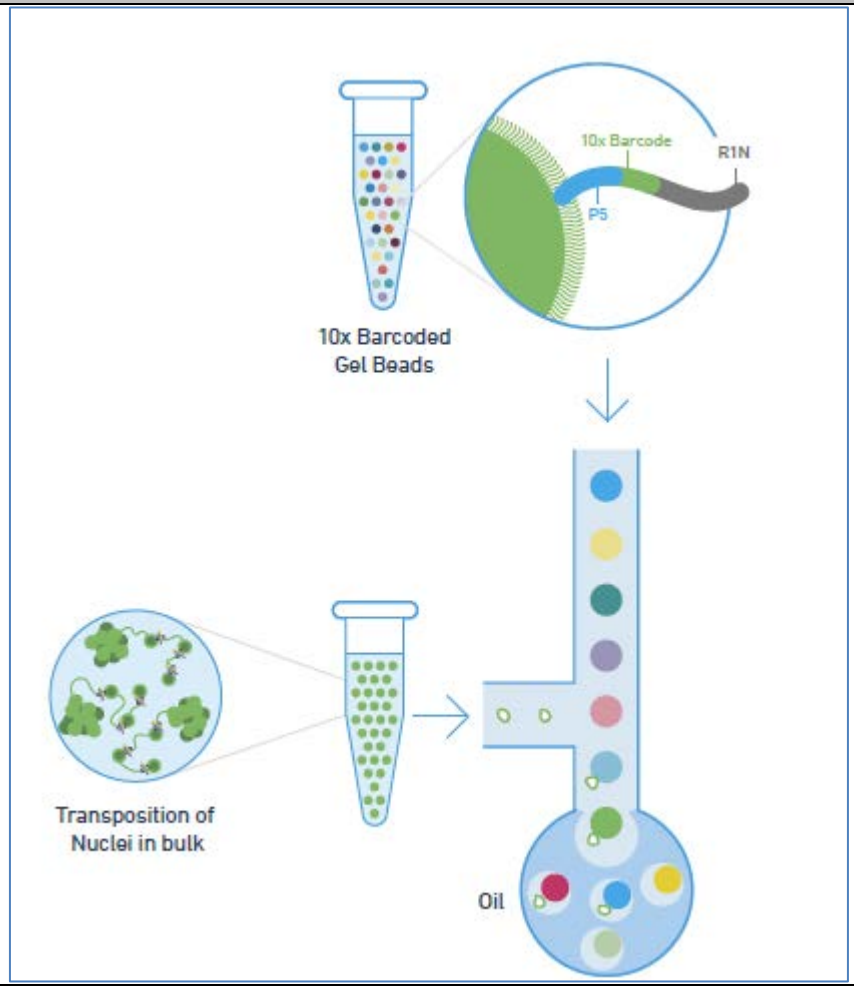
’115 Claim Language	Infringement Support
	Step 1 GEM Generation & Barcoding GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads v1.1, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell. Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.  Ex. 8 at 16.

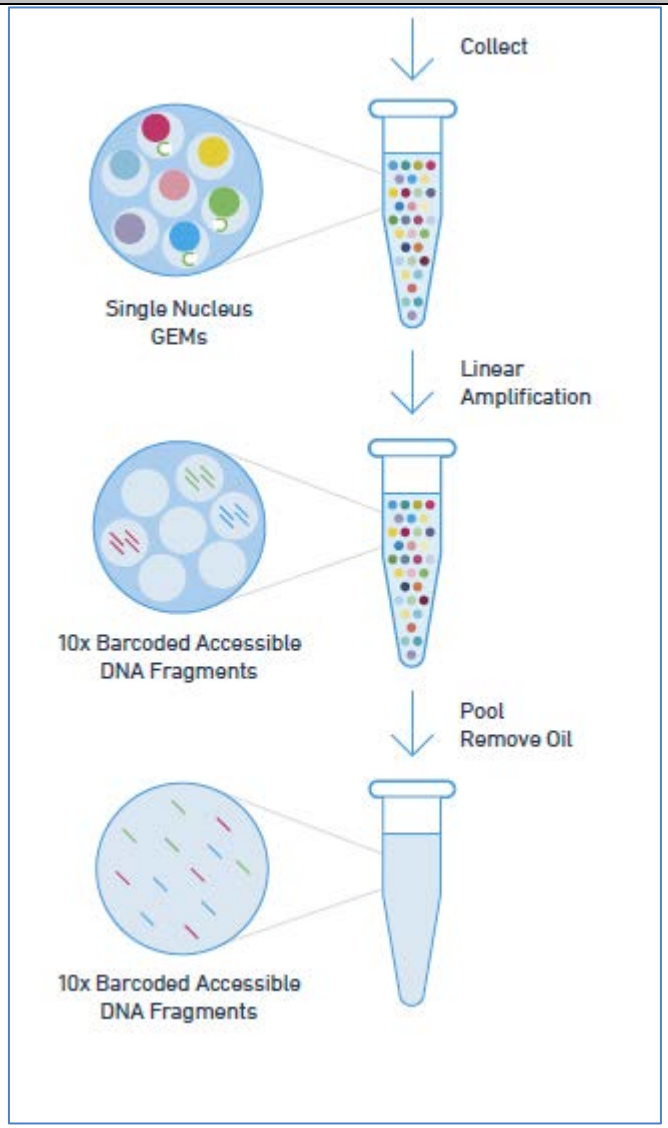
’115 Claim Language	Infringement Support
	Step 2 GEM Generation & Barcoding GEMs are generated by combining barcoded Gel Beads, transposed nuclei, a Master Mix, and Partitioning Oil on a Chromium Next GEM Chip H. To achieve single nuclei resolution, the nuclei are delivered at a limiting dilution, such that the majority (~90-99%) of generated GEMs contains no nuclei, while the remainder largely contain a single nucleus. Upon GEM generation, the Gel Bead is dissolved. Oligonucleotides containing (i) an Illumina® P5 sequence, (ii) a 16 nt 10x Barcode and (iii) a Read 1 (Read 1N) sequence are released and mixed with DNA fragments and Master Mix. Thermal cycling of the GEMs produces 10x barcoded single-stranded DNA. After incubation, the GEMs are broken and pooled fractions are recovered.  Ex. 9 at 13.
8. The composition of claim 7, wherein the second partition further comprises a DNA	The product manuals for each of 10X’s Next GEM products explain that upon GEM generation, the Gel Bead is dissolved releasing the reagents required to attach barcodes to genetic material. The barcodes are mixed with the genetic material and “a Master Mix containing reverse

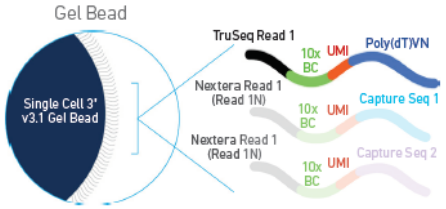
'115 Claim Language	Infringement Support
polymerase enzyme or a reverse transcriptase enzyme.	transcription (RT) reagents and poly(dT) RT primers" so that transcription can occur in order to attach the barcodes to the sample. Ex. 8 at 16. Step 1 GEM Generation & Barcoding GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads v1.1, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell. Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.    *Id.*

