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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
90/012,894	06/17/2013	6,440,706 B1	LT00831 REX	8442

11332 7590 07/03/2013

Banner & Witcoff, Ltd.
Attorneys for client 001107
1100 13th Street N.W.
Suite 1200
Washington, DC 20005-4051

EXAMINER	
CAMPELL, BRUCE R	
ART UNIT	**PAPER NUMBER**
3991	

MAIL DATE	DELIVERY MODE
07/03/2013	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

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THIRD PARTY REQUESTER'S CORRESPONDENCE ADDRESS
LIFE TECHNOLOGIES CORPORATION
ATTN: IP DEPARTMENT
5791 VAN ALLEN WAY
CARLSBAD, CA 92008

Date:

EX PARTE REEXAMINATION COMMUNICATION TRANSMITTAL FORM

REEXAMINATION CONTROL NO. : 90012894
PATENT NO. : 6440706
ART UNIT : 3991

Enclosed is a copy of the latest communication from the United States Patent and Trademark Office in the above identified ex parte reexamination proceeding (37 CFR 1.550(f)).

Where this copy is supplied after the reply by requester, 37 CFR 1.535, or the time for filing a reply has passed, no submission on behalf of the ex parte reexamination requester will be acknowledged or considered (37 CFR 1.550(g)).

Ex Parte Reexamination Interview Summary – Pilot Program for Waiver of Patent Owner's Statement	**Control No.** 90/012,894	**Patent For Which Reexamination is Requested** 6,440,706
	Examiner	**Art Unit** 3991

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address. --

All participants (USPTO official and patent owner):

(1) Karen Ward, CRU (3)

(2) Franklin Wolfe, 19724 (4)

Date of Telephonic Interview: June 27, 2013.

The USPTO official requested waiver of the patent owner's statement pursuant to the pilot program for waiver of patent owner's statement in *ex parte* reexamination proceedings.*

☐ The patent owner **agreed** to waive its right to file a patent owner's statement under 35 U.S.C. 304 in the event reexamination is ordered for the above-identified patent.

☐ The patent owner **did not agree** to waive its right to file a patent owner's statement under 35 U.S.C. 304 at this time.

The patent owner is not required to file a written statement of this telephone communication under 37 CFR 1.560(b) or otherwise. However, any disagreement as to this interview summary must be brought to the immediate attention of the USPTO, and no later than one month from the mailing date of this interview summary. Extensions of time are governed by 37 CFR 1.550(c).

*For more information regarding this pilot program, see *Pilot Program for Waiver of Patent Owner's Statement in Ex Parte Reexamination Proceedings*, 75 *Fed. Reg.* 47269 (August 5, 2010), available on the USPTO Web site at http://www.uspto.gov/patents/law/notices/2010.jsp.

☒ USPTO personnel were unable to reach the patent owner.

The patent owner may contact the USPTO personnel at the telephone number provided below if the patent owner decides to waive the right to file a patent owner's statement under 35 U.S.C. 304.

/Karen Ward/ 571-272-7932

Signature and telephone number of the USPTO official who contacted or attempted to contact the patent owner.

cc: Requester (if third party requester)

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90/012,894	06/17/2013	6,440,706 B1	LT00831 REX	8442

11332 7590 08/28/2013

Banner & Witcoff, Ltd.
Attorneys for client 001107
1100 13th Street N.W.
Suite 1200
Washington, DC 20005-4051

EXAMINER	
CAMPELL, BRUCE R	
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08/28/2013	PAPER

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CARLSBAD, CA 92008

EX PARTE REEXAMINATION COMMUNICATION TRANSMITTAL FORM

REEXAMINATION CONTROL NO. *90/012,894*.

PATENT NO. *6,440,706 B1 E*.

ART UNIT *3991*.

Enclosed is a copy of the latest communication from the United States Patent and Trademark Office in the above identified *ex parte* reexamination proceeding (37 CFR 1.550(f)).

Where this copy is supplied after the reply by requester, 37 CFR 1.535, or the time for filing a reply has passed, no submission on behalf of the *ex parte* reexamination requester will be acknowledged or considered (37 CFR 1.550(g)).

Request for Ex Parte Reexamination

A request for *ex parte* reexamination of claims 1-12, 14-16, 19-32, 38-44, 46-48 and 51-64 of U.S. Patent 6,440,706 was filed on June 17, 2013 by a third party requester.

Decision on Request

A substantial new question of patentability (SNQ) affecting claims 1-12, 14-16, 19-32, 38-44, 46-48 and 51-64 of U.S. Patent 6,440,706 is raised by the request for ex parte reexamination.

Scope of the Claims

In reexamination, patent claims are construed broadly. In re Yamamoto, 740 F.2d 1569, 1571, 222 USPQ 934, 936 (Fed. Cir. 1984) (claims given "their broadest reasonable interpretation consistent with the specification"). The independent claims subject to reexamination read as follows:

1. A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of:

diluting nucleic acid template molecules in a biological sample to form a set comprising a plurality of assay samples;

amplifying the template molecules within the assay samples to form a population of amplified molecules in the assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence;

comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

38. A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of:

amplifying template molecules within a set comprising a plurality of assay samples to form a population of amplified molecules in each of the assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence, wherein at least one-fiftieth of the assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required to determine the presence of the selected genetic sequence;

comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

Claim Interpretation

The biological sample of claim 1 can either be comprised of cells, tissues, bodily fluids, etc. or cell free, as recited in dependent claims 6 and 24. In either case, nucleic acids are distributed throughout the sample. Therefore any process in which the sample is diluted is considered "diluting nucleic acid template molecules in a biological sample." The “ratio of a selected genetic sequence” is interpreted as the ratio of the selected genetic sequence to the reference genetic sequence.

With regard to the limitation in claim 38 “the assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required to determine the presence of the selected genetic sequence,” it is impossible to ascertain a value for N because this number can only be determined after the method has been performed. The "plain English" meaning of this limitation is that, for example, if the selected sequence is present in the biological sample at a level of 1 copy in 50, then the assay samples should contain at least 50 total copies (“selected” + “reference”) of the genetic sequence to ensure a reasonable likelihood that there is a selected genetic sequence present in the sample to be amplified and detected. This assumes that a single copy of a sequence is sufficient to be detected after amplification and detection, which may or may not be true, depending on experimental conditions (how many amplification cycles, detection method used, etc.). It appears that this information can only be derived *ex*

post facto, or at least after preliminary experiments have been performed with similar biological samples. For purposes of interpreting the prior art, if a reference shows that a selected genetic sequence was detected in an assay sample, then clearly that assay sample contained enough template nucleic acid molecules to enable detection of the selected genetic sequence and this claim limitation is met, whether or not "N" is specifically disclosed.

Documents Submitted by Requester

Li *et al.,* "Amplification and analysis of DNA sequences in single human sperm and diploid cells." Nature 335(6189):414-7 (1988)

Zhang *et al.,* "Whole genome amplification from a single cell: implications for genetic analysis." *PNAS USA,* 89(13):5847-51 (1992)

Jeffreys *et al.,* "Amplification of human minisatellites by the polymerase chain reaction: towards DNA fingerprinting of single cells." *Nucl. Acids. Res.,* vol 16, no. 23, pages 10953-10971 (1988)

Kalinina *et al.,* "Nanoliter scale PCR with TaqMan detection," *Nucl. Acids. Res.* vol 25, 1999-2004 (1997)

Chou *et al.,* "Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications," *Nucleic Acids Res.,* 20(7): 1717-1723 (April 11, 1992)

Burg, *et al.,* "Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii,* by polymerase chain reaction." J. *Clin. Microbiol.* 27, 1787-1792 (1989)

Trumper *et al.,* "Single-Cell Analysis of Hodgkin and Reed-Sternberg Cells: Molecular Heterogeneity of Gene Expression and p53 Mutations," *Blood, 81 :* 3097-3115 (1993)

Kanzler *et al.,* "Molecular Single Cell Analysis Demonstrates the Derivation of Peripheral Blood-Derived Cell Line (L 1236) From the Hodgkin/Reed-Sternberg Cells of a Hodgkin's Lymphoma Patient," *Blood,* 87:3429-3436 (1996)

Gravel *et al.,* "Single-cell analysis of the t(14; 18)(q32;q21) chromosomal translocation in Hodgkin's disease demonstrates the absence of this

translocation in neoplastic Hodgkin and Reed-Sternberg cells," *Blood* 91(8):2866-74 (Apr 15, 1998)

Marcucci *et al.,* "Detection of Unique ALLA.(MLL) Fusion Transcripts in Normal Human Bone Marrow and Blood: Distinct Origin of Normal versus Leukemic ALL 1 Fusion Transcripts," *Cancer Res,* 58:790-793. (February 15, 1998)

Flint *et al.,* "NR2A Subunit Expression Shortens NMDA Receptor Synaptic Currents in Developing Neocortex," J. *Neurosci.,* 17(7):2469-2476 (April 1, 1997)

Ponten *et al.,* "Genomic analysis of single cells from human basal cell cancer using laser-assisted capture microscopy," *Mutation Research Genomics* 382, 45-55 (1997)

Review of the '706 patent file shows that Li and Zhang were cited in an information disclosure statement but not applied in any rejection. None of the other references were considered during prosecution.

Requester's Proposed SNQs

Requester proposes 24 SNQs (summarized in Request, pp. 1-2).

1. Requester considers claims 1-3, 7-11, 14-16, 19, 21, 22, 27 and 32 unpatentable over **Li** (proposed SNQs 1 and 4).

Li discloses a method in which a ratio of genetic sequences (β-globin) was obtained from a tissue culture flask containing co-cultured cells (the biological sample) of an individual homozygous for the β^S allele ("selected genetic sequence," which causes sickle cell anemia) and another individual homozygous for the β^A allele (normal, "reference genetic sequence"). The nucleic acid template molecules, contained within the cultured cells, were diluted by isolating single cells from the culture. Thirty seven single cells (assay samples) were lysed, and the released DNA was subjected to polymerase chain reaction (PCR) to amplify the portion of the globin gene containing

the sickle cell mutation. Amplified DNA was hybridized with allele specific probes. It was found that 19 of the samples contained the normal allele, 12 contained the sickle cell allele, and 6 samples did not hybridize with either probe. These numerical values were "compared," which inherently ascertains a ratio between the two values (19:12). See pp. 414-415, Fig. 1.

In another experiment (p. 415, Fig. 2), the biological sample was semen obtained from a subject heterozygous for a polymorphism in the LDLr gene. Eighty individual sperm cells were lysed and the DNA subjected to PCR followed by hybridization with allele specific probes. A total of 55% of sperm cells ("assay samples") gave a hybridization signal. It was found that 22 assay samples contained one allele and 21 samples contained the other, a ratio of 22:21. Either allele can be considered the "selected genetic sequence" or the "reference genetic sequence."

A reasonable examiner would consider the disclosure of Li important in determining whether claims 1-3, 7-11, 14-16, 19, 21, 22, 27 and 32 are patentable. Accordingly, Li raises a SNQ regarding claims 1-3, 7-11, 14-16, 19, 21, 22, 27 and 32.

2. Requester considers claims 38, 39, 46 and 51 unpatentable over **Zhang** (proposed SNQ 14).

Zhang discloses a method similar to that of Li. In Zhang's method (p. 5847), a biological sample (semen) was diluted into 18 assay samples by selecting and isolating 18 single sperm cells. Each cell was lysed and the released DNA was pre-amplified by repeated primer extension reactions with a set of random 15-mer primers (primer-extension preamplification, or PEP). The PEP process was estimated to produce at least 30 copies of every sequence capable of amplification (p. 5848, col. 1). After PEP, aliquots of each sample were subjected to a two-step hemi-nested PCR process to determine the genotype at each of 12 different loci. PCR was first performed using a first pair of primers designed to amplify the genetic sequence of interest, then an aliquot of the sample was removed and subjected to a second PCR using one primer from the

first pair and a second primer internal to the previously amplified sequence of interest. The second set of primers were chosen so that the two possible alleles would produce amplified fragments of different lengths. This method ensures specificity of the PCR and allows discrimination between the two reaction products (hence, alleles present in the template molecules) by gel electrophoresis of the final PCR product to determine fragment length (p. 5847, col. 2). Each of the 12 loci were successfully amplified in at least 15 of the 18 sperm cells (assay samples; see Table 2). The genotype of each cell was determined for two loci (results for 9 cells shown in Fig. 3). Each of the two APOC2 alleles was found in 9 cells, the expected 1:1 ratio for this heterozygous sperm donor. Similarly, analysis of the sex linked STS gene/pseudogene showed that 9 cells carried an X chromosome and 8 carried a Y chromosome (the 18th cell did not yield detectable STS sequence). Independent assortment of these two loci was also observed (p. 5848, col. 2).

A reasonable examiner would consider the disclosure of Zhang important in determining whether claims 38, 39, 46 and 51 are patentable. Accordingly, Zhang raises a SNQ regarding claims 38, 39, 46 and 51.

3. Requester considers claims 4-6, 12, 20, 23-26, and 28-31 unpatentable over **Li** in combination with one or more of **Zhang**, **Jeffreys**, **Kalinina**, **Chou**, **Burg**, **Trümper**, **Kanzler**, **Gravel**, **Marcucci**, **Flint** and **Pontén** (proposed SNQs 2, 3 and 5-13).

Li and **Zhang** are discussed above.

Jeffreys discloses methods for amplification of human minisatellite DNA for the purpose of producing DNA fingerprints of individuals. In one method, a biological sample is split into multiple assay samples by isolating single cells, then analyzed in much the same way as in Li and Zhang (pp. 10955-10956). In an alternative method, isolated (cell free) DNA was diluted into multiple assay samples, each containing 6 pg DNA. This amount was estimated to be equivalent to the amount of DNA in a single

cell. It was concluded that single DNA molecules could be faithfully amplified (pp. 10960-10962). In the experiment shown in Fig. 4, each assay sample was subjected to PCR with 4 sets of primers (in a single reaction), the primers designed to amplify two alleles for each of 2 minisatellites. Successful amplification was obtained, with a mean failure rate of 63% per allele per reaction, equating to an estimated 0.46 successful amplification events per 6 pg sample (because statistically one would not expect the template sequence to be present in every sample; p. 10961).

Kalinina discloses a method for PCR amplification and detection using TaqMan probes. Samples diluted to contain approximately 1 template molecule are subjected to TaqMan PCR in sealed capillary tubes containing a few nanoliters of reactants, then presence of PCR product is determined by measuring the probe fluorescence (entire document, see especially p. 2000). The method is considered especially useful for assays meant to determine the presence or absence of PCR product (i.e. not quantitative analysis; p. 2004, last paragraph).

Chou discloses a method for “hot start” PCR. The method uses a wax barrier to separate one or more PCR components from the remainder of the reactants until heat is applied to melt the wax (entire document). This method reduces amplification due to mispriming and primer oligomerization, and is said to be especially useful for PCR with a sample containing a low number of template molecules (p. 1722, col. 1).

Burg discloses a method for PCR detection of a single cell of *Toxoplasma gondii*. Cells are lysed and PCR is performed for 60 cycles (p. 1790, col. 1; Fig. 4).

Trümper isolated single cells from lymph nodes of patients diagnosed with Hodgkin’s disease. Cells were lysed, cDNA was produced by reverse transcription and PCR performed on the cDNA (see methods, pp. 3098-3100). One cell was found to have a mutation in exon 7 of the p53 gene, at a known "hot spot."

Kanzler isolated single cells from bone marrow of patients diagnosed with Hodgkin's disease (p. 3429). PCR analysis identified three gene rearrangements (abstract). Kanzler suggests, "Using tumor clone-specific primers … residual tumor cells may be detected after therapy" (p. 3434, col. 2).

Gravel used single cell PCR analysis to determine the presence or absence of a chromosomal translocation, t(14;18)(q32;q21), in cells from bone of patients diagnosed with Hodgkin's disease (see methods, pp. 2866-2868).

Marcucci discloses a chromosome segment which is subject to partial tandem duplication, which is a common defect found in acute myeloid leukemia.

Flint used single cell reverse transcription and PCR to study gene expression in developing neocortex tissues (abstract).

Pontén performed single cell PCR on cells derived from a single tumor and showed that the tumor contained multiple p53 mutations. Some cells contained more than one mutation of the p53 gene (see overview on p. 52).

A reasonable examiner would consider the disclosure of Li, in combination with one or more of Zhang, Jeffreys, Kalinina, Chou, Burg, Trümper, Kanzler, Gravel, Marcucci, Flint and Pontén, important in determining whether claims 4-6, 12, 20, 23-26, and 28-31 are patentable. Accordingly, Li in combination with one or more of Zhang, Jeffreys, Kalinina, Chou, Burg, Trümper, Kanzler, Gravel, Marcucci, Flint and Pontén raises a SNQ regarding claims 4-6, 12, 20, 23-26, and 28-31.

4. Requester considers claims 40-44, 47, 48 and 52-64 unpatentable over **Zhang** in combination with one or more of **Li**, **Kalinina**, **Chou**, **Burg**, **Trümper**, **Kanzler**, **Gravel**, **Marcucci**, **Flint** and **Pontén** (proposed SNQs 15-24).

Each reference is discussed above.

A reasonable examiner would consider the disclosure of Zhang in combination with Li, Kalinina, Chou, Burg, Trümper, Kanzler, Gravel, Marcucci, Flint and Pontén important in determining whether claims 40-44, 47, 48 and 52-64 are patentable. Accordingly, Zhang in combination with Li, Kalinina, Chou, Burg, Trümper, Kanzler, Gravel, Marcucci, Flint and Pontén raises a SNQ regarding claims 40-44, 47, 48 and 52-64.

Conclusion

In view of the analysis above, the request for reexamination is **GRANTED**. Claims 1-12, 14-16, 19-32, 38-44, 46-48 and 51-64 of US Patent 6,440,706 will be reexamined.

Duty to Disclose

The patent owner is reminded of the continuing responsibility under 37 CFR 1.565(a) to apprise the Office of any litigation activity, or other prior or concurrent proceeding, involving Patent No. 6,440,706 throughout the course of this reexamination proceeding. The third party requester is also reminded of the ability to similarly apprise the Office of any such activity or proceeding throughout the course of this reexamination proceeding. See MPEP §§ 2207, 2282 and 2286.

Waiver of Right to File Patent Owner Statement

In a reexamination proceeding, Patent Owner may waive the right under 37 C.F.R. 1.530 to file a Patent Owner Statement. The waiver document must contain a statement that Patent Owner waives the right under 37 C.F.R. 1.530 to file a Patent Owner Statement and proof of service in the manner provided by 37 C.F.R. 1.248, if the request for reexamination was made by a third party requester (see 37 C.F.R 1.550(f)).

Amendment in Reexamination Proceedings

Patent owner is notified that any proposed amendment to the specification and/or claims in this reexamination proceeding must comply with 37 CFR 1.530(d)-(j), must be formally presented pursuant to 37 CFR 1.52(a) and (b), and must contain any fees required by 37 CFR 1.20(c).

Service of Papers

After the filing of a request for reexamination by a third party requester, any document filed by either the patent owner or the third party requester must be served on the other party (or parties where two or more third party requester proceedings are merged) in the reexamination proceeding in the manner provided in 37 CFR 1.248. See 37 CFR 1.550(f).

Correspondence

Any inquiry concerning this communication or earlier communications from the examiner should be directed to BRUCE CAMPELL whose telephone number is 571-272-0974. The examiner can normally be reached on Monday - Thursday from 8:00 to 5:00. The examiner can also be reached on alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Deborah Jones, can be reached on 571-272-1535. The fax phone number for the organization where this application or proceeding is assigned is 571-273-9900.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

All correspondence relating to this ex parte reexamination proceeding should be directed:

By EFS: Registered users may submit via the electronic filing system EFS-Web at

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By Mail to: Mail Stop *Ex Parte* Reexam
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/Bruce Campell/
Patent Reexamination Specialist
Central Reexamination Unit 3991

Conferee:

/Padmashri Ponnaluri/
Patent Reexamination Specialist
CRU-3991

/Deborah D Jones/
Supervisory Patent Examiner, Art Unit 3991

Order Granting / Denying Request For Ex Parte Reexamination	Control No.	Patent Under Reexamination
	90/012,894	6,440,706 B1 E
	Examiner	Art Unit
	BRUCE CAMPELL	3991

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

The request for *ex parte* reexamination filed *17 June 2013* has been considered and a determination has been made. An identification of the claims, the references relied upon, and the rationale supporting the determination are attached.

Attachments: a)☐ PTO-892, b)☒ PTO/SB/08, c)☐ Other: _____

1. ☒ The request for *ex parte* reexamination is GRANTED.

RESPONSE TIMES ARE SET AS FOLLOWS:

For Patent Owner's Statement (Optional): TWO MONTHS from the mailing date of this communication (37 CFR 1.530 (b)). **EXTENSIONS OF TIME ARE GOVERNED BY 37 CFR 1.550(c).**

For Requester's Reply (optional): TWO MONTHS from the **date of service** of any timely filed Patent Owner's Statement (37 CFR 1.535). **NO EXTENSION OF THIS TIME PERIOD IS PERMITTED.** If Patent Owner does not file a timely statement under 37 CFR 1.530(b), then no reply by requester is permitted.

2. ☐ The request for *ex parte* reexamination is DENIED.

This decision is not appealable (35 U.S.C. 303(c)). Requester may seek review by petition to the Commissioner under 37 CFR 1.181 within ONE MONTH from the mailing date of this communication (37 CFR 1.515(c)). **EXTENSION OF TIME TO FILE SUCH A PETITION UNDER 37 CFR 1.181 ARE AVAILABLE ONLY BY PETITION TO SUSPEND OR WAIVE THE REGULATIONS UNDER 37 CFR 1.183.**

In due course, a refund under 37 CFR 1.26 (c) will be made to requester:

a) ☐ by Treasury check or,

b) ☐ by credit to Deposit Account No. _____, or

c) ☐ by credit to a credit card account, unless otherwise notified (35 U.S.C. 303(c)).

/Bruce Campell/ Patent Reexamination Specialist Central Reexamination Unit 3991		

cc:Requester (if third party requester)

EXHIBIT 2

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)					
				Application Number	Unknown
				Filing Date	June 17, 2013
				First Named Inventor	Bert Vogelstein
				Art Unit	Unknown
				Examiner Name	Unknown
Sheet	1	of	2	**Docket Number**	LT00831 REX

U.S.PATENTS

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

U.S.PATENT APPLICATION PUBLICATIONS

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2]	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]

NON-PATENT LITERATURE DOCUMENTS

Examiner Initial*	Cite No	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[5]
/B.C./	C1	BURG, J. L. et al., "Direct and Sensitive Detection of a Pathogenic Protozoan, Toxoplasma gondii, by Polymerase Chain Reaction", Journal of Clinical Microbiology, Vol. 27, No. 8, 1989, 1787-1792	
/B.C./	C2	CHOU, QUIN et al., "Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications", Nucleic Acids Research, Vol. 20, No. 7, Oxford University Press, 1992, 1717-1723	
/B.C./	C3	FLINT, ALEXANDER C. et al., "NR2A Subunit Expression Shortens NMDA Receptor Synaptic Currents in Developing Neocortex", The Journal of Neuroscience, Vol. 17, No. 7, 1997, 2469-2476	
/B.C./	C4	GRAVEL, SYLVIA et al., "Single-Cell Analysis of the t(14;18)(q32;q21) Chromosomal Translocation in Hodgkin's Disease Demonstrates the Absence of This Translocation in Neoplastic Hodgkin and Reed-Sternberg Cells", Blood, Vol. 91, No. 8, 1998, 2866-2874	
/B.C./	C5	JEFFREYS, ALEC et al., "Amplification of human minisatellites by the polymerase chain reaction: towards DNA fingerprinting of single cells", Nucl. Acids Res., Vol. 16, No. 23, 1988, 10953-10971	
/B.C./	C6	KALININA, OLGA et al., "Nanoliter Scale PCR with TaqMan Detection", Nucleic Acids Research, Vol. 25, No. 10, 1997, 1999-2004	
/B.C./	C7	KANZLER, H et al., "Molecular single cell analysis demonstrates the derivation of a peripheral blood-derived cell line (L1236) from the Hodgkin/Reed-Sternberg cells of a Hodgkin's lymphoma patient", Blood, Vol. 87, No. 8, 1996, 3429-3436	
/B.C./	C8	MARCUCCI, GUIDO et al., "Detection of Unique ALL1 (MLL) Fusion Transcripts in Normal Human Bone Marrow and Blood: Distinct Origin of Normal versus Leukemic ALL1 Fusion Transcripts", Cancer Research, Vol. 58, 1998, 700-793	
/B.C./	C9	PONTEN, FREDRIK et al., "Genomic analysis of single cells from human basal cell cancer using laser-assisted capture microscopy", Mutation Research Genomics, Vol. 382, No. 1-2, 1997, 45-55	

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)					
				Application Number	Unknown
				Filing Date	June 17, 2013
				First Named Inventor	Bert Vogelstein
				Art Unit	Unknown
				Examiner Name	Unknown
Sheet	2	of	2	**Docket Number**	LT00831 REX

/B.C./	C10	TRÜMPER, LORENZ H. et al., "Single-Cell Analysis of Hodgkin and Reed-Sternberg Cells: Molecular Heterogeneity of Gene Expression and p53 Mutations", Blood, Vol. 81, No. 11, 1993, 3097-3115	

EXAMINER SIGNATURE

Examiner Signature	/Bruce Campell/	Date Considered	08/12/2013

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter the office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.
[4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

Reexamination	Application/Control No.	Applicant(s)/Patent Under Reexamination
	90012894	6,440,706 B1 ET AL.
	Certificate Date	Certificate Number
		C1

Requester Correspondence Address:	☐ Patent Owner	☒ Third Party

LIFE TECHNOLOGIES CORPORATION
ATTN: IP DEPARTMENT
5791 VAN ALLEN WAY
CARLSBAD, CA 92008

LITIGATION REVIEW ☒	/BC/ (examiner initials)	06/27/2013 (date)
Case Name		Director Initials
Esoterix Genetic Laboratories, Llc et al v. Life Technologies		
U.S. District - North Carolina Middle 1:12cv1173		

COPENDING OFFICE PROCEEDINGS	
TYPE OF PROCEEDING	NUMBER
1. none	

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	90012894	6,440,706 B1 ET AL.
	Examiner	Art Unit
	BRUCE CAMPELL	3991

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
reviewed prosecution history of 09/613,826google: gene amplification in cancer review	7/12/137/24/13	/BC//BC/

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
90/012,894	06/17/2013	6,440,706 B1	LT00831 REX	8442

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Banner & Witcoff, Ltd.
Attorneys for client 001107
1100 13th Street N.W.
Suite 1200
Washington, DC 20005-4051

EXAMINER	
CAMPELL, BRUCE R	
ART UNIT	PAPER NUMBER
3991	

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11/27/2013	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

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EX PARTE REEXAMINATION COMMUNICATION TRANSMITTAL FORM

REEXAMINATION CONTROL NO. *90/012,894*.

PATENT NO. *6,440,706 B1* .

ART UNIT *3991*.

Enclosed is a copy of the latest communication from the United States Patent and Trademark Office in the above identified *ex parte* reexamination proceeding (37 CFR 1.550(f)).

Where this copy is supplied after the reply by requester, 37 CFR 1.535, or the time for filing a reply has passed, no submission on behalf of the *ex parte* reexamination requester will be acknowledged or considered (37 CFR 1.550(g)).

Office Action in Ex Parte Reexamination	**Control No.** 90/012,894	**Patent Under Reexamination** 6,440,706 B1 E
	Examiner BRUCE CAMPELL	**Art Unit** 3991

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

a☐ Responsive to the communication(s) filed on _____ . b☐ This action is made FINAL.
c☐ A statement under 37 CFR 1.530 has not been received from the patent owner.

A shortened statutory period for response to this action is set to expire 2 month(s) from the mailing date of this letter. Failure to respond within the period for response will result in termination of the proceeding and issuance of an *ex parte* reexamination certificate in accordance with this action. 37 CFR 1.550(d). **EXTENSIONS OF TIME ARE GOVERNED BY 37 CFR 1.550(c).** If the period for response specified above is less than thirty (30) days, a response within the statutory minimum of thirty (30) days will be considered timely.

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☒ Notice of References Cited by Examiner, PTO-892.
2. ☐ Information Disclosure Statement, PTO/SB/08.
3. ☐ Interview Summary, PTO-474.
4. ☐ _____.

Part II SUMMARY OF ACTION

1a. ☒ Claims *1-12,14-16,19-32,38-44,46-48 and 51-64* are subject to reexamination.

1b. ☒ Claims *13,17,18,33-37,45,49,50* are not subject to reexamination.

2. ☐ Claims _____ have been canceled in the present reexamination proceeding.

3. ☐ Claims _____ are patentable and/or confirmed.

4. ☒ Claims *1-12, 14-16, 19-32, 38-44, 46-48, 51-64* are rejected.

5. ☐ Claims _____ are objected to.

6. ☐ The drawings, filed on _____ are acceptable.

7. ☐ The proposed drawing correction, filed on _____ has been (7a)☐ approved (7b)☐ disapproved.

8. ☐ Acknowledgment is made of the priority claim under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some* c)☐ None of the certified copies have

1☐ been received.

2☐ not been received.

3☐ been filed in Application No. _____ .

4☐ been filed in reexamination Control No. _____.

5☐ been received by the International Bureau in PCT application No. _____.

* See the attached detailed Office action for a list of the certified copies not received.

9. ☐ Since the proceeding appears to be in condition for issuance of an *ex parte* reexamination certificate except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte* Quayle, 1935 C.D. 11, 453 O.G. 213.

10. ☐ Other: _____

cc: Requester (if third party requester)

Ex Parte Reexamination

Detailed Non-Final Office Action

This is a reexamination of U.S. Patent 6,440,706, issued August 22, 2002. A Request pursuant to 37 CFR 1.510 for ex parte reexamination of claims 1-12, 14-16, 19-32, 38-44, 46-48 and 51-64 of U.S. Patent 6,440,706 was filed on June 17, 2013 by a third party requester. An Order granting the request was mailed August 28, 2013.

Patent Owner Statement

No patent owner statement has been received.

Status of the Claims

Claims 1-12, 14-16, 19-32, 38-44, 46-48 and 51-64 of U.S. Patent 6,440,706 are subject to reexamination.

Scope of the Claims

In reexamination, patent claims are construed broadly. In re Yamamoto, 740 F.2d 1569, 1571, 222 USPQ 934, 936 (Fed. Cir. 1984) (claims given "their broadest reasonable interpretation consistent with the specification"). The independent claims subject to reexamination read as follows:

1. A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of:

diluting nucleic acid template molecules in a biological sample to form a set comprising a plurality of assay samples;

amplifying the template molecules within the assay samples to form a population of amplified molecules in the assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence;

comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

38. A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of:

amplifying template molecules within a set comprising a plurality of assay samples to form a population of amplified molecules in each of the assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence, wherein at least one-fiftieth of the assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required to determine the presence of the selected genetic sequence;

comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

Claim Interpretation

The biological sample of claim 1 can either be comprised of cells, tissues, bodily fluids, etc. or cell free, as recited in dependent claims 6 and 24. In either case, nucleic acids are distributed throughout the sample. Therefore any process in which the sample is diluted is considered "diluting nucleic acid template molecules in a biological sample." The “ratio of a selected genetic sequence” is interpreted as the ratio of the selected genetic sequence to the reference genetic sequence.

With regard to the limitation in claim 38 “the assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required to determine the presence of the selected genetic sequence,” it is impossible to ascertain a value for N because this number can only be determined after the method has been performed. The "plain English" meaning of this limitation is that, for example, if the selected sequence is present in the biological sample at a level of 1 copy in 50, then the assay samples should contain at least 50 total copies (“selected” + “reference”) of the genetic sequence

to ensure a reasonable likelihood that there is a selected genetic sequence present in the sample to be amplified and detected. This assumes that a single copy of a sequence is sufficient to be detected after amplification and detection, which may or may not be true, depending on experimental conditions (how many amplification cycles, detection method used, etc.). It appears that this information can only be derived *ex post facto*, or at least after preliminary experiments have been performed with similar biological samples. For purposes of interpreting the prior art, if a reference shows that a selected genetic sequence was detected in an assay sample, then clearly that assay sample contained enough template nucleic acid molecules to enable detection of the selected genetic sequence and this claim limitation is met, whether or not “N” is specifically disclosed.

With regard to "a polymerase which is activated only after heating," as recited in claims 20 and 52, the specification does not disclose a polymerase which requires heat to become capable of catalytic activity. This limitation is interpreted to mean that the polymerase is separated from one or more reactants until heat is applied, thereby bringing enzyme and reactants in contact and allowing polymerization to begin.

Documents Submitted by Requester

Li *et al.,* "Amplification and analysis of DNA sequences in single human sperm and diploid cells."Nature 335(6189):414-7 (1988)

Zhang *et al.,* "Whole genome amplification from a single cell: implications for genetic analysis." *PNAS USA,* 89(13):5847-51 (1992)

Jeffreys *et al.,* "Amplification of human minisatellites by the polymerase chain reaction: towards DNA fingerprinting of single cells." *Nucl. Acids. Res.,* vol 16, no. 23, pages 10953-10971 (1988)

Kalinina *et al.,* "Nanoliter scale PCR with TaqMan detection," *Nucl. Acids. Res.* vol 25, 1999-2004 (1997)

Chou *et al.,* "Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications," *Nucleic Acids Res.,* 20(7): 1717-1723 (April 11, 1992)

Burg, *et al.,* "Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii,* by polymerase chain reaction." J. *Clin. Microbiol.* 27, 1787-1792 (1989)

Trumper *et al.,* "Single-Cell Analysis of Hodgkin and Reed-Sternberg Cells: Molecular Heterogeneity of Gene Expression and p53 Mutations," *Blood, 81 :* 3097-3115 (1993)

Kanzler *et al.,* "Molecular Single Cell Analysis Demonstrates the Derivation of Peripheral Blood-Derived Cell Line (L 1236) From the Hodgkin/Reed-Sternberg Cells of a Hodgkin's Lymphoma Patient," *Blood,* 87:3429-3436 (1996)

Gravel *et al.,* "Single-cell analysis of the t(14; 18)(q32;q21) chromosomal translocation in Hodgkin's disease demonstrates the absence of this translocation in neoplastic Hodgkin and Reed-Sternberg cells," *Blood* 91(8):2866-74 (Apr 15, 1998)

Marcucci *et al.,* "Detection of Unique ALLA.(MLL) Fusion Transcripts in Normal Human Bone Marrow and Blood: Distinct Origin of Normal versus Leukemic ALL 1 Fusion Transcripts," *Cancer Res,* 58:790-793. (February 15, 1998)

Flint *et al.,* "NR2A Subunit Expression Shortens NMDA Receptor Synaptic Currents in Developing Neocortex," J. *Neurosci.,* 17(7):2469-2476 (April 1, 1997)

Ponten *et al.,* "Genomic analysis of single cells from human basal cell cancer using laser-assisted capture microscopy," *Mutation Research Genomics* 382, 45-55 (1997)

Documents Cited by Examiner

M Schwab, “Amplification of oncogenes in human cancer cells.” Bioessays 20(6): 473-479 (1998)

Claim Rejections – 35 U.S.C. §§ 102 and 103

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-3, 7-9, 15, 16, 19, 21, 22, 27, 32, 38-41, 47, 48, 51, 53, 54, 59 and 64 are rejected under 35 U.S.C. 102(b) as being anticipated by Li.

Li discloses a method in which a ratio of genetic sequences (β-globin) was obtained from a tissue culture flask containing co-cultured cells (the biological sample) of an individual homozygous for the β^S allele ("selected genetic sequence," which causes sickle cell anemia) and another individual homozygous for the β^A allele (normal, "reference genetic sequence"). The nucleic acid template molecules, contained within the cultured cells, were diluted by isolating single cells from the culture. Thirty seven single cells (assay samples) were lysed, and the released DNA was subjected to polymerase chain reaction (PCR) to amplify the portion of the globin gene containing the sickle cell mutation. Amplified DNA was hybridized with allele specific probes. It was found that 19 of the samples contained the normal allele, 12 contained the sickle cell allele, and 6 samples did not hybridize with either probe. These numerical values were "compared," which inherently ascertains a ratio between the two values (19:12). This experiment (pp. 414-415, Fig. 1) meets all the limitations of claim 1.

In another experiment (p. 415, Fig. 2), the biological sample was semen obtained from a subject heterozygous for a polymorphism in the LDLr gene. Eighty individual sperm cells were lysed and the DNA subjected to PCR followed by hybridization with allele specific probes. A total of 55% of sperm cells ("assay samples") gave a hybridization signal. It was found that 22 assay samples contained one allele and 21

samples contained the other, a ratio of 22:21. Either allele can be considered the "selected genetic sequence" or the "reference genetic sequence." Therefore this experiment also meets all the limitations of claim 1.

With regard to claim 2, the fact that the selected genetic sequences were detected in some of the assay samples shows that the additional claim limitation was met (see claim interpretation above).

Claim 38 is essentially the same as claim 2, except it does not require the dilution step recited in claim 1, and only 1/50 (rather than 1/10) of the assay samples must comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required to determine the presence of the selected genetic sequence. Therefore claim 38, being broader, is anticipated for the same reasons as claim 2.

With regard to claim 3, 84% and 55% of assay samples produced detectable amplification product in Figs. 1 and 2, respectively.

With regard to claims 7-9 and 39-41, Li discloses a third experiment in which the number of assay samples (individual sperm cells) was greater than 100 (pp. 415-416, Table 1).

With regard to claims 15, 16, 47 and 48, amplified DNA in the assay samples was hybridized with 2 or more allele specific probes.

With regard to claims 19, 21, 22, 51, 53 and 54, the experiments described in Figs. 1 and 2 each used a single pair of PCR primers. Fig. 1 used 50 cycles of PCR amplification.

With regard to claims 27, 32, 59 and 64, it is arbitrary which sequence is the "selected" sequence and which is the "reference" sequence. In Fig. 1, one of the detected sequences is the β^A (wild type) globin sequence, meeting the limitations of claims 27 and 59. In the third experiment described on pp. 415-416, sequences from two different chromosomes were detected, meeting the limitations of claims 32 and 64.

Claims 1, 2, 7, 14, 19, 27, 32, 38, 39, 46, 51, 59 and 64 are rejected under 35 U.S.C. 102(b) as being anticipated by Zhang.

Zhang discloses a method similar to that of Li. In Zhang's method (p. 5847), a biological sample (semen) was diluted into 18 assay samples by selecting and isolating 18 single sperm cells. Each cell was lysed and the released DNA was pre-amplified by repeated primer extension reactions with a set of random 15-mer primers (primer-extension preamplification, or PEP). The PEP process was estimated to produce at least 30 copies of every sequence capable of amplification (p. 5848, col. 1). After PEP, aliquots of each sample were subjected to a two-step hemi-nested PCR process to determine the genotype at each of 12 different loci. PCR was first performed using a first pair of primers designed to amplify the genetic sequence of interest, then an aliquot of the sample was removed and subjected to a second PCR using one primer from the first pair and a second primer internal to the previously amplified sequence of interest. The second set of primers was chosen so that the two possible alleles would produce amplified fragments of different lengths. This method ensures specificity of the PCR and allows discrimination between the two reaction products (hence, alleles present in the template molecules) by gel electrophoresis of the final PCR product to determine fragment length (p. 5847, col. 2). Each of the 12 loci were successfully amplified in at least 15 of the 18 sperm cells (assay samples; see Table 2). The genotype of each cell was determined for two loci (results for 9 cells shown in Fig. 3). Each of the two APOC2 alleles was found in 9 cells, the expected 1:1 ratio for this heterozygous sperm donor. Similarly, analysis of the sex linked STS gene/pseudogene showed that 9 cells carried an X chromosome and 8 carried a Y chromosome (the 18^{th} cell did not yield detectable STS sequence). Independent assortment of these two loci was also observed (p. 5848, col. 2). Therefore the method of Zhang anticipates claim 1.

With regard to claim 2, the fact that the selected genetic sequences were detected in some of the assay samples shows that the additional claim limitation was met (see claim interpretation above).

Claim 38 is essentially the same as claim 2, except it does not require the dilution step recited in claim 1, and only 1/50 (rather than 1/10) of the assay samples must

comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required to determine the presence of the selected genetic sequence. Therefore claim 38, being broader, is anticipated for the same reasons as claim 2.

With regard to claims 7 and 39, the number of assay samples (individual sperm cells) was greater than 10 (18).

With regard to claims 14 and 46, amplified DNA in the assay samples was analyzed by gel electrophoresis.

With regard to claims 19 and 51, while there are two amplification steps, each amplification employs a single pair of primers.

With regard to claims 27, 32, 59 and 64, it is arbitrary which APOC2 allele is the "selected" sequence and which is the "reference" sequence. Absent evidence to the contrary, each of the STS sequences is assumed to be wild type (one for the X chromosome, the other for the Y chromosome), meeting the limitations of claims 27 and 59, as well as claims 32 and 64. Furthermore, with regard to claims 32 and 64, the APOC2 locus is on chromosome 19 (p. 5848, col. 2).

Claims 4-6 are rejected under 35 U.S.C. 103(a) as being unpatentable over either one of Li or Zhang as applied to claim 1, furhter in view of Jeffreys.

Li and Zhang are relied on as described above. Each reference discloses a method in which a biological sample is diluted into a plurality of assay samples containing a single cell, DNA from each cell is amplified, the presence or absence of two different DNA sequences is determined, and the relative frequency (ratio) of the two sequences is determined. Li and Zhang do not disclose a method wherein the biological sample is cell free.

Jeffreys discloses methods for amplification of human minisatellite DNA for the purpose of producing DNA fingerprints of individuals. In one method, a biological sample is split into multiple assay samples by isolating single cells, then analyzed in much the same way as in Li and Zhang (pp. 10955-10956). In an alternative method, isolated (cell free) DNA was diluted into multiple assay samples, each containing 6 pg

DNA. This amount was estimated to be equivalent to the amount of DNA in a single cell. It was concluded that single DNA molecules could be faithfully amplified (pp. 10960-10962). In the experiment shown in Fig. 4, each assay sample was subjected to PCR with 4 sets of primers (in a single reaction), the primers designed to amplify two alleles for each of 2 minisatellites. Successful amplification was obtained, with a mean failure rate of 63% per allele per reaction, equating to an estimated 0.46 successful amplification events per 6 pg sample (because statistically one would not expect the template sequence to be present in every sample; p. 10961).

It would have been obvious to one of ordinary skill in the art to modify the method of Li or Zhang by obtaining DNA from a cell free sample, then diluting it into multiple assay samples which each contain approximately as much DNA as a single cell, as taught by Jeffreys. One would have been motivated to do this in order to analyze DNA from sources which do not contain intact cells (e.g. forensic samples) and/or to eliminate the labor intensive process of isolating single cells. With regard to claims 4 and 5, Jeffreys estimates that with one genome equivalent of DNA per sample, about 46% of PCRs were successful. One would be motivated to ensure that every sample yielded a successful PCR, to avoid wasting time and reagents. It would have been obvious to increase (e.g. double or triple) the amount of DNA in each sample to ensure that each PCR yielded an amplification product, which would still be less than 10 genome equivalents per sample, or less than 10 reference sequence template molecules per sample (in the case of a gene having a single copy per haploid genome). Thus the invention as a whole was clearly prima facie obvious to one of ordinary skill in the art at the time the invention was made.

Claims 12 and 44 are rejected under 35 U.S.C. 103(a) as being unpatentable over either one of Li or Zhang as applied to claims 1 and 38 and further in view of Kalinina.

Li and Zhang are relied on as described above. Together, the references teach a method in which a cell free biological sample is diluted into a plurality of assay samples containing the DNA in an amount equivalent to a single cell, DNA from each assay

sample is amplified, the presence or absence of two different DNA sequences is determined, and the relative frequency (ratio) of the two sequences is determined. Neither Li nor Zhang disclose a method wherein amplification and analysis are performed in the same receptacle.

Kalinina discloses a method for PCR amplification and detection using TaqMan probes. Samples diluted to contain approximately 1 template molecule are subjected to TaqMan PCR in sealed capillary tubes containing a few nanoliters of reactants, then presence of PCR product is determined by measuring the probe fluorescence (entire document, see especially p. 2000). The method is considered especially useful for assays meant to determine the presence or absence of PCR product (i.e. not quantitative analysis; p. 2004, last paragraph).

It would have been obvious to one of ordinary skill in the art to modify the method of Li or Zhang by conducting nanoliter scale TaqMan PCR in a sealed capillary as taught by Kalinina. One would have been motivated to do this in view of the readily apparent advantages of doing so. Nanoliter scale PCR would reduce the amount (and cost) of reagents required, and fluorescence detection would eliminate the need for radioactive probes (Li method) or gel electrophoresis (Zhang method). Kalinina suggests that the process could be automated (abstract), and explicitly states that the method should be useful to determine the presence or absence of PCR product in samples diluted to contain approximately one template molecule (p. 2004). Thus the invention as a whole was clearly prima facie obvious to one of ordinary skill in the art at the time the invention was made.

Claims 20 and 52 are rejected under 35 U.S.C. 103(a) as being unpatentable over either one of Li or Zhang as applied to claims 1 and 38 and further in view of Chou.

Li and Zhang are relied on as described above. Each reference discloses a method in which a biological sample is diluted into a plurality of assay samples containing a single cell, DNA from each cell is amplified, the presence or absence of two different DNA sequences is determined, and the relative frequency (ratio) of the

two sequences is determined. Li and Zhang do not disclose a method wherein the DNA polymerase is activated after heating.

Chou discloses a method for "hot start" PCR. The method uses a wax barrier to separate one or more PCR components from the remainder of the reactants until heat is applied to melt the wax (entire document). This method reduces amplification due to mispriming and primer oligomerization, and is said to be especially useful for PCR with a sample containing a low number of template molecules (p. 1722, col. 1).

It would have been obvious to one of ordinary skill in the art to modify the method of Li or Zhang by using the hot start PCR method. One would have been motivated to do so in order to increase the specificity of the PCR as taught by Chou. Thus the invention as a whole was clearly prima facie obvious to one of ordinary skill in the art at the time the invention was made.

Claims 23 and 55 are rejected under 35 U.S.C. 103(a) as being unpatentable over either one of Li or Zhang as applied to claims 1 and 38 and further in view of Burg.

Li and Zhang are relied on as described above. Each reference discloses a method in which a biological sample is diluted into a plurality of assay samples containing a single cell, DNA from each cell is amplified, the presence or absence of two different DNA sequences is determined, and the relative frequency (ratio) of the two sequences is determined. Li and Zhang do not disclose a method wherein the PCR is performed for 60 cycles.

Burg discloses a method for PCR detection of a single cell of *Toxoplasma gondii*. Cells are lysed and PCR is performed for 60 cycles (p. 1790, col. 1; Fig. 4).

It would have been obvious to one of ordinary skill in the art to modify the method of Li or Zhang by performing the PCR for 60 amplification cycles. One would have been motivated to do so, given the knowledge that this method is effective for detecting target DNA sequences from a single cell as taught by Burg. Thus the invention as a whole was clearly prima facie obvious to one of ordinary skill in the art at the time the invention was made.

Claims 24, 29, 30, 56, 61 and 62 are rejected under 35 U.S.C. 103(a) as being unpatentable over either one of Li or Zhang as applied to claims 1 and 38 and further in view of Trümper.

Li and Zhang are relied on as described above. Each reference discloses a method in which a biological sample is diluted into a plurality of assay samples containing a single cell, DNA from each cell is amplified, the presence or absence of two different DNA sequences is determined, and the relative frequency (ratio) of the two sequences is determined. Li and Zhang do not disclose a method wherein the biological sample is derived from stool, blood or lymph nodes, nor do they disclose a method wherein the template molecules to be amplified are on cDNA..

Trümper isolated single cells from lymph nodes of patients diagnosed with Hodgkin's disease. Cells were lysed, cDNA was produced by reverse transcription and PCR performed on the cDNA (see methods, pp. 3098-3100). One cell was found to have a mutation in exon 7 of the p53 gene, at a known "hot spot." This mutation is considered to be a "rare exon sequence" as recited in claims 29 and 61.

It would have been obvious to one of ordinary skill in the art to use the method of Li or Zhang to study cells from lymph node tissue, for example to determine the percentage of Hodgkins cells having the p53 mutation found by Trümper. In this case, the mutant p53 sequence would be the selected genetic sequence and the wild type sequence would be the reference sequence. This is exactly the type of analysis suggested by Li, which states, "the ability to study DNA sequences in individual diploid cells will make it possible to study cell-to-cell variation in developmental processes involving DNA rearrangements or other genetic alterations. It is also likely that analysis of messenger RNAs in single cells would be possible if efficient reverse transcription could be carried out before PCR was initiated" (p. 417, col. 2). Thus the invention as a whole was clearly prima facie obvious to one of ordinary skill in the art at the time the invention was made.

Claims 31 and 63 are rejected under 35 U.S.C. 103(a) as being unpatentable over either one of Li or Zhang as applied to claims 1 and 38 and further in view of Pontén.

Li, Zhang are relied on as described above; together they teach a method for measuring the relative number (ratio) of cells in a sample which have a mutation in the p53 gene. Neither Li nor Zhang teach a method in which the selected genetic sequence and the reference genetic sequence each comprise a different mutation.

Pontén performed single cell PCR on cells derived from a single tumor and showed that the tumor contained multiple p53 mutations. Some cells contained more than one mutation of the p53 gene (see overview on p. 52).

It would have been obvious to one of ordinary skill in the art to modify the method of Li or Zhang by performing PCR with multiple primer sets capable of amplifying different portions of the p53 gene known to contain mutation-prone sequences of interest, as disclosed by Pontén. As noted above, it is arbitrary which sequence is the "selected" sequence and which is the "reference" sequence. One would have been motivated to determine the relative abundance (ratio) of p53 mutations in tumors to investigate, for example, possible correlations between different p53 mutations and tumor phenotype (invasiveness, susceptibility to anti-cancer drugs, etc.). Thus the invention as a whole was clearly prima facie obvious to one of ordinary skill in the art at the time the invention was made.

Claims 10, 11, 25, 28, 42, 43, 57 and 60 are rejected under 35 U.S.C. 103(a) as being unpatentable over either one of Li or Zhang as applied to claims 1 and 38 and further in view of Kanzler.

Li and Zhang are relied on as described above. Each reference discloses a method in which a biological sample is diluted into a plurality of assay samples containing a single cell, DNA from each cell is amplified, the presence or absence of two different DNA sequences is determined, and the relative frequency (ratio) of the two sequences is determined. Li and Zhang do not disclose a method wherein the biological sample is derived from blood or bone marrow of a leukemia or lymphoma

patient who has received anti-cancer therapy. Li and Zhang also do not disclose a method wherein the number of assay samples is greater than 500 or 1,000, nor a method wherein the selected genetic sequence is part of a sequence which is amplified during neoplastic development.

Kanzler isolated single cells from bone marrow of patients diagnosed with Hodgkin's disease (p. 3429). PCR analysis identified three gene rearrangements (abstract). Kanzler suggests, "Using tumor clone-specific primers … residual tumor cells may be detected after therapy" (p. 3434, col. 2).

It would have been obvious to one of ordinary skill in the art to use the method of Li or Zhang to study cells from bone marrow of a Hodgkin's lymphoma patient who has received anti-cancer therapy as suggested by Kanzler. In this case, one or more of the rearranged DNA sequences noted by Kanzler would be the selected genetic sequence(s) and the wild type sequence(s) would be the reference sequence, and the analysis would determine the percentage (ratio) of cancerous cells remaining after therapy. Ideally, there should be no cancerous cells remaining after therapy, but every cell cannot be tested. It is readily apparent that the more cells are tested and found to be non-cancerous, the greater the likelihood that all cancerous cells have been eradicated by the anti-cancer therapy. It would therefore be obvious to increase the number of cells analyzed to 500, 1,000 or more, as recited in claims 10, 11, 42 and 43. This type of analysis is suggested by Li, which states, "the ability to study DNA sequences in individual diploid cells will make it possible to study cell-to-cell variation in developmental processes involving DNA rearrangements or other genetic alterations" (p. 417, col. 2). Thus the invention as a whole was clearly prima facie obvious to one of ordinary skill in the art at the time the invention was made.

Claims 26 and 58 are rejected under 35 U.S.C. 103(a) as being unpatentable over either one of Li or Zhang as applied to claims 1 and 38 and further in view of Gravel.

Li and Zhang are relied on as described above. Each reference discloses a method in which a biological sample is diluted into a plurality of assay samples

containing a single cell, DNA from each cell is amplified, the presence or absence of two different DNA sequences is determined, and the relative frequency (ratio) of the two sequences is determined. Li and Zhang do not disclose a method wherein the selected genetic sequence is a translocated allele.

Gravel used single cell PCR analysis to determine the presence or absence of a chromosomal translocation, t(14;18)(q32;q21), in cells from bone of patients diagnosed with Hodgkin's disease (see methods, pp. 2866-2868).

It would have been obvious to one of ordinary skill in the art to use the method of Li or Zhang to search for chromosomal translocations as was done by Gravel. In this case, the wild type (non-translocated) sequence would be the reference sequence, and the analysis would determine the percentage (ratio) of cells in a sample having the translocation. This type of analysis is suggested by Li, which states, "the ability to study DNA sequences in individual diploid cells will make it possible to study cell-to-cell variation in developmental processes involving DNA rearrangements or other genetic alterations" (p. 417, col. 2). There would have been a reasonable expectation of success, since Gravel had already used single cell PCR to detect a translocation. Thus the invention as a whole was clearly prima facie obvious to one of ordinary skill in the art at the time the invention was made.

Claims 28 and 60 are rejected under 35 U.S.C. 103(a) as being unpatentable over either one of Li or Zhang as applied to claims 1 and 38 and further in view of Schwab.

Li and Zhang are relied on as described above. Each reference discloses a method in which a biological sample is diluted into a plurality of assay samples containing a single cell, DNA from each cell is amplified, the presence or absence of two different DNA sequences is determined, and the relative frequency (ratio) of the two sequences is determined. Li and Zhang do not disclose a method wherein the selected genetic sequence is one which is amplified during neoplastic development.

Schwab is a review article which summarizes what was known about gene amplification in different types of cancer at the time the invention was made (entire

document). For example, “Amplified MYCN has been found only in more aggressive variants of neuroblastoma, where it connotes a dire prognosis. Clinically, it has emerged as a powerful independent marker to predict poor patient outcome” (p. 475, col. 2). Regarding amplification of ERBB2 in breast cancer, “amplification was found to be a significant predictor of both overall survival and time to relapse and appears to be superior to all other prognostic parameters except for positive lymph nodes” (p. 476. Col. 1).

It would have been obvious to one of ordinary skill in the art to use the method of Li or Zhang to search for amplified oncogene sequences such as those disclosed by Schwab. In this case, the wild type (unamplified) sequence would be the reference sequence, and the analysis would determine the percentage (ratio) of cells in a sample having the amplified version. This type of analysis is suggested by Li, which states, “the ability to study DNA sequences in individual diploid cells will make it possible to study cell-to-cell variation in developmental processes involving DNA rearrangements or other genetic alterations" (p. 417, col. 2). One would have been motivated to do so to help predict the prognosis for patients, to search for metastatic cells in surrounding tissues, to conduct basic research in oncogenesis, etc. Thus the invention as a whole was clearly prima facie obvious to one of ordinary skill in the art at the time the invention was made.

Documents Not Relied Upon

Flint used single cell reverse transcription and PCR to study gene expression in developing neocortex tissues. Flint is cumulative to Trümper, which also utilizes single cell RT-PCR.

Marcucci is cited in the request as disclosing an amplicon which is amplified during neoplastic development, as recited in claims 28 and 60. However Marcucci discloses a chromosome segment which is subject to partial tandem duplication, which is a common defect found in acute myeloid leukemia. Since the duplication is only

partial and results in a different gene product (see p. 790), this cannot fairly be characterized as gene amplification.

Conclusion

Claims 1-12, 14-16, 19-32, 38-44, 46-48 and 51-64 are rejected. Claims 13, 17, 18, 33-37, 45, 49 and 50 are not subject to reexamination.

Extensions of Time

Extensions of time under 37 CFR 1.136(a) will not be permitted in these proceedings because the provisions of 37 CFR 1.136 apply only to "an applicant" and not to parties in a reexamination proceeding. Additionally, 35 U.S.C. 305 requires that reexamination proceedings "will be conducted with special dispatch" (37 CFR 1.550(a)). Extension of time in *ex parte* reexamination proceedings are provided for in 37 CFR 1.550(c).

Patent owner is notified that any proposed amendment to the specification and/or claims in this reexamination proceeding must comply with 37 CFR 1.530(d)-(j), must be formally presented pursuant to 37 CFR 1.52(a) and (b), and must contain any fees required by 37 CFR 1.20(c).

