CHAIRMAN

UNITED STATES INTERNATIONAL TRADE COMMISSION

Washington, D.C. 20436

May 19, 2020

The President
The White House
Washington, D.C. 20500

Dear Mr. President:

In accordance with subsection (j) of section 337 of the Tariff Act of 1930, as amended (19 U.S.C. § 1337) ("section 337"), and the July 21, 2005 Memorandum for the United States Trade Representative (70 *Fed. Reg.* 43251), I am transmitting to you and the United States Trade Representative copies of the Commission's limited exclusion order, as described below, and the record upon which the Commission based its determination.

On May 19, 2020, the United States International Trade Commission issued a limited exclusion order pursuant to section 337 in USITC Investigation No. 337-TA-1120, *Certain Human Milk Oligosaccharides and Methods of Producing the Same.* The limited exclusion order prohibits the unlicensed importation of human milk oligosaccharides and methods of producing the same that infringe one or more of claims 1-3, 5, 8, 10, 12, 18, and 24-28 of U.S. Patent No. 9,970,018 that are manufactured abroad by or on behalf of, or imported by or on behalf of respondent Jennewein Biotechnologie GmbH of Rheinbreitbach, Germany ("Respondent").

The Commission concluded that the statutory public interest factors enumerated in subsection (d)(1) of section 337 do not preclude the issuance of this remedy. The Commission also determined that during the period of Presidential review, the products described above, manufactured abroad or imported by, for, or on behalf of Respondent, may be imported and sold in the United States with the posting of a bond in the amount of five (5) percent of the entered value.

Confidential Removed SDB

Sincerely,

David S. Johanson
Chairman

Enclosures

CHAIRMAN

UNITED STATES INTERNATIONAL TRADE COMMISSION

Washington, D.C. 20436

May 19, 2020

The Honorable Steven T. Mnuchin
Secretary of the Treasury
Washington, D.C. 20220

Dear Mr. Secretary:

In accordance with subsection (d) of section 337 of the Tariff Act of 1930, as amended (19 U.S.C. § 1337) ("section 337"), I am transmitting to you a copy of the Commission's limited exclusion order, as described below, and the record upon which the Commission based its determination.

On May 19, 2020, the United States International Trade Commission issued a limited exclusion order pursuant to section 337 in USITC Investigation No. 337-TA-1120, *Certain Human Milk Oligosaccharides and Methods of Producing the Same.* The limited exclusion order prohibits the unlicensed importation of human milk oligosaccharides and methods of producing the same that infringe one or more of claims 1-3, 5, 8, 10, 12, 18, and 24-28 of U.S. Patent No. 9,970,018 that are manufactured abroad by or on behalf of, or imported by or on behalf of respondent Jennewein Biotechnologie GmbH of Rheinbreitbach, Germany ("Respondent").

The Commission concluded that the statutory public interest factors enumerated in subsection (d)(1) of section 337 do not preclude the issuance of this remedy. The Commission also determined that, during the period of Presidential review, the products described above, manufactured abroad or imported by, for, or on behalf of Respondent, may be imported and sold in the United States with the posting of a bond in the amount of five (5) percent of the entered value.

Sincerely,

David S. Johanson
Chairman

Enclosures

cc: Charles Steuart, Chief
IPR & Restricted Merchandise Branch
Office of International Trade
Regulation and Rulings
U.S. Customs and Border Protection
90 K Street, NE
Washington, D.C. 20229-1177

CHAIRMAN

UNITED STATES INTERNATIONAL TRADE COMMISSION

Washington, D.C. 20436

May 19, 2020

The Honorable Robert Lighthizer
United States Trade Representative
Washington, D.C. 20508

Dear Ambassador Lighthizer:

In accordance with subsection (j) of section 337 of the Tariff Act of 1930, as amended (19 U.S.C. § 1337) ("section 337"), and the July 21, 2005 Memorandum for the United States Trade Representative (70 *Fed. Reg.* 43251), I am transmitting to you and the President copies of the Commission's limited exclusion order, as described below, and the record upon which the Commission based its determination.

On May 19, 2020, the United States International Trade Commission issued a limited exclusion order pursuant to section 337 in USITC Investigation No. 337-TA-1120, *Certain Human Milk Oligosaccharides and Methods of Producing the Same.*

The limited exclusion order prohibits the unlicensed importation of human milk oligosaccharides and methods of producing the same that infringe one or more of claims 1-3, 5, 8, 10, 12, 18, and 24-28 of U.S. Patent No. 9,970,018 that are manufactured abroad by or on behalf of, or imported by or on behalf of respondent Jennewein Biotechnologie GmbH of Rheinbreitbach, Germany ("Respondent").

The Commission concluded that the statutory public interest factors enumerated in subsection (d)(1) of section 337 do not preclude the issuance of this remedy. The Commission also determined that during the period of Presidential review, the products described above, manufactured abroad or imported by, for, or on behalf of Respondent, may be imported and sold in the United States with the posting of a bond in the amount of five (5) percent of the entered value.

Sincerely,

David S. Johanson
Chairman

Enclosures

cc: Shannon M. Nestor, Esq.
Juli C. Schwartz, Esq.
Office of the General Counsel
Office of the United States Trade Representative

UNITED STATES INTERNATIONAL TRADE COMMISSION

Washington, D.C.

In the Matter of **CERTAIN HUMAN MILK OLIGOSACCHARIDES AND METHODS OF PRODUCING THE SAME**	**Investigation No. 337-TA-1120**

NOTICE OF COMMISSION FINAL DETERMINATION FINDING A VIOLATION OF SECTION 337; ISSUANCE OF A LIMITED EXCLUSION ORDER; TERMINATION OF THE INVESTIGATION

AGENCY: U.S. International Trade Commission.

ACTION: Notice.

SUMMARY: Notice is hereby given that the U.S. International Trade Commission has found a violation of section 337 of the Tariff Act of 1930 ("section 337"), as amended, in this investigation. The Commission has issued a limited exclusion order ("LEO") prohibiting the importation by respondent Jennewein Biotechnologie GmbH ("Jennewein") of Rheinbreitbach, Germany of certain human milk oligosaccharides that infringe complainant's asserted claims. The investigation is terminated.

FOR FURTHER INFORMATION CONTACT: Houda Morad, Office of the General Counsel, U.S. International Trade Commission, 500 E Street SW., Washington, DC 20436, telephone (202) 708-4716. Copies of non-confidential documents filed in connection with this investigation are or will be available for inspection during official business hours (8:45 a.m. to 5:15 p.m.) in the Office of the Secretary, U.S. International Trade Commission, 500 E Street SW., Washington, D.C. 20436, telephone (202) 205-2000. General information concerning the Commission may also be obtained by accessing its Internet server at *https://www.usitc.gov*. The public record for this investigation may be viewed on the Commission's electronic docket (EDIS) at *https://edis.usitc.gov*. Hearing-impaired persons are advised that information on this matter can be obtained by contacting the Commission's TDD terminal on (202) 205-1810.

SUPPLEMENTARY INFORMATION: The Commission instituted this investigation on June 21, 2018, based on a complaint, as amended and supplemented, filed on behalf of Glycosyn LLC of Waltham, Massachusetts ("Glycosyn"). *See* 83 Fed. Reg. 28865 (June 21, 2018). The complaint, as amended and supplemented, alleges violations of section 337 of the Tariff Act of 1930, as amended, 19 U.S.C. 1337 ("section 337"), based upon the importation into the United States, the sale for importation, and the sale within the United States after importation of certain human milk oligosaccharides by reason of infringement of claims 1-40 of U.S. Patent No. 9,453,230 ("the '230 patent") and claims 1-28 of U.S. Patent No. 9,970,018 ("the '018 patent").

See id. The notice of investigation named Jennewein as a respondent in this investigation. *See id.* The Office of Unfair Import Investigations ("OUII") is also named as a party to the investigation. *See id.*

The ALJ conducted an evidentiary hearing on May 14-17, 2019, and on September 9, 2019, issued the FID finding a violation of section 337 based on the infringement of claims 1-3, 5, 8, 10, 12, 18, and 24-28 of the '018 patent (hereinafter, the "Asserted Claims"). In addition, the FID finds that the Asserted Claims are neither invalid under 35 U.S.C. §§ 103 and 112, nor unenforceable for inequitable conduct. Furthermore, the FID finds that the domestic industry requirement is satisfied. All asserted claims in the '230 patent were withdrawn during the investigation. The FID also contains a recommended determination ("RD") recommending that the Commission issue an LEO barring entry of articles that infringe the '018 patent. The RD also recommends that the Commission impose a 5 percent bond during the period of Presidential review. Furthermore, as directed by the Commission, the RD provides findings with respect to the public interest and recommends that the Commission determine that the public interest factors do not preclude entry of the proposed LEO. Glycosyn does not seek and the RD does not recommend issuance of a cease and desist order.

On October 9 and 10, 2019, respectively, Glycosyn and Jennewein filed statements on the public interest pursuant to Commission Rule 210.50. On October 23, 2019, non-party DuPont Nutrition & Health filed a public interest submission pursuant to the Commission's notice requesting public interest comments. *See* 84 Fed. Reg. 49335 (Sept. 19, 2019).

On January 30, 2020, the Commission issued a notice determining to review the FID in part. *See* 85 Fed. Reg. 6573 (Feb. 5, 2020). The Commission's notice requested written submissions in response to certain questions relating to issues under review and on issues of remedy, the public interest, and bonding. On February 18, 2020, the parties, including OUII, filed written submissions in response to the notice, and on February 25, 2020, the parties filed responses to each other's submissions. On February 18, 2020, non-party Abbott Laboratories filed a written submission concerning the public interest.

Having examined the record of this investigation, including the FID, the RD, and the parties' and non-parties' submissions, the Commission has determined to affirm with modification the FID's determination of a violation of section 337 with respect to claims 1-3, 5, 8, 10, 12, 18, and 24-28 of the '018 patent. Specifically, as explained in the Commission Opinion filed concurrently herewith, the Commission has determined to affirm with modification the FID's findings with respect to infringement by the accused Jennewein bacterial strains and to reverse the FID's decision not to adjudicate infringement with respect to Jennewein's TTFL12 bacterial strain. As to the TTFL12 strain, the Commission has determined that it does not infringe the Asserted Claims. All findings in the FID that are not inconsistent with the Commission's determination are affirmed.

The Commission has determined that the appropriate remedy is an LEO against Jennewein's infringing products. The Commission has also determined that the public interest factors enumerated in subsection 337(d)(1) (19 U.S.C. 1337(d)(1)) do not preclude the issuance of the LEO. The Commission has further determined to set a bond during the period of

Presidential review at five (5) percent of the entered value of Jennewein's infringing products (19 U.S.C. 1337(j)).

The Commission's order and opinion were delivered to the President and to the United States Trade Representative on the day of their issuance.

The Commission's vote for these determinations took place on May 19, 2020.

The authority for the Commission's determination is contained in section 337 of the Tariff Act of 1930, as amended (19 U.S.C. 1337), and in part 210 of the Commission's Rules of Practice and Procedure (19 CFR part 210).

By order of the Commission.

Lisa R. Barton
Secretary to the Commission

Issued: May 19, 2020

UNITED STATES INTERNATIONAL TRADE COMMISSION
Washington, D.C.

In the Matter of **CERTAIN HUMAN MILK OLIGOSACCHARIDES AND METHODS OF PRODUCING THE SAME**	**Investigation No. 337-TA-1120**

LIMITED EXCLUSION ORDER

The United States International Trade Commission ("Commission") has determined that there is a violation of Section 337 of the Tariff Act of 1930, as amended (19 U.S.C. § 1337), in the unlawful importation, sale for importation, or sale within the United States after importation by Jennewein Biotechnologie GmbH ("Jennewein" or "Respondent") of certain 2'-fucosyllactose oligosaccharides that infringe one or more of claims 1-3, 5, 8, 10, 12, 18, and 24-28 ("the Asserted Claims") of U.S. Patent No. 9,970,018 ("the '018 patent").

Having reviewed the record of this investigation, including the written submissions of the parties, the Commission has made its determination on the issues of remedy, public interest, and bonding. The Commission has determined that the appropriate form of relief includes a limited exclusion order prohibiting the unlicensed entry into the United States of 2'-fucosyllactose oligosaccharides manufactured abroad by or on behalf of, or imported by or on behalf of, Respondent or any of its affiliated companies, parents, subsidiaries, or other related business entities, or its successors or assigns. The exclusion order does not apply to Respondent's TTFL12 bacterial strain and 2'-fucosyllactose oligosaccharides produced by that strain, which, as the Commission determined, do not infringe the Asserted Claims.

The Commission has also determined that the public interest factors enumerated in 19 U.S.C. § 1337(d) do not preclude the issuance of the limited exclusion order, and that the bond

during the period of Presidential review shall be in the amount of five (5) percent of the entered value of the covered articles.

Accordingly, the Commission hereby ORDERS that:

1. 2'-fucosyllactose oligosaccharides that infringe one or more of the Asserted Claims that are manufactured abroad by or on behalf of, or imported by or on behalf of, Respondent, or its affiliated companies, parents, subsidiaries, or other related business entities, or its successors or assigns ("covered articles"), are excluded from entry for consumption into the United States, entry for consumption from a foreign-trade zone, or withdrawal from a warehouse for consumption, for the remaining term of the '018 patent, except under license of the patent owner or as provided by law.

2. This Order does not apply to Respondent's TTFL12 bacterial strain and 2'-fucosyllactose oligosaccharides produced by that strain, which, as the Commission determined, do not infringe the Asserted Claims.

3. Notwithstanding paragraph 1 of this Order, covered articles are entitled to entry into the United States for consumption, entry for consumption from a foreign trade zone, or withdrawal from a warehouse for consumption, under bond in the amount of five (5) percent of the entered value of the infringing products pursuant to subsection (j) of section 337 of the Tariff Act of 1930, as amended (19 U.S.C. § 1337(j)), and the Presidential Memorandum for the United States Trade Representative of July 21, 2005, (70 FR 43251), from the day after this Order is received by the United States Trade Representative, and until such time as the United States Trade representative notifies the Commission that this Order is approved or disapproved but, in any event, not later than sixty (60) days after the date of receipt of this Order. All entries of

covered articles made pursuant to this paragraph are to be reported to U.S. Customs and Border Protection ("CBP"), in advance of the date of the entry, pursuant to procedures CBP establishes.

4. At the discretion of CBP and pursuant to the procedures it establishes, persons seeking to import 2'-fucosyllactose oligosaccharides, that are potentially subject to this Order may be required to certify that they are familiar with the terms of this Order, that they have made appropriate inquiry, and thereupon state that, to the best of their knowledge and belief, the products being imported are not excluded from entry under paragraph 1 of this Order. At its discretion, CBP may require persons who have provided the certification described in this paragraph to furnish such records or analyses as are necessary to substantiate this certification.

5. In accordance with 19 U.S.C. § 1337(l), the provisions of this Order shall not apply to 2'-fucosyllactose oligosaccharides that are imported by or for the use of the United States, or imported for and to be used for, the United States with the authorization or consent of the Government.

6. The Commission may modify this Order in accordance with the procedures described in Rule 210.76 of the Commission's Rules of Practice and Procedure (19 C.F.R. § 210.76).

7. The Secretary shall serve copies of this Order upon each party of record in this Investigation and upon CBP.

8. Notice of this Order shall be published in the Federal Register.

By order of the Commission.

Lisa R. Barton
Secretary to the Commission

Issued: May 19, 2020

U 7679494

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

May 16, 2018

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THIS OFFICE OF:

U.S. PATENT: *9,970,018*
ISSUE DATE: *May 15, 2018*

By Authority of the
Under Secretary of Commerce for Intellectual Property
and Director of the United States Patent and Trademark Office

M. Tarver

M. TARVER
Certifying Officer

U 7679494

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

May 16, 2018

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THIS OFFICE OF:

U.S. PATENT: ***9,970,018***
ISSUE DATE: ***May 15, 2018***

By Authority of the
Under Secretary of Commerce for Intellectual Property
and Director of the United States Patent and Trademark Office

M. Tarver

M. TARVER
Certifying Officer

US009970018B2

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

(10) **Patent No.: US 9,970,018 B2**
(45) **Date of Patent: *May 15, 2018**

(54) **BIOSYNTHESIS OF HUMAN MILK OLIGOSACCHARIDES IN ENGINEERED BACTERIA**

(71) Applicant: **Glycosyn LLC**, Woburn, MA (US)

(72) Inventors: **Massimo Merighi**, Somerville, MA (US); **John M. McCoy**, Reading, MA (US); **Matthew Ian Heidtman**, Brighton, MA (US)

(73) Assignee: **Glycosyn LLC**, Woburn, MA (US)

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

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **15/712,074**

(22) Filed: **Sep. 21, 2017**

(65) **Prior Publication Data**

US 2018/0080034 A1 Mar. 22, 2018

Related U.S. Application Data

(60) Continuation of application No. 15/442,131, filed on Feb. 24, 2017, which is a continuation of application No. 14/033,664, filed on Sep. 23, 2013, now Pat. No. 9,587,241, which is a division of application No. 13/398,526, filed on Feb. 16, 2012, now Pat. No. 9,453,230.

(60) Provisional application No. 61/443,470, filed on Feb. 16, 2011.

(51) **Int. Cl.**

C12P 19/18	(2006.01)
C12N 15/70	(2006.01)
C12P 19/26	(2006.01)
C12P 19/00	(2006.01)
C07H 13/04	(2006.01)
C07H 3/06	(2006.01)
C12N 9/38	(2006.01)
C12N 9/10	(2006.01)
C12N 9/00	(2006.01)

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

CPC ***C12N 15/70*** (2013.01); ***C07H 3/06*** (2013.01); ***C07H 13/04*** (2013.01); ***C12N 9/00*** (2013.01); ***C12N 9/1051*** (2013.01); ***C12N 9/2471*** (2013.01); ***C12P 19/00*** (2013.01); ***C12P 19/18*** (2013.01); ***C12P 19/26*** (2013.01); *Y02P 20/52* (2015.11)

(58) **Field of Classification Search**

CPC C12P 19/18

See application file for complete search history.