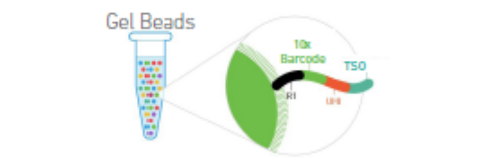
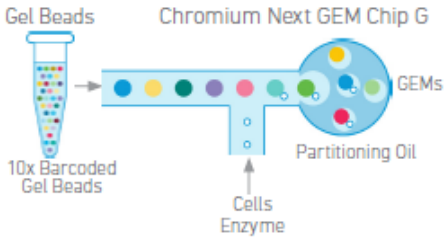
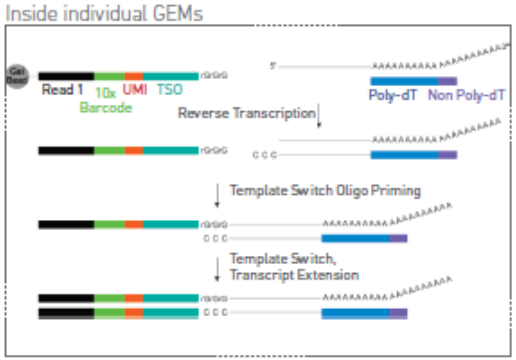
’115 Claim Language	Infringement Support
	 Ex. 7 at 15.
9. The composition of claim 7, wherein the polynucleotides comprise DNA.	The 10X technology brochure explains that the goal of the Next GEM platform is to “encapsulate your sample into hundreds to tens of thousands of uniquely addressable partitions in minutes.” Ex. 2 at 2. Samples “can be high molecular weight (HMW) DNA, individual cells, cells labeled using Feature Barcoding technology, nuclei, nuclei treated with transposase, or Cell Beads.” *Id*. The end result of this process is to use the identifying barcodes that are attached to samples within the partitions (i.e. GEMs) “to map reads back to their original HMW DNA, single cell, or single nucleus of origin.” *Id*.

'115 Claim Language	**Infringement Support**
	 *Id.*
10. The composition of claim 9, wherein the DNA is genomic DNA.	The 10X Next GEM technology brochure explains that samples may be nuclei or nuclei treated with transposase. Ex. 2 at 2. This results in the encapsulation of DNA from cell nuclei (*i.e.* genomic DNA) to be encapsulated in GEMs. Ex. 2 at 5.

’115 Claim Language	Infringement Support
	

’115 Claim Language	Infringement Support
	

'115 Claim Language	**Infringement Support**
	Id.
11. The composition of claim 7, wherein the polynucleotides comprise RNA.	The genetic material used in the 10X process may be derived from samples of high molecular weight DNA, individual cells, cells labelled using Feature Barcoding technology, nuclei, nuclei treated with transposase, or cell beads. Ex. 2 at 2. At least in cases where single cells are used to obtain the genetic sample, the cell is lysed and the cDNA from the cell is denatured to produce mRNA in order to attach barcodes. The 10X product manuals confirm that barcoded cDNA molecules are produced from mRNA. Single Cell 3' v3.1 Gel Beads In addition to the poly(dT) primer that enables the production of barcoded, full-length cDNA from poly-adenylated mRNA, the Single Cell 3' v3.1 Gel Beads also include two additional primer sequences (Capture Sequence 1 and Capture Sequence 2), that enable capture and priming of Feature Barcoding technology compatible targets or analytes of interest. Only the poly(dT) primers are used in this protocol for generating Single Cell 3' Gene Expression libraries.  Ex. 7 at 14.

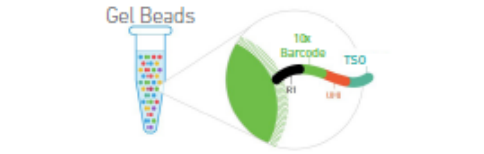
'115 Claim Language	Infringement Support
	Step 1 GEM Generation & Barcoding GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads v1.1, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell. Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.    Ex. 8 at 16.
12. The composition of claim 7, wherein the polynucleotides comprise cDNA	The genetic material used in the 10X process may be derived from samples of high molecular weight DNA, individual cells, cells labelled using Feature Barcoding technology, nuclei, nuclei treated with transposase, or cell beads. Ex. 2 at 2. At least in cases where single cells are used to

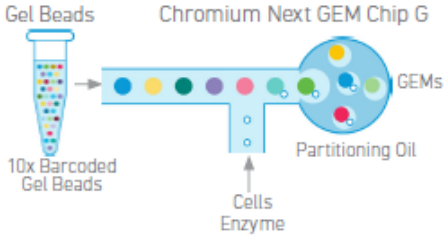
'115 Claim Language	Infringement Support
	obtain the genetic sample, the cell is lysed and the cDNA from the cell is denatured to produce mRNA in order to attach barcodes. 10X's product manuals for its Next GEM platform confirm that once the cell is encapsulated by the GEM, GEMs are incubated to produce "barcoded, full-length cDNA." Ex. 8 at 16.

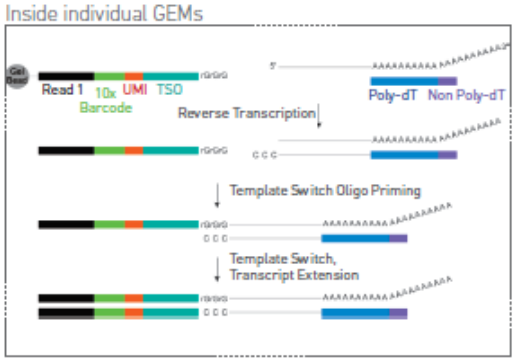
Step 1
GEM Generation & Barcoding

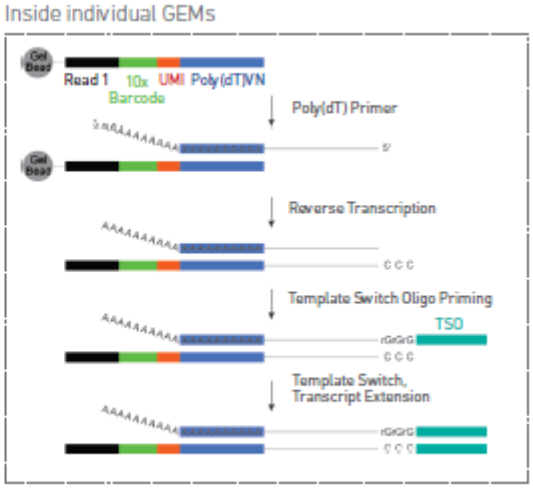
GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads v1.1, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell.

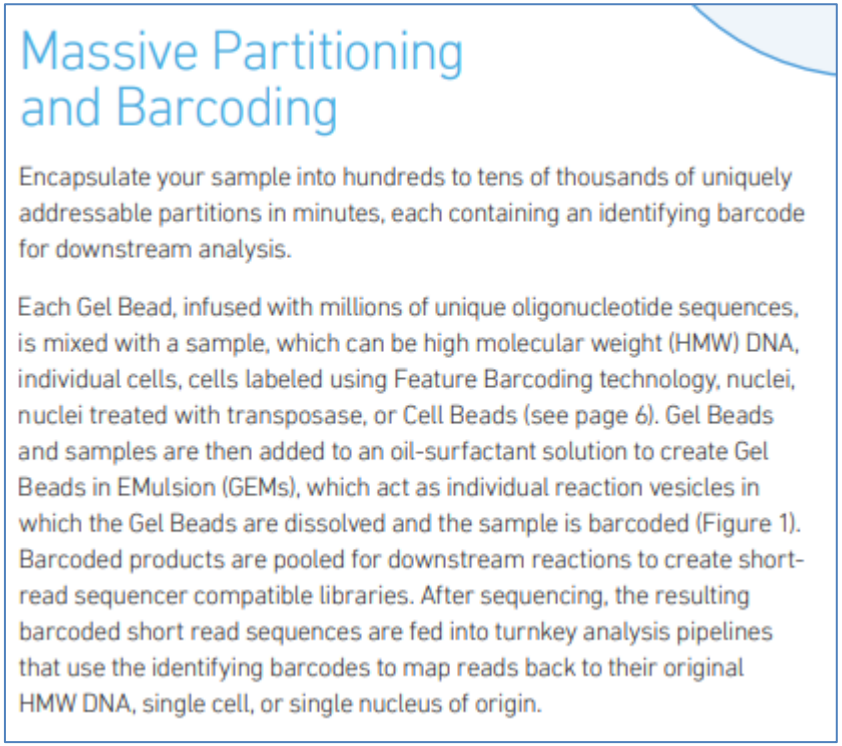
Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.