In order to ensure full consideration of any amendments, affidavits or declarations, or other documents as evidence of patentability, such documents must be submitted in response to this Office action. Submissions after the next Office action, which is intended to be a final action, will be governed by the requirements of 37 CFR 1.116, after final rejection and 37 CFR 41.33 after appeal, which will be strictly enforced.

Duty to Disclose

The patent owner is reminded of the continuing responsibility under 37 CFR 1.565(a) to apprise the Office of any litigation activity, or other prior or concurrent proceeding, involving U.S. Patent No. 6,440,706 throughout the course of this reexamination proceeding. The third party requester is also reminded of the ability to similarly apprise the Office of any such activity or proceeding throughout the course of this reexamination proceeding. See MPEP §§ 2207, 2282 and 2286.

Correspondence

Any inquiry concerning this communication or earlier communications from the examiner should be directed to BRUCE CAMPELL whose telephone number is (571)272-7064. The examiner can normally be reached on Monday - Thursday from 8:00 to 5:00. The examiner can also be reached on alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Deborah Jones, can be reached on 571-272-1535. The fax phone number for the organization where this proceeding is assigned is 571-273-9900.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

All correspondence relating to this ex parte reexamination proceeding should be directed:

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Commissioner for Patents
United States Patent & Trademark Office
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Alexandria, VA 22313-1450

By FAX to: (571) 273-9900
Central Reexamination Unit

By hand: Customer Service Window
Randolph Building
401 Dulany Street
Alexandria, VA 22314

/Bruce Campell/
Patent Reexamination Specialist
Central Reexamination Unit 3991

/Padmashri Ponnaluri/
Patent Reexamination Specialist
Central Reexamination Unit 3991

/Deborah D Jones/
Supervisory Patent Examiner, Art Unit 3991

Notice of References Cited	Application/Control No. 90/012,894	Applicant(s)/Patent Under Reexamination 6,440,706 B1 .	
	Examiner BRUCE CAMPELL	Art Unit 3991	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
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FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	M Schwab, “Amplification of oncogenes in human cancer cells.” Bioessays 20(6): 473-479 (1998)
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Reexamination	Application/Control No.	Applicant(s)/Patent Under Reexamination
	90012894	6,440,706 B1 ET AL.
	Certificate Date	Certificate Number
		C1

Requester Correspondence Address:	☐ Patent Owner	☒ Third Party

LIFE TECHNOLOGIES CORPORATION
ATTN: IP DEPARTMENT
5791 VAN ALLEN WAY
CARLSBAD, CA 92008

LITIGATION REVIEW ☒	/BC/ (examiner initials)	06/27/2013 (date)
Case Name		Director Initials
Esoterix Genetic Laboratories, Llc et al v. Life Technologies		
U.S. District - North Carolina Middle 1:12cv1173		

COPENDING OFFICE PROCEEDINGS	
TYPE OF PROCEEDING	NUMBER
1. none	

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	90012894	6,440,706 B1 ET AL.
	Examiner	Art Unit
	BRUCE CAMPELL	3991

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
reviewed prosecution history of 09/613,826	7/12/13	/BC/
google: gene amplification in cancer review	7/24/13	/BC/

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	90012894
Filing Date	2013-06-17
First Named Inventor	
Art Unit	3991
Examiner Name	Bruce R. Campell
Attorney Docket Number	001107.00989

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	90012894
	Filing Date	2013-06-17
	First Named Inventor	
	Art Unit	3991
	Examiner Name	Bruce R. Campell
	Attorney Docket Number	001107.00989

	1	Supplemental Joint Claim Construction Statement filed in Civil Action No. 12-cv-1173-CCE-JEP on October 28, 2013 (filed with exhibits A, B, and C)	☐
	2	Defendants' Responsive Claim Construction Brief filed in Case No. 1:12-CV-1173 on November 26, 2013	☐
	3	Deposition of David Sherman, Ph.D., dated October 17, 2013	☐
	4	Supplemental Joint Claim Construction Statement Exhibit C filed in Civil Action No. 12-cv-1173-CCE-JEP on October 28, 2013 (filed with Supplemental Joint Claim Construction Statement filed in Civil Action No. 12-cv-1173-CCE-JEP on October 28, 2013)	☐
	5	Plaintiffs' Responsive Claim Construction Brief filed in filed in Civil Action No. 12-cv-1173-CCE-JEP on November 26, 2013	☐
	6	Plaintiffs' Proposed Construction of Disputed Terms, Supporting Evidence, and Rebuttal Evidence Exhibit B, filed in filed in Civil Action No. 12-cv-1173-CCE-JEP on October 28, 2013 (filed with Supplemental Joint Claim Construction Statement filed in Civil Action No. 12-cv-1173-CCE-JEP on October 28, 2013)	☐
	7	Declaration of David H. Sherman in Support of Esoterix Genetic Laboratories' Claim Construction Brief filed in Civil Action Nos. 12-cv-411-CCE-JEP and 12-cv-1173-CCE-JEP, executed September 27, 2013	☐
	8	Defendants' Opening Claim Construction Brief filed in Case No. 1:12-CV-1173 on November 5, 2013	☐
	9	Exhibit A filed in Civil Action No. 12-cv-1173-CCE-JEP on October 28, 2013 (filed with Supplemental Joint Claim Construction Statement filed in Civil Action No. 12-cv-1173-CCE-JEP on October 28, 2013)	☐
	10	Plaintiffs' Opening Claim Construction Brief filed in Civil Action No. 12-cv-1173-CCE-JEP on November 5, 2013	☐

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	90012894
	Filing Date	2013-06-17
	First Named Inventor	
	Art Unit	3991
	Examiner Name	Bruce R. Campell
	Attorney Docket Number	001107.00989

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	90012894
	Filing Date	2013-06-17
	First Named Inventor	
	Art Unit	3991
	Examiner Name	Bruce R. Campell
	Attorney Docket Number	001107.00989

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☒ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☐ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature		Date (YYYY-MM-DD)	
Name/Print	Sarah A. Kagan	Registration Number	32141

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

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9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt

EFS ID:	18030312
Application Number:	90012894
International Application Number:	
Confirmation Number:	8442
Title of Invention:	Digital Amplification
First Named Inventor/Applicant Name:	6,440,706 B1
Customer Number:	11332
Filer:	Sarah Anne Kagan./Jennifer Hazzard
Filer Authorized By:	Sarah Anne Kagan.
Attorney Docket Number:	001107.00989
Receipt Date:	27-JAN-2014
Filing Date:	17-JUN-2013
Time Stamp:	13:29:31
Application Type:	Reexam (Third Party)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Form (SB08)	IDSSB08.PDF	612652 f43086f0dc59eae4b5c8239cb9912f11e31e049f	no	5

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re *Ex Parte* Reexamination:	)	Group Art Unit: 3991
U.S. Patent No. 6,440,706	)	Docket No. 001107.00989
Control No. 90/012,894	)	Confirmation No: 8442
Reexam Filing Date: June 17, 2013	)	Examiner: Bruce R. Campell

For: DIGITAL AMPLIFICATION

RESPONSIVE AMENDMENT TO OFFICE ACTION

U.S. Patent and Trademark Office
Customer Service Window
Randolph Building
401 Dulany Street
Alexandria, VA 22314

Commissioner:

This paper is in response to the Non-Final Office Action mailed November 27, 2013 ("Office Action"). Johns Hopkins University ("the Patent Owner") respectfully requests reconsideration of the rejections made in the Office Action in view of the following remarks.

Amendments to the Claims are reflected in the Listing of Claims, which begins on page 2 of this paper.

Remarks begin on page 9 of this paper.

Conclusion begins on page 30 of this paper.

LISTING OF THE CLAIMS

Please amend the following claims as indicated by the status identifier. Patent claims under reexamination but not amended are indicated as "original." Patent claims not subject to reexamination are not shown.

1. (Original) A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of:

diluting nucleic acid template molecules in a biological sample to form a set comprising a plurality of assay samples;

amplifying the template molecules within the assay samples to form a population of amplified molecules in the assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence;

comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

2. (Original) The method of claim 1 wherein the step of diluting is performed until at least one-tenth of the assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required for the step of analyzing to determine the presence of the selected genetic sequence.

3. (Original) The method of claim 1 wherein the step of diluting is performed until between 0.1 and 0.9 of the assay samples yield an amplification product when subjected to a polymerase chain reaction.

4. (Original) The method of claim 1 wherein the step of diluting is performed until all of the assay samples yield an amplification product when subjected to a polymerase chain reaction and each assay sample contains less than 10 nucleic acid template molecules containing the reference genetic sequence.

5. (Original) The method of claim 1 wherein the step of diluting is performed until all of the assay samples yield an amplification product when subjected to a polymerase chain reaction and each assay sample contains less than 100 nucleic acid template molecules containing the reference genetic sequence.

6. (Original) The method of claim 1 wherein the biological sample is cell-free.

7. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 10.

8. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 50.

9. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 100.

10. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 500.

11. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 1000.

12. (Original) The method of claim 1 wherein the step of amplifying and the step of analyzing are performed on assay samples in the same receptacle.

13. (Not subject to reexamination)

14. (Original) The method of claim 1 wherein the step of analyzing employs gel electrophoresis.

15. (Original) The method of claim 1 wherein the step of analyzing employs

hybridization to at least one nucleic acid probe.

16. (Original) The method of claim 1 wherein the step of analyzing employs hybridization to at least two nucleic acid probe.

17. (Not subject to reexamination)

18. (Not subject to reexamination)

19. (Original) The method of claim 1 wherein the step of amplifying employs a single pair of primers.

20. (Original) The method of claim 1 wherein the step of amplifying employs a polymerase which is activated only after heating.

21. (Original) The method of claim 1 wherein the step of amplifying employs at least 40 cycles of heating and cooling.

22. (Original) The method of claim 1 wherein the step of amplifying employs at least 50 cycles of heating and cooling.

23. (Original) The method of claim 1 wherein the step of amplifying employs at least 60 cycles of heating and cooling.

24. (Original) The method of claim 1 wherein the biological sample is selected from the group consisting of stool, blood, and lymph nodes.

25. (Original) The method of claim 1 wherein the biological sample is blood or bone marrow of a leukemia or lymphoma patient who has received anti-cancer therapy.

26. (Original) The method of claim 1 wherein the selected genetic sequence is a

translocated allele.

27. (Original) The method of claim 1 wherein the selected genetic sequence is a wild-type allele.

28. (Original) The method of claim 1 wherein the selected genetic sequence is within an amplicon which is amplified during neoplastic development.

29. (Original) The method of claim 1 wherein the selected genetic sequence is a rare exon sequence.

30. (Original) The method of claim 1 wherein the nucleic acid template molecules comprise cDNA of RNA transcripts and the selected genetic sequence is present on a cDNA of a first transcript and the reference genetic sequence is present on a cDNA of a second transcript.

31. (Original) The method of claim 1 wherein the selected genetic sequence comprises a first mutation and the reference genetic sequence comprises a second mutation.

32. (Original) The method of claim 1 wherein the selected genetic sequence and the reference genetic sequence are on distinct chromosomes.

33-37. (Not subject to reexamination)

38. (Currently amended) A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of:

amplifying template molecules <u>in a biological sample</u> within a set comprising a plurality of assay samples to form a population of amplified molecules in each of the assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence, wherein at least one-fiftieth of the

assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required to determine the presence of the selected genetic sequence;

comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

39. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 10.

40. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 50.

41. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 100.

42. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 500.

43. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 1000.

44. (Original) The method of claim 38 wherein the step of amplifying and the step of analyzing are performed on assay samples in the same receptacle.

45. (Not subject to reexamination)

46. (Original) The method of claim 38 wherein the step of analyzing employs gel electrophoresis.

47. (Original) The method of claim 38 wherein the step of analyzing employs hybridization to at least one nucleic acid probe.

48. (Original) The method of claim 38 wherein the step of analyzing employs hybridization to at least two nucleic acid probe.

49. (Not subject to reexamination)

50. (Not subject to reexamination)

51. (Original) The method of claim 38 wherein the step of amplifying employs a single pair of primers.

52. (Original) The method of claim 38 wherein the step of amplifying employs a polymerase which is activated only after heating.

53. (Original) The method of claim 38 wherein the step of amplifying employs at least 40 cycles of heating and cooling.

54. (Original) The method of claim 38 wherein the step of amplifying employs at least 50 cycles of heating and cooling.

55. (Original) The method of claim 38 wherein the step of amplifying employs at least 60 cycles of heating and cooling.

56. (Original) The method of claim 38 wherein the template molecules are obtained from a body sample selected from the group consisting of stool, blood, and lymph nodes.

57. (Original) The method of claim 38 wherein the template molecules are obtained from a body sample of a leukemia or lymphoma patient who has received anti-cancer therapy, said body sample being selected from the group consisting of blood and bone marrow.

58. (Original) The method of claim 38 wherein the selected genetic sequence is a

translocated allele.

59. (Original) The method of claim 38 wherein the selected genetic sequence is a wild-type allele.

60. (Original) The method of claim 38 wherein the selected genetic sequence is within an amplicon which is amplified during neoplastic development.

61. (Original) The method of claim 38 wherein the selected genetic sequence is a rare exon sequence.

62. (Original) The method of claim 38 wherein the nucleic acid template molecules comprise cDNA of RNA transcripts and the selected genetic sequence is present on a cDNA of a first transcript and the reference genetic sequence is present on a cDNA of a second transcript.

63. (Original) The method of claim 38 wherein the selected genetic sequence comprises a first mutation and the reference genetic sequence comprises a second mutation.

64. (Original) The method of claim 38 wherein the selected genetic sequence and the reference genetic sequence are on distinct chromosomes.

Remarks

Claims 1-12, 14-16, 19-32, 38-44, 46-48, and 51-64 are pending and subject to re-examination of U.S. Patent No. 6,440,706 ("the '706 patent"). Claims 13, 17, 18, 33-37, 45, 49, and 50 are not subject to reexamination.

Claim 38 has been amended herein for purposes of clarity, by providing proper antecedent basis for the last three words of the claim. Support for the amendment can be found throughout the specification, for example, at Col. 2, lines 4-11; Col. 4, lines 12-33; and Col. 6, lines 45-49 of the '706 patent. The scope of the claim is not enlarged by this amendment, and no new matter is added.

I. Overview

U.S. Patent No. 6,440,706 issued on August 27, 2002. The issued claims of the '706 patent are directed to methods for determining the composition of a biological sample as a whole. In particular, the methods determine the ratio of a selected genetic sequence in a population of genetic sequences in the biological sample. Col. 1, lines 65-67 of the '706 patent. The thrust of the invention is to separate or isolate the components of a mixed population of genetic sequences down to a level where each of the genetic sequences are more readily detected. For this reason, the disclosed methods are particularly useful for the detection of a rare or non-predominant genetic sequence within a mixed population of genetic sequences.

In the disclosed methods, the population of genetic sequences are contained within nucleic acid template molecules that are obtained from a biological sample. The specification discloses that the biological samples:

> which can be used as the *starting material* for the analyses may be from any tissue or body sample from which DNA or mRNA can be isolated. Preferred sources include stool, blood, and lymph nodes. Preferably the biological sample is a cell-free lysate.

Col. 6, lines 45-49 of the '706 patent (emphasis added). The specification does not teach that the claimed method be performed on "individual cells" or "single cells," but rather is concerned with

the detection of genetic sequences within a mixed population of genetic sequences without requiring the isolation of single cells as was described in the prior art.

The specification teaches that the nucleic acid template molecules obtained from the biological sample are diluted to form a set comprising a plurality of assay samples. Figure 1A; Col. 2, lines 7-11 of the '706 patent. The nucleic acid template molecules in each of the assay samples are then amplified, and the amplified molecules are analyzed to determine the number of assay samples across the set that contain the selected genetic sequence and the number of assay samples across the set that contain a reference genetic sequence. Col. 2, lines 11-15 of the '706 patent. By comparing the two numbers, the ratio of the selected genetic sequence to the population of sequences can be determined, which ratio reflects the composition of the biological sample as a whole. Col. 2, lines 15-17 of the '706 patent.

It is critical that the analysis step in the described methods is performed across the set of assay samples, and therefore, this ratio provides information with respect to the composition of the population of genetic sequences in the biological sample, *as a whole*. This type of analysis is different from, and provides very different information from, an analysis of a single intact cell of the biological sample.

II. Novelty

A. Li et al. (1988, *Nature 335*(6189):414-17)

Claims 1-3, 7-9, 15, 16, 19, 21, 22, 27, 32, 38-41, 47, 48, 51, 53, 54, 59 and 64 were rejected under §102(b) as allegedly anticipated by Li et al. (1988, *Nature 335*(6189):414-17; "Li"). Claims 1 and 38 are independent claims. Claims 2, 3, 7-9, 15, 16, 19, 21, 22, 27, and 32 depend directly or indirectly from claim 1, and claims 39-41, 47, 48, 51, 53, 54, 59, and 64 depend directly or indirectly from claim 38. Claim 1 and claims dependent on claim 1 will be discussed first, and then claim 38 and claims dependent on claim 38 will be discussed.

To anticipate a claim, the cited reference must disclose each and every element of the claims. *Verdegaal Bros. v. Union Oil Co.*, 814 F.2d 628, 631 (Fed. Cir. 1987). Here, Li does not anticipate the claims because Li fails to disclose each element of the claims.

Claims 1-3, 7-9, 15, 16, 19, 21, 22, 27, and 32

Claim 1 includes four steps. The first step requires the dilution of *nucleic acid template molecules in a biological sample* to form a *set comprising a plurality of assay samples*. The second step requires amplifying the template molecules within each of the assay samples to form a population of amplified molecules in the assay samples of the set. The third step requires analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence. And the final step requires comparing the first number to the second number to ascertain a ratio which reflects the *composition of the biological sample*.

The Office Action first cites Li's experiment with lymphocytes described at Pages 414-415 as anticipating claim 1. Office Action, Page 6. In this lymphocyte experiment, Li made an artificial mixture of tissue culture cells from two individuals and then micromanipulated the mixture to isolate individual cells. The individual cells were separately lysed, and their nucleic acids were used in amplification reactions and analyzed. With respect to the first step of claim 1, the Office Action asserts that Li discloses that the "nucleic acid template molecules, contained within the cultured cells, were diluted *by isolating single cells from the culture*." Office Action, Page 6 (emphasis added).

The Patent and Trademark Office, in a re-examination, must construe claims using the broadest reasonable interpretation *consistent with the specification. In re Yamamoto,* 740 F.2d 1569, 1571 (Fed. Cir. 1984) (emphasis added). The Office Action erred in the construction of the dilution step of claim 1 which recites "diluting nucleic acid template molecules in a biological sample to form a set comprising a plurality of assay samples." The preposition "in" refers to the nucleic acid template molecule. That is, "in" denotes where the nucleic acid template molecules are obtained or derived from, rather than into what the molecules are diluted. The specification is clear and not only supports, but mandates this construction. Relevant portions of the specification include, but are not limited to, the following:

Figure 1A showing that isolated DNA template molecules are diluted.

> Thus there is a need in the art for methods for accurately and quantitatively detecting genetic sequences in mixed populations of sequences. Col. 1, lines 59-61 of the '706 patent.
>
> It is an object of the present invention to provide methods for determining the presence of a selected genetic sequence in a population of genetic sequences. Col. 1, lines 65-67 of the '706 patent.
>
> The first number is then compared to the second number to ascertain a ratio which reflects the composition of the biological sample. Col. 2, lines 15-17 of the '706 patent.
>
> The invention thus provides the art with the means to obtain quantitative assessments of particular DNA or RNA sequences in mixed populations of sequences using digital (binary) signals. Col. 2, lines 48-51 of the '706 patent.
>
> The method requires analyzing a large number of amplified products simply and reliably. Techniques for such assessments were developed, with the output providing a digital readout of the fraction of mutant alleles in the analyzed population. Col. 4, lines 8-12 of the '706 patent.
>
> Desirably each assay sample prior to amplification will contain less than a hundred or less than ten template molecules. Col. 4, lines 31-33 of the '706 patent.
>
> Digital amplification can be used to detect mutations present at relatively low levels in the samples to be analyzed. Col. 4, lines 34-35 of the '706 patent.
>
> In one preferred embodiment each diluted sample has on average one half a template molecule. Col. 5, lines 40-41 of the '706 patent.

Thus, it is clear that the specification clearly aims to quantify the proportion of two genetic sequences relative to each other in a mixed population of genetic sequences in a biological sample. The biological sample is the *starting material* from which the nucleic acid template molecules containing the genetic sequences are obtained, and the "set comprising a plurality of assay samples" is produced by diluting the *nucleic acid template molecules* of the biological sample into different assay samples.

The step of "diluting nucleic acid template molecules in a biological sample" is understood by one of skill in the art as a step in which isolated nucleic acid template molecules, obtained from a biological sample, are diluted by a process of placing the nucleic acid template molecules from the biological sample in a larger volume of liquid. Shendure Declaration at paragraph 10. This is the plain meaning of the phrase. Moreover this understanding is consistent

with the teachings of the specification. See, for example, Col. 9, lines 24-27 of the '706 patent ("Principles underlying experiment. The experiment is outlined in FIG. 1A. First, the DNA is diluted into multiwell plates so that there is on average, one template molecule per two wells, and PCR is performed."). Based on the specification, the assay samples are not isolated cells or DNA released from single isolated cells.

The Patent and Trademark Office's construction of the term "diluting" as encompassing single cell micromanipulation followed by lysis is inconsistent with the teachings of the specification. There is no discussion in the patent of performing a single cell analysis. In fact, the use of a single cell in performance of the method runs contrary to preferred embodiments discussed in the specification. The specification contemplates a preferred dilution level where half of the assay samples have one template molecule. Col. 5, lines 40-43 of the '706 patent. This level of dilution could not be achieved by single cell micromanipulation and lysis of lymphocytes. Shendure Declaration at paragraph 11. "A claim interpretation that excludes a preferred embodiment from the scope of the claim is rarely, if ever, correct." *On-Line Techs., Inc. v. Bodenseewerk Perkin-Elmer GmbH*, 386 F.3d 1133, 1138 (Fed. Cir. 2004). Thus a proper construction of claim 1, step 1, fails to encompass single cell isolation and subsequent lysis.

Moreover, if one posits that the lysis step alone is a dilution, without considering the single cell micromanipulation, that too would not fulfill step 1. Simply lysing a single cell, whether a diploid or a haploid cell, would not yield from that cell *a set comprising a plurality of assay samples* as recited in the claims. Thus this construction is also unsupported and untenable.

Therefore, Li fails to disclose dilution of nucleic acid template molecules that are in a biological sample in order to form a set comprising a plurality of assay samples, as recited in claim 1, step 1. Because Li fails to disclose the generation of a set comprising a plurality of assay samples containing diluted nucleic acid template molecules, Li also fails to disclose the second step ("amplifying the template molecules within each of the assay samples") and the third step ("analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence") of claim 1. The fourth step of claim 1 involves "comparing the first number to the second number" from the third step. Therefore,

because Li fails to disclose the third step of claim 1, Li also fails to disclose the fourth step of claim 1.

The Patent and Trademark Office similarly asserts that Li's experiment with single human sperm anticipates claim 1. Office Action, Pages 6-7. In that experiment, individual sperm were micromanipulated and lysed, and the lysates were subjected to amplification. For the same reasons as with the lymphocyte experiment, Li's experiment with isolated sperm fails to disclose step 1 of claim 1. Li does not dilute nucleic acid template molecules to form a set comprising a plurality of assay samples. Rather, the assay samples in Li each comprise DNA sequences from a single sperm cell that has been lysed. Because Li fails to disclose a set comprising a plurality of assay samples containing diluted nucleic acid template molecules, Li also fails to disclose the remaining steps of claim 1.

Claims 2, 3, 7-9, 15, 16, 19, 21, 22, 27, and 32 depend directly or indirectly from claim 1, and must therefore incorporate all of the limitations of claim 1. Therefore, these claims are novel over Li for at least the same reasons discussed above with respect to claim 1. The Patent Owner respectfully requests that the rejection of claims 1-3, 7-9, 15, 16, 19, 21, 22, 27, and 32 under 35 U.S.C. § 102(b) based on Li be withdrawn.

Claims 38-41, 47, 48, 51, 53, 54, 59, and 64

The Office Action asserts that Li discloses the limitations of claim 38 because "the selected genetic sequences were detected in some of the assay samples." Office Action, Page 7. The Patent Owner respectfully disagrees with this assertion.

Claim 38 includes three steps. The first step requires amplifying template molecules in a biological sample within a set comprising a plurality of assay samples to form a population of amplified molecules in each of the assay samples of the set. The second step requires analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence, wherein at least one-fiftieth of the assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required to determine the presence of the selected

genetic sequence. The third step requires comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

The recitation of "amplifying template molecules in a biological sample within a set comprising a plurality of assay samples" in step 1 of claim 38 requires that the nucleic acid template molecules be obtained from a biological sample as a whole, rather than portions thereof (*e.g.*, single cells). As taught in the specification, each of the assay samples is prepared identically from the biological sample such that each sample differs only by the statistical fluctuations inherent in the sampling of the template molecules to make the assay samples. Col. 4, lines 59-63 of the '706 patent. The specification describes the assay samples as being prepared in parallel. Col. 9, 24-30; Col. 11, lines 4-6 of the '706 patent. The specification further teaches that the determined ratios of numbers of assay samples are dependent on the relative fraction of mutant genes within the template population of the biological sample. Col. 11, lines 14-17 of the '706 patent. Thus, consistent with the specification, claim 38, step 1, is directed to analysis of a biological sample as a whole by amplification of nucleic acid template molecules in a set comprising a plurality of assay samples. When the ratio is ascertained among the entire set of assay samples, it reflects the composition of the biological sample as a whole, rather than determining the composition of each assay sample individually.

Moreover, claim 38 recites amplifying template molecules from a biological sample within a set comprising a plurality of assay samples. This recitation requires that the template molecules are obtained from a single biological sample. Moreover, as taught in the specification each of the assay samples are prepared identically from that biological sample such that they differ only by the statistical fluctuations inherent in the sampling of the template molecules to make the assay samples. Col. 4, lines 59-63. The specification describes the assay samples as replicate wells. Col. 11, lines 4-6. The specification further teaches that the determined ratios of assay samples are dependent on the relative fraction of mutant genes within the template population of the biological sample. Col. 11, lines 14-17. Thus, consistent with the intention manifested in the specification, claim 38, step 1, is directed to analysis of a single biological sample by amplification of a set of assay samples that are replicates of each other. When the ratio is ascertained it reflects the composition of the single biological sample of nucleic acid template molecules.

Li fails to disclose step 1 of claim 38 because Li fails to disclose a set comprising a plurality of assay samples comprising template molecules obtained or derived from a biological sample. Li's assay samples use 1) nucleic acid sequences from single cells isolated from a mixture of two homogenous cell lines that had been co-cultivated or 2) nucleic acid sequences from individual sperm isolated from a semen sample (*i.e.*, the assay samples are not representative of a biological sample as a whole). Li does not describe a biological sample comprising a population of template molecules that are separated, as described in the specification. Construction of claim 38 consistent with the specification requires a biological sample as a whole as the source of the nucleic acid template molecules.

Because Li fails to disclose the generation of a set comprising a plurality of assay samples containing nucleic acid template molecules from a single biological sample, Li fails to disclose the first step ("amplifying the template molecules in the assay samples") and the second step ("analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence") of claim 38. The third step of claim 38 involves "comparing the first number to the second number" from the second step. Therefore, because Li fails to disclose the third step of claim 38, Li also fails to disclose the third step of claim 38.

Claims 39-41, 47, 48, 51, 53, 54, 59, and 64 depend directly or indirectly from claim 38, and therefore, incorporate all of the limitations of claim 38. These claims are novel over Li for at least the same reasons discussed above with respect to claim 38. Accordingly, the Patent Owner respectfully requests that the rejection of claims 38-41, 47, 48, 51, 53, 54, 59, and 64 under 35 U.S.C. § 102(b) based on Li be withdrawn.

B. Zhang et al. (1992, *PNAS 89*(13):5847-51)

Claims 1, 2, 7, 14, 19, 27, 32, 38, 39, 46, 51, 59 and 64 were rejected under §102(b) as allegedly being anticipated by Zhang et al. (1992, *PNAS 89*(13):5847-51; "Zhang"). The Patent Owner respectfully traverses this rejection.

Zhang describes a method for producing amounts of DNA from a single cell that may be

used for multiple sequence analyses, the methods using an initial amplification step of DNA from a single cell, followed by dilution of the amplification product, and a second amplification with different primers to analyze a particular sequence. See, *e.g.,* Zhang, Page 5847, Col. 1, first paragraph. As acknowledged by the Office Action, Zhang, like Li, isolated individual human sperm and then lysed the single, isolated sperm to yield nucleic acid molecules from the single isolated sperm. Office Action, Page 8. Zhang did not dilute nucleic acid template molecules in a biological sample (*i.e.*, obtained or derived from a tissue or body sample) and instead separated and lysed a single sperm to acquire template molecules.

As described in detail above for Li, a proper construction of claim 1, step 1 does not encompass single cell isolation and subsequent lysis. Therefore, Zhang, like Li, fails to disclose step 1 of claim 1 because Zhang isolated single sperm and then lysed the individual sperm, rather than diluting nucleic acid template molecules in a biological sample. As noted above, simply lysing a single isolated sperm does not yield a set comprising a plurality of assay samples as recited in the claims. Similarly, as described above for Li, Zhang fails to disclose step 1 of claim 38 which requires a single biological sample as a whole as the source of the nucleic acid template molecules in a set comprising a plurality of assay samples.

Because Zhang fails to disclose the generation of a set comprising a plurality of assay samples containing nucleic acid template molecules from a single biological sample as a whole, Zhang also fails to disclose the remaining steps of claims 1 and 38.

Claims 2, 7, 14, 19, 27, 32, 39, 46, 51, 59, and 64 depend from Claims 1 and 38 and incorporate all the limitations of those claims. Therefore, the claims are novel over Zhang for at least the same reasons as discussed above for independent claims 1 and 38. The Patent Owner respectfully requests that the rejection of claims 1, 2, 7, 14, 19, 27, 32, 38, 39, 46, 51, 59, and 64 under 35 U.S.C. § 102(b) based on Zhang be withdrawn.

II. Nonobviousness

A. Li or Zhang, in view of Jeffreys et al. (1988, *Nucleic Acids Research 16*(23):10953-71)

Claims 4-6 were rejected under § 103(a) as allegedly being obvious over either Li or

Zhang and further in view of Jeffreys et al. (1988, *Nucleic Acids Research 16*(23):10953-71; "Jeffreys"). The Patent Owner traverses this rejection.

All three of the rejected claims are dependent on claim 1. Claims 4 and 5 further recite dilution until all assay samples yield an amplification product (both claims), and that each assay sample contains less than 10 (claim 4) or less than 100 nucleic acid template molecules containing the reference sequence (claim 5). Claim 6 recites that the biological sample is cell-free.

Li and Zhang are both cited as disclosing micromanipulation of single cells into separate assay samples, amplification of DNA from each cell, determination of the presences or absence of two different DNA sequences, and the determination of a ratio of the two sequences. Office Action, Page 9. The Office Action acknowledges that Li and Zhang fail to disclose a biological sample that is cell free. Jeffreys is cited as disclosing dilution of DNA from a cell-free sample.

The Office Action first cites an experiment in Jeffreys in which single cells were isolated and then analyzed similar to the methods of Li and Zhang. Office Action, Page 9. Next, the Office Action cited an experiment in which "isolated (cell free) DNA was diluted into multiple assay samples, each containing 6 pg of DNA." Office Action, Pages 9-10. The Office Action asserts that each assay sample was amplified with four sets of primers to amplify two alleles for minisatellites, and successful amplification was obtained with an estimated 0.46 successful amplification events per 6 pg sample. The Office Action concludes that it would have been obvious to combine Li or Zhang with Jeffreys in order to analyze DNA from sources which do not contain intact cells and/or to eliminate the labor intensive process of isolating single cells. Office Action, Page 10.

To establish a proper *prima facie* case of obviousness, the following criteria must be established: (1) the prior art reference, or references when combined, must disclose or suggest all the claim limitations (*See In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991)); (2) the Patent Office must provide an apparent reason to combine the known elements in the claims (*See KSR International Co. v. Teleflex Inc.*, 550 U.S. 398 (2007)); and (3) there must be a reasonable expectation of success in combining the teachings of the reference(s) (*See id.*). Here, the Office Action has not established a *prima facie* case of obviousness because the cited references do not

disclose or suggest all of the claim limitations and one of ordinary skill in the art would not have been motivated to combine the cited references.

As discussed above, both Li and Zhang fail to disclose each of the steps of claims 1 and 38. For example, both Li and Zhang at least fail to disclose or suggest step 1 of claim 1 – the dilution of nucleic acid template molecules in a biological sample. Clearly, the first experiment by Jeffreys does not cure the deficiencies of Li and Zhang because it too uses the same single cell analysis. With respect to the second experiment, although Jeffreys does discuss PCR from 6 or 60 pg of genomic DNA (Figure 4), this experiment was performed merely as a proof of concept that it is possible to achieve faithful amplification of nucleic acids from isolated single cells. Jeffreys, Page 10960, last paragraph. In addition, none of the references disclose or suggest a set comprising a plurality of assay samples comprising nucleic acid template molecules obtained or derived from a biological sample as taught in claims 1 and 38.

One of ordinary skill in the art would not be motivated to combine Jeffreys with either Li or Zhang to meet the limitation of any of claims 4-6. The combination has been made improperly using hindsight knowledge gained from the present invention. It is impermissible to use the claimed invention as an instruction manual or "template" to piece together the teachings of the prior art so that the claimed invention is rendered obvious. *In re Fritch*, 972 F.2d 1260, 1266 (Fed. Cir. 1992). Further, this proposed combination would have destroyed the intended purpose of each of Li and Zhang. Zhang and Li disclose micromanipulation of isolated single cells or sperm to form individual assay samples. This micromanipulation method serves to ensure that all chromosomes within a single cell or sperm remain together throughout the analysis of the cell or sperm. Thus, Zhang discloses the typing of individual sperm cells for 12 loci (Table 2) located on multiple chromosomes. Li focuses on the benefits of a single cell analysis to achieve accurate measurements of genetic distances of less than 1 cM (Li, Page 416, Col. 2, lines 7-11), to genetically map species that cannot be bred or have long generation times (Li, Page 417, sentence spanning Col. 1 and 2). Li also focuses on the benefit of a single cell analysis for studying cell-to-cell variations in development (Li, Page 417, Col. 2, lines 12-15).

The single cell analysis of Li or Zhang is intentional. In fact, Zhang actually teaches away from using a cell-free sample. Zhang, Page 5850, Col. 2, lines 60-70. Specifically, Zhang

expresses concern over the potential for sampling errors, particularly in the context of small, cell-free, forensic or ancient DNA samples. Therefore, it would not have been obvious to modify the intentional single cell analysis, as the rejection proposes, by using cell-free samples. This proposed modification would destroy the information that Li and Zhang were trying to collect regarding each individual cell or sperm. Shendure Declaration at paragraph 12. If a proposed modification to an invention would render invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. *See* MPEP § 2143.01, citing *In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984).

Jeffreys also fails to disclose the specific limitation of claims 4 and 5 wherein all samples yield an amplification product. Although the Office Action asserts that it would have been obvious to one of ordinary skill in the art to double or triple the amount of DNA to achieve this recitation, this assertion is not supported by the facts. Although the asserted motivation was to save time and reagents, doubling or tripling the amount of DNA would not be possible in the case of rare forensic samples (part of the asserted motivation.) Moreover, doubling and tripling would contradict the very purpose of primary references Li and Zhang, who scrupulously worked to have just one cell's DNA in each sample. Shendure Declaration at paragraph 13. Thus, one of ordinary skill in the art would not have been motivated to combine Jeffreys with Li or Zhang or to increase the amount of DNA template.

For at least these reasons, claims 4-6 are not obvious over Li, Zhang, and Jeffreys. The Patent Owner respectfully requests that this rejection under 35 U.S.C.§ 103(a) be withdrawn.

B. Li or Zhang, in view of Kalinina et al. (1997, *Nucleic Acids Research 25:*1999-2004)

Claims 12 and 44 were rejected under § 103(a) as allegedly being obvious over either Li or Zhang and further in view of Kalinina et al. (1997, *Nucleic Acids Research 25:*1999-2004; "Kalinina"). Claims 12 and 44 are dependent on claims 1 and 38, respectively, and both claims 12 and 44 further recite that the amplification and analysis is performed in the same receptacle.

Li and Zhang are both cited as disclosing the dilution of a cell free biological sample into a plurality of assay samples (Office Action, Page 10), amplification of DNA from each sample, determination of the presences or absence of two different DNA sequences, and determination of

a ratio of the two sequences. Office Action, Pages 10-11. The Office Action acknowledges that Li and Zhang fail to disclose a method wherein amplification and analysis are performed in the same receptacle. The Office Action cites Kalinina as disclosing amplification and analysis of single molecules of template DNA in the same receptacle.

The Office Action has not established a *prima facie* case of obviousness because the cited references fail to disclose or suggest all of the claim limitations. As an initial matter, neither Li nor Zhang disclose the dilution of a cell free biological sample as asserted by the Office Action here. Rather, as described above and as acknowledged by the Office Action (See, e.g, Office Action, page 9), Li and Zhang disclose micromanipulation of single cells into separate assay samples. This micromanipulation method was intentional and serves to ensure that all chromosomes within a single cell or sperm remain together throughout the analysis of the cell or sperm. Although Kalinina discloses the dilution of genomic DNA to 0-42 pg, it would not have been obvious to modify the methods of Li and Zhang to conduct the methods on a nanoliter scale as asserted by the Examiner.

The Office Action's combination of references is the result of selective extraction of portions of references with the benefit of hindsight, using the subject claims as a model. It is improper for the Patent and Trademark Office to use the claim as a framework and to employ individual naked parts of separate prior art references as a mosaic to recreate a facsimile of the claimed invention. *See W.L. Gore & Assoc. v. Garlock*, 721 F.2d 1550, 1552-53 (Fed. Cir. 1983). To do so "is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher." *Id.* at 1553. Again, the proposed combination would have destroyed the intended purpose of each of Li and Zhang, which disclose micromanipulation of isolated single cells or sperm to form individual assay samples. This micromanipulation method serves to ensure that all chromosomes within a single cell or sperm remain together throughout the analysis of the cell or sperm. Thus, the single cell analysis of Li or Zhang is intentional and essential to the intended purpose. As noted above, Zhang actually teaches away from using a cell-free sample, expressing concern over the potential for sampling errors, particularly in the context of small, cell-free, forensic, or ancient DNA samples. Zhang, Page 5850, Col. 2, lines 60-70. Therefore, it would not have been obvious to modify the intentional single cell analysis, as the rejection proposes, by using cell-free samples in a single

receptacle. This proposed modification would destroy the information that Li and Zhang were trying to collect regarding each cell or sperm.

For at least these reasons, claims 12 and 44 are not obvious over Li, Zhang, and Kalinina. The Patent Owner respectfully requests that this rejection under 35 U.S.C. § 103(a) be withdrawn.

C. Li or Zhang, in view of Chou et al. (1992, *Nucleic Acids Research 20*(7):1717-23)

Claims 20 and 52 stand rejected under § 103(a) as being obvious over either Li or Zhang and further in view of Chou et al. (1992, *Nucleic Acids Research 20*(7):1717-23; "Chou"). Claims 20 and 52 depend from claims 1 and 38, respectively, and further recite the use of a heat-activatable polymerase.

Li and Zhang are both cited as disclosing micromanipulation of single cells into separate assay samples, amplification of DNA from each cell, determination of the presence or absence of two different DNA sequences, and the determination of a ratio of the two sequences. Office Action, Pages 11-12. The Office Action acknowledged that Li and Zhang fail to disclose a method in which the DNA polymerase is activated after heating. Chou is cited as disclosing a heat-activatable polymerase.

The Office Action has not established a *prima facie* case of obviousness because the cited references fail to disclose or suggest all of the claim limitations. As discussed above, both Li and Zhang fail to disclose or suggest each of the steps of claims 1 and 38. Both Li and Zhang at least fail to disclose step 1 of claim 1 – the dilution of nucleic acid template molecules in a biological sample. In addition, none of the references disclose a set comprising a plurality of assay samples comprising template molecules obtained or derived from a biological sample as required in step 1 of claims 1 and 38. Chou fails to cure the deficiencies of these references as Chou fails to disclose or suggest the dilution and/or amplification of template molecules in a biological sample within a set comprising a plurality of assay samples.

For at least these reasons, claims 20 and 52 are not obvious over Li, Zhang, and Chou. Therefore, the Patent Owner respectfully requests that this rejection under 35 U.S.C.§ 103(a) should be withdrawn.

D. Li or Zhang, in view of Burg et al. (1989, *J. Clin. Microbiol. 27*:1787-92)

Claims 23 and 55 were rejected under § 103(a) as allegedly being obvious over either Li or Zhang and further in view of Burg et al. (1989, *J. Clin. Microbiol. 27:*1787-92; "Burg"). Claims 23 and 55 depend from Claims 1 and 38, respectively, and further recite at least 60 cycles of heating and cooling.

Li and Zhang are both cited as disclosing micromanipulation of single cells into separate assay samples, amplification of DNA from each cell, determination of the presence or absence of two different DNA sequences, and the determination of a ratio of the two sequences. Office Action, Page 12. The Office Action acknowledges that Li and Zhang fail to disclose a method wherein the PCR is performed for 60 cycles. Burg is cited as disclosing at least 60 cycles of heating and cooling to amplify DNA of a single cell of Toxoplasma.

The Office Action has not established a *prima facie* case of obviousness because the cited references fail to disclose or suggest all of the steps of claims 1 and 38. Both Li and Zhang at least fail to disclose step 1 of claim 1 – the dilution of nucleic acid template molecules in a biological sample. In addition, neither of the references discloses a set comprising a plurality of assay samples with template molecules obtained or derived from a biological sample as required in step 1 of claims 1 and 38. Burg fails to cure the deficiencies of these references as Burg fails to disclose or suggest the dilution and/or amplification of template molecules in a biological sample within a set comprising a plurality of assay samples.

For at least these reasons, claims 23 and 55 are not obvious over Li, Zhang, and Burg. The Patent Owner respectfully requests that this rejection under 35 U.S.C. § 103(a) be withdrawn.

E. Li or Zhang, in view of Trümper et al. (1993, *Blood 81:*3097-115)

Claims 24, 29, 30, 56, 61 and 62 were rejected under § 103(a) as allegedly being obvious over either Li or Zhang, and further in view of Trümper et al. (1993, *Blood 81*:3097-115; "Trümper"). These dependent claims recite a biological sample which is stool, blood, or lymph nodes (claims 24 and 56), a selected sequence which is a rare exon (claims 29 and 61), and the template molecules are cDNA molecules (claims 30 and 62).

Li and Zhang are both cited as disclosing micromanipulation of single cells into separate assay samples, amplification of DNA from each cell, determination of the presence or absence of two different DNA sequences, and the determination of a ratio of the two sequences. Office Action, Page 13. The Office Action acknowledges that Li and Zhang fail to disclose a method wherein the biological sample is derived from stool, blood, or lymph nodes or a method wherein the template molecules to be amplified are cDNA. Trümper is cited as disclosing analysis of isolated single cells from lymph nodes followed by RT-PCR to detect a p53 mutation.

The Office Action has not established a *prima facie* case of obviousness because the cited references fail to disclose or suggest all of the steps of claims 1 and 38. Both Li and Zhang at least fail to disclose step 1 of claim 1 – the dilution of nucleic acid template molecules in a biological sample. In addition, neither of the references discloses a set comprising a plurality of assay samples with template molecules obtained or derived from a biological sample as required in step 1 of claims 1 and 38. Trümper fails to cure the deficiencies of these references as Trümper fails to disclose the dilution and/or amplification of nucleic acid template molecules in a biological sample within a set comprising a plurality of assay samples. Rather, Trümper, like Li and Zhang, involves the analysis of isolated single cells.

For at least these reasons, claims 24, 29, 30, 56, 61 and 62 are not obvious over the teachings of Li, Zhang, and Trümper. The Patent Owner respectfully requests that this rejection under 35 U.S.C. § 103(a) be withdrawn.

F. Li or Zhang, in view of Pontén et al. (1997, *Mutation Research Genomics 382*:45-55)

Claims 31 and 63 were rejected under § 103(a) as allegedly being obvious over either Li or Zhang and further in view of Pontén et al. (1997, *Mutation Research Genomics 382:*45-55; "Pontén"). Claims 31 and 63 depend from claims 1 and 38, respectively, and recite that the two analyzed sequences comprise a first and a second mutation.

Li and Zhang are both cited as disclosing micromanipulation of single cells into separate assay samples, amplification of DNA from each cell, determination of the presence or absence of two different DNA sequences, and the determination of a ratio of the two sequences. Office Action, Page 14. The Office Action acknowledges that Li and Zhang fail to disclose a method in

which the selected genetic sequence and the reference genetic sequence comprise a different mutation. Pontén is cited as disclosing single cell PCR analysis of tumor cells and detection of two different point mutations in p53.

The Office Action has not established a *prima facie* case of obviousness because the cited references fail to disclose or suggest all of the steps of claims 1 and 38. Both Li and Zhang at least fail to disclose step 1 of claim 1 – the dilution of nucleic acid template molecules in a biological sample. In addition, neither of the references teaches a set comprising a plurality of assay samples comprising template molecules obtained or derived from a biological sample as required in step 1 of claims 1 and 38. Pontén fails to cure the deficiencies of Li and Zhang as Pontén neither discloses or suggests the dilution and/or amplification of template molecules in a biological sample within a set of a plurality of assay samples. Pontén, like Li and Zhang, involves the analysis of isolated single cells.

Since all three references utilize a single cell isolation technique, none of them disclose or suggest the dilution of nucleic acid templates from a biological sample to form a plurality of samples, nor amplification of a template from the biological sample within a set comprising a plurality of assay samples for the reasons discussed above.

For at least these reasons, claims 31 and 63 are not obvious over Li, Zhang, and Pontén. Therefore, the Patent Owner respectfully requests that this rejection under 35 U.S.C. § 103(a) be withdrawn.

G. Li or Zhang, in view of Kanzler et al. (1996, *Blood 87*:3429-36)

Claims 10, 11, 25, 28, 42, 43, 57 and 60 were rejected under § 103(a) as allegedly being obvious over either Li or Zhang and further in view of Kanzler et al. (1996, *Blood 87:*3429-36; "Kanzler"). Claims 10, 11, 25, and 28 depend directly or indirectly from claim 1, and claims 42, 43, 57, and 60 depend directly or indirectly from claim 38. Claims 10, 11, 42 and 43 recite the use of sets of assay samples of at least 500 or at least 5000 samples. Claims 25 and 57 recite blood or bone marrow of a leukemia or lymphoma patient who received anti-cancer therapy. Claims 28 and 60 recite a selected genetic sequence which is within an amplicon amplified during neoplastic development.

Li and Zhang are again cited as disclosing micromanipulation of single cells into separate assay samples, amplification of DNA from each cell, determination of the presence or absence of two different DNA sequences, and the determination of a ratio of the two sequences. Office Action, Page 14. The Office Action acknowledges that Li and Zhang fail to disclose a method in which the biological sample is derived from blood or bone marrow of a leukemia or lymphoma patient who has received anti-cancer therapy. The Office Action also acknowledges that Li and Zhang fail to disclose a method wherein the number of assay samples is greater than 500 or 1000 or wherein the selected genetic sequence is part of a sequence that is amplified during neoplastic development. Kanzler is cited as disclosing, like Li and Zhang, single cell analysis.

The Office Action has not established a *prima facie* case of obviousness because the cited references taken together fail to disclose or suggest all of the steps of claims 1 and 38. Each of the three references at least fails to disclose or suggest step 1 of claim 1 – the dilution of nucleic acid template molecules in a biological sample because each of the references utilizes a single cell isolation technique. In addition, none of the references disclose or suggest a set comprising a plurality of assay samples comprising template molecules obtained or derived from a biological sample as required in step 1 of claims 1 and 38 for the same reason.

For at least these reasons, claims 10, 11, 25, 28, 42, 43, 57 and 60 are not obvious over Li, Zhang, and Kanzler. The Patent Owner respectfully requests that this rejection under 35 U.S.C. § 103(a) be withdrawn.

H. Li or Zhang, in view of Gravel et al. (1998, *Blood 91*(8):2866-74)

Claims 26 and 58 were rejected under § 103(a) as allegedly being obvious over either Li or Zhang and further in view of Gravel et al. (1998, *Blood 91*(8):2866-74; "Gravel"). Claims 26 and 58 depend from claims 1 and 38, respectively, and further recite that the selected sequence is a translocated allele.

Li and Zhang are again cited as disclosing micromanipulation of single cells into separate assay samples, amplification of DNA from each cell, determination of the presence or absence of two different DNA sequences, and the determination of a ratio of the two sequences. Office Action, Pages 15-16. The Office Action acknowledges that Li and Zhang fail to disclose a

method in which the selected genetic sequence is a translocated allele. Gravel, like Li and Zhang, is cited as disclosing a single cell analysis, but for the detection of a chromosomal translocation in cells from bone of patients diagnosed with Hodgkin's Disease.

The Office Action has not established a *prima facie* case of obviousness because the cited references taken together fail to disclose or suggest all of the steps of claims 1 and 38. Each of the three references at least fails to disclose or suggest step 1 of claim 1 – the dilution of nucleic acid template molecules in a biological sample because each reference utilizes single cell micromanipulation, which does not meet the requirement for claim 1 step 1 as discussed in detail above. In addition, none of the references disclose a set comprising a plurality of assay samples comprising template molecules obtained or derived from a biological sample as required in step 1 of claims 1 and 38.

For at least these reasons, claims 26 and 58 are not obvious over Li, Zhang, and Gravel. Therefore, the Patent Owner respectfully requests that this rejection under 35 U.S.C. § 103(a) be withdrawn.

I. Li or Zhang, in view of Schwab et al. (1998, *Bioessays 20*(6):473-79)

Claims 28 and 60 were rejected under § 103(a) as allegedly being obvious over either Li or Zhang, and further in view of Schwab (1998, *Bioessays 20*(6):473-79; "Schwab"). Claims 28 and 60 depend from claims 1 and 38, and additionally, they recite an amplicon which is amplified during neoplastic development.

Li and Zhang are again cited by the Office Action as disclosing micromanipulation of single cells into separate assay samples, amplification of DNA from each cell, determination of the presence or absence of two different DNA sequences, and the determination of a ratio of the two sequences. Office Action, Pages 16. The Office Action acknowledges that Li and Zhang fail to disclose or suggest a method in which the selected genetic sequence is one that is amplified during neoplastic development. Schwab is a review article cited for disclosing amplification of certain markers as prognostic markers for cancer.

The Office Action has not established a *prima facie* case of obviousness because the cited references taken together fail to disclose or suggest all of the steps of claims 1 and 38. As

discussed in detail above, Li and Zhang at least fail to disclose or suggest step 1 of claim 1 – the dilution of nucleic acid template molecules in a biological sample – because each reference utilizes single cell micromanipulation, which does not meet the requirement for claim 1 step 1 as discussed in detail above. In addition, Li and Zhang fail to disclose or suggest a set comprising a plurality of assay samples comprising template molecules obtained or derived from a biological sample as required in step 1 of claims 1 and 38. Schwab fails to cure the deficiencies of Li and Zhang because Schwab merely discloses the presence of amplification of certain markers associated with cancer and fails to disclose particular methods for the dilution and/or amplification of nucleic acid template molecules in a biological sample within a set comprising a plurality of assay samples.

In addition, the proposed combination of references by the Patent and Trademark Office suggests determining the "percentage (ratio) of *cells* in a sample having the amplified version" (emphasis added). But the claims are directed to determining the ratio of genetic *sequences* in a population of genetic sequences of a biological sample. This is distinct from a cell-by-cell analysis.

The Office Action also posits a hypothetical experiment in which the wild-type sequence is the sequence that is not amplified in neoplastic development. This experiment would not yield any useful result. The same product would be amplified from the wild-type sequence and from the selected sequence because the selected genetic sequence is "within the amplicon," *i.e.,* it does not span the amplicon breakpoint. Shendure Declaration at paragraph 14.

For at least these reasons, claims 28 and 60 are not obvious over Li, Zhang, and Schwab. The Patent Owner respectfully requests that this rejection under 35 U.S.C.§ 103(a) be withdrawn.

CONCLUSION

For at least the reasons set forth above, all claims in this reexamination are patentable and should be confirmed. Therefore, the issuance of a Reexamination Certificate confirming the patentability of all claims is respectfully requested. The absence of additional comments regarding the Office Action does not signify the Patent Owner's agreement with or concession of any characterization or requirement. If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 202 824 3000.

No fees are believed to be due with respect to the filing of this response. However, should any such fees be due, the Commissioner is hereby authorized to charge any such fees in connection with this paper to Deposit Account No. 190733.

Respectfully submitted,

By: /Sarah A. Kagan/
Sarah A. Kagan
Registration No. 32,141

Dated: 27 January 2014

Banner & Witcoff, Ltd.
Customer No. 11332

CERTIFICATE OF SERVICE

The undersigned certifies that, in accordance with 37 C.F.R. § 1.550(f) and concurrently with the electronic filing of this request to the United States Patent and Trademark Office, a complete copy of this Responsive Amendment to Office Action and declaration under rule 132, has been mailed via first class mail on January 27, 2014 to the third party requester:

Life Technologies Corporation
Attn: IP Department
5791 Van Allen Way
Carlsbad, CA 92008

/Sarah A. Kagan/
Sarah A. Kagan
Registration No. 32,141

Dated: 27 January 2014

Banner & Witcoff, Ltd.
Customer No. 11332

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re *Ex Parte* Reexamination:	)	Group Art Unit: 3991
	)	
U.S. Patent No. 6,440,706	)	Docket No. 001107.00989
	)	
Control No. 90/012,894	)	Confirmation No: 8442
	)	
Reexam Filing Date: June 17, 2013	)	Examiner: Bruce R. Campell

For: DIGITAL AMPLIFICATION

DECLARATION OF JAY SHENDURE

1. My name is Jay Shendure. I make this declaration based on my personal knowledge. I am over 21 and otherwise competent to make this declaration.
2. I am currently an Associate Professor in the Department of Genome Sciences at the University of Washington School of Medicine in Seattle, Washington. I have held this position since 2011. Prior to that I was an Assistant Professor in the Department of Genome Sciences at the University of Washington. I held this position from 2007 to 2011. I am also an Affiliate Professor with the Division of Human Biology at the Fred Hutchinson Cancer Research Center in Seattle, Washington; a position I have held since 2010. A copy of my *Curriculum vitae* is attached as Exhibit A.
3. I obtained my M.D. from Harvard Medical School in Boston, Massachusetts in 2007 and, previous to that, obtained my Ph.D. in Genetics from Harvard University in 2005.

4. As can be gleaned from my *Curriculum vitae*, I have been engaged in genetics and genomics research since about 1995. My current research is focused on the development of new technologies for genomics and molecular biology. Throughout my career I have followed new developments in the field by reading of the scientific literature, active research, and interactions with colleagues. Because of my training and experience, I consider myself knowledgeable in various aspects of genomics, technology development, and nucleic acid sequencing. This includes technologies that are used to analyze DNA sequences and variations in DNA sequences.
5. I have been informed that Johns Hopkins University (JHU) owns U.S. patent 6,440,706 ("'706 patent") and has licensed it to Esoterix Genetics Laboratories (EGL), a subsidiary of Laboratory Corporation of America Holdings (LabCorp) (Esoterix), and to Exact Sciences.
6. I have never been employed by JHU or by EGL, LabCorp or Exact Sciences.
7. I have reviewed the '706 patent, including original claims 1-12, 14-16, 19-32, 38-44, 46-48, 51-64, attached as Exhibit B, the re-examination office action mailed November 27, 2013, and the cited references:

    - Li *et al.*, "Amplification and analysis of DNA sequences in single human sperm and diploid cells," *Nature 335*(6189):414-7 (1988);
    - Zhang *et al.*, "Whole genome amplification from a single cell: implications for genetic analysis," *PNAS USA 89*(13):5847-51 (1992);
    - Jeffreys *et al.*, "Amplification of human minisatellites by the polymerase chain reaction: towards DNA fingerprinting of single cells," *Nucl. Acids. Res. 16*(23):10953-10971 (1988).

8. The statements that I make include my opinions and the bases for them. Although I am being compensated for my time in preparing this declaration, the opinions are my own, and I have no stake in the outcome of the reexamination proceeding. My compensation does not depend in any way on the outcome of the re-examination.