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(Continued)

Primary Examiner — Rebecca E Prouty

(74) *Attorney, Agent, or Firm* — Mintz Levin Cohn Ferris Glovsky and Popeo, P.C.; Ingrid A. Beattie

(57) **ABSTRACT**

The invention provides compositions and methods for engineering bacteria to produce fucosylated oligosaccharides, and the use thereof in the prevention or treatment of infection.

28 Claims, 24 Drawing Sheets

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FIG. 1

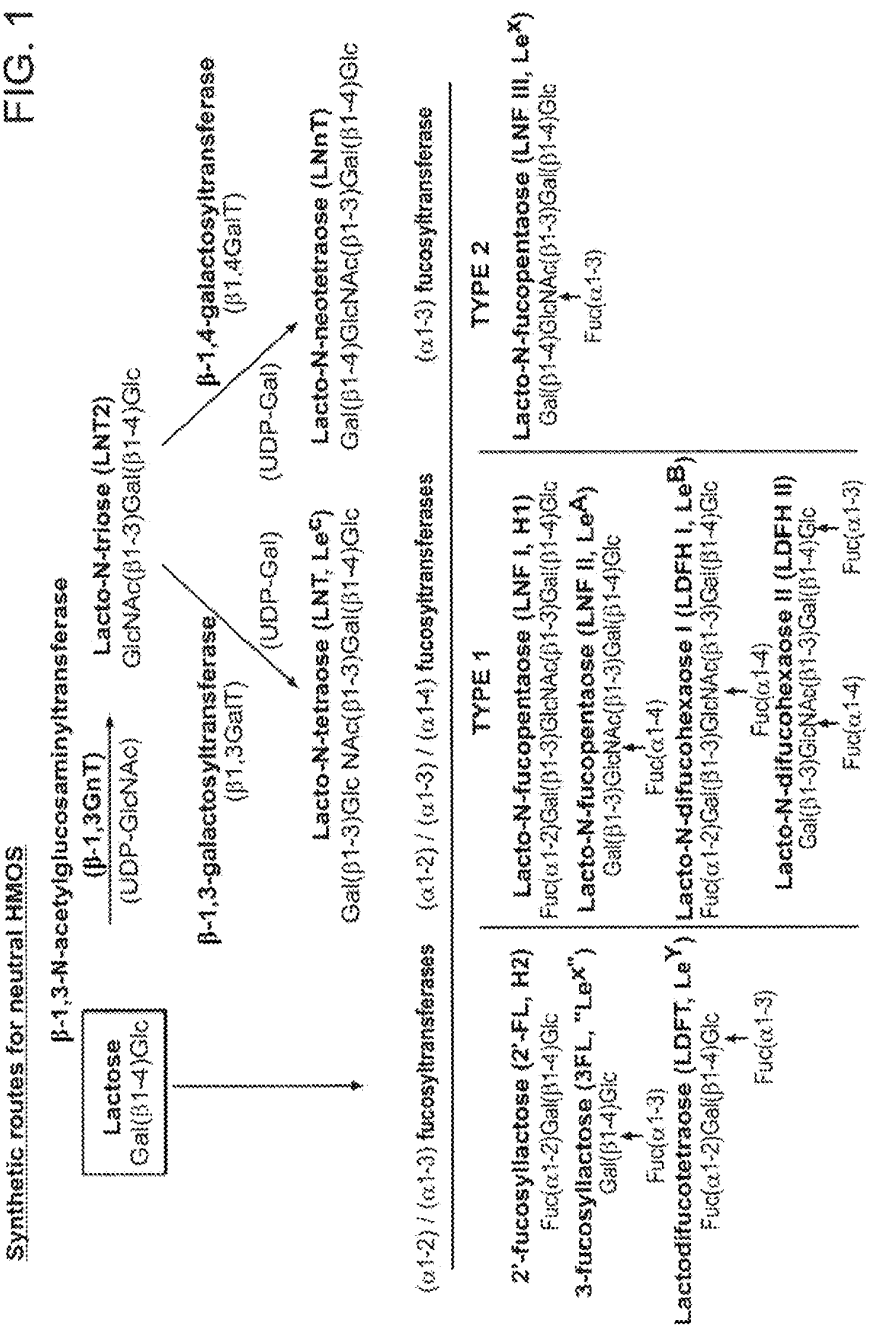


FIG. 2

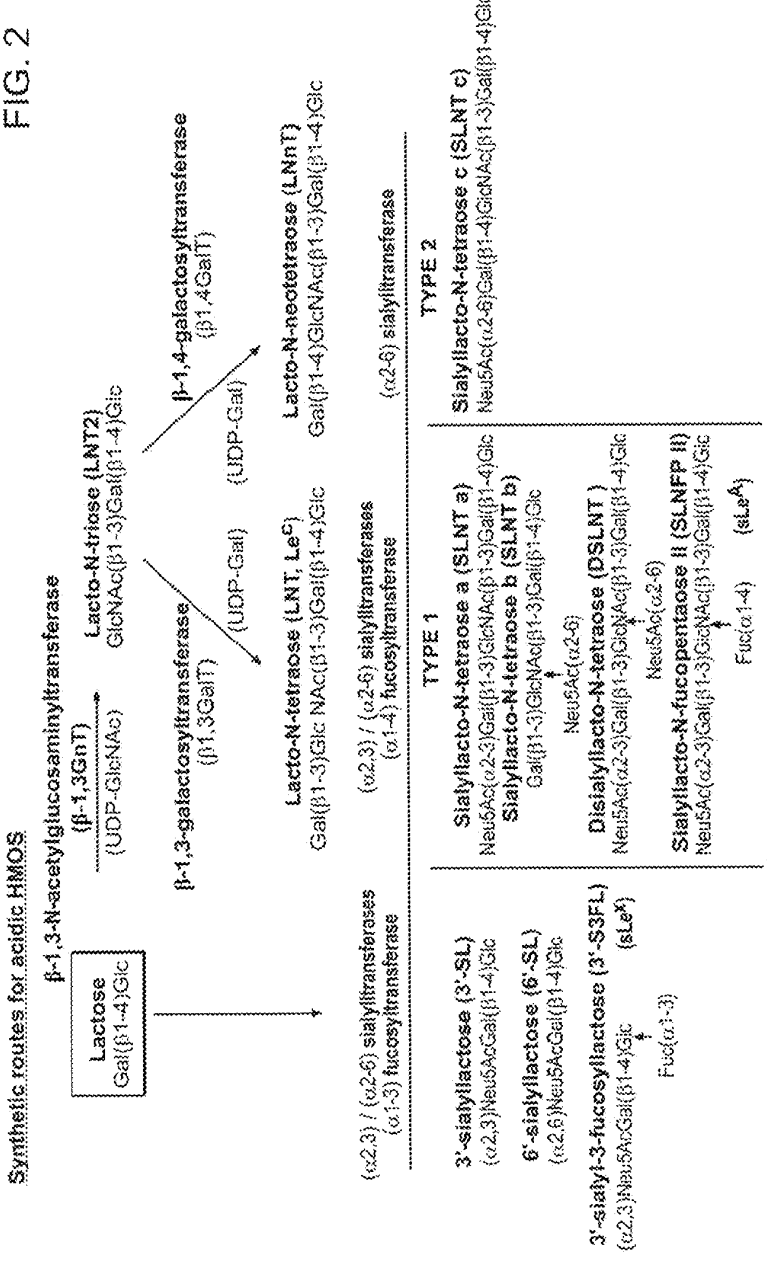


FIG. 3

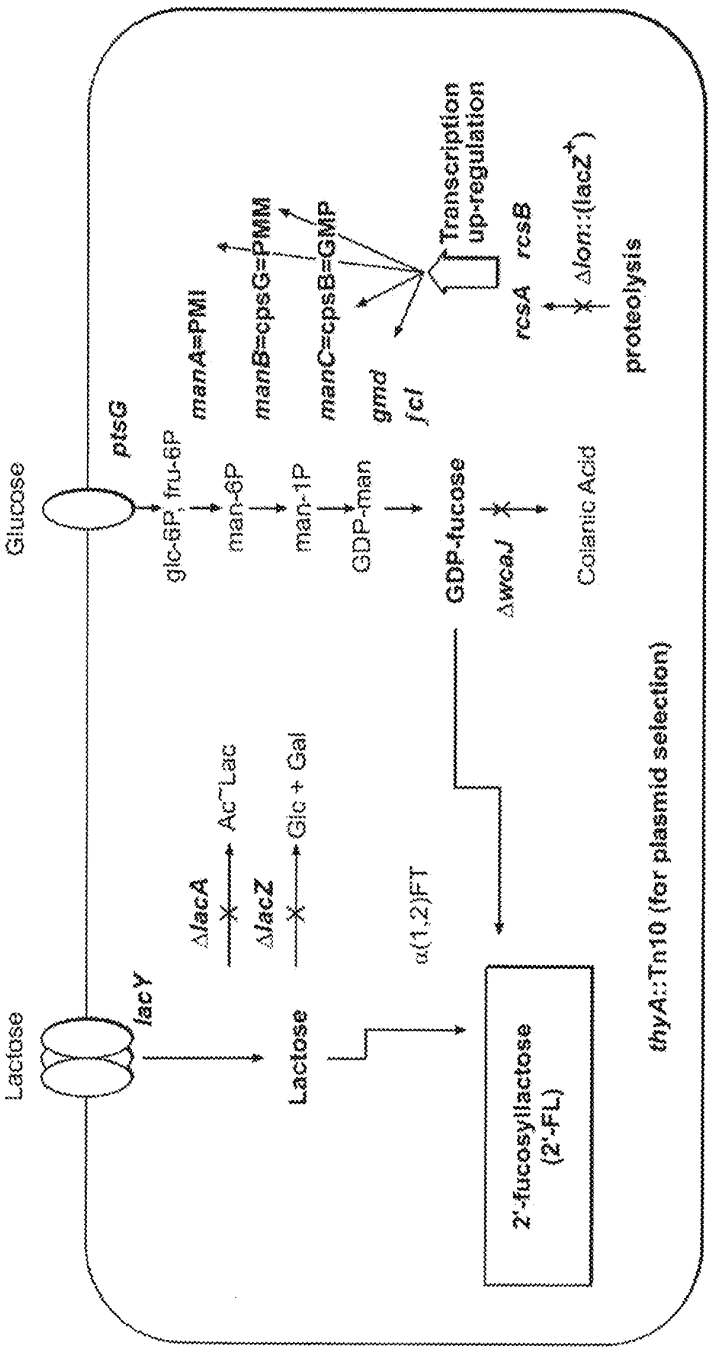


FIG. 4

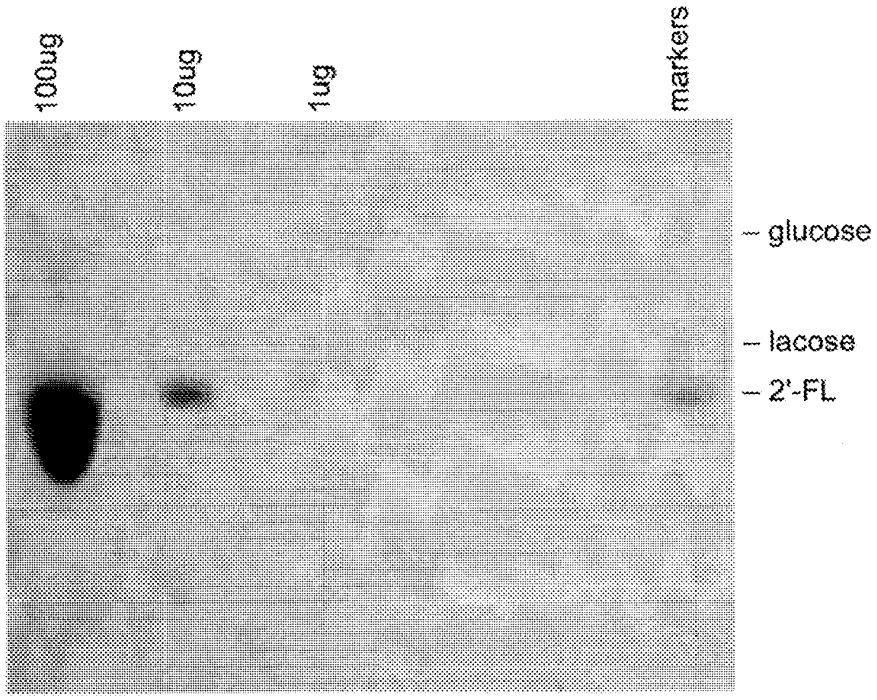
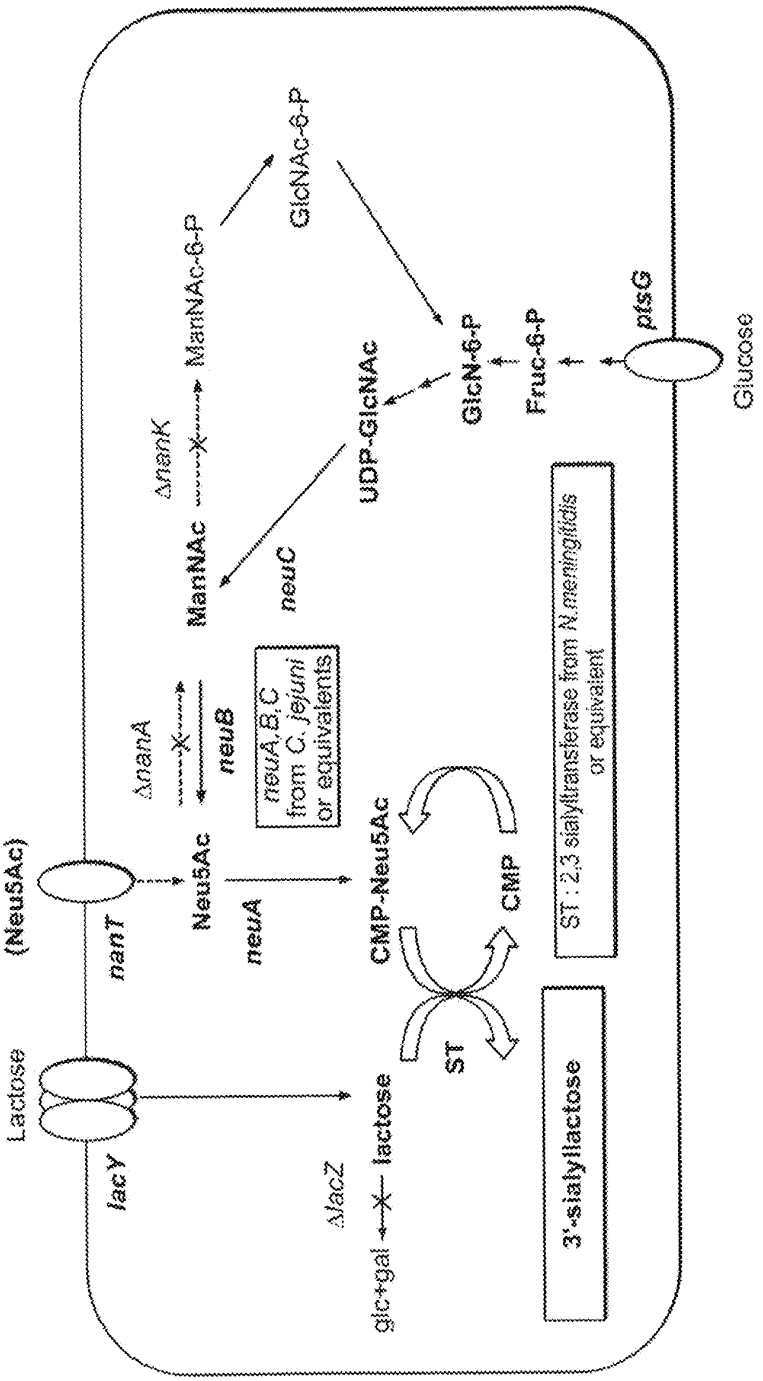