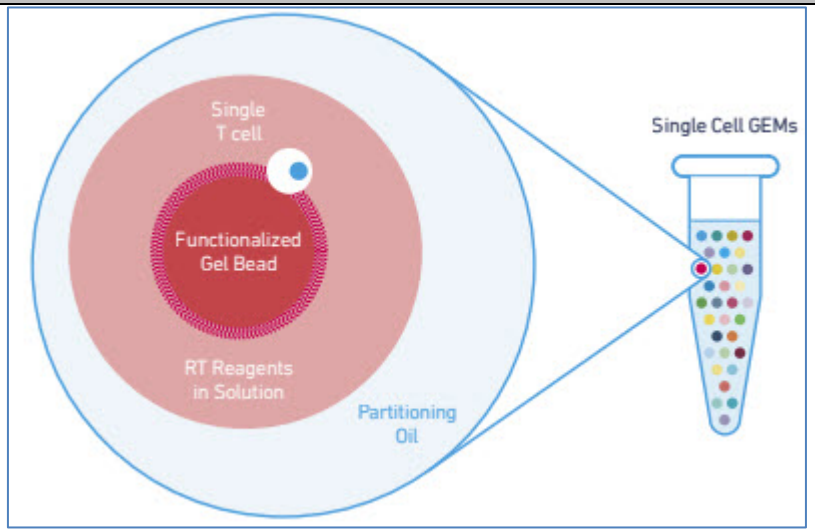


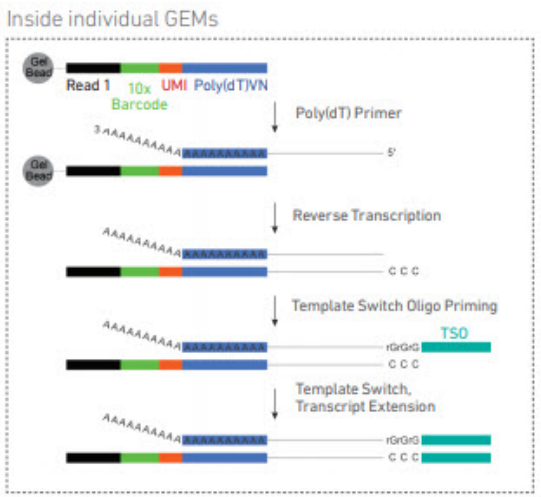


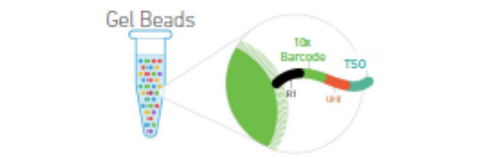
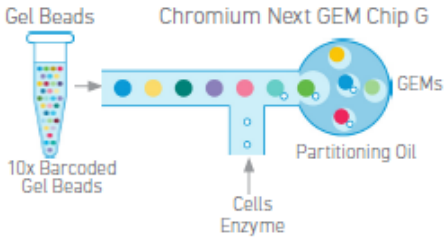
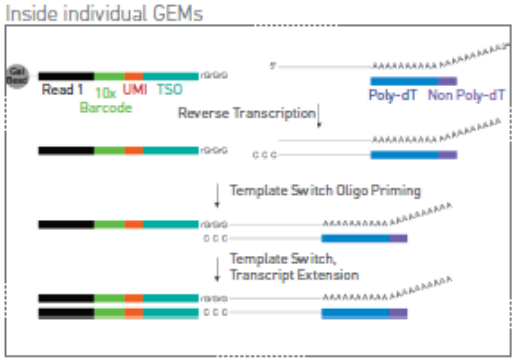


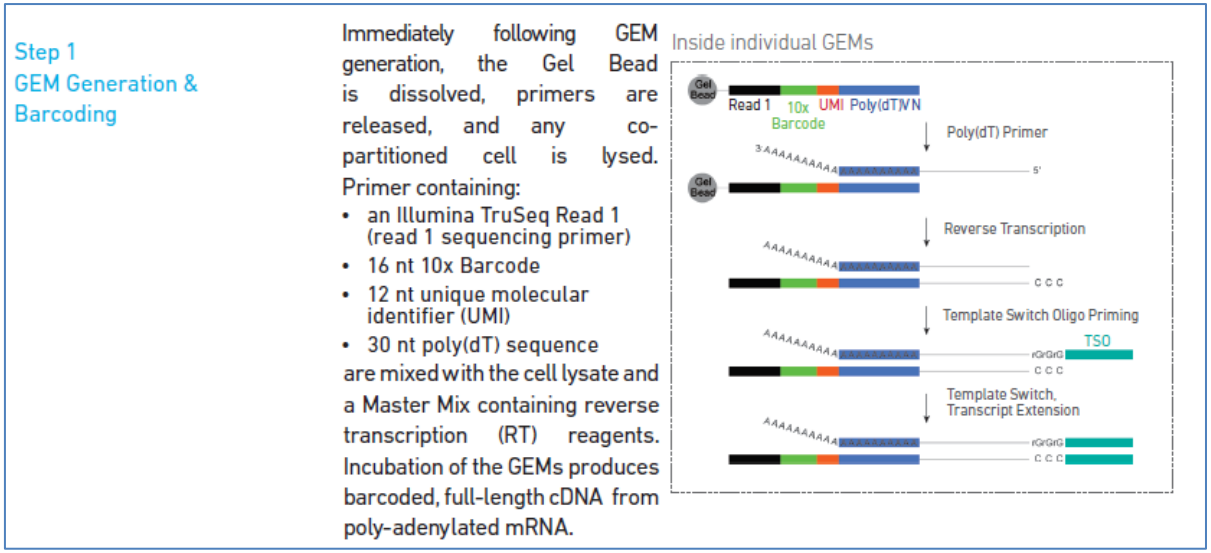
'115 Claim Language	Infringement Support
	Id. Step 1 GEM Generation & Barcoding Immediately following GEM generation, the Gel Bead is dissolved, primers are released, and any co-partitioned cell is lysed. Primer containing: • an Illumina TruSeq Read 1 (read 1 sequencing primer) • 16 nt 10x Barcode • 12 nt unique molecular identifier (UMI) • 30 nt poly(dT) sequence are mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents. Incubation of the GEMs produces barcoded, full-length cDNA from poly-adenylated mRNA.  Ex. 7 at 15.
13. The composition of claim 7, wherein the partitions are less than 10 nL in volume	The 10x product manuals explain that in the Next GEM sequencing platform, samples are "partitioned into nanoliter-scale Gel Beads-in-Emulsions (GEMs)" Ex. 9 at 13; *see also* Ex. 7 at 14 (indicating that samples are barcoded "by partitioning thousands of cells into nanoliter-scale" GEMs).
14. A device comprising a plurality of second partitions, wherein:	10X's Next GEM platform includes a microfluidic droplet generator, which creates a plurality of aqueous microcapsules. The microcapsules ("GEMs") are created by inserting an aqueous solution including a Gel Bead and a sample into an oil emulsion.
a. at least one second partition of the plurality of second partitions contains a first	10X's Next GEM platform includes a microfluidic droplet generator, which creates a plurality of aqueous microcapsules. The microcapsules are created by inserting a Gel Bead and genetic material (e.g. a cell, cell nucleus, DNA) into an aqueous solution, and then adding the aqueous solution