9. I understand that obviousness is assessed from the standpoint of the hypothetical person of ordinary skill in the relevant art. I believe that such a person would have training in molecular biology techniques, such as PCR and related laboratory procedures, having a bachelor's degree in biological or chemical sciences, and have at least three years of experience in a laboratory, or alternatively have a Master's degree in a biological or chemical sciences and have at least one year of laboratory experience.

10. Diluting nucleic acid template molecules would be understood by one of skill in the art as a step in which nucleic acid template molecules are diluted by a process of placing them in a larger volume of liquid.

11. The specification contemplates a preferred dilution level where half of the diluted samples have one template molecule. Col. 9, lines 40-44. This level of dilution could not be achieved by single cell micromanipulation and lysis of lymphocytes.

12. Li and Zhang taught genetic analysis of single cells. It would not have been obvious to modify their methods by using cell-free samples because this would largely destroy the linkage within and entirely destroy the linkage between chromosomes in a

single cell. Conventional methods for DNA preparation fragment genomic DNA considerably, such that the ability to assess recombination rates between two loci (the contemplated goal of Li) would be destroyed, except at very short distances. Additionally such fragmentation would entirely destroy the ability to assess independent assortment of chromosomes, as was actually tested by Li. A hypothetical modification of the methods of Li and Zhang to use a cell-free sample without the isolation of single cells would destroy the information that Li and Zhang were trying to collect.

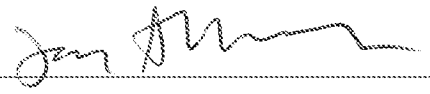
13. I do not think that it would have been obvious to one of ordinary skill in the art to double or triple the amount of DNA used in the PCR reactions described by Jeffreys in order to save time and reagents because doubling or tripling the amount of DNA would not be possible in the case of rare forensic samples, for example, where the amount of DNA is limited. Moreover, doubling and tripling the amount of DNA used in the PCR reactions would contradict the purposes of Li and Zhang, who scrupulously worked to have the DNA from only a single cell in each PCR reaction.

14. I understand that the Patent and Trademark Office proposes that one could detect genomic amplification if one selected a genetic sequence that is within an amplicon that is amplified during neoplastic development, as recited in claims 28 and 60. (Reexamination office action at page 17, first full paragraph.) The Patent and Trademark Office further proposes that one would use a wild-type of that sequence as a reference sequence. I do not believe that such an experiment would be operable for detecting or quantifying amplifications associated with the neoplastic development

because both the amplified sequence and the wild-type reference sequence would have the same nucleic acid sequence and, thus, would not be distinguishable from each other using the method of claims 28 and 60.

15. I am also aware that the Patent and Trademark Office proposed that this same modification would have been motivated by Li's suggestion to study cell-to-cell variations involving DNA rearrangements and genetic alterations. I do not agree. A ratio of cells is not the same as a ratio of genetic sequences in a population of genetic sequences. These ratios can be different and provide different information.

16. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the claims or the patent.



Jay Shendure

January 27, 2017

Date

Updated January 18, 2014

Current Position

Associate Professor
Department of Genome Sciences
University of Washington
School of Medicine
Foege Building S-250, Box 355065
3720 15th Ave NE
Seattle WA 98195-5065

Contact Information

E-mail: shendure@uw.edu
Lab website: http://krishna.gs.washington.edu
Office phone: (206) 685-8543

Education

- 2007 M.D., Harvard Medical School (Boston, Massachusetts)
- 2005 Ph.D. in Genetics, Harvard University (Cambridge, Massachusetts)
  Research Advisor: George M. Church
  Thesis entitled "*Multiplex Genome Sequencing and Analysis*"
- 1996 A.B., *summa cum laude* in Molecular Biology, Princeton University (Princeton, NJ)
  Research Advisor: Lee M. Silver

Professional Experience

- 2011 – Present Associate Professor (with tenure)
  Department of Genome Sciences, University of Washington, Seattle, WA
- 2010 – Present Affiliate Professor
  Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA
- 2007 – 2011 Assistant Professor
  Department of Genome Sciences, University of Washington, Seattle, WA
- 1998 – 2007 Medical Scientist Training Program (MSTP) Candidate
  Department of Genetics, Harvard Medical School, Boston, WA
- 1997 – 1998 Research Scientist
  Vaccine Division, Merck Research Laboratories, Rahway, NJ
- 1996 – 1997 Fulbright Scholar to India
  Department of Pediatrics, Sassoon General Hospital, Pune, India

Honors and Awards

- 2014 HudsonAlpha Prize for Life Sciences
  HudsonAlpha Institute for Biotechnology
- 2013 FEDERAprijs
  Federation of Dutch Medical Scientific Societies
- 2013 NIH Director's Pioneer Award
  National Institutes of Health

- 2012 Curt Stern Award
  American Society of Human Genetics
- 2010 Lowell Milken Young Investigator (2010-2013)
  Prostate Cancer Foundation
- 2008 Science in Medicine New Investigator Lecture
  University of Washington
- 2008 3rd Annual Tomorrow's PIs
  Genome Technology Magazine
- 2007 James Tolbert Shipley Prize
  Harvard Medical School
- 2006 TR35 Young Innovator Award
  M.I.T. Technology Review
- 1998 Medical Science Training Program Fellowship
  National Institutes of Health
- 1996 Fulbright Scholarship
  U.S. State Department
- 1996 *summa cum laude*
  Princeton University
- 1996 Honorary Major in Anthropology
  Princeton University
- 1996 Sigma Chi Book Award for Molecular Biology Senior Thesis ("*The Genetics of Alcohol Consumption: QTLs Affecting Ethanol Consumption in Inbred Mice*")
  Princeton University
- 1996 Senior Prize for Best Thesis in Anthropology ("*Homunculi, Polyps and the Generation of Beings: Interpreting Theory Change in Biology*")
  Princeton University
- 1996 Phi Beta Kappa
  Princeton University
- 1992 National Merit Scholar
  Solon High School

Editorial Boards, Consortium Leadership & Scientific Advisory Boards

- 2014 – Present Editorial Board of *Human Molecular Genetics*
- 2011 – Present Editorial Board of *Human Genetics*
- 2011 – Present Editorial Board of *Biotechniques*
- 2010 – Present Editorial Advisory Board of *Genome Biology*
- 2009 – Present Editorial Board of *Genome Research*
- 2009 – 2012 Associate Editor of *American Journal of Human Genetics*

- 2012 – Present Member, *Autism Sequencing Consortium (ASC)*
- 2012 – Present Steering Committee, *NHGRI Centers for Mendelian Genomics (CMG)*
- 2009 – 2012 Steering Committee, *NHLBI Exome Sequencing Project (ESP)*

- 2012 – Present Scientific Advisory Board, *Department of Energy - Joint Genome Institute (DOE-JGI)*
- 2009 – Present Technology Development Advisory Group, *International Barcode of Life (iBOL)*
- 2011 – Present External Advisory Committee, *Genomics and Pathology Services at Washington University in St Louis (GPS @ WUSTL)*

Other Activities

- 2012 – Present Faculty of 1000 (F1000), Medical Genetics
- 2011 Guest Editor, *Genome Biology* (special issue on exome sequencing)
- 2009 – Present Member, Fred Hutchinson / University of Washington Cancer Consortium
- 2009 Program Committee, American Association for Cancer Research, 101st Annual Meeting
- 2009 – 2012 Convener, NHLBI Exome Sequencing Project (ESP) Family Studies Working Group

Commercial Advisory Roles

- 2013 – Present Scientific Advisory Board of *Ingenuity Systems*
- 2013 – Present Scientific Advisory Board of *Rubicon Genomics*
- 2013 – Present Scientific Advisory Board of *GenePeeks*
- 2013 – Present Scientific Advisory Board of *Gen9*
- 2010 – Present Consultant to *Ariosa Diagnostics*
- 2010 – Present Scientific Advisory Board of *Adaptive Biotechnologies*
- 2009 – Present Scientific Advisory Board of *Good Start Genetics*
- 2009 – Present Scientific Advisory Board of *Stratos Genomics*
- 2012 Consultant to *Merck Research Laboratories*
- 2010 – 2011 Scientific Advisory Board of *Halo Genomics*
- 2008 – 2009 Consultant to *Complete Genomics*
- 2006 Consultant to *Highland Capital Partners*
- 2004 – 2005 Consultant to *Agencourt Biosciences*

Faculty Administrative Responsibilities (University of Washington)

- 2013 – 2014 Chair, Seminar Series Committee (Genome Sciences)
- 2012 – 2013 Co-chair, Scientific Discovery Subcommittee for Curriculum Renewal
- 2008 – 2013 Member, Faculty Search Committee (Medical Genetics)
- 2011 – 2012 Member, Faculty Search Committee (Genome Sciences)
- 2010 – 2011 Member, Faculty Search Committee (Genome Sciences)
- 2008 – 2009 Member, Faculty Search Committee (Genome Sciences)
- 2010 Co-organizer, Symposium & Panel Discussion – "*New Discoveries in Medicine: Implications for the Cost and Quality of American Healthcare*." (Genome Sciences)
- 2009 Organizer, Departmental Retreat (Genome Sciences)
- 2009 Member, U.W. "Two Years to Two Decades" (2y2d) initiative, Discovery focus group
- 2008 – 2009 Member, Seminar Series Committee (Genome Sciences)

Reviewer (ad hoc)

Nature
Science
Cell
New England Journal of Medicine
Nature Genetics
Nature Biotechnology
Nature Medicine
Nature Methods
Nature Reviews Genetics
Science Translational Medicine
Analytical Chemistry
Bioinformatics
Biotechniques
BMC Genomics
Cell Stem Cell
Cellular & Molecular Biology Letters
Genomics
Human Mutation
Mammalian Genome
Nature Protocols

Proceedings of the National Academy of Sciences	Neuron
PLoS Genetics	Nucleic Acids Research
Genome Research	PLoS Computational Biology
American Journal of Human Genetics	Trends in Genetics
Genome Biology	Genetics in Medicine

Grant Review & Other Service

- 2013 Grant reviewer, National Institute of Child Health and Human Development Special Emphasis Panel for U01 Male Contraceptive Development Program
- 2013 Abstract reviewer, 63th Annual Meeting of American Society of Human Genetics
- 2013 Grant reviewer, The Wellcome Trust
- 2011 Grant reviewer, W. M. Keck Foundation
- 2011 Grant reviewer, Lasker Clinical Research Scholars Program
- 2010 Grant reviewer, UK Medical Research Council, Molecular and Cellular Medicine Board
- 2009 Grant reviewer, National Science Foundation
- 2009 Grant reviewer, NIH ARRA Challenge Grants (Genes, Genomes and Genetics IRG)
- 2009 Grant reviewer, Ontario Research Fund (GL2 Competition)
- 2008 Grant reviewer, Genome BritishColumbia

Postdoctoral Fellows Trained (University of Washington)

- 2014 – Present Ron Hause, Ph.D.
- 2013 – Present Jacob Kitzman, Ph.D.
- 2012 – Present Martin Kircher, Ph.D.
- 2011 – Present Stephen Salipante, M.D., Ph.D.
- 2009 – 2013 Jerrod Schwartz, Ph.D. (current position: GoogleX)
- 2009 – 2013 Brian O'Roak, Ph.D. (joint trainee with Evan Eichler; current position: Assistant Professor, Department of Molecular & Medical Genetics, Oregon Health & Science University)
- 2007 – 2009 Emily Turner, Ph.D. (current position: Senior Scientist, Genetics & Solid Tumors, Department of Laboratory Medicine, University of Washington)

Graduate Students Trained (University of Washington)

- 2013 – Present Aaron McKenna (Genome Sciences)
- 2012 – Present Matthew Snyder (Genome Sciences)
- 2011 – Present Joshua Burton (Genome Sciences)
- 2010 – Present Akash Kumar (Medical Scientist Training Program, Genome Sciences)
- 2010 – Present Andrew Adey (Molecular & Cellular Biology)
- 2009 – 2013 Jacob Kitzman (Genome Sciences; dissertation entitled "New technologies for sequencing and interpreting genomes"; current position: Postdoctoral Fellow, Shendure Lab)
- 2009 – 2012 Joseph Hiatt (Medical Scientist Training Program, Genome Sciences; dissertation entitled "Molecular tagging to overcome limitations of massively parallel sequencing"; current position: completing medical school)
- 2007 – 2012 Sarah Ng (Genome Sciences; dissertation entitled "Next Generation Mendelian Genetics"; current position: Research Fellow, Institute of Molecular and Cell Biology, Singapore)
- 2007 – 2012 Rupali Patwardhan (Genome Sciences; dissertation entitled "Massively parallel functional dissection of regulatory elements"; current position: Software Engineer, Facebook)

Rotation Students Supervised (University of Washington)

• Vijay Ramani	Genome Sciences	Winter 2014
• Seungsoo Kim	Genome Sciences	Winter 2014
• Jason Klein	MSTP program	Summer 2013
• Hugh Haddox	Molecular & Cellular Biology	Spring 2013
• Aaron McKenna	Genome Sciences	Winter 2013
• Greg Findlay	MSTP program	Summer 2012
• Matthew Snyder	Genome Sciences	Spring 2012
• Jorgen Nelson	Genome Sciences	Winter 2012
• Elyse Hope	Genome Sciences	Winter 2012
• Meara Davies	Molecular & Cellular Biology	Fall 2011
• Josh Burton	Genome Sciences	Winter 2011
• Jenny Wagner	Genome Sciences	Winter 2011
• Andrew Adey	Molecular & Cellular Biology	Fall 2009
• David Young	MSTP program	Summer 2009
• Akash Kumar	MSTP program	Summer 2009
• Jacob Kitzman	Genome Sciences	Spring 2009
• Keisha Carlson	Genome Sciences	Winter 2009
• Jarrett Egerston	Genome Sciences	Winter 2009
• Matthew Maurano	Genome Sciences	Fall 2008
• Joseph Hiatt	MSTP program	Summer 2008
• Sayer Herrin	Genome Sciences	Winter 2008
• Rupali Patwardhan	Genome Sciences	Winter 2008
• Sarah Ng	Genome Sciences	Fall 2007

Graduate Student Committees (in addition to trainees)

• 2013 – Present	Jorgen Nelson	U.W. Genome Sciences	Advisor: David Baker
• 2013 – Present	David Young	U.W. Genome Sciences	Advisor: Stan Fields
• 2012 – Present	Niklas Krumm	U.W. Genome Sciences	Advisor: Evan Eichler
• 2012 – Present	Andrew Laszlo	U.W. Physics	Advisor: Jens Gundlach
• 2012 – Present	Benjamin Vernot	U.W. Genome Sciences	Advisor: Josh Akey
• 2011 – Present	Jennifer Andrie	U.W. Genome Sciences	Advisor: Josh Akey
• 2010 – Present	Russell Berg	U.W. Molecular & Cellular Biology	Advisor: Lalita Ramakrishnan
• 2010 – Present	Leslie Emery	U.W. Genome Sciences	Advisor: Josh Akey
• 2010 – 2013	Peter Sudmant	U.W. Genome Sciences	Advisor: Evan Eichler
• 2010 – 2013	Thomas White	U.W. Molecular & Cellular Biology	Advisor: Peter Nelson
• 2010 – 2013	Benjamin Whiddon	U.W. Genome Sciences	Advisor: Richard Palmiter
• 2009 – 2013	Cailyn Spurrell	U.W. Genome Sciences	Advisor: Mary-Claire King
• 2008 – 2013	Alan Rubin	U.W. Genome Sciences	Advisor: Phil Green
• 2009 – 2012	Joshua Bishop	U.W. Electrical Engineering	Advisor: Eric Klavins
• 2011 – 2012	Lucas Gray	U.W. Biochemistry	Advisor: Alan Weiner
• 2009 – 2012	Kyle Minch	U.W. Molecular & Cellular Biology	Advisor: David Sherman
• 2011	Sung Hang	U.W. Neurobiology and Behavior	Advisor: William Catterall
• 2010	Carlos Araya	U.W. Genome Sciences	Advisor: Stanley Fields
• 2008 – 2010	Steven Josefowicz	U.W. Immunology	Advisor: Sasha Rudensky
• 2008 – 2010	Kevin Schutz	U.W. Genome Sciences	Advisor: Stan Fields

- 2008 – 2010 Marcia Paddock U.W. Immunology Advisor: Andy Scharenberg

Other Trainee Committees

- 2010 – Present Michael Cho, M.D. K08 Advisory Committee Advisor: Ed Silverman

Courses Taught

- 2012 – 2013 CONJOINT 511 – "Genetic Anatomy" (University of Washington)
  Medical school 1st year elective; co-taught w/ Marshall Horwitz and John Clark
- 2012 – 2013 HUBIO 554 – "Genetics" (University of Washington)
  Medical school 2nd year pre-clinical curriculum; co-chaired with Heather Mefford
- 2008 – 2013 GENOME 550 – "Methods and Logic in Genetics" (University of Washington)
  Graduate seminar course; co-taught with Bob Waterston
- 2010 – 2012 GENOME 373 – "Genome Informatics" (University of Washington)
  Undergraduate lecture course; co-taught with Jim Thomas or Elhanan Borenstein
- 2001 – 2003 "Principles of Pharmacology" (Harvard Medical School)
  Teaching assistant, 1st year medical school course

Other Teaching or Outreach Activities

- Dec 2013 Guest session leader for BIOL 485 "Senior Seminar in Cellular, Molecular and Developmental Biology" (UW)
- Nov 2013 Keynote speaker, UW Postdoc Association Symposium
- Nov 2013 Speaker, Pacific Science Center "Science Café" series
- Oct 2013 Guest session leader for MCB 517 "The Developmental Basis of Human Disease" (UW)
- Aug 2013 Co-organizer, UW Center for Mendelian Genomics (CMG) Data Analysis Workshop
- Jul 2013 Speaker, UW Genome Sciences summer research internship program
- Jun 2013 Guest session leader for MEBI 590 "Biomedical and Health Informatics Lecture Series" (UW)
- Apr 2013 Guest speaker, UW MSTP Dinner/Recruitment meeting
- Apr 2013 Guest session leader for EPI 590 "Introduction to Laboratory Methods in Population Research" (UW)
- Oct 2012 Speaker, Seattle Sequencing Interest Group
- Jul 2012 Speaker, "Science on Tap" series
- Jul 2012 Speaker, UW Genome Sciences summer research internship program
- Apr 2012 Guest session leader for GENOME 580 "Ethics in Biomedical Research and Teaching" (UW)
- Apr 2011 Guest session leader for GENOME 580 "Ethics in Biomedical Research and Teaching" (UW)
- Apr 2011 Guest session leader for EPI 590 "Introduction to Laboratory Methods in Population Research" (UW)
- Oct 2010 Lecturer for Medical Genetics "Introduction to Human & Medical Genetics" course (UW)
- Apr 2010 Moderator for UW Genome Sciences 2010 Panel Discussion on ""New Discoveries in Medicine: Implications for the Cost and Quality of American Healthcare"
- Nov 2009 Panelist for Lasker Foundation / UW Dept. of Genome Sciences Round Table: "Personal Genomes: Promise or Hype?"
- Sep 2009 Panelist for "The Two Body Question and Faculty with children" at HHMI Future Faculty Workshop

- Apr 2009 Guest session leader for GENOME 580 "Ethics in Biomedical Research and Teaching" (UW)
- Apr 2009 Guest speaker, UW MSTP Dinner/Recruitment meeting
- Apr 2009 Guest session leader for EPI 590 "Introduction to Laboratory Methods in Population Research" (UW)
- Feb 2009 Guest speaker, Rainier Scholars program (UW)
- Jul 2008 Talk at StarNet 2008 Summer Workshop, UW Genome Sciences Education Outreach
- Jul 2008 Talk at "Wednesdays at the Genome" UW Genome Sciences Public Lecture Series
- Oct 2008 Chalk Talk Workshop, UW Women in Genome Sciences (WiGS)
- May 2008 Guest session leader for GENOME 580 "Ethics in Biomedical Research and Teaching" (UW)

Active Patents & Published Patent Applications

- Polony fluorescent in situ sequencing beads (issued; 7,425,431)
- Sequence tag directed subassembly of short sequencing reads into long sequencing reads (issued; 8,383,345)
- Massively parallel contiguity mapping (application; 20130203605)
- Methods for retrieval of sequence-verified DNA constructs (application; 20120283110)
- Nanogrid rolling circle DNA sequencing (application; 20090018024)
- Multiplex decoding of sequence tags in barcodes (application; 20080269068)
- Wobble sequencing (application; 20070207482)
- Nucleic acid memory device (application; 20100099080)

Peer-Reviewed Research Articles *(* denotes equal contributors; # denotes corresponding author(s))*

1. Melo JA, **Shendure J**, Pociask K, Silver LM#. Identification of sex-specific quantitative trait loci controlling alcohol preference in C57BL/ 6 mice. *Nature Genetics* 1996 Jun;13(2):147-53.
2. **Shendure J***, Melo JA*, Pociask K, Derr R, Silver LM#. Sex-restricted non-Mendelian inheritance of mouse chromosome 11 in the offspring of crosses between C57BL/6J and (C57BL/6J x DBA/2J)F1 mice. *Mammalian Genome* 1998 Oct;9(10):812-5.
3. Peirce JL*, Derr R*, **Shendure J**, Kolata T, Silver LM#. A major influence of sex-specific loci on alcohol preference in C57Bl/6 and DBA/2 inbred mice. *Mammalian Genome* 1998 Dec;9(12):942-8.
4. Liang X#, Munshi S, **Shendure J**, Mark G 3rd, Davies ME, Freed DC, Montefiori DC, Shiver JW. Epitope insertion into variable loops of HIV-1 gp120 as a potential means to improve immunogenicity of viral envelope protein. *Vaccine* 1999 Jul 16;17(22):2862-72.
5. Aach J*, Bulyk ML, Church GM#, Comander J, Derti A, **Shendure J***. Computational comparison of two draft sequences of the human genome. *Nature* 2001 Feb 15;409(6822):856-9.
6. Badarinarayana V, Estep PW 3rd, **Shendure J**, Edwards J, Tavazoie S, Lam F, Church GM#. Selection analyses of insertional mutants using subgenic-resolution arrays. *Nature Biotechnology* 2001 Nov;19(11):1060-5.
7. Weber G*, **Shendure J***, Tanenbaum DM, Church GM, Meyerson M#. Identification of foreign gene sequences by transcript filtering against the human genome. *Nature Genetics* 2002 Feb;30(2):141-2.
8. **Shendure J**, Church GM#. Computational discovery of sense-antisense transcription in the human and mouse genomes. *Genome Biology* 2002 Aug 22;3(9):RESEARCH0044.
9. Mitra RD, Butty VL, **Shendure J**, Williams BR, Housman DE, Church GM#. Digital genotyping and haplotyping with polymerase colonies. *Proceedings of the National Academy of Sciences* 2003 May 13;100(10):5926-31.
10. Zhu J*, **Shendure J***, Mitra RD, Church GM#. Single molecule profiling of alternative pre-mRNA splicing. *Science* 2003 Aug 8;301(5634):836-8.

11. Mitra RD, **Shendure J**, Olejnik J, Edyta-Krzymanska-Olejnik, Church GM[#]. Fluorescent in situ sequencing on polymerase colonies. *Analytical Biochemistry* 2003 Sep 1;320(1):55-65.
12. Zhu Z[#], **Shendure J**, Church GM[#]. Discovering functional transcription-factor combinations in the human cell cycle. *Genome Research* 2005 Jun;15(6):848-55.
13. **Shendure J**[*#], Porreca GJ[*#], Reppas NB, Lin X, McCutcheon JP, Rosenbaum AM, Wang MD, Zhang K, Mitra RD, Church GM. Accurate Multiplex Polony Sequencing of an Evolved Bacterial Genome. *Science* 2005 Sep 9;309(5741):1728-32.
14. Zhang K[#], Zhu J, **Shendure J**, Porreca GJ, Aach JD, Mitra RD, Church GM[#]. Polony haplotyping of individual human chromosome molecules. *Nature Genetics* 2006 Mar;38(3):382-7.
15. Turner DJ, **Shendure J**, Porreca G, Church G, Green P, Tyler-Smith C, Hurles ME[#]. Assaying chromosomal inversions by single-molecule haplotyping. *Nature Methods* 2006 Jun;3(6):439-45.
16. Moskowitz I, Kim JB, Moore M, Wolf C, Peterson MA, **Shendure J**, Norbrega M, Yokota Y, Berul C, Izumo S, Seidman JG*, Seidman CE*[#]. A Genetic Pathway Including Id2, Tbx5, and Nkx2-5 Required for Cardiac Conduction System Development. *Cell* 2007 Jun 29;129(7):1365-76.
17. Porreca GJ*, Zhang K*, Li JB, Xie B, Austin D, Vassallo SL, LeProust EM, Peck BJ, Emig CJ, Dahl F, Gao Y, Church GM*[#], **Shendure J**[*#]. Multiplex Amplification of Large Sets of Human Exons. *Nature Methods* 2007 Nov;4(11):931-6.
18. Higgins AW, Alkuraya FS, Bosco AF, Brown KK, Bruns GA, Donovan DJ, Eisenman R, Fan Y, Farra CG, Ferguson HL, Gusella JF, Harris DJ, Herrick SR, Kelly C, Kim HG, Kishikawa S, Korf BR, Kulkarni S, Lally E, Leach NT, Lemyre E, Lewis J, Ligon AH, Lu W, Maas RL, MacDonald ME, Moore SD, Peters RE, Quade BJ, Quintero-Rivera F, Saadi I, Shen Y, **Shendure J**, Williamson RE, Morton CC[#]. Characterization of apparently balanced chromosomal rearrangements from the developmental genome anatomy project. *American Journal of Human Genetics* 2008 Mar;82(3):712-22.
19. Turner EH, Lee C, Ng SB, Nickerson DA, **Shendure J**[#]. Massively parallel exon capture and library-free resequencing across 16 genomes. *Nature Methods* 2009 May;6(5):315-6.
20. Brkanac Z[#], Spencer D, **Shendure J**, Robertson PD, Matsushita M, Vu T, Bird TD, Olson MV, Raskind WH. IFRD1 is a candidate gene for SMNA on chromosome 7q22-q23. *American Journal of Human Genetics* 2009 May;84(5):692-7.
21. Ng SB[#], Turner EH, Robertson PD, Flygare SD, Bigham AW, Lee C, Shaffer T, Wong M, Bhattacharjee A, Eichler EE, Bamshad M, Nickerson DA, **Shendure J**[#]. Targeted capture and massively parallel sequencing of 12 human exomes. *Nature* 2009 Aug 16.
22. Vasta V, Ng SB, Turner EH, **Shendure J**[#], Hahn SH[#]. Next generation sequence analysis for mitochondrial disorders. *Genome Medicine* 2009 Oct 23;1(10):100.
23. Ng SB*, Buckingham KJ*, Lee C, Bigham AW, Tabor HK, Dent KM, Huff CD, Shannon PT, Jabs EW, Nickerson DA, **Shendure J**[#], Bamshad MJ[#]. Exome sequencing identifies the cause of a mendelian disorder. *Nature Genetics* 2010 Jan;42(1):30-5.
24. Patwardhan RP[#], Lee C, Litvin O, Young DL, Pe'er D, **Shendure J**[#]. High-resolution analysis of DNA regulatory elements by synthetic saturation mutagenesis. *Nature Biotechnology* 2009 Dec;27(12):1173-5.
26. Hiatt JB*[#], Patwardhan RP*, Turner EH, Lee C, **Shendure J**[#]. Parallel, tag-directed assembly of locally derived short sequence reads. *Nature Methods* 2010 Feb;7(2):119-22.
27. Thomas JH[#], Emerson RO, **Shendure J**. Extraordinary molecular evolution in the PRDM9 fertility gene. *PLoS One*. 2009 Dec 30;4(12):e8505.
28. Roach JC*, Glusman G*, Smit AF*, Huff CD*, Hubley R, Shannon PT, Rowen L, Pant KP, Goodman N, Bamshad M, **Shendure J**, Drmanac R, Jorde LB[#], Hood L[#], Galas DJ. Analysis of Genetic Inheritance in a Family Quartet by Whole-Genome Sequencing. *Science* Apr 30;328(5978):636-9.
29. Cooper GM[#], Goode DL, Ng SB, Sidow A, Bamshad MJ, **Shendure J**, Nickerson DA. Single-nucleotide evolutionary constraint scores highlight disease-causing mutations. *Nature Methods* 2010 Apr;7(4):250-1.
30. Yang F, Babak T, **Shendure J**, Disteche CM[#]. Global survey of escape from X inactivation by RNA-sequencing in mouse. *Genome Research* 2010 May;20(5):614-22.
31. Duan Z*, Andronescu M*, Schutz K, McIlwain S, Kim YJ, Lee C, **Shendure J**, Fields S, Blau CA[#], Noble WS[#]. A three-dimensional model of the yeast genome. *Nature* 2010 May 20;465(7296):363-7.

32. Ng SB*, Bigham AW*, Buckingham KJ, Hannibal MC, McMillin MJ, Gildersleeve HI, Beck AE, Tabor HK, Cooper GM, Mefford HC, Lee C, Turner EH, Smith JD, Rieder MJ, Yoshiura KI, Matsumoto N, Ohta T, Niikawa N, Nickerson DA, Bamshad MJ#, **Shendure J#**. Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. *Nature Genetics* 2010 Sep;42(9):790-3.
33. Rios J, Stein E, **Shendure J**, Hobbs HH#, Cohen JC#. Identification by whole-genome resequencing of gene defect responsible for severe hypercholesterolemia. *Human Molecular Genetics* 2010 Nov 15;19(22):4313-8.
34. Sudmant PH*, Kitzman JO*, Antonacci F, Alkan C, Malig M, Tsalenko A, Sampas N, Bruhn L, **Shendure J**; 1000 Genomes Project, Eichler EE#. Diversity of human copy number variation and multicopy genes. *Science* 2010 Oct 29;330(6004):641-6.
35. Adey A* Morrison HG*, Asan*, Xun X*, Kitzman JO, Turner EH, Stackhouse B, Mackenzie AP, Caruccio NC, Zhang X#, **Shendure J#**. Rapid, low-input, low-bias construction of shotgun fragment libraries by high-density in vitro transposition. *Genome Biology* 2010 Dec 8;11(12):R119.
36. Kitzman JO#, Mackenzie AP, Adey A, Hiatt JB, Patwardhan RP, Sudmant PH, Ng SB, Alkan C, Qiu R, Eichler EE, **Shendure J#**. Haplotype-resolved genome sequencing of a Gujarati Indian individual. *Nature Biotechnology* 2011 Jan;29(1):59-63.
37. Muthappan V*, Lee A*, Lamprecht T, Akileswaran L, Dintzis S, Lee C, Magrini V, Mardis E, **Shendure J**, Van Gelder R#. Biome representational in silico karyotyping. *Genome Research* 2011 Apr;21(4):626-33.
38. Gallagher LA, **Shendure J**, Manoil C#. Genome-Scale Identification of Resistance Functions in Pseudomonas aeruginosa Using Tn-seq. *MBio* 2011 Jan 18;2(1). pii: e00315-10.
39. O'Roak BJ, Deriziotis P, Lee C, Vives L, Schwartz JJ, Girirajan S, Karakoc E, Mackenzie AP, Ng SB, Baker C, Rieder MJ, Nickerson DA, Bernier R, Fisher SE, **Shendure J#**, Eichler EE#. Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations. *Nature Genetics* 2011 Jun;43(6):585-9.
40. Hannibal MC*, Buckingham KJ*, Ng SB*, Ming JE, Beck AE, McMillin MJ, Gildersleeve HI, Bigham AW, Tabor HK, Mefford HC, Cook J, Yoshiura K, Matsumoto T, Matsumoto N, Miyake N, Tonoki H, Naritomi K, Kaname T, Nagai T, Ohashi H, Kurosawa K, Hou JW, Ohta T, Liang D, Sudo A, Morris CA, Banka S, Black GC, Clayton-Smith J, Nickerson DA, Zackai EH, Shaikh TH, Donnai D, Niikawa N, **Shendure J**, Bamshad MJ#. Spectrum of MLL2 (ALR) mutations in 110 cases of Kabuki syndrome. *American Journal of Medical Genetics* 2011 Jul;155A(7):1511-6.
41. Cosart T#, Beja-Pereira A#, Chen S, Ng SB, **Shendure J**, Luikart G. Exome-wide DNA capture and next generation sequencing in domestic and wild species. *BMC Genomics* 2011 Jul 5;12:347.
42. Regalado ES, Guo DC, Villamizar C, Avidan N, Gilchrist D, McGillivray B, Clarke L, Bernier F, Santos-Cortez RL, Leal SM, Bertoli-Avella AM, **Shendure J**, Rieder MJ, Nickerson DA; NHLBI GO Exome Sequencing Project, Milewicz DM#. Exome Sequencing Identifies SMAD3 Mutations as a Cause of Familial Thoracic Aortic Aneurysm and Dissection With Intracranial and Other Arterial Aneurysms. *Circulation Research* 2011 Sep 2;109(6):680-6.
43. Kumar A, White TA, Mackenzie AP, Clegg N, Lee C, Dumpit RF, Coleman I, Ng SB, Salipante SJ, Rieder MJ, Nickerson DA, Corey E, Lange PH, Morrissey C, Vessella RL, Nelson PS#, **Shendure J#**. Exome sequencing identifies a spectrum of mutation frequencies in advanced and lethal prostate cancers. *Proceedings of the National Academy of Sciences* 2011 Oct 11;108(41):17087-92.
44. Ventura M, Catacchio CR, Alkan C, Marques-Bonet T, Sajjadian S, Graves TA, Hormozdiari F, Navarro A, Malig M, Baker C, Lee C, Turner EH, Chen L, Kidd JM, Archidiacono N, **Shendure J**, Wilson RK, Eichler EE#. Gorilla genome structural variation reveals evolutionary parallelisms with chimpanzee. *Genome Research* 2011 Oct;21(10):1640-9.
45. George RD#, McVicker G, Diederich R, Ng SB, Mackenzie AP, Swanson WJ, **Shendure J#**, Thomas JH#. Trans genomic capture and sequencing of primate exomes reveals new targets of positive selection. *Genome Research* 2011 Oct;21(10):1686-94.
46. Fairfield H, Gilbert GJ, Barter M, Corrigan RR, Curtain M, Ding Y, D'Ascenzo M, Gerhardt DJ, He C, Huang W, Richmond T, Rowe L, Probst FJ, Bergstrom DE, Murray SA, Bult C, Richardson J, Kile BT, Gut I, Hager J, Sigurdsson S, Mauceli E, Di Palma F, Lindblad-Toh K, Cunningham ML, Cox TC, Justice MJ, Spector

MS, Lowe SW, Albert T, Donahue LR, Jeddeloh J, **Shendure J**, Reinholdt LG[#]. Mutation discovery in mice by whole exome sequencing. *Genome Biology* 2011 Sep 14;12(9):R86.
47. Deng X, Hiatt JB, Nguyen DK, Ercan S, Sturgill D, Hillier LW, Schlesinger F, Davis CA, Reinke VJ, Gingeras TR, **Shendure J**, Waterston RH, Oliver B, Lieb JD, Disteche CM[#]. Evidence for compensatory upregulation of expressed X-linked genes in mammals, Caenorhabditis elegans and Drosophila melanogaster. *Nature Genetics* 2011 Oct 23;43(12):1179-85.
48. Hondowicz BD, Schwedhelm KV, Kas A, Tasch MA, Rawlings C, Ramchurren N, McIntosh M, D'Amico LA, Sanda S, Standifer NE, **Shendure J**, Stone B[#]. Discovery of T cell antigens by high-throughput screening of synthetic minigene libraries. *PLoS One* 2012;7(1):e29949.
49. Rivière JB*, van Bon BW*, Hoischen A, Kholmanskikh SS, O'Roak BJ, Gilissen C, Gijsen S, Sullivan CT, Christian SL, Abdul-Rahman OA, Atkin JF, Chassaing N, Drouin-Garraud V, Fry AE, Fryns JP, Gripp KW, Kempers M, Kleefstra T, Mancini GM, Nowaczyk MJ, van Ravenswaaij-Arts CM, Roscioli T, Marble M, Rosenfeld JA, Siu VM, de Vries BB, **Shendure J**, Verloes A, Veltman JA, Brunner HG, Ross ME, Pilz DT[#], Dobyns WB[#]. De novo mutations in the actin genes ACTB and ACTG1 cause Baraitser-Winter syndrome. *Nature Genetics* 2012 Feb 26;44(4):440-4, S1-2.
50. Patwardhan RP*, Hiatt JB*, Witten DM, Kim MJ, Smith RP, May D, Lee C, Andrie JM, Lee SI, Cooper GM, Ahituv N[#], Pennacchio LA[#], **Shendure J**[#]. Massively parallel functional dissection of mammalian enhancers in vivo. *Nature Biotechnology* 2012 Feb 26;30(3):265-70.
51. Adey A, **Shendure J**[#]. Ultra-low-input, tagmentation-based whole-genome bisulfite sequencing. *Genome Research* 2012 Jun;22(6):1139-43.
52. O'Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP, Levy R, Ko A, Lee C, Smith JD, Turner EH, Stanaway IB, Vernot B, Malig M, Baker C, Reilly B, Akey JM, Borenstein E, Rieder MJ, Nickerson DA, Bernier R, **Shendure J**[#], Eichler EE[#]. Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. *Nature* 2012 Apr 4;485(7397):246-50.
53. Tabor HK, Stock J, Brazg T, McMillin MJ, Dent KM, Yu JH, **Shendure J**, Bamshad MJ. Informed consent for whole genome sequencing: A qualitative analysis of participant expectations and perceptions of risks, benefits, and harms. *American Journal of Medical Genetics (A)* 2012 Apr 24.
54. Bernier FP, Caluseriu O, Ng S, Schwartzentruber J, Buckingham KJ, Innes AM, Jabs EW, Innis JW, Schuette JL, Gorski JL, Byers PH, Andelfinger G, Siu V, Lauzon J, Fernandez BA, McMillin M, Scott RH, Racher H; FORGE Canada Consortium, Majewski J, Nickerson DA, **Shendure J**, Bamshad MJ[#], Parboosingh JS[#]. Haploinsufficiency of SF3B4, a Component of the Pre-mRNA Spliceosomal Complex, Causes Nager Syndrome. *American Journal of Human Genetics* 2012 May 4;90(5):925-33.
55. Kitzman JO[#], Snyder MW, Ventura M, Lewis AP, Qiu R, Simmons LE, Gammill HS, Rubens CE, Santillan DA, Murray JC, Tabor HK, Bamshad MJ, Eichler EE, **Shendure J**[#]. Noninvasive whole-genome sequencing of a human fetus. *Science Translational Medicine* 2012 Jun 6;4(137):137ra76.
56. Boileau C[#], Guo DC, Hanna N, Regalado ES, Detaint D, Gong L, Varret M, Prakash SK, Li AH, d'Indy H, Braverman AC, Grandchamp B, Kwartler CS, Gouya L, Santos-Cortez RL, Abifadel M, Leal SM, Muti C, **Shendure J**, Gross MS, Rieder MJ, Vahanian A, Nickerson DA, Michel JB; National Heart, Lung, and Blood Institute (NHLBI) Go Exome Sequencing Project, Jondeau G, Milewicz DM[#]. TGFB2 mutations cause familial thoracic aortic aneurysms and dissections associated with mild systemic features of Marfan syndrome. *Nature Genetics* 2012 Jul 8;44(8):916-921.
57. Rivière JB, Mirzaa GM, O'Roak BJ, Beddaoui M, Alcantara D, Conway RL, St-Onge J, Schwartzentruber JA, Gripp KW, Nikkel SM, Worthylake T, Sullivan CT, Ward TR, Butler HE, Kramer NA, Albrecht B, Armour CM, Armstrong L, Caluseriu O, Cytrynbaum C, Drolet BA, Innes AM, Lauzon JL, Lin AE, Mancini GM, Meschino WS, Reggin JD, Saggar AK, Lerman-Sagie T, Uyanik G, Weksberg R, Zirn B, Beaulieu CL; Finding of Rare Disease Genes (FORGE) Canada Consortium, Majewski J, Bulman DE, O'Driscoll M, **Shendure J**, Graham JM Jr, Boycott KM, Dobyns WB[#]. De novo germline and postzygotic mutations in AKT3, PIK3R2 and PIK3CA cause a spectrum of related megalencephaly syndromes. *Nature Genetics* 2012 Jun 24;44(8):934-40.
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Reviews, Perspectives and Book Chapters

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Active Research Support

1DP1HG007811 (NIH/OD) 09/23/13 – 07/31/18
Interpreting genetic variants of uncertain significance (Shendure)
This project aims to develop novel experimental and computational paradigms for predicting the functional consequences of all possible single residue variants in clinically significant genes, thereby informing the interpretation of variants newly observed in patients.
Role: PI

ETOP2013 (DOE/JGI) 10/01/13 – 09/30/15
Accurate gene synthesis with tag-directed retrieval of sequence-verified DNA molecules (Shendure)
The goals of this project include the implementation and further development of dial-out PCR and other technologies for synthetic biology at the DOE's Joint Genome Institute.
Role: PI

1R01HG006768 (NIH/NHGRI) 04/01/12 – 3/31/15
Massively parallel, in vivo functional testing of regulatory elements (MPI: Ahituv, Shendure)
The major goal of this project is to develop novel, multiplexed assays that can easily be adopted by other researchers to clone and simultaneously test tens-of-thousands of candidate regulatory elements for their in vivo functional potential.
Role: PI (MPI award)

1U54HG006493 (NIH/NHGRI) 12/05/11 – 11/30/15
UW Center for Mendelian Genomics (MPI: Bamshad, Nickerson, Shendure)
The goal of the proposed research is to establish the UW Center for Mendelian Genomics (UW-CMG) that will apply exome sequencing and analysis to discover the candidate genes and sequence variants underlying rare Mendelian disorders and other human health-related Mendelian phenotypes.
Role: PI (MPI award)

1R01HG006283 (NIH/NHGRI) 08/15/11 – 07/31/14
Massively parallel contiguity mapping (Shendure)
The aim of this grant is to develop massively parallel methods that facilitate the recovery of contiguity information in genomic DNA at various scales, thereby facilitating high-quality de novo genome assembly and haplotype-resolved human genome sequencing.
Role: PI

1R21CA160080 (NCI/NIH) 07/01/11 – 06/30/14
Ultrasensitive identification and precise quantitation of low frequency somatic mutations by molecular counting (Shendure)
The goal of the proposed research will be to develop novel, robust molecular technologies for sensitively and specifically identifying low frequency mutations in the context of genetically heterogeneous, stromally contaminated cancer samples.
Role: PI

1R01CA160674-01A1 (NIH/NCI) 06/06/12 – 03/31/17
Clonally Expanded Mutations Identify Cancer Precursors in Chronic Inflammation (MPI: Loeb, Brentnall)
The major goal of this project is to develop better methods for identifying early cancers with greater ease and at less cost using state-of-the-art DNA sequencing technology that can be rapidly commercialized for translation to patient care settings.
Role: Co-investigator

1R01MH101221-01 (NIH/NIMH) 08/01/13 – 06/30/17
Sporadic Mutations and Autism Spectrum Disorders (Eichler)
The major goal of this project is to identify genes responsible for autism spectrum disorder (ASD) and developmental delay.
Role: Co-Investigator

SFARI 191889EE (Simons Foundation) 01/01/12 – 12/31/13
Whole Exome Sequencing of Simons Simplex Collection Quads (Eichler)
The goal of this project is to complete exome sequencing of the Simons Simplex Collection.
Role: Co-investigator

Completed Research Support

5U54AI057141-08REV (NIH/NIAID) 03/01/11 – 02/28/14

NW Research Center for Excellence in Biodefense and Emerging Infectious Diseases (Miller)
The major goal is to develop and implement methods for the whole genome sequencing and epidemiological analysis of clinical isolates of gram-negative bacteria at unprecedented speed and low cost.
Role: PI of Developmental Project

University of Washington Cystic Fibrosis Foundation 10/01/11 – 09/30/13
Studying Cystic Fibrosis Infections Using Massively Parallel Sequencing Technology (Shendure)
We test the hypotheses that CF P. aeruginosa populations are highly diverse, and that population composition is stable in the absence of overt changes in symptoms. We will measure diversity using whole genome sequencing of isolate pools to measure allelic variation. We will also test the hypothesis that the abundance of variant alleles changes at the onset of exacerbations, during antibiotic treatment, and upon restoration of the "well" state.
Role: PI

1R011AG039700 (NIH/NIMH) 05/01/11 – 04/30/16
Next Generation Mendelian Genetics in Familial Alzheimer Disease (Brkanac)
The goal of this proposal is to apply novel analytic approaches to identify families in which Alzheimer disease (AD) is likely to have a single gene etiology and to utilize next generation sequencing technologies to find these genes.
Role: Co-investigator

1R01HL110879-01 (NIH/NHLBI) 09/01/11 – 05/31/15
Investigating bacterial-host interactions driving CF Pulmonary Exacerbations (MPI: Bruce, Singh)
The major goal is to test the hypothesis that at the onset of exacerbations, changes in the composition of infecting *P. aeruginosa* populations elicit host responses leading to lung inflammation and injury.
Role: Co-investigator

5R01NS069719 (NIH/NINDS) 04/01/10 – 03/31/14
Next Generation Gene Discovery in Neurogenetics (Raskind)
This proposal seeks to perform massively parallel whole exome sequencing and array comparative genomic hybridization to identify candidate genes for Mendelian neurogenetics disorders.
Role: Co-investigator

W81XWH-10-1-0589 (Department of Defense) 07/01/10 – 08/14/13
Global Characterization of Protein Altering Mutations in Prostate Cancer (Shendure)
The goal of this proposal is to perform comprehensive identification of protein-coding alterations in both primary and metastatic prostate tumors.
Role: PI (synergy award with Nelson at FHCRC)

5P01CA078902 (NIH/NCI) 02/01/09 – 01/31/14
Identification of Canine Minor Histocompatability Antigens (Storb)
The major goal of this subproject is to develop a novel genomics-driven approach for identifying minor histocompatability antigens in a canine transplantation model.
Role: PI of Project 1

5RC2HG005608 (NIH/NHGRI) 09/30/09 – 08/31/12
Next Generation Mendelian Genetics (MPI: Bamshad, Nickerson, Raskind, Shendure)
The goal of this proposal is to sequence and identify the candidate genes responsible for more than 20 Mendelian diseases/disorders.
Role: PI (MPI award)

5UC2HL102926 (NIH/NHLBI) 09/30/09 – 06/30/12
Northwest Genomics Center (MPI: Green, Nickerson, Rieder, Shendure)
The goal of the Northwest Genomics Center is to apply next-generation exome sequencing to medically relevant DNA sample cohorts selected by the NHLBI.
Role: PI (MPI award)

5R01HL094976 (NIH/NHLBI) 09/30/08 – 06/30/12
SeattleSeq (MPI: Eichler, Green, Nickerson, Shendure)
The major goal of this project is to develop a high-throughput pipeline for the comprehensive capture and high-throughput sequencing of all protein-coding sequences in individual human genomes.
Role: PI (MPI award)

Young Investigator Award (Prostate Cancer Foundation) 04/01/10 – 03/31/13
Methods & Tools for Next-Generation Analysis of Prostate Cancer Genomes (Shendure)
The aim of this grant is to develop and deploy methods that enable the efficient characterization of primary and metastatic prostate cancer genomes in large numbers of samples.
Role: PI

3U54AI057141-06S1880509 (NIH/NIAID) 09/12/09 – 02/29/12
Massively parallel genome sequencing of antibiotic-resistant emerging pathogens (Shendure)
The goal of this proposal is to sequence the genomes of over 1,000 antibiotic-resistant bacterial strains representing emerging pathogens.
Role: PI

1R21HG004749 (NIH/NHGRI) 07/23/08 – 06/30/10
Molecular Tools for Genome Partitioning (Shendure)
The major goal of this project is to develop and optimize methods for selective capture of gene families or long contiguous genomic regions.
Role: PI

5R01NS069605 (NIH/NINDS) 02/15/10 – 02/14/14
A Genomic Approach to Epilepsy (Mefford)
The aim of this grant to identify novel candidate genes and pathways for epilepsy through a combination of genome-wide approaches including array comparative genomic hybridization and exome sequencing.
Role: Co-investigator

5R01HG004348 (NIH/NHGRI) 07/01/11 – 06/30/12
Advances in Computational Gene Finding (Korf)
The goal of the proposed research will be to use fosmid-pool-based sequencing to provide contiguity informative validation data for the Assemblathon competition for de novo genome assemblies of the snake, parrot and cichlid genomes.
Role: Co-investigator

2P50HG003233 (NIH/NHGRI) 05/01/09 – 04/30/14
Center for the Epigenetics of Common Human Disease (Feinberg)
The major goal of the UW component of this program is to develop and apply technology for large-scale targeted profiling of DNA methylation in epidemiological samples.
Role: Co-investigator

5R01HD065285 (NIH/NICHD) 09/30/09 – 08/31/12
Genomic Identification of Autism Loci (Eichler)

The aim of this grant is to explore the hypothesis that autism is caused by highly-penetrant, rare mutations using emerging technologies that screen regions for autism-specific copy-number variation (CNV) mutations and exonic point mutations.
Role: Co-investigator

1RC2HG005921 (NIH/NHGRI) 08/20/10 – 01/31/12
A Genome-wide Mutation Resource for C. elegans (Waterston)
The aim of this grant is to construct a community resource of several thousand chemically mutagenized C. elegans strains that have been whole genome sequenced.
Role: Co-investigator

SFARI 191889 (Simons Foundation) 12/01/10 – 11/30/11
Exome Sequencing of Simons Simplex Collection (SSC) Trios (Eichler)
The goal of this project is to perform exome sequencing of 400 SSC autism trios in collaboration with Matt State at Yale University to discover pathogenic SNPs associated with disease.
Role: Co-investigator

1RC2CA148317 (NIH/NCI) 09/30/09 – 09/29/11
An infrastructure for cancer virus discovery from next-generation sequencing data (Meyerson)
The aim of this grant is to develop automated pipelines for identifying virus-derived sequences in next-generation sequencing data from all public sources by computational subtraction.
Role: Co-investigator

1RC1AG035681 (NIH/NIA) 09/30/09 – 09/29/11
Mutational Cloning in Familial Dementia and Alzheimer's Disease (Raskind)
The goal of this proposal is to apply whole exome sequencing in well-characterized pedigrees to identify functional mutations leading to familial dementia and/or Alzheimer's disease.
Role: Co-investigator

1RC2CA148232 (NIH/NCI) 09/30/09 – 09/29/11
Application of RiboTag-seq to Exploration of Tumor Microenvironments (Morris)
The aim of this grant is to develop and apply methods for tagging of ribosome-associated RNAs to study cell-type specific gene expression in complex tissues.
Role: Co-investigator

1I01BX000531 (Department of Veterans Affairs) 10/01/09 – 09/30/13
Genetic Risk Factors for Parkinson's Disease (Zabetian)
The major goal of this project is to validate findings from an ongoing genome-wide association study on PD using next generation sequencing and brain/CSF proteomic analyses.
Role: Consultant

Invited Talks or Workshops

Jan 2014	*Keynote speaker,* UCLA Center for Neurobehavioral Genetics Annual Retreat (Los Angeles, CA)
Dec 2013	*Workshop participant,* NCI Center for Cancer Genomics Think Tank (Bethesda, MD)
Nov 2013	*Speaker*, NIH / NCI Innovative Molecular Analysis Technologies (IMAT) Grantee Meeting (Bethesda, MD)
Oct 2013	*Invited speaker,* FederaDAG: Next Generation DNA Sequencing: impact on clinical care and society (Utrecht, Netherlands)
Oct 2013	*Invited seminar,* Nijmegen Centre for Molecular Life Sciences (Nijmegen, Netherlands)

Oct 2013	*Participant & speaker,* NHGRI Sequencing Network Meeting (Washington DC)
July 2013	*Invited seminar,* Fred Hutchinson Cancer Research Center, Computational Biology Seminar Series (Seattle, WA)
July 2013	*Invited speaker,* The Human Genetics & Genomics Gordon Research Conference, Bryant University (Smithfield, RI)
June 2013	*Keynote speaker,* Functional Genomics Data Society (FGED) 15th International Conference (Seattle, WA)
May 2013	*Invited seminar,* Department of Cellular and Molecular Medicine, University of California, San Diego (San Diego, CA)
May 2013	*Invited seminar,* McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine (Baltimore, MD)
Apr 2013	*Invited seminar*, Institute for Genomics & Systems Biology, University of Chicago (Chicago, IL)
Apr 2013	*Speaker*, NIH / NHGRI Advanced Sequencing Technology Grantee Meeting (San Diego, CA)
Mar 2013	*Invited seminar*, HudsonAlpha Institute for Biotechnology (Huntsville, AL)
Mar 2013	*Invited seminar,* Seminars in Integrative Genomics, Vanderbilt University (Nashville, TN)
Mar 2013	*Plenary speaker,* 2013 Annual Meeting of the Association of Biomolecular Resource Facilities (Palm Springs, CA)
Feb 2013	*Plenary speaker,* Advances in Genome Biology and Technology (AGBT) (Marco Island, FL)
Jan 2013	*Keynote speaker,* The Eleventh Asia Pacific Bioinformatics Conference (Vancouver, BC)
Dec 2012	*Invited seminar,* Dept. of Molecular and Medical Genetics, Oregon Health & Science University (Portland, OR)
Nov 2012	*Invited speaker*, CSHL Personal Genomes meeting (Cold Spring Harbor, NY)
Nov 2012	*Invited participant in closing symposium,* 62th Annual Meeting of American Society of Human Genetics, "Human Genetics 2012 and Beyond: Present Progress and Future Frontiers" (San Francisco, CA)
Nov 2012	*Invited session moderator & speaker,* 62th Annual Meeting of American Society of Human Genetics, "Genomic Approaches to Mendelian Disorders" (San Francisco, CA)
Nov 2012	*Curt Stern Award: Presentation and Lecture,* 62th Annual Meeting of American Society of Human Genetics (San Francisco, CA)
Nov 2012	*Invited speaker,* Institute of Translational Health Sciences 'Omics Workshop - "Lessons Learned and the Path Forward" University of Washington, South Lake Union (Seattle, WA)
Oct 2012	*Participant & speaker,* NHGRI Sequencing Network Meeting (Houston, TX)
Sep 2012	*Invited speaker,* Nature Genetics "Genomics of Common Disease" meeting (Washington DC)
Sep 2012	*Workshop co-organizer & attendee,*" Implicating Sequence Variants in Human Disease" (Washington DC)
Aug 2012	*Invited speaker*, 43rd Annual Meeting of the Environmental Mutagen Society (Seattle, WA)
Jul 2012	*Invited speaker,* 1000 Genomes Community Meeting (Ann Arbor, MI)
Jun 2012	*Invited seminar*, Department of Pathology, University of Washington (Seattle, WA)
Jun 2012	*Invited speaker,* ESHG European Human Genetics Conference 2012 (Nürnberg, Germany)
Jun 2012	*Invited seminar,* UCLA Molecular Biology Institute (Los Angeles, CA)
May 2012	*Grand Rounds,* Division of Hematology, University of Washington Medical Center (Seattle, WA)
May 2012	*Invited seminar,* Institute for Systems Biology (Seattle, WA)
Apr 2012	*Invited speaker,* Chemical & Engineering News Webinar
Apr 2012	*Invited seminar,* NIH / NHGRI Division of Intramural Research (Bethesda, MD)
Apr 2012	*Speaker*, NIH / NHGRI Advanced Sequencing Technology Grantee Meeting (San Diego, CA)
Mar 2012	*Distinguished Lecture Series,* Duke University Program in Genetics and Genomics (Chapel Hill, NC)
Mar 2012	*Co-organizer & speaker,* NIH / NIDDK "Workshop on Rare Syndromic Body Fat Disorders-What Can They Teach Us?" (Bethesda, MD)
Feb 2012	*Invited seminar*, Program in Medical & Population Genetics, Broad Institute of M.I.T. and Harvard (Cambridge, MA)