FIG. 5



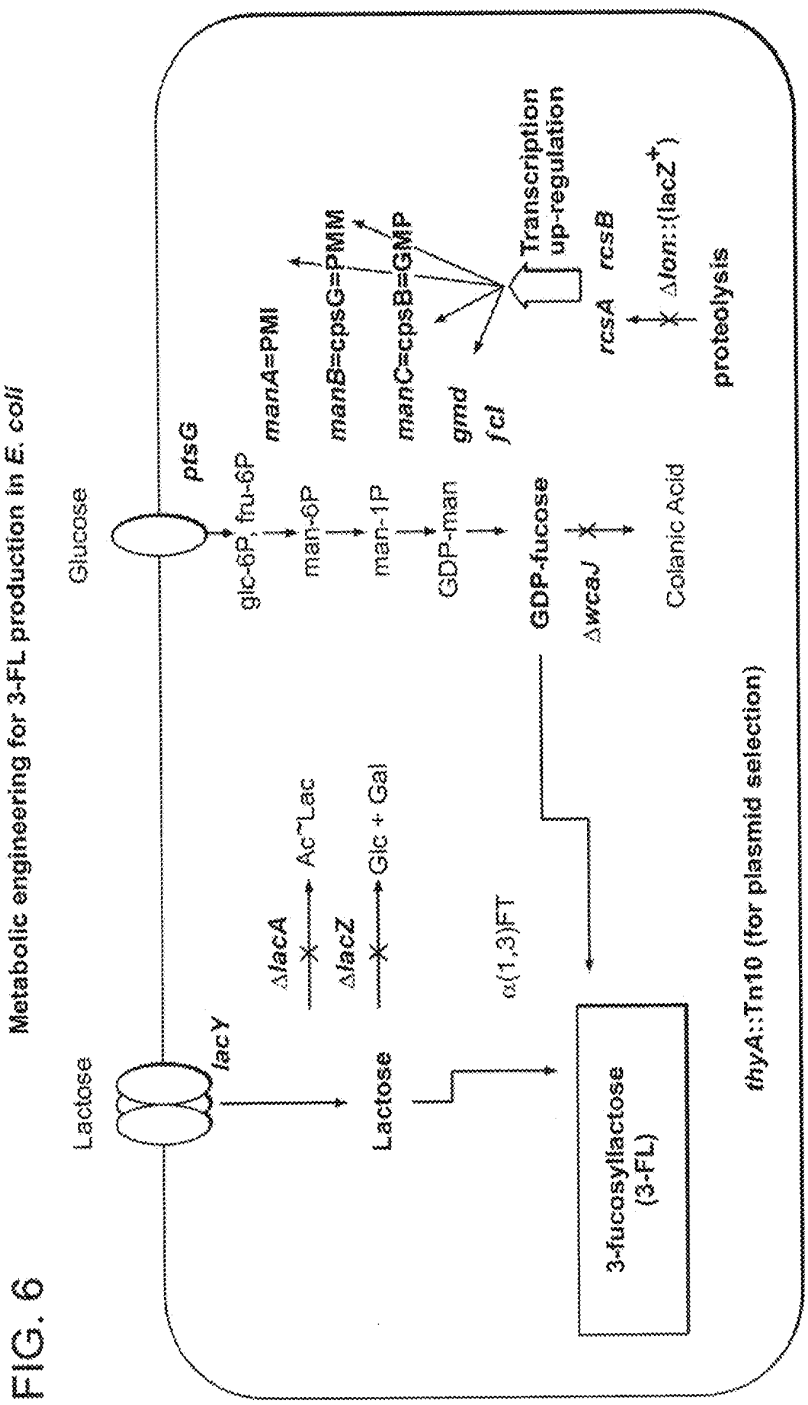


FIG. 6

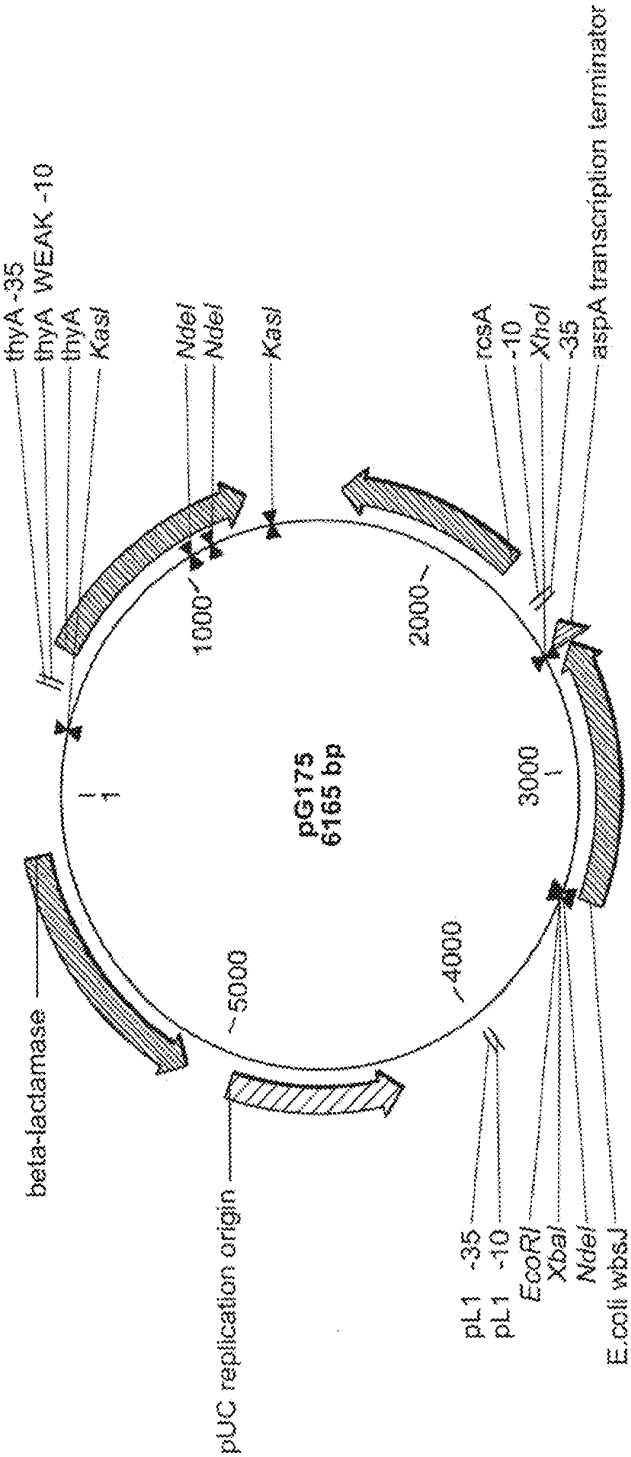
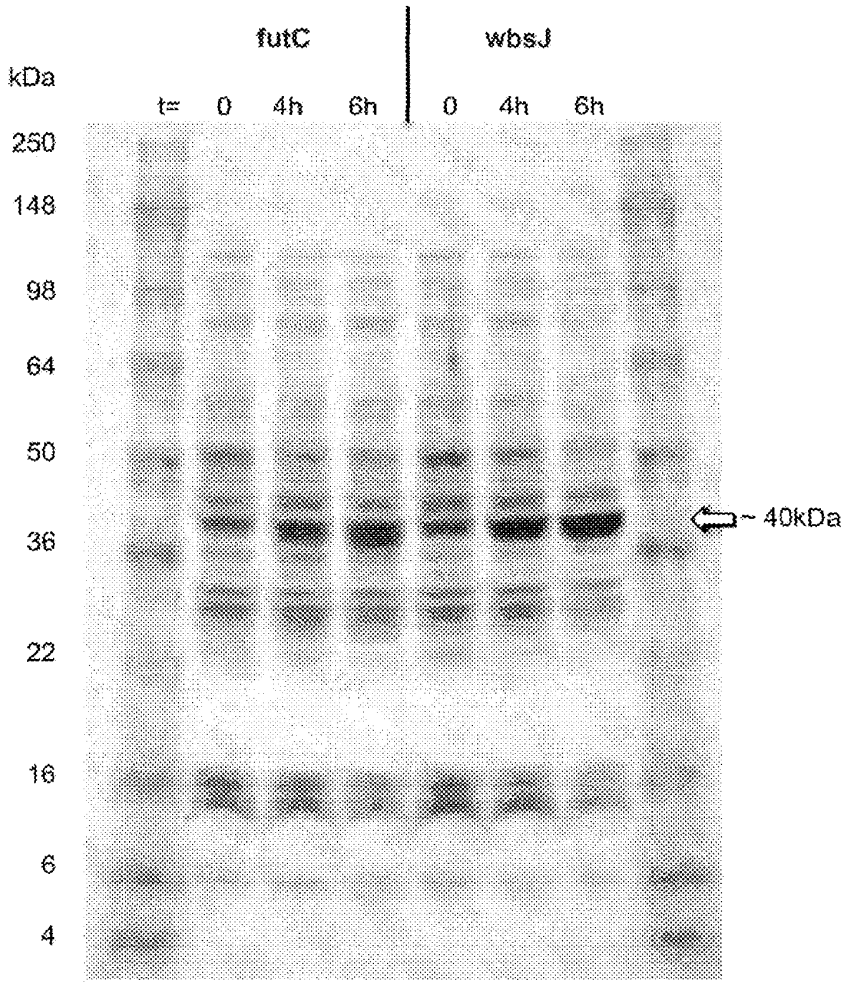


FIG. 7

FIG. 8



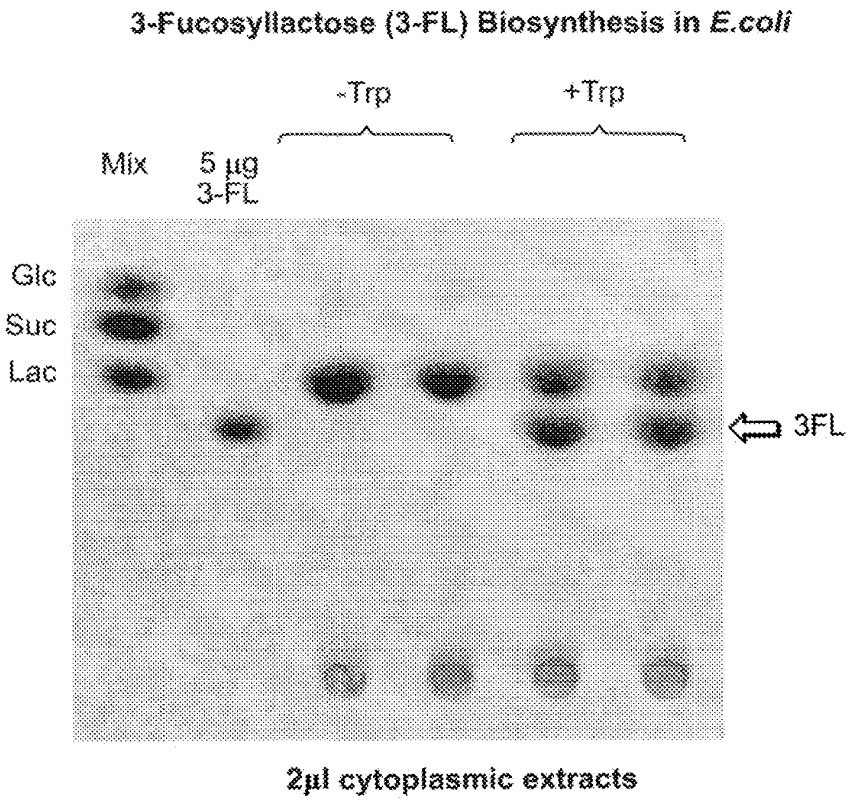


FIG. 9

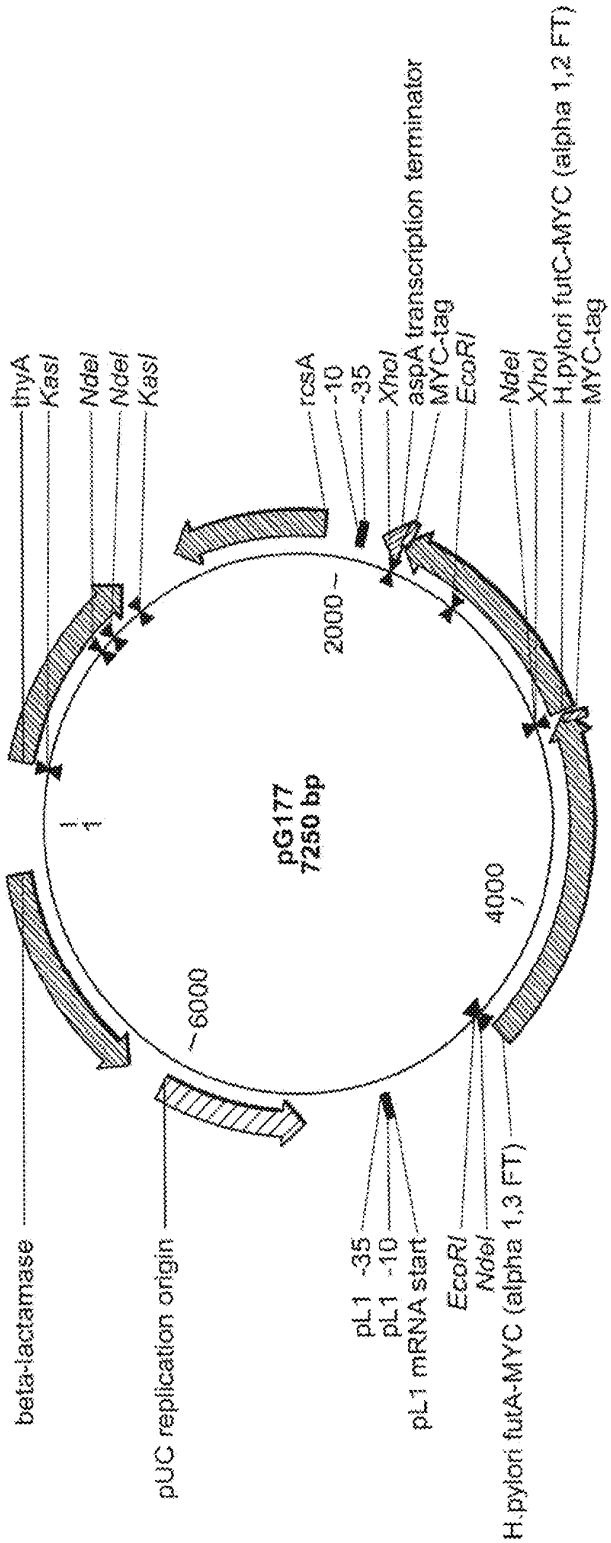
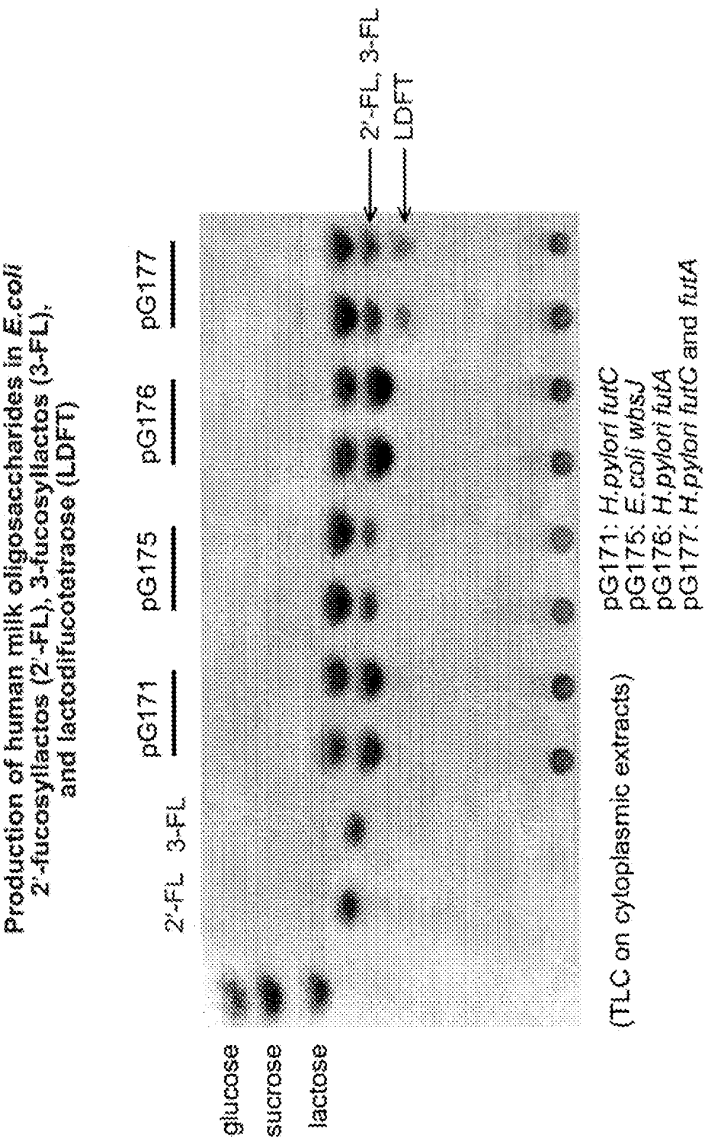