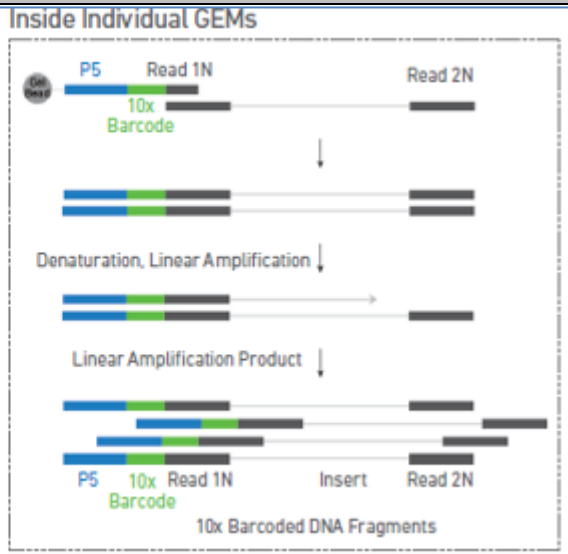
'115 Claim Language	**Infringement Support**
partition comprising an oligonucleotide barcode, and the first partition has a first volume and the at least one second partition has a second volume, wherein the second volume is at least twice as large as the first volume; and	comprising the Gel Bead and sample into an oil surfactant solution. Gel Beads are "infused with millions of unique oligonucleotide sequences." Ex. 2 at 2. This process creates "Gel Beads in Emulsion (GEMs), which act as individual reaction vesicles" to attach barcodes to genetic material. *Id.*  Massive Partitioning and Barcoding Encapsulate your sample into hundreds to tens of thousands of uniquely addressable partitions in minutes, each containing an identifying barcode for downstream analysis. Each Gel Bead, infused with millions of unique oligonucleotide sequences, is mixed with a sample, which can be high molecular weight (HMW) DNA, individual cells, cells labeled using Feature Barcoding technology, nuclei, nuclei treated with transposase, or Cell Beads (see page 6). Gel Beads and samples are then added to an oil-surfactant solution to create Gel Beads in EMulsion (GEMs), which act as individual reaction vesicles in which the Gel Beads are dissolved and the sample is barcoded (Figure 1). Barcoded products are pooled for downstream reactions to create short-read sequencer compatible libraries. After sequencing, the resulting barcoded short read sequences are fed into turnkey analysis pipelines that use the identifying barcodes to map reads back to their original HMW DNA, single cell, or single nucleus of origin. *Id.* 10X's technology brochure depicts the creation of multiple GEMs.

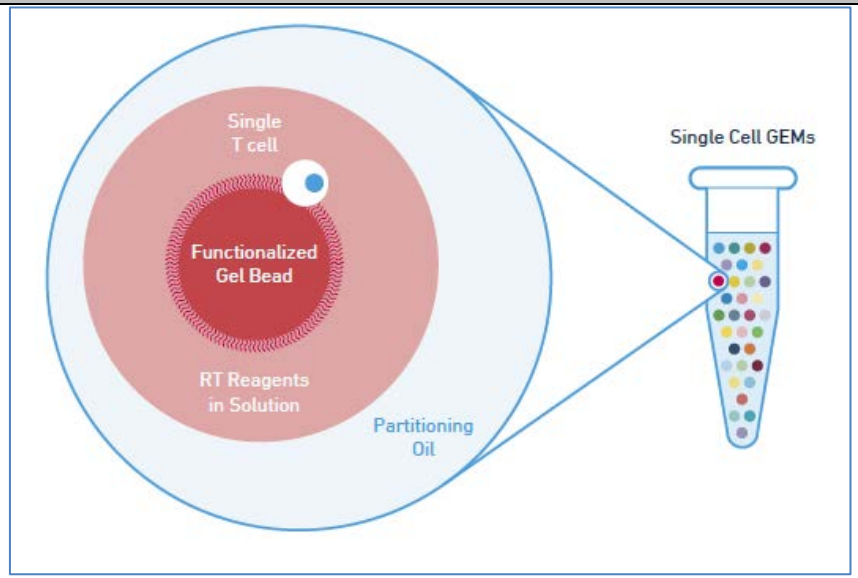
’115 Claim Language	Infringement Support
	 *Id.* As depicted in the above image, the technology brochure for 10X’s Next GEM system depicts GEMs with volumes that are at least twice as large of the volumes of the Gel Beads. *Id.* Furthermore, GEMs need to be large enough so that individual barcoding reactions can occur in each GEM. This reaction includes cell lysis, dissolving the Gel Bead, and denaturing and transcribing DNA. The reaction that takes place inside the GEM is depicted in 10X’s product manuals. *See e.g.* Ex. 7 at 15.

’115 Claim Language	Infringement Support
	Step 1 GEM Generation & Barcoding Immediately following GEM generation, the Gel Bead is dissolved, primers are released, and any co-partitioned cell is lysed. Primer containing: • an Illumina TruSeq Read 1 (read 1 sequencing primer) • 16 nt 10x Barcode • 12 nt unique molecular identifier (UMI) • 30 nt poly(dT) sequence are mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents. Incubation of the GEMs produces barcoded, full-length cDNA from poly-adenylated mRNA.  *Id.*

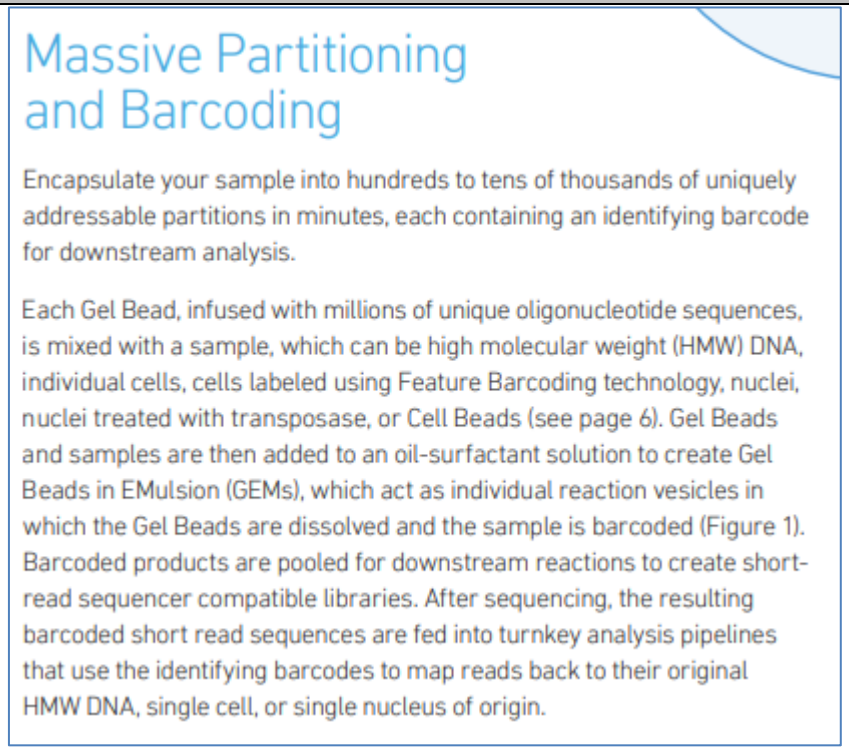
'115 Claim Language	Infringement Support
	Step 1 GEM Generation & Barcoding GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads v1.1, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell. Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.    Ex. 8 at 16.
b. said first partition is degradable upon the application of a stimulus to	10X's technology brochure explains that the Gel Beads (*i.e.* first partitions) are dissolved once the Bead and the genetic material are encapsulated in a GEM (*i.e.* a second partition). The Gel Beads are designed to dissolve "[i]mmediately following GEM generation." Ex. 7 at 15.