Feb 2012	*Invited seminar,* Division of Genetics, Brigham and Women's Hospital, Harvard Medical School (Boston, MA)
Jan 2012	*Invited seminar,* Cystic Fibrosis Seminar Series, Seattle Children's Research Institute / University of Washington (Seattle, WA)
Jan 2012	*Grand Rounds,* Department of Pathology, Brigham and Women's Hospital, Harvard Medical School (Boston, MA)
Dec 2011	*Invited seminar*, Department of Biology, University of Pennsylvania (Philadelphia, PA)
Oct 2011	*Guest speaker,* Fred Hutchinson Cancer Research Center, 8th Human Biology Division Retreat (Seattle, WA)
Oct 2011	*Keynote address,* "The Genome and Beyond", BioTechniques Virtual Symposium
Oct 2011	*Chair & organizer,* IPAM (Institute for Pure & Applied Mathematics): Mathematical and Computational Approaches in High-Throughput Genomics; Workshop I: Next-generation Sequencing Technology and Algorithms for Primary Data Analysis (Los Angeles, CA)
Sep 2011	*Invited speaker & session chair,* Beyond the Genome 2011 (Rockville, MD)
Sep 2011	*Invited speaker,* NHLBI Symposium: Genomics: Gene Discovery and Clinical Applications for Cardiovascular, Lung, and Blood Diseases (Bethesda, MD)
Jul 2011	*Workshop speaker*, Illumina Sequencing Expert Panel 2011 (Woodinville, WA)
Jul 2011	*Invited speaker,* "Revolution of Genome Science", 9th International Workshop on Advanced Genomics (Tokyo, Japan)
Jul 2011	*Invited speaker,* University of Tokyo, "Cutting Edge of Human Genome Science", 4th Symposium of the IMSUT & RCAST Global COE (Tokyo, Japan)
Apr 2011	*Invited seminar,* Princeton University and Lewis-Sigler Institute, Quantitative and Computational Biology seminar series (Princeton, NJ)
Mar 2011	*Invited speaker,* Genome 10K Workshop (Santa Cruz, CA)
Feb 2011	*Invited seminar,* Stanford University, Frontiers in Biology Seminar Series (Palo Alto, CA)
Jan 2011	*Invited seminar,* Institute for Molecular Medicine, UT Houston (Houston, TX)
Dec 2010	*Invited speaker,* Illumina Webinar
Dec 2010	*Invited seminar,* UCSF Biomedical Sciences Seminar Series (San Francisco, CA)
Dec 2010	*Invited seminar,* Amgen, Molecular and Computational Toxicology Seminar Series (Seattle, WA)
Nov 2010	*Invited speaker,* American Heart Association, Scientific Sessions 2010, "Whole Genome Sequencing and Integrative Genomics" session (Chicago, IL)
Nov 2010	*Invited speaker,* American Heart Association, Scientific Sessions 2010, "Whole Exome Resequencing: Methods and Early Findings" session (Chicago, IL)
Nov 2010	*Invited session moderator & speaker,* 60th Annual Meeting of American Society of Human Genetics, "Exome Sequencing and Human Genetics" (Washington DC)
Oct 2010	*Invited seminar,* Department of Global Health, University of Washington, Pathobiology Seminar Series (Seattle, WA)
Oct 2010	*Invited speaker,* Beyond the Genome 2010 (Boston, MA)
Sep 2010	*Invited speaker,* Prostate Cancer Foundation, 17th Annual Scientific Retreat (Washington DC)
Jul 2010	*Invited speaker,* Illumina PNW User Group Meeting (Seattle, WA)
Jul 2010	*Invited speaker,* BioC 2010 (Seattle, WA)
Jul 2010	*Workshop attendee,* Planning the Future of Genomics: Foundational Research and Applications in Genomic Medicine, NHGRI (Warrenton, VA)
Jul 2010	*Invited speaker,* 13th International MGED Meeting (Boston, MA)
Jul 2010	*Invited speaker,* Merck (Boston, MA)
Jul 2010	*Evening lecture,* 51st Annual Short Course on Medical and Experimental Mammalian Genetics, The Jackson Laboratory (Bar Harbor, ME)
Jun 2010	*Invited seminar,* PNW Prostate Cancer SPORE Seminar Series (Seattle, WA)
May 2010	*Colloquium co-convener & speaker*, American Society for Microbiology 110th General Meeting, "Ultra-Deep Sequencing in Infectious Diseases" (San Diego, CA)
May 2010	*Invited speaker,* University of Washington, Computational Molecular Biology Spring Symposium

(Seattle, WA)
May 2010 *Invited seminar,* University of Washington, Department of Medical Genetics Seminar Series (Seattle, WA)
May 2010 *Session co-chair & speaker,* The Biology of Genomes, Cold Spring Harbor Laboratories, "High Throughput Genomics & Genetics" (Cold Spring Harbor, NY)
May 2010 *Workshop participant,* NIH Director's "Big Think" Meeting (Bethesda, MD)
Apr 2010 *Invited speaker,* 4th International Conference on Primate Genomics (Seattle, WA)
Jan 2010 *Invited seminar,* Washington University in St. Louis, Department of Genetics (St. Louis, MO)
Jan 2010 *Invited seminar,* University of Chicago, Department of Human Genetics (Chicago, IL)
Dec 2009 *Invited speaker,* Simons Foundation, workshop on sequencing (New York City, NY)
Dec 2009 *Invited speaker,* Cardiovascular Center Breakfast Club, University of Washington (Seattle, WA)
Oct 2009 *Plenary speaker,* 59th Annual Meeting of American Society of Human Genetics (Honolulu, HI)
Sep 2009 *Invited speaker,* Grand Rounds in Laboratory Medicine, University of Washington (Seattle, WA)
Sep 2009 *Invited speaker,* CSHL Personal Genomes meeting (Cold Spring Harbor, NY)
Aug 2009 *Invited speaker,* eMERGE Network Steering Committee meeting (Seattle, WA)
Aug 2009 *Invited seminar,* McDermott Center, Excellence in Human Genetics Lecture Series, UT Southwestern (Dallas, TX)
Jun 2009 *Invited speaker,* Genomic Tools and Technologies Summit, Cambridge Healthtech Institute (San Francisco, CA)
May 2009 *Invited speaker,* Northwest Institute of Genetic Medicine, 2009 Retreat (Seattle, WA)
Mar 2009 *Invited seminar,* University of Michigan, Center for Translational Pathology (Ann Arbor, MI)
Mar 2009 *Invited speaker,* Next-Generation Sequencing meeting, Cambridge Healthtech Institute (San Diego, CA)
Feb 2009 *Invited speaker,* Advances in Genome Biology and Technology (AGBT) (Marco Island, FL)
Feb 2009 *Invited speaker,* Advances in Genome Biology and Technology (AGBT), pre-meeting workshop (Marco Island, FL)
Dec 2008 *Invited seminar,* Puget Sound Blood Center Research (Seattle, WA)
Oct 2008 *Invited speaker,* Discovery2Diagnostics conference (San Diego, CA)
Sep 2008 *New Investigator Science in Medicine Lecture*, University of Washington (Seattle, WA)
Sep 2008 *Keynote address,* Institute for Systems Biology, Annual Retreat (Seabeck, WA)
Sep 2008 *Invited speaker,* Nature Genetics "Genomics of Common Disease" meeting (Cambridge, MA)
Aug 2008 *Invited seminar,* BC Cancer Agency, Genome Sciences Centre (Vancouver, BC)
Mar 2008 *Invited seminar,* Fred Hutchinson Cancer Research Center, Computational Biology Working Group Seminar Series (Seattle, WA)
Mar 2008 *Invited seminar,* University of Washington, Department of Medical Genetics Seminar Series (Seattle, WA)
Mar 2008 *Invited speaker,* Joint Genome Institute (JGI) User 3rd Annual Meeting (Walnut Creek, CA)
Feb 2008 *Invited speaker,* Association of Biomolecular Resource Facilities (ABRF) Annual Meeting (Salt Lake City, UT)
Feb 2008 *Plenary speaker,* Advances in Genome Biology and Technology (AGBT) (Marco Island, FL)
Nov 2007 *Invited seminar,* Stanford University, Frontiers in Biology Seminar Series (Palo Alto, CA)
Nov 2007 *Invited speaker,* 1st Annual Parallel Sequencing Genomics Meeting, Stanford Genome Technology Center, Stanford University (Palo Alto, CA)
Sep 2007 *Invited seminar,* Fred Hutchinson Cancer Research Center, Program in Prostate Cancer Research Seminar Series, (Seattle, WA)
May 2007 *Invited speaker,* Stanford Genome Technology Center, Stanford University (Palo Alto, CA)
Mar 2007 *Invited seminar,* Institute for Molecular Pediatric Sciences, University of Chicago (Chicago, IL)
Mar 2007 *Invited speaker,* Next Generation Sequencing: Applications and Case Studies, Cambridge Healthtech Institute (San Diego, CA)
Feb 2007 *Invited seminar,* Department of Genetics, University of Pennsylvania (Philadelphia, PA)
Feb 2007 *Invited seminar,* Department of Bioengineering, University of California, Berkeley (Berkeley, CA)

Feb 2007	*Invited seminar*, Division of Genetics, Brigham and Women's Hospital, Harvard Medical School (Boston, MA)
Feb 2007	*Invited seminar*, Department of Pathology, Massachusetts General Hospital, Harvard Medical School (Boston, MA)
Feb 2007	*Invited seminar*, Department of Genome Sciences, University of Washington (Seattle, WA)
Feb 2007	*Invited seminar*, Broad Institute of M.I.T. and Harvard (Cambridge, MA)
Jan 2007	*Invited seminar*, Department of Molecular & Cell Biology, University of California, Berkeley (Berkeley, CA)
Jan 2007	*Invited seminar*, National Human Genome Research Institute, National Institutes of Health (Bethesda, MD)
Jan 2007	*Workshop speaker*, Workshop on Systems Biology and Information Medicine in a Global Society, Princeton University (Princeton, NJ)
Jan 2007	*Invited seminar*, Institute for Systems Biology (Seattle, WA)
Mar 2006	*Invited seminar*, Biological Physics & Biophysical Chemistry Seminar, State University of New York, Stony Brook (Stony Brook, NY)

LISTING OF THE CLAIMS for US 6,440,706

Please amend the following claims as indicated by the status identifier. Patent claims under reexamination but not amended are indicated as "original." Patent claims not subject to reexamination are not shown.

1. (Original) A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of:

diluting nucleic acid template molecules in a biological sample to form a set comprising a plurality of assay samples;

amplifying the template molecules within the assay samples to form a population of amplified molecules in the assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence;

comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

2. (Original) The method of claim 1 wherein the step of diluting is performed until at least one-tenth of the assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required for the step of analyzing to determine the presence of the selected genetic sequence.

3. (Original) The method of claim 1 wherein the step of diluting is performed until between 0.1 and 0.9 of the assay samples yield an amplification product when subjected to a polymerase chain reaction.

4. (Original) The method of claim 1 wherein the step of diluting is performed until all of the assay samples yield an amplification product when subjected to a polymerase chain reaction and each assay sample contains less than 10 nucleic acid template molecules containing the reference genetic sequence.

5. (Original) The method of claim 1 wherein the step of diluting is performed until all of the assay samples yield an amplification product when subjected to a polymerase chain reaction and each assay sample contains less than 100 nucleic acid template molecules containing the reference genetic sequence.

6. (Original) The method of claim 1 wherein the biological sample is cell-free.

7. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 10.

8. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 50.

9. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 100.

10. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 500.

11. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 1000.

12. (Original) The method of claim 1 wherein the step of amplifying and the step of analyzing are performed on assay samples in the same receptacle.

13. (Not subject to reexamination)

14. (Original) The method of claim 1 wherein the step of analyzing employs gel electrophoresis.

15. (Original) The method of claim 1 wherein the step of analyzing employs

hybridization to at least one nucleic acid probe.

16. (Original) The method of claim 1 wherein the step of analyzing employs hybridization to at least two nucleic acid probe.

17. (Not subject to reexamination)

18. (Not subject to reexamination)

19. (Original) The method of claim 1 wherein the step of amplifying employs a single pair of primers.

20. (Original) The method of claim 1 wherein the step of amplifying employs a polymerase which is activated only after heating.

21. (Original) The method of claim 1 wherein the step of amplifying employs at least 40 cycles of heating and cooling.

22. (Original) The method of claim 1 wherein the step of amplifying employs at least 50 cycles of heating and cooling.

23. (Original) The method of claim 1 wherein the step of amplifying employs at least 60 cycles of heating and cooling.

24. (Original) The method of claim 1 wherein the biological sample is selected from the group consisting of stool, blood, and lymph nodes.

25. (Original) The method of claim 1 wherein the biological sample is blood or bone marrow of a leukemia or lymphoma patient who has received anti-cancer therapy.

26. (Original) The method of claim 1 wherein the selected genetic sequence is a

translocated allele.

27. (Original) The method of claim 1 wherein the selected genetic sequence is a wild-type allele.

28. (Original) The method of claim 1 wherein the selected genetic sequence is within an amplicon which is amplified during neoplastic development.

29. (Original) The method of claim 1 wherein the selected genetic sequence is a rare exon sequence.

30. (Original) The method of claim 1 wherein the nucleic acid template molecules comprise cDNA of RNA transcripts and the selected genetic sequence is present on a cDNA of a first transcript and the reference genetic sequence is present on a cDNA of a second transcript.

31. (Original) The method of claim 1 wherein the selected genetic sequence comprises a first mutation and the reference genetic sequence comprises a second mutation.

32. (Original) The method of claim 1 wherein the selected genetic sequence and the reference genetic sequence are on distinct chromosomes.

33-37. (Not subject to reexamination)

38. (Currently amended) A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of:

amplifying template molecules in a biological sample within a set comprising a plurality of assay samples to form a population of amplified molecules in each of the assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence, wherein at least one-fiftieth of the

assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required to determine the presence of the selected genetic sequence;

comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

39. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 10.

40. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 50.

41. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 100.

42. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 500.

43. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 1000.

44. (Original) The method of claim 38 wherein the step of amplifying and the step of analyzing are performed on assay samples in the same receptacle.

45. (Not subject to reexamination)

46. (Original) The method of claim 38 wherein the step of analyzing employs gel electrophoresis.

47. (Original) The method of claim 38 wherein the step of analyzing employs hybridization to at least one nucleic acid probe.

48. (Original) The method of claim 38 wherein the step of analyzing employs hybridization to at least two nucleic acid probe.

49. (Not subject to reexamination)

50. (Not subject to reexamination)

51. (Original) The method of claim 38 wherein the step of amplifying employs a single pair of primers.

52. (Original) The method of claim 38 wherein the step of amplifying employs a polymerase which is activated only after heating.

53. (Original) The method of claim 38 wherein the step of amplifying employs at least 40 cycles of heating and cooling.

54. (Original) The method of claim 38 wherein the step of amplifying employs at least 50 cycles of heating and cooling.

55. (Original) The method of claim 38 wherein the step of amplifying employs at least 60 cycles of heating and cooling.

56. (Original) The method of claim 38 wherein the template molecules are obtained from a body sample selected from the group consisting of stool, blood, and lymph nodes.

57. (Original) The method of claim 38 wherein the template molecules are obtained from a body sample of a leukemia or lymphoma patient who has received anti-cancer therapy, said body sample being selected from the group consisting of blood and bone marrow.

58. (Original) The method of claim 38 wherein the selected genetic sequence is a

translocated allele.

59. (Original) The method of claim 38 wherein the selected genetic sequence is a wild-type allele.

60. (Original) The method of claim 38 wherein the selected genetic sequence is within an amplicon which is amplified during neoplastic development.

61. (Original) The method of claim 38 wherein the selected genetic sequence is a rare exon sequence.

62. (Original) The method of claim 38 wherein the nucleic acid template molecules comprise cDNA of RNA transcripts and the selected genetic sequence is present on a cDNA of a first transcript and the reference genetic sequence is present on a cDNA of a second transcript.

63. (Original) The method of claim 38 wherein the selected genetic sequence comprises a first mutation and the reference genetic sequence comprises a second mutation.

64. (Original) The method of claim 38 wherein the selected genetic sequence and the reference genetic sequence are on distinct chromosomes.

Electronic Acknowledgement Receipt

EFS ID:	18036562
Application Number:	90012894
International Application Number:	
Confirmation Number:	8442
Title of Invention:	Digital Amplification
First Named Inventor/Applicant Name:	6,440,706 B1
Customer Number:	11332
Filer:	Sarah Anne Kagan.
Filer Authorized By:	
Attorney Docket Number:	001107.00989
Receipt Date:	27-JAN-2014
Filing Date:	17-JUN-2013
Time Stamp:	17:03:08
Application Type:	Reexam (Patent Owner)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Amendment/Req. Reconsideration-After Non-Final Reject	00989Rsp.pdf	189797 bb057ff137f8faa05230f2edd0bb7f4c3048d17f	no	30

Warnings:	
Information:	

2	Affidavit-traversing rejectns or objectns rule 132	shendure132dec00989binderReprint.pdf	1817469 81b4f534be4f4d547ba7486390c21b829363f218	no	36

Warnings:	
Information:	
Total Files Size (in bytes):	2007266

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re *Ex Parte* Reexamination:	)	Group Art Unit: 3991
	)	
U.S. Patent No. 6,440,706	)	Docket No. 001107.00989
	)	
Control No. 90/012,894	)	Confirmation No: 8442
	)	
Reexam Filing Date: June 17, 2013	)	Examiner: Bruce R. Campell

For: DIGITAL AMPLIFICATION

CERTIFICATE OF SERVICE

The undersigned certifies that a complete copy of the Information Disclosure Statement and associated disclosed documents filed in the U.S. Patent and Trademark Office on January 27, 2014, has been mailed via first class mail to the third party requester this day at the following address:

Life Technologies Corporation
Attn: IP Department
5791 Van Allen Way
Carlsbad, CA 92008

/Sarah A. Kagan/
Sarah A. Kagan
Registration No. 32,141

Dated: 30 January 2014

Banner & Witcoff, Ltd.
Customer No. 11332

Electronic Acknowledgement Receipt

EFS ID:	18072377
Application Number:	90012894
International Application Number:	
Confirmation Number:	8442
Title of Invention:	Digital Amplification
First Named Inventor/Applicant Name:	6,440,706 B1
Customer Number:	11332
Filer:	Sarah Anne Kagan.
Filer Authorized By:	
Attorney Docket Number:	001107.00989
Receipt Date:	30-JAN-2014
Filing Date:	17-JUN-2013
Time Stamp:	16:07:31
Application Type:	Reexam (Patent Owner)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	00989CertServ.pdf	78568 6d5f2a4e5e09d00bb3adafb7ed3ac18ef7432ec6	no	1

Warnings:	
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Total Files Size (in bytes):	78568

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Electronic Acknowledgement Receipt

EFS ID:	18636036
Application Number:	90012894
International Application Number:	
Confirmation Number:	8442
Title of Invention:	Digital Amplification
First Named Inventor/Applicant Name:	6,440,706 B1
Customer Number:	11332
Filer:	Sarah Anne Kagan./Jennifer Hazzard
Filer Authorized By:	Sarah Anne Kagan.
Attorney Docket Number:	001107.00989
Receipt Date:	01-APR-2014
Filing Date:	17-JUN-2013
Time Stamp:	09:46:12
Application Type:	Reexam (Third Party)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	Exhibit-510Levinson.pdf	129466 cb6105797b9ce98515a0d223f2f56dba8e8df11d	no	34

Warnings:	
Information:	

2	Non Patent Literature	Exhibit-511Li1988.pdf	129981 c18bcaeb8d95c9f140191a1db2eb835678133250	no	34
Warnings:					
Information:					
3	Non Patent Literature	Exhibit-512Lia.pdf	8362655 10e7056c02b453612582f61d71e3ab61e3f2a9bb	no	44
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4	Non Patent Literature	Exhibit-513Liu.pdf	178264 a40b6d01f84a8559bcfb786669cdd2dc1312ccc9	no	36
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5	Non Patent Literature	Exhibit-514Munier.pdf	142957 23a65332459a197d4744368e77dbc9d9da5c5fbf	no	39
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6	Non Patent Literature	Exhibit-515Sheehy.pdf	126653 22bb6ae195327e87aa6f560661b1435ba755ee94	no	32
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7	Non Patent Literature	Exhibit-516Simmonds.pdf	115237 6488293f4c7891259481ab48db2215af435e3afb	no	29
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8	Non Patent Literature	Exhibit-517Stark.pdf	113674 b186137d2fef5751a9075e9dfa09467d1020047d	no	28
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9	Non Patent Literature	Exhibit-518Sykes.pdf	93693 6a31bc2fc929b07bb5399171fc1763b65125aa26	no	21
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10	Non Patent Literature	Exhibit-519Zhang1992.pdf	7306043 1bf038fe18c116918e8f2b4ff47c040599d5d057	no	36
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11	Non Patent Literature	Exhibit-520Zhang1993.pdf	116879 eaf335f1fcd331fe6953c31ac3edfb0003789c73	no	30
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13	Non Patent Literature	Exhibit-522Gaynor.pdf	364991 0db1f7244ce3ca9097d4825e3684a6a2cb9276f5	no	44
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14	Non Patent Literature	Exhibit-523Jena.pdf	171680 da735c514d6731626514c0c2ac916acab8997d8b	no	40
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15	Non Patent Literature	Exhibit-61Baker.pdf	222598 2982ae5cb9da6c3f62e5a3d62dececb8681f4944	no	49
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16	Non Patent Literature	Exhibit-62Bischoff.pdf	215195 41bc6ee74c827bee989d3646fbec0245d9e3c55d	no	36
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17	Non Patent Literature	Exhibit-63Brisco.pdf	183961 567f7809f879959bb81ef4afe20fcb86425e352e	no	54
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18	Non Patent Literature	Exhibit-64Dreesen.pdf	160267 b84b8eaf482d1681e5bc717640108f3005149bfb	no	36
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19	Non Patent Literature	Exhibit-65Gravel.pdf	123786 34e52b9c9c6106214a418a2556926cc3bd95950f	no	31
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Information:					
21	Non Patent Literature	Exhibit-67Jeffreys.pdf	427807 9d4667e327ecc893d3c04eb80641c65d032cf1c4	no	59
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22	Non Patent Literature	Exhibit-68Kunst.pdf	4906995 1396b954821f584bbb3ff632450d04a27a293c2e	no	25
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23	Non Patent Literature	Exhibit-69Leeflang.pdf	5621618 e6418d833e2c0e6f64a046c9ce0f0616a8b4d905	no	32
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Information:					
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25	Non Patent Literature	Exhibit-611Li1988.pdf	119315 a245e139f59554ef7fff2fbda5b73695c4771f9f	no	30
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26	Non Patent Literature	Exhibit-612Lia.pdf	8179466 7ec49817f5d97a3931936fac99fd62e8f9cc3824	no	42
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27	Non Patent Literature	Exhibit-613Liu.pdf	164756 25668f7b3f0b07594ece46ba23c46eb7d0cb318f	no	31
Warnings:					
Information:					
28	Non Patent Literature	Exhibit-614Munier.pdf	98525 6fd65204c70a79c7428057462d6cc5e173580114	no	23
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Information:					

29	Non Patent Literature	Exhibit-615Sheehy.pdf	112155 1e5ef805c79dd18ef2491cc8b25fae5737fdb080	no	27
Warnings:					
Information:					
30	Non Patent Literature	Exhibit-616Simmonds.pdf	92165 e9fb9b3d3f19fc4076204c667bf9210a20301a64	no	21
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31	Non Patent Literature	Exhibit-617Stark.pdf	105316 9bbe21d80d53f29f37fa068fdf5fe00e8943e9b9	no	25
Warnings:					
Information:					
32	Non Patent Literature	Exhibit-618Sykes.pdf	88210 5f0408f5c0bb35896f7d010cb7df35f5e7bb8441	no	18
Warnings:					
Information:					
33	Non Patent Literature	Exhibit-619Zhang1992.pdf	199879 f435eeb4ee74088ca37f718b113c9be5558de1cb	no	31
Warnings:					
Information:					
34	Non Patent Literature	Exhibit-620Zhang1993.pdf	94661 8479f9e84705d487fecb74d3e45ef8041a36a480	no	21
Warnings:					
Information:					
			Total Files Size (in bytes):	39020297	

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PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	90012894
Filing Date	2013-06-17
First Named Inventor	
Art Unit	3991
Examiner Name	Bruce R. Campell
Attorney Docket Number	001107.00989

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Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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	Filing Date	2013-06-17
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	Art Unit	3991
	Examiner Name	Bruce R. Campell
	Attorney Docket Number	001107.00989

	1	Defendants Life Technologies Corporation, Applied Biosystems, LLC, and Ion Torrent Systems, Inc.'s Preliminary Non-Infringement and Patent invalidity Contensions pursuant to Local Rule 103.3, filed in Case No. Case No. 1:12-CV-1173 on August 22, 2013	☐
	2	Deposition of Michael Metzker, Ph.D., dated October 25, 2013	☐
	3	Declaration of Michael Metzker, Ph.D. executed September 27, 2013	☐
	4	BAKER et al., "Male Mice Defective in the DNA Mismatch Repair Gene PMS2 Exhibit Abnormal Chromosome Synapsis in Meiosis," Cell, vol. 82, 309-319, July 28, 1995	☐
	5	BISCHOFF et al., "Single cell analysis demonstrating somatic mosaicism involving 11p in a patient with paternal isodisomy and Beckwith-Wiedemann syndrome," Human Molecular Genetics, 1995, vol. 4, no. 3, 395-399	☐
	6	BRISCO et al., "Detection and quantitation of neoplastic cells in acute lymphoblastic leukaemia, by use of teh polymerase chain reaction," British Journal of Haematology, 1991, 79, 211-217	☐
	7	DREESEN et al., "Preimplantation genetic diagnosis of spinal muscular atrophy," Molecular Human Reproduction, vol. 4, no. 9, pp. 881-885, 1998	☐
	8	FLINT et al., "NR2A Subunit Expression Shortens NMDA Receptor Synaptic Currents in Developing Neocortex," The Journal of Neuroscience, April 1, 1997, 17(7):2469-2476	☐
	9	GAYNOR et al., "Use of Flow Cytometry and RT-PCR for Detecting Gene Expression by Single Cells," BioTechniques, vol. 21, no. 2 (1996)	☐
	10	GRAVEL et al., "Single-Cell Analysis of the t(14;18)(q32;q21) Chromosomal Translocation in Hodgkin's Disease Demonstrates the Absence of This Translocation in Neoplastic Hodgkin and Reed-Sternberg Cells," Blood, 1998, 91:2866-2874	☐
	11	GREWAL et al., "The mutation properties of spinal and bulbar muscular atrophy disease alleles," Neurogenetics (1998) 1:249-252	☐

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	Art Unit	3991
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	Attorney Docket Number	001107.00989

	12	JEFFREYS et al., "Complex gene conversion events in germline mutation at human minisatellites," Nature Genetics, vol. 6, February 1994	☐
	13	JENA et al., "Amplification of genes, single transcripts and cDNA libraries from one cell and direct sequence analysis of amplified products derived from one molecule," Journal of Immunological Methods 190 (1996) 199-213	☐
	14	KUNST et al., "The effect of FMR1 CFF repeat interruptions on mutation frequency as measured by sperm typing," J. Med. Genet., 1997; 34:627-631	☐
	15	LEEFLANG et al., "Single sperm analysis of the trinucleotide repeats in the Huntington's disease gene: quantification of the mutation frequency spectrum," Human Molecular Genetics, 1995, vol. 4, no. 9, 1519-1526	☐
	16	LEVINSON et al., "Molecular Characterization of Transgene-induced Immunodeficiency in B-less Mice Using a Novel Quantitative Limiting Dilution Polymerase Chain Reaction Method," J. Exp. Med, vol. 178, July 1993, 317-329	☐
	17	LI et al., "Amplification and analysis of DNA sequences in single human sperm and diploid cells," Nature, vol. 335, September 29, 1988	☐
	18	LIA et al., "Somatic instability of the CTG repeat in mice transgenic for the myotonic dystrophy region is age dependent but not correlated to the relative intertissue transcription levels and proliferative capacities," Human Molecular Genetics, 1998, vol. 7, no. 8, 1285-1291	☐
	19	LIU et al., "Efficiency and accuracy of polymerase-chain-reaction assay for cystic fibrosis allele F508 in single cell," The Lancet, vol. 339, May 16, 1992	☐
	20	SHEEHY et al., "Concurrent evolution of regions of the envelope and polymerase genes of human immunodeficiency virus type 1 observed during zidovudine (AZT) therapy," Journal of General Virology, (1996), 76, 1071-1081	☐
	21	SIMMONDS et al., "Human immunodeficiency virus-infected individuals contain provirus in small numbers of peripheral mononuclear cells and at low copy numbers," Journal of Virology, February 1990, vol. 64, no. 2, p. 864-872	☐
	22	STARK et al., "Single-cell PCR performed with neurofibroma Schwann cells reveals the presence of both alleles of the neurofibromatosis type 1 (NF1) gene," Hum Genet (1995) 96:619-523	☐

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	Attorney Docket Number	001107.00989

	23	SYKES et al., "Quantitation of Targets for PCR by Use of Limiting Dilution," BioTechniques, vol. 13, no. 3 (1992)	☐
	24	ZHANG et al., "Whole genome amplification from a single cell: Implications for genetic analysis," Proc. Natl. Acad. Sci., vol. 89, pp. 5847-5851, July 1992	☐
	25	ZHANG et al., "Selection for Specific Sequences in the External Envelope Protein of Human Immunodeficiency Virus Type 1 upon Primary Infection," Journal of Virology, June 1993, vol. 67, no. 6, p. 3345-3356	☐
	26	Exhibit 4.1 (Baker)	☐
	27	Exhibit 4.10 (Levinson)	☐
	28	Exhibit 4.11 (Li 1988)	☐
	29	Exhibit 4.12 (Lia)	☐
	30	Exhibit 4.13 (Liu)	☐
	31	Exhibit 4.14 (Munier)	☐
	32	Exhibit 4.15 (Sheehy)	☐
	33	Exhibit 4.16 (Simmonds)	☐

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	Art Unit	3991
	Examiner Name	Bruce R. Campell
	Attorney Docket Number	001107.00989

	34	Exhibit 4.17 (Stark)	☐
	35	Exhibit 4.18 (Sykes)	☐
	36	Exhibit 4.19 (Zhang 1992)	☐
	37	Exhibit 4.2 (Bischoff)	☐
	38	Exhibit 4.20 (Zhang 1993)	☐
	39	Exhibit 4.21 (Flint)	☐
	40	Exhibit 4.22 ((Gaynor)	☐
	41	Exhibit 4.23 (Jena)	☐
	42	Exhibit 4.3 (Brisco)	☐
	43	Exhibit 4.4 (Dreesen)	☐
	44	Exhibit 4.5 (Gravel)	☐

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	Attorney Docket Number	001107.00989

	45	Exhibit 4.6 (Grewal)	☐
	46	Exhibit 4.7 (Jeffreys)	☐
	47	Exhibit 4.8 (Kunst)	☐
	48	Exhibit 4.9 (Leeflang)	☐
	49	Exhibit 5.1 (Baker)	☐
	50	Exhibit 5.10 (Levinson)	☐

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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 a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

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	First Named Inventor	
	Art Unit	3991
	Examiner Name	Bruce R. Campell
	Attorney Docket Number	001107.00989

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Sarah A. Kagan/	Date (YYYY-MM-DD)	2014-03-27
Name/Print	Sarah A. Kagan	Registration Number	32141

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The information provided by you in this form will be subject to the following routine uses:

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5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

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7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	90012894
	Filing Date	2013-06-17
	First Named Inventor	
	Art Unit	3991
	Examiner Name	Bruce R. Campell
	Attorney Docket Number	001107.00989

	1	Exhibit 5.11 (Li 1988)	☐
	2	Exhibit 5.12 (Lia)	☐
	3	Exhibit 5.13 (Liu)	☐
	4	Exhibit 5.14 (Munier)	☐
	5	Exhibit 5.15 (Sheehy)	☐
	6	Exhibit 5.16 (Simmonds)	☐
	7	Exhibit 5.17 (Stark)	☐
	8	Exhibit 5.18 (Sykes)	☐
	9	Exhibit 5.19 (Zhang 1992)	☐
	10	Exhibit 5.2 (Bischoff)	☐
	11	Exhibit 5.20 (Zhang 1993)	☐

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	Filing Date	2013-06-17
	First Named Inventor	
	Art Unit	3991
	Examiner Name	Bruce R. Campell
	Attorney Docket Number	001107.00989

	12	Exhibit 5.21 (Flint)	☐
	13	Exhibit 5.22 (Gaynor)	☐
	14	Exhibit 5.23 (Jena)	☐
	15	Exhibit 5.3 (Brisco)	☐
	16	Exhibit 5.4 (Dreesen)	☐
	17	Exhibit 5.5 (Gravel)	☐
	18	Exhibit 5.6 (Grewal)	☐
	19	Exhibit 5.7 (Jeffreys)	☐
	20	Exhibit 5.8 (Kunst)	☐
	21	Exhibit 5.9 (Leeflang)	☐
	22	Exhibit 6.1 (Baker)	☐

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	First Named Inventor	
	Art Unit	3991
	Examiner Name	Bruce R. Campell
	Attorney Docket Number	001107.00989

	23	Exhibit 6.10 (Levinson)	☐
	24	Exhibit 6.11 (Li 1988)	☐
	25	Exhibit 6.12 (Lia)	☐
	26	Exhibit 6.13 (Liu)	☐
	27	Exhibit 6.14 (Munier)	☐
	28	Exhibit 6.15 (Sheehy)	☐
	29	Exhibit 6.16 (Simmonds)	☐
	30	Exhibit 6.17 (Stark)	☐
	31	Exhibit 6.18 (Sykes)	☐
	32	Exhibit 6.19 (Zhang 1992)	☐
	33	Exhibit 6.2 (Bischoff)	☐

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	34	Exhibit 6.20 (Zhang 1993)	☐
	35	Exhibit 6.3 (Brisco)	☐
	36	Exhibit 6.4 (Dreesen)	☐
	37	Exhibit 6.5 (Gravel)	☐
	38	Exhibit 6.6 (Grewal)	☐
	39	Exhibit 6.7 (Jeffreys)	☐
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	Application Number	90012894
	Filing Date	2013-06-17
	First Named Inventor	
	Art Unit	3991
	Examiner Name	Bruce R. Campell
	Attorney Docket Number	001107.00989

CERTIFICATION STATEMENT

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A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Sarah A. Kagan/	Date (YYYY-MM-DD)	2014-03-27
Name/Print	Sarah A. Kagan	Registration Number	32141

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EFS ID:	18635983
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International Application Number:	
Confirmation Number:	8442
Title of Invention:	Digital Amplification
First Named Inventor/Applicant Name:	6,440,706 B1
Customer Number:	11332
Filer:	Sarah Anne Kagan./Jennifer Hazzard
Filer Authorized By:	Sarah Anne Kagan.
Attorney Docket Number:	001107.00989
Receipt Date:	01-APR-2014
Filing Date:	17-JUN-2013
Time Stamp:	09:40:24
Application Type:	Reexam (Third Party)

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1	Information Disclosure Statement (IDS) Form (SB08)	IDS.PDF	615121 bea30716a33afab6a352637804556d4f07240400	no	8

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4	Non Patent Literature	Metzker-Deposition.pdf	890336 38e0ba7515fb299037e4003b8d0fd31b66839ba3	no	129

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5	Non Patent Literature	Metzker-Declaration.pdf	2200153 3bcb8ce137d969b2a5084934003e1bf7aa7ecdb5	no	51

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30	Non Patent Literature	Exhibit-42Bischoff.pdf	356589 de6bad8271d46c897d5f7e1305c7d215c18b1e17	no	85
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56	Non Patent Literature	Exhibit-55Gravel.pdf	121218 122ae3758a61186541608e7b6b8c455fb7dbc115	no	31
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
90/012,894	06/17/2013	6,440,706 B1	001107.00989	8442

11332 7590 05/09/2014

Banner & Witcoff, Ltd.
Attorneys for client 001107
1100 13th Street N.W.
Suite 1200
Washington, DC 20005-4051

EXAMINER	
CAMPELL, BRUCE R	
ART UNIT	PAPER NUMBER
3991	

MAIL DATE	DELIVERY MODE
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Enclosed is a copy of the latest communication from the United States Patent and Trademark Office in the above identified *ex parte* reexamination proceeding (37 CFR 1.550(f)).

Where this copy is supplied after the reply by requester, 37 CFR 1.535, or the time for filing a reply has passed, no submission on behalf of the *ex parte* reexamination requester will be acknowledged or considered (37 CFR 1.550(g)).

Ex Parte Reexamination

Detailed Final Office Action

This is a reexamination of U.S. Patent 6,440,706, issued August 22, 2002. A Request pursuant to 37 CFR 1.510 for ex parte reexamination of claims 1-12, 14-16, 19-32, 38-44, 46-48 and 51-64 of U.S. Patent 6,440,706 was filed on June 17, 2013 by a third party requester. An Order granting the request was mailed on August 28, 2013. A non-final Office action was mailed on November 27, 2013. Patent Owner filed a response including a declaration of Jay Shendure on January 27, 2014.

Status of the Claims

Claims 1-12, 14-16, 19-32, 38-44, 46-48 and 51-64 of U.S. Patent 6,440,706 are subject to reexamination. Claim 38 has been amended in the response filed on January 27, 2014.

Scope of the Claims

In reexamination, patent claims are construed broadly. In re Yamamoto, 740 F.2d 1569, 1571, 222 USPQ 934, 936 (Fed. Cir. 1984) (claims given "their broadest reasonable interpretation consistent with the specification"). The independent claims subject to reexamination read as follows:

1. A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of:

diluting nucleic acid template molecules in a biological sample to form a set comprising a plurality of assay samples;

amplifying the template molecules within the assay samples to form a population of amplified molecules in the assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence;

comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

38. (amended) A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of:

amplifying template molecules in a biological sample within a set comprising a plurality of assay samples to form a population of amplified molecules in each of the assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence, wherein at least one-fiftieth of the assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required to determine the presence of the selected genetic sequence;

comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

Claim Interpretation

The biological sample of claim 1 can either be comprised of cells, tissues, bodily fluids, etc. or cell free, as recited in dependent claims 6 and 24. In either case, nucleic acids are distributed throughout the sample. Therefore any process in which the sample is diluted is considered "diluting nucleic acid template molecules in a biological sample." The “ratio of a selected genetic sequence” is interpreted as the ratio of the selected genetic sequence to the reference genetic sequence. “Template molecule” is not explicitly defined in the ‘706 patent specification. Absent any definition, “template” is interpreted to mean the sequences capable of amplification in the assay.

Patent Owner argues (Response filed January 27, 2014, pp. 9-13) that the “biological sample” refers to isolated nucleic acids and that the claims exclude methods in which single cells are analyzed, as in the Li and Zhang references. This argument is not persuasive because it is not supported by the specification or the claims. Claim 1 requires “diluting nucleic acid template molecules in a biological sample” and claim 24 recites that the biological sample is stool, blood or lymph nodes, which are clearly not

isolated nucleic acids. The claims do not recite a step in which nucleic acids are purified in any way prior to dilution. Patent Owner cites the '706 patent specification, which recites, "Biological samples which can be used as the starting material for the analyses may be from any tissue or body sample from which DNA or mRNA can be isolated," but does not explain why this precludes the use of semen or cultured cells (as disclosed in Li, pp. 414-415 and Zhang, p. 5847) as starting material for analyses. The '706 patent does not require that "assay samples" consist of purified nucleic acids; "assay sample" is not defined at all in the specification. Therefore there is no reason why a group of single cells isolated from a biological sample cannot be construed as a set of assay samples. Patent Owner is correct in stating that the quoted portion of the specification does not require isolation of single cells, but neither is such a step excluded. Patent Owner attempts to prove its point by quoting 8 sections from the specification (Response, p. 12), but does not explain how they exclude the methods of Li and Zhang; the first 7 citations clearly do not and the eighth is only describing a preferred embodiment. Regarding the eighth citation, a more complete quotation is, "In one preferred embodiment each diluted sample has on average one half a template molecule. This is the same as one half of the diluted samples having one template molecule." In the case of Zhang analyzing sperm cells for sequences specific for X and Y chromosomes, approximately half of the cells (assay samples) should contain the X template and half the Y template, so even this preferred embodiment can be met by methods in which single cells are isolated. Patent Owner's argument that "a claim interpretation that excludes a preferred embodiment from the scope of the claim is rarely, if ever, correct" is not persuasive because Examiner's broad interpretation does not exclude the preferred embodiment. A particular embodiment appearing in the written description may not be read into a claim when the claim language is broader than the embodiment (MPEP 2111.01 (II)).

With regard to the limitation in claim 38 "the assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required to determine the presence of the selected genetic sequence," it is impossible to ascertain a value for N because this

number can only be determined after the method has been performed. The "plain English" meaning of this limitation is that, for example, if the selected sequence is present in the biological sample at a level of 1 copy in 50, then the assay samples should contain at least 50 total copies (“selected” + “reference”) of the genetic sequence to ensure a reasonable likelihood that there is a selected genetic sequence present in the sample to be amplified and detected. This assumes that a single copy of a sequence is sufficient to be detected after amplification and detection, which may or may not be true, depending on experimental conditions (how many amplification cycles, detection method used, etc.). It appears that this information can only be derived *ex post facto*, or at least after preliminary experiments have been performed with similar biological samples. For purposes of interpreting the prior art, if a reference shows that a selected genetic sequence was detected in an assay sample, then clearly that assay sample contained enough template nucleic acid molecules to enable detection of the selected genetic sequence and this claim limitation is met, whether or not “N” is specifically disclosed.

With regard to "a polymerase which is activated only after heating," as recited in claims 20 and 52, the specification does not disclose a polymerase which requires heat to become capable of catalytic activity. This limitation is interpreted to mean that the polymerase is separated from one or more reactants until heat is applied, thereby bringing enzyme and reactants in contact and allowing polymerization to begin.

Documents Submitted by Requester

Li *et al.,* "Amplification and analysis of DNA sequences in single human sperm and diploid cells." Nature 335(6189):414-7 (1988)

Zhang *et al.,* "Whole genome amplification from a single cell: implications for genetic analysis." *PNAS USA,* 89(13):5847-51 (1992)

Jeffreys *et al.,* "Amplification of human minisatellites by the polymerase chain reaction: towards DNA fingerprinting of single cells." *Nucl. Acids. Res.,* vol 16, no. 23, pages 10953-10971 (1988)

Kalinina *et al.,* "Nanoliter scale PCR with TaqMan detection," *Nucl. Acids. Res.* vol 25, 1999-2004 (1997)

Chou *et al.,* "Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications," *Nucleic Acids Res.,* 20(7): 1717-1723 (April 11, 1992)

Burg, *et al.,* "Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii,* by polymerase chain reaction." J. *Clin. Microbiol.* 27, 1787-1792 (1989)

Trümper *et al.,* "Single-Cell Analysis of Hodgkin and Reed-Sternberg Cells: Molecular Heterogeneity of Gene Expression and p53 Mutations," *Blood, 81 :* 3097-3115 (1993)

Kanzler *et al.,* "Molecular Single Cell Analysis Demonstrates the Derivation of Peripheral Blood-Derived Cell Line (L 1236) From the Hodgkin/Reed-Sternberg Cells of a Hodgkin's Lymphoma Patient," *Blood,* 87:3429-3436 (1996)

Gravel *et al.,* "Single-cell analysis of the t(14; 18)(q32;q21) chromosomal translocation in Hodgkin's disease demonstrates the absence of this translocation in neoplastic Hodgkin and Reed-Sternberg cells," *Blood* 91(8):2866-74 (Apr 15, 1998)

Pontén *et al.,* "Genomic analysis of single cells from human basal cell cancer using laser-assisted capture microscopy," *Mutation Research Genomics* 382, 45-55 (1997)

Documents Cited by Examiner

M Schwab, “Amplification of oncogenes in human cancer cells.” Bioessays 20(6): 473-479 (1998)

http://en.wikipedia.org/wiki/Semen_analysis, downloaded 4/17/2014 (“Wikipedia”)

S Gelmini et al., “Quantitative polymerase chain reaction-based homogeneous assay with fluorogenic probes to measure c-erbB-2 oncogene amplification.” Clinical Chemistry 43(5): 752-758 (1997)

Maintained Claim Rejections – 35 U.S.C. §§ 102 and 103

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-3, 7-9, 15, 16, 19, 21, 22, 27, 32, 38-41, 47, 48, 51, 53, 54, 59 and 64 are rejected under pre-AIA 35 U.S.C. 102(b) as being anticipated by Li.

Li discloses a method in which a ratio of genetic sequences (β-globin) was obtained from a tissue culture flask containing co-cultured cells (the biological sample) of an individual homozygous for the β^S allele ("selected genetic sequence," which causes sickle cell anemia) and another individual homozygous for the β^A allele (normal, "reference genetic sequence"). The nucleic acid template molecules, contained within the cultured cells, were diluted by isolating single cells from the culture. Thirty seven single cells (assay samples) were lysed, and the released DNA was subjected to polymerase chain reaction (PCR) to amplify the portion of the globin gene containing the sickle cell mutation. Amplified DNA was hybridized with allele specific probes. It was found that 19 of the samples contained the normal allele, 12 contained the sickle cell allele, and 6 samples did not hybridize with either probe. These numerical values were "compared," which inherently ascertains a ratio between the two values (19:12). This experiment (pp. 414-415, Fig. 1) meets all the limitations of claim 1.

In another experiment (p. 415, Fig. 2), the biological sample was semen obtained from a subject heterozygous for a polymorphism in the LDLr gene. Eighty individual sperm cells were lysed and the DNA subjected to PCR followed by hybridization with

allele specific probes. A total of 55% of sperm cells ("assay samples") gave a hybridization signal. It was found that 22 assay samples contained one allele and 21 samples contained the other, a ratio of 22:21. Either allele can be considered the "selected genetic sequence" or the "reference genetic sequence." Therefore this experiment also meets all the limitations of claim 1.

With regard to claim 2, the fact that the selected genetic sequences were detected in some of the assay samples shows that the additional claim limitation was met (see claim interpretation above).

Claim 38 is essentially the same as claim 2, except it does not require the dilution step recited in claim 1, and only 1/50 (rather than 1/10) of the assay samples must comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required to determine the presence of the selected genetic sequence. Therefore claim 38, being broader, is anticipated for the same reasons as claim 2.

With regard to claim 3, 84% and 55% of assay samples produced detectable amplification product in Figs. 1 and 2, respectively.

With regard to claims 7-9 and 39-41, Li discloses a third experiment in which the number of assay samples (individual sperm cells) was greater than 100 (pp. 415-416, Table 1).

With regard to claims 15, 16, 47 and 48, amplified DNA in the assay samples was hybridized with 2 or more allele specific probes.

With regard to claims 19, 21, 22, 51, 53 and 54, the experiments described in Figs. 1 and 2 each used a single pair of PCR primers. Fig. 1 used 50 cycles of PCR amplification.

With regard to claims 27, 32, 59 and 64, it is arbitrary which sequence is the "selected" sequence and which is the "reference" sequence. In Fig. 1, one of the detected sequences is the β^A (wild type) globin sequence, meeting the limitations of claims 27 and 59. In the third experiment described on pp. 415-416, sequences from two different chromosomes were detected, meeting the limitations of claims 32 and 64.

Claims 1, 2, 7, 14, 19, 27, 32, 38, 39, 46, 51, 59 and 64 are rejected under pre-AIA 35 U.S.C. 102(b) as being anticipated by Zhang.

Zhang discloses a method similar to that of Li. In Zhang's method (p. 5847), a biological sample (semen) was diluted into 18 assay samples by selecting and isolating 18 single sperm cells. Each cell was lysed and the released DNA was pre-amplified by repeated primer extension reactions with a set of random 15-mer primers (primer-extension preamplification, or PEP). The PEP process was estimated to produce at least 30 copies of every sequence capable of amplification (p. 5848, col. 1). After PEP, aliquots of each sample were subjected to a two-step hemi-nested PCR process to determine the genotype at each of 12 different loci. PCR was first performed using a first pair of primers designed to amplify the genetic sequence of interest, then an aliquot of the sample was removed and subjected to a second PCR using one primer from the first pair and a second primer internal to the previously amplified sequence of interest. The second set of primers was chosen so that the two possible alleles would produce amplified fragments of different lengths. This method ensures specificity of the PCR and allows discrimination between the two reaction products (hence, alleles present in the template molecules) by gel electrophoresis of the final PCR product to determine fragment length (p. 5847, col. 2). Each of the 12 loci were successfully amplified in at least 15 of the 18 sperm cells (assay samples; see Table 2). The genotype of each cell was determined for two loci (results for 9 cells shown in Fig. 3). Each of the two APOC2 alleles was found in 9 cells, the expected 1:1 ratio for this heterozygous sperm donor. Similarly, analysis of the sex linked STS gene/pseudogene showed that 9 cells carried an X chromosome and 8 carried a Y chromosome (the 18th cell did not yield detectable STS sequence). Independent assortment of these two loci was also observed (p. 5848, col. 2). Therefore the method of Zhang anticipates claim 1.

With regard to claim 2, the fact that the selected genetic sequences were detected in some of the assay samples shows that the additional claim limitation was met (see claim interpretation above).

Claim 38 is essentially the same as claim 2, except it does not require the dilution step recited in claim 1, and only 1/50 (rather than 1/10) of the assay samples must comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required to determine the presence of the selected genetic sequence. Therefore claim 38, being broader, is anticipated for the same reasons as claim 2.

With regard to claims 7 and 39, the number of assay samples (individual sperm cells) was greater than 10 (18).

With regard to claims 14 and 46, amplified DNA in the assay samples was analyzed by gel electrophoresis.

With regard to claims 19 and 51, while there are two amplification steps, each amplification employs a single pair of primers.

With regard to claims 27, 32, 59 and 64, it is arbitrary which APOC2 allele is the "selected" sequence and which is the "reference" sequence. Absent evidence to the contrary, each of the STS sequences is assumed to be wild type (one for the X chromosome, the other for the Y chromosome), meeting the limitations of claims 27 and 59, as well as claims 32 and 64. Furthermore, with regard to claims 32 and 64, the APOC2 locus is on chromosome 19 (p. 5848, col. 2).

Claims 4-6 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over either one of Li or Zhang as applied to claim 1, further in view of Jeffreys.

Li and Zhang are relied on as described above. Each reference discloses a method in which a biological sample is diluted into a plurality of assay samples containing a single cell, DNA from each cell is amplified, the presence or absence of two different DNA sequences is determined, and the relative frequency (ratio) of the two sequences is determined. Li and Zhang do not disclose a method wherein the biological sample is cell free.

Jeffreys discloses methods for amplification of human minisatellite DNA for the purpose of producing DNA fingerprints of individuals. In one method, a biological sample is split into multiple assay samples by isolating single cells, then analyzed in

much the same way as in Li and Zhang (pp. 10955-10956). In an alternative method, isolated (cell free) DNA was diluted into multiple assay samples, each containing 6 pg DNA. This amount was estimated to be equivalent to the amount of DNA in a single cell. It was concluded that single DNA molecules could be faithfully amplified (pp. 10960-10962). In the experiment shown in Fig. 4, each assay sample was subjected to PCR with 4 sets of primers (in a single reaction), the primers designed to amplify two alleles for each of 2 minisatellites. Successful amplification was obtained, with a mean failure rate of 63% per allele per reaction, equating to an estimated 0.46 successful amplification events per 6 pg sample (because statistically one would not expect the template sequence to be present in every sample; p. 10961).

It would have been obvious to one of ordinary skill in the art to modify the method of Li or Zhang by obtaining DNA from a cell free sample, then diluting it into multiple assay samples which each contain approximately as much DNA as a single cell, as taught by Jeffreys. One would have been motivated to do this in order to analyze DNA from sources which do not contain intact cells (e.g. forensic samples) and/or to eliminate the labor intensive process of isolating single cells. With regard to claims 4 and 5, Jeffreys estimates that with one genome equivalent of DNA per sample, about 46% of PCRs were successful. One would be motivated to ensure that every sample yielded a successful PCR, to avoid wasting time and reagents. It would have been obvious to increase (e.g. double or triple) the amount of DNA in each sample to ensure that each PCR yielded an amplification product, which would still be less than 10 genome equivalents per sample, or less than 10 reference sequence template molecules per sample (in the case of a gene having a single copy per haploid genome). Thus the invention as a whole was clearly prima facie obvious to one of ordinary skill in the art at the time the invention was made.

Claims 12 and 44 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over either one of Li or Zhang as applied to claims 1 and 38 and further in view of Kalinina.

Li and Zhang are relied on as described above. Together, the references teach a method in which a cell free biological sample is diluted into a plurality of assay samples containing the DNA in an amount equivalent to a single cell, DNA from each assay sample is amplified, the presence or absence of two different DNA sequences is determined, and the relative frequency (ratio) of the two sequences is determined. Neither Li nor Zhang disclose a method wherein amplification and analysis are performed in the same receptacle.

Kalinina discloses a method for PCR amplification and detection using TaqMan probes. Samples diluted to contain approximately 1 template molecule are subjected to TaqMan PCR in sealed capillary tubes containing a few nanoliters of reactants, then presence of PCR product is determined by measuring the probe fluorescence (entire document, see especially p. 2000). The method is considered especially useful for assays meant to determine the presence or absence of PCR product (i.e. not quantitative analysis; p. 2004, last paragraph). Kalinina discusses "conventional" TaqMan assays, which also allow amplification and analysis to be performed in the same receptacle, in reviewing the state of the art (p. 1999).

It would have been obvious to one of ordinary skill in the art to modify the method of Li or Zhang by conducting TaqMan PCR in a sealed receptacle as taught by Kalinina. One would have been motivated to do this in view of the readily apparent advantages of doing so. Nanoliter scale PCR would reduce the amount (and cost) of reagents required, and fluorescence detection (by either conventional or nanoliter scale assay) would eliminate the need for radioactive probes (Li method) or gel electrophoresis (Zhang method). Kalinina suggests that the process could be automated (abstract), and explicitly states that the method should be useful to determine the presence or absence of PCR product in samples diluted to contain approximately one template molecule (p. 2004). Thus the invention as a whole was clearly prima facie obvious to one of ordinary skill in the art at the time the invention was made.

Claims 20 and 52 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over either one of Li or Zhang as applied to claims 1 and 38 and further in view of Chou.

Li and Zhang are relied on as described above. Each reference discloses a method in which a biological sample is diluted into a plurality of assay samples containing a single cell, DNA from each cell is amplified, the presence or absence of two different DNA sequences is determined, and the relative frequency (ratio) of the two sequences is determined. Li and Zhang do not disclose a method wherein the DNA polymerase is activated after heating.

Chou discloses a method for "hot start" PCR. The method uses a wax barrier to separate one or more PCR components from the remainder of the reactants until heat is applied to melt the wax (entire document). This method reduces amplification due to mispriming and primer oligomerization, and is said to be especially useful for PCR with a sample containing a low number of template molecules (p. 1722, col. 1).

It would have been obvious to one of ordinary skill in the art to modify the method of Li or Zhang by using the hot start PCR method. One would have been motivated to do so in order to increase the specificity of the PCR as taught by Chou. Thus the invention as a whole was clearly prima facie obvious to one of ordinary skill in the art at the time the invention was made.

Claims 23 and 55 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over either one of Li or Zhang as applied to claims 1 and 38 and further in view of Burg.

Li and Zhang are relied on as described above. Each reference discloses a method in which a biological sample is diluted into a plurality of assay samples containing a single cell, DNA from each cell is amplified, the presence or absence of two different DNA sequences is determined, and the relative frequency (ratio) of the two sequences is determined. Li and Zhang do not disclose a method wherein the PCR is performed for 60 cycles.

Burg discloses a method for PCR detection of a single cell of *Toxoplasma gondii*. Cells are lysed and PCR is performed for 60 cycles (p. 1790, col. 1; Fig. 4).

It would have been obvious to one of ordinary skill in the art to modify the method of Li or Zhang by performing the PCR for 60 amplification cycles. One would have been motivated to do so, given the knowledge that this method is effective for detecting target DNA sequences from a single cell as taught by Burg. Thus the invention as a whole was clearly prima facie obvious to one of ordinary skill in the art at the time the invention was made.

Claims 24, 29, 30, 56, 61 and 62 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over either one of Li or Zhang as applied to claims 1 and 38 and further in view of Trümper.

Li and Zhang are relied on as described above. Each reference discloses a method in which a biological sample is diluted into a plurality of assay samples containing a single cell, DNA from each cell is amplified, the presence or absence of two different DNA sequences is determined, and the relative frequency (ratio) of the two sequences is determined. Li and Zhang do not disclose a method wherein the biological sample is derived from stool, blood or lymph nodes, nor do they disclose a method wherein the template molecules to be amplified are on cDNA..

Trümper isolated single cells from lymph nodes of patients diagnosed with Hodgkin's disease. Cells were lysed, cDNA was produced by reverse transcription and PCR performed on the cDNA (see methods, pp. 3098-3100). One cell was found to have a mutation in exon 7 of the p53 gene, at a known "hot spot." This mutation is considered to be a "rare exon sequence" as recited in claims 29 and 61.