FIG. 10

FIG. 11



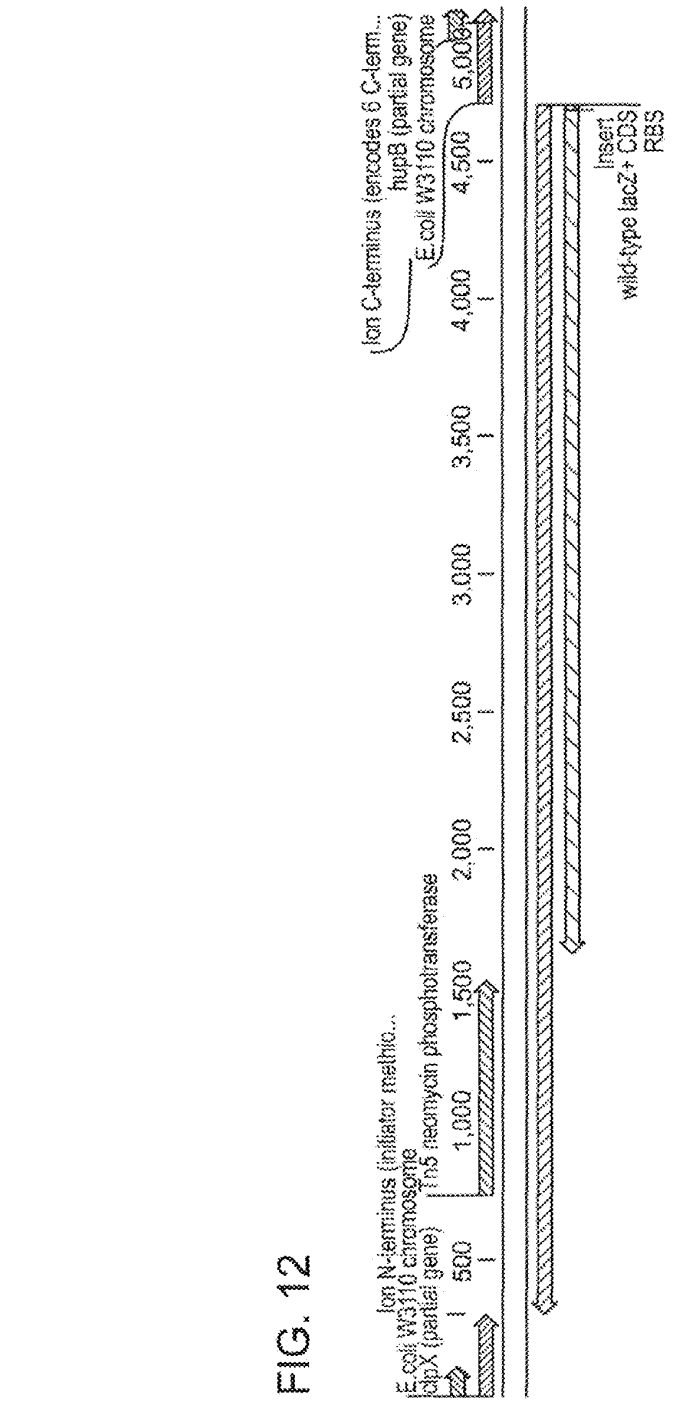


FIG. 12

FIG. 13A

```
LOCUS       W3110_delta_lon::Kan::lacZ_with_RBS      5049 bp    DNA
linear   BCT 19-FEB-2009
DEFINITION  Escherichia coli str. K-12 substr. W3110 strain K-12.
ACCESSION   AC_000091
VERSION     AC_000091.1  GI:89106884
KEYWORDS
SOURCE      Escherichia coli str. K-12 substr. W3110 (unknown)
  ORGANISM  Escherichia coli str. K-12 substr. W3110
            Bacteria; Proteobacteria; Gammaproteobacteria;
Enterobacteriales;
            Enterobacteriaceae; Escherichia.
FEATURES             Location/Qualifiers
     gene            1..112
                     /gene="clpX"
     CDS             1..112
                     /gene="clpX"
                     /note="ECK0432:JW0428:b0438"
                     /codon_start=1
                     /transl_table=11
                     /product="ATPase and specificity subunit of ClpX-ClpP
ATP-dependent serine protease"
                     /protein_id="AP_001089.1"
                     /db_xref="GI:89107308"
/translation="MTDKRKDGSGNLLYCSFCGKSQHEVRKLIAGPSVYICDECVDLC
NDIIREEIRDVAPHRENSALPTPHEIRNHLDDYVIGQEQAKKVLAVAVYNHYKRLRNG
DTSNGVELGKSNILLIGPTGSGKTLLAETLARLLDVPFTMADATTLTEAGYVGEDVEN
IIQKLLQKCDYDVQKAQRGIVYIDEIDKISRKSDNPSITRDVSGEGVQQALLKLIEGT
VAAVPPQGGRKHPQQEFLQVDTSKILFICGGAFAGLDKVISHRVETGSGIGFGATVKA
KSDKASEGELLAQVEPEDLIKFGLIPEFIGRLPVVATLNELSEEALIQILKEPKNALT
KQYQALFNLEGVDLEFEDEALDAIAKKAMARKTGARGLRSIVEAALLDTMYDLPSMED
VEKVVIDESVIDGQSRPLLIYGKPEAQQASGE" (SEQ ID NO: 9)
                     /label="clpX (partial gene)"
     host_DNA        1..302
                     /label="E. coli W3110 chromosome"
     source          1..302
                     /organism="Escherichia coli str. K-12 substr. W3110"
                     /mol_type="genomic DNA"
                     /strain="K-12"
                     /sub_strain="W3110"
                     /db_xref="taxon:316407"
     primer          258..302
                     /label="lon-R1 Lambda red homology region"
     gene            300..302
                     /gene="lon"
     CDS             300..302
                     /gene="lon"
                     /note="ECK0433:JW0429:b0439"
                     /codon_start=1
                     /transl_table=11
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                     /db_xref="GI:89107309"
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DHDKRIMLVAQKEASIDEPGVNDLFTVGVVASILQMLKLPDGTVKVLVEGLQRARIGS
LSDNGEHFSARAEYLSPTIDERDQEVLVRTALSQFEGYIKLNKKIPPEVLMSLNSID
DPARLADTIAAHMPLKLADKQSVLEMSDVNERLEYLMAMMESEIDLLQVEKRIRNRVK
KQMEKSQREYYLNEQMKAIQKELGEMDDAPDENEALRKKIDAARMPKEAKEKAEAELQ
```

FIG. 13B

KLKMMSPMSAEATVVRGYIDWMVQVPWNARSKVEKDLRQAQFILDTDHYGLERVKDRI
LDYLAVQSRVNKIKGFTACLVGPPGVGKTSLGQSIAKATGRKYVRMALGGVRDEAEIR
GHRRTYIGSMPGKLIQKMAKVGVKNPLFLLDEIDKMSSDMRGDPASALLEVLDPEQNV
AFSDHYLEVDYDLSDVMFVATSNSMNIPAPLLDRMEVIRLSGYTEDEKTNIAKRHLLP
KQIERNALKKGELVVDDSAIIGIIRYYTREAGVRGLEREISKLCRKAVKQLLLDKSLK
HIEINGDNLHDYLGVQRFDYGRADNENRVGQVTGLAWTEVGGDLLTIETACVPGKGKL
TYTGSLGEVMQESIQAALTVVRARAEKLGIKPDFYEKRDIHVHVPEGATPKDGPSAGI
AMCTALVSCLTGNPVRADVAMTGEITLRGQVLPIGGLKEKLLAARRGGIKTVLIPFEN
KRDLSEIPDNVIADLDIHPVKRIEEVLTLALQNEPSGMQVVTAK" (SEQ ID NO: 10)

```
                     /label="lon N-terminus (initiator methionine codon
only remains)"
     repeat_unit     303..330
                     /label="Flp site"
     primer_bind     303..322
                     /label="P4 wanner=P3 Baba and Mori"
     source          complement(303..1605)
                     /organism="Template plasmid pKD13"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:170483"
     misc_feature    complement(303..4704)
                     /note="originates from KanR-lac2RBS-"
                     /label=insert
     repeat_unit     331..364
                     /label="34 nt Flp site scar"
     repeat_unit     331..342
                     /label="Flp site"
     repeat_unit     complement(353..364)
                     /label="FLP site"
     Region          365..1506
                     /label="excised region upon pCP20 introduction"
     KAN_resistance  732..1526
                     /note="kanamycin resistance"
                     /codon_start=1
                     /transl_table=11
                     /product="Tn5 neomycin phosphotransferase"
                     /protein_id="AAL02037.1"
                     /db_xref="GI:15554336"
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PVLFVKTDLSGALNELQDEAARLSWLATTGVPCAAVLDVVTEAGRDWLLLGEVPGQDL
LSSHLAPAEKVSIMSDAMRRLHTLDPATCPFDHQAKHRIERARTRMEAGLVDQDDLDE
EHQGLAPAELFARLKARMPDGEDLVVTHGDACLPNIMVENGRFSGFIDCGRLGVADRY
QDIALATRDIAEELGGEWADRFLVLYGIAAPDSQRIAFYRLLDEFF" (SEQ ID NO: 11)
     repeat_unit     1538..1550
                     /label="Flp site"
     misc_feature    1539..1586
                     /note="natural FRT site"
     repeat_unit     1553..1564
                     /label="Flp site"
     repeat_unit     complement(1575..1586)
                     /label="FLP site"
     primer_bind     complement(1586..1605)
                     /label="P1 Wanner=P2 Baba et al"
     repeat_unit     1587..1605
                     /label="Flp site downstream scar Baba et al"
     Site            join(1615^1616,1610..1617)
                     /note="Name: NotI"
                     /note="Pattern: gcggccgc"
                     /note="cut 0 on positive strand: 3083^3084"
                     /note="cut 0 on negative strand: 3087^3088"
                     /note="inhibited by: 5'; N4-methylcytosine"
                     /note="site type: other"
```

FIG. 13C

```
                     /note="restriction site"
                     /label=NotI
     source          1618..4704
                     /organism="Escherichia coli W3110"
                     /mol_type="genomic DNA"
                     /strain="K-12"
                     /sub_strain="W3110"
                     /db_xref="taxon:316407"
                     /note="synonym: Escherichia coli str. K12 substr.
W3110"
     wild_type_CDS   complement(1618..4692)
                     /gene="lacZ"
                     /note="ECK0341:JW0335:b0344"
                     /codon_start=1
                     /transl_table=11
                     /product="beta-D-galactosidase"
                     /protein_id="BAE76126.1"
                     /db_xref="GI:85674486"
/translation="MTMITDSLAVVLQRRDWENPGVTQLNRLAAHPPFASWRNSEEAR
TDRPSQQLRSLNGEWRFAWFPAPEAVPESWLECDLPEADTVVVPSNWQMHGYDAPIYT
NVTYPITVNPPFVPTENPTGCYSLTFNVDESWLQEGQTRIIFDGVNSAFHLWCNGRWV
GYGQDSRLPSEFDLSAFLRAGENRLAVMVLRWSDGSYLEDQDMWRMSGIFRDVSLLHK
PTTQISDFHVATRFNDDFSRAVLEAEVQMCGELRDYLRVTVSLWQGETQVASGTAPF
GEIIDERGGYADRVTLRLNVENPKLWSAEIPNLYRAVVELHTADGTLIEAEACDVGFR
EVRIENGLLLLNGKPLLIRGVNRHEHHPLHGQVMDEQTMVQDILLMKQNNFNAVRCSH
YPNHPLWYTLCDRYGLYVVDEANIETHGMVPMNRLTDDPRWLPAMSERVTRMVQRDRN
HPSVIIWSLGNESGHGANHDALYRWIKSVDPSRPVQYEGGGADTTATDIICPMYARVD
EDQPFPAVPKWSIKKWLSLPGETRPLILCEYAHAMGNSLGGFAKYWDAFRQYPRLQGG
FVWDWVDQSLIKYDENGNPWSAYGGDFGDTPNDRQFCMNGLVFADRTPHPALTEAKHQ
QQFFQFRLSGQTIEVTSEYLFRHSDNELLHWMVALDGKPLASGEVPLDVAPQGKQLIE
LPELPQPESAGQLWLTVRVVQPNATAWSEAGHISAWQQWRLAENLSVTLPAASHAIPH
LTTSEMDFCIELGNKRWQFNRQSGFLSQMWIGDKKQLLTPLRDQFTRAPLDNDIGVSE
ATRIDPNAWVERWKAAGHYQAEAALLQCTADTLADAVLITTAHAWQHQGKTLFISRKT
YRIDGSGQMAITVDVEVASDTPHPARIGLNCQLAQVAERVNWLGLGPQENYPDRLTAA
CFDRWDLPLSDMYTPYVFPSENGLRCGTRELNYGPHQWRGDFQFNISRYSQQQLMETS
HRHLLHAEEGTWLNIDGFHMGIGGDDSWSPSVSAEFQLSAGRYHYQLVWCQK"(SEQ ID NO: 12)
                     /label="wild-type lacZ+ CDS"
     epitope_tag     complement(4671..4704)
                     /label="LacFRpnlnoRBS cloning primer"
     RBS             complement(4693..4704)
     gene            <4705..4725
                     /gene="lon"
     CDS             <4705..4725
                     /gene="lon"
                     /note="ECK0433:JW0429:b0439"
                     /codon_start=1
                     /transl_table=11
                     /product="DNA-binding ATP-dependent protease La"
                     /protein_id="AP_001089.1"
                     /db_xref="GI:89107309"
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LSDNGEHFSARAEYLESPTIDERFQEVLVRTAISCFEGYIKLNKKIPPEVLTSLNSID
DPARLADTIAAHMPIKLADKQSVLEMSDVNERLEYLMAMMESEIDLLQVEKRIRNRVK
KQMEKSQREYYLNEQMKAIQKELGGMDDAPDENEALKRKIDAAKMPKEAKDKAEAELQ
KLKNMSPMSAEATVVRGYIDWMVQVPWNARSKVKKDLRQAQEILDTDHYGLEEVKDRI
LEYLAVQSRVNKIKGPILCLVGPPGVGKTSLGQSIAKATGRKYVRMALGGVRDEAEIR
GHRRTYIGSMPGKLIQKMAKVGVKNPLFLLDEIDKMSSDMRGDPASALLEVLDPEQNV
AFSDHYLEVDYDLSDVMFVATSNSMNIPRPLLDRMEVIRLSGYTEDEKLNIAKRHLLP
KQIERNALKKGELTVDDSAIIGIIRYYTREAGVRGLEREISKLCRKAVKQLLLDKSLK
```

FIG. 13D

```
HIEINGDNLHDYLGVQRFDYCRADNENRVGQVTGLAMTEVGGDLLTIETACVPGKGKL
TYTGSLGEVMQESIQAALTVVRANAEKLGINPDFYEKRDIHVHVPEGATPKDGPSAGI
AMCTALVSCLTGNPVRADVAMTGEITLRGQVLPIGGLKEKLLAARRCGIKTVLIPFEN
KRDLEEIPDNVIADLDIHPVKRISEVLTLALQNEPDGMQVVTAK" (SEQ ID NO: 13)
                     /label="lon C-terminus (encodes 6 C-terminal
residues)"
     host_DNA        4705..5049
                     /label="E. coli W3110 chromosome"
     source          <4705..>5049
                     /organism="Escherichia coli str. K-12 substr. W3110"
                     /mol_type="genomic DNA"
                     /strain="K-12"
                     /sub_strain="W3110"
                     /db_xref="taxon:316407"
     primer          complement(4705..4749)
                     /label="lon-B2 homology region for lambda red"
     gene            4934..>5049
                     /gene="hupB"
     CDS             4934..>5049
                     /gene="hupB"
                     /note="BCR0434:JW0430:b0440"
beta subunit"        /codon_start=1
                     /transl_table=11
                     /product="HU, DNA-binding transcriptional regulator,
                     /protein_id="NP_001090.1"
                     /db_xref="GI:89TC7310"
/translation="MNKSQLIDKIAAGADISKAAAGRALDAIIASVTESLKEGDDVAL
VGFGTFAVKERAARTGRNPQTGKEITIAAAKVPSFRAGKALKDAVN" (SEQ ID NO: 14)
                     /label="hupB (partial gene)"
ORIGIN
```

FIG. 13E

```
1801 ACTGTAGCGG CTGATGTTGA ACTGGAAGTC GCCGCGCCAC TGGTGTGGGC CATAATTCAA
1861 TTCGCGCGTC CCGCAGCGCA GACCGTTTTC GCTCGGGAAG ACGTACGGGG TATACATGTC
1921 TGACAATGGC AGATCCCAGC GGTCAAAACA GGCGGCAGTA AGGCGGTCGG GATAGTTTTC
1981 TTGCGGCCCT AATCCGAGCC AGTTTACCCG CTCTGCTACC TGCGCCAGCT GGCAGTTCAG
2041 GCCAATCCGC GCCGGATGCG GTGTATCGCT CGCCACTTCA ACATCAACGG TAATCGCCAT
2101 TTGACCACTA CCATCAATCC GGTAGGTTTT CCGGCTGATA AATAAGGTTT TCCCCTGATG
2161 CTGCCACGCG TGAGCGGTCG TAATCAGCAC CGCATCAGCA AGTGTATCTG CCGTGCACTG
2221 CAACAACGCT GCTTCGGCCT GGTAATGGCC CGCCGCCTCC CAGCGTTCGA CCCAGGCGTT
2281 AGGGTCAATG CGGGTCGCTT CACTTACGCC AATGTCGTTA TCCAGCGGTG CACGGGTGAA
2341 CTGATCGCGC AGCGGCGTCA GCAGTTGTTT TTTATCGCCA ATCCACATCT GTGAAAGAAA
2401 GCCTGACTGG CGGTTAAATT GCCAACGCTT ATTACCCAGC TGGATGCAAA AATCCATTTC
2461 GCTGGTGGTC AGATGCGGGA TGGCGTGGGA CGCGGCGGGG AGCGTCACAC TGAGGTTTTC
2521 CGCCAGACGC CACTGCTGCC AGGCGCTGAT GTGCCCGGCT TCTGACCATG CGGTCGCGTT
2581 CGGTTGCACT ACGCGTACTG TGAGCCAGAG TTGCCCGGCG CTCTCCGGCT GCGGTAGTTC
2641 AGGCAGTTCA ATCAACTGTT TACCTTGTGG AGCGACATCC AGAGGCACTT CACCGCTTGC
2701 CAGCGGCTTA CCATCCAGCG CCACCATCCA GTGCAGGAGC TCGTTATCGC TATGACGGAA
2761 CAGGTATTCG CTGGTCACTT CGATGGTTTG CCCGGATAAA CGGAACTGGA AAAACTGCTG
2821 CTGGTGTTTT GCTTCGGTCA GCGCTGGATG CGGCGTGCGG TCGGCAAAGA CCAGACCGTT
2881 CATACAGAAC TGGCGATCGT TCGGCGTATC GCCAAAATCA CCGCCGTAAG CCGACCACGG
2941 GTTGCCGTTT TCATCATATT TAATCAGCGA CTGATCCACC CAGTCCCAGA CGAAGCCGCC
3001 CTGTAAACGG GGATACTGAC GAAACGCCTG CCAGTATTTA GCGAAACCGC CAAGACTGTT
3061 ACCCATCGCG TGGGCGTATT CGCAAAGGAT CAGCGGCGCG GTCTCTCCAG GTAGCGAAAG
3121 CCATTTTTTG ATGGACCATT TCGGCACAGC CGGGAAGGGC TGGTCTTCAT CCACGCGCGC
3181 GTACATCGGG CAAATAATAT CGGTGGCCGT GGTGTCGGCT CCGCCGCCTT CATACTGCAC
3241 CGGGCGGGAA GGATCGACAG ATTTGATCCA GCGATACAGC GCGTCGTGAT TAGCGCCGTG
3301 GCCTGATTCA TTCCCCAGCG ACCAGATGAT CACACTCGGG TGATTACGAT CGCGCTGCAC
3361 CATTCGCGTT ACGCGTTCGC TCATCGCCGG TAGCCAGCGC GGATCATCGG TCAGACGATT
3421 CATTGGCACC ATGCCGTGGG TTTCAATATT GGCTTCATCC ACCACATACA GGCCGTAGCG
3481 GTCGCACAGC GTGTACCACA GCGGATGGTT CGGATAATGC GAACAGCGCA CGGCGTTAAA
3541 GTTGTTCTGC TTCATCAGCA GGATATCCTG CACCATCGTC TGCTCATCCA TGACCTGACC
3601 ATGCAGAGGA TGATGCTCGT GACGGTTAAC GCCTCGAATC AGCAACGGCT TGCCGTTCAG
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3721 AATCAGCGTG CCGTCGGCGG TGTGCAGTTC AACCACCGCA CGATAGAGAT TCGGGATTTC
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3841 ACCACGCTCA TCGATAATTT CACCGCCGAA AGGCGCGGTG CCGCTGGCGA CCTGCGTTTC
3901 ACCCTGCCAT AAAGAAACTG TTACCCGTAG GTAGTCACGC AACTCGCCGC ACATCTGAAC
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4021 GCTGATTTGT GTAGTCGGTT TATGCAGCAA CGAGACGTCA CGGAAAATGC CGCTCATCCG
4081 CCACATATCC TGATCTTCCA GATAACTGCC GTCACTCCAG CGCAGCACCA TCACCGCGAG
4141 GCGGTTTTCT CCGCCGCGTA AAAATGCGCT CAGGTCAAAT TCAGACGGCA AACGACTGTC
4201 CTGGCCGTAA CCGACCCAGC GCCCGTTGCA CCACAGATGA AACGCCGAGT TAACGCCATC
4261 AAAAATAATT CGCGTCTGGC CTTCCTGTAG CCAGCTTTCA TCAACATTAA ATGTGAGCGA
4321 GTAACAACCC GTCGGATTCT CCGTGGGAAC AAACGGCGGA TTGACCGTAA TGGGATAGGT
4381 CACGTTGGTG TAGATGGGCG CATCGTAACC GTGCATCTGC CAGTTTGAGG GGACGACGAC
4441 AGTATCGGCC TCAGGAAGAT CGCACTCCAG CCAGCTTTCC GGCACCGCTT CTGGTGCCGG
4501 AAACCAGGCA AAGCGCCATT CGCCATTCAG GCTGCGCAAC TGTTGGGAAG GGCGATCGGT
4561 GCGGGCCTCT TCGCTATTAC GCCAGCTGGC GAAAGGGGGA TGTGCTGCAA GGCGATTAAG
4621 TTGGGTAACG CCAGGGTTTT CCCAGTCACG ACGTTGTAAA ACGACGGCCA GTGAATCCGT
4681 AATCATGGTC ATagtaggtt tcctCAGGTT GTGACTGCAA AATAGTGACC TCGCGCAAAA
4741 TGCACTAATA AAAACAGGGC TGGCAGGCTA ATTCGGGCTT GCCAGCCTTT TTTTGTCTCG
4801 CTAAGTTAGA TGGCGGATCG GGCTTGCCCT TATTAAGGGG TGTTGTAAGG GGATGGCTGG
4861 CCTGATATAA CTGCTGCGCG TTCGTACCTT GAAGGATTCA AGTGCGATAT AAATTATAAA
4921 GAGGAAGACA AGAGTGAATA AATCTCAATT CATCGACAAG ATTGCTCCAG GGGCTCATAT
4981 CTCTAAAGCT GCGGCTGGCC GTGCGTTAGA TGCTATTATT GCTTCCGTAA CTGAATCTCT
5041 GAAAGAAGG (SEQ ID NO: 15)
```