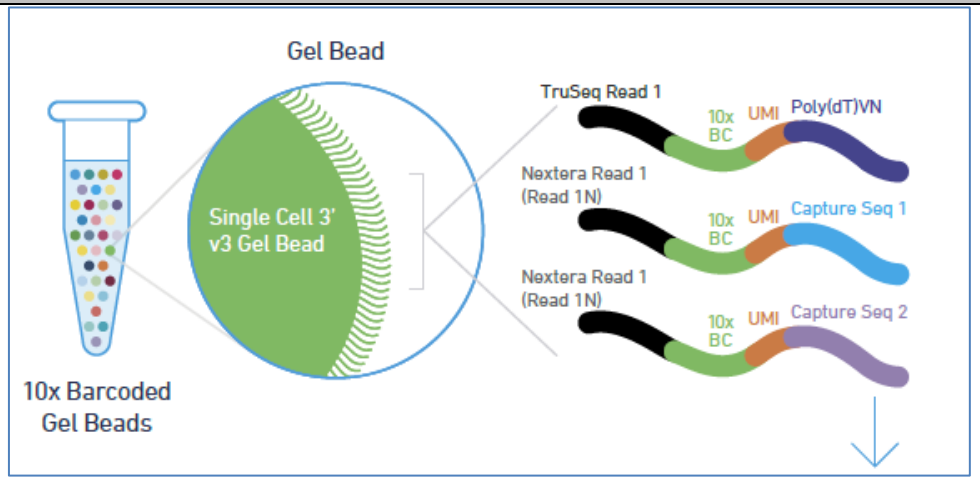
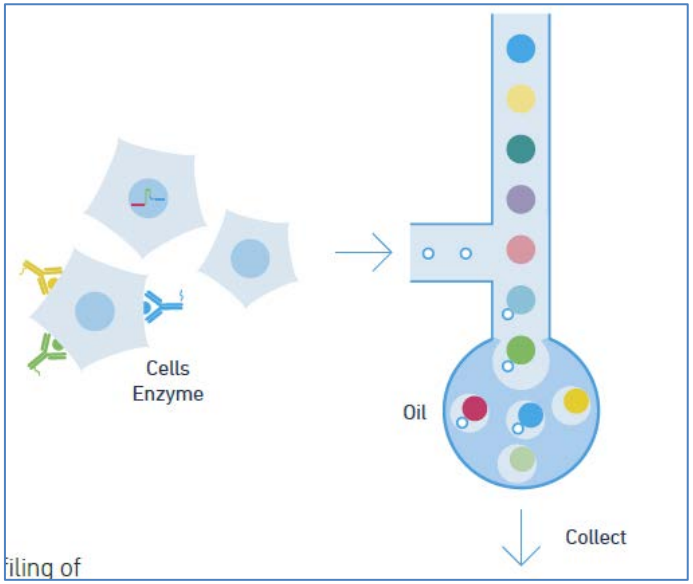
’115 Claim Language	Infringement Support
said first partition such that contents of a first partition is mixed with contents of a second partition.	 *Id.* Once the Gel Bead is dissolved, the contents of the Bead (primers, barcodes, and unique molecular identifiers, for example) are released into the GEM so that they can mix with the genetic material in order to attach barcodes. *See* Ex. 9 at 13.

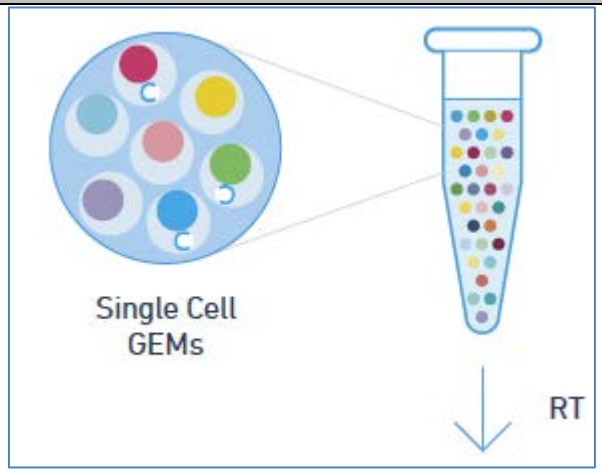
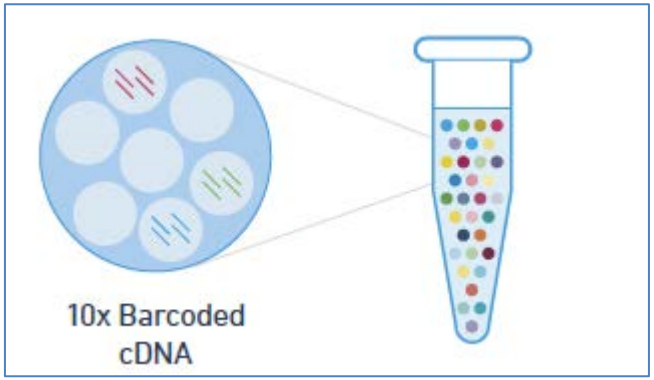
'115 Claim Language	**Infringement Support**
	Upon GEM generation, the Gel Bead is dissolved. Oligonucleotides containing (i) an Illumina® P5 sequence, (ii) a 16 nt 10x Barcode and (iii) a Read 1 (Read 1N) sequence are released and mixed with DNA fragments and Master Mix. Thermal cycling of the GEMs produces 10x barcoded single-stranded DNA. After incubation, the GEMs are broken and pooled fractions are recovered.  *Id*.
15. The device of claim 14, wherein said second partition is a droplet.	10X's technology brochure confirms that "A GEM is a 'Gel bead in Emulsion' droplet that encapsulates each micro-reaction within the Chromium System. Ex. 2 at 2.

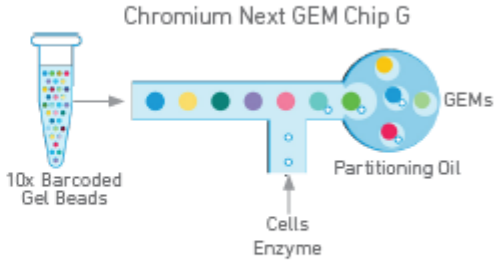
’115 Claim Language	Infringement Support
	 Figure 1 A GEM is a "Gel bead in EMulsion" droplet that encapsulates each micro-reaction within the Chromium System. Here we show a Single Cell GEM with a single T cell, reagents, and barcoded gel bead all partitioned within a single droplet. *Id.*
18. The device of claim 14, wherein the first partition is a droplet.	10X’s technology brochure indicates that barcodes are contained by a “gel bead.” A gel bead is a type of droplet.
19. The device of claim 14, wherein the first partition and the second partition are droplets.	10X’s technology brochure indicates that barcodes are contained by a “gel bead.” A gel bead is a type of droplet.

'115 Claim Language	**Infringement Support**
20. The device of claim 19, wherein the droplets are less than 10 nL in volume.	The 10x product manuals explain that in the Next GEM sequencing platform, samples are "partitioned into nanoliter-scale Gel Beads-in-Emulsions (GEMs)" Ex. 9 at 13; *see also* Ex. 7 at 14 (indicating that samples are barcoded "by partitioning thousands of cells into nanoliter-scale" GEMs).
21. The device of claim 14, wherein the second partition comprises a sample comprising polynucleotides.	The 10X technology brochure explains that the goal of the Next GEM platform is to "encapsulate your sample into hundreds to tens of thousands of uniquely addressable partitions in minutes." Ex. 2 at 2. Samples "can be high molecular weight (HMW) DNA, individual cells, cells labeled using Feature Barcoding technology, nuclei, nuclei treated with transposase, or Cell Beads." *Id*. The end result of this process is to use the identifying barcodes that are attached to samples within the partitions (i.e. GEMs) "to map reads back to their original HMW DNA, single cell, or single nucleus of origin." *Id*.