It would have been obvious to one of ordinary skill in the art to use the method of Li or Zhang to study cells from lymph node tissue, for example to determine the percentage of Hodgkins cells having the p53 mutation found by Trümper. In this case, the mutant p53 sequence would be the selected genetic sequence and the wild type sequence would be the reference sequence. This is exactly the type of analysis suggested by Li, which states, "the ability to study DNA sequences in individual diploid

cells will make it possible to study cell-to-cell variation in developmental processes involving DNA rearrangements or other genetic alterations. It is also likely that analysis of messenger RNAs in single cells would be possible if efficient reverse transcription could be carried out before PCR was initiated" (p. 417, col. 2). Thus the invention as a whole was clearly prima facie obvious to one of ordinary skill in the art at the time the invention was made.

Claims 31 and 63 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over either one of Li or Zhang as applied to claims 1 and 38 and further in view of Pontén.

Li, Zhang are relied on as described above; together they teach a method for measuring the relative number (ratio) of cells in a sample which have a mutation in the p53 gene. Neither Li nor Zhang teach a method in which the selected genetic sequence and the reference genetic sequence each comprise a different mutation.

Pontén performed single cell PCR on cells derived from a single tumor and showed that the tumor contained multiple p53 mutations. Some cells contained more than one mutation of the p53 gene (see overview on p. 52).

It would have been obvious to one of ordinary skill in the art to modify the method of Li or Zhang by performing PCR with multiple primer sets capable of amplifying different portions of the p53 gene known to contain mutation-prone sequences of interest, as disclosed by Pontén. As noted above, it is arbitrary which sequence is the "selected" sequence and which is the "reference" sequence. One would have been motivated to determine the relative abundance (ratio) of p53 mutations in tumors to investigate, for example, possible correlations between different p53 mutations and tumor phenotype (invasiveness, susceptibility to anti-cancer drugs, etc.). Thus the invention as a whole was clearly prima facie obvious to one of ordinary skill in the art at the time the invention was made.

Claims 10, 11, 25, 28, 42, 43, 57 and 60 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over either one of Li or Zhang as applied to claims 1 and 38 and further in view of Kanzler.

Li and Zhang are relied on as described above. Each reference discloses a method in which a biological sample is diluted into a plurality of assay samples containing a single cell, DNA from each cell is amplified, the presence or absence of two different DNA sequences is determined, and the relative frequency (ratio) of the two sequences is determined. Li and Zhang do not disclose a method wherein the biological sample is derived from blood or bone marrow of a leukemia or lymphoma patient who has received anti-cancer therapy. Li and Zhang also do not disclose a method wherein the number of assay samples is greater than 500 or 1,000, nor a method wherein the selected genetic sequence is part of a sequence which is amplified during neoplastic development.

Kanzler isolated single cells from bone marrow of patients diagnosed with Hodgkin's disease (p. 3429). PCR analysis identified three gene rearrangements (abstract). Kanzler suggests, "Using tumor clone-specific primers … residual tumor cells may be detected after therapy" (p. 3434, col. 2).

It would have been obvious to one of ordinary skill in the art to use the method of Li or Zhang to study cells from bone marrow of a Hodgkin's lymphoma patient who has received anti-cancer therapy as suggested by Kanzler. In this case, one or more of the rearranged DNA sequences noted by Kanzler would be the selected genetic sequence(s) and the wild type sequence(s) would be the reference sequence, and the analysis would determine the percentage (ratio) of cancerous cells remaining after therapy. Ideally, there should be no cancerous cells remaining after therapy, but every cell cannot be tested. It is readily apparent that the more cells are tested and found to be non-cancerous, the greater the likelihood that all cancerous cells have been eradicated by the anti-cancer therapy. It would therefore be obvious to increase the number of cells analyzed to 500, 1,000 or more, as recited in claims 10, 11, 42 and 43. This type of analysis is suggested by Li, which states, "the ability to study DNA sequences in individual diploid cells will make it possible to study cell-to-cell variation in

developmental processes involving DNA rearrangements or other genetic alterations" (p. 417, col. 2). Thus the invention as a whole was clearly prima facie obvious to one of ordinary skill in the art at the time the invention was made.

Claims 26 and 58 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over either one of Li or Zhang as applied to claims 1 and 38 and further in view of Gravel.

Li and Zhang are relied on as described above. Each reference discloses a method in which a biological sample is diluted into a plurality of assay samples containing a single cell, DNA from each cell is amplified, the presence or absence of two different DNA sequences is determined, and the relative frequency (ratio) of the two sequences is determined. Li and Zhang do not disclose a method wherein the selected genetic sequence is a translocated allele.

Gravel used single cell PCR analysis to determine the presence or absence of a chromosomal translocation, t(14;18)(q32;q21), in cells from bone of patients diagnosed with Hodgkin's disease (see methods, pp. 2866-2868).

It would have been obvious to one of ordinary skill in the art to use the method of Li or Zhang to search for chromosomal translocations as was done by Gravel. In this case, the wild type (non-translocated) sequence would be the reference sequence, and the analysis would determine the percentage (ratio) of cells in a sample having the translocation. This type of analysis is suggested by Li, which states, "the ability to study DNA sequences in individual diploid cells will make it possible to study cell-to-cell variation in developmental processes involving DNA rearrangements or other genetic alterations" (p. 417, col. 2). There would have been a reasonable expectation of success, since Gravel had already used single cell PCR to detect a translocation. Thus the invention as a whole was clearly prima facie obvious to one of ordinary skill in the art at the time the invention was made.

Claims 28 and 60 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over either one of Li or Zhang as applied to claims 1 and 38 and further in view of Schwab.

Li and Zhang are relied on as described above. Each reference discloses a method in which a biological sample is diluted into a plurality of assay samples containing a single cell, DNA from each cell is amplified, the presence or absence of two different DNA sequences is determined, and the relative frequency (ratio) of the two sequences is determined. Li and Zhang do not disclose a method wherein the selected genetic sequence is one which is amplified during neoplastic development.

Schwab is a review article which summarizes what was known about gene amplification in different types of cancer at the time the invention was made (entire document). For example, “Amplified MYCN has been found only in more aggressive variants of neuroblastoma, where it connotes a dire prognosis. Clinically, it has emerged as a powerful independent marker to predict poor patient outcome” (p. 475, col. 2). Regarding amplification of ERBB2 in breast cancer, “amplification was found to be a significant predictor of both overall survival and time to relapse and appears to be superior to all other prognostic parameters except for positive lymph nodes” (p. 476. Col. 1).

It would have been obvious to one of ordinary skill in the art to use the method of Li or Zhang to search for amplified oncogene sequences such as those disclosed by Schwab. In this case, the wild type (unamplified) sequence would be the reference sequence, and the analysis would determine the percentage (ratio) of cells in a sample having the amplified version. This type of analysis is suggested by Li, which states, “the ability to study DNA sequences in individual diploid cells will make it possible to study cell-to-cell variation in developmental processes involving DNA rearrangements or other genetic alterations" (p. 417, col. 2). One would have been motivated to do so to help predict the prognosis for patients, to search for metastatic cells in surrounding tissues, to conduct basic research in oncogenesis, etc. Thus the invention as a whole was clearly prima facie obvious to one of ordinary skill in the art at the time the invention was made.

Response to Arguments

Patent Owner's remarks and the Shendure declaration, submitted on January 27, 2014, have been considered but are not found persuasive.

§ 102(b) rejection over Li

Patent Owner's arguments regarding the scope of the claims (Response, pp. 11-13) have been addressed in the "claim interpretation" section above; no further comment is deemed necessary.

With regard to claims 1-3, 7-9, 15, 16, 19, 21, 22, 27 and 32, Patent Owner argues (Response, pp. 12-14) that Li does not dilute nucleic acid template molecules, citing the Shendure declaration (¶ 10). This argument is not persuasive because each of the two experiments reported by Li included a dilution step. The caption of Fig. 1 discloses, "After washing three times single cells were selected from a cell suspension (1-3 X 10^6 ml^{-1})…Each single cell sample was delivered into a 0.5 ml plastic microfuge tube containing 10 µl autoclaved distilled water." Thus each microfuge tube contained an assay sample of 1 diploid genome (contained within a cell, until the cells were lysed) at a concentration of 100 diploid genomes per ml. This is at least a 10,000 fold dilution from the suspension (biological sample) containing 1-3 X 10^6 diploid genomes (cells) per ml. The caption of Fig. 2 discloses, "0.5 ml semen was mixed with 3 ml of 40% sucrose," which is clearly a dilution, then following purification, "[s]ingle sperm were isolated in the same way as individual diploid cells using a sperm suspension at a concentration of 1 X 10^5 ml^{-1}," which is a 1,000 fold dilution to single cells at a concentration of 100 cells (haploid genomes) per ml. The Shendure declaration cites the '706 patent specification co. 9, lines 40-44 and states that this level of dilution cannot be achieved by single cell micromanipulation and lysis of lymphocytes (¶11). The declaration is not persuasive since the cited limitations are not in the present claims, and because it makes no attempt to explain why Li's procedures are not "diluting

nucleic acid template molecules in a biological sample" as recited in claim 1. Moreover declarant's opinion is not supported by any objective evidence.

With regard to claims 38-41, 47, 48, 51, 53, 54, 59 and 64, Patent Owner argues (Response, pp. 15-16) that claim 38 requires that nucleic acid be isolated in bulk from the biological sample, then diluted into assay samples. This argument is not persuasive because it is not supported by the plain language of the claim, which contains no step for extracting (or purifying or isolating) template nucleic acids from the biological sample. Furthermore, the claim as amended now recites that the template molecules are "in a biological sample" and the biological sample can contain whole cells, as discussed above. Li starts with biological samples (cell suspensions) and creates sets of assay samples (individual cells) therefrom, then amplifies the nucleic acids within the set of assay samples. Patent Owner points to Example 4 as support for its position, but this is not persuasive because it is improperly reading limitations from the specification into the claim and also because the '706 patent clearly states, "specific examples…are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention" (col. 7, lines 54-56).

Patent Owner's argument (Response, p. 16) that the 22 individual sperm cells (assay samples) isolated from the semen (biological sample) are not representative of the biological sample as a whole is not persuasive absent any explanation of why Patent Owner believes this to be the case. This argument also does not relate to the 37 diploid cells isolated from cell culture in the other experiment reported by Li.

§ 102(b) rejection over Zhang

Patent Owner argues (p. 17) that Zhang did not dilute the biological sample. This argument is not persuasive because Zhang sorted individual sperm cells into microtiter plate wells containing 5 µl of solution (200 cells per ml). Since semen typically contains about 15,000,000 cells per ml (see Wikipedia, p. 2), this is a dilution of the biological sample.

Patent Owner reiterates the arguments made regarding the rejection over Li (Response, p. 17), which are not persuasive for the reasons stated above.

§ 103 rejection over Li or Zhang, in view of Jeffreys

Patent Owner reiterates the arguments made regarding the rejections over Li and Zhang (Response, p. 19), which are not persuasive for the reasons stated above.

Patent Owner's argument (Response, p. 19) that the experiment reported in Jeffreys Fig. 4 is "merely…a proof of concept" is not persuasive because a reference is relevant for all it discloses (MPEP 2123). Patent Owner's assertion that Jeffreys does not disclose or suggest a set comprising a plurality of assay samples comprising nucleic acid template molecules obtained or derived from a biological sample is incorrect. The data in Fig. 4A were generated by dividing human DNA (biological sample) into 16 aliquots containing 6 pg DNA (a set of assay samples), which were each subjected to PCR amplification with allele-specific primers.

Patent Owner's allegation of hindsight (Response, p. 19) is not persuasive absent any further explanation. The experiment disclosed in Jeffreys Fig. 4 uses the first 3 steps of the claimed method. The final step of the method is simply data analysis, which Li and Zhang show were well known in the art. Patent Owner further argues (Response, pp. 19-20), citing the Shendure declaration (¶ 12), that combining the references would destroy the information Li and Zhang were trying to obtain, pointing to gene mapping and preimplantation genetic diagnostic applications described in the references. Patent Owner has again failed to consider all that the references teach. The experiment depicted in Li Fig.1 is a method for determining the relative abundance of normal and sickle cell anemia cells in a mixed population of cells. One skilled in the art would recognize that this is the same as, or analogous to, "determining the ratio of a selected genetic sequence in a population of genetic sequences" as recited in the claims. Jeffreys showed that diluting bulk DNA into samples equivalent to a single genome could also produce usable results (i.e. successfully amplify a single copy of a DNA sequence). Jeffreys' "cell free" method can be applied to the sickle cell anemia

experiment of Li because answering that particular question does not require maintaining chromosomal linkages as argued by Patent Owner. One of ordinary skill in the art would immediately recognize the advantages (savings in time and labor) of avoiding the isolation of single cells, and would also recognize the general applicability of the method, i.e. the relative proportions of cell types "A" and "B" in a population of cells could be readily determined by the method of Jeffreys, so long as genetic markers for "A" and "B" were available.

Patent Owner's argument (Response, p. 20) regarding increasing the amount of template nucleic acids in each assay sample (claims 4 and 5) is not persuasive. The rejection does not state that the Jeffreys method would be useful for "rare" samples as alleged by Patent Owner and the Shendure declaration (¶ 13). The rejection states that one "would have been motivated to do this in order to analyze DNA from sources which do not contain intact cells." Furthermore, even if there were a limited amount of starting material, the primer-extension preamplification (PEP) method of Zhang provides a means for increasing the amount of starting DNA available for analysis (Zhang p. 5850, col. 2).

§ 103 rejection over Li or Zhang in view of Kalinina

Patent Owner reiterates (Response, p. 21) its arguments regarding Li and Zhang (use of single cells, alleged failure to dilute samples), which are not persuasive for the reasons cited above. Patent Owner further argues hindsight (p. 21). This argument is not persuasive. Li and Zhang each performed amplification (PCR) using microfuge tubes in a thermal cycler. Claims 12 and 44 simply require that analysis of the amplified products be performed in the same receptacle as amplification. Kalinina discloses two methods for doing so. One is the "conventional" TaqMan assay described at p. 1999, col. 1, which could be used with the single cell lysates of either Li or Zhang. The other is Kalinina's nanoliter scale method, which could be used with aliquots of Zhang's pre-amplified DNA. Patent Owner does not explain why application of these known analytical methods to the samples prepared by Li and Zhang would only be obvious in

hindsight. Patent Owner's argument (Response, pp. 21-22) regarding "teaching away" from using cell free samples is not persuasive because the TaqMan methods disclosed by Kalinina can be used with samples prepared from single cells as done by Li and Zhang.

§ 103 rejection over Li or Zhang in view of Chou

Patent Owner argues (Response, p. 22) that Chou does not cure the "deficiencies" of Li and Zhang. Since Li and Zhang are not deficient, other than failing to teach the hot start PCR method of Chou, for the reasons discussed above, this argument is not persuasive.

§ 103 rejection over Li or Zhang in view of Burg

Patent Owner argues (Response, p. 23) that Burg does not cure the "deficiencies" of Li and Zhang. Since Li and Zhang are not deficient, other than using fewer than 60 cycles of PCR, for the reasons discussed above, this argument is not persuasive.

§ 103 rejection over Li or Zhang in view of Trümper

Patent Owner argues (Response, p. 24) that Trümper does not cure the "deficiencies" of Li and Zhang. Since Li and Zhang are not deficient for the reasons discussed above, this argument is not persuasive.

§ 103 rejection over Li or Zhang in view of Pontén

Patent Owner argues (Response, pp. 24-25) that Pontén does not cure the "deficiencies" of Li and Zhang. Since Li and Zhang are not deficient for the reasons discussed above, this argument is not persuasive.

§ 103 rejection over Li or Zhang in view of Kanzler

Patent Owner argues (Response, pp. 25-26) that Kanzler does not cure the "deficiencies" of Li and Zhang. Since Li and Zhang are not deficient for the reasons discussed above, this argument is not persuasive.

§ 103 rejection over Li or Zhang in view of Gravel

Patent Owner argues (Response, pp. 26-27) that Gravel does not cure the "deficiencies" of Li and Zhang. Since Li and Zhang are not deficient for the reasons discussed above, this argument is not persuasive.

§ 103 rejection over Li or Zhang in view of Schwab

Patent Owner argues (Response, pp. 27-28) that Schwab does not cure the "deficiencies" of Li and Zhang. Since Li and Zhang are not deficient for the reasons discussed above, this argument is not persuasive. Patent Owner further argues that the claims are directed to determining the ratio of genetic sequences in a population of genetic sequences of a biological sample, which is distinct from a cell-by-cell analysis. This argument is not persuasive because determining the percentage of cells in a biological sample that have an amplified gene will yield "a ratio which reflects the composition of the biological sample," which is all the claims require. Patent Owner argues that it is impossible to determine whether a genetic sequence is amplified using a probe that hybridizes within the amplified sequence, citing the Shendure declaration (¶ 14). This is incorrect. For example, Gelmini discloses a method for determining whether the c-*erb*B-2 oncogene is amplified, using quantitative TaqMan PCR with a probe binding within the c-*erb*B-2 oncogene (entire document). Gelmini also teaches that other methods for quantitative PCR are known in the art (p. 752).

Conclusion

Claims 1-12, 14-16, 19-32, 38-44, 46-48 and 51-64 are rejected. Claims 13, 17, 18, 33-37, 45, 49 and 50 are not subject to reexamination.

Extensions of Time

THIS ACTION IS MADE FINAL.

A shortened statutory period for response to this action is set to expire 2 months from the mailing date of this action.

Extensions of time under 37 CFR 1.136(a) do not apply in reexamination proceedings. The provisions of 37 CFR 1.136 apply only to "an applicant" and not to parties in a reexamination proceeding. Further, in 35 U.S.C. 305 and in 37 CFR 1.550(a), it is required that reexamination proceedings "will be conducted with special dispatch within the Office."

Extensions of time in reexamination proceedings are provided for in 37 CFR 1.550(c). A request for extension of time must be filed on or before the day on which a response to this action is due, and it must be accompanied by the petition fee set forth in 37 CFR 1.17(g). The mere filing of a request will not effect any extension of time. An extension of time will be granted only for sufficient cause, and for a reasonable time specified.

The filing of a timely first response to this final rejection will be construed as including a request to extend the shortened statutory period for an additional month, which will be granted even if previous extensions have been granted. In no event however, will the statutory period for response expire later than SIX MONTHS from the mailing date of the final action. See MPEP § 2265.

Duty to Disclose

The patent owner is reminded of the continuing responsibility under 37 CFR 1.565(a) to apprise the Office of any litigation activity, or other prior or concurrent proceeding, involving U.S. Patent No. 6,440,706 throughout the course of this reexamination proceeding. The third party requester is also reminded of the ability to similarly apprise the Office of any such activity or proceeding throughout the course of this reexamination proceeding. See MPEP §§ 2207, 2282 and 2286.

Correspondence

Any inquiry concerning this communication or earlier communications from the examiner should be directed to BRUCE CAMPELL whose telephone number is (571)272-0974. The examiner can normally be reached on Monday - Thursday from 8:00 to 5:00. The examiner can also be reached on alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Deborah Jones, can be reached on 571-272-1535.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

All correspondence relating to this ex parte reexamination proceeding should be directed:

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Alexandria, VA 22314

/Bruce Campell/
Patent Reexamination Specialist
Central Reexamination Unit 3991

/Padmashri Ponnaluri/
Patent Reexamination Specialist
Central Reexamination Unit 3991

/Deborah D Jones/
Supervisory Patent Examiner, Art Unit 3991

Office Action in Ex Parte Reexamination	Control No. 90/012,894	Patent Under Reexamination 6,440,706 B1 E	
	Examiner BRUCE CAMPELL	Art Unit 3991	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

a. ☒ Responsive to the communication(s) filed on *1/27/2014* .
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

b. ☒ This action is made FINAL.

c. ☐ A statement under 37 CFR 1.530 has not been received from the patent owner.

A shortened statutory period for response to this action is set to expire *2* month(s) from the mailing date of this letter. Failure to respond within the period for response will result in termination of the proceeding and issuance of an *ex parte* reexamination certificate in accordance with this action. 37 CFR 1.550(d). **EXTENSIONS OF TIME ARE GOVERNED BY 37 CFR 1.550(c).** If the period for response specified above is less than thirty (30) days, a response within the statutory minimum of thirty (30) days will be considered timely.

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☒ Notice of References Cited by Examiner, PTO-892.
2. ☒ Information Disclosure Statement, PTO/SB/08.
3. ☐ Interview Summary, PTO-474.
4. ☐ _____.

Part II SUMMARY OF ACTION

1a. ☒ Claims *1-12,14-16,19-32,38-44,46-48 and 51-64* are subject to reexamination.

1b. ☒ Claims *13,17,18,33-37,45,49 and 50* are not subject to reexamination.

2. ☐ Claims _____ have been canceled in the present reexamination proceeding.

3. ☐ Claims _____ are patentable and/or confirmed.

4. ☒ Claims *1-12,14-16,19-32,38-44,46-48 and 51-64* are rejected.

5. ☐ Claims _____ are objected to.

6. ☐ The drawings, filed on _____ are acceptable.

7. ☐ The proposed drawing correction, filed on _____ has been (7a) ☐ approved (7b) ☐ disapproved.

8. ☐ Acknowledgment is made of the priority claim under 35 U.S.C. § 119(a)-(d) or (f).

a) ☐ All b) ☐ Some* c) ☐ None of the certified copies have

1 ☐ been received.

2 ☐ not been received.

3 ☐ been filed in Application No. _____ .

4 ☐ been filed in reexamination Control No. _____.

5 ☐ been received by the International Bureau in PCT application No. _____.

* See the attached detailed Office action for a list of the certified copies not received.

9. ☐ Since the proceeding appears to be in condition for issuance of an *ex parte* reexamination certificate except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte* Quayle, 1935 C.D. 11, 453 O.G. 213.

10. ☐ Other: _____

cc: Requester (if third party requester)

Notice of References Cited	Application/Control No. 90/012,894	Applicant(s)/Patent Under Reexamination 6,440,706 B1	
	Examiner BRUCE CAMPELL	Art Unit 3991	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
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NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	http://en.wikipedia.org/wiki/Semen_analysis, downloaded 4/17/2014
	V	S Gelmini et al., "Quantitative polymerase chain reaction-based homogeneous assay with fluorogenic probes to measure c-erbB-2 oncogene amplification." Clinical Chemistry 43(5): 752-758 (1997)
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

PTO/SB/08a (01-10)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	90012894
Filing Date	2013-06-17
First Named Inventor	
Art Unit	3991
Examiner Name	Bruce R. Campell
Attorney Docket Number	001107.00989

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	1	Defendants Life Technologies Corporation, Applied Biosystems, LLC, and Ion Torrent Systems, Inc.'s Preliminary Non-Infringement and Patent invalidity Contensions pursuant to Local Rule 103.3, filed in Case No. Case No. 1:12-CV-1173 on August 22, 2013	☐
	2	Deposition of Michael Metzker, Ph.D., dated October 25, 2013	☐
	3	Declaration of Michael Metzker, Ph.D. executed September 27, 2013	☐
	4	BAKER et al., "Male Mice Defective in the DNA Mismatch Repair Gene PMS2 Exhibit Abnormal Chromosome Synapsis in Meiosis," Cell, vol. 82, 309-319, July 28, 1995	☐
	5	BISCHOFF et al., "Single cell analysis demonstrating somatic mosaicism involving 11p in a patient with paternal isodisomy and Beckwith-Wiedemann syndrome," Human Molecular Genetics, 1995, vol. 4, no. 3, 395-399	☐
	6	BRISCO et al., "Detection and quantitation of neoplastic cells in acute lymphoblastic leukaemia, by use of teh polymerase chain reaction," British Journal of Haematology, 1991, 79, 211-217	☐
	7	DREESEN et al., "Preimplantation genetic diagnosis of spinal muscular atrophy," Molecular Human Reproduction, vol. 4, no. 9, pp. 881-885, 1998	☐
	8	FLINT et al., "NR2A Subunit Expression Shortens NMDA Receptor Synaptic Currents in Developing Neocortex," The Journal of Neuroscience, April 1, 1997, 17(7):2469-2476	☐
	9	GAYNOR et al., "Use of Flow Cytometry and RT-PCR for Detecting Gene Expression by Single Cells," BioTechniques, vol. 21, no. 2 (1996)	☐
	10	GRAVEL et al., "Single-Cell Analysis of the t(14;18)(q32;q21) Chromosomal Translocation in Hodgkin's Disease Demonstrates the Absence of This Translocation in Neoplastic Hodgkin and Reed-Sternberg Cells," Blood, 1998, 91:2866-2874	☐
	11	GREWAL et al., "The mutation properties of spinal and bulbar muscular atrophy disease alleles," Neurogenetics (1998) 1:249-252	☐

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	12	JEFFREYS et al., "Complex gene conversion events in germline mutation at human minisatellites," Nature Genetics, vol. 6, February 1994	☐
	13	JENA et al., "Amplification of genes, single transcripts and cDNA libraries from one cell and direct sequence analysis of amplified products derived from one molecule," Journal of Immunological Methods 190 (1996) 199-213	☐
	14	KUNST et al., "The effect of FMR1 CFF repeat interruptions on mutation frequency as measured by sperm typing," J. Med. Genet., 1997; 34:627-631	☐
	15	LEEFLANG et al., "Single sperm analysis of the trinucleotide repeats in the Huntington's disease gene: quantification of the mutation frequency spectrum," Human Molecular Genetics, 1995, vol. 4, no. 9, 1519-1526	☐
	16	LEVINSON et al., "Molecular Characterization of Transgene-induced Immunodeficiency in B-less Mice Using a Novel Quantitative Limiting Dilution Polymerase Chain Reaction Method," J. Exp. Med, vol. 178, July 1993, 317-329	☐
	17	LI et al., "Amplification and analysis of DNA sequences in single human sperm and diploid cells," Nature, vol. 335, September 29, 1988	☐
	18	LIA et al., "Somatic instability of the CTG repeat in mice transgenic for the myotonic dystrophy region is age dependent but not correlated to the relative intertissue transcription levels and proliferative capacities," Human Molecular Genetics, 1998, vol. 7, no. 8, 1285-1291	☐
	19	LIU et al., "Efficiency and accuracy of polymerase-chain-reaction assay for cystic fibrosis allele F508 in single cell," The Lancet, vol. 339, May 16, 1992	☐
	20	SHEEHY et al., "Concurrent evolution of regions of the envelope and polymerase genes of human immunodeficiency virus type 1 observed during zidovudine (AZT) therapy," Journal of General Virology, (1996), 76, 1071-1081	☐
	21	SIMMONDS et al., "Human immunodeficiency virus-infected individuals contain provirus in small numbers of peripheral mononuclear cells and at low copy numbers," Journal of Virology, February 1990, vol. 64, no. 2, p. 864-872	☐
	22	STARK et al., "Single-cell PCR performed with neurofibroma Schwann cells reveals the presence of both alleles of the neurofibromatosis type 1 (NF1) gene," Hum Genet (1995) 96:619-523	☐

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /B.C./

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	Attorney Docket Number	001107.00989

	23	SYKES et al., "Quantitation of Targets for PCR by Use of Limiting Dilution," BioTechniques, vol. 13, no. 3 (1992)	☐
	24	ZHANG et al., "Whole genome amplification from a single cell: Implications for genetic analysis," Proc. Natl. Acad. Sci., vol. 89, pp. 5847-5851, July 1992	☐
	25	ZHANG et al., "Selection for Specific Sequences in the External Envelope Protein of Human Immunodeficiency Virus Type 1 upon Primary Infection," Journal of Virology, June 1993, vol. 67, no. 6, p. 3345-3356	☐
	26	Exhibit 4.1 (Baker)	☐
	27	Exhibit 4.10 (Levinson)	☐
	28	Exhibit 4.11 (Li 1988)	☐
	29	Exhibit 4.12 (Lia)	☐
	30	Exhibit 4.13 (Liu)	☐
	31	Exhibit 4.14 (Munier)	☐
	32	Exhibit 4.15 (Sheehy)	☐
	33	Exhibit 4.16 (Simmonds)	☐

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	Attorney Docket Number	001107.00989

	34	Exhibit 4.17 (Stark)	☐
	35	Exhibit 4.18 (Sykes)	☐
	36	Exhibit 4.19 (Zhang 1992)	☐
	37	Exhibit 4.2 (Bischoff)	☐
	38	Exhibit 4.20 (Zhang 1993)	☐
	39	Exhibit 4.21 (Flint)	☐
	40	Exhibit 4.22 ((Gaynor)	☐
	41	Exhibit 4.23 (Jena)	☐
	42	Exhibit 4.3 (Brisco)	☐
	43	Exhibit 4.4 (Dreesen)	☐
	44	Exhibit 4.5 (Gravel)	☐

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /B.C./

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	Art Unit	3991
	Examiner Name	Bruce R. Campell
	Attorney Docket Number	001107.00989

	45	Exhibit 4.6 (Grewal)	☐
	46	Exhibit 4.7 (Jeffreys)	☐
	47	Exhibit 4.8 (Kunst)	☐
	48	Exhibit 4.9 (Leeflang)	☐
	49	Exhibit 5.1 (Baker)	☐
	50	Exhibit 5.10 (Levinson)	☐

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EXAMINER SIGNATURE

Examiner Signature	/Bruce Campell/	Date Considered	04/18/2014

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

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	First Named Inventor	
	Art Unit	3991
	Examiner Name	Bruce R. Campell
	Attorney Docket Number	001107.00989

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Sarah A. Kagan/	Date (YYYY-MM-DD)	2014-03-27
Name/Print	Sarah A. Kagan	Registration Number	32141

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PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	90012894
Filing Date	2013-06-17
First Named Inventor	
Art Unit	3991
Examiner Name	Bruce R. Campell
Attorney Docket Number	001107.00989

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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Examiner Initials*	Cite No	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, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

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Attorney Docket Number	001107.00989

	1	Supplemental Joint Claim Construction Statement filed in Civil Action No. 12-cv-1173-CCE-JEP on October 28, 2013 (filed with exhibits A, B, and C)	☐
	2	Defendants' Responsive Claim Construction Brief filed in Case No. 1:12-CV-1173 on November 26, 2013	☐
	3	Deposition of David Sherman, Ph.D., dated October 17, 2013	☐
	4	Supplemental Joint Claim Construction Statement Exhibit C filed in Civil Action No. 12-cv-1173-CCE-JEP on October 28, 2013 (filed with Supplemental Joint Claim Construction Statement filed in Civil Action No. 12-cv-1173-CCE-JEP on October 28, 2013)	☐
	5	Plaintiffs' Responsive Claim Construction Brief filed in filed in Civil Action No. 12-cv-1173-CCE-JEP on November 26, 2013	☐
	6	Plaintiffs' Proposed Construction of Disputed Terms, Supporting Evidence, and Rebuttal Evidence Exhibit B, filed in filed in Civil Action No. 12-cv-1173-CCE-JEP on October 28, 2013 (filed with Supplemental Joint Claim Construction Statement filed in Civil Action No. 12-cv-1173-CCE-JEP on October 28, 2013)	☐
	7	Declaration of David H. Sherman in Support of Esoterix Genetic Laboratories' Claim Construction Brief filed in Civil Action Nos. 12-cv-411-CCE-JEP and 12-cv-1173-CCE-JEP, executed September 27, 2013	☐
	8	Defendants' Opening Claim Construction Brief filed in Case No. 1:12-CV-1173 on November 5, 2013	☐
	9	Exhibit A filed in Civil Action No. 12-cv-1173-CCE-JEP on October 28, 2013 (filed with Supplemental Joint Claim Construction Statement filed in Civil Action No. 12-cv-1173-CCE-JEP on October 28, 2013)	☐
	10	Plaintiffs' Opening Claim Construction Brief filed in Civil Action No. 12-cv-1173-CCE-JEP on November 5, 2013	☐

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	First Named Inventor	
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	Examiner Name	Bruce R. Campell
	Attorney Docket Number	001107.00989

EXAMINER SIGNATURE			
Examiner Signature	/Bruce Campell/	Date Considered	04/18/2014

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

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	Attorney Docket Number	001107.00989

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OR

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☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

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Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	90012894	6,440,706 B1 ET AL.
	Examiner	Art Unit
	BRUCE CAMPELL	3991

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
reviewed prosecution history of 09/613,826google: gene amplification in cancer review	7/12/137/24/13	/BC//BC/

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

Reexamination	Application/Control No.	Applicant(s)/Patent Under Reexamination
	90012894	6,440,706 B1 ET AL.
	Certificate Date	Certificate Number
		C1

Requester Correspondence Address:	☐ Patent Owner	☒ Third Party

LIFE TECHNOLOGIES CORPORATION
ATTN: IP DEPARTMENT
5791 VAN ALLEN WAY
CARLSBAD, CA 92008

LITIGATION REVIEW ☒	/BC/ (examiner initials)	06/27/2013 (date)
Case Name		Director Initials
Esoterix Genetic Laboratories, Llc et al v. Life Technologies		
U.S. District - North Carolina Middle 1:12cv1173		

COPENDING OFFICE PROCEEDINGS	
TYPE OF PROCEEDING	NUMBER
1. none	

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	1	Exhibit 5.11 (Li 1988)	☐
	2	Exhibit 5.12 (Lia)	☐
	3	Exhibit 5.13 (Liu)	☐
	4	Exhibit 5.14 (Munier)	☐
	5	Exhibit 5.15 (Sheehy)	☐
	6	Exhibit 5.16 (Simmonds)	☐
	7	Exhibit 5.17 (Stark)	☐
	8	Exhibit 5.18 (Sykes)	☐
	9	Exhibit 5.19 (Zhang 1992)	☐
	10	Exhibit 5.2 (Bischoff)	☐
	11	Exhibit 5.20 (Zhang 1993)	☐

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	Attorney Docket Number	001107.00989

	12	Exhibit 5.21 (Flint)	☐
	13	Exhibit 5.22 (Gaynor)	☐
	14	Exhibit 5.23 (Jena)	☐
	15	Exhibit 5.3 (Brisco)	☐
	16	Exhibit 5.4 (Dreesen)	☐
	17	Exhibit 5.5 (Gravel)	☐
	18	Exhibit 5.6 (Grewal)	☐
	19	Exhibit 5.7 (Jeffreys)	☐
	20	Exhibit 5.8 (Kunst)	☐
	21	Exhibit 5.9 (Leeflang)	☐
	22	Exhibit 6.1 (Baker)	☐

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	23	Exhibit 6.10 (Levinson)	☐
	24	Exhibit 6.11 (Li 1988)	☐
	25	Exhibit 6.12 (Lia)	☐
	26	Exhibit 6.13 (Liu)	☐
	27	Exhibit 6.14 (Munier)	☐
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EXAMINER SIGNATURE

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re *Ex Parte* Reexamination of
U.S. Patent No. 6,440,706

Examiner: Bruce R. Campell

Issued: August 27, 2002

Art Unit: 3991

Reexam Control No.: 90/012,894

Reexam Filing Date: June 17, 2013

Confirmation No.: 8442

For: DIGITAL AMPLIFICATION

NOTIFICATION OF ACTION (EXTENSION OF STAY) IN CONCURRENT LITIGATION

Mail Stop *Ex Parte* Reexam
Attn: Central Reexamination Unit
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Examiner:

Third-Party Requester hereby provides notice that the Court, upon mutual request by both parties, has extended the stay of the concurrent litigation proceeding at least until September 29, 2014 (*Esoterix Genetic Laboratories, LLC v. Life Technologies Corporation* (Civil Action No. 1:12-cv-01173-CCE-JEP)). A copy of the Court Order extending the stay is attached.

Dated: 5/13/14

Respectfully submitted,

By: /Ashita Doshi/
Ashita A. Doshi
Reg. No. 57,327

Life Technologies Corporation
5791 Van Allen Way
Carlsbad, California 92008
(760) 845-2798

U.S. Patent No.: 6,440,706
Reexam No.: 90/012,894
Filing Date: June 17, 2013
Title: DIGITAL AMPLIFICATION
Inventor: BERT VOGELSTEIN
Issue Date: August 27, 2002
Examiner: Bruce R. Campell

CERTIFICATE OF SERVICE

I hereby certify that a true and accurate copy of the above-identified Notification of Action (Extension of Stay) in Concurrent Litigation by Third Party Requester Life Technologies Corporation was served on the patent owner through its attorney/agent of record on May 14, 2014 by First Class mail to the following address:

Banner & Witcoff, Ltd.
1100 13th Street N.W.
Suite 1200
Washington DC 20005-4051

Dated: May 14, 2014

Respectfully submitted,

By: /Elizabeth Morgan/
Elizabeth Morgan
Patent Paralegal

Life Technologies Corporation
2130 Woodward St., Bldg. 1
Austin, TX 78744
Customer No.: 52059

From: ECF@ncmd.uscourts.gov [mailto:ECF@ncmd.uscourts.gov]
Sent: Tuesday, April 29, 2014 11:00 AM
To: ecf@ncmd.uscourts.gov
Subject: Activity in Case 1:12-cv-01173-CCE-JEP ESOTERIX GENETIC LABORATORIES, LLC et al v. LIFE TECHNOLOGIES CORPORATION, et al Order

U.S. District Court

North Carolina Middle District

Notice of Electronic Filing

The following transaction was entered on 4/29/2014 at 2:00 PM EST and filed on 4/29/2014

Case Name:	ESOTERIX GENETIC LABORATORIES, LLC et al v. LIFE TECHNOLOGIES CORPORATION, et al
Case Number:	1:12-cv-01173-CCE-JEP
Filer:	
Document Number:	No document attached

Docket Text:
TEXT ORDER: Consistent with the request of the parties, (see Doc. 87), the stay in this case is extended through September 29, 2014. The parties shall confer in August 2014 and shall, no later than August 28, 2014, inform the Court of the status of the patent re-examinations, in a joint submission if possible. To the extent the parties agree that the stay should be extended or allowed to expire, they will inform the Court in the status report. To the extent they do not agree, each party may file a brief no longer than ten pages supporting its position. SO ORDERED. Signed by JUDGE CATHERINE C. EAGLES on April 29, 2014. (EAGLES, CATHERINE)

1:12-cv-01173-CCE-JEP Notice has been electronically mailed to:

ALLISON O. VAN LANINGHAM avanlaningham@vldlitigation.com, creynolds@turningpointlit.com, dgordon@vldlitigation.com

ANNE S. TOKER annetoker@quinnemanuel.com

JOHN STEVEN GARDNER sgardner@kilpatricktownsend.com, radkins@kilpatricktownsend.com, sjarrell@kilpatricktownsend.com

KATHERINE NOLAN-STEVAUX katherine.nolan-stevaux@lifetech.com

KATRINA M. QUICKER kquicker@bakerlaw.com, vdotson@bakerlaw.com

LESLIE THOMAS GRAB lgrab@kilpatricktownsend.com, jgiordano-coltart@kilpatricktownsend.com

MATIAS FERRARIO MFerrario@kilpatricktownsend.com, amjohnson@kilpatricktownsend.com

PAUL K. SUN , JR paul_sun@elliswinters.com, elizabeth.worthy@elliswinters.com

PETER J. ARMENIO peterarmenio@quinnemanuel.com

STEPHEN MCDANIEL RUSSELL , JR srussell@vldlitigation.com, dgordon@vldlitigation.com

SUSAN A. CAHOON scahoon@kilpatricktownsend.com

1:12-cv-01173-CCE-JEP Notice will not be electronically mailed to:

Electronic Acknowledgement Receipt

EFS ID:	19025963
Application Number:	90012894
International Application Number:	
Confirmation Number:	8442
Title of Invention:	Digital Amplification
First Named Inventor/Applicant Name:	6,440,706 B1
Customer Number:	11332
Filer:	Ashita Amu Doshi/Elizabeth Morgan
Filer Authorized By:	Ashita Amu Doshi
Attorney Docket Number:	001107.00989
Receipt Date:	14-MAY-2014
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Application Type:	Reexam (Third Party)

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Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		LT00831REX-notification-EXT-stay-concurrent-litigation-w-serv-cert-5-14-14.pdf	188243 f0dddcbbe9ed230a313ee2fb2388add6a11fc776	yes	4

	Multipart Description/PDF files in .zip description		
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	Notice of concurrent proceeding(s)	1	1
	Reexam Certificate of Service	2	2
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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re *Ex Parte* Reexamination:	)	Group Art Unit: 3991
	)	
U.S. Patent No. 6,440,706	)	Docket No. 001107.00989
	)	
Control No. 90/012,894	)	Confirmation No: 8442
	)	
Reexam Filing Date: June 17, 2013	)	Examiner: Bruce R. Campell

For: DIGITAL AMPLIFICATION

RESPONSIVE AMENDMENT TO FINAL OFFICE ACTION

U.S. Patent and Trademark Office
Customer Service Window
Randolph Building
401 Dulany Street
Alexandria, VA 22314

Sir:

This paper is in response to the final Office Action mailed May 9, 2014.

Amendments to the Claims are reflected in the Listing of Claims, which begins on page 2 of this paper.

Remarks/Arguments begin on page 9 of this paper.

IN THE CLAIMS

Please amend the following claims as indicated by the status identifier. Patent claims under reexamination but not amended are indicated as "original." Patent claims not subject to reexamination are not shown.

1. (Amended) A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of:

diluting isolated nucleic acid template molecules [in] isolated from a biological sample to form a set comprising a plurality of assay samples;

amplifying the template molecules within the assay samples to form a population of amplified molecules in the assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence;

comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

2. (Original) The method of claim 1 wherein the step of diluting is performed until at least one-tenth of the assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required for the step of analyzing to determine the presence of the selected genetic sequence.

3. (Amended) The method of claim 1 wherein the step of diluting is performed until between 0.1 and 0.9 of the assay samples yield an amplification product of at least one of the selected and reference genetic sequences when subjected to a polymerase chain reaction.

4. (Original) The method of claim 1 wherein the step of diluting is performed until all of the assay samples yield an amplification product when subjected to a polymerase chain reaction and each assay sample contains less than 10 nucleic acid template molecules containing the reference genetic sequence.

5. (Original) The method of claim 1 wherein the step of diluting is performed until all of the assay samples yield an amplification product when subjected to a polymerase chain reaction and each assay sample contains less than 100 nucleic acid template molecules containing the reference genetic sequence.

6. (Original) The method of claim 1 wherein the biological sample is cell-free.

7. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 10.

8. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 50.

9. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 100.

10. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 500.

11. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 1000.

12. (Original) The method of claim 1 wherein the step of amplifying and the step of analyzing are performed on assay samples in the same receptacle.

13. (Not subject to reexamination)

14. (Original) The method of claim 1 wherein the step of analyzing employs gel electrophoresis.

15. (Original) The method of claim 1 wherein the step of analyzing employs hybridization to at least one nucleic acid probe.

16. (Original) The method of claim 1 wherein the step of analyzing employs hybridization to at least two nucleic acid probe.

17-18. (Not subject to reexamination)

19. (Original) The method of claim 1 wherein the step of amplifying employs a single pair of primers.

20. (Original) The method of claim 1 wherein the step of amplifying employs a polymerase which is activated only after heating.

21. (Original) The method of claim 1 wherein the step of amplifying employs at least 40 cycles of heating and cooling.

22. (Original) The method of claim 1 wherein the step of amplifying employs at least 50 cycles of heating and cooling.

23. (Original) The method of claim 1 wherein the step of amplifying employs at least 60 cycles of heating and cooling.

24. (Original) The method of claim 1 wherein the biological sample is selected from the group consisting of stool, blood, and lymph nodes.

25. (Original) The method of claim 1 wherein the biological sample is blood or bone marrow of a leukemia or lymphoma patient who has received anti-cancer therapy.

26. (Original) The method of claim 1 wherein the selected genetic sequence is a

translocated allele.

27. (Original) The method of claim 1 wherein the selected genetic sequence is a wild-type allele.

28. (Original) The method of claim 1 wherein the selected genetic sequence is within an amplicon which is amplified during neoplastic development.

29. (Original) The method of claim 1 wherein the selected genetic sequence is a rare exon sequence.

30. (Original) The method of claim 1 wherein the nucleic acid template molecules comprise cDNA of RNA transcripts and the selected genetic sequence is present on a cDNA of a first transcript and the reference genetic sequence is present on a cDNA of a second transcript.

31. (Original) The method of claim 1 wherein the selected genetic sequence comprises a first mutation and the reference genetic sequence comprises a second mutation.

32. (Original) The method of claim 1 wherein the selected genetic sequence and the reference genetic sequence are on distinct chromosomes.

33-37. (Not subject to reexamination)

38. (Twice amended) A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of:

distributing cell-free nucleic acid template molecules from a biological sample to form a set comprising a plurality of assay samples;

amplifying the nucleic acid template molecules [within a set comprising a plurality of assay samples] to form a population of amplified molecules in [each of the] individual assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first

number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence, wherein at least one-fiftieth of the assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required to determine the presence of the selected genetic sequence;

comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

39. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 10.

40. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 50.

41. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 100.

42. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 500.

43. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 1000.

44. (Original) The method of claim 38 wherein the step of amplifying and the step of analyzing are performed on assay samples in the same receptacle.

45. (Not subject to reexamination)

46. (Original) The method of claim 38 wherein the step of analyzing employs gel electrophoresis.

47. (Original) The method of claim 38 wherein the step of analyzing employs hybridization to at least one nucleic acid probe.

48. (Amended) The method of claim 38 wherein the step of analyzing employs hybridization to at least two nucleic acid [probe] probes.

49-50. (Not subject to reexamination)

51. (Original) The method of claim 38 wherein the step of amplifying employs a single pair of primers.

52. (Original) The method of claim 38 wherein the step of amplifying employs a polymerase which is activated only after heating.

53. (Original) The method of claim 38 wherein the step of amplifying employs at least 40 cycles of heating and cooling.

54. (Original) The method of claim 38 wherein the step of amplifying employs at least 50 cycles of heating and cooling.

55. (Original) The method of claim 38 wherein the step of amplifying employs at least 60 cycles of heating and cooling.

56. (Original) The method of claim 38 wherein the template molecules are obtained from a body sample selected from the group consisting of stool, blood, and lymph nodes.

57. (Original) The method of claim 38 wherein the template molecules are obtained from a body sample of a leukemia or lymphoma patient who has received anti-cancer therapy, said body sample being selected from the group consisting of blood and bone marrow.

58. (Original) The method of claim 38 wherein the selected genetic sequence is a translocated allele.

59. (Original) The method of claim 38 wherein the selected genetic sequence is a wild-type allele.

60. (Original) The method of claim 38 wherein the selected genetic sequence is within an amplicon which is amplified during neoplastic development.

61. (Original) The method of claim 38 wherein the selected genetic sequence is a rare exon sequence.

62. (Original) The method of claim 38 wherein the nucleic acid template molecules comprise cDNA of RNA transcripts and the selected genetic sequence is present on a cDNA of a first transcript and the reference genetic sequence is present on a cDNA of a second transcript.

63. (Original) The method of claim 38 wherein the selected genetic sequence comprises a first mutation and the reference genetic sequence comprises a second mutation.

64. (Original) The method of claim 38 wherein the selected genetic sequence and the reference genetic sequence are on distinct chromosomes.

Remarks

Status of claims

Claims 1-12, 14-16, 19-32, 38-44, 46-48, and 51-64 are pending and subject to re-examination. Claims 1, 3, 38, and 48 are amended.

Claims 13, 17, 18, 33-37, 45, 49, and 50 are not subject to re-examination.

Amendments do not expand the scope of the patent claims

The amended claims do not enlarge the scope of the patent claims because they each include all limitations of an issued patent claim.

Clams 1 and 38 are amended to clarify the nucleic acid template molecules previously recited. Claim 38 has been amended to include an additional initial step. Claim 3 has been amended to add a clarifying recitation regarding the amplification products produced. Claim 48 is amended to correct a grammatical error. No claim recitations have been removed, obviated, or vitiated. Therefore all claims are narrower than at least one patent claim.

Support for amendments

The amendment to claim 1 to recite isolated nucleic acid template molecules is supported at col. 6, lines 45-49. The amendment to claim 3 is supported at col. 3, lines 27-32, at col. 4, lines 12-32, and col. 6, lines 3-8. The amendment to claim 38 to recite distribution of cell-free nucleic acid template molecules is supported at col. 4, lines 12-32 and col. 6, lines 45-49.

All amendments are supported fully by the specification and do not add new matter

Interview

We thank the re-examination examiners for agreeing to the interview scheduled for July 10, 2014.

1. Novelty

a. Li (*Nature 225:*414-417, 1988)

Claims 1-3, 7-9, 15, 16, 19, 21, 22, 27, 32, 38-41, 47, 48, 51, 53, 54, 59 and 64 stand rejected under §102(b) as anticipated by Li. Claims 1 and 38 are the only independent claims of the rejected claim set.

The Patent and Trademark Office cites Li's experiment with lymphocytes described at pages 414-415 as anticipating claim 1. (Final office action at page 7.) Li micromanipulated an artificial mixture of tissue culture cells from two individuals to isolate individual cells. The individual cells were separately lysed, their nucleic acids templates amplified and analyzed. Li does not teach dilution of isolated nucleic acid template molecules that are isolated from a biological sample in order to form a set comprising a plurality of assay samples, as recited in claim 1, step 1, as amended.

Moreover, Li does not teach step 4 of claim 1. Li does not teach any comparison of the number of assay samples of a selected genetic sequence to a reference genetic sequence to ascertain a ratio which reflects the composition of the biological sample. With the benefit of hindsight knowledge gained from the present invention, the Patent and Trademark Office may have produced such a comparison or ratio, but Li did not teach it. Li was merely showing that: DNA contamination was insignificant, and no sample hybridized with both probes (indicating that a single cell only was introduced into each tube, and that DNA from lysed cells present in the co-cultivation mixture did not adhere to individual cells.) Page 414, col. 2, lines 21-26.

The Patent and Trademark Office similarly asserts that Li's experiment with single human sperm anticipates claim 1. Individual sperm were micromanipulated, their nucleic acids released, and amplified. For the same reasons as with the lymphocyte experiment, this teaching of Li does not disclose all limitations of claim 1 as amended. Li does not teach diluting isolated nucleic acid template molecules isolated from a biological sample to form a plurality of assay samples, as recited in claim 1, step 1.

Li also does not anticipate independent claim 38 because Li does not teach distributing

cell-free nucleic acid template molecules to form a set comprising a plurality of assay samples, as recited in step 1. Li's multiple assays are formed by distribution of whole cells, rather than cell-free nucleic acid template molecules. Li therefore does not disclose distributing cell-free nucleic acid template molecules to form a set comprising a plurality of assay samples.

For these reasons, none of independent claims 1 and 38 or their dependent claims 2-3, 7-9, 15, 16, 19, 21, 22, 27, 32, 39-41, 47, 48, 51, 53, 54, 59 and 64 are anticipated by Li.

Please withdraw this rejection.

b. Zhang (*Proc. Natl. Academy Sciences USA 89:*5847-51, 1992)

Claims 1, 2, 7, 14, 19, 27, 32, 38, 39, 46, 51, 59 and 64 stand rejected under §102(b) as anticipated by Zhang. Zhang, like Li, separated human sperm and then lysed single, isolated sperm to yield nucleic acid molecules. Thus, as for Li, Zhang fails to teach step 1 of claim 1 (diluting isolated nucleic acid template molecules isolated from a biological sample) as amended. Similarly, as for Li, Zhang fails to teach step 1 of claim 38 which requires distribution of cell-free nucleic acid template molecules from a biological sample to form a set comprising a plurality of assay samples.

None of dependent claims 2, 7, 14, 19, 27, 32, 39, 46, 51, 59 and 64 are anticipated by Zhang for at least the same reasons as for independent claims 1 and 38.

Please withdraw this rejection.

2. Non-obviousness

a. Li or Zhang in view of Jeffreys (*Nucleic Acid Research 16:*10953-71, 1988)

Claims 4-6 stand rejected under §103(a) as obvious over either Li or Zhang and further in view of Jeffreys. All three of the rejected claims are dependent on claim 1.

Claims 4 and 5 recite dilution until all assay samples yield an amplification product, and that each assay sample contains less than 10 (claim 4) or less than 100 (claim 5) nucleic acid template molecules containing the reference sequence. Claim 6 recites that the biological sample is

cell-free.

Li and Zhang are both cited as teaching micromanipulation of single cells into separate assay samples. Both fail to teach dilution of isolated nucleic acid template molecules as recited in step 1 of claim 1.

Jeffreys is cited as teaching dilution of DNA from a cell-free sample.

To establish a proper *prima facie* case of obviousness, the following criteria must be established: (1) the prior art reference, or references when combined, must disclose or suggest all the claim limitations (*See In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991)); (2) the Patent Office must provide an apparent reason to combine the known elements in the claims (*See KSR International Co. v. Teleflex Inc.*, 550 U.S. 398 (2007)); and (3) there must be a reasonable expectation of success in combining the teachings of the reference(s) (*See id.*) However, it is impermissible to use the claimed invention as an instruction manual or "template" to piece together the teachings of the prior art so that the claimed invention is rendered obvious. *In re Fritch,* 972 F.2d 1260, 1266 (Fed. Cir. 1992).

Contrary to the assertion of the Patent and Trademark Office, it would have not have been obvious to combine Jeffreys with either Li or Zhang to meet the limitations of any of claims 4-6. The combination has been made improperly using hindsight knowledge obtained from the present invention. The proposed combination would have destroyed the intended purpose of each of Li and Zhang. Zhang and Li both teach micromanipulation of isolated, single cells or sperm to form individual assay samples. This micromanipulation method serves to ensure that all chromosomes within a cell or sperm remain together throughout the assay in a single assay sample. For example, Zhang teaches typing individual sperm cells for 12 loci (Table 2) located on multiple chromosomes. Li focuses on the benefits of a single cell analysis to achieve accurate measurements of genetic distances of less than 1 cM, (page 416, col. 2, lines 7-11) to genetically map species that cannot be bred or have long generation times (page 417, sentence spanning col. 1 and 2). Similarly, Li teaches the benefit of single-cell analysis for studying cell-to-cell variations in development. (Page 417, col. 2, lines 12-15). The focus on the benefits of single-cell analysis would have led one of ordinary skill in the art away from combining Li or Zhang's teachings with Jeffreys' technique using a cell-free sample. Jeffreys' technique would have obviated the advantages that Li and Zhang taught for their

single-cell methods by separating the chromosomes of a single sperm or cell and mixing them with the chromosomes of other sperm or cells.

Zhang explicitly articulates concerns associated with using a cell-free sample. Page 5850, col. 2, lines 60-70. Zhang expresses concern over sampling errors, particularly in the context of small, cell-free, forensic or ancient DNA samples.

Thus one of ordinary skill in the art would not have intentionally destroyed the primary references' intentional functional "linkage" of chromosomes (*i.e.,* keeping a single cell's set of chromosomes together in a single assay sample) by diluting cell-free nucleic acid templates as the rejection proposes. This proposed modification would destroy the information that Li and Zhang were trying to collect. January 27, 2014 declaration of Jay Shendure, MD, PhD, ("Shendure Declaration") at ¶12.

Jeffreys also does not teach the specific limitation of claims 4 and 5 in which each sample yields an amplification product. Jeffreys teaches thatonly 46% of PCR reactions were successful. Although the Patent and Trademark Office asserts that it would have been obvious to one of ordinary skill in the art to double or triple the amount of DNA to achieve this recitation, this is not supported by the facts. Although the Patent and Trademark Office's asserted motivation was to save time and reagents, doubling or tripling the amount of DNA would not be possible in the case of rare forensic samples (part of the Patent and Trademark Office's asserted motivation). Moreover, doubling and tripling would contradict the very purpose of primary references Li and Zhang, who scrupulously worked to have just one cell's DNA in each sample. Shendure Declaration at ¶13. Thus one of ordinary skill in the art would not have been motivated to combine Jeffreys with Li or Zhang or to increase the amount of DNA template in each sample.

Please withdraw this rejection

b. Li or Zhang in view of Kalinina (*Nucleic Acid Research 25:*1999-2004, 1997)

Claims 12 and 44 stand rejected under §103(a) as obvious over either Li or Zhang and further in view of Kalinina. Claims 12 and 44 are dependent on claims 1 and 38, respectively. Claims 12 and 44 further recite amplification and analysis in the same receptacle.

Li and Zhang teach analysis of single cells, not dilution of isolated template molecules from a biological sample, or distribution of cell-free nucleic acid template molecules from a biological sample to form a plurality of assay samples, as recited in claims 1 and 38, respectively. Kalinina is cited to teach amplifying and analyzing in the same receptacle single molecules of template DNA

It is improper for the Patent and Trademark Office to use the claims as a framework and to employ individual naked parts of separate prior art references as a mosaic to recreate a facsimile of the claimed invention. *See W.L. Gore & Assoc. v. Garlock*, 721 F.2d 1550, 1552-53 (Fed. Cir. 1983).