E.coli strains of the current invention

E100 (GI724)	ampC::(P_{trpB}λcI+)	$lacI^q{}_{lac}$ P_{L8}				
E183	ampC::(P_{trpB}λcI+)	$P_{lacI}{}^q$(ΔlacI-lacZ)$_{158}$lacY$^+$				
E205	ampC::(P_{trpB}λcI+)	$P_{lacI}{}^q$(Δlacl-lacZ)$_{158}$lacY$^+$	ΔwcaJ			
E214	ampC::(P_{trpB}λcI+)	$P_{lacI}{}^q$(Δlacl-lacZ)$_{158}$lacY$^+$	ΔwcaJ	thyA$_{748}$::Tn10		
E390	ampC::(P_{trpB}λcI+)	$P_{lacI}{}^q$(Δlacl-lacZ)$_{158}$lacY$^+$	ΔwcaJ	thyA$_{748}$::Tn10	Δlon::(kan,lacZ$^+$)	
E403	ampC::(P_{trpB}λcI+)	$P_{lacI}{}^q$(Δlacl-lacZ)$_{158}$lacY$^+$	ΔwcaJ	thyA$_{748}$::Tn10	Δlon::(kan,lacZ$^+$)	ΔlacA

FIG. 14

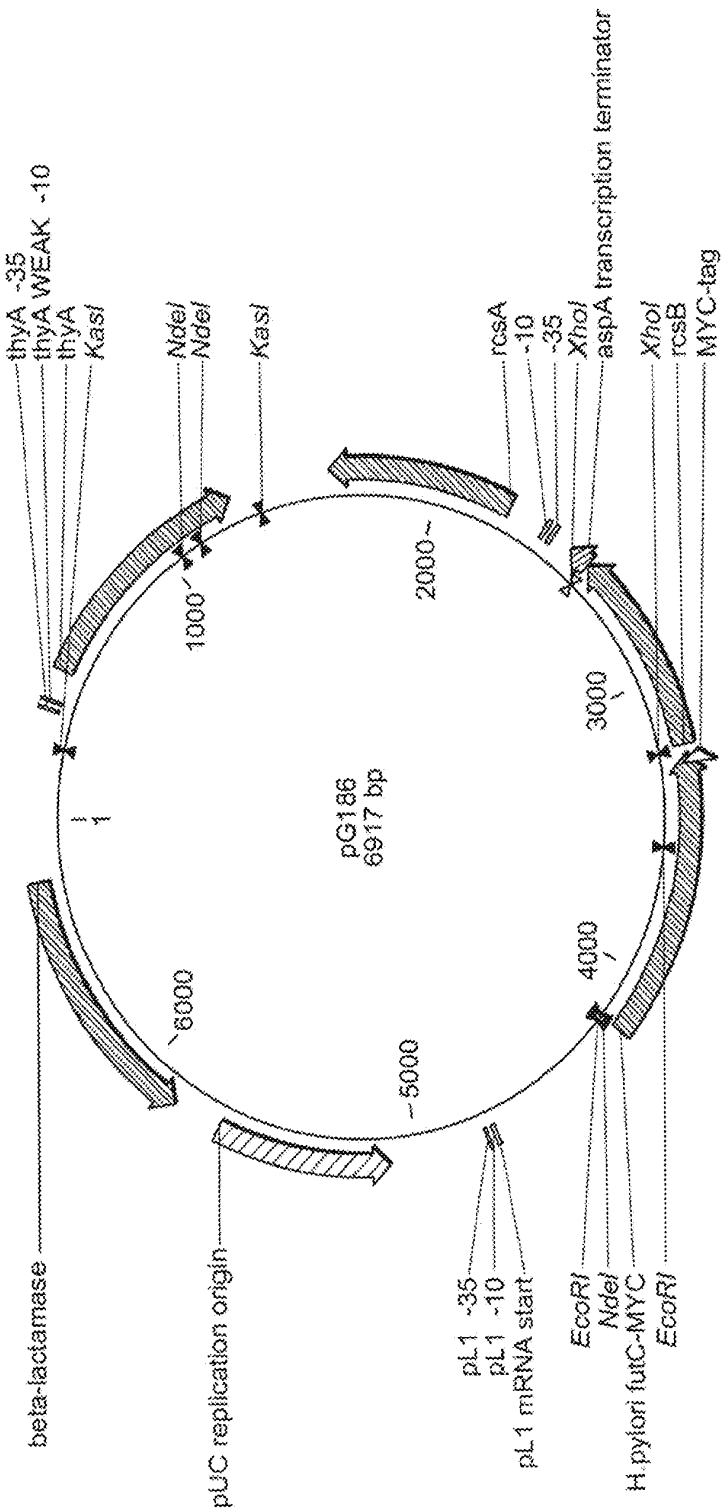


FIG. 15

FIG. 16

Comparison of expression levels at 37C of *futC and wcfW in E.coli* strain E390

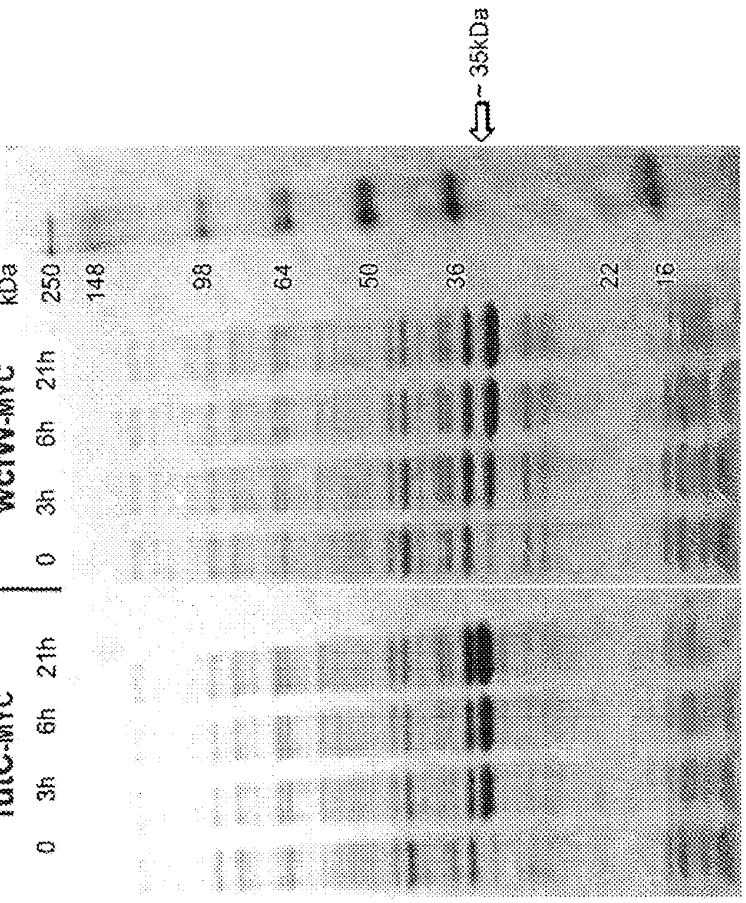


FIG. 17

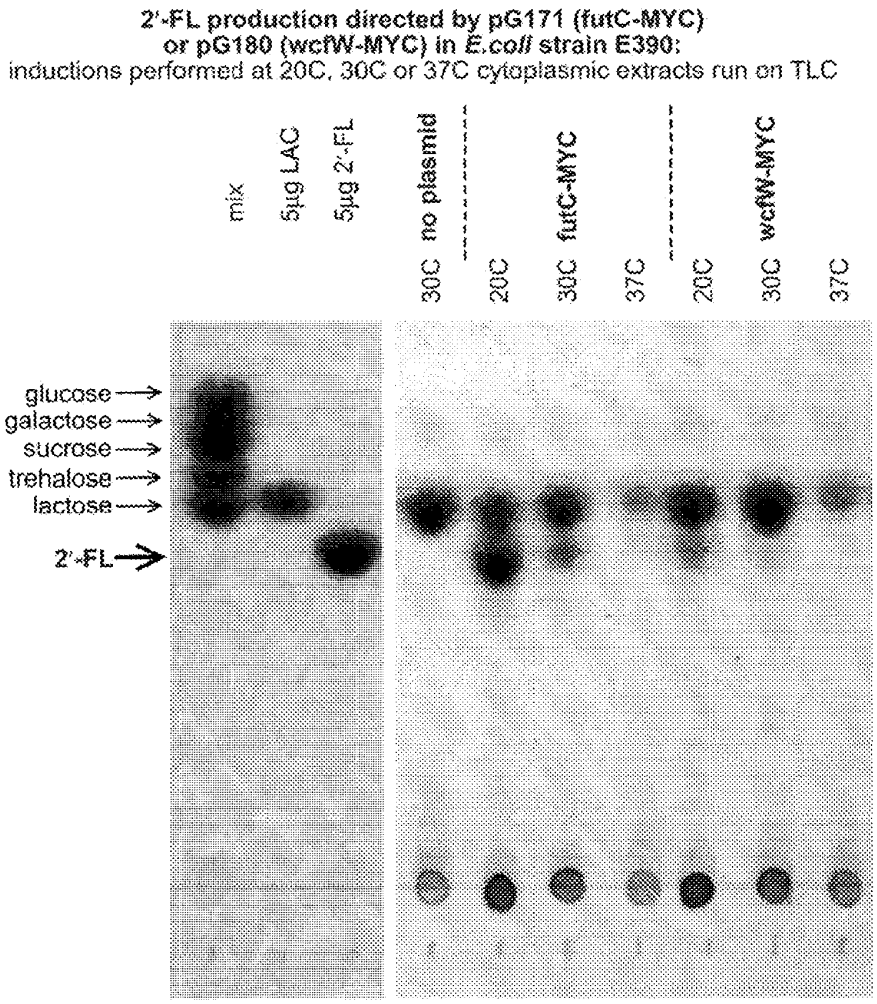
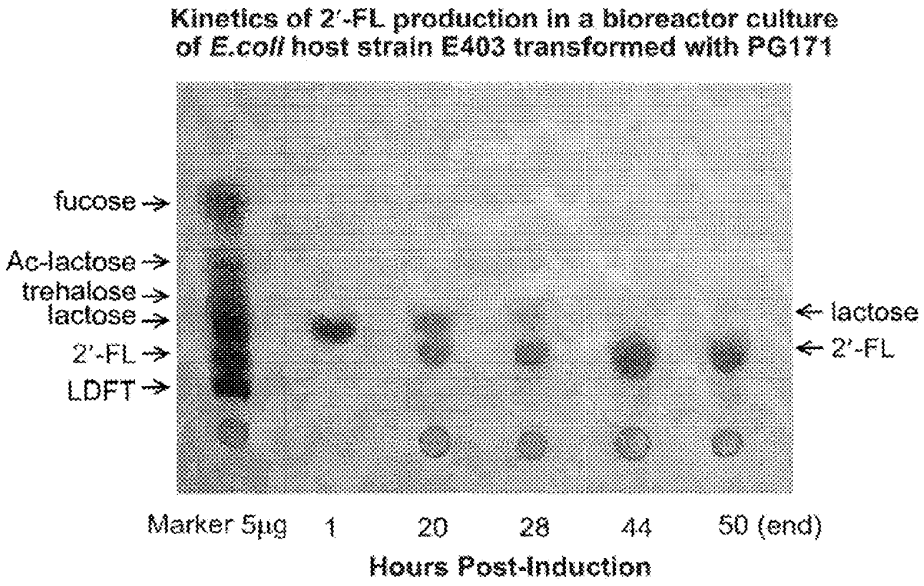


FIG. 18



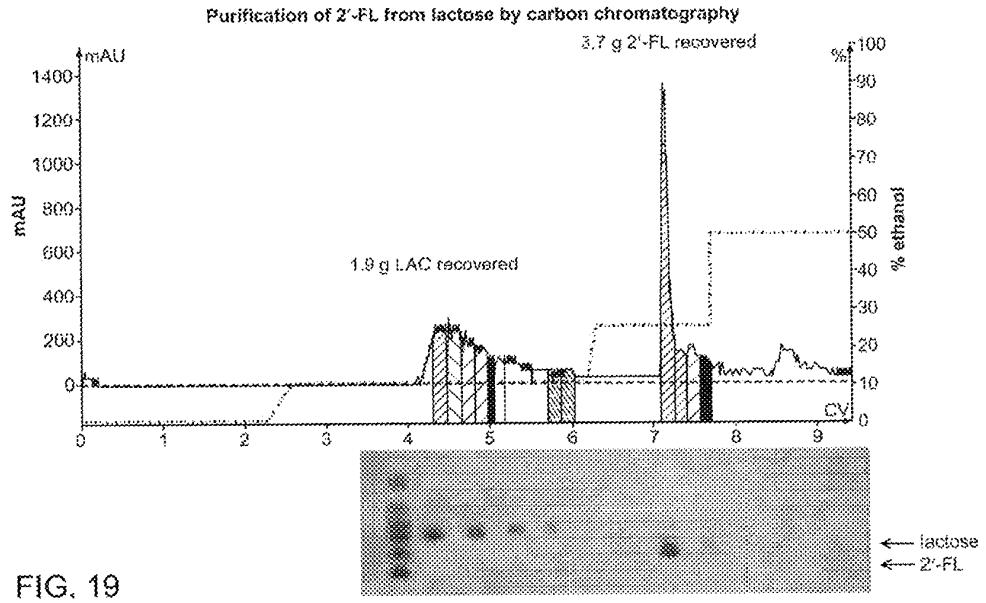
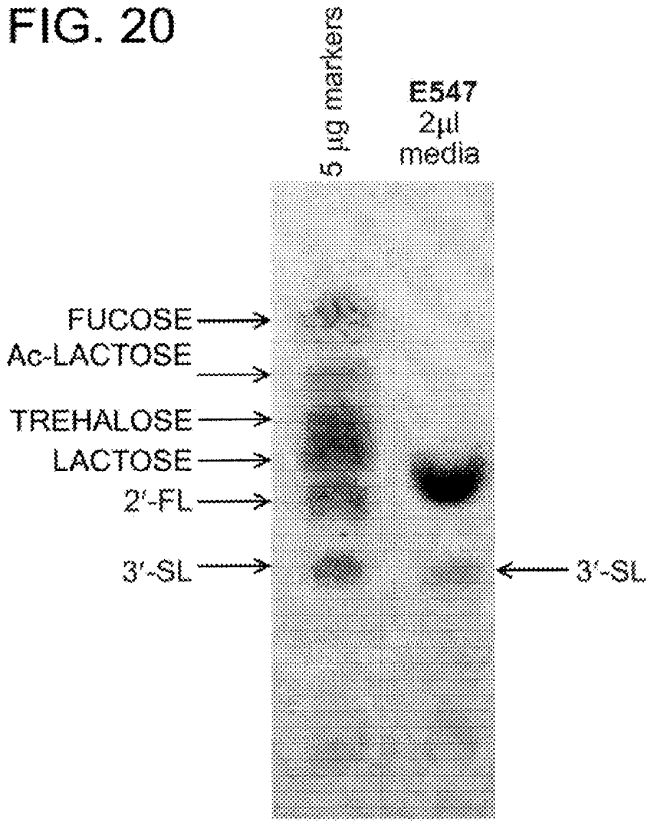


FIG. 19

FIG. 20



BIOSYNTHESIS OF HUMAN MILK OLIGOSACCHARIDES IN ENGINEERED BACTERIA

RELATED APPLICATIONS

This application is a continuation of U.S. Ser. No. 15/442,131, filed Feb. 24, 2017, which is a continuation of U.S. Ser. No. 14/033,664 filed Sep. 23, 2013, now U.S. Pat. No. 9,587,241 issued Feb. 15, 2017, which is a divisional of U.S. Ser. No. 13/398,526 filed Feb. 16, 2012, now U.S. Pat. No. 9,453,230 issued Sep. 27, 2016, and claims the benefit of priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 61/443,470, filed Feb. 16, 2011, the entire contents of each of which are incorporated herein by reference.