’115 Claim Language	Infringement Support
	 *Id.* Samples comprising polynucleotides, including DNA and single cells, are encapsulated into GEMs for barcoding, as depicted in 10X’s technology brochure and product manuals.

’115 Claim Language	Infringement Support
	 

’115 Claim Language	Infringement Support
	  Ex. 2 at 3.

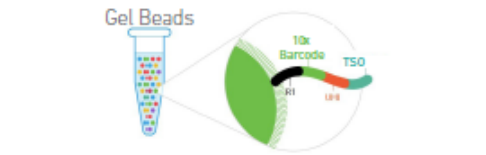
’115 Claim Language	Infringement Support
	Step 1 GEM Generation & Barcoding GEMs are generated by combining barcoded Single Cell 3' v3.1 Gel Beads, a Master Mix containing cells, and Partitioning Oil onto Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90-99%) of generated GEMs contain no cell, while the remainder largely contain a single cell.  Ex. 7 at 15.

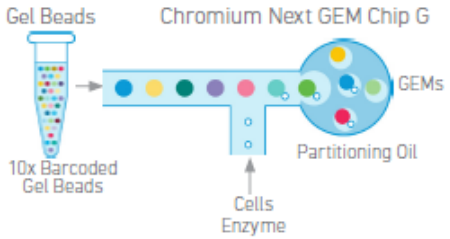
'115 Claim Language	Infringement Support

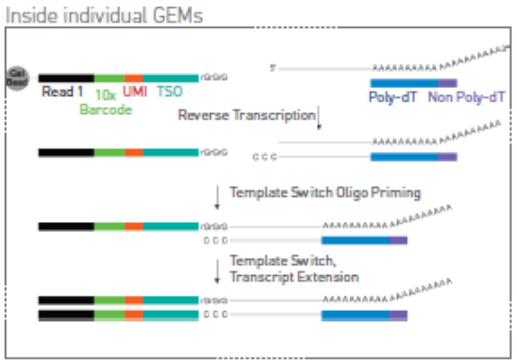
Step 1
GEM Generation & Barcoding

GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads v1.1, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell.

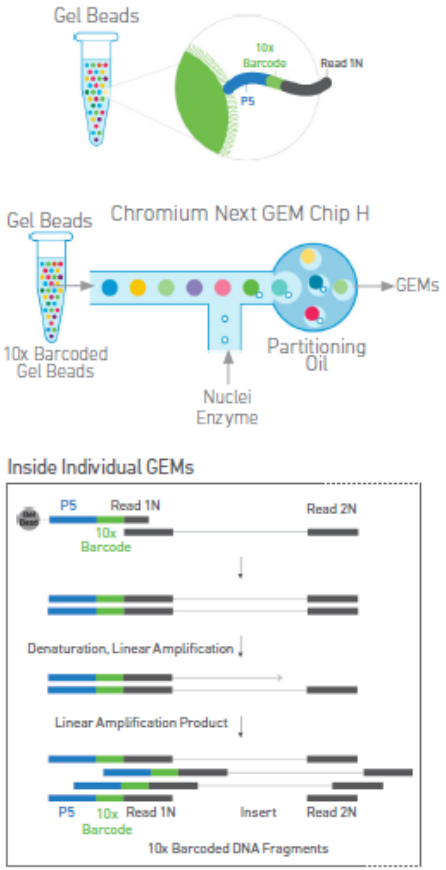
Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.







Ex. 8 at 16.

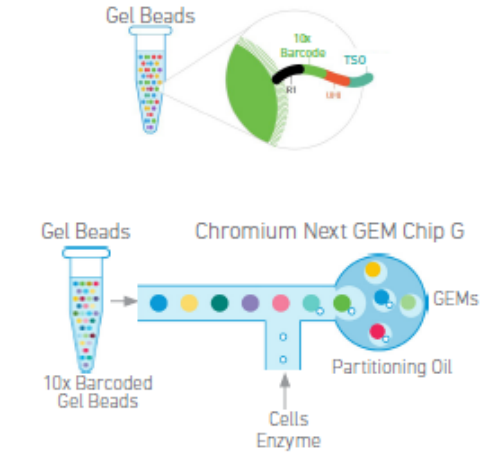
'115 Claim Language	Infringement Support
	Step 2 GEM Generation & Barcoding GEMs are generated by combining barcoded Gel Beads, transposed nuclei, a Master Mix, and Partitioning Oil on a Chromium Next GEM Chip H. To achieve single nuclei resolution, the nuclei are delivered at a limiting dilution, such that the majority (~90-99%) of generated GEMs contains no nuclei, while the remainder largely contain a single nucleus. Upon GEM generation, the Gel Bead is dissolved. Oligonucleotides containing (i) an Illumina® P5 sequence, (ii) a 16 nt 10x Barcode and (iii) a Read 1 (Read 1N) sequence are released and mixed with DNA fragments and Master Mix. Thermal cycling of the GEMs produces 10x barcoded single-stranded DNA. After incubation, the GEMs are broken and pooled fractions are recovered.  Ex. 9 at 13.
22. The device of claim 21, wherein the second partition further comprises a DNA polymerase enzyme or a reverse transcriptase enzyme.	The product manuals for each of 10X's Next GEM products explain that upon GEM generation, the Gel Bead is dissolved releasing the reagents required to attach barcodes to genetic material. The barcodes are mixed with the genetic material and "a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers" so that transcription can occur in order to attach the barcodes to the sample. Ex. 8 at 16.

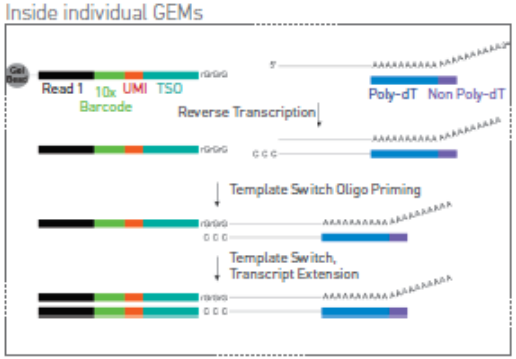
'115 Claim Language	Infringement Support

Step 1
GEM Generation & Barcoding

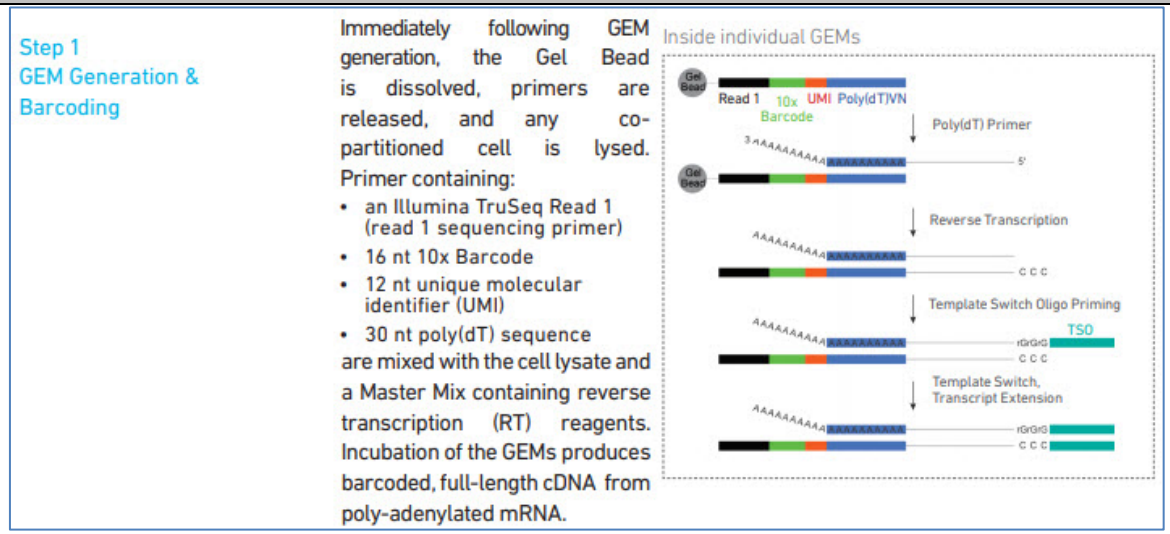
GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads v1.1, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell.