The combination of Kalinina with the methods of Li and Zhang does not in any event remedy the deficiencies of Li and Zhang. Kalinina does not suggest comparing two different numbers of two different template molecules by determining a number of assay samples. The Patent and Trademark Office's combination of reference teachings is the result of selective extraction of portions of the references with the benefit of hindsight, using the subject claims as a model.

Please withdraw this rejection.

c. Li or Zhang in view of Chou (*Nucleic Acids Research 20:* 1717-23, 1992)

Claims 20 and 52 stand rejected under §103(a) as obvious over either Li or Zhang and further in view of Chou. Claims 20 and 52 depend from claims 1 and 38 , respectively, and further recite use of a heat-activatable polymerase. Chou is cited as teaching a heat-activatable polymerase. Chou does not, however, remedy the deficiencies of Li or Zhang in teaching the elements of independent claims 1 and 38 from which claims 20 and 52 depend. None of the three references teaches the dilution and/or distribution of isolated or cell-free nucleic acid template molecules from a biological sample to form a set comprising a plurality of assay samples.

Please withdraw this rejection.

d. Li or Zhang in view of Burg (*J. Clin. Microbiol. 27:*1787-92, 1989)

Claims 23 and 55 stand rejected under §103(a) as obvious over either Li or Zhang and further in view of Burg. Claims 23 and 55 depend from claims 1 and 38 respectively and further recite at least 60 cycles of heating and cooling. Burg is cited as teaching at least 60 cycles of heating and cooling to amplify DNA of a single cell of Toxoplasma. Burg does not, however, remedy the deficiencies of Li or Zhang in teaching the elements of independent claims 1 and 38, in particular, in step 1. None of the three references teaches the dilution and/or distribution of isolated or cell-free nucleic acid template molecules to form a set comprising a plurality of assay samples.

Please withdraw this rejection.

e. Li or Zhang in view of Trümper (*Blood 81:*3097-4115, 1993)

Claims 24, 29, 30, 56, 61 and 62 stand rejected under §103(a) as obvious over either Li or Zhang and further in view of Trümper. These claims, dependent on claims 1 and 38, further recite a biological sample which is stool, blood, or lymph nodes (claims 24 and 56), a selected sequence which is a rare exon (claims 29 and 61), and the template molecules are cDNA molecules (claims 30 and 62).

Trümper is cited as teaching lysis of isolated cells from lymph nodes followed by RT-PCR to detect a p53 mutation.

Because Trümper taught isolating single cells, it does not remedy the deficiency of Li and Zhang, who also taught isolating single cells. None of the references teach diluting isolated nucleic acid template molecules isolated from a biological sample, or distributing cell-free nucleic acid template molecules from a biological sample, as recited in independent claims 1 and 38. Thus the combination of references does not render any of claims 24, 29, 30, 56, 61 and 62 obvious due to failure to teach or suggest a non-single cell, nucleic acid analysis.

Please withdraw this rejection.

f. Li or Zhang in view of Pontén (*Mut. Res. Genomics 382:*44-55, 1997)

Claims 31 and 63 stand rejected under §103(a) as obvious over either Li or Zhang and further in view of Pontén. Claims 31 and 63, dependent on claims 1 and 38, respectively, further recite that the two analyzed sequences comprise a first and a second mutation. Pontén is cited as teaching single-cell PCR analysis of tumor cells and detection of two different point mutations in p53, at codon 245 and at codon 266. Since all three cited references used a single-cell isolation technique, none of them teaches or suggests the dilution of isolated nucleic acid templates from a biological sample to form a plurality of samples, nor the distribution of cell free nucleic acid templates from a biological sample to form a plurality of assay samples. Such single-cell methods as taught by the references are antipodal to the claimed method. The claimed methods would not have been obvious.

Please withdraw this rejection.

g. Li or Zhang in view of Kanzler (*Blood 87:*3429-36, 1996)

Claims 10, 11, 25, 28, 42, 43, 57 and 60 stand rejected under §103(a) as being unpatentable over either Li or Zhang and further in view of Kanzler. Claims 10, 11, 42 and 43 are dependent on claims 1 and 38 and further recite use of sets of assay samples of at least 500 or at least 1000 samples. Claims 25 and 57 are dependent on claims 1 and 38 and further recite blood or bone marrow of a leukemia or lymphoma patient who received anti-cancer therapy as the biological sample. Claims 28 and 60 are dependent on claims 1 and 38 and further recite a selected genetic sequence which is within an amplicon amplified during neoplastic development.

Kanzler is cited as teaching, like Li and Zhang, single-cell analysis. Kanzler taught micromanipulation of single cells. Kanzler does not remedy the deficiencies of Li and Zhang. None of the three references teach diluting isolated nucleic acid template molecules isolated from a biological sample or distributing cell-free nucleic acid template molecule from a biological sample to form a plurality of assay samples.

Please withdraw this rejection.

h. Li or Zhang in view of Gravel (*Blood 91:*2866-74, 1998)

Claims 26 and 58 stand rejected under §103(a) as being unpatentable over either Li or Zhang and further in view of Gravel. These claims depend from claims 1 and 38, respectively, and further recite that the selected sequence is a translocated allele.

Gravel, like Li and Zhang, is cited as teaching a single-cell analysis. Thus Gravel does not cure the defect of Li and Zhang in teaching the methods of independent claims 1 and 38, particularly the first steps of each of them.

Thus Gravel's analysis of a translocated allele in combination with Li or Zhang are not sufficient to render the subject matter of dependent claims 26 and 58 obvious. None of the references, alone or in the asserted combination teaches or suggests the dilution of isolated nucleic acid template molecules isolated from a biological sample or the distribution of cell-free nucleic acid template molecules from a biological sample to form a plurality of assay samples.

Please withdraw this rejection.

i. Li or Zhang in view of Schwab (*Bioessays 20:*473-479, 1998)

Claims 28 and 60 stand rejected under §103(a) as being unpatentable over either Li or Zhang and further in view of Schwab.

Claims 28 and 60 depend on claims 1 and 38, respectively. Additionally, they recite an amplicon which is amplified during neoplastic development. Schwab is cited for teaching amplified *MYCN* and *ERBB2* as prognostic markers. However, the combinations of cited teachings would not render the claimed invention obvious. Li and Zhang teach single-cell analysis. They do not teach step 1 of either claim 1 or claim 38, as amended, because they both employ a step of single-cell dilution or distribution. The Schwab reference does not remedy the basic deficiency of the primary references.

Please withdraw this rejection.

Respectfully submitted,

By: /Sarah A. Kagan/
Sarah A. Kagan
Registration No. 32,141

Dated: July 9, 2014

Banner & Witcoff, Ltd.
Customer No. 11332

Electronic Acknowledgement Receipt

EFS ID:	19531785
Application Number:	90012894
International Application Number:	
Confirmation Number:	8442
Title of Invention:	Digital Amplification
First Named Inventor/Applicant Name:	6,440,706 B1
Customer Number:	11332
Filer:	Sarah Anne Kagan./Jennifer Hazzard
Filer Authorized By:	Sarah Anne Kagan.
Attorney Docket Number:	001107.00989
Receipt Date:	09-JUL-2014
Filing Date:	17-JUN-2013
Time Stamp:	15:20:30
Application Type:	Reexam (Patent Owner)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		Response-to-FOA-90012894.pdf	166691 f8ec8b954d2b42a62d21a4a08cc99fadaaeca35a	yes	18

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Response After Final Action	1	1
	Claims	2	8
	Applicant Arguments/Remarks Made in an Amendment	9	18
Warnings:			
Information:			
	Total Files Size (in bytes):	166691	

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re *Ex Parte* Reexamination:	)	Group Art Unit: 3991
	)	
U.S. Patent No. 6,440,706	)	Docket No. 001107.00989
	)	
Control No. 90/012,894	)	Confirmation No: 8442
	)	
Reexam Filing Date: June 17, 2013	)	Examiner: Bruce R. Campell

For: DIGITAL AMPLIFICATION

CERTIFICATE OF SERVICE

The undersigned certifies that a complete copy of the Responsive Amendment to Final Office Action filed in the U.S. Patent and Trademark Office on July 9, 2014, has been mailed via first class mail to the third party requester this day at the following address:

Life Technologies Corporation
Attn: IP Department
5791 Van Allen Way
Carlsbad, CA 92008

/Sarah A. Kagan/
Sarah A. Kagan
Registration No. 32,141

Dated: July 9, 2014

Banner & Witcoff, Ltd.
Customer No. 11332

Electronic Acknowledgement Receipt

EFS ID:	19532792
Application Number:	90012894
International Application Number:	
Confirmation Number:	8442
Title of Invention:	Digital Amplification
First Named Inventor/Applicant Name:	6,440,706 B1
Customer Number:	11332
Filer:	Sarah Anne Kagan./Jennifer Hazzard
Filer Authorized By:	Sarah Anne Kagan.
Attorney Docket Number:	001107.00989
Receipt Date:	09-JUL-2014
Filing Date:	17-JUN-2013
Time Stamp:	15:52:20
Application Type:	Reexam (Patent Owner)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Reexam Certificate of Service	Certificate-of-Service.pdf	78531 841ebe310df23540264e4613825646b1882c6300	no	1

Warnings:	
Information:	

Total Files Size (in bytes):	78531

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UNITED STATES PATENT AND TRADEMARK OFFICE

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United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
90/012,894	06/17/2013	6,440,706 B1	001107.00989	8442

11332 7590 07/22/2014

Banner & Witcoff, Ltd.
Attorneys for client 001107
1100 13th Street N.W.
Suite 1200
Washington, DC 20005-4051

EXAMINER	
CAMPELL, BRUCE R	
ART UNIT	PAPER NUMBER
3991	

MAIL DATE	DELIVERY MODE
07/22/2014	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

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Alexandria, VA 22313-1450
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LIFE TECHNOLOGIES CORPORATION

ATTN: IP DEPARTMENT

5791 VAN ALLEN WAY

CARLSBAD, CA 92008

EX PARTE REEXAMINATION COMMUNICATION TRANSMITTAL FORM

REEXAMINATION CONTROL NO. *90/012,894*.

PATENT NO. *6,440,706 B1*.

ART UNIT *3991*.

Enclosed is a copy of the latest communication from the United States Patent and Trademark Office in the above identified *ex parte* reexamination proceeding (37 CFR 1.550(f)).

Where this copy is supplied after the reply by requester, 37 CFR 1.535, or the time for filing a reply has passed, no submission on behalf of the *ex parte* reexamination requester will be acknowledged or considered (37 CFR 1.550(g)).

Ex Parte Reexamination Interview Summary	**Control No.**	**Patent Under Reexamination**
	90/012,894	6,440,706 B1
	Examiner	**Art Unit**
	BRUCE CAMPELL	3991

All participants (USPTO personnel, patent owner, patent owner's representative):

(1) *BRUCE CAMPELL*	(3) *Sarah Kagan, Joseph Skerpon*
(2) *Deborah Jones, Padmashri Ponnaluri*	(4) *Kathryn Wade, Tina McEwan*

Date of Interview: *10 July 2014*

Type: a)☐ Telephonic b)☐ Video Conference
c)☒ Personal (copy given to: 1)☐ patent owner 2)☐ patent owner's representative)

Exhibit shown or demonstration conducted: d)☐ Yes e)☒ No.
If Yes, brief description: _____

Agreement with respect to the claims f)☐ was reached. g)☒ was not reached. h)☐ N/A.
Any other agreement(s) are set forth below under "Description of the general nature of what was agreed to…"

Claim(s) discussed: *all*.

Identification of prior art discussed: *Li, Zhang, Jeffreys*.

Description of the general nature of what was agreed to if an agreement was reached, or any other comments:
Discussed the amendment filed 7/9/2014. It was agreed that the amendments, if entered, overcome the 102 rejections over Li and Zhang. Amendment alone appears insufficient to overcome 103 rejection over Li, Zhang and Jeffreys(as presently applied to claims 4-6, but would be applicable to all claims in combination with other references of record), since Jeffreys discloses every physical step of the method of claim 1. Exrs will fully consider PO's submission after final. PO intends to file evidence (declaration) to support argument of non-obviousness. .

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims patentable, if available, must be attached. Also, where no copy of the amendments that would render the claims patentable is available, a summary thereof must be attached.)

A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION MUST INCLUDE PATENT OWNER'S STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. (See MPEP § 2281). IF A RESPONSE TO THE LAST OFFICE ACTION HAS ALREADY BEEN FILED, THEN PATENT OWNER IS GIVEN **ONE MONTH** FROM THIS INTERVIEW DATE TO PROVIDE THE MANDATORY STATEMENT OF THE SUBSTANCE OF THE INTERVIEW (37 CFR 1.560(b)). THE REQUIREMENT FOR PATENT OWNER'S STATEMENT CAN NOT BE WAIVED. **EXTENSIONS OF TIME ARE GOVERNED BY 37 CFR 1.550(c).**

/Bruce Campell/ Patent Reexamination Specialist Central Reexamination Unit 3991	/Padmashri Ponnaluri/ Patent Reexamination Specialist Central Reexamination Unit 3991	/Deborah Jones/ Supervisory Patent Reexamination Specialist Central Reexamination Unit 3991

cc: Requester (if third party requester)

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
90/012,894	06/17/2013	6,440,706 B1	001107.00989	8442

11332 7590 07/22/2014

Banner & Witcoff, Ltd.
Attorneys for client 001107
1100 13th Street N.W.
Suite 1200
Washington, DC 20005-4051

EXAMINER	
CAMPELL, BRUCE R	
ART UNIT	PAPER NUMBER
3991	

MAIL DATE	DELIVERY MODE
07/22/2014	PAPER

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LIFE TECHNOLOGIES CORPORATION

ATTN: IP DEPARTMENT

5791 VAN ALLEN WAY

CARLSBAD, CA 92008

EX PARTE REEXAMINATION COMMUNICATION TRANSMITTAL FORM

REEXAMINATION CONTROL NO. *90/012,894*.

PATENT NO. *6,440,706 B1 E*.

ART UNIT *3991*.

Enclosed is a copy of the latest communication from the United States Patent and Trademark Office in the above identified *ex parte* reexamination proceeding (37 CFR 1.550(f)).

Where this copy is supplied after the reply by requester, 37 CFR 1.535, or the time for filing a reply has passed, no submission on behalf of the *ex parte* reexamination requester will be acknowledged or considered (37 CFR 1.550(g)).

Ex Parte Reexamination Advisory Action	Control Number	Patent Under Reexamination	
	90/012,894	6,440,706 B1 E	
	Examiner	Art Unit	AIA (First Inventor to File) Status
	BRUCE CAMPELL	3991	No

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

THE PROPOSED RESPONSE FILED *09 July 2014* FAILS TO OVERCOME ALL OF THE REJECTIONS IN THE FINAL REJECTION MAILED *09 May 2014*. Therefore, unless a timely appeal is filed, or other appropriate action by the patent owner is taken to overcome all of the outstanding rejection(s), this *ex parte* reexamination proceeding WILL BE TERMINATED and a Notice of Intent to Issue *Ex Parte* Reexamination Certificate will be mailed in due course. Any finally rejected claims, or claims objected to, will be CANCELLED.

THE PERIOD FOR RESPONSE IS EXTENDED TO RUN *4* MONTHS FROM THE MAILING DATE OF THE FINAL REJECTION.

(Extensions of time are governed by 37 CFR 1.550(c))

1. ☐ Appellant's Brief is due two months from the date of the Notice of Appeal filed on _____ (or within the extended period for response set forth above, whichever is later). See 37 CFR 1.191(d) and 37 CFR 1.192(a).

2. ☒ The proposed amendment(s) will not be entered because:
   (a) ☒ they raise new issues that would require further consideration and/or search (see NOTE below);
   (b) ☐ they raise the issue of new matter (see NOTE below);
   (c) ☐ they are not deemed to place the proceeding in better form for appeal by materially reducing or simplifying the issues for appeal; and/or
   (d) ☐ they present additional claims without canceling a corresponding number of finally rejected claims.
   NOTE: *See Continuation Sheet*

3. ☐ Patent owner's proposed response filed _____ has overcome the following rejection(s):_____

4. ☐ The proposed new or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).

5. ☐ An affidavit(s)/declaration(s) under **37 CFR 1.130(b)** was/were filed on _____.

6. ☐ The a)☐ affidavit/declaration, b)☐ exhibit, or c)☐ request for reconsideration has been considered but does NOT overcome the rejection(s) because: __ .

7. ☐ The affidavit/declaration or exhibit will NOT be considered because it is not directed SOLELY to issues which were newly raised by the Examiner in the final rejection.

8. ☒ For purposes of Appeal, the proposed amendment(s) a)☒ will not be entered or b)☐ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.

   The status of the claim(s) is (or will be) as follows:
   Claim(s) patentable and/or confirmed: _____
   Claim(s) objected to: _____
   Claim(s) rejected: *1-12,14-16,19-32,38-44,46-49 and 51-64*
   Claim(s) not subject to reexamination: *13,17,18,33-37,45,49,50*

9. ☐ The drawing correction filed on _____ a)☐ has b)☐ has not been approved by the Examiner.

10. ☐ Note the attached Information Disclosure Statement(s), PTO-1449, Paper No(s) _____.

11. ☐ Other:_____

Bruce Campell
Primary Examiner
Art Unit: 3991

cc: Requester (if third party requester)

Continuation of 2. (d) NOTE: The proposed amendment would have overcome the § 102 rejections over Li and Zhang. However further consideration is required because the § 103 rejection over Li or Zhang in combination with Jeffreys, presently applied to claims 4-6, could render the claims obvious because Jeffreys performs the "diluting," "amplifying" and "analyzing" steps recited in claims 1 and 38. Patent Owner's response does not explain why it would not have been obvious to use this method to determine the ratio of genetic sequences in a population of sequence, given the fact that Jeffreys showed that PCR could detect target sequences in a "genome equivalent" (6 pg) of DNA obtained by diluting bulk DNA. It is noted that in the interview of 7/10/2014 Patent Owner indicated that it intends to submit evidence (declaration) addressing this question..

/Bruce Campell/
Patent Reexamination Specialist

/Padmashri Ponnaluri/
Patent Reexamination Specialist

/Deborah Jones/
Supervisory Patent Reexamination Specialist
Central Reexamination Unit 3991

IN THE CLAIMS

Please amend the following claims as indicated by the status identifier. Patent claims under reexamination but not amended are indicated as "original." Patent claims not subject to reexamination are not shown.

1. (Amended) A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of:

diluting isolated nucleic acid template molecules [in] isolated from a biological sample to form a set comprising a plurality of assay samples;

amplifying the template molecules within the assay samples to form a population of amplified molecules in the assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence;

comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

2. (Original) The method of claim 1 wherein the step of diluting is performed until at least one-tenth of the assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required for the step of analyzing to determine the presence of the selected genetic sequence.

3. (Amended) The method of claim 1 wherein the step of diluting is performed until between 0.1 and 0.9 of the assay samples yield an amplification product of at least one of the selected and reference genetic sequences when subjected to a polymerase chain reaction.

4. (Original) The method of claim 1 wherein the step of diluting is performed until all of the assay samples yield an amplification product when subjected to a polymerase chain reaction and each assay sample contains less than 10 nucleic acid template molecules containing the reference genetic sequence.

5. (Original) The method of claim 1 wherein the step of diluting is performed until all of the assay samples yield an amplification product when subjected to a polymerase chain reaction and each assay sample contains less than 100 nucleic acid template molecules containing the reference genetic sequence.

6. (Original) The method of claim 1 wherein the biological sample is cell-free.

7. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 10.

8. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 50.

9. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 100.

10. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 500.

11. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 1000.

12. (Original) The method of claim 1 wherein the step of amplifying and the step of analyzing are performed on assay samples in the same receptacle.

13. (Not subject to reexamination)

14. (Original) The method of claim 1 wherein the step of analyzing employs gel electrophoresis.

15. (Original) The method of claim 1 wherein the step of analyzing employs hybridization to at least one nucleic acid probe.

16. (Original) The method of claim 1 wherein the step of analyzing employs hybridization to at least two nucleic acid probe.

17-18. (Not subject to reexamination)

19. (Original) The method of claim 1 wherein the step of amplifying employs a single pair of primers.

20. (Original) The method of claim 1 wherein the step of amplifying employs a polymerase which is activated only after heating.

21. (Original) The method of claim 1 wherein the step of amplifying employs at least 40 cycles of heating and cooling.

22. (Original) The method of claim 1 wherein the step of amplifying employs at least 50 cycles of heating and cooling.

23. (Original) The method of claim 1 wherein the step of amplifying employs at least 60 cycles of heating and cooling.

24. (Original) The method of claim 1 wherein the biological sample is selected from the group consisting of stool, blood, and lymph nodes.

25. (Original) The method of claim 1 wherein the biological sample is blood or bone marrow of a leukemia or lymphoma patient who has received anti-cancer therapy.

26. (Original) The method of claim 1 wherein the selected genetic sequence is a

translocated allele.

27. (Original) The method of claim 1 wherein the selected genetic sequence is a wild-type allele.

28. (Original) The method of claim 1 wherein the selected genetic sequence is within an amplicon which is amplified during neoplastic development.

29. (Original) The method of claim 1 wherein the selected genetic sequence is a rare exon sequence.

30. (Original) The method of claim 1 wherein the nucleic acid template molecules comprise cDNA of RNA transcripts and the selected genetic sequence is present on a cDNA of a first transcript and the reference genetic sequence is present on a cDNA of a second transcript.

31. (Original) The method of claim 1 wherein the selected genetic sequence comprises a first mutation and the reference genetic sequence comprises a second mutation.

32. (Original) The method of claim 1 wherein the selected genetic sequence and the reference genetic sequence are on distinct chromosomes.

33-37. (Not subject to reexamination)

38. (Twice amended) A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of:

distributing cell-free nucleic acid template molecules from a biological sample to form a set comprising a plurality of assay samples;

amplifying the nucleic acid template molecules [within a set comprising a plurality of assay samples] to form a population of amplified molecules in [each of the] individual assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first

number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence, wherein at least one-fiftieth of the assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required to determine the presence of the selected genetic sequence;

comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

39. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 10.

40. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 50.

41. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 100.

42. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 500.

43. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 1000.

44. (Original) The method of claim 38 wherein the step of amplifying and the step of analyzing are performed on assay samples in the same receptacle.

45. (Not subject to reexamination)

46. (Original) The method of claim 38 wherein the step of analyzing employs gel electrophoresis.

47. (Original) The method of claim 38 wherein the step of analyzing employs hybridization to at least one nucleic acid probe.

48. (Amended) The method of claim 38 wherein the step of analyzing employs hybridization to at least two nucleic acid [probe] probes.

49-50. (Not subject to reexamination)

51. (Original) The method of claim 38 wherein the step of amplifying employs a single pair of primers.

52. (Original) The method of claim 38 wherein the step of amplifying employs a polymerase which is activated only after heating.

53. (Original) The method of claim 38 wherein the step of amplifying employs at least 40 cycles of heating and cooling.

54. (Original) The method of claim 38 wherein the step of amplifying employs at least 50 cycles of heating and cooling.

55. (Original) The method of claim 38 wherein the step of amplifying employs at least 60 cycles of heating and cooling.

56. (Original) The method of claim 38 wherein the template molecules are obtained from a body sample selected from the group consisting of stool, blood, and lymph nodes.

57. (Original) The method of claim 38 wherein the template molecules are obtained from a body sample of a leukemia or lymphoma patient who has received anti-cancer therapy, said body sample being selected from the group consisting of blood and bone marrow.

58. (Original) The method of claim 38 wherein the selected genetic sequence is a translocated allele.

59. (Original) The method of claim 38 wherein the selected genetic sequence is a wild-type allele.

60. (Original) The method of claim 38 wherein the selected genetic sequence is within an amplicon which is amplified during neoplastic development.

61. (Original) The method of claim 38 wherein the selected genetic sequence is a rare exon sequence.

62. (Original) The method of claim 38 wherein the nucleic acid template molecules comprise cDNA of RNA transcripts and the selected genetic sequence is present on a cDNA of a first transcript and the reference genetic sequence is present on a cDNA of a second transcript.

63. (Original) The method of claim 38 wherein the selected genetic sequence comprises a first mutation and the reference genetic sequence comprises a second mutation.

64. (Original) The method of claim 38 wherein the selected genetic sequence and the reference genetic sequence are on distinct chromosomes.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re *Ex Parte* Reexamination:	)	Group Art Unit: 3991
	)	
U.S. Patent No. 6,440,706	)	Docket No. 001107.00989
	)	
Control No. 90/012,894	)	Confirmation No: 8442
	)	
Reexam Filing Date: June 17, 2013	)	Examiner: Bruce R. Campell

For: DIGITAL AMPLIFICATION

PATENT OWNER'S INTERVIEW SUMMARY

Examiners Campell, Jones, and Ponnaluri graciously conducted an interview with representatives of the patent owner and a licensee on July 10, 2014. During the interview the patent owner presented the various amendments to the claims that were submitted on July 9, 2014. An advance copy of the amendments had been provided to the examiners on July 7, for their review. The following amendments were raised for comment:

i. Claim 1
    1. Specify ***isolated*** nucleic acid template molecule is diluted
    2. Clarify that nucleic acid template molecule is ***from*** a biological sample
ii. Claim 3—clarify that 0.1 to 0.9 of assay samples have ***at least one of*** selected and reference templates amplified
iii. Claim 38—add step of distributing cell-free nucleic acid template molecule
iv. Claim 48—correct grammar (singular/plural)

The patent owner indicated that the amendments distinguish the claims over the references, particularly with regard to the rejections of certain claims for anticipation and obviousness based on Li (*Nature 225:*414-417, 1988) and Zhang (*Proc. Natl. Academy Sciences USA 89:*5847-51, 1992) and other claims for

obviousness over Li or Zhang in view of Jeffreys (*Nucleic Acid Research 16:*10953-71, 1988).

The patent owner emphasized that different goals and purposes of the prior art from the nonobvious goals and purposes of the claimed methods. In almost all cases the prior art taught qualitative methods, whereas the claimed methods are directed to quantitative methods. The prior art taught methods involving a very small number of assay samples which were sufficient for the qualitative determinations sought. The success of the prior art in their qualitative determinations would not have motivated the ordinary skilled artisan (nor have made obvious the changes needed) to change to a quantitative assessment based on statistics or to change to a method involving large numbers of assay samples, such as greater than 500 or 1000 assay samples.

The examiners suggested that the patent owner may improve the record by submitting declarations demonstrating that the claimed method is commercially used and is considered in the art as a breakthrough technology.

The patent owner acknowledges receipt of the Examiner's Interview Summary mailed July 22, 2014. The patent owner agrees with the report of the proceedings, but not with the interpretation of the claims and the interpretation of the prior art.

/Sarah A. Kagan/
Sarah A. Kagan
Registration No. 32,141

Dated: July 22, 2014

Banner & Witcoff, Ltd.
Customer No. 11332

Electronic Acknowledgement Receipt

EFS ID:	19646197
Application Number:	90012894
International Application Number:	
Confirmation Number:	8442
Title of Invention:	Digital Amplification
First Named Inventor/Applicant Name:	6,440,706 B1
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Filer:	Sarah Anne Kagan./Jennifer Hazzard
Filer Authorized By:	Sarah Anne Kagan.
Attorney Docket Number:	001107.00989
Receipt Date:	22-JUL-2014
Filing Date:	17-JUN-2013
Time Stamp:	14:03:54
Application Type:	Reexam (Patent Owner)

Payment information:

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1	Miscellaneous Incoming Letter	Interview-Summary.pdf	116617 59a43950eaf0bcc6e2db6f275101579478c2ca87	no	2

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re *Ex Parte* Reexamination:	)	Group Art Unit: 3991
	)	
U.S. Patent No. 6,440,706	)	Docket No. 001107.00989
	)	
Control No. 90/012,894	)	Confirmation No: 8442
	)	
Reexam Filing Date: June 17, 2013	)	Examiner: Bruce R. Campell

For: DIGITAL AMPLIFICATION

RESPONSIVE AMENDMENT TO FINAL OFFICE ACTION

U.S. Patent and Trademark Office
Customer Service Window
Randolph Building
401 Dulany Street
Alexandria, VA 22314

Sir:

This paper responds to the final Office Action mailed May 9, 2014, and the comments made in the Advisory Action mailed July 22, 2014.

Amendments to the Claims are reflected in the Listing of Claims, which begins on page 2 of this paper.

Remarks/Arguments begin on page 9 of this paper.

Two **declarations under rule 132** accompany this submission.

A **notice of appeal** accompanies this submission.

IN THE CLAIMS

Please amend the following claims as indicated by the status identifier. Patent claims under reexamination but not amended are indicated as "original." Patent claims not subject to reexamination are not shown.

1. (Amended) A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of:

diluting isolated nucleic acid template molecules [in] isolated from a biological sample to form a set comprising a plurality of assay samples;

amplifying the template molecules within the assay samples to form a population of amplified molecules in the assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence;

comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

2. (Original) The method of claim 1 wherein the step of diluting is performed until at least one-tenth of the assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required for the step of analyzing to determine the presence of the selected genetic sequence.

3. (Amended) The method of claim 1 wherein the step of diluting is performed until between 0.1 and 0.9 of the assay samples yield an amplification product of at least one of the selected and reference genetic sequences when subjected to a polymerase chain reaction.

4. (Original) The method of claim 1 wherein the step of diluting is performed until all of the assay samples yield an amplification product when subjected to a polymerase chain reaction and each assay sample contains less than 10 nucleic acid template molecules containing the reference genetic sequence.

5. (Original) The method of claim 1 wherein the step of diluting is performed until all of the

assay samples yield an amplification product when subjected to a polymerase chain reaction and each assay sample contains less than 100 nucleic acid template molecules containing the reference genetic sequence.

6. (Original) The method of claim 1 wherein the biological sample is cell-free.

7. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 10.

8. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 50.

9. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 100.

10. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 500.

11. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 1000.

12. (Original) The method of claim 1 wherein the step of amplifying and the step of analyzing are performed on assay samples in the same receptacle.

13. (Not subject to reexamination)

14. (Original) The method of claim 1 wherein the step of analyzing employs gel electrophoresis.

15. (Original) The method of claim 1 wherein the step of analyzing employs hybridization to at least one nucleic acid probe.

16. (Amended) The method of claim 1 wherein the step of analyzing employs hybridization to at least two nucleic acid [probe] probes.

17-18. (Not subject to reexamination)

19. (Original) The method of claim 1 wherein the step of amplifying employs a single pair of primers.

20. (Original) The method of claim 1 wherein the step of amplifying employs a polymerase which is activated only after heating.

21. (Original) The method of claim 1 wherein the step of amplifying employs at least 40 cycles of heating and cooling.

22. (Original) The method of claim 1 wherein the step of amplifying employs at least 50 cycles of heating and cooling.

23. (Original) The method of claim 1 wherein the step of amplifying employs at least 60 cycles of heating and cooling.

24. (Original) The method of claim 1 wherein the biological sample is selected from the group consisting of stool, blood, and lymph nodes.

25. (Original) The method of claim 1 wherein the biological sample is blood or bone marrow of a leukemia or lymphoma patient who has received anti-cancer therapy.

26. (Original) The method of claim 1 wherein the selected genetic sequence is a translocated allele.

27. (Original) The method of claim 1 wherein the selected genetic sequence is a wild-type allele.

28. (Original) The method of claim 1 wherein the selected genetic sequence is within an amplicon which is amplified during neoplastic development.

29. (Original) The method of claim 1 wherein the selected genetic sequence is a rare exon sequence.

30. (Original) The method of claim 1 wherein the nucleic acid template molecules comprise cDNA of RNA transcripts and the selected genetic sequence is present on a cDNA of a first transcript and the reference genetic sequence is present on a cDNA of a second transcript.

31. (Original) The method of claim 1 wherein the selected genetic sequence comprises a first mutation and the reference genetic sequence comprises a second mutation.

32. (Original) The method of claim 1 wherein the selected genetic sequence and the reference genetic sequence are on distinct chromosomes.

33-37. (Not subject to reexamination)

38. (Twice amended) A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of:

distributing cell-free nucleic acid template molecules from a biological sample to form a set comprising a plurality of assay samples;

amplifying the nucleic acid template molecules [within a set comprising a plurality of assay samples] to form a population of amplified molecules in [each of the] individual assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence, wherein at least one-fiftieth of the assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required to determine the presence of the selected genetic sequence;

comparing the first number to the second number to ascertain a ratio which reflects the

composition of the biological sample.

39. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 10.

40. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 50.

41. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 100.

42. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 500.

43. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 1000.

44. (Original) The method of claim 38 wherein the step of amplifying and the step of analyzing are performed on assay samples in the same receptacle.

45. (Not subject to reexamination)

46. (Original) The method of claim 38 wherein the step of analyzing employs gel electrophoresis.

47. (Original) The method of claim 38 wherein the step of analyzing employs hybridization to at least one nucleic acid probe.

48. (Amended) The method of claim 38 wherein the step of analyzing employs hybridization to at least two nucleic acid [probe] probes.

49-50. (Not subject to reexamination)

51. (Original) The method of claim 38 wherein the step of amplifying employs a single pair of primers.

52. (Original) The method of claim 38 wherein the step of amplifying employs a polymerase which is activated only after heating.

53. (Original) The method of claim 38 wherein the step of amplifying employs at least 40 cycles of heating and cooling.

54. (Original) The method of claim 38 wherein the step of amplifying employs at least 50 cycles of heating and cooling.

55. (Original) The method of claim 38 wherein the step of amplifying employs at least 60 cycles of heating and cooling.

56. (Original) The method of claim 38 wherein the template molecules are obtained from a body sample selected from the group consisting of stool, blood, and lymph nodes.

57. (Original) The method of claim 38 wherein the template molecules are obtained from a body sample of a leukemia or lymphoma patient who has received anti-cancer therapy, said body sample being selected from the group consisting of blood and bone marrow.

58. (Original) The method of claim 38 wherein the selected genetic sequence is a translocated allele.

59. (Original) The method of claim 38 wherein the selected genetic sequence is a wild-type allele.

60. (Original) The method of claim 38 wherein the selected genetic sequence is within an amplicon which is amplified during neoplastic development.

61. (Original) The method of claim 38 wherein the selected genetic sequence is a rare exon sequence.

62. (Original) The method of claim 38 wherein the nucleic acid template molecules comprise cDNA of RNA transcripts and the selected genetic sequence is present on a cDNA of a first transcript and the reference genetic sequence is present on a cDNA of a second transcript.

63. (Original) The method of claim 38 wherein the selected genetic sequence comprises a first mutation and the reference genetic sequence comprises a second mutation.

64. (Original) The method of claim 38 wherein the selected genetic sequence and the reference genetic sequence are on distinct chromosomes.

Remarks

Status of claims

Claims 1-12, 14-16, 19-32, 38-44, 46-48, and 51-64 are pending and subject to re-examination. Claims 1, 3, 16, 38, and 48 are amended, as previously proposed but not entered. Claim 16 is additionally amended to correct a grammatical error.

Claims 13, 17, 18, 33-37, 45, 49, and 50 are not subject to re-examination.

Support for amendments

The amendment to claim 1 to recite isolated nucleic acid template molecules is supported at col. 6, lines 45-49. The amendment to claim 3 is supported at col. 3, lines 27-32, at col. 4, lines 12-32, and col. 6, lines 3-8. The amendment to claim 38 to recite distribution of cell-free nucleic acid template molecules is supported at col. 4, lines 12-32 and col. 6, lines 45-49.

All amendments are supported fully by the specification and do not add new matter.

Amendments do not expand the scope of the patent claims

The amended claims do not enlarge the scope of the patent claims because they each include all limitations of an issued patent claim.

Clams 1 and 38 are amended to clarify the nucleic acid template molecules previously recited. Claim 38 has been amended to include an additional initial step. Claim 3 has been amended to add a clarifying recitation regarding the amplification products produced. Claims 16 and 48 are amended to correct a grammatical error. No claim recitations have been removed, obviated, or vitiated. Therefore all claims are narrower than at least one patent claim.

1. Novelty

a. Li (*Nature 225:*414-417, 1988)

Claims 1-3, 7-9, 15, 16, 19, 21, 22, 27, 32, 38-41, 47, 48, 51, 53, 54, 59 and 64 stand rejected under §102(b) as anticipated by Li. Claims 1 and 38 are the only independent claims of the rejected claim set.

The Patent and Trademark Office cites Li's experiment with lymphocytes described at pages 414-415 as anticipating claim 1. (Final office action at page 7.) Li micromanipulated an artificial mixture of tissue culture cells from two individuals to isolate individual cells. The individual cells were separately lysed, their nucleic acids templates amplified and analyzed. Li does not teach dilution of isolated nucleic acid template molecules that are isolated from a biological sample in order to form a set comprising a plurality of assay samples, as recited in claim 1, step 1, as amended.

Moreover, Li does not teach step 4 of claim 1. Li does not teach any comparison of the number of assay samples of a selected genetic sequence to a reference genetic sequence to ascertain a ratio which reflects the composition of the biological sample. With the benefit of hindsight knowledge gained from the present invention, the Patent and Trademark Office may have produced such a comparison or ratio, but Li did not teach it. Li was merely showing that: DNA contamination was insignificant, and no sample hybridized with both probes (indicating that a single cell only was introduced into each tube, and that DNA from lysed cells present in the co-cultivation mixture did not adhere to individual cells.) Page 414, col. 2, lines 21-26.

The Patent and Trademark Office similarly asserts that Li's experiment with single human sperm anticipates claim 1. Individual sperm were micromanipulated, their nucleic acids released, and amplified. For the same reasons as with the lymphocyte experiment, this teaching of Li does not disclose all limitations of claim 1 as amended. Li does not teach diluting isolated nucleic acid template molecules isolated from a biological sample to form a plurality of assay samples, as recited in claim 1, step 1.

Li also does not anticipate independent claim 38 because Li does not teach distributing cell-free nucleic acid template molecules to form a set comprising a plurality of assay samples, as recited in step 1. Li's multiple assays are formed by distribution of whole cells, rather than cell-free nucleic acid template molecules. Li therefore does not disclose distributing cell-free nucleic acid template molecules

to form a set comprising a plurality of assay samples.

For these reasons, none of independent claims 1 and 38 or their dependent claims 2-3, 7-9, 15, 16, 19, 21, 22, 27, 32, 39-41, 47, 48, 51, 53, 54, 59 and 64 are anticipated by Li.

Please withdraw this rejection.

b. Zhang (*Proc. Natl. Academy Sciences USA 89:*5847-51, 1992)

Claims 1, 2, 7, 14, 19, 27, 32, 38, 39, 46, 51, 59 and 64 stand rejected under §102(b) as anticipated by Zhang. Zhang, like Li, separated human sperm and then lysed single, isolated sperm to yield nucleic acid molecules. Thus, as for Li, Zhang fails to teach step 1 of claim 1 (diluting isolated nucleic acid template molecules isolated from a biological sample) as amended. Similarly, as for Li, Zhang fails to teach step 1 of claim 38 which requires distribution of cell-free nucleic acid template molecules from a biological sample to form a set comprising a plurality of assay samples.

None of dependent claims 2, 7, 14, 19, 27, 32, 39, 46, 51, 59 and 64 are anticipated by Zhang for at least the same reasons as for independent claims 1 and 38.

Please withdraw this rejection.

2. Non-obviousness

a. Li or Zhang in view of Jeffreys (*Nucleic Acid Research 16:*10953-71, 1988)

Claims 4-6 stand rejected under §103(a) as obvious over either Li or Zhang and further in view of Jeffreys. All three of the rejected claims are dependent on claim 1.

Claims 4 and 5 recite dilution until all assay samples yield an amplification product, and that each assay sample contains less than 10 (claim 4) or less than 100 (claim 5) nucleic acid template molecules containing the reference sequence. Claim 6 recites that the biological sample is cell-free.

Li and Zhang are both cited as teaching micromanipulation of single cells into separate assay samples. Both fail to teach dilution of isolated (or cell free) nucleic acid template molecules as recited in step 1 of claim 1.

Jeffreys is cited as teaching dilution of DNA from a cell-free sample.

To establish a proper *prima facie* case of obviousness, the following criteria must be established: (1) the prior art reference, or references when combined, must disclose or suggest all the claim limitations (*See In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991)); (2) the Patent Office must provide an apparent reason to combine the known elements in the claims (*See KSR International Co. v. Teleflex Inc.*, 550 U.S. 398 (2007)); and (3) there must be a reasonable expectation of success in combining the teachings of the reference(s) (*See id.*) However, it is impermissible to use the claimed invention as an instruction manual or "template" to piece together the teachings of the prior art so that the claimed invention is rendered obvious. *In re Fritch,* 972 F.2d 1260, 1266 (Fed. Cir. 1992).

Contrary to the assertion of the Patent and Trademark Office, it would not have been obvious to combine Jeffreys with either Li or Zhang to meet the limitations of any of claims 4-6. The combination has been made improperly using hindsight knowledge obtained from the present invention. The proposed combination would have destroyed the intended purpose of each of Li and Zhang. Zhang and Li both teach micromanipulation of isolated, single cells or sperm to form individual assay samples. This micromanipulation method serves to ensure that all chromosomes within a cell or sperm remain together throughout the assay in a single assay sample. For example, Zhang teaches typing individual sperm cells for 12 loci (Table 2) located on multiple chromosomes. Li focuses on the benefits of a single cell analysis to achieve accurate measurements of genetic distances of less than 1 cM, (page 416, col. 2, lines 7-11) to genetically map species that cannot be bred or have long generation times (page 417, sentence spanning col. 1 and 2). Similarly, Li teaches the benefit of single-cell analysis for studying cell-to-cell variations in development. (Page 417, col. 2, lines 12-15). The focus on the benefits of single-cell analysis would have led one of ordinary skill in the art *away* from combining Li or Zhang's teachings with Jeffreys' technique using a cell-free sample. Jeffreys' technique would have destroyed the advantages that Li and Zhang taught for their single-cell methods by separating the chromosomes of a single sperm or cell and mixing them with the chromosomes of other sperm or cells.

Zhang teaches away from cell-free sampling. Zhang explicitly articulates concerns associated with using a cell-free sample. Page 5850, col. 2, lines 60-70. Zhang expresses concern over sampling errors, particularly in the context of small, cell-free, forensic or ancient DNA samples.

Thus one of ordinary skill in the art would not have intentionally destroyed the primary

references' intentional functional "linkage" of chromosomes (*i.e.,* keeping a single cell's set of chromosomes together in a single assay sample) by diluting cell-free nucleic acid templates as the rejection proposes. This proposed modification would have destroyed the information that Li and Zhang were trying to collect. January 27, 2014 declaration of Jay Shendure, MD, PhD, ("Shendure Declaration") at ¶12.

These reasons for not combining the references apply with equal force to the independent claims, 1 and 38 as amended.

Jeffreys does not teach the specific limitation of claims 4 and 5 in which each sample yields an amplification product. Jeffreys teaches that only 46% of PCR reactions on the diluted isolated DNA were successful. Li and Zhang are not relevant in this regard because neither of them dilute isolated or cell-free nucleic acid template molecules isolated from a biological sample, so perforce they do not teach a degree of dilution. Although the Patent and Trademark Office asserts that it would have been obvious to one of ordinary skill in the art to double or triple the amount of DNA to achieve this recitation, this is not supported by the record. Although the Patent and Trademark Office's asserted motivation was to save time and reagents, doubling or tripling the amount of DNA would not be possible in the case of rare forensic samples (part of the Patent and Trademark Office's asserted motivation). Moreover, doubling and tripling would contradict the very purpose of primary references Li and Zhang, who scrupulously worked to have just one cell's DNA in each sample. Shendure Declaration at ¶13. Jeffreys similarly wanted to have just one cell's worth of DNA present per assay. Thus one of ordinary skill in the art would not have been motivated to combine Jeffreys with Li or Zhang *and then* to increase the amount of DNA template in each sample.

Additionally, the Patent Owner provides evidence of secondary considerations of non-obviousness in the form of two declarations under 37 C.F.R. § 1.132. Evidence of commercial success, long-felt but unsolved needs, failure of others, and unexpected results "may also serve to 'guard against slipping into use of hindsight' and to resist the temptation to read into the prior art the teachings of the invention in issue." *Graham v. John Deere*, 383 U.S. 1, 36 (1966) (quoting *Monroe Auto Equip. Co. v. Heckethorn Mfg. & Supply Co.*, 332 F.2d 406, 412 (6th Cir. 1964). Furthermore, "such evidence must *always* be considered in connection with the determination of obviousness." *In re Fielder*, 471 F.2d 640, 644) (C.C.P.A. 1973); see also, M.P.E.P. § 716.01(a).

The declaration under rule 132 of Dr. Shih (Exhibit A), provides evidence of non-obviousness. Dr. Shih's declaration introduces evidence regarding the reception of the invention in the technological art and adoption by commercial entities. The declaration demonstrates that those of skill in the art consider digital PCR to be a significant advance in the art. Many articles have cited the original publication of the invention. ¶15. Expensive machines have been developed by commercial vendors to efficiently carry out the method. ¶¶25, 27, and 28. The method has been compared to the prior quantitative amplification method and the claimed method has been found to yield results that are more precise and less ambiguous. ¶17, 27. The method achieves a finer degree of quantitative discrimination. ¶21. The method achieves a higher degree of precision. ¶22. It makes possible the precise evaluation of balance/imbalance between mutant and wild-type alleles. ¶20. Annual meetings have been organized on the topic of digital PCR by at least three different organizations. ¶¶16, 17. The method solves a need in the art: its precision is needed in the screening and detection of aneuploidy. ¶¶20, 21. It achieves the long sought goal of non-invasive detection of Down syndrome. ¶18.

The declaration under rule 132 of Mr. Lapidus (Exhibit B) constitutes additional evidence of the reception of the invention by one of skill in the art at the time of the invention. Mr. Lapidus considers digital PCR to be a brilliant innovation that made a tremendous impact on the field, particularly for generating quantitative data concerning rare genetic sequences. ¶ 11. At the time of the invention, Mr. Lapidus and others skilled in the art were genuinely surprised by the success of the method. ¶ 11. Furthermore, Mr. Lapidus notes that digital PCR addressed a previously unmet need as evidenced by numerous publications that related to how to determine mutant to wild-type genetic ratios and the like. None of these publications, however, described digital enumeration by spatial separation, as used in digital PCR. ¶¶ 11, 12. Additionally, Mr. Lapidus notes that digital PCR was a substantial improvement over other methods in use at the time for determining the ratio of rare or mutant alleles. ¶ 13. Mr. Lapidus is also aware of a number of companies that have marketed or are currently marketing products for use in digital PCR. ¶ 14.

Thus, even if, *arguendo,* the combination of cited references properly formed a *prima facie* case of obviousness, which the Patent Owner does not concede, the secondary considerations such as commercial success, long-felt but unsolved needs, failure of others, and unexpected results indicate the nonobviousness of digital PCR. The secondary considerations would outweigh a *prima facie* case of obviousness.

Please withdraw this rejection.

b. Li or Zhang in view of Kalinina (*Nucleic Acid Research 25:*1999-2004, 1997)

Claims 12 and 44 stand rejected under §103(a) as obvious over either Li or Zhang and further in view of Kalinina. Claims 12 and 44 are dependent on claims 1 and 38, respectively. Claims 12 and 44 further recite amplification and analysis in the same receptacle.

Li and Zhang teach analysis of single cells, not dilution of isolated template molecules from a biological sample, or distribution of cell-free nucleic acid template molecules from a biological sample to form a plurality of assay samples, as recited in claims 1 and 38, respectively. Kalinina is cited to teach amplifying and analyzing in the same receptacle single molecules of template DNA.

It is improper for the Patent and Trademark Office to use the claims as a framework and to employ individual naked parts of separate prior art references as a mosaic to recreate a facsimile of the claimed invention. *See W.L. Gore & Assoc. v. Garlock*, 721 F.2d 1550, 1552-53 (Fed. Cir. 1983).

The combination of Kalinina with the methods of Li and Zhang does not in any event remedy the deficiencies of Li and Zhang. Kalinina does not suggest comparing two different numbers of two different template molecules by determining a number of assay samples. The Patent and Trademark Office's combination of reference teachings is the result of selective extraction of portions of the references with the benefit of hindsight, using the subject claims as a model.

Please withdraw this rejection.

c. Li or Zhang in view of Chou (*Nucleic Acids Research 20:* 1717-23, 1992)

Claims 20 and 52 stand rejected under §103(a) as obvious over either Li or Zhang and further in view of Chou. Claims 20 and 52 depend from claims 1 and 38 , respectively, and further recite use of a heat-activatable polymerase. Chou is cited as teaching a heat-activatable polymerase. Chou does not, however, remedy the deficiencies of Li or Zhang in teaching the elements of independent claims 1 and 38 from which claims 20 and 52 depend. None of the three references teaches the dilution and/or distribution of isolated or cell-free nucleic acid template molecules from a biological sample to form a set comprising a plurality of assay samples.

Please withdraw this rejection.

d. Li or Zhang in view of Burg (*J. Clin. Microbiol. 27:*1787-92, 1989)

Claims 23 and 55 stand rejected under §103(a) as obvious over either Li or Zhang and further in view of Burg. Claims 23 and 55 depend from claims 1 and 38 respectively and further recite at least 60 cycles of heating and cooling. Burg is cited as teaching at least 60 cycles of heating and cooling to amplify DNA of a single cell of Toxoplasma. Burg does not, however, remedy the deficiencies of Li or Zhang in teaching the elements of independent claims 1 and 38, in particular, in step 1. None of the three references teaches the dilution and/or distribution of isolated or cell-free nucleic acid template molecules to form a set comprising a plurality of assay samples.

Please withdraw this rejection.

e. Li or Zhang in view of Trümper (*Blood 81:*3097-4115, 1993)

Claims 24, 29, 30, 56, 61 and 62 stand rejected under §103(a) as obvious over either Li or Zhang and further in view of Trümper. These claims, dependent on claims 1 and 38, further recite a biological sample which is stool, blood, or lymph nodes (claims 24 and 56), a selected sequence which is a rare exon (claims 29 and 61), and the template molecules are cDNA molecules (claims 30 and 62).

Trümper is cited as teaching lysis of isolated cells from lymph nodes followed by RT-PCR to detect a p53 mutation.

Because Trümper taught isolating single cells, it does not remedy the deficiency of Li and Zhang, who also taught isolating single cells. None of the references teach diluting isolated nucleic acid template molecules isolated from a biological sample, or distributing cell-free nucleic acid template molecules from a biological sample, as recited in independent claims 1 and 38. Thus the combination of references does not render any of claims 24, 29, 30, 56, 61 and 62 obvious due to failure to teach or suggest a non-single cell, nucleic acid analysis.

Please withdraw this rejection.

f. Li or Zhang in view of Pontén (*Mut. Res. Genomics 382:*44-55, 1997)

Claims 31 and 63 stand rejected under §103(a) as obvious over either Li or Zhang and further in view of Pontén. Claims 31 and 63, dependent on claims 1 and 38, respectively, further recite that the two analyzed sequences comprise a first and a second mutation. Pontén is cited as teaching single-cell PCR analysis of tumor cells and detection of two different point mutations in p53, at codon 245 and at codon 266. Since all three cited references used a single-cell isolation technique, none of them teaches or suggests the dilution of isolated nucleic acid templates from a biological sample to form a plurality of samples, nor the distribution of cell free nucleic acid templates from a biological sample to form a plurality of assay samples. Such single-cell methods as taught by the references are antipodal to the claimed method. The claimed methods would not have been obvious.

Please withdraw this rejection.

g. Li or Zhang in view of Kanzler (*Blood 87:*3429-36, 1996)

Claims 10, 11, 25, 28, 42, 43, 57 and 60 stand rejected under §103(a) as being obvious over either Li or Zhang and further in view of Kanzler. Claims 10, 11, 42 and 43 are dependent on claims 1 and 38 and further recite use of sets of assay samples of at least 500 or at least 1000 samples. Claims 25 and 57 are dependent on claims 1 and 38 and further recite blood or bone marrow of a leukemia or lymphoma patient who received anti-cancer therapy as the biological sample. Claims 28 and 60 are dependent on claims 1 and 38 and further recite a selected genetic sequence which is within an amplicon amplified during neoplastic development.

Kanzler is cited as teaching, like Li and Zhang, single-cell analysis. Kanzler taught micromanipulation of single cells. Kanzler does not remedy the deficiencies of Li and Zhang. None of the three references teach diluting isolated nucleic acid template molecules isolated from a biological sample or distributing cell-free nucleic acid template molecule from a biological sample to form a plurality of assay samples.

Please withdraw this rejection.

h. Li or Zhang in view of Gravel (*Blood 91:*2866-74, 1998)

Claims 26 and 58 stand rejected under §103(a) as being obvious over either Li or Zhang and further in view of Gravel. These claims depend from claims 1 and 38, respectively, and further recite that the selected sequence is a translocated allele.

Gravel, like Li and Zhang, is cited as teaching a single-cell analysis. Thus Gravel does not cure the defect of Li and Zhang in teaching the methods of independent claims 1 and 38, particularly the first steps of each of them.

Thus Gravel's analysis of a translocated allele in combination with Li or Zhang are not sufficient to render the subject matter of dependent claims 26 and 58 obvious. None of the references, alone or in the asserted combination teaches or suggests the dilution of isolated nucleic acid template molecules isolated from a biological sample or the distribution of cell-free nucleic acid template molecules from a biological sample to form a plurality of assay samples.

Please withdraw this rejection.

i. Li or Zhang in view of Schwab (*Bioessays 20:*473-479, 1998)

Claims 28 and 60 stand rejected under §103(a) as being obvious over either Li or Zhang and further in view of Schwab.

Claims 28 and 60 depend on claims 1 and 38, respectively. Additionally, they recite an amplicon which is amplified during neoplastic development. Schwab is cited for teaching amplified *MYCN* and *ERBB2* as prognostic markers. However, the combinations of cited teachings would not render the claimed invention obvious. Li and Zhang teach single-cell analysis. They do not teach step 1 of either claim 1 or claim 38, as amended, because they both employ a step of single-cell dilution or distribution. The Schwab reference does not remedy the basic deficiency of the primary references.

Please withdraw this rejection.

Conclusion

For at least the reasons stated above and in the Shih Declaration and in the Lapidus Declaration, and for the reasons stated in the previously submitted response and in the the previously

submitted Shendure Declaration, all claims in this reexamination are patentable and should be confirmed. Therefore, we request that the Patent and Trademark Office issue a certificate of reexamination confirming the patentability of all claims. The absence of additional comments regarding the Office Action does not indicate agreement with or concession of any characterization or requirement. If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 202 824 3000.

No additional fees are believed to be due with respect to the filing of this response. However, should any such fees be due, the Commissioner is hereby authorized to charge any such fees in connection with this paper to Deposit Account No. 19-0733.

Respectfully submitted,

By: /Sarah A Kagan/
Sarah A. Kagan
Registration No. 32,141

Dated: September 8, 2014

Banner & Witcoff, Ltd.
Customer No. 11332

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re *Ex Parte* Reexamination:	)	Group Art Unit: 3991
U.S. Patent No. 7,915,015	)	Docket No. 001107.00988
Control No. 90/012,896	)	Confirmation No: 8361
Reexam Filing Date: June 17, 2013	)	Examiner: Bruce R. Campell
For: DIGITAL AMPLIFICATION	)	

DECLARATION OF STANLEY N. LAPIDUS

I, Stanley N. Lapidus, declare:

1. I am the President, CEO, and Founder of SynapDx located at Four Hartwell Place, Lexington, MA 02421.
2. I have a Bachelor of Science degree in Electrical Engineering from The Cooper Union for the Advancement of Science and Art.
3. A true and correct copy of my curriculum vitae is attached to this Declaration as Exhibit 1.
4. I have been retained as an expert consultant by Esoterix Genetic Laboratories in connection with the reexamination of U.S. Patent No. 7,915,015 (the '015 patent).
5. I am inventor on certain patents at issue in a related litigation matter in the United States District Court for the Middle District of North Carolina, Greensboro Division (*Esoterix Genetics Laboratories v. Life Technologies Corporation*, Case No. 12-CV-411).
6. Laboratory Corporation of America is a minority investor in SynapDx.
7. I was a Founder and former President and CEO of Exact Sciences Corporation, and I currently own a small number of shares in Exact, which I purchased on the open market.
8. I have reviewed the '015 patent, including the claims, which I understand is related to determining an allelic imbalance of a first allelic form of a marker and a second allelic form of a marker in a biological sample using a method generally referred to as digital polymerase chain reaction (PCR), a term coined by Dr. Bert Vogelstein and Dr. Kenneth Kinzler and

adopted by the industry.