INCORPORATED-BY-REFERENCE OF SEQUENCE LISTING

The contents of the text file named "37847-505C03US_Sequence_Listing.txt", which was created on Sep. 21, 2017 and is 94 KB in size, are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

The invention provides compositions and methods for producing purified oligosaccharides, in particular certain fucosylated and/or sialylated oligosaccharides that are typically found in human milk.

BACKGROUND OF THE INVENTION

Human milk contains a diverse and abundant set of neutral and acidic oligosaccharides (human milk oligosaccharides, HMOS). Many of these molecules are not utilized directly by infants for nutrition, but they nevertheless serve critical roles in the establishment of a healthy gut microbiome, in the prevention of disease, and in immune function. Prior to the invention described herein, the ability to produce HMOS inexpensively at large scale was problematic. For example, HMOS production through chemical synthesis was limited by stereo-specificity issues, precursor availability, product impurities, and high overall cost. As such, there is a pressing need for new strategies to inexpensively manufacture large quantities of HMOS for a variety of commercial applications.

SUMMARY OF THE INVENTION

The invention described herein features efficient and economical methods for producing fucosylated and sialylated oligosaccharides. The method for producing a fucosylated oligosaccharide in a bacterium comprises the following steps: providing a bacterium that comprises a functional β-galactosidase gene, an exogenous fucosyltransferase gene, a GDP-fucose synthesis pathway, and a functional lactose permease gene; culturing the bacterium in the presence of lactose; and retrieving a fucosylated oligosaccharide from the bacterium or from a culture supernatant of the bacterium.

To produce a fucosylated oligosaccharide by biosynthesis, the bacterium utilizes an endogenous or exogenous guanosine diphosphate (GDP)-fucose synthesis pathway. By "GDP-fucose synthesis pathway" is meant a sequence of reactions, usually controlled and catalyzed by enzymes, which results in the synthesis of GDP-fucose. An exemplary GDP-fucose synthesis pathway in *Escherichia coli* is set forth below. In the GDP-fucose synthesis pathway set forth below, the enzymes for GDP-fucose synthesis include: 1) manA=phosphomannose isomerase (PMI), 2) manB=phosphomannomutase (PMM), 3) manC=mannose-1-phosphate guanylyltransferase (GMP), 4) gmd=GDP-mannose-4,6-dehydratase (GMD), 5) fcl=GDP-fucose synthase (GFS), and 6) ΔwcaJ=mutated UDP-glucose lipid carrier transferase.

Glucose→Glc-6-P→Fru-6-P→[1] Man-6-P→[2] Man-1-P→[3] GDP-Man→[4,5] GDP-Fuc→[6] Colanic acid.

The synthetic pathway from fructose-6-phosphate, a common metabolic intermediate of all organisms, to GDP-fucose consists of 5 enzymatic steps: 1) PMI (phosphomannose isomerase), 2) PMM (phosphomannomutase), 3) GMP (mannose-1-phosphate guanylyltransferase), 4) GMD (GDP-mannose-4,6-dehydratase), and 5) GFS (GDP-fucose synthase). Individual bacterial species possess different inherent capabilities with respect to GDP-fucose synthesis. *Escherichia coli*, for example, contains enzymes competent to perform all five steps, whereas *Bacillus licheniformis* is missing enzymes capable of performing steps 4 and 5 (i.e., GMD and GFS). Any enzymes in the GDP-synthesis pathway that are inherently missing in any particular bacterial species are provided as genes on recombinant DNA constructs, supplied either on a plasmid expression vector or as exogenous genes integrated into the host chromosome.

The invention described herein details the manipulation of genes and pathways within bacteria such as the enterobacterium *Escherichia coli* K12 (*E. coli*) or probiotic bacteria leading to high level synthesis of HMOS. A variety of bacterial species may be used in the oligosaccharide biosynthesis methods, for example *Erwinia herbicola* (*Pantoea agglomerans*), *Citrobacter freundii, Pantoea citrea, Pectobacterium carotovorum*, or *Xanthomonas campestris*. Bacteria of the genus *Bacillus* may also be used, including *Bacillus subtilis, Bacillus licheniformis, Bacillus coagulans, Bacillus thermophilus, Bacillus laterosporus, Bacillus megaterium, Bacillus mycoides, Bacillus pumilus, Bacillus lentus, Bacillus cereus*, and *Bacillus circulans*. Similarly, bacteria of the genera *Lactobacillus* and *Lactococcus* may be modified using the methods of this invention, including but not limited to *Lactobacillus acidophilus, Lactobacillus salivarius, Lactobacillus plantarum, Lactobacillus helveticus, Lactobacillus delbrueckii, Lactobacillus rhamnosus, Lactobacillus bulgaricus, Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus casei, Lactobacillus reuteri, Lactobacillus jensenii*, and *Lactococcus lactis. Streptococcus thermophiles* and *Proprionibacterium freudenreichii* are also suitable bacterial species for the invention described herein. Also included as part of this invention are strains, modified as described here, from the genera *Enterococcus* (e.g., *Enterococcus faecium* and *Enterococcus thermophiles*), *Bifidobacterium* (e.g., *Bifidobacterium longum, Bifidobacterium infantis*, and *Bifidobacterium bifidum*), *Sporolactobacillus* spp., *Micromomospora* spp., *Micrococcus* spp., *Rhodococcus* spp., and *Pseudomonas* (e.g., *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*). Bacteria comprising the characteristics described herein are cultured in the presence of lactose, and a fucosylated oligosaccharide is retrieved, either from the bacterium itself or from a culture supernatant of the bacterium. The fucosylated oligosaccharide is purified for use in therapeutic or nutritional products, or the bacteria are used directly in such products.

The bacterium also comprises a functional β-galactosidase gene. The β-galactosidase gene is an endogenous β-galactosidase gene or an exogenous β-galactosidase gene.

For example, the β-galactosidase gene comprises an *E. coli* lacZ gene (e.g., GenBank Accession Number V00296 (GI: 41901), incorporated herein by reference). The bacterium accumulates an increased intracellular lactose pool, and produces a low level of β-galactosidase.

A functional lactose permease gene is also present in the bacterium. The lactose permease gene is an endogenous lactose permease gene or an exogenous lactose permease gene. For example, the lactose permease gene comprises an *E. coli* lacY gene (e.g., GenBank Accession Number V00295 (GI:41897), incorporated herein by reference). Many bacteria possess the inherent ability to transport lactose from the growth medium into the cell, by utilizing a transport protein that is either a homolog of the *E. coli* lactose permease (e.g., as found in *Bacillus licheniformis*), or a transporter that is a member of the ubiquitous PTS sugar transport family (e.g., as found in *Lactobacillus casei* and *Lactobacillus rhamnosus*). For bacteria lacking an inherent ability to transport extracellular lactose into the cell cytoplasm, this ability is conferred by an exogenous lactose transporter gene (e.g., *E. coli* lacY) provided on recombinant DNA constructs, and supplied either on a plasmid expression vector or as exogenous genes integrated into the host chromosome.

The bacterium comprises an exogenous fucosyltransferase gene. For example, the exogenous fucosyltransferase gene encodes α(1,2) fucosyltransferase and/or α(1,3) fucosyltransferase. An exemplary α(1,2) fucosyltransferase gene is the wcfW gene from *Bacteroides fragilis* NCTC 9343 (SEQ ID NO: 4). An exemplary α(1,3) fucosyltransferase gene is the *Helicobacter pylori* 26695 futA gene. One example of the *Helicobacter pylori* futA gene is presented in GenBank Accession Number HV532291 (GI:365791177), incorporated herein by reference.

Alternatively, a method for producing a fucosylated oligosaccharide by biosynthesis comprises the following steps: providing an enteric bacterium that comprises a functional β-galactosidase gene, an exogenous fucosyltransferase gene, a mutation in a colanic acid synthesis gene, and a functional lactose permease gene; culturing the bacterium in the presence of lactose; and retrieving a fucosylated oligosaccharide from the bacterium or from a culture supernatant of the bacterium.

To produce a fucosylated oligosaccharide by biosynthesis, the bacterium comprises a mutation in an endogenous colanic acid (a fucose-containing exopolysaccharide) synthesis gene. By "colanic acid synthesis gene" is meant a gene involved in a sequence of reactions, usually controlled and catalyzed by enzymes that result in the synthesis of colanic acid. Exemplary colanic acid synthesis genes include an rcsA gene (e.g., GenBank Accession Number M58003 (GI: 1103316), incorporated herein by reference), an rcsB gene, (e.g., GenBank Accession Number E04821 (GI:2173017), incorporated herein by reference), a weal gene, (e.g., GenBank Accession Number (amino acid) BAA15900 (GI: 1736749), incorporated herein by reference), a wzxC gene, (e.g., GenBank Accession Number (amino acid) BAA15899 (GI:1736748), incorporated herein by reference), a wcaD gene, (e.g., GenBank Accession Number (amino acid) BAE76573 (GI:85675202), incorporated herein by reference), a wza gene, (e.g., GenBank Accession Number (amino acid) BAE76576 (GI:85675205), incorporated herein by reference), a wzb gene, and (e.g., GenBank Accession Number (amino acid) BAE76575 (GI:85675204), incorporated herein by reference), and a wzc gene (e.g., GenBank Accession Number (amino acid) BAA15913 (GI: 1736763), incorporated herein by reference).

This is achieved through a number of genetic modifications of endogenous *E. coli* genes involved either directly in colanic acid precursor biosynthesis, or in overall control of the colanic acid synthetic regulon. Specifically, the ability of the host *E. coli* strain to synthesize colanic acid, an extracellular capsular polysaccharide, is eliminated by the deletion of the wcaJ gene, encoding the UDP-glucose lipid carrier transferase. In a wcaJ null background, GDP-fucose accumulates in the *E. coli* cytoplasm. Over-expression of a positive regulator protein, RcsA, in the colanic acid synthesis pathway results in an increase in intracellular GDP-fucose levels. Over-expression of an additional positive regulator of colanic acid biosynthesis, namely RcsB, is also utilized, either instead of or in addition to over-expression of RcsA, to increase intracellular GDP-fucose levels. Alternatively, colanic acid biosynthesis is increased following the introduction of a null mutation into the *E. coli* lon gene (e.g., GenBank Accession Number L20572 (GI:304907), incorporated herein by reference). Lon is an adenosine-5'-triphosphate (ATP)-dependant intracellular protease that is responsible for degrading RcsA, mentioned above as a positive transcriptional regulator of colanic acid biosynthesis in *E. coli*. In a lon null background, RcsA is stabilized, RcsA levels increase, the genes responsible for GDP-fucose synthesis in *E. coli* are up-regulated, and intracellular GDP-fucose concentrations are enhanced.

For example, the bacterium further comprises a functional, wild-type *E. coli* lacZ$^+$ gene inserted into an endogenous gene, for example the lon gene in *E. coli*. In this manner, the bacterium may comprise a mutation in a lon gene.

The bacterium also comprises a functional β-galactosidase gene. The β-galactosidase gene is an endogenous β-galactosidase gene or an exogenous β-galactosidase gene. For example, the β-galactosidase gene comprises an *E. coli* lacZ gene. The endogenous lacZ gene of the *E. coli* is deleted or functionally inactivated, but in such a way that expression of the downstream lactose permease (lacY) gene remains intact.

The bacterium comprises an exogenous fucosyltransferase gene. For example, the exogenous fucosyltransferase gene encodes α(1,2) fucosyltransferase and/or α(1,3) fucosyltransferase. An exemplary α(1,2) fucosyltransferase gene is the wcfW gene from *Bacteroides fragilis* NCTC 9343 (SEQ ID NO: 4). An exemplary α(1,3) fucosyltransferase gene is the *Helicobacter pylori* 26695 futA gene. One example of the *Helicobacter pylori* futA gene is presented in GenBank Accession Number HV532291 (GI:365791177), incorporated herein by reference.

A functional lactose permease gene is also present in the bacterium. The lactose permease gene is an endogenous lactose permease gene or an exogenous lactose permease gene. For example, the lactose permease gene comprises an *E. coli* lacY gene.

The bacterium may further comprise an exogenous rcsA and/or rcsB gene (e.g., in an ectopic nucleic acid construct such as a plasmid), and the bacterium optionally further comprises a mutation in a lacA gene (e.g., GenBank Accession Number X51872 (GI:41891), incorporated herein by reference).

Bacteria comprising the characteristics described herein are cultured in the presence of lactose, and a fucosylated oligosaccharide is retrieved, either from the bacterium itself or from a culture supernatant of the bacterium. The fucosylated oligosaccharide is purified for use in therapeutic or nutritional products, or the bacteria are used directly in such products.

The bacteria used herein to produce HMOS are genetically engineered to comprise an increased intracellular guanosine diphosphate (GDP)-fucose pool, an increased intracellular lactose pool (as compared to wild type) and to comprise fucosyl transferase activity. Accordingly, the bacterium contains a mutation in a colanic acid (a fucose-containing exopolysaccharide) synthesis pathway gene, such as a wcaJ gene, resulting in an enhanced intracellular GDP-fucose pool. The bacterium further comprises a functional, wild-type *E. coli* lacZ$^+$ gene inserted into an endogenous gene, for example the lon gene in *E. coli*. In this manner, the bacterium may further comprise a mutation in a lon gene. The endogenous lacZ gene of the *E. coli* is deleted or functionally inactivated, but in such a way that expression of the downstream lactose permease (lacY) gene remains intact. The organism so manipulated maintains the ability to transport lactose from the growth medium, and to develop an intracellular lactose pool for use as an acceptor sugar in oligosaccharide synthesis, while also maintaining a low level of intracellular beta-galactosidase activity useful for a variety of additional purposes. The bacterium may further comprise an exogenous rcsA and/or rcsB gene (e.g., in an ectopic nucleic acid construct such as a plasmid), and the bacterium optionally further comprises a mutation in a lacA gene. Preferably, the bacterium accumulates an increased intracellular lactose pool, and produces a low level of beta-galactosidase.

The bacterium possesses fucosyl transferase activity. For example, the bacterium comprises one or both of an exogenous fucosyltransferase gene encoding an α(1,2) fucosyltransferase and an exogenous fucosyltransferase gene encoding an α(1,3) fucosyltransferase. An exemplary α(1,2) fucosyltransferase gene is the wcfW gene from *Bacteroides fragilis* NCTC 9343 (SEQ ID NO: 4). Prior to the present invention, this wcfW gene was not known to encode a protein with an α(1,2) fucosyltransferase activity, and further was not suspected to possess the ability to utilize lactose as an acceptor sugar. Other α(1,2) fucosyltransferase genes that use lactose as an acceptor sugar (e.g., the *Helicobacter pylori* 26695 futC gene or the *E. coli* O128:B12 wbsJ gene) may readily be substituted for *Bacteroides fragilis* wcfW. One example of the *Helicobacter pylori* futC gene is presented in GenBank Accession Number EF452503 (GI: 134142866), incorporated herein by reference.

An exemplary α(1,3) fucosyltransferase gene is the *Helicobacter pylori* 26695 futA gene, although other α(1,3) fucosyltransferase genes known in the art may be substituted (e.g., α(1,3) fucosyltransferase genes from *Helicobacter hepaticus* Hh0072, *Helicobacter bilis, Campylobacter jejuni*, or from *Bacteroides* species). The invention includes a nucleic acid construct comprising one, two, three or more of the genes described above. For example, the invention includes a nucleic acid construct expressing an exogenous fucosyltransferase gene (encoding α(1,2) fucosyltransferase or α(1,3) fucosyltransferase) transformed into a bacterial host strain comprising a deleted endogenous β-galactosidase (e.g., lacZ) gene, a replacement functional β-galactosidase gene of low activity, a GDP-fucose synthesis pathway, a functional lactose permease gene, and a deleted lactose acetyltransferase gene.

Also within the invention is an isolated *E. coli* bacterium as described above and characterized as comprising a defective colanic acid synthesis pathway, a reduced level of β-galactosidase (LacZ) activity, and an exogenous fucosyl transferase gene. The invention also includes: a) methods for phenotypic marking of a gene locus in a β-galactosidase negative host cell by utilizing a β-galactosidase (e.g., lacZ) gene insert engineered to produce a low but readily detectable level of β-galactosidase activity, b) methods for readily detecting lytic bacteriophage contamination in fermentation runs through release and detection of cytoplasmic β-galactosidase in the cell culture medium, and c) methods for depleting a bacterial culture of residual lactose at the end of production runs. a), b) and c) are each achieved by utilizing a functional β-galactosidase (e.g., lacZ) gene insert carefully engineered to direct the expression of a low, but detectable level of β-galactosidase activity in an otherwise β-galactosidase negative host cell.

A purified fucosylated oligosaccharide produced by the methods described above is also within the invention. A purified oligosaccharide, e.g., 2'-FL, 3FL, LDFT, is one that is at least 90%, 95%, 98%, 99%, or 100% (w/w) of the desired oligosaccharide by weight.

Purity is assessed by any known method, e.g., thin layer chromatography or other electrophoretic or chromatographic techniques known in the art. The invention includes a method of purifying a fucosylated oligosaccharide produced by the genetically engineered bacterium described above, which method comprises separating the desired fucosylated oligosaccharide (e.g., 2'-FL) from contaminants in a bacterial cell extract or lysate, or bacterial cell culture supernatant. Contaminants include bacterial DNA, protein and cell wall components, and yellow/brown sugar caramels sometimes formed in spontaneous chemical reactions in the culture medium.