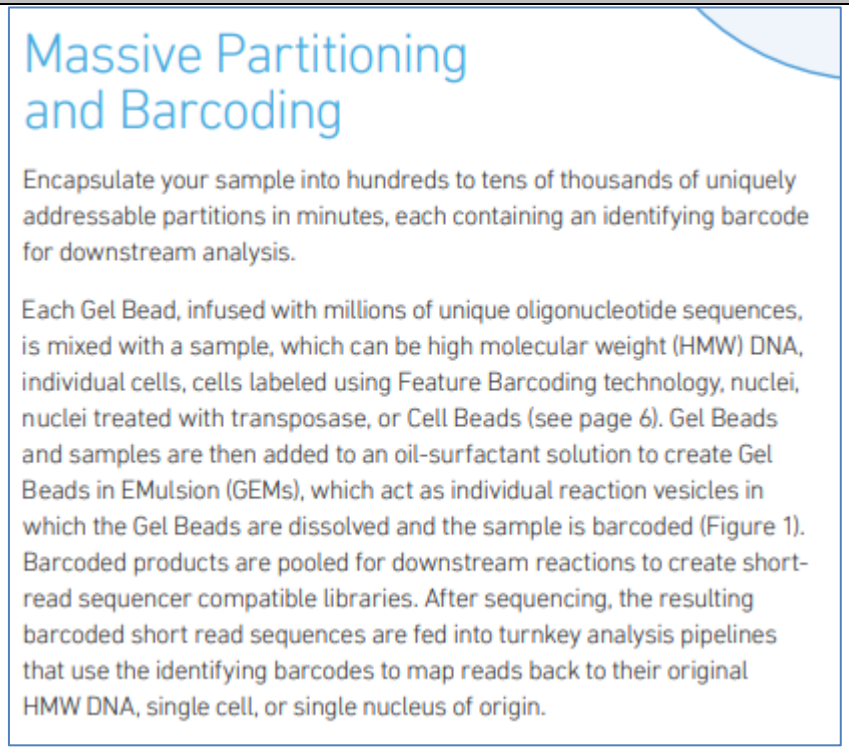
Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.

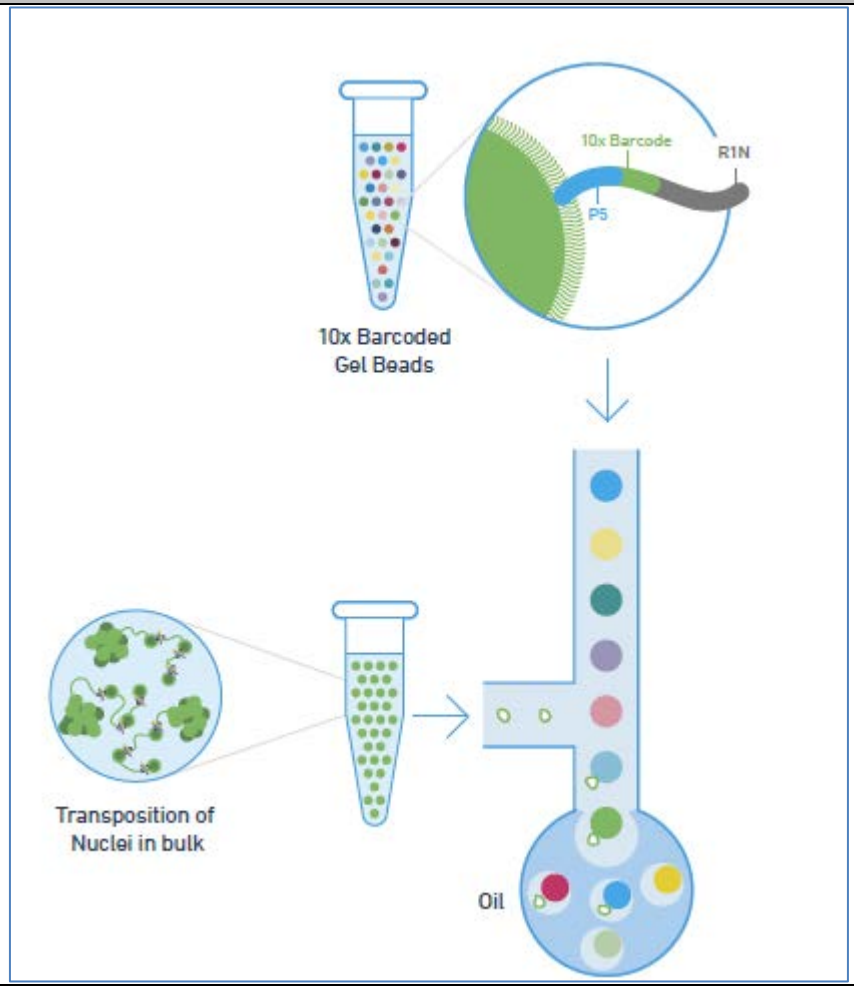


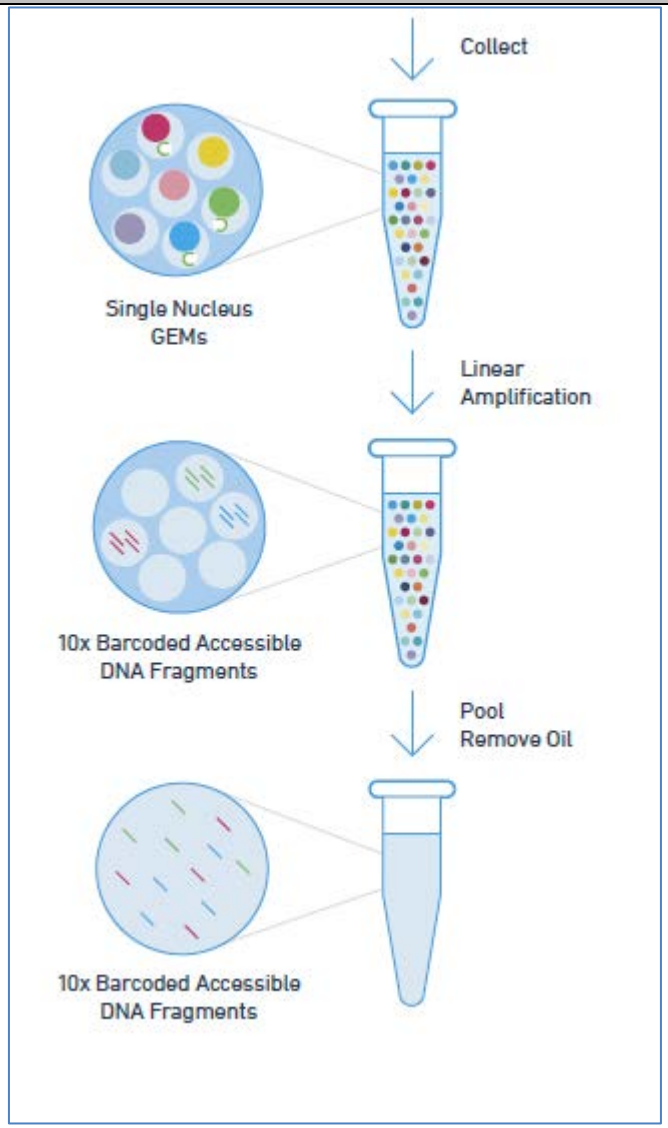


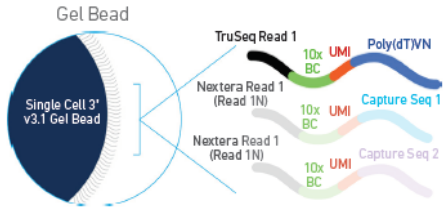
Id.