9. I have been actively engaged in the field of biotechnology research and development for over 27 years. I was recently elected to the College of Fellows of the American Institute of Medical and Biological Engineering, an organization whose Fellows are said to represent the top 2% of the medical and biological engineering community.
10. I was conducting research in this area at the time that Drs. Vogelstein and Kinzler invented and first presented their research on digital PCR.
11. Digital PCR was a brilliant innovation that made a tremendous impact on the field, particularly for generating quantitative data concerning rare genetic sequences. When Drs. Vogelstein and Kinzler first described digital PCR, I, and others skilled in the art, were genuinely surprised by the success of the method and even considered it to be an audacious method to try. Digital PCR was not obvious at the time of its invention to those of us skilled in the art. Researchers in this area immediately appreciated the significance of this invention and its capabilities.
12. Digital PCR met a previously unmet need in the art. Many publications were directed to how to determine mutant to wild-type genetic ratios and the like, but none suggested digital PCR, which allowed for the quantification of rare sequences, including rare mutations or alleles, in a population of sequences, through the use of digital enumeration by spatial separation.
13. Digital PCR was a substantial improvement over other methods used at the time to determine the ratio of mutant or rare sequences to wild type sequences in a sample. Methods in use at the time included cytometry, fluorescence in situ hybridization (FISH), counting, amplification-refractory mutation system (ARMS), and gel-based methods. These methods are distinctly different than digital PCR, which worked better than the methods in use at the time Drs. Vogelstein and Kinzler invented digital PCR.
14. Digital PCR is still in use today. A number of companies have marketed or are currently marketing products for use in digital PCR methods, including, for example, Life Technologies.
15. All statements made herein of my own knowledge are true, and all statements made on

information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the patent.

Full Name of Declarant: STANLEY N. LAPIDUS

Declarant's Signature: 

Date: August 25, 2014

Stanley N. Lapidus

7 Marston Drive
Bedford, NH 03110
slapidus@lapidx.com
+1 603 494 2832

Education

BS Engineering, Cooper Union, New York City, 1970

Employment history

SynapDx Corp. 2009-present

Founder and CEO
Founder and leader of neurodevelopmental diagnostics testing laboratory

Helicos BioSciences Corp. 2003-present

Co-founder, President, CEO, Chairman, BOD member
Co-founded and led single molecule sequencing company

EXACT Sciences Corp. 1995-2006

Founder, President, Chairman, BOD member
Founded and led colorectal cancer early detection company

Cytyc Corp. 1987-1994

Founder, President
Founded and led pap smear diagnostic company.

Itran Corp. 1983-1987

Founder, President
Founded and led industrial machine-vision company

Gentech Inc. 1979-1983

Founder President
Principal at contract engineering company

Raytheon Medical Electronics 1976-1978

Engineering Group Leader, Engineering Manager
Led and managed engineering team at nuclear medicine instrument manufacturer

Elscint Ltd. 1971-1976

Design Engineer, Engineering Group Leader
Designed and led design of nuclear medicine instrumentation

Academic Appointments

Instructor, Harvard/MIT of division of Health Sciences Technology, MIT Sloan School of Management 2002-present

Research Assistant Professor, Department of Pathology, Tufts University School of Medicine 1994-present

Board Appointments

Harvard School of Public Health Center for Cancer Prevention, Advisory Board Member 1995-2000

EXACT Sciences, Director 1995-2006

Cooper Union School of Engineering, Advisory Board Member 1999-present

Precision Therapeutics Director 2001-2013

Harvard MIT Division of Health Sciences and Technology, Advisor 2001-present

Cooper Union Board of Trustees 2002-2012

Helicos Biosciences, Director 2003-2011

T2 Biosystems, Inc., Director 2008 – present

Advisory Board of Technology Fund of Boston Children's Hospital 2009 – present

Daktari DX, Corp. Director 2009-present

Institutes of Medicine, Committee on the Evolution of Translational Omics 2011 – 2012

Fractyl Laboratories Director 2013-present

Honors

Elected as Fellow at American Institute of Medical and Biological Engineering. 2014

Patents and Publications*

Inventor of >30 issued US patents and 20+ pending patent applications.

Patent	Date	Inventor	Title	Subject Matter

	Number	Issued			
1	4,093,857	6-Jun-78	**Lapidus, Stanley N.**	Radiographic normalizing system	Uniformity correction for gamma cameras
2	4,281,249	28-Jul-81	**Lapidus, Stanley N.**	Stepped scanner imaging system	Whole-body gamma camera imaged using step and repeat
3	4,570,217	11-Feb-86	Allen; Bruce S. (East Kingston, NH), Dunalvey; Michael R. (Needham, MA), King; Bruce A. (Bolton, MA), DuPrie; Harold J. (Andover, MA), Hudnall; Richard E. (Nashua, NH), **Lapidus; Stanely N.** (Bedford, NH), Gilbert; Daniel R. (Dracut, MA), Carlson; Anne M. (Wakefield, MA), Thakrar; Kiran (Salem, NH), Doig; Robert C. (Salem, NH), Kimerer; Brian S. (Reading, MA), Sirois; Andrew F. (Lawrence, MA), Poirer; Bruce A. (Bradford, MA), Hunt; Philip G. (Derry, NH), Dziezanowski; Joseph J. (Brighton, MA), Bromberg; Michael A. (Nashua, NH), Brown; Michael (Salem, NH), Friedel; Seymour A. (Merrimack, NH)	Man machine interface	Computer hardware for high-speed graphics
4	4,581,762	8-Apr-86	**Lapidus; Stanley N.** (Bedford, NH), Dziezanowski; Joseph J. (Weare, NH), Friedel; Seymour A. (Goffstown, NH), Greenberg; Michael P. (Manchester, NH)	Vision inspection system	A vision inspection system operable with foreground illumination provides user identification of selected regions of a known object for later comparison to an unknown object.
5	5,143,627	1-Sep-92	**Lapidus; Stanley N.** (Bedford, NH), Polk, Jr.; Lewis T. (Bedford, MA), Farber; Fredric L. (Chestnut Hill, MA), Barlas; J. Morgan (Malden, MA), Hurley; Anne A. (Carver, MA)	Method and apparatus for preparing cells for examination	An apparatus and method provide automated collection and transfer of particles from a liquid suspension to a glass slide for visual examination.
6	5,185,084	9-Feb-93	**Lapidus; Stanley N.** (Bedford, NH), Kamen; Dean (Bedford, NH), Villeneuve; Richard R. (Bedford, NH), Polk, Jr.; Lewis T. (Bedford, MA)	Method and apparatus for control of flow through a filter chamber by measured chamber equilibration pressure	A method and apparatus for the controlled instrumentation processing of cells and other paricles with a filter device measures a parameter of the flow through the filter device of a fluid carrying the particles.
7	5,240,606	31-Aug-93	**Lapidus; Stanley N.** (Bedford, NH), Polk, Jr.; Lewis T. (Bedford, MA), Farber; Fredric L. (Chestnut Hill, MA), Barlas; J. Morgan (Malden, MA), Hurley; Anne A. (Carver, MA)	Apparatus for preparing cells for examination	An apparatus and method provide automated collection and transfer of particles from a liquid suspension to a glass slide for visual examination.
8	5,266,495	30-Nov-93	**Lapidus; Stanley N.**	Method and apparatus for controlled instrumentation of particles with a filter device	A method and apparatus for the controlled instrumentation processing of cells and other particles with a filter device measures a parameter of the flow through the filter device of a fluid carrying the particles.

9	5,269,918	14-Dec-93	**Lapidus; Stanley N.** (Bedford, NH), Polk, Jr.; Lewis T. (Bedford, MA), O'Lari; Arlen M. (Chelmsford, MA)	Clinical cartridge apparatus	A cartridge-like holder or carrier for automatic operation with a specimen processor has a frame for removable and replaceable alignment in operative engagement with the specimen processor and has multiple supports, each of which removably and replaceably supports an implement such as a container of a biological specimen having cellular particles suspended in a liquid, a filter device for use in collecting cellular particles from the liquid in the sample container, a viewing screen onto which the collected cellular particles can be transferred from the filter device and, further, an output container for receiving the viewing screen with the cellular particles thereon.
10	5,670,325	23-Sep-97	**Lapidus; Stanley N.** (Bedford, NH), Shuber; Anthony P. (Milford, MA), Ulmer; Kevin M. (Cohasset, MA)	Method for the detection of clonal populations of transformed cells in a genomically heterogeneous cellular sample	Methods are provided for detecting the presence of mutant sequences in a subpopulation of gene sequences in a biological sample.
11	5,741,650	21-Apr-98	**Lapidus; Stanley N.** (Bedford, NH), Shuber; Anthony P. (Milford, MA), Ulmer; Kevin M. (Cohasset, MA)	Methods for detecting colon cancer from stool samples	The present invention provides methods for screening for the presence of a subpopulation of cancerous or precancerous cells in a heterogeneous cellular sample, such as a stool sample.
12	5,928,870	27-Jul-99	**Lapidus; Stanley N.** (Bedford, NH), Shuber; Anthony P. (Milford, MA)	Methods for the detection of loss of heterozygosity	Methods are provided for detecting loss of heterozygosity in a nucleic acid sample.
13	5,952,178	14-Sep-99	**Lapidus; Stanley N.** (Bedford, NH), Shuber; Anthony P. (Milford, MA)	Methods for disease diagnosis from stool samples	The present invention provides methods for preparing a stool sample in order to screen for the presence of indicators of a disease, for example a subpopulation of cancerous or precancerous cells.
14	6,010,909	4-Jan-00	**Lapidus; Stanley N.** (Bedford, NH)	Method and apparatus for controlled instrumentation of particles with a filter device	A method and apparatus for the controlled instrumentation processing of cells and other particles with a filter device measures a parameter of the flow through the filter device of a fluid carrying the particles.
15	6,020,137	1-Feb-00	**Lapidus; Stanley N.** (Bedford, NH), Shuber; Anthony P. (Milford, MA)	Methods for the detection of loss of heterozygosity	Methods are provided for detecting loss of heterozygosity in a pooled nucleic acid sample obtained from a patient population.
16	6,100,029	8-Aug-00	**Lapidus; Stanley N.** (Bedford, NH), Shuber; Anthony P. (Milford, MA)	Methods for the detection of chromosomal aberrations	Methods are provided for detecting fetal chromosomal aberrations by detecting statistically-significant differences between normal and aberrant chromosomes.
17	6,143,529	7-Nov-00	**Lapidus; Stanley N.** (Bedford, NH), Shuber; Anthony P. (Milford, MA)	Methods for improving sensitivity and specificity of screening assays	Methods of the invention comprise assays for markers indicative of cancer or precancer.
18	6,146,828	14-Nov-00	**Lapidus; Stanley N.** (Bedford, NH), Shuber; Anthony P. (Milford, MA)	Methods for detecting differences in RNA expression levels and uses therefor	Methods are disclosed for the detection and diagnosis of disease by determining differences in the number of RNA molecules in a patient sample compared to an expected number

19	6,203,993	20-Mar-01	Shuber; Anthony P. (Milford, MA), **Lapidus; Stanley N.** (Bedford, NH), Daley; George Q. (Weston, MA)	Methods for the detection of nucleic acids	Methods are provided for identifying nucleic acids. Methods of the invention are useful for identifying and analyzing nucleic acids, especially variants of single nucleotide polymorphisms, that are indicative of disease or the predisposition for disease.
20	6,214,558	10-Apr-01	**Lapidus; Stanley N.** (Bedford, NH), Shuber; Anthony P. (Milford, MA)	Methods for the detection of chromosomal aberrations	Methods are provided for detecting fetal chromosomal aberrations by detecting statistically-significant differences between normal and aberrant chromosomes
21	6,225,125	1-May-01	**Lapidus; Stanley N.** (Bedford, NH)	Method and apparatus for controlled instrumentation of particles with a filter device	A method and apparatus for the controlled instrumentation processing of cells and other particles with a filter device measures a parameter of the flow through the filter device of fluid carrying the particles.
22	6,268,136	31-Jul-01	Shuber; Anthony P. (Milford, MA), **Lapidus; Stanley N.** (Bedford, NH), Radcliffe; Gail E. (Worcester, MA)	Methods for stool sample preparation	The present invention provides methods for the preparation of stool samples to increase the yield of relevant DNA, and further provides methods for isolating and analyzing target DNA for characteristics indicative of colorectal cancer.
23	6,300,077	9-Oct-01	Shuber; Anthony P. (Milford, MA), **Lapidus; Stanley N.** (Bedford, NH)	Methods for the detection of nucleic acids	Methods are provided for identifying nucleic acids. Methods of the invention are useful for identifying and analyzing nucleic acids, especially variants of single nucleotide polymorphisms, that are indicative of disease or the predisposition for disease.
24	6,303,304	16-Oct-01	Shuber; Anthony P. (Milford, MA), **Lapidus; Stanley N.** (Bedford, NH)	Methods for disease diagnosis from stool samples	The present invention provides methods for preparing a stool sample in order to screen for the presence of indicators of a disease, for example a subpopulation of cancerous or precancerous cells.
25	6,351,857	5-Mar-02	Slaon [sic, should be Sloan], III; Walker M. (Berlin, MA), **Lapidus; Stanley N**. (Bedford, NH)	Stool specimen collector	An apparatus for obtaining a stool specimen. The apparatus comprises a housing, a collection bag, a slider to close the collection bag and a draw string to move the slider.
26	6,406,857	18-Jun-02	Shuber; Anthony P. (Milford, MA), **Lapidus; Stanley N.** (Bedford, NH), Radcliffe; Gail E. (Worcester, MA)	Methods for stool sample preparation	The present invention provides methods for the preparation of stool samples to increase the yield of relevant DNA, and further provides methods for isolating and analyzing target DNA for characteristics indicative of colorectal cancer.
27	6,415,455	9-Jul-02	Slaon [sic, should be Sloan], III; Walker M. (Berlin, MA), **Lapidus; Stanley N**. (Bedford, NH)	Stool specimen collector	The invention provides an apparatus for obtaining a stool specimen. The apparatus comprises a housing, a collection bag, a slider to close the collection bag and a draw string to move the slider.
28	6,566,101	20-May-03	Shuber; Anthony P. (Milford, MA), **Lapidus; Stanley N.** (Bedford, NH)	Primer extension methods for detecting nucleic acids	Methods are provided for selective nucleic acid sequence detection in single base primer extension reactions of high sensitivity. These methods are useful for detecting small amounts of mutant nucleic acid in a heterogeneous biological sample. These methods are particularly useful for identifying individuals with gene mutations indicative of early colorectal cancer.

29	7,269,560	30-Jan-07	**Lapidus; Stanley N** (Bedford, NH), Buzby; Philip Richard (Brockton, MA), Harris; Timothy (Ocean County, NJ)	Short cycle methods for sequencing polynucleotides	The invention provides methods for sequencing a polynucleotide comprising stopping an extension cycle in a sequence by synthesis reaction before the reaction has run to near or full completion.
30	7,491,498	17-Feb-09	**Lapidus; Stanley N** (Bedford, NH), Buzby; Philip Richard (Brockton, MA), Harris; Timothy (Ocean County, NJ)	Short cycle methods for sequencing polynucleotides	The invention provides methods for sequencing a polynucleotide comprising stopping an extension cycle in a sequence by synthesis reaction before the reaction has run to near or full completion.
31	7,666,593	23-Feb-10	**Lapidus; Stanley** (Bedford, NH)	Single molecule sequencing of captured nucleic acids	The invention provides methods and devices for detecting, enumerating or identifying target nucleic acid molecules using immobilized capture probes and single molecule sequencing techniques.
32	7,897,345	1-Mar-11	**Lapidus; Stanley N** (Bedford, NH), Buzby; Philip Richard (Brockton, MA), Harris; Timothy (Ocean County, NJ)	Short cycle methods for sequencing polynucleotides	The invention provides methods for sequencing a polynucleotide comprising stopping an extension cycle in a sequence by synthesis reaction before the reaction has run to near or fill completion.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re *Ex Parte* Reexamination:	)	Group Art Unit: 3991
	)	
U.S. Patent Nos. 6,440,706;	)	Docket No. 001107.00989
7,824,889; and	)	001107.00990
7,915,015	)	001107.00988
	)	
	)	
Control No. 90/012,894, 90/012,895, 90/012,896	)	Confirmation No: 8361
	)	
Reexam Filing Date: June 17, 2013	)	Examiner: Bruce R. Campell

For: DIGITAL AMPLIFICATION

DECLARATION OF IE-MING SHIH

1. My name is Ie-Ming Shih. I make this declaration based on my personal knowledge. I am over 21 and otherwise competent to make this declaration.
2. I am currently the Richard W. TeLinde Distinguished Professor in the Department of Gynecology and Obstetrics at the Johns Hopkins University Medical School in Baltimore, MD. I have secondary appointments in the departments of Oncology and Pathology. A copy of my Curriculum vitae is attached as Exhibit 1 that details my training background and research experience.
3. As my *Curriculum vitae* indicates, I obtained my M.D. from Taipei Medical University in Taiwan, obtained my Ph.D. in pathology from University of Pennsylvania. Thereafter I finished my residency training in anatomic pathology and did further clinical and research fellowships at the Johns Hopkins Medical Institutions until I became a member of the faculty.
4. As can be gleaned from my *Curriculum vitae*, I have been engaged in medical

research since about 1989. My current research is focused on cancer genes and markers of gynecological cancers. Throughout my career I have followed new developments in the field by reading of the scientific literature, active research, and interactions with colleagues. Because of my training and experience, I consider myself knowledgeable in various aspects of nucleic acid amplification. This includes technologies that are used to analyze DNA sequences and variations in DNA sequences.

5. In 2004 I co-authored a review article on digital PCR that appeared in Expert Reviews in Molecular Diagnostics, appended as Exhibit 3. I draw from that review as well, as applications of digital PCR more current than at that time with which I am familiar.

6. I have also been informed that Johns Hopkins University (JHU) owns U.S. patents 7,915,015 ("'015 patent") 7,824,889 ("'889 patent") and 6,440,706 ("'706 patent") and has licensed them to LabCorp (Esoterix), and Exact Sciences.

7. I have reviewed the '015 patent, the '889 patent, and the '706 patent, including the original claims and the amendments filed July 9, 2014, (attached as Exhibit 2).

8. I have been asked to review and summarize the state of the digital PCR field. The statements that I make include my reading and interpretation of the statements as represented in the exhibits. The readings and interpretations are my own, and I have no stake in the outcome of the re-examination proceedings.

9. I understand that the "digital PCR" methods described in the claims of the three subject patents involve (1) analysis of two different analytes and (2) comparing

the number of assay samples containing one of the analytes to the number of assay samples containing the other analyte. While I understand that the inventors coined and applied the term "digital PCR" to their methods, I understand that many in this field subsequently adopted the term "digital PCR" and use it more broadly. I have attempted in this declaration to refer only to examples of digital PCR that share the two features stated above, rather than the broader usage.

10. As an illustration of the different ways that the term is often used in the field, Day *et al., Methods 59:*101-107, 2013, describes two types of digital PCR as those which (1) calculate absolute abundance of a target sequence and those which (2) obtain a relative abundance by comparing to an internal reference sequence. Exhibit 12, paragraph spanning pages 101-102. Day refers to the latter type as the more common use. *Ibid.* The latter type is what I understand is described in the claims of the three subject patents.

11. The study of DNA sequence variation is important for many areas of research. Prior to digital PCR, conventional PCR did not allow the identification and quantification of rare molecular genetic changes because conventional PCR amplifies a pool of DNA templates from the starting material. Digital PCR is useful for amplifying a single DNA template from limiting dilution samples, therefore transforming the exponential, analog signals from conventional PCR to linear, digital signals, allowing statistical analysis of the PCR products. Digital PCR has been applied in the quantification of muant alleles and detection of allelic imbalance in clinical specimens, providing a useful molecular diagnostic tool for cancer detection. Exhibit 3, page 46, col. 2, text box. Digital PCR has also been applied in the

quantification of muant alleles and detection of allelic imbalance in fetal abnormalities.

12. In 2004, in our review article, we noted twelve different examples in twelve different scientific publications in the scientific literature in which digital PCR had been used for molecular analysis of clinical samples. These involved detection of cancer mutations, detection of allelic imbalance, detection of loss of heterozygosity, quantitative detection of tumor suppressor gene expression. Exhibit 3, Table 1.

13. The digital PCR technique is especially powerful in experiments requiring quantitative investigation of individual alleles in DNA samples isolated from a mixed cell population. Exhibit 3, page 46, col. 1, last full paragraph.

14. Vogelstein and Kinzler published their original scientific paper on digital PCR in *Proc. Natl. Acad.* Sciences *USA 96*: 9236-9241 (1999). Exhibit 19. I understand that the paper served as the basis for the application underlying the three subject patents, as its text and figures appear to have been incorporated entirely in the application. Exhibit 18.

Recognition in the Art

15. According to Google Scholar™, the original digital PCR publication of inventors Vogelstein and Kinzler, *Proc. Natl. Acad. Sciences USA 96*: 9236-9241 (1999), has been cited in 532 scholarly publications in its archive from 2009-2014. Exhibit 4. That is an indication of its unusually high impact in the scientific community. According to the Altmetric™ score, this article was in the 88th

percentile of a sample of the 1888361 tracked articles of a similar age published within six weeks on either side in al journals. Exhibit 4, page 2.

16. I am aware of a number of scientific conferences on digital PCR that have been organized in the US and in Europe. One, put on by Cambridge Health Tech Institute, October 6-8, 2014, the third annual such conference, describes digital PCR as "**creating waves** across the diagnostic landscape" in its conference announcement. Exhibit 5, emphasis added. One of the featured presentations at last year's conference was titled "Use of digital PCR in Oncology: **Changing the paradigm** for systemic therapy." Exhibit 6, emphasis added. The organizers of the 2013 digital PCR conference in San Diego, CA, stated that digital PCR "has already shown potential to be a **disruptive technology** in many areas of diagnostics." Exhibit 7, emphasis added. The existence of these conferences as well as the descriptions they use are indications of the high importance of digital PCR in the scientific community.

17. Another conference, put on by an organization called Global Engage, will hold its second annual event in Europe on "qPCR and digital PCR." Exhibit 8. The first such congress in 2013 reportedly had 150 attendees, and over 200 attendees are expected in 2014. Global Engage indicates that "increasing numbers of real-time PCR users [are] purchasing digital PCR [machines] due to its reduction in cost, absolute quantification, improved sensitivity, precision and greater robustness." Exhibit 8. This reflects the growing adoption of digital PCR (broadly used) in the scientific and diagnostic communities.

18. Hahn *et al., Expert Rev.* Mol. *Diag. 9:*613-621, 2009, describes non-invasive detection of Down syndrome as a "long-sought goal." It further teaches that application of digital PCR or shot-gun sequencing to analysis of cell-free fetal DNA may be the fulfillment of this goal. Exhibit 16, abstract, lines 1-3. Hahn further refers to these techniques as providing a paradigm shift in prenatal diagnosis. Exhibit 16, abstract lines 3-6.

19. Tsui *et al., Current Opinion in Hematology 19:* 462-468, 2012, reviews analyses of fetal nucleic acid in maternal plasma. Exhibit 17. Tsui indicates that digital PCR has enabled high quantitative precision for maternal plasma DNA analyses. Abstract, lines 7-9. Tsui further touts the importance of digital PCR in detecting fetal monogenic diseases, stating, "To obtain an analytical precision that would allow discrimination of the small concentration differences between the mutant and wild-type DNA, quantification based on molecular counting, such as digital PCR, has been employed. Exhibit 17, page 463, col. 2, lines 11-16. Tsui refers to this as a "technically challenging" determination to which digital PCR has provided one approach to address. See "Key Points" at Exhibit 17, page 463.

Advantages

20. A later review article than mine, by Vlkova *et al., Med. Sci. Monit. 16:*RA85-91, 2010, describes digital PCR in Figure 2. Exhibit 9. Vlkova indicates the advantages of Digital PCR over real-time PCR. Vlkova asserts that "DigPCR outperforms real-time PCR in precision which is needed especially in the screening and detection of aneuploidy. Digital PCR has been proven an effective

approach in noninvasive prenatal diagnostics of trisomy 21." Exhibit 9. RA87, last paragraph, citations omitted. Vlkova also asserts that for detecting monogenic diseases the "advantage lies in the digital relative mutation dosage approach. Effective quantification of allele frequency by digital PCR makes possible the precise evaluation of balance/imbalance between mutant and wild-type alleles." Exhibit 9, RA88, first paragraph, citation omitted.

21. Lo *et al., Proc. Natl. Acad. Sciences USA 104:*13116-13121, 2007, explored the use of digital PCR "to achieve finer degree of quantitative discrimination" than possible with real-time PCR. Exhibit 10 at page 13116, col. 2, last paragraph. The technique could successfully detect aneuploidy even when the fetal fraction is a minor population of a sample. Exhibit 10, page 13121, column 1, lines 3-4.

22. Lun *et al., Clin. Chem 54:*1664-1672, 2008, demonstrated a higher degree of precision of the digital PCR to real-time PCR for detection of amounts of X and Y chromosomes using the *ZFX/ZFY* loci. Exhibit 11, page 1664, column 1, paragraph 3.

23. Sedlak *et al., Expert Rev. Mol. Diag. 14:*501-507, 2014, teaches that digital PCR is superior to qPCR (quantitative or real-time PCR) for ratiometric assays. Exhibit 15, page 504, col. 2, lines 43-46. Sedlak uses the assay to detect both replicating viral DNA and chromosomally integrated viral DNA. Exhibit 15, page 502, col. 2, first full paragraph.

24. Day emphasizes the sensitivity and ability to achieve quantitation of rare variants of digital PCR. Exhibit 12, page 102, first full paragraph. Day lists the positive

attributes of digital PCR as including rare variant detection, estimating copy number variation, minimal template requirements, ease of analysis, and integration with next generation sequencing. Section 3, spanning pages 102-103.

Commercial Activities

25. It is my understanding that, a number of apparatus manufacturers have developed products to carry out digital PCR. These include Fluidigm Corp. Life Technologies, Bio-Rad Laboratories, and RainDance. These and other platforms for PCR are compared in Table 1 of Day *et al., Methods 59:*101-107, 2013. Exhibit 12.

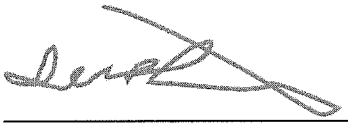
26. Global Engage in announcing its digital PCR and qPCR conference, reported that "the gene amplification market [is] predicted to grow to $1.9 billion by 2015." Exhibit 8. This prediction is not limited to digital PCR, or relative digital PCR, but may nonetheless suggest substantial commercial activities.

27. Baker, Nature Methods 9:541-544, 2012 surveys the commercial digital PCR offerings. Exhibit 13. The machines offered by Fluidigm and Life Technologies can run either digital PCR or qPCR (real-time PCR). Exhibit 13, page 542-543, spanning paragraph and page 543, second full paragraph. Digital PCR is more accurate and less ambiguous but more expensive than qPCR. Exhibit 13, page 541, col. 3, last paragraph. The RainDance and Bio-Rad machines perform only digital PCR but not qPCR. Exhibit 13, page 543, col. 2, last paragraph. Baker compares the four instruments in Exhibit 13, Table 1.

28. Roche also markets an apparatus which employs digital PCR for genotyping.

Exhibit 14. The Light Cycler™ is used to detect *IDH1* mutations. See Fig. 4. Although many different techniques are part of the workflow, Roche describes the digital PCR as the "all important second step" which "allows relative quantification of mutant tumor cell DNA in a blood sample." Page 5, "Conclusion," col. 2, lines 3-5.

29. I declare that all statements (prepared by Sarah A. Kagan) made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the claims or the patent.

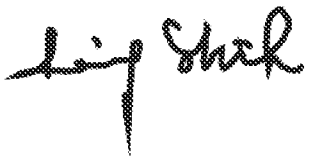


Ie-Ming Shih

August 6, 2014
Date

CURRICULUM VITAE

The Johns Hopkins University School of Medicine



(Typed Name): Ie-Ming Shih (Date of this version): April, 2014

DEMOGRAPHIC AND PERSONAL INFORMATION

Current Appointments

Professor, Department of Pathology with secondary appointment in the Departments of Oncology (*Cancer Biology Program*) and Gynecology/Obstetrics and, Johns Hopkins Medical Institutions
Faculty in the Graduate (Ph.D.) Program in Pathobiology, Johns Hopkins University School of Medicine, Baltimore, Maryland
Faculty in the Institute for NanoBioTechnology (INBT), Johns Hopkins University

Personal Data

Country of birth place: Dai-Chia Town, Tai-Chuan County, Taiwan
Nationality/citizenship: United States of America
Contact information:
Address: 1550 Orleans Street, RM: 305, Baltimore, Maryland 21231
Office phone: 410-502-7774
Fax: 410-502-7943
E-mail: ishih@jhmi.edu, shihie@yahoo.com

EDUCATION AND TRAINING

Year	Degree	Institution	Discipline
1981-1988	M.D.	Taipei Medical University	Medicine
1989- 1993	Ph.D.	University of Pennsylvania	Pathology
1993-1994	Postdoctoral Fellow	The Wistar Institute	Cancer Biology
1994-1997	Resident	Johns Hopkins Hospital	Pathology
1997-1998	Clinical Fellow	Johns Hopkins Hospital	Gynecologic Pathology
1998-2000	Research Fellow	Johns Hopkins Oncology Ctr. (w/ Dr. Bert Vogelstein)	Cancer Genetics

PROFESSIONAL EXPERIENCE

2000-2001 **Instructor**, Department of Pathology

	Johns Hopkins Medical Institutions, Baltimore, MD
2001-2003	**Assistant Professor**, Department of Pathology Johns Hopkins Medical Institutions, Baltimore, MD
2003-2008	**Associate Professor**, Departments of Pathology, Oncology and Gynecology and Obstetrics Johns Hopkins Medical Institutions, Baltimore, MD
2008-	**Professor**, Departments of Pathology, Oncology and Gynecology/Obstetrics Johns Hopkins Medical Institutions, Baltimore, MD
2014-	**Richard W. TeLinde Distinguished Professor** Department of Gynecology and Obstetrics Johns Hopkins University School of Medicine
	Co-director of the Female Malignancy Program, Sidney Kimmel Cancer Center, Johns Hopkins Medical Institutions, Baltimore, MD

RESEARCH ACTIVITIES

Peer-Reviewed Research Articles

1. **Shih IM**, Chiang HS, Yang LL, Wang TL. Antimotility effects of Chinese herbal medicines on human sperm. J Formos Med Assoc, 89:466-9, 1990. PMID: 1977862

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5. Mancianti ML, Gyorfi T, **Shih IM**, Valyi-Nagy I, Levengood G, Menssen HD, Halpern A, Elder DE, Herlyn M. Growth regulation of cultured human nevus cells. J Invest Dermatol, 100:281S-287S, 1993. PMID: 8440904

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11. **Shih IM**, Wang TL, Westra WH. Diagnostic and biologic implications of Mel-CAM expression in spindle cell neoplasms. Clin Cancer Res, 2:569-575, 1996. PMID: 9816205

12. **Shih IM**, Kurman RJ. Expression of melanoma cell adhesion molecule in intermediate trophoblast. Lab Invest, 75: 377-388, 1996. (with cover illustration) PMID: 8804361

13. **Shih IM**, Speicher D, Hsu MY, Levine E, Herlyn M. Melanoma cell-cell interactions are mediated through heterophilic Mel-CAM/ligand adhesion. Cancer Res, 57: 3835-3840, 1997. PMID: 9288796

14. **Shih IM**, Hsu MY, Palazzo JP, Herlyn M. The cell-cell adhesion receptor Mel-CAM acts as a tumor suppressor in breast carcinoma. Am J Pathol, 151:745-751, 1997. PMID: 9284823

15. **Shih IM**, Kurman RJ. New concepts in trophoblastic growth and differentiation with practical application for the diagnosis of gestational trophoblastic disease. Verh Dtsch Ges Path, 81: 266-272, 1997. PMID: 9474880

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24. **Shih IM.** The role of CD146 (Mel-CAM) in biology and pathology. J Pathol, 189:4-11,1999. PMID: 10451481

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Book Chapters

1. **Shih IM**, Mazur MT, Kurman RJ. Chapter 49: Gestational trophoblastic disease. In Sternberg's Diagnostic Surgical Pathology. Edited by Stacey E. Mills. pp 2049-2070, Fifth edition. Lippincott Williams & Wilkins Publishers, New York, 2009.

2. **Shih IM**, Mazur MT, Kurman RJ. Chapter 20: Gestational trophoblastic disease. In Blaustein's Pathology of Female Genital Tract. Edited by Robert J. Kurman. Sixth edition. Springer-Verlag, New York, pp1075-1135, 2011.

3. **Shih IM**, Sokoll L, Chan DW. Tumor markers of ovarian cancer. In "Tumor markers-physiology, pathobiology and clinical applications" Edited by E.P. Diamandis et al. American Association for Clinical Chemistry Press. Washington DC, First edition, pp239-252, 2002.

4. Chang H-W, **Shih IM**. Digital Single-Nucleotide polymorphism analysis for allelic imbalance. In Methods in Molecular Medicine: Pancreatic Cancer (volume: 103). Edited by G. H. Su, Humana Press, Totowa, NJ, USA, pp 137-142, 2004.

5. Yen, JM, **Shih IM**, Velculescu VE, Wang TL. Amplification in DNA copy numbers as a mechanism of acquired drug reisistance. In Cancer drug resistance. Edited by Teicher BA, Human press, Totowa, New Jersey. pp 531-540, 2006.

6. **Shih IM**, Kurman RJ. Ovarian serous carcinogenesis- a proposed model. In Molecular Pathology of Gynecological Cancer. Edited by Giordano A, Bovicelli A, and Kurman RJ, Humana press, Totowa, New Jersey. pp 17-28, 2006.

7. **Shih IM**, Kurman RJ. Pathogenesis of gestational trophoblastic lesions. In Molecular Pathology of Gynecological Cancer. Edited by Giordano A, Bovicelli A, and Kurman RJ, Humana press, Totowa, New Jersey. pp 157-166, 2006.

8. Sturgeon CM, Duffy MJ, Hofmann BR, Stenman U-H, Lilja H, Brünner N, Chan DW, Sokoll L, Babaian R, Bast RC, Bosl GJ, Dowell B, Esteva FJ, Haglund C, Harbeck N, Hayes DF, Holten-Andersen M, Klee GG, Lamerz R, Looijenga LH, Molina R, Nielsen HJ, Rittenhouse H, Semjonow A, **Shih IM**, Sibley P, Sölétormos G, Stephan C and Diamandis EP. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for Use of Tumor Markers in Testicular, Prostate, Colorectal, Breast and Ovarian Cancers. American Association for Clinical Chemistry press.

9. Jinawath N. **Shih IM**. Biology and Pathology of Ovarian Cancer. In Early Diagnosis of Cancer Series: Ovarian Cancer. Edited by Bristow R. and Armstrong D. (series editor: Yang, SC). Elsevier, Amsterdam, Netherlands, pp17-32, 2009.

10. Guan B, Wang TL, **Shih IM**. Recent advances in cancer genomics and cancer-associated genes discovery. In: An Omics Perspective of Cancer. WCS Cho (ed.), p11-29, Springer-Verlag, New York, 2010.

11. **Shih IM**. Gestational trophoblastic lesions. In Gynecologic Pathology, a volume in the series of Foundations in Diagnostic Pathology. Edited by Nucci MR, Oliva E. (Series editor: Goldblum JR), pp645-655. Elsevier Churchill Linvingstone, 2009.

12. Park J. **Shih IM**, Wang TL. Targeting the Notch signaling pathway in cancer stem cells. In: Cancer Stem Cells. Edited by William Farrar. pp128-137, Cambridge University Press (CUUS668), 2009.

13. Sfakianos G P, Secord AA, **Shih IM**. Chapter 13: Epithelial ovarian cancers: low malignant potential and non-serous ovarian histologies. In: Gynecologic oncology: clinical practice and surgical atlas. pp 237-256. McGraw-Hill Professional, New York, NY, 2012.

14. Kurman RJ, Bagby C. **Shih IM**. Chapter 37: Molecular diagnostics of gynecologic neoplasms. In: Principles of Molecular Diagnostics and Personalized Cancer Therapy. Ed by Tan D. Lippincott Williams & Wilkins.

15. Chen L, Tian Y, Yu G, Miller DJ, **Shih IM**, and Wang Y. Discriminant and network analysis to study origin of cancer. In: Statistical Diagnostics of Cancer: Analyzing High Dimensional Genetics and Genomics Data. Edited by Frank Emmert-Streib and Matthias Dehmer, Wiley-Blackwell, 2012.

16. WHO classification of tumours of female reproductive organs. Ed by Kurman, Carcangiu, Herrington, Young. 4th edition, WHO (IARC) press, Lyon, France, 2014.

Others

1. **Shih IM**. Placental site trophoblastic tumor. In Encyclopedia of Cancer, 2nd edition, Springer-Verlag, Editor: Manfred Schwab, Berlin and Heidelberg, GmbH & Co, 2009. http://www.springerreference.com/docs/featured/978-3-540-47648-1_5715.html

2. Chen L, Xuan J, Gu J, Wang Y, Zhang Z, Wang TL, **Shih IM**. Integrative network analysis to identify aberrant pathway networks in ovarian cancer. Pac Symp Biocomput, 31-42, 2012.

Inventions, Patents, Copyrights

- US patent #6419896: Non-invasive approach for assessing tumor in living animals. Inventors: Vogelstein B, Kinzler WK and Shih I-M
- US patent #20110171741: DNA integrity assay (DIA) for cancer diagnostics, using confocal fluorescence spectroscopy. Inventors: Tza-Hui Wang, Kelvin J. Liu, Ie-Ming Shih
- US patent in process (11/604,183): Application of Rsf-1 expression to predict clinical outcome in cancer patients. Inventors: Shih I-M and Wang T-L
- International patent in progress (PCT/US2008/011948): Detection of cancer by measuring genomic DNA copy number and strand length in cell-free DNA. Inventors: Shih I-M

Extramural Funding

Current awarded Grants

4/1/2011 – 3/31/2016	Notch3 signaling in ovarian cancer RO1 CA148826 (PI: TL Wang) NCI/NIH Role: co-investigator; 0.5 calendar months Purpose: To investigate the molecular mechanism of Notch3 signaling in the pathogenesis of ovarian high-grade serous carcinoma.
10/01/2011 – 09/30/2016	Prevention of Ovarian High-Grade Serous Carcinoma by Elucidating Its Early Changes OC100517 (Director: RJ Kurman; co-Director: I-M Shih) Consortium Award, US Department of Defense (USAMRMC), Directed Medical Research Programs (CDMRP) Role: Co-director and co-investigator; 3.0 calendar months Purpose: To determine the origin and pathogenesis in the development of ovarian high-grade serous carcinomas by employing cancer genetics, cell biology, animal models and epidemiologic studies through multi-institutional research effort. The consortium includes five research projects and three cores.
07/01/2011 - 06/30/2016	Multiplexed Detection of Cell Free DNA Biomarkers for Cancer RO1 CA155305 (PI: TZ Wang) NCI/NIH Role: co-investigator; 1.0 calendar months Purpose: To analyze the potential application of multiplexed detection of cell free DNA as biomarkers for cancer detection.
09/01/2011 - 08/30/2016	Proteome characterization center: a genoproteomics pipeline for cancer biomarker. Clinical Proteomic Technologies for Cancer Initiative. U24CA160036 (PI: D Chan) NCI/NIH Role: co-investigator; 1.0 calendar months Purpose: To identify, verify and characterize biomarkers for ovarian cancer by combining genomics and proteomic approaches. To establish the clinical proteomic technology center and to validate, verify and characterized of ovarian cancer biomarkers using genoproteomic approaches.
12/01/2011 - 11/30/2014	Tumor suppressor role of ARID1A R21 CA165807 (PI: IM Shih) NCI/NIH Role: principal investigator; 1.0 calendar months Purpose: To determine the tumor suppressor roles of ARID1A and its molecular mechanisms in developing gynecological cancer.

Recent Completed Research Grants

12/01/2004 - 11/30/2012	Molecular Diagnostics for Malignant Effusion 2R01 CA103937 (PI: I-M Shih) NCI/NIH Role: principal investigator; 1.0 calendar months Purpose: To study the functional role of NAC-1 in the development of ovarian carcinoma.
4/01/2008 - 1/31/2013	The Roles of HBXAP Gene in Ovarian Cancer 1R01 CA129080 (PI: I-M Shih) NCI/NIH Role: principal investigator; 1.0 calendar months Purpose: To study the molecular mechanism of HBXAP gene product in the progression of ovarian carcinoma.
04/01/2007 - 01/31/2012	Pathogenesis of Ovarian Serous Borderline Tumors RO1 CA116184 (PI: R.J. Kurman) NCI/NIH Role: co-Director, project 1 leader; 0.5 calendar months Purpose: To study the molecular genetic profiles of implants that is associated with ovarian serous borderline tumors. To develop biomarkers to better diagnose the implant and correlate the molecular genetic profiles and biomarker expression with clinical behavior in patients.
07/01/2002- 06/30/2007	Development of a New Technology in Analyzing Allelic Imbalance in Plasma DNA as a Tool for Early Cancer Detection R21/R33 CA97527 (PI: Shih) NCI/NIH Role: principal investigator; 4.0 calendar months Purpose: To develop an innovative molecular method to better diagnose human cancer using cell-free circulating DNA in patients.
07/01/2008 - 06/30/2012	Notch3 Signaling Pathway in the Ovarian Carcinoma GMC-113937 (PI: TL Wang) American Cancer Society Role: co-investigator; 1.0 calendar month Purpose: This project is to characterize the role of Notch3 signaling pathway in ovarian tumorigenesis and identify Notch3 down-stream target genes in ovarian cancer.
06/01/2009 – 05/31/2012	High-throughput intracellular microrheology: a new tool for cancer research 1R21CA137686 (PI: D Wirtz/IM Shih) NCI/NIH Role: Co-PI Purpose: To apply a high-throughput intracellular microrheology in studying ovarian cancer

07/01/2002- 06/30/2006	Diverse Pathways in the Development of Ovarian Serous Tumors OC010017 (PI: RJ Kurman) US Department of Defense (USAMRMC), Directed Medical Research Programs (CDMRP) Role: Project #1 leader; 3.0 calendar months Purpose: To study the molecular pathways that is involved in the development of different types of ovarian serous carcinoma by using several new technologies including SAGE.
09/01/2003- 08/30/2004	Molecular genetic changes in the development of cervical cancer P50CA098252- SPORE (PI: TC Wu) NIH/NCI Role: co-investigator; 1.0 calendar month Purpose: The development project/pilot study in this SPORE of cervical cancer is to investigate the DNA copy number changes involved in the development of cervical cancer.
12/28/2005- 12/27/2006	Marker Discovery for Ovarian Cancer Research agreement Developmental Center of Biotechnology, Taiwan (PI: Shih) Role: principal investigator; 1.0 calendar month Purpose: To identify biomarkers for potential use in ovarian cancer diagnosis and therapy.
10/01/2006 - 09/30/2007	Characterization of Rsf-1 in human cancer China Medical University, Taiwan Research agreement (PI: Shih) Role: principal investigator; no salary requested Purpose: To study the molecular etiology of Rsf-1 expression in oral cancer in Taiwanese patients.
1/1/2008 - 12/31/2009	Notch3 signaling in the pathogenesis of ovarian cancer Ovarian Cancer Research Foundation (OCRF, New York) Individual Investigator Award (PI: T.L. Wang) Role: co-investigator; 0.6 calendar month Purpose: To characterize the Notch3 signaling pathway in the tumor progression of ovarian cancer. Specifically, the proposal is to determine how the Notch3 pathway goes awry in normal ovaries and the molecular mechanisms in which Notch3 pathway aberration contributes to ovarian cancer.
01/01/2009 – 12/31/2010	Screening of Chinese herbal medicine extracts in cancer therapy Research Agreement (PI: IM Shih) China Medical University, Taichung city, Taiwan Role: Principal; investigator

	Purpose: To screen candidate Chinese herbal extracts to inhibit specific cancer-associated targets for potential molecularly targeted therapy.
12/11/2006 - 12/31/2007	Molecular Markers for Clinical Outcome Prediction Oncotech, Inc. Research Agreement (PI: Shih) Role: principal investigator; 0.60 calendar month Purpose: To assess the clinical potential of Rsf-1 and NAC-1 immunohistochemistry in predicting clinical outcome in ovarian cancer patients.
04/01/2008 - 03/31/2010	Nanobiosensing Method for Point Mutation Detection of Cancer 1R21CA120742 (PI: TZ Wang) NCI/NIH Role: co-investigator; 0.60 calendar month Purpose: To develop a nanobiosensing technical platform to detect point sequence mutation of Kras and Braf genes using a relatively small amount of DNA samples without PCR.
07/01/2007 - 06/31/2009	Characterization of Chromatin Remodeling Gene, Rsf-1, in Pathogenesis of Ovarian Cancer Johns Hopkins-Weizmann Inst. (PI: Shih) Role: principal investigator; 0.60 calendar month Purpose: To study the biological function of Rsf-1 gene in the development of ovarian cancer.
01/01/2005 -12/31/2008	Identification and Characterization of Genomic Amplifications in Ovarian Serous Carcinoma OC04-0060 (PI: T.L. Wang) US Department of Defense (USAMRMC), Directed Medical Research Programs (CDMRP), New Investigator Research award Role: co-investigator; 1.0 calendar month Purpose: To identify and characterize ovarian cancer genome using digital karyotyping and SNP array.
07/01/2009 -- 06/30/2011	Elucidation of molecular alterations in precursor lesions of ovarian serous carcinoma OC080469 (Director: RJ Kurman; Co-director: IM Shih) Role: Co-director Purpose: To establish ovarian cancer research consortiums to facilitate identify and characterize early lesions of ovarian cancer through multiple institution collaborations

EDUCATIONAL ACTIVITIES

Classroom Instruction (Johns Hopkins University School of Medicine)

- Gynecological Pathology and laboratory/small group, Pathology course for medical students, 1994-
- Graduate course in Pathobiology and Disease Mechanisms, Section of Ovarian Tumors, 2002-
- Graduate course in Functional Anatomy ("Female Reproductive Organ"), for graduate students, Johns Hopkins University, 2006-
- Graduate course in Pathobiology ("Gynecological Pathology") for graduate students, Johns Hopkins University, 2005-

Clinical Instruction (the Johns Hopkins Hospital)

- Microscopic and gross teachings for medical students, residents and fellows rotating to gynecologic pathology, 1999-
- Didactic course on Gynecologic Pathology for residents and fellows, 2002-

CME course speaker

- "Molecular pathways of ovarian cancer". At the Current Concepts in the Multidisciplinary Management of Ovarian Cancer, the Sidney Kimmel Cancer Center and the office of Continuing Medical Education, Johns Hopkins University, Baltimore, September, 2004.
- "Molecular genetics and target-based therapy for low-grade serous cancers of the ovary". At the Current Concepts in the Multidisciplinary Management of Ovarian Cancer, the office of Continuing Medical Education, Johns Hopkins University, Baltimore, September, 2005.
- "Gynecologic neoplasms- trophoblastic tumors and ovarian epithelial neoplasms". Symposium of the Taiwanese Association of Pathology, August 2006.
- "Update in gestational trophoblastic disease". Surgical Pathology Update, Leipzig, Germany, June, 2007.

Mentoring

Research Fellows

- 2000-2002, Hsueh-Wei Chang, PhD, currently Chairman and Professor of the Department of Biological Science and Environmental Biology, Kaohsiung Medical University, Taiwan
- 2001-2003, Gad Singer, M.D., Professor at the Institute of Pathology, Baden, Switzerland
- 2002-2004, Brant G. Wang, MD, PhD, research fellow; currently an attending pathologist at the Washington Medical Center, Washington DC
- 2003-2004, Gudrun Pohl, MD, assistant professor at the University of Vienna, Austria
- 2003-2004, Chung-Liang Ho, MD, PhD, Associate Professor, National Chenug-Kung University School of Medicine, Tainan, Taiwan
- 2003, Ariane Aigelsreiter, MD, visiting research fellow, Austria
- 2003-2004, Reiko Dehari, MD, Visiting research fellow, Japan
- 2003-2004, Chih-Yi Hsu, MD, Visiting research fellow, currently a faculty t the National Yang-Ming University School of Medicine/VGH -Taipei, Taiwan
- 2004-2005, Tsung-Hsuan Lai, MD, Director of Reproductive Endocrinology and Infertility division, Department of Ob and Gyn, Taipei Cathay General Hospital, Taipei, Taiwan

- 2004-2006, Kentaro Nakayma, MD, PhD, Associate Professor, Shimane National University School of Medicine, Japan
- 2005-2007, Jim Sheu, PhD, Professor at the Institute of Biomedical Sciences, National Sun Yat-Sen University, Taiwan
- 2005-2006, Ritu Salani, MD, Assistant Professor and attending physician at the Ohio State University Health System, division of Gynecologic Oncology
- 2007 and 2008, Ayse Ayhan, MD, PhD, attending/consulting pathologist at the Seirei Mikatahara General Hospital, Hamamatsu, Japan
- 2005-2007, Tsui-Lien Mao, MD, research fellow, currently an assistant professor at the National Taiwan University College of Medicine, Taipei, Taiwan
- 2007, Artit Jinawath, MD, PhD, research fellow/visiting resident, Thailand
- 2006-2008, Natini, Jinawath, MD, PhD, research fellow, currently a medical cytogenetics fellow at the Johns Hopkins Hospital
- 2006-2008, Jung Hye Choi, PhD, Assistant Professor at Kyung Hee University, Seoul, South Korea
- 2006-2008, Kuan-Ting Kuo, MD, Assistant Professor at the National Taiwan University Hospital, Taipei, Taiwan
- 2007-2008, Stefanie Ueda, MD, Assistant Profession, Department of Obstetrics and Gynecology, University of California at San Francisco, CA
- 2008-2010, Michelle Thiaville, PhD, Assistant Professor, Department of Biological Science, Nicholls State University, Louisiana
- 2008-2010, Pradeep K. panuganti, MD, currently a resident in Texas Tech University of Health Sciences
- 2010, Daichi Maeda, MD, PhD, Assistant Professor, Department of Pathology, University of Tokyo, Japan
- 2010-2012, Stephanie Gaillard, Assistant Professor, Duke University
- 2009-2012, Alex Stoeck, PhD, Research Scientist Leader, Merck Co.
- 2011-2012, Chen-Hsuan Wu, MD, Instructor, Kaohsiung Chang Gung Memorial Hospital, and Chang Gung University college of medicine, Kaohsiung, Taiwan
- 2012-2013, Laura Ardighieri, MD, a fellow at the Anatomia Patologicaat Spedali Civili Brescia, Italy
- 2009-2013, Elisabetta Kuhn, MD, staff scientist, International Agency for Research on Cancer (IARC), Lyon, France
- 2007-2013, Bin Guan, PhD, NIDDK, NIH
- 2012-2014, Tae Mogami, MD, PhD, Department of Gynecology, Yokolohoma City University Medical Center, Japan

Graduate and Undergraduate Students (Johns Hopkins University except Ms. Mahle)

- 2008-2012, KaiLee Yap, pathobiology graduate student (thesis student), currently a postdoc fellow at the University of Chicago.
- 2010-2012, Min Gao, exchange/visiting graduate student from Shandong University/Zilu hospital, China.
- 2008-2010, Chen Xu, exchange/visiting graduate student from China Scholarship council, currently attending physician in the Department of Urology, the first affiliated hospital, Sun Yat Sen University, China
- 2005- 2009, Joon Park, pathobiology graduate student (thesis student), currently a Senior Scientist, Samsung Advanced Institution for Technology, Seoul, South Korea.

- 2009-2010, Elizabeth Chen, currently medical student in Uniformed Services University of Health Sciences, Bethesda, Maryland.
- 2007-2008, Vivek Murthy, currently a medical student at NYU.
- 2003-2005, Robert J. Oldt III, currently a medical student at UMDNJ, NY.
- 2005, Jim M. Yen, MD, currently a medical resident at the Medical Center of the University of South California, CA.
- 2005, Eric Cheng, currently a medical student at UMDNJ, NY.
- 2005, Ilena Neuberger, currently a medical student at UMDNJ, NY.
- 2007, Rebecca Bush, currently a medical student in Washington University School of Medicine, MO.
- 2007, David Chu, currently a medical student in University of Pittsburg, PA.
- 2007, Mandy Mahle, Queens University of Charlotte, NC, currently, a Biochemistry major
- 2007-2009, Kevin Lee, currently a medical student in Albany Medical College, NY.
- 2007-2009, Paul Markowiski, previously lab assistant, currently a medical student in Robert Wood Johnson Medical School, NJ.
- Marilina Mascaró, visiting PhD student, Facultad de Farmacia Bioquimica, Catedra de Immunologia, Buenos Aires, Argentina

Ph.D. Student Qualification Committee:

- MD/PhD candidates in Cellular & Molecular Medicine Graduate Program:
  Saurubh Saha, Harith Rajagopalan, Chetan Bettego, Jordan Cummins
- PhD candidates in Cellular & Molecular Medicine Graduate Program:
  Ian Cheong, Carlo Rago and Jihye Yun
- Pharmacology Graduate Program:
  Xin Huang, Meng Li, Kibem Kim
- Pathobiology Graduate Program:
  Yin Yeh, Shaaretha Pelly, Sophie Lin Zhirong; Kah Suan Lim; Byung-Hak Kang, Shu-Han Yu
- Graduate Board Exam, Department of Chemical and Molecular Engineering, Johns Hopkins Univeristy:
  Serving as the Chair of the Exam committee for Melissa Thompson, CK Wang.

Ph.D. Student Thesis Committee:

- Melissa Thompson, PhD candidate, Department of Chemical and Molecular Engineering, Johns Hopkins University (Homewood campus), 2007- current
- Melissa Landek, PhD candidate, Pathobiology Graduate Program, Johns Hopkins Medical Institutions, 2008
- Hsin Chih Yeh, PhD candidate, Department of Bioengineering, Johns Hopkins University, 2008
- Christopher Puleo, PhD candidate, Department of Bioengineering, Johns Hopkins University, 2009
- Vasudev Bailey, PhD candidate, Department of Bioengineering, Johns Hopkins University, 2010
- Kelvin Liu, PhD candidate, Department of Bioengineering, Johns Hopkins University, 2011
- Yi Zhang, PhD candidate, Department of Bioengineering, Johns Hopkins University, 2013

Participation in mentoring Gynecologic Pathology Fellows (*Johns Hopkins Hospital*):

- 2003 – 2005, Monica Srodon, M.D.
  Staff pathologist
  Greensboro Pathology Associates
  Greensboro, NC
- 2004 – 2006, Saeid Movahedi-Lankarani, M.D.
  Staff pathologist
  Hospital Pathology Associates
  St. Paul, MN
- 2006 – 2007, Dengfeng Cao, M.D., Ph.D.
  Assistant Professor
  Department of Pathology & Immunology
  Washington University School of Medicine
  St. Louis, MO
- 2006 – 2007, Kara Judson, M.D.
  Attending pathologist
  Lenox Hill Hospital
  New York, NY
- 2005 – Current, Anna Yemelyanova, M.D.
  (Current Fellow)
- 2007 – Current, Thomas McConnell, M.D.
  (Current Fellow)
- 2007 – 2008, Emanuela Veras, M.D.
  Memorial Sloan-Kettering Cancer Center

Awards Received by Dr. Shih's Trainees

- **Collen's Dream Foundation for ovarian cancer research award,** 2014, Hiroyasu Kashima, MD, research fellow
- **Keio University School of Medicine Young Investigator Award, Japan,** 2014, Yusuke Kobayashi, research fellow
- **Young Investigator Award in Basic Science, Department of Pathology, JHU,** 2014, Fun Yuyu, postdoctoral fellow
- **Ovarian Cancer Research Foundation (OCRF) award, 2013,** Fun Yuyu, postdoctoral fellow
- **Oppo's Foundation for Ovarian Cancer Young Investigator Award,** 2013, Felix Zeppernick, research fellow
- **Scholar-in-Training Award, American Association for Cancer Research,** 2013, Ren-Chin Wu, graduate student
- **HERA Research Award,** 2013, Fnu Yuyu, PhD, research fellow
- **Collen's Dream Foundation for ovarian cancer research award,** 2013, Felix Zeppernick, MD, research fellow
- **YW Loke Award,** 2012, Yusuke Kobayashi, MD, PhD, research fellow, award from International Federation of Placenta Associations
- **HERA Research Award,** 2012, Elizabeth Kuhn, MD, research fellow
- **Scholar-in-Training Award, American Association for Cancer Research**, 2011, Kai-Lee Yap, graduate student

- **Ovarian Cancer Research Foundation (OCRF) Award**, 2011, Bin Guan, PhD, postdoctoral fellow
- **American Society of Clinical Oncology Young Investigator Research Grant,** 2011, Stephanie Gaillard, MD, PhD, research fellow
- **Scholar-in-Training Award by Aflac, Inc.,** 2011, Kai-Lee Yap, PhD graduate student
- **HERA Research Award,** 2011, Alex Stoeck, PhD, research fellow
- **Pathology Young Investigator Award,** 2011, Kai-Lee Yap, PhD graduate student
- **Pathology Young Investigator Award,** 2011, Elisabetta Kuhn, MD research fellow
- **Pathology Young Investigator Award,** 2011, Alex Stoeck, PhD research fellow
- **International Society of Gynecologic Pathology Fellowship Award,** 2011, Laura Ardigheri, research fellow, 2011
- **HERA Research Award,** 2010, Bin Guan, PhD, research fellow
- **UICC, ICRETT award**. 2010, Marilina Mascaró, visiting PhD student, Argentina
- **Pathology Young Investigator Award,** 2010, Kai-Lee Yap, PhD graduate student
- **HERA Research Award,** 2008, Stefanie Ueda, MD, research fellow
- **Pathology Department Young Investigator First Price Award in Basic Science,** 2008, Joon Park, Johns Hopkins Medical Institutions
- **HERA Research Award,** 2007, Natini Jinawath, MD, PhD, research fellow
- **Provost's undergraduate research award,** 2007, Chanont Vasoontara, Johns Hopkins University
- **Ovarian Cancer Research Fund (OCRF)**, 2006, Ritu Salani, MD, research fellow
- **Best Abstract Award,** 2006, Ritu Salani, MD, research fellow, International Gynecologic Cancer Society biannual meeting, Santa Monica
- **Provost's undergraduate research award**, 2006, Rebecca Busch, JHU undergraduate student
- **HERA Research Award,** 2005, Kentaro Nakayama, MD, PhD, research fellow
- **First Place Award for Research Fellow in Basic Research, Johns Hopkins Oncology,** 2005, Jim Sheu, PhD, research fellow
- **International Union Against Cancer Technology Transfer Fellowship,** 2004, Gudrum Pohl, MD, research fellow
- **HERA Research Award,** 2003, Brant Wang, MD, PhD, research fellow
- **Yong Investigator Award of the International Society of Gynecologic Pathologists**, 2004, Gad Singer, MD, research fellow
- **Howard Hughes Undergraduate Research Award**, 2003, Robert J. Oldt III, JHU undergraduate student
- **Provost's undergraduate research award**, 2002, Robert J. Oldt III, JHU undergraduate student

CLINICAL ACTIVITIES

Certification

- The American Board of Pathology --- Anatomic Pathology, 1997
- Medical Licensure: Maryland, 1997

Clinical Service Responsibilities (20% of total effort) at the Johns Hopkins Hospital

- **Attending Physician**- diagnostic pathology in routine gynecologic specimens

- **Consultant Pathologist**- gynecologic pathology, specifically gestational trophoblastic diseases (nationally and internationally)

ADMINISTRATIVE AND ORGANIZATIONAL ACTIVITIES

Administrative Appointments

- Co-director, the Female Reproductive Cancer Program (in development), Kimmel Cancer Center, Johns Hopkins Medical Institutions, 2011- current. Mainly involved in program development, research planning and educational activities.
- Planning Committee, the 7th Biennial Meeting of Asia-Pacific International Academy of Pathology, 2009-2011
- Johns Hopkins Oncology Center Tissue Core oversight committee, 2013-
- Johns Hopkins Professor Promotion Committee, 2013-
- Symposium organizer, Johns Hopkins Annual Ovarian Cancer Symposium, 2009-current.
- President of International Association of Chinese Pathologists, 2006-2007; received the *Excellent Service Award*, March 2, 2008
- President of North American Taiwanese Medical Association-Baltimore chapter, 2006-2008
- Faculty promotion committee, Department of Pathology, Johns Hopkins Medical Institutions, 2004
- PhD student qualification/thesis committees, 2002-current
- Pathology residency advisory committee, 2009-current

Editorial Board Appointments

- Editor-in-Chief, Current Obstetrics and Gynecology Report (2012-)
- Cancer Research (2013-2015)
- The Journal of Pathology (2012-)
- Guest Editor, Journal of Oncology special issue in ovarian cancer targeted therapy, 2011
- International Journal of Gynecologic Pathology
- ISRN Pathology
- International Journal of Molecular Sciences (Molecular Pathology section)
- Journal of the Formosan Medical Association
- Frontiers in Women's Cancer

Journal Peer Review Activities

- Proceedings of National Academy of Science
- Cancer Research
- Clinical Cancer Research
- Oncogene
- Journal of Clinical Investigation
- Journal of Biological Chemistry
- International Journal of Cancer
- Gynecologic Oncology
- Cancer Letters
- Modern Pathology
- Placenta
- The American Journal of Pathology
- Laboratory Investigation

- Human Pathology
- The Journal of Obstetrics and Gynecology Research
- British Journal of Cancer
- International Journal of Gynecologic Pathology
- Gastroenterology
- Annals of Oncology
- American Journal of Obstetrics and Gynecology
- International Journal of Gynecologic Cancer

Professional Societies Membership

- American Association for Cancer Research, 2004-present
- American Society for Investigative Pathology, 2002-present
- International Association of Gynecologic Pathologists, 1998-present
- United States and Canadian Academy of Pathology, 1998-present
- International Society for the Study of Trophoblastic Disease, 2000-present
- Society for the Study of Reproduction, 2000-present
- American Medical Association, 1998
- International Federation of Placental Associations, 1996-present

Panelist in Study Sections and Grant Review Committees

- National Institute of Health, National Cancer Institute, member of Omnibus- Cancer Biology 1 study section, 2013
- National Institute of Health, National Cancer Institute, member of P50 SPORE study section, 2012-
- National Institute of Health, National Cancer Institute, , Ad Hoc member of Provocative Question study section, 2012
- National Institute of Health, National Cancer Institute, member of Cancer Molecular Pathobiology Study section (CAMP), 2006-2011 (*Recipient of "Brain Award" and "Humanitarian Award")
- National Institute of Health, National Cancer Institute, Ad Hoc member of R15 Academic Research Enhancement Award Study Section, 2011.
- National Institute of Health, National Cancer Institute, site visit adviser, EDRN Early Detection Network, Cancer Biomarkers Research Group, July 15, 2008
- National Institute of Health, National Cancer Institute, member of ZRG1 Onc-L (12)B Cancer Diagnostic & Treatment Study Section, March 2005, October 2005, March 2006, June 2006, February 2007 (member)
- The Wellcome Trust, London, United Kingdom, Research proposal reviewer, 1998 (Ad Hoc)
- National Institute of Health, National Cancer Institute, study section of IMAT, R21: "new innovative technology in cancer", 2002 (Ad Hoc)
- Israel Science Foundation (ISF), Research proposal reviewer, 2004 (Ad Hoc)
- US Department of Defense (USAMRMC/CDMRP) ovarian cancer research program, member of the review committee, April, 2005 (Ad Hoc)
- Cancer Research UK, April 2005, July 2008 (Ad Hoc)
- Netherlands Organization for Health Research and Development (ZonMw), Netherland, grant proposal reviewer for 80-007029-98-07041, March 2006 (Ad Hoc)
- Research Grants Council of Hong Kong, panel member and external reviewer, March 2006, December 2007

- US Department of Defense ovarian cancer research program-concept awards, member of the review committee, April, 2006 (Ad Hoc)
- Cancer Research UK, requested by the Translational Research in Clinical Trials Committee, July 2006 (Ad Hoc)
- U.S. Civilian Research Development Foundation, Arlington, Virginia, October 2006 (Ad Hoc)
- Swiss Nationals Science Foundation, Berne, Switzerland, January, 2007 (Ad Hoc)
- Kansas Masonic Foundation, Kansas Masonic Cancer Research Institute, 2007 (Ad Hoc)
- Invited reviewer requested by the Ministry of Science & Technology, Life Sciences Division, Israel, for Taiwanese Israeli scientific and technological cooperation, 2007
- Invited reviewer requested by the Sheffield Hospital Charitable Trust Medical Research Committee, UK, 2008
- Maryland Industrial Partnerships (MIPS) Program, University of Maryland College Park, 2008
- US Department of Defense (USAMRMC/CDMRP) ovarian cancer research program, member of the review committee, April, 2009 (Ad Hoc)
- American Institute of Biological Sciences (AIBS), May, 2010 (Ad Hoc)
- Calgary Laboratory Services Health Services Research Funding Competition, June, 2010 (Ad Hoc)
- National Medical Research Council, Singapore, January 2011.

Organizer, chair and moderator in conference organizations

- *Chair Moderator,* Poster Section In 4th Conference of the International Federation of Placenta Associations. Tokyo, Japan, 1998.
- *Symposium section chair,* Gestational trophoblastic disease. In XXVI International Congress of the International Academy of Pathology, Montreal, Canada, September 2006.
- *Moderator*, Pathobiology platform section, annual (the 97th) meeting of the United States and Canadian Academy of Pathology (USCAP), Denver, Colorado, March 2008.
- *Symposium organizer,* Ovarian Cancer Symposium- Elucidating Early Ovarian Carcinogenesis: Implications for Early Detection and Treatment. Sponsored by Department of Defense. Baltimore, Maryland, May 28-29, 2009.
- *Moderator,* Gynecologic Pathology platform section, annual (the 99th) meeting of the United States and Canadian Academy of Pathology (USCAP), Washington DC, March 2010.
- *Moderator,* Gynecologic Pathology platform section, annual (the 100th) meeting of the United States and Canadian Academy of Pathology (USCAP), San Antonio, TX, March 2011.
- *Section convener,* gynecologic pathology section, in the (scheduled) 7th Asia-Pacific International Academy of Pathology, Taipei, Taiwan, May 20-24, 2011.

Advisory boards, committees and consultation groups

- **Scientific Advisory Committee,** Ovarian Cancer Research Foundation (OCRF), New York, 2013-.
- **NCI Ovarian Task Force of Gynecologic Cancer Steering Committee**, 2012-2015

- **International Society of Gynecologic Pathology/World Health Organization (WHO) Nomenclature Committee for gynecological neoplasm**, 2012
- **External advisory board**, Ovarian Cancer SPORE at Fox Chase Cancer Center, 2013
- **International Society of Gynecologic Pathology Nomenclature Committee**: Gestational trophoblastic disease subcommittee, 2011-
- **Panelist** of an NIH sponsored consensus meeting for ovarian borderline tumor, Bethesda, 2003
- **Committee member** in the *National Academy for Clinical Biochemistry*-ovarian cancer marker Laboratory Medicine Practice Guidelines (tumor markers). 2003

Ad Hoc member in Award/Fellowship Committee

- Wittgenstein Award, funded by the Austrian Science Fund (FWF), 2007
- Moldovan Young Scientist Scholarship Program, United States Civilian Research & Development Foundation, 2007

RECOGNITION

Awards and Honors

- *The Best Intern Award,* McKay Memorial Hospital, Taiwan, 1988
- *TeLinde Research Award,* Division of Gynecologic Pathology, Department of Pathology, the Johns Hopkins Hospital, 1996-1998
- *Young Investigator Award,* The 13th Rochester Trophoblast Conference, Banff, Canada, 1996
- *Junior Achievement Award,* NIH/FDA Chinese American Association and Washington DC Chapter of Society of Chinese Bioscientists in America, 1998
- *Young Investigator Award,* International Society of Gynecological Pathologists, 2000.
- *Clinician Scientist Award,* Johns Hopkins University School of Medicine, 2002.

Invited Talks and Panels

- *Invited Speaker*, "Pathology of benign and malignant lesions of intermediate trophoblast". In 4th Conference of the International Federation of Placental Associations. Tokyo, Japan, 1998.
- *Invited Speaker* "Molecular surrogates of tumor progression in body fluids". Bowling Green State University, Ohio, 2001.
- *Invited Speaker,* "Molecular Landscape of Ovarian cancer and its implication for early diagnosis". Chang-Gung Memorial Hospital, Taiwan, 2002.
- *Invited Speaker,* "Gestational trophoblastic diseases", Taipei Medical University, Taiwan, 2002.
- *Invited Speaker,* "Molecular Landscape of Ovarian cancer". National Cancer Institute/NIH, 2002.
- *Invited Lecturer,* "Gestational trophoblastic diseases", Pathology Laboratory, National Cancer Institute/NIH, 2002.
- *Invited Speaker,* "Circulating tumor-released DNA as the marker for early detection of cancer". Pathology Grand Round, MD Anderson Cancer Center, January 2003.
- *Invited Lecturer,* "Pathology of gestational trophoblastic diseases", MD Anderson Cancer Center, January 2003.

- *Invited Speaker,* "Digital PCR and clinical applications". At the 11th annual meeting of "Nuclei acid-based technologies" Baltimore, June 2003.
- *Invited Speaker,* "New technologies in exploring disorders of human implantation and trophoblast". Perinatology research branch, NICHD, Detroit, May, 2003.
- *Invited Speaker,* "Pathology of intermediate trophoblastic lesions". NICHD, Detroit, May, 2003.
- *Invited Speaker,* "Allelic imbalance in detecting ovarian and other types of cancer". At the 4th Principal Investigator Meeting of "Innovative Molecular Analysis Technologies (IMAT) Program" sponsored by NIH. San Diego, June 2003.
- *Invited Speaker,* "Molecular Genetic Markers for Cancer Detection in Blood". At the Cambridge Healthtech Institute's 11th Annual Molecular Medicine Tri-Conference, San Francisco, March 2004.
- *Invited Speaker,* "Molecular pathways of ovarian cancer-translational cancer research by analyzing cancer genome". Division of epidemiology and genetics, NCI/NIH, Rockville, Maryland, September 16, 2004.
- *Invited Speaker,* "DNA preparation for cancer genomic study-the pathologist's views". Lecture in the G.O.T. (Getting Optimal Targets) summit series, Genomic and Proteomic Sample Preparation, Boston, May 3-4, 2005.
- *Invited Speaker,* "Identification of novel genes for cancer therapy and diagnosis by exploring cancer genome". 10th Annual Meeting of Chinese Biopharmaceutical Association, Rockville, Maryland, June 18, 2005.
- *Guest Speaker,* "Exploring ovarian cancer genome- new insights and old challenges". Fox Chase Cancer Center, Philadelphia, Pennsylvania, August 9, 2005.
- *Invited Speaker,* "Relationship of serous borderline tumor and carcinoma". The annual companion meeting of the International Association for Gynecologic Pathologists. Atlanta, Georgia, Feb. 12, 2006.
- *Invited Speaker,* "Identification of novel molecular targets for ovarian cancer therapy". University of Oslo. Olso, Norway, Feb. 27, 2006.
- *Invited Speaker,* "Translating Ovarian Cancer Genome- New Genes for Prognostic Prediction and Targeted Therapy". Pathology Grand Round, University of British Columbia, Vancouver, Canada, March 13, 2006.
- *Invited Speaker,* "Trophoblastic tumors and tumor-like lesions". Department of Pathology, Vancouver Hospital, Canada, March 13, 2006.
- *Invited Speaker, "Gestational trophoblastic tumor-an intellectual Odyssey".* Second Investigative Pathology Conference, Cleveland Clinics, Cleveland, Ohio, June 3, 2006
- *Invited Speaker,* "Applications of HLA-G expression in the diagnosis of human neoplastic diseases". Forth International conference on HLA-G, Paris, France, July 12, 2006.
- *Invited Speaker,* "Trophoblastic tumors- molecular classification and pathogenesis". Biennial Meeting of International Gynecological Cancer Society, Santa Monica, October 17, 2006.
- *Invited Speaker,* "Analyzing ovarian cancer genome- from gene discovery to therapeutic targets". Sloan Kettering Memorial Hospital, New York, December 11, 2006.
- *Distinguished Visiting Professor*, "Ovarian cancer- molecular pathways, diagnostic markers and therapeutic targets". Pathology Grand Round, Emory University, March 9, 2007.
- *Distinguished Visiting Professor*, "New concept in ovarian cancer- the dualistic pathway and its implications". Pathology Grand Round, Yale University School of Medicine, April 19, 2007.

- *Invited Speaker,* "Translational Research and New Diagnosis in Ovarian Cancer". The 12th Taiwan Joint Cancer Conference (Gynecologic Oncology section), Taipei, Taiwan, May 5, 2007.
- *Invited Speaker,* "Genomic analysis of ovarian cancer from marker discovery to translational applications". Taipei Medical University, Taipei, Taiwan, May 3, 2007.
- *Invited Speaker,* "Analyzing Ovarian Cancer Genome for Marker Discovery". International Symposium on Biomarkers Discovery in Human Cancers, Tainan, Taiwan, May 7, 2007.
- *Invited Speaker,* "Analyzing ovarian cancer genome for therapeutic target discovery". 12th annual meeting of SCBA, University of Maryland Shady Groove Conference Center, MD, June 2, 2007.
- *Invited Speaker,* "Update in gestational trophoblastic disease". Surgical Pathology Update, Leipzig, Germany, June 15, 2007.
- *Invited Speaker,* "The roles of NAC-1 in chemoresistance in ovarian carcinoma". The Montebello Conference, Norway, June 18, 2007.
- *Invited Speaker,* "Exploring ovarian cancer genome- from marker discovery to therapeutic targeting". Symposium of Toronto Ovarian Cancer Research Network/University of Toronto Health Network, Toronto, Canada, November 2, 2007.
- *Invited Speaker,* "Biological and clinical significance of Rsf-1 gene amplification in ovarian cancer". Grand Round at the Cancer Institute of New Jersey, April 2, 2008.
- *Invited Speaker,* "Analyzing cancer genome to identify new cancer-associated genes in ovarian cancer". In the series of Molecular Pathology seminar, University of Maryland at Baltimore, Baltimore, April 11, 2008.
- *Invited Speaker,* "Molecular etiology of drug resistance in ovarian cancer". Symposium on Ovarian Cancer Research, Medical University of South Carolina, Charleston, South Carolina, May 2, 2008.
- *Invited Speaker,* "Identifying new cancer genes through analyzing cancer genomics- Rsf-1 amplification in ovarian cancer". National Health Research Institution, Taiwan, August 5, 2008.
- *Invited Speaker,* "Early detection and treatment of ovarian cancer: shifting from early stage to minimal volume of disease based on a new model of carcinogenesis". 7th Biennial Ovarian Cancer Symposium, Marsha Rivkin Center for Ovarian Cancer Research, Charleston, Seattle, Washington, September 4-5, 2008
- *Invited Speaker,* "Functional genomic analysis of ovarian cancer", in honor of Dr. Meenhard Herlyn's achievement in cancer research, The Wistar Institute, Philadelphia, PA, August 10, 2009
- *Invited Speaker,* "Notch3 signaling in ovarian cancer", Institute of Genomic Medicine, China Medical University, Taiwan, August 21, 2009
- *Invited Speaker,* "Targeted therapy in ovarian cancer", Ovarian Cancer SPORE meeting, Fox Chase Cancer Center, Philadelphia, PA, September 26, 2009
- *Invited Speaker,* 7th International Seminar at Lake Hamana- Surgical and Molecular Pathology of the Endometrium, Placenta, and Ovary. "Pathology of gestational trophoblastic diseases", and "Molecular pathogenesis of ovarian cancer", Hamamatsu, Shizuoka, Japan, November 7, 8, 2009
- *Invited Speaker,* "Gestational trophoblastic diseases", Grand Round in the Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY, December 7, 2009
- *Invited Speaker,* "The origin and pathogenesis of epithelial ovarian cancer- a proposed unifying theory", Grand Round, Department of Gynecologic Oncology, MD Anderson Cancer Center, Houston, TX, February 1, 2010

- *Invited Speaker*, "Definition and characterization of low-grade and high-grade ovarian serous carcinomas", 2nd Annual European Gynecologic Oncology Congress, Athens, Greece, February 12-13, 2010
- *Invited Speaker*, "Clear cell carcinoma of the ovary", Gynecologic Pathology Specialty Conference, United States & Canadian Academy of Pathology, 99th annual meeting. Washington DC, March 20-26, 2010
- *Invited Speaker*, "Molecular pathology of ovarian clear cell carcinoma", University of British Columbia, Vancouver, Canada, June 24, 2010
- *Invited Speaker*, "The origin and pathogenesis of epithelial ovarian cancer- a proposed unifying theory", Fox Chase Cancer Center, Philadelphia, July 15, 2010
- *Invited Speaker*, "The origin and pathogenesis of epithelial ovarian cancer- a proposed unifying theory", Department of Pathology, Chang-Gang Memorial Hospital at Kaohsiung, Taiwan, August 12, 2010
- *Invited Speaker*, "The biological roles of NAC1 in cancer pathogenesis", Department of Developmental Biology and Regeneration Medicine, Mount Sinai School of Medicine, New York City, New York, September 2, 2010
- *Invited Speaker*, "Chromatin remodeling in ovarian cancer", Department of Molecular and Cellular Biology, Rutgers University, New Jersey, January 11, 2011
- *Invited Speaker*, "Genomic analysis of gynecological cancer", National Cancer Research Center, Tokyo, Japan, June 30, 2011
- *Invited Keynote Speaker*, "Ovarian cancer is an imported disease- fiction or fact", The 10th annual meeting of targeted therapy in gynecologic oncology, Izumo, Shimane, Japan, July 2, 2011
- *Invited Keynote Speaker*, "Pathogenesis of ovarian clear cell carcinoma", The 10th annual meeting of targeted therapy in gynecologic oncology, Izumo, Shimane, Japan, July 2, 2011
- *Invited Speaker*, "Diagnosis of biological implication of serous tubal intraepithelial carcinoma", Chang-Kung Memorial Hospital, Kaohsiung, Taiwan, July 6, 2011
- *Invited Speaker*, "Ovarian cancer genetics- latest insight", The Boehringer Ingelheim Conversations in Oncology, Vienna, Austria, October 28-29, 2011
- *Invited Speaker*, "Integrated molecular analysis of ovarian cancer", Virginia Polytechnic Institute and State University, Arlington, Virginia, February 22, 2012.
- *Invited Speaker,* "Intertumoral heterogeneity- how many types of cancers do my patients have?" In the symposium of "Intratumoral and intertumoral heterogeneity in ovarian cancer", American Association for Cancer Research (AACR) annual meeting, Chicago, April 2, 2012
- *Invited Speaker*, "Genomic landscape in gynecologic cancer and its biological and translation implications", Department of Pathology and Laboratory Medicine, University of California at Irvine, April 16, 2012.
- *Lecture,* "Molecular analysis of serous tubal intraepithelial carcinoma", the 3rd Johns Hopkins Ovarian Cancer Symposium, Baltimore, Maryland, May 18, 2012.
- *Invited Keynote Speaker*, "Endometriosis-related ovarian cancer", The 16th Korea-Japan, the 2nd Korea-Taiwan-Japan Joint Conference for Gynecological Pathology, Kumamoto University, Kumamoto City, Japan, May 26, 2012.
- *Invited Speaker*, "Genomic landscape in gynecologic cancer- a road map to new therapeutics", Bristol-Myers Squibb Lectureship, Kumamoto City, Japan, May 27, 2012.
- *Invited Speaker*, "Genomic landscape in gynecologic cancer- a road map to new therapeutics", Kyoto University, Kyoto, Japan, May 29, 2012.

- *Invited Keynote Speaker*, "Genomic analysis of gynecological cancer and their clinical implications", In annual meeting of Korean Division of International Association of Pathologists, Seoul, South Korea, October 18, 2012.
- *Invited Speaker*, "The tumor suppressor role of ARID1A in human cancer", Kyung Hee University, Seoul, South Korea, October 18, 2012.
- *Invited Speaker*, "The tumor suppressor role of ARID1A in human cancer", Korean National Cancer Center, Seoul, South Korea, October 19, 2012.
- *Invited Speaker*, "The origin of ovarian cancer- clear cell carcinoma", International Society of Gynecologic Pathologists companion meeting of United States and Canadian Association of Pathology annual meeting, Baltimore, Maryland, March 3, 2013.
- *Invited Speaker*, "Genomic landscape of ovarian cancer and its translational implications", The Wistar Institute, Philadelphia, April 15, 2013.
- *Invited Speaker*, "Molecular alterations in serous tubal intraepithelial carcinoma", 4th Ovarian Cancer Symposium, the Memorial Sloan Kettering Cancer Center, New York, May 15, 2013.
- *Invited Speaker,* "Emerging therapeutics in gynecologic cancer", China Medical University, Taichung, Taiwan, July 7, 2013
- *Invited Speaker,* "Bokhman's dualistic model of endometrial carcinoma- revisited", Chang-Kung Memorial Hospital, Kaohsiung, Taiwan, July 8, 2013
- *Invited Speaker*, "Genomic analysis and pathogenesis of uterine carcinoma", Taipei Veterans General Hospital, Taipei, Taiwan, July 11, 2013.
- *Invited Speaker*, "The Genomic landscape and origin of ovarian cancer", The 18th Taiwan Joint Cancer conference, Taipei, Taiwan, July 13, 2013.
- *Invited Lecturer*, "The origin and pathogenesis of ovarian cancer", The 2013 International Diagnostic Pathology Course, Tokyo, Japan, July 14, 2013.
- *Invited Speaker,* "Ovarian cancer is an imported disease- fiction or fact?" Charite Hospital (Mitt campus), Berlin, Germany, September 11, 2013
- *Invited Lecturer,* "Various topics in gynecologic pathology and oncology", Nederland Master Class in ovarian cancer. Berlin, Germany, September 12, 2013
- *Invited Lecturer,* "Understanding the molecular mechanisms in the development of chemoresistance in cancer", Rush University Medical Center, Chicago, October 30, 2013
- *Invited Speaker*, "Ovarian cancer is an imported disease – translational implication and beyond", Ovarian Cancer SPORE meeting, MD Anderson Cancer Center, Houston, TX, May 28, 2014
- *Invited Speaker,* "Molecular pathogenesis of high-grade serous carcinoma". Symposium of the National Gynecologic Oncology Group (NGR, GOG). Chicago, July 9, 2014

OTHER NONPROFESSIONAL ACTIVITIES

Photography website: http://www.shih-photography.com

United States Patent **6,440,706**
Vogelstein , et al. **August 27, 2002**

Digital amplification

Claims

What is claimed is:

1. A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of: diluting nucleic acid template molecules in a biological sample to form a set comprising a plurality of assay samples; amplifying the template molecules within the assay samples to form a population of amplified molecules in the assay samples of the set; analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence; comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

2. The method of claim 1 wherein the step of diluting is performed until at least one-tenth of the assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required for the step of analyzing to determine the presence of the selected genetic sequence.

3. The method of claim 1 wherein the step of diluting is performed until between 0.1 and 0.9 of the assay samples yield an amplification product when subjected to a polymerase chain reaction.

4. The method of claim 1 wherein the step of diluting is performed until all of the assay samples yield an amplification product when subjected to a polymerase chain reaction and each assay sample contains less than 10 nucleic acid template molecules containing the reference genetic sequence.

5. The method of claim 1 wherein the step of diluting is performed until all of the assay samples yield an amplification product when subjected to a polymerase chain reaction and each assay sample contains less than 100 nucleic acid template molecules containing the reference genetic sequence.

6. The method of claim 1 wherein the biological sample is cell-free.

7. The method of claim 1 wherein the number of assay samples within the set is greater than 10.

8. The method of claim 1 wherein the number of assay samples within the set is greater than 50.

9. The method of claim 1 wherein the number of assay samples within the set is greater than 100.

10. The method of claim 1 wherein the number of assay samples within the set is greater than 500.

11. The method of claim 1 wherein the number of assay samples within the set is greater than 1000.

12. The method of claim 1 wherein the step of amplifying and the step of analyzing are performed on assay samples in the same receptacle.

13. The method of claim 1 wherein a molecular beacon probe is used in the step of analyzing, wherein a molecular beacon probe is an oligonucleotide with a stem-loop structure having a photoluminescent dye at one of the 5' or 3' ends and a quenching agent at the opposite 5' or 3' end.

14. The method of claim 1 wherein the step of analyzing employs gel electrophoresis.

15. The method of claim 1 wherein the step of analyzing employs hybridization to at least one nucleic acid probe.

16. The method of claim 1 wherein the step of analyzing employs hybridization to at least two nucleic acid probe.

17. The method of claim 13 wherein two molecular beacon probes are used, each having a different photoluminescent dye.

18. The method of claim 13 wherein the molecular beacon probe detects a wild-type selected genetic sequence better than a mutant selected genetic sequence.

19. The method of claim 1 wherein the step of amplifying employs a single pair of primers.

20. The method of claim 1 wherein the step of amplifying employs a polymerase which is activated only after heating.

21. The method of claim 1 wherein the step of amplifying employs at least 40 cycles of heating and cooling.

22. The method of claim 1 wherein the step of amplifying employs at least 50 cycles of heating and cooling.

23. The method of claim 1 wherein the step of amplifying employs at least 60 cycles of heating and cooling.

24. The method of claim 1 wherein the biological sample is selected from the group consisting of stool, blood, and lymph nodes.

25. The method of claim 1 wherein the biological sample is blood or bone marrow of a leukemia or lymphoma patient who has received anti-cancer therapy.

26. The method of claim 1 wherein the selected genetic sequence is a translocated allele.

27. The method of claim 1 wherein the selected genetic sequence is a wild-type allele.

28. The method of claim 1 wherein the selected genetic sequence is within an amplicon which is amplified during neoplastic development.

29. The method of claim 1 wherein the selected genetic sequence is a rare exon sequence.

30. The method of claim 1 wherein the nucleic acid template molecules comprise cDNA of RNA transcripts and the selected genetic sequence is present on a cDNA of a first transcript and the reference genetic sequence is present on a cDNA of a second transcript.

31. The method of claim 1 wherein the selected genetic sequence comprises a first mutation and the reference genetic sequence comprises a second mutation.

32. The method of claim 1 wherein the selected genetic sequence and the reference genetic sequence are on distinct chromosomes.

33. A molecular beacon probe comprising: an oligonucleotide with a stem-loop structure having a photoluminescent dye at one of the 5' or 3' ends and a quenching agent at the opposite 5' or 3' end, wherein the loop consists of 16 base pairs, wherein the loop has a T.sub.m of 50-51.degree. C. and the stem consists of 4 base pairs having a sequence 5'-CACG-3'.

34. The probe of claim 33 wherein the molecular beacon probe detects a wild-type selected genetic sequence better than a mutant selected genetic sequence.

35. The probe of claim 33 wherein the molecular beacon probe detects a mutant genetic sequence better than a wild-type genetic sequence.

36. A molecular beacon probe comprising: an oligonucleotide with a stem-loop structure having a photoluminescent dye at one of the 5' or 3' ends and a quenching agent at the opposite 5' or 3' end, wherein the loop consists of 19-20 base pairs, wherein the loop has a T.sub.m of 54-56.degree. C. and the stem consists of 4 base pairs having a sequence 5'-CACG-3'.

37. A pair of molecular beacon probes comprising: a first molecular beacon probe which is an oligonucleotide with a stem-loop structure having a first photoluminescent dye at one of the 5' or 3' ends and a quenching agent at the opposite 5' or 3' end, wherein the loop consists of 16 base pairs having a T.sub.m of 50-51.degree. C. and the stem consists of 4 base pairs having a sequence 5'-CACG-3'; and a second molecular beacon probe which is an oligonucleotide with a stem-loop structure having a second photoluminescent dye at one of the 5' or 3' ends and a quenching agent at the opposite 5' or 3' end, wherein the loop consists of 19-20 base pairs having a T.sub.m of 54-56.degree. C. and the stem consists of 4 base pairs having a sequence 5'-CACG-

3'; wherein the first and the second photoluminescent dyes are distinct.

38. A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of: amplifying template molecules within a set comprising a plurality of assay samples to form a population of amplified molecules in each of the assay samples of the set; analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence, wherein at least one-fiftieth of the assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required to determine the presence of the selected genetic sequence; comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

39. The method of claim 38 wherein the number of assay samples within the set is greater than 10.

40. The method of claim 38 wherein the number of assay samples within the set is greater than 50.

41. The method of claim 38 wherein the number of assay samples within the set is greater than 100.

42. The method of claim 38 wherein the number of assay samples within the set is greater than 500.

43. The method of claim 38 wherein the number of assay samples within the set is greater than 1000.

44. The method of claim 38 wherein the step of amplifying and the step of analyzing are performed on assay samples in the same receptacle.

45. The method of claim 38 wherein a molecular beacon probe is used in the step of analyzing, wherein a molecular beacon probe is an oligonucleotide with a stem-loop structure having a photoluminescent dye at one of the 5' or 3' ends and a quenching agent at the opposite 5' or 3' end.

46. The method of claim 38 wherein the step of analyzing employs gel electrophoresis.

47. The method of claim 38 wherein the step of analyzing employs hybridization to at least one nucleic acid probe.

48. The method of claim 38 wherein the step of analyzing employs hybridization to at least two nucleic acid probe.

49. The method of claim 45 wherein two molecular beacon probes are used, each having a different photoluminescent dye.

50. The method of claim 45 wherein the molecular beacon probe detects a wild-type selected genetic sequence better than a mutant selected genetic sequence.

51. The method of claim 38 wherein the step of amplifying employs a single pair of primers.

52. The method of claim 38 wherein the step of amplifying employs a polymerase which is activated only after heating.

53. The method of claim 38 wherein the step of amplifying employs at least 40 cycles of heating and cooling.

54. The method of claim 38 wherein the step of amplifying employs at least 50 cycles of heating and cooling.

55. The method of claim 38 wherein the step of amplifying employs at least 60 cycles of heating and cooling.

56. The method of claim 38 wherein the template molecules are obtained from a body sample selected from the group consisting of stool, blood, and lymph nodes.

57. The method of claim 38 wherein the template molecules are obtained from a body sample of a leukemia or lymphoma patient who has received anti-cancer therapy, said body sample being selected from the group consisting of blood and bone marrow.

58. The method of claim 38 wherein the selected genetic sequence is a translocated allele.

59. The method of claim 38 wherein the selected genetic sequence is a wild-type allele.

60. The method of claim 38 wherein the selected genetic sequence is within an amplicon which is amplified during neoplastic development.

61. The method of claim 38 wherein the selected genetic sequence is a rare exon sequence.

62. The method of claim 38 wherein the nucleic acid template molecules comprise cDNA of RNA transcripts and the selected genetic sequence is present on a cDNA of a first transcript and the reference genetic sequence is present on a cDNA of a second transcript.

63. The method of claim 38 wherein the selected genetic sequence comprises a first mutation and the reference genetic sequence comprises a second mutation.

64. The method of claim 38 wherein the selected genetic sequence and the reference genetic sequence are on distinct chromosomes.

Digital amplification

Claims

The invention claimed is:

1. A method for determining an allelic imbalance in a biological sample, comprising the steps of: amplifying template molecules within a set comprising a plurality of assay samples to form a population of amplified molecules in each of the assay samples of the set, wherein the template molecules are obtained from a biological sample; analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain a selected genetic sequence on a first chromosome and a second number of assay samples which contain a reference genetic sequence on a second chromosome, wherein between 0.1 and 0.9 of the assay samples yield an amplification product; comparing the first number of assay samples to the second number of assay samples to ascertain an allelic imbalance in the biological sample.

2. The method of claim 1 wherein the step of amplifying employs real-time polymerase chain reactions.

3. The method of claim 2 wherein the real-time polymerase chain reactions comprise a dual-labeled fluorogenic probe.

4. The method of claim 1 wherein the selected genetic sequence and the reference genetic sequence are non-polymorphic markers.

5. The method of claim 1 wherein the biological sample is from blood.

6. The method of claim 1 wherein the selected genetic sequence is a non-polymorphic marker.

7. The method of claim 1 wherein the reference genetic sequence is a non-polymorphic marker.

8. The method of claim 1 wherein between 0.1 and 0.6 of the assay samples yield an amplification product.

9. The method of claim 1 wherein between 0.3 and 0.5 of the assay samples yield an amplification product.

10. The method of claim 1 wherein between 0.1 and 0.9 of the assay samples yield an amplification product as determined by amplification of the selected genetic sequence.

11. The method of claim 1 wherein between 0.1 and 0.9 of the assay samples yield an amplification product as determined by amplification of the reference genetic sequence.

12. The method of claim 1 wherein between 0.1 and 0.6 of the assay samples yield an amplification product as determined by amplification of the selected genetic sequence.

13. The method of claim 1 wherein between 0.1 and 0.6 of the assay samples yield an amplification product as determined by amplification of the reference genetic sequence.

14. The method of claim 1 wherein between 0.3 and 0.5 of the assay samples yield an amplification product as determined by amplification of the selected genetic sequence.

15. The method of claim 1 wherein between 0.3 and 0.5 of the assay samples yield an amplification product as determined by amplification of the reference genetic sequence.

16. The method of claim 1 wherein the set comprises at least 500 assay samples.

17. The method of claim 1 wherein the set comprises at least 1000 assay samples.

18. The method of claim 1 wherein the amplified molecules in each of the assay samples in the first and second numbers of assay samples are homogeneous such that the first number of assay samples do not contain the reference genetic sequence and the second number of assay samples do not contain the selected genetic sequence.

19. A method for determining an allelic imbalance in a biological sample, comprising the steps of: distributing nucleic acid template molecules from a biological sample to form a set comprising a plurality of assay samples; amplifying the template molecules within the assay samples to form a population of amplified molecules in the assay samples of the set; analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain a selected genetic sequence on a first chromosome and a second number of assay samples which contain a reference genetic sequence on a second chromosome; comparing the first number of assay samples to the second number of assay samples to ascertain an allelic imbalance between the first chromosome and the second chromosome in the biological sample.

20. The method of claim 19 wherein between 0.1 and 0.9 of the assay samples yield an amplification product.

21. The method of claim 20 wherein between 0.1 and 0.9 of the assay samples yield a homogeneous amplification product.

22. The method of claim 19 wherein the biological sample is blood.

Digital amplification

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Claims

The invention claimed is:

1. A method for determining an allelic imbalance in a biological sample, comprising the steps of: amplifying template molecules within a set comprising a plurality of assay samples to form a population of amplified molecules in each of the assay samples of the set, wherein the template molecules are obtained from the biological sample; analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain a first allelic form of a marker and a second number of assay samples which contain a second allelic form of the marker, wherein between 0.1 and 0.9 of the assay samples yield an amplification product; comparing the first number to the second number to ascertain an allelic imbalance in the biological sample; and identifying an allelic imbalance in the biological sample.

2. The method of claim 1 wherein the step of amplifying employs real-time polymerase chain reactions.

3. The method of claim 2 wherein the real-time polymerase chain reactions comprise a dual-labeled fluorogenic probe.

4. The method of claim 1 wherein between 0.1 and 0.9 of the assay samples yield an amplification product as determined by amplification of the first allelic form of the marker.

5. The method of claim 1 wherein between 0.1 and 0.9 of the assay samples yield an amplification product as determined by amplification of the second allelic form of the marker.

6. The method of claim 1 wherein the amplified molecules in each of the assay samples within the first and second numbers of assay samples are homogeneous such that the first number of assay samples do not contain the second allelic form of the marker and the second number of assay samples do not contain the first allelic form of the marker.

7. The method of claim 1 wherein the sample is from blood.

8. A method for determining an allelic imbalance in a biological sample, comprising the steps of: distributing nucleic acid template molecules from a biological sample to form a set comprising a plurality of assay samples; amplifying the template molecules within the assay samples to form a population of amplified molecules in the assay samples of the set; analyzing the amplified

molecules in the assay samples of the set to determine a first number of assay samples which contain a first allelic form of a marker and a second number of assay samples which contain a second allelic form of the marker; comparing the first number of assay samples to the second number of assay samples to ascertain an allelic imbalance between the first allelic form and the second allelic form in the biological sample.

9. The method of claim 8 wherein the sample is from blood.

10. The method of claim 1 or 8 wherein between 0.1 and 0.6 of the assay samples yield an amplification product.

11. The method of claim 1 or 8 wherein between 0.3 and 0.5 of the assay samples yield an amplification product.

12. The method of claim 1 or 8 wherein the set comprises at least 500 assay samples.

13. The method of claim 1 or 8 wherein the set comprises at least 1000 assay samples.

14. The method of claim 8 wherein the step of amplifying employs real-time polymerase chain reactions.

15. The method of claim 14 wherein the real-time polymerase chain reactions comprise a dual-labeled fluorogenic probe.

16. The method of claim 8 wherein between 0.1 and 0.9 of the assay samples yield an amplification product as determined by amplification of the first allelic form of the marker.

17. The method of claim 8 wherein between 0.1 and 0.9 of the assay samples yield an amplification product as determined by amplification of the second allelic form of the marker.

18. The method of claim 8 wherein the amplified molecules in each of the assay samples within the first and second numbers of assay samples are homogeneous such that the first number of assay samples do not contain the second allelic form of the marker and the second number of assay samples do not contain the first allelic form of the marker.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re *Ex Parte* Reexamination:	)	Group Art Unit: 3991
	)	
U.S. Patent No. 6,440,706	)	Docket No. 001107.00989
	)	
Control No. 90/012,894	)	Confirmation No: 8442
	)	
Reexam Filing Date: June 17, 2013	)	Examiner: Bruce R. Campell

For: DIGITAL AMPLIFICATION

RESPONSIVE AMENDMENT TO FINAL OFFICE ACTION

IN THE CLAIMS

Please amend the following claims as indicated by the status identifier. Patent claims under reexamination but not amended are indicated as "original." Patent claims not subject to reexamination are not shown.

1. (Amended) A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of:

diluting isolated nucleic acid template molecules [in] isolated from a biological sample to form a set comprising a plurality of assay samples;

amplifying the template molecules within the assay samples to form a population of amplified molecules in the assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence;

comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

2. (Original) The method of claim 1 wherein the step of diluting is performed until at least one-tenth of the assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required for the step of analyzing to determine the presence of the selected genetic sequence.

3. (Amended) The method of claim 1 wherein the step of diluting is performed until between 0.1 and 0.9 of the assay samples yield an amplification product of at least one of the selected and reference genetic sequences when subjected to a polymerase chain reaction.

4. (Original) The method of claim 1 wherein the step of diluting is performed until all of the assay samples yield an amplification product when subjected to a polymerase chain reaction and each assay sample contains less than 10 nucleic acid template molecules containing the reference genetic sequence.

5. (Original) The method of claim 1 wherein the step of diluting is performed until all of the assay samples yield an amplification product when subjected to a polymerase chain reaction and each assay sample contains less than 100 nucleic acid template molecules containing the reference genetic sequence.

6. (Original) The method of claim 1 wherein the biological sample is cell-free.

7. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 10.

8. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 50.

9. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 100.

10. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 500.

11. (Original) The method of claim 1 wherein the number of assay samples within the set is greater than 1000.

12. (Original) The method of claim 1 wherein the step of amplifying and the step of analyzing are performed on assay samples in the same receptacle.

13. (Not subject to reexamination)

14. (Original) The method of claim 1 wherein the step of analyzing employs gel electrophoresis.

15. (Original) The method of claim 1 wherein the step of analyzing employs hybridization to at least one nucleic acid probe.

16. (Original) The method of claim 1 wherein the step of analyzing employs hybridization to at least two nucleic acid probe.

17-18. (Not subject to reexamination)

19. (Original) The method of claim 1 wherein the step of amplifying employs a single pair of primers.

20. (Original) The method of claim 1 wherein the step of amplifying employs a polymerase which is activated only after heating.

21. (Original) The method of claim 1 wherein the step of amplifying employs at least 40 cycles of heating and cooling.

22. (Original) The method of claim 1 wherein the step of amplifying employs at least 50 cycles of heating and cooling.

23. (Original) The method of claim 1 wherein the step of amplifying employs at least 60 cycles of heating and cooling.

24. (Original) The method of claim 1 wherein the biological sample is selected from the group consisting of stool, blood, and lymph nodes.

25. (Original) The method of claim 1 wherein the biological sample is blood or bone marrow of a leukemia or lymphoma patient who has received anti-cancer therapy.

26. (Original) The method of claim 1 wherein the selected genetic sequence is a

translocated allele.

27. (Original) The method of claim 1 wherein the selected genetic sequence is a wild-type allele.

28. (Original) The method of claim 1 wherein the selected genetic sequence is within an amplicon which is amplified during neoplastic development.

29. (Original) The method of claim 1 wherein the selected genetic sequence is a rare exon sequence.

30. (Original) The method of claim 1 wherein the nucleic acid template molecules comprise cDNA of RNA transcripts and the selected genetic sequence is present on a cDNA of a first transcript and the reference genetic sequence is present on a cDNA of a second transcript.

31. (Original) The method of claim 1 wherein the selected genetic sequence comprises a first mutation and the reference genetic sequence comprises a second mutation.

32. (Original) The method of claim 1 wherein the selected genetic sequence and the reference genetic sequence are on distinct chromosomes.

33-37. (Not subject to reexamination)

38. (Twice amended) A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of:

distributing cell-free nucleic acid template molecules from a biological sample to form a set comprising a plurality of assay samples;

amplifying the nucleic acid template molecules [within a set comprising a plurality of assay samples] to form a population of amplified molecules in [each of the] individual assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first

number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence, wherein at least one-fiftieth of the assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required to determine the presence of the selected genetic sequence;

comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

39. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 10.

40. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 50.

41. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 100.

42. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 500.

43. (Original) The method of claim 38 wherein the number of assay samples within the set is greater than 1000.

44. (Original) The method of claim 38 wherein the step of amplifying and the step of analyzing are performed on assay samples in the same receptacle.

45. (Not subject to reexamination)

46. (Original) The method of claim 38 wherein the step of analyzing employs gel electrophoresis.

47. (Original) The method of claim 38 wherein the step of analyzing employs hybridization to at least one nucleic acid probe.

48. (Amended) The method of claim 38 wherein the step of analyzing employs hybridization to at least two nucleic acid [probe] probes.

49-50. (Not subject to reexamination)

51. (Original) The method of claim 38 wherein the step of amplifying employs a single pair of primers.

52. (Original) The method of claim 38 wherein the step of amplifying employs a polymerase which is activated only after heating.

53. (Original) The method of claim 38 wherein the step of amplifying employs at least 40 cycles of heating and cooling.

54. (Original) The method of claim 38 wherein the step of amplifying employs at least 50 cycles of heating and cooling.

55. (Original) The method of claim 38 wherein the step of amplifying employs at least 60 cycles of heating and cooling.

56. (Original) The method of claim 38 wherein the template molecules are obtained from a body sample selected from the group consisting of stool, blood, and lymph nodes.

57. (Original) The method of claim 38 wherein the template molecules are obtained from a body sample of a leukemia or lymphoma patient who has received anti-cancer therapy, said body sample being selected from the group consisting of blood and bone marrow.

58. (Original) The method of claim 38 wherein the selected genetic sequence is a translocated allele.

59. (Original) The method of claim 38 wherein the selected genetic sequence is a wild-type allele.

60. (Original) The method of claim 38 wherein the selected genetic sequence is within an amplicon which is amplified during neoplastic development.

61. (Original) The method of claim 38 wherein the selected genetic sequence is a rare exon sequence.

62. (Original) The method of claim 38 wherein the nucleic acid template molecules comprise cDNA of RNA transcripts and the selected genetic sequence is present on a cDNA of a first transcript and the reference genetic sequence is present on a cDNA of a second transcript.

63. (Original) The method of claim 38 wherein the selected genetic sequence comprises a first mutation and the reference genetic sequence comprises a second mutation.

64. (Original) The method of claim 38 wherein the selected genetic sequence and the reference genetic sequence are on distinct chromosomes.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re *Ex Parte* Reexamination:	)	Group Art Unit: 3991
	)	
U.S. Patent No. 7,824,889	)	Docket No. 001107.00990
	)	
Control No. 90/012,895	)	Confirmation No: 7285
	)	
Reexam Filing Date: June 17, 2013	)	Examiner: Bruce R. Campell

For: DIGITAL AMPLIFICATION

RESPONSIVE AMENDMENT TO FINAL OFFICE ACTION

IN THE CLAIMS

Please amend the claims as shown below with the standard markings for re-examination proceedings. Patent claims under reexamination but not amended are indicated as "original." Patent claims not subject to reexamination are not shown.

1. (Amended) A method for determining an allelic imbalance in a biological sample, comprising the steps of:

distributing isolated nucleic acid template molecules to form a set comprising a plurality of assay samples, wherein the nucleic acid template molecules are isolated from the biological sample;

amplifying the template molecules within [a] the set [comprising a plurality of assay samples] to form a population of amplified molecules in [each of the] individual assay samples of the set [, wherein the template molecules are obtained from a biological sample];

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain a selected genetic sequence on a first chromosome and a second number of assay samples which contain a reference genetic sequence on a second chromosome, wherein between 0.1 and 0.9 of the assay samples yield an amplification product of at least one of the selected and the reference genetic sequences;

comparing the first number of assay samples to the second number of assay samples to ascertain an allelic imbalance in the biological sample.

2. (Original) The method of claim 1 wherein the step of amplifying employs real-time polymerase chain reactions.

3. (Original) The method of claim 2 wherein the real-time polymerase chain reactions comprise a dual-labeled fluorogenic probe.

4. (Original) The method of claim 1 wherein the selected genetic sequence and the reference genetic sequence are non-polymorphic markers.

5. (Original) The method of claim 1 wherein the biological sample is from blood.

6. (Original) The method of claim 1 wherein the selected genetic sequence is a non-polymorphic marker.

7. (Original) The method of claim 1 wherein the reference genetic sequence is a non-polymorphic marker.

8. (Amended) The method of claim 1 wherein between 0.1 and 0.6 of the assay samples yield an amplification product of at least one of the selected and the reference genetic sequences.

9. (Amended) The method of claim 1 wherein between 0.3 and 0.5 of the assay samples yield an amplification product of at least one of the selected and the reference genetic sequences.

10. (Amended) The method of claim 1 further comprising the step of isolating nucleic acid template molecules from the biological sample prior to the step of distributing [wherein between 0.1 and 0.9 of the assay samples yield an amplification product as determined by amplification of the selected genetic sequence].

11. (Amended) The method of claim 19 further comprising the step of isolating nucleic acid template molecules from the biological sample to form cell-free nucleic acid template molecules prior to the step of distributing [wherein between 0.1 and 0.9 of the assay samples yield an amplification product as determined by amplification of the reference genetic sequence].

12. (Amended) The method of claim 1 wherein between 0.1 and 0.6 of the assay samples yield [an] a homogeneous amplification product of at least one of the selected and the reference genetic sequences [as determined by amplification of the selected genetic sequence].

13. (Amended) The method of claim [1] 19 wherein between 0.1 and 0.6 of the assay samples yield an amplification of at least one of the selected and the reference genetic sequences [product as determined by amplification of the reference genetic sequence].

14. (Amended) The method of claim 1 wherein between 0.3 and 0.5 of the assay samples

yield [an] a homogeneous amplification product of at least one of the selected and the reference genetic sequences [as determined by amplification of the selected genetic sequence].

15. (Amended) The method of claim [1] 19 wherein between 0.3 and 0.5 of the assay samples yield an amplification product of at least one of the selected and the reference genetic sequences [as determined by amplification of the reference genetic sequence].

16. (Original) The method of claim 1 wherein the set comprises at least 500 assay samples.

17. (Original) The method of claim 1 wherein the set comprises at least 1000 assay samples.

18. (Original) The method of claim 1 wherein the amplified molecules in each of the assay samples in the first and second numbers of assay samples are homogeneous such that the first number of assay samples do not contain the reference genetic sequence and the second number of assay samples do not contain the selected genetic sequence.

19. (Amended) A method for determining an allelic imbalance in a biological sample, comprising the steps of:

distributing cell-free nucleic acid template molecules from a biological sample to form a set comprising a plurality of assay samples;

amplifying the template molecules within the assay samples to form a population of amplified molecules in the assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain a selected genetic sequence on a first chromosome and a second number of assay samples which contain a reference genetic sequence on a second chromosome;

comparing the first number of assay samples to the second number of assay samples to ascertain an allelic imbalance between the first chromosome and the second chromosome in the biological sample.

20. (Amended) The method of claim 19 wherein between 0.1 and 0.9 of the assay samples yield an amplification product of at least one of the selected and the reference genetic sequences.

21. (Amended) The method of claim 20 wherein between 0.1 and 0.9 of the assay samples yield a homogeneous amplification product of at least one of the selected and the reference genetic sequences.

22. (Original) The method of claim 19 wherein the biological sample is blood.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re *Ex Parte* Reexamination:	)	Group Art Unit: 3991
	)	
U.S. Patent No. 7,915,015	)	Docket No. 001107.00988
	)	
Control No. 90/012,896	)	Confirmation No: 8361
	)	
Reexam Filing Date: June 17, 2013	)	Examiner: Bruce R. Campell

For: DIGITAL AMPLIFICATION

RESPONSIVE AMENDMENT TO FINAL OFFICE ACTION

IN THE CLAIMS

Please amend the following claims as indicated by the status identifier. Patent claims under reexamination but not amended are indicated as "original." Patent claims not subject to reexamination are not shown.

1. (Amended) A method for determining an allelic imbalance in a biological sample, comprising the steps of:

distributing isolated nucleic acid template molecules to form a set comprising a plurality of assay samples, wherein the nucleic acid template molecules are isolated from the biological sample;

amplifying the isolated nucleic acid template molecules within [a] the set [comprising a plurality of assay samples] to form a population of amplified molecules in [each of the] individual assay samples of the set[, wherein the template molecules are obtained from the biological sample];

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain a first allelic form of a marker and a second number of assay samples which contain a second allelic form of the marker, wherein between 0.1 and 0.9 of the assay samples yield an amplification product of at least one of the first and second allelic forms of the marker;

comparing the first number to the second number to ascertain an allelic imbalance in the biological sample; and

identifying an allelic imbalance in the biological sample.

2. (Original) The method of claim 1 wherein the step of amplifying employs real-time polymerase chain reactions.

3. (Original) The method of claim 2 wherein the real-time polymerase chain reactions comprise a dual-labeled fluorogenic probe.

4. (Amended) The method of claim 1 further comprising the step of isolating template nucleic acid molecules from the biological sample prior to the step of distributing [wherein

between 0.1 and 0.9 of the assay samples yield an amplification product as determined by amplification of the first allelic form of the marker].

5. (Amended) The method of claim 1 wherein the step of distributing the isolated nucleic acid template molecules is performed by diluting [wherein between 0.1 and 0.9 of the assay samples yield an amplification product as determined by amplification of the second allelic form of the marker].

6. (Original) The method of claim 1 wherein the amplified molecules in each of the assay samples within the first and second numbers of assay samples are homogeneous such that the first number of assay samples do not contain the second allelic form of the marker and the second number of assay samples do not contain the first allelic form of the marker.

7. (Original) The method of claim 1 wherein the sample is from blood.

8. (Amended) A method for determining an allelic imbalance in a biological sample, comprising the steps of:

distributing cell-free nucleic acid template molecules from a biological sample to form a set comprising a plurality of assay samples;

amplifying the template molecules within the assay samples to form a population of amplified molecules in the assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain a first allelic form of a marker and a second number of assay samples which contain a second allelic form of the marker;

comparing the first number of assay samples to the second number of assay samples to ascertain an allelic imbalance between the first allelic form and the second allelic form in the biological sample.

9. (Original) The method of claim 8 wherein the sample is from blood.

10. (Amended) The method of claim 1 or 8 wherein between 0.1 and 0.6 of the assay

samples yield an amplification product of at least one of the first and second allelic forms of the marker.

11. (Amended) The method of claim 1 or 8 wherein between 0.3 and 0.5 of the assay samples yield an amplification product of at least one of the first and second allelic forms of the marker.

12. (Original) The method of claim 1 or 8 wherein the set comprises at least 500 assay samples.

13. (Original) The method of claim 1 or 8 wherein the set comprises at least 1000 assay samples.

14. (Original) The method of claim 8 wherein the step of amplifying employs real-time polymerase chain reactions.

15. (Original) The method of claim 14 wherein the real-time polymerase chain reactions comprise a dual-labeled fluorogenic probe.

16. (Amended) The method of claim 8 wherein the step of distributing is performed by diluting [between 0.1 and 0.9 of the assay samples yield an amplification product as determined by amplification of the first allelic form of the marker] .

17. (Amended) The method of claim 8 further comprising the step of isolating cell-free nucleic acid template molecules from the biological sample prior to the step of distributing [wherein between 0.1 and 0.9 of the assay samples yield an amplification product as determined by amplification of the second allelic form of the marker].

18. (Original) The method of claim 8 wherein the amplified molecules in each of the assay samples within the first and second numbers of assay samples are homogeneous such that

the first number of assay samples do not contain the second allelic form of the marker and the second number of assay samples do not contain the first allelic form of the marker.