The oligosaccharides are purified and used in a number of products for consumption by humans as well as animals, such as companion animals (dogs, cats) as well as livestock (bovine, equine, ovine, caprine, or porcine animals, as well as poultry). For example, a pharmaceutical composition comprising purified 2'-fucosyllactose (2'-FL), 3-fucosyllactose (3FL), lactodifucotetraose (LDFT), or 3'-sialyl-3-fucosyllactose (3'-S3FL) and an excipient is suitable for oral administration. Large quantities of 2'-FL, 3FL, LDFT, or 3'-S3FL are produced in bacterial hosts, e.g., an *E. coli* bacterium comprising a heterologous α(1,2)fucosyltransferase, a heterologous α(1,3) fucosyltransferase, or a heterologous sialyltransferase, or a combination thereof. An *E. coli* bacterium comprising an enhanced cytoplasmic pool of each of the following: lactose, GDP-fucose, and CMP-Neu5Ac, is useful in such production systems. In the case of lactose and GDP-fucose, endogenous *E. coli* metabolic pathways and genes are manipulated in ways that result in the generation of increased cytoplasmic concentrations of lactose and/or GDP-fucose, as compared to levels found in wild type *E. coli*. For example, the bacteria contain at least 10%, 20%, 50%, 2×, 5×, 10× or more of the levels in a corresponding wild type bacteria that lacks the genetic modifications described above. In the case of CMP-Neu5Ac, endogenous Neu5Ac catabolism genes are inactivated and exogenous CMP-Neu5Ac biosynthesis genes introduced into *E. coli* resulting in the generation of a cytoplasmic pool of CMP-Neu5Ac not found in the wild type bacterium. A method of producing a pharmaceutical composition comprising a purified HMOS is carried out by culturing the bacterium described above, purifying the HMOS produced by the bacterium, and combining the HMOS with an excipient or carrier to yield a dietary supplement for oral administration. These compositions are useful in methods of preventing or treating enteric and/or respiratory diseases in infants and adults. Accordingly, the compositions are administered to a subject suffering from or at risk of developing such a disease.

The invention therefore provides methods for increasing intracellular levels of GDP-fucose in *Escherichia coli* by manipulating the organism's endogenous colanic acid biosynthesis pathway. This is achieved through a number of genetic modifications of endogenous *E. coli* genes involved either directly in colanic acid precursor biosynthesis, or in overall control of the colanic acid synthetic regulon. The invention also provides for increasing the intracellular concentration of lactose in *E. coli*, for cells grown in the presence of lactose, by using manipulations of endogenous *E. coli* genes involved in lactose import, export, and catabolism. In particular, described herein are methods of increasing intracellular lactose levels in *E. coli* genetically engineered to produce a human milk oligosaccharide by incorporating a lacA mutation into the genetically modified *E. coli*. The lacA mutation prevents the formation of intracellular acetyl-lactose, which not only removes this molecule as a contaminant from subsequent purifications, but also eliminates *E. coli*'s ability to export excess lactose from its cytoplasm, thus greatly facilitating purposeful manipulations of the *E. coli* intracellular lactose pool.

Also described herein are bacterial host cells with the ability to accumulate a intracellular lactose pool while simultaneously possessing low, functional levels of cytoplasmic β-galactosidase activity, for example as provided by the introduction of a functional recombinant *E. coli* lacZ gene, or by a β-galactosidase gene from any of a number of other organisms (e.g., the lac4 gene of *Kluyveromyces lactis* (e.g., GenBank Accession Number M84410 (GI:173304), incorporated herein by reference). Low, functional levels of cytoplasmic β-galactosidase include β-galactosidase activity levels, of between 0.05 and 200 units, e.g., between 0.05 and 5 units, between 0.05 and 4 units, between 0.05 and 3 units, or between 0.05 and 2 units (for unit definition see: Miller J H, Laboratory CSH. Experiments in molecular genetics. Cold Spring Harbor Laboratory Cold Spring Harbor, N.Y.; 1972; incorporated herein by reference). This low level of cytoplasmic β-galactosidase activity, while not high enough to significantly diminish the intracellular lactose pool, is nevertheless very useful for tasks such as phenotypic marking of desirable genetic loci during construction of host cell backgrounds, for detection of cell lysis due to undesired bacteriophage contaminations in fermentation processes, or for the facile removal of undesired residual lactose at the end of fermentations.

In one aspect, the human milk oligosaccharide produced by engineered bacteria comprising an exogenous nucleic acid molecule encoding an α(1,2) fucosyltransferase, is 2'-FL (2'-fucosyllactose). Preferably, the α(1,2)fucosyltransferase utilized is the previously completely uncharacterized wcfW gene from *Bacteroides fragilis* NCTC 9343 of the present invention, alternatively the futC gene of *Helicobacter pylori* 26695 or the wbsJ gene of *E. coli* strain O128:B12, or any other α(1,2) fucosyltransferase capable of using lactose as the sugar acceptor substrate may be utilized for 2'-FL synthesis. In another aspect the human milk oligosaccharide produced by engineered bacteria comprising an exogenous nucleic acid molecule encoding an α(1,3) fucosyltransferase, is 3FL (3-fucosyllactose), wherein the bacterial cell comprises an exogenous nucleic acid molecule encoding an exogenous α(1,3) fucosyltransferase. Preferably, the bacterial cell is *E. coli*. The exogenous α(1,3) fucosyltransferase is isolated from, e.g., *Helicobacter pylori, H. hepaticus, H bilis, C. jejuni*, or a species of *Bacteroides*. In one aspect, the exogenous α(1,3) fucosyltransferase comprises *H. hepaticus* Hh0072, *H. pylori* 11639 FucTa, or *H. pylori* UA948 FucTa (e.g., GenBank Accession Number AF194963 (GI:28436396), incorporated herein by reference). The invention also provides compositions comprising *E. coli* genetically engineered to produce the human milk tetrasaccharide lactodifucotetraose (LDFT). The *E. coli* in this instance comprise an exogenous nucleic acid molecule encoding an α(1,2) fucosyltransferase and an exogenous nucleic acid molecule encoding an α(1,3) fucosyltransferase. In one aspect, the *E. coli* is transformed with a plasmid expressing an α(1,2) fucosyltransferase and/or a plasmid expressing an α(1,3) fucosyltransferase. In another aspect, the *E. coli* is transformed with a plasmid that expresses both an α(1,2) fucosyltransferase and an α(1,3) fucosyltransferase. Alternatively, the *E. coli* is transformed with a chromosomal integrant expressing an α(1,2) fucosyltransferase and a chromosomal integrant expressing an α(1,3) fucosyltransferase. Optionally, the *E. coli* is transformed with plasmid pG177.

Also described herein are compositions comprising a bacterial cell that produces the human milk oligosaccharide 3'-S3FL (3'-sialyl-3-fucosyllactose), wherein the bacterial cell comprises an exogenous sialyl-transferase gene encoding α(2,3)sialyl-transferase and an exogenous fucosyltransferase gene encoding α(1,3) fucosyltransferase. Preferably, the bacterial cell is *E. coli*. The exogenous fucosyltransferase gene is isolated from, e.g., *Helicobacter pylori, H. hepaticus, H. bilis, C. jejuni*, or a species of *Bacteroides*. For example, the exogenous fucosyltransferase gene comprises *H. hepaticus* Hh0072, *H. pylori* 11639 FucTa, or *H. pylori* UA948 FucTa. The exogenous sialyltransferase gene utilized for 3'-S3FL production may be obtained from any one of a number of sources, e.g., those described from *N. meningitidis* and *N. gonorrhoeae*. Preferably, the bacterium comprises a GDP-fucose synthesis pathway.

Additionally, the bacterium contains a deficient sialic acid catabolic pathway. By "sialic acid catabolic pathway" is meant a sequence of reactions, usually controlled and catalyzed by enzymes, which results in the degradation of sialic acid. An exemplary sialic acid catabolic pathway in *Escherichia coli* is described herein. In the sialic acid catabolic pathway described herein, sialic acid (Neu5Ac; N-acetylneuraminic acid) is degraded by the enzymes NanA (N-acetylneuraminic acid lyase) and NanK (N-acetylmannosamine kinase). For example, a deficient sialic acid catabolic pathway is engineered in *Escherichia coli* by way of a null mutation in endogenous nanA (N-acetylneuraminate lyase) (e.g., GenBank Accession Number D00067 (GI: 216588), incorporated herein by reference) and/or nanK (N-acetylmannosamine kinase) genes (e.g., GenBank Accession Number (amino acid) BAE77265 (GI:85676015), incorporated herein by reference). Other components of sialic acid metabolism include: (nanT) sialic acid transporter; (ManNAc-6-P) N-acetylmannosamine-6-phosphate; (GlcNAc-6-P) N-acetylglucosamine-6-phosphate; (GlcN-6-P) Glucosamine-6-phosphate; and (Fruc-6-P) Fructose-6-phosphate.

Moreover, the bacterium (e.g., *E. coli*) also comprises a sialic acid synthetic capability. For example, the bacterium comprises a sialic acid synthetic capability through provision of an exogenous UDP-GlcNAc 2-epimerase (e.g., neuC of *Campylobacter jejuni* or equivalent (e.g., GenBank Accession Number (amino acid) AAG29921 (GI: 11095585), incorporated herein by reference)), a Neu5Ac synthase (e.g., neuB of *C. jejuni* or equivalent, e.g., GenBank Accession Number (amino acid) AAG29920 (GI: 11095584), incorporated herein by reference)), and/or a CMP-Neu5Ac synthetase (e.g., neuA of *C. jejuni* or equiva-

lent, e.g., GenBank Accession Number (amino acid) ADN91474 (GI:307748204), incorporated herein by reference).

Additionally, the bacterium also comprises a functional β-galactosidase gene and a functional lactose permease gene. Bacteria comprising the characteristics described herein are cultured in the presence of lactose, and a 3'-sialyl-3-fucosyllactose is retrieved, either from the bacterium itself or from a culture supernatant of the bacterium.

Also provided are methods for producing a 3'-sialyl-3-fucosyllactose (3'-S3FL) in an enteric bacterium, wherein the enteric bacterium comprises a mutation in an endogenous colanic acid synthesis gene, a functional lacZ gene, a functional lactose permease gene, an exogenous fucosyltransferase gene encoding α(1,3) fucosyltransferase, and an exogenous sialyltransferase gene encoding an α(2,3)sialyl transferase. Additionally, the bacterium contains a deficient sialic acid catabolic pathway. For example, the bacterium comprises a deficient sialic acid catabolic pathway by way of a null mutation in endogenous nanA (N-acetylneuraminate lyase) and/or nanK (N-acetylmannosamine kinase) genes. The bacterium also comprises a sialic acid synthetic capability. For example, the bacterium comprises a sialic acid synthetic capability through provision of an exogenous UDP-GlcNAc 2-epimerase (e.g., neuC of *C. jejuni* or equivalent), a Neu5Ac synthase (e.g., neuB of *C. jejuni* or equivalent), and/or a CMP-Neu5Ac synthetase (e.g., neuA of *C. jejuni* or equivalent). Bacteria comprising the characteristics described herein are cultured in the presence of lactose, and a 3'-sialyl-3-fucosyllactose is retrieved, either from the bacterium itself or from a culture supernatant of the bacterium.

Also provided is a method for phenotypic marking of a gene locus in a host cell, whose native β-galactosidase gene is deleted or inactivated, by utilizing an inserted recombinant β-galactosidase (e.g., lacZ) gene engineered to produce a low, but detectable level of β-galactosidase activity. Similarly, the invention also provides methods for depleting a bacterial culture of residual lactose in a β-galactosidase negative host cell, whose native β-galactosidase gene is deleted or inactivated, by utilizing an inserted recombinant β-galactosidase (e.g., lacZ) gene engineered to produce a low but detectable level of β-galactosidase activity. Finally, also provided is a method for detecting bacterial cell lysis in a culture of a β-galactosidase negative host cell, whose native β-galactosidase gene is deleted or inactivated, by utilizing an inserted recombinant β-galactosidase. (e.g., lacZ) gene engineered to produce a low but detectable level of β-galactosidase activity.

Methods of purifying a fucosylated oligosaccharide produced by the methods described herein are carried out by binding the fucosylated oligosaccharide from a bacterial cell lysate or bacterial cell culture supernatant of the bacterium to a carbon column, and eluting the fucosylated oligosaccharide from the column. Purified fucosylated oligosaccharide are produced by the methods described herein.

Optionally, the invention features a vector, e.g., a vector containing a nucleic acid. The vector can further include one or more regulatory elements, e.g., a heterologous promoter. The regulatory elements can be operably linked to a protein gene, fusion protein gene, or a series of genes linked in an operon in order to express the fusion protein. In yet another aspect, the invention comprises an isolated recombinant cell, e.g., a bacterial cell containing an aforementioned nucleic acid molecule or vector. The nucleic acid sequence can be optionally integrated into the genome.

The term "substantially pure" in reference to a given polypeptide, polynucleotide or oligosaccharide means that the polypeptide, polynucleotide or oligosaccharide is substantially free from other biological macromolecules. The substantially pure polypeptide, polynucleotide or oligosaccharide is at least 75% (e.g., at least 80, 85, 95, or 99%) pure by dry weight. Purity can be measured by any appropriate calibrated standard method, for example, by column chromatography, polyacrylamide gel electrophoresis, thin layer chromatography (TLC) or HPLC analysis.

Polynucleotides, polypeptides, and oligosaccharides of the invention are purified and/or isolated. Purified defines a degree of sterility that is safe for administration to a human subject, e.g., lacking infectious or toxic agents. Specifically, as used herein, an "isolated" or "purified" nucleic acid molecule, polynucleotide, polypeptide, protein or oligosaccharide, is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or chemical precursors or other chemicals when chemically synthesized. For example, Purified HMOS compositions are at least 60% by weight (dry weight) the compound of interest. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight the compound of interest. Purity is measured by any appropriate calibrated standard method, for example, by column chromatography, polyacrylamide gel electrophoresis, thin layer chromatography (TLC) or HPLC analysis. For example, a "purified protein" refers to a protein that has been separated from other proteins, lipids, and nucleic acids with which it is naturally associated. Preferably, the protein constitutes at least 10, 20, 50, 70, 80, 90, 95, 99-100% by dry weight of the purified preparation.

By "isolated nucleic acid" is meant a nucleic acid that is free of the genes which flank it in the naturally-occurring genome of the organism from which the nucleic acid is derived. The term covers, for example: (a) a DNA which is part of a naturally occurring genomic DNA molecule, but is not flanked by both of the nucleic acid sequences that flank that part of the molecule in the genome of the organism in which it naturally occurs; (b) a nucleic acid incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner, such that the resulting molecule is not identical to any naturally occurring vector or genomic DNA; (c) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; and (d) a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein. Isolated nucleic acid molecules according to the present invention further include molecules produced synthetically, as well as any nucleic acids that have been altered chemically and/or that have modified backbones. For example, the isolated nucleic acid is a purified cDNA or RNA polynucleotide.

A "heterologous promoter", when operably linked to a nucleic acid sequence, refers to a promoter which is not naturally associated with the nucleic acid sequence.

The terms "express" and "over-express" are used to denote the fact that, in some cases, a cell useful in the method herein may inherently express some of the factor that it is to be genetically altered to produce, in which case the addition of the polynucleotide sequence results in over-expression of the factor. That is, more factor is expressed by the altered cell than would be, under the same conditions, by a wild type cell. Similarly, if the cell does not inherently express the factor that it is genetically altered to produce, the term used would be to merely "express" the factor since the wild type cell did not express the factor at all.

The terms "treating" and "treatment" as used herein refer to the administration of an agent or formulation to a clinically symptomatic individual afflicted with an adverse condition, disorder, or disease, so as to effect a reduction in severity and/or frequency of symptoms, eliminate the symptoms and/or their underlying cause, and/or facilitate improvement or remediation of damage. The terms "preventing" and "prevention" refer to the administration of an agent or composition to a clinically asymptomatic individual who is susceptible to a particular adverse condition, disorder, or disease, and thus relates to the prevention of the occurrence of symptoms and/or their underlying cause.

The invention provides a method of treating, preventing, or reducing the risk of infection in a subject comprising administering to said subject a composition comprising a human milk oligosaccharide, purified from a culture of a recombinant strain of the current invention, wherein the HMOS binds to a pathogen and wherein the subject is infected with or at risk of infection with the pathogen. In one aspect, the infection is caused by a Norwalk-like virus or *Campylobacter jejuni*. The subject is preferably a mammal in need of such treatment. The mammal is, e.g., any mammal, e.g., a human, a primate, a mouse, a rat, a dog, a cat, a cow, a horse, or a pig. In a preferred embodiment, the mammal is a human. For example, the compositions are formulated into animal feed (e.g., pellets, kibble, mash) or animal food supplements for companion animals, e.g., dogs or cats, as well as livestock or animals grown for food consumption, e.g., cattle, sheep, pigs, chickens, and goats. Preferably, the purified HMOS is formulated into a powder (e.g., infant formula powder or adult nutritional supplement powder, each of which is mixed with a liquid such as water or juice prior to consumption) or in the form of tablets, capsules or pastes or is incorporated as a component in dairy products such as milk, cream, cheese, yogurt or kefir, or as a component in any beverage, or combined in a preparation containing live microbial cultures intended to serve as probiotics, or in prebiotic preparations intended to enhance the growth of beneficial microorganisms either in vitro or in vivo. For example, the purified sugar (e.g., 2'-FL) can be mixed with a *Bifidobacterium* or *Lactobacillus* in a probiotic nutritional composition. (i.e. *Bifidobacteria* are beneficial components of a normal human gut flora and are also known to utilize HMOS for growth.

By the terms "effective amount" and "therapeutically effective amount" of a formulation or formulation component is meant a nontoxic but sufficient amount of the formulation or component to provide the desired effect.

The transitional term "comprising," which is synonymous with "including," "containing," or "characterized by," is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. By contrast, the transitional phrase "consisting of" excludes any element, step, or ingredient not specified in the claim. The transitional phrase "consisting essentially of" limits the scope of a claim to the specified materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All published foreign patents and patent applications cited herein are incorporated herein by reference. Genbank and NCBI submissions indicated by accession number cited herein are incorporated herein by reference. All other published references, documents, manuscripts and scientific literature cited herein are incorporated herein by reference. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a schematic illustration showing the synthetic pathway of the major neutral fucosyl-oligosaccharides found in human milk.

FIG. **2** is a schematic illustration showing the synthetic pathway of the major sialyloligosaccharides found in human milk.

FIG. **3** is a schematic demonstrating metabolic pathways and the changes introduced into them to engineer 2'-fucosyllactose (2'-FL) synthesis in *Escherichia coli* (*E. coli*). Specifically, the lactose synthesis pathway and the GDP-fucose synthesis pathway are illustrated. In the GDP-fucose synthesis pathway: manA=phosphomannose isomerase (PMI), manB=phosphomannomutase (PMM), manC=mannose-1-phosphate guanylyltransferase (GMP), gmd=GDP-mannose-4,6-dehydratase, fcl=GDP-fucose synthase (GFS), and ΔwcaJ=mutated UDP-glucose lipid carrier transferase.

FIG. **4** is a photograph of a thin layer chromatogram of purified 2'-FL produced in *E. coli*.

FIG. **5** is a schematic demonstrating metabolic pathways and the changes introduced into them to engineer 3'-sialyllactose (3'-SL) synthesis in *E. coli*. Abbreviations include: (Neu5Ac) N-acetylneuraminic acid, sialic acid; (nanT) sialic acid transporter; (ΔnanA) mutated N-acetylneuraminic acid lyase; (ManNAc) N-acetylmannosamine; (ΔnanK) mutated N-acetylmannosamine kinase; (ManNAc-6-P) N-acetylmannosamine-6-phosphate; (GlcNAc-6-P) N-acetylglucosamine-6-phosphate; (GlcN-6-P) Glucosamine-6-phosphate; (Fruc-6-P) Fructose-6-phosphate; (neuA), CMP-N-acetylneuraminic acid synthetase; (CMP-Neu5Ac) CMP-N-acetylneuraminic acid; and (neuB), N-acetylneuraminic acid synthase.

FIG. **6** is a schematic demonstrating metabolic pathways and the changes introduced into them to engineer 3-fucosyllactose (3-FL) synthesis in *E. coli*.

FIG. **7** is a plasmid map of pG175, which expresses the *E. coli* α(1,2)fucosyltransferase gene wbsJ.

FIG. **8** is a photograph of a western blot of lysates of *E. coli* containing pG175 and expressing wbsJ, and of cells containing pG171, a pG175 derivative plasmid carrying the *H. pylori* 26695 futC gene in place of wbsJ and which expresses futC.

FIG. **9** is a photograph of a thin layer chromatogram of 3FL produced in *E. coli* containing the plasmid pG176 and induced for expression of the *H. pylori* 26695 α(1,3)fucosyltransferase gene futA by tryptophan addition.

FIG. **10** is a plasmid map of pG177, which contains both the *H. pylori* 26695 α(1,2)fucosyltransferase gene futC and the *H. pylori* 26695 α(1,3)fucosyltransferase gene futA, configured as an operon.

FIG. **11** is a photograph of a thin layer chromatogram of 2'-FL, 3FL, and LDFT (lactodifucotetraose) produced in *E. coli*, directed by plasmids pG171, pG175 (2'-FL), pG176 (3FL), and pG177 (LDFT, 2'-FL and 3FL).

FIG. **12** is a diagram showing the replacement of the lon gene in *E. coli* strain E390 by a DNA fragment carrying both a kanamycin resistance gene (derived from transposon Tn5) and a wild-type *E. coli* lacZ+ coding sequence.

FIG. **13**A-E is a DNA sequence with annotations (in GenBank format) of the DNA insertion into the lon region diagrammed in FIG. **12** (SEQ ID NOs 9-15).

FIG. **14** is a table containing the genotypes of several *E. coli* strains of the current invention.

FIG. **15** is a plasmid map of pG186, which expresses the α(1,2)fucosyltransferase gene futC in an operon with the colanic acid pathway transcription activator gene rcsB.

FIG. **16** is a photograph of a western blot of lysates of *E. coli* containing pG180, a pG175 derivative plasmid carrying the *B. fragilis* wcfW gene in place of wbsJ and which expresses wcfW, and of cells containing pG171, a pG175 derivative plasmid carrying the *H. pylori* 26695 futC gene in place of wbsJ and which expresses futC.

FIG. **17** is a photograph of a thin layer chromatogram of 2'-FL produced in *E. coli* by cells carrying plasmids pG180 or pG171 and induced for expression of wcfW or futC respectively.

FIG. **18** is a photograph of a thin layer chromatogram showing the kinetics and extent of 2'-FL production in a 10 L bioreactor of *E. coli* host strain E403 transformed with plasmid pG171.

FIG. **19** is a column chromatogram and a TLC analysis of the resolution on a carbon column of a sample of 2'-FL made in *E. coli* from a lactose impurity.

FIG. **20** is a photograph of a thin layer chromatogram showing 3'-SL in culture medium produced by *E. coli* strain E547, containing plasmids expressing a bacterial α(2,3) sialyltransferase and neuA, neuB and neuC.

DETAILED DESCRIPTION OF THE INVENTION

Human milk glycans, which comprise both oligosaccharides (HMOS) and their glycoconjugates, play significant roles in the protection and development of human infants, and in particular the infant gastrointestinal (GI) tract. Milk oligosaccharides found in various mammals differ greatly, and their composition in humans is unique (Hamosh M., 2001 Pediatr Clin North Am, 48:69-86; Newburg D. S., 2001 Adv Exp Med Biol, 501:3-10). Moreover, glycan levels in human milk change throughout lactation and also vary widely among individuals (Morrow A. L. et al., 2004 J Pediatr, 145:297-303; Chaturvedi P et al., 2001 Glycobiology, 11:365-372). Previously, a full exploration of the roles of HMOS was limited by the inability to adequately characterize and measure these compounds. In recent years sensitive and reproducible quantitative methods for the analysis of both neutral and acidic HMOS have been developed (Erney, R., Hilty, M., Pickering, L., Ruiz-Palacios, G., and Prieto, P. (2001) *Adv Exp Med Biol* 501, 285-297. Bao, Y., and Newburg, D. S. (2008) *Electrophoresis* 29, 2508-2515). Approximately 200 distinct oligosaccharides have been identified in human milk, and combinations of a small number of simple epitopes are responsible for this diversity (Newburg D. S., 1999 Curr_Med Chem, 6:117-127; Ninonuevo M. et al., 2006 J Agric Food Chem, 54:7471-74801). HMOS are composed of 5 monosaccharides: D-glucose (Glc), D-galactose (Gal), N-acetylglucosamine (GlcNAc), L-fucose (Fuc), and sialic acid (N-acetyl neuraminic acid, Neu5Ac, NANA). HMOS are usually divided into two groups according to their chemical structures: neutral compounds containing Glc, Gal, GlcNAc, and Fuc, linked to a lactose (Galβ1-4Glc) core, and acidic compounds including the same sugars, and often the same core structures, plus NANA (Charlwood J. et al., 1999 Anal_Biochem, 273:261-277; Martin-Sosa et al., 2003 J Dairy Sci, 86:52-59; Parkkinen J. and Finne J., 1987 Methods Enzymol, 138:289-300; Shen Z. et al., 2001 J Chromatogr A, 921:315-321). Approximately 70-80% of oligosaccharides in human milk are fucosylated, and their synthetic pathways are believed to proceed in a manner similar to those pathways shown in FIG. **1** (with the Type I and Type II subgroups beginning with different precursor molecules). A smaller proportion of the oligosaccharides in human milk are sialylated, or are both fucosylated and sialylated. FIG. **2** outlines possible biosynthetic routes for sialylated (acidic) HMOS, although their actual synthetic pathways in humans are not yet completely defined.

Interestingly, HMOS as a class, survive transit through the intestine of infants very efficiently, a function of their being poorly transported across the gut wall and of their resistance to digestion by human gut enzymes (Chaturvedi, P., Warren, C. D., Buescher, C. R., Pickering, L. K. & Newburg, D. S. Adv Exp Med Biol 501, 315-323 (2001)). One consequence of this survival in the gut is that HMOS are able to function as prebiotics, i.e. they are available to serve as an abundant carbon source for the growth of resident gut commensal microorganisms (Ward, R. E., Niñonuevo, M., Mills, D. A., Lebrilla, C. B., and German, J. B. (2007) *Mol Nutr Food Res* 51, 1398-1405). Recently, there is burgeoning interest in the role of diet and dietary prebiotic agents in determining the composition of the gut microflora, and in understanding the linkage between the gut microflora and human health (Roberfroid, M., Gibson, G. R., Hoyles, L., McCartney, A. L., Rastall, R., Rowland, I., Wolvers, D., Watzl, B., Szajewska, H., Stahl, B., Guarner, F., Respondek, F., Whelan, K., Coxam, V., Davicco, M. J., Léotoing, L., Wittrant, Y., Delzenne, N. M., Cani, P. D., Neyrinck, A. M., and Meheust, A. (2010) *Br J Nutr* 104 Suppl 2, S1-63).

A number of human milk glycans possess structural homology to cell receptors for enteropathogens, and serve roles in pathogen defense by acting as molecular receptor "decoys". For example, pathogenic strains of *Campylobacter* bind specifically to glycans in human milk containing the H-2 epitope, i.e., 2'-fucosyl-N-acetyllactosamine or 2'-fucosyllactose (2'-FL); *Campylobacter* binding and infectivity are inhibited by 2'-FL and other glycans containing this H-2 epitope (Ruiz-Palacios, G. M., Cervantes, L. E., Ramos, P., Chavez-Munguia, B., and Newburg, D. S. (2003) *J Biol Chem* 278, 14112-14120). Similarly, some diarrheagenic *E. coli* pathogens are strongly inhibited in vivo by HMOS containing 2'-linked fucose moieties. Several major strains of human caliciviruses, especially the noroviruses, also bind to 2'-linked fucosylated glycans, and this binding is inhibited by human milk 2'-linked fucosylated glycans. Consumption of human milk that has high levels of these 2'-linked fucosyloligosaccharides has been associated with lower risk of norovirus, *Campylobacter*, ST of *E. coli*-associated diarrhea, and moderate-to-severe diarrhea of all causes in a Mexican cohort of breastfeeding children (Newburg D. S. et al., 2004 Glycobiology, 14:253-263; Newburg D. S. et al., 1998 Lancet, 351:1160-1164). Several pathogens are also known to utilize sialylated glycans as their host receptors, such as influenza (Couceiro, J. N., Paulson, J. C. & Baum, L. G. Virus Res 29, 155-165 (1993)), parainfluenza (Amonsen, M., Smith, D. F., Cummings, R. D. & Air, G. M. J Virol 81, 8341-8345 (2007), and rotoviruses (Kuhlenschmidt, T. B., Hanafin, W. P., Gelberg, H. B. & Kuhlenschmidt, M. S. Adv Exp Med Biol 473, 309-317 (1999)).

The sialyl-Lewis X epitope is used by *Helicobacter pylori* (Mandavi, J., Sondén, B., Hurtig, M., Olfat, F. O., et al. Science 297, 573-578 (2002)), *Pseudomonas aeruginosa* (Scharfman, A., Delmotte, P., Beau, J., Lamblin, G., et al. Glycoconj J 17, 735-740 (2000)), and some strains of noroviruses (Rydell, G. E., Nilsson, J., Rodriguez-Diaz, J., Ruvën-Clouet, N., et al. Glycobiology 19, 309-320 (2009)).

While studies suggest that human milk glycans could be used as prebiotics and as antimicrobial anti-adhesion agents, the difficulty and expense of producing adequate quantities of these agents of a quality suitable for human consumption has limited their full-scale testing and perceived utility. What has been needed is a suitable method for producing the appropriate glycans in sufficient quantities at reasonable cost. Prior to the invention described herein, there were attempts to use several distinct synthetic approaches for glycan synthesis. Novel chemical approaches can synthesize oligosaccharides (Flowers, H. M. Methods Enzymol 50, 93-121 (1978); Seeberger, P. H. Chem Commun (Camb) 1115-1121 (2003)), but reactants for these methods are expensive and potentially toxic (Koeller, K. M. & Wong, C. H. Chem Rev 100, 4465-4494 (2000)). Enzymes expressed from engineered organisms (Albermann, C., Piepersberg, W. & Wehmeier, U. F. Carbohydr Res 334, 97-103 (2001); Bettler, E., Samain, E., Chazalet, V., Bosso, C., et al. Glycoconj J 16, 205-212 (1999); Johnson, K. F. Glycoconj J 16, 141-146 (1999); Palcic, M. M. Curr Opin Biotechnol 10, 616-624 (1999); Wymer, N. & Toone, E. J. Curr Opin Chem Biol 4, 110-119 (2000)) provide a precise and efficient synthesis (Palcic, M. M. Curr Opin Biotechnol 10, 616-624 (1999)); Crout, D. H. & Vic, G. Curr Opin Chem Biol 2, 98-111 (1998)), but the high cost of the reactants, especially the sugar nucleotides, limits their utility for low-cost, large-scale production. Microbes have been genetically engineered to express the glycosyltransferases needed to synthesize oligosaccharides from the bacteria's innate pool of nucleotide sugars (Endo, T., Koizumi, S., Tabata, K., Kakita, S. & Ozaki, A. Carbohydr Res 330, 439-443 (2001); Endo, T., Koizumi, S., Tabata, K. & Ozaki, A. Appl Microbiol Biotechnol 53, 257-261 (2000); Endo, T. & Koizumi, S. Curr Opin Struct Biol 10, 536-541 (2000); Endo, T., Koizumi, S., Tabata, K., Kakita, S. & Ozaki, A. Carbohydr Res 316, 179-183 (1999); Koizumi, S., Endo, T., Tabata, K. & Ozaki, A. Nat Biotechnol 16, 847-850 (1998)). However, low overall product yields and high process complexity have limited the commercial utility of these approaches.

Prior to the invention described herein, which enables the inexpensive production of large quantities of neutral and acidic HMOS, it had not been possible to fully investigate the ability of this class of molecule to inhibit pathogen binding, or indeed to explore their full range of potential additional functions.

Prior to the invention described herein, chemical syntheses of HMOS were possible, but were limited by stereospecificity issues, precursor availability, product impurities, and high overall cost (Flowers, H. M. Methods Enzymol 50, 93-121 (1978); Seeberger, P. H. Chem Commun (Camb) 1115-1121 (2003); Koeller, K. M. & Wong, C. H. Chem Rev 100, 4465-4494 (2000)). Also, prior to the invention described herein, in vitro enzymatic syntheses were also possible, but were limited by a requirement for expensive nucleotide-sugar precursors. The invention overcomes the shortcomings of these previous attempts by providing new strategies to inexpensively manufacture large quantities of human milk oligosaccharides for use as dietary supplements. The invention described herein makes use of an engineered bacterium *E. coli* (or other bacteria) engineered to produce 2'-FL, 3FL, LDFT, or sialylated fucosyl-oligosaccharides in commercially viable levels, for example the methods described herein enable the production of 2'-fucosylactose at >50 g/L in bioreactors.

Example 1. Engineering of *E. coli* to Generate Host Strains for the Production of Fucosylated Human Milk Oligosaccharides

The *E. coli* K12 prototroph W3110 was chosen as the parent background for fucosylated HMOS biosynthesis. This strain had previously been modified at the ampC locus by the introduction of a tryptophan-inducible P_{trpB}-cI+ repressor construct (McCoy, J. & Lavallie, E. Current protocols in molecular biology/edited by Frederick M. Ausubel . . . [et al.] (2001)), enabling economical production of recombinant proteins from the phage λ P_L promoter (Sanger, F., Coulson, A. R., Hong, G. F., Hill, D. F. & Petersen, G. B. J Mol Biol 162, 729-773 (1982)) through induction with millimolar concentrations of tryptophan (Mieschendahl, M., Petri, T. & Hänggi, U. Nature Biotechnology 4, 802-808 (1986)). The strain GI724, an *E. coli* W3110 derivative containing the tryptophan-inducible P_{trpB}-cI+ repressor construct in ampC, was used at the basis for further *E. coli* strain manipulations (FIG. **14**).

Biosynthesis of fucosylated HMOS requires the generation of an enhanced cellular pool of both lactose and GDP-fucose (FIG. **3**). This enhancement was achieved in strain GI724 through several manipulations of the chromosome using λ Red recombineering (Court, D. L., Sawitzke, J. A. & Thomason, L. C. Annu Rev Genet 36, 361-388 (2002)) and generalized P1 phage transduction (Thomason, L. C., Costantino, N. & Court, D. L. Mol Biol Chapter 1, Unit 1.17 (2007)). FIG. **14** is a table presenting the genotypes of several *E. coli* strains constructed for this invention. The ability of the *E. coli* host strain to accumulate intracellular lactose was first engineered in strain E183 (FIG. **14**) by simultaneous deletion of the endogenous β-galactosidase gene (lacZ) and the lactose operon repressor gene (lacI). During construction of this deletion in GI724 to produce E183, the lacIq promoter was placed immediately upstream of the lactose permease gene, lacY. The modified strain thus maintains its ability to transport lactose from the culture medium (via LacY), but is deleted for the wild-type copy of the lacZ (β-galactosidase) gene responsible for lactose catabolism. An intracellular lactose pool is therefore created when the modified strain is cultured in the presence of exogenous lactose.

Subsequently, the ability of the host *E. coli* strain to synthesize colanic acid, an extracellular capsular polysaccharide, was eliminated in strain E205 (FIG. **14**) by the deletion of the wcaJ gene, encoding the UDP