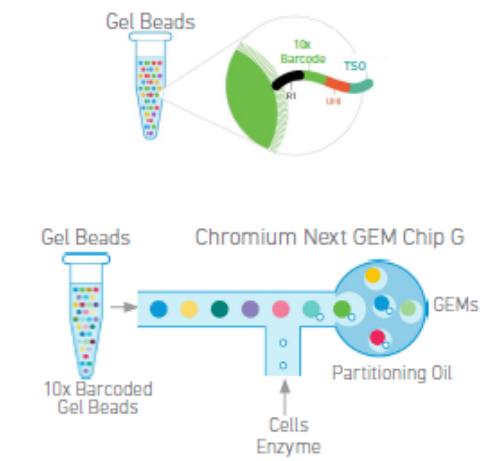
’115 Claim Language	Infringement Support
	 Ex. 7 at 15.
23. The device of claim 21, wherein the polynucleotides comprise DNA.	The 10X technology brochure explains that the goal of the Next GEM platform is to “encapsulate your sample into hundreds to tens of thousands of uniquely addressable partitions in minutes.” Ex. 2 at 2. Samples “can be high molecular weight (HMW) DNA, individual cells, cells labeled using Feature Barcoding technology, nuclei, nuclei treated with transposase, or Cell Beads.” *Id*. The end result of this process is to use the identifying barcodes that are attached to samples within the partitions (i.e. GEMs) “to map reads back to their original HMW DNA, single cell, or single nucleus of origin.” *Id*.

'115 Claim Language	**Infringement Support**
	 *Id.*
24. The device of claim 23, wherein the DNA is genomic DNA.	The 10X Next GEM technology brochure explains that samples may be nuclei, nuclei treated with transposase. Ex. 2 at 2. This results in the encapsulation of DNA from cell nuclei (i.e. genomic DNA) to be encapsulated in GEMs. Ex. 2 at 5.

’115 Claim Language	Infringement Support
	

’115 Claim Language	Infringement Support
	

'115 Claim Language	Infringement Support
	Id.
25. The device of claim 21, wherein the polynucleotides comprise RNA.	The genetic material used in the 10X process may be derived from samples of high molecular weight DNA, individual cells, cells labelled using Feature Barcoding technology, nuclei, nuclei treated with transposase, or cell beads. Ex. 2 at 2. At least in cases where single cells are used to obtain the genetic sample, the cell is lysed and the cDNA from the cell is denatured to produce mRNA in order to attach barcodes. Ex. 7 at 14. The 10X product manuals confirm that barcoded cDNA molecules are produced from mRNA. Single Cell 3' v3.1 Gel Beads In addition to the poly(dT) primer that enables the production of barcoded, full-length cDNA from poly-adenylated mRNA, the Single Cell 3' v3.1 Gel Beads also include two additional primer sequences (Capture Sequence 1 and Capture Sequence 2), that enable capture and priming of Feature Barcoding technology compatible targets or analytes of interest. Only the poly(dT) primers are used in this protocol for generating Single Cell 3' Gene Expression libraries.  Ex. 7 at 14.

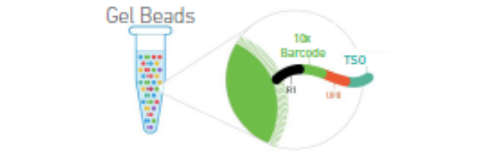
'115 Claim Language	Infringement Support
	Step 1 GEM Generation & Barcoding GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads v1.1, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell. Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.  Ex. 8 at 16.
26. The device of claim 21, wherein the polynucleotides comprise cDNA.	The genetic material used in the 10X process may be derived from samples of high molecular weight DNA, individual cells, cells labelled using Feature Barcoding technology, nuclei, nuclei treated with transposase, or cell beads. Ex. 2 at 2. At least in cases where single cells are used to

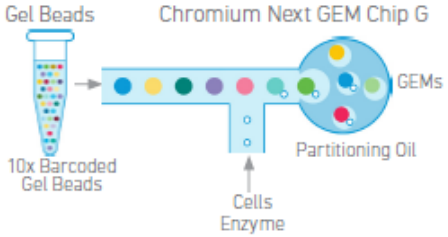
'115 Claim Language	Infringement Support
	obtain the genetic sample, the cell is lysed and the cDNA from the cell is denatured to produce mRNA in order to attach barcodes. 10X's product manuals for its Next GEM platform confirm that once the cell is encapsulated by the GEM, GEMs are incubated to produce "barcoded, full-length cDNA." Ex. 8 at 16.

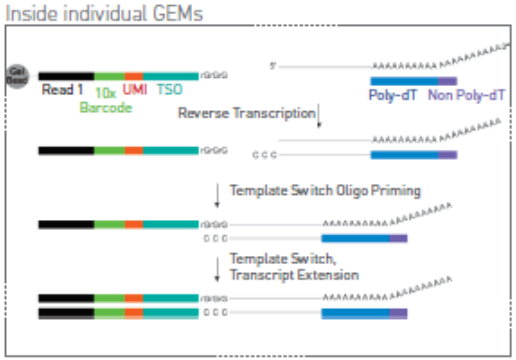
Step 1
GEM Generation & Barcoding

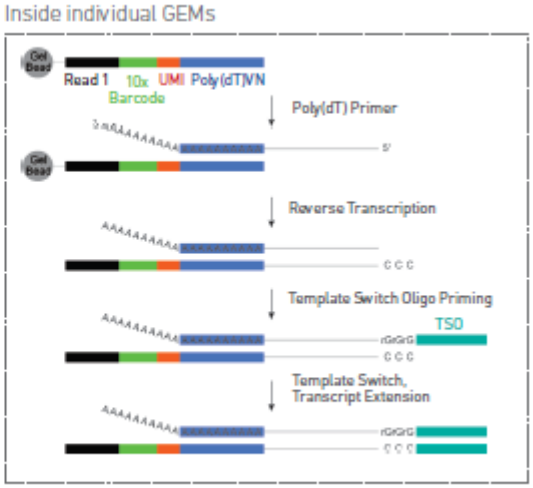
GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads v1.1, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell.

Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.







'115 Claim Language	Infringement Support
	Id. Step 1 GEM Generation & Barcoding Immediately following GEM generation, the Gel Bead is dissolved, primers are released, and any co-partitioned cell is lysed. Primer containing: • an Illumina TruSeq Read 1 (read 1 sequencing primer) • 16 nt 10x Barcode • 12 nt unique molecular identifier (UMI) • 30 nt poly(dT) sequence are mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents. Incubation of the GEMs produces barcoded, full-length cDNA from poly-adenylated mRNA.  Ex. 7 at 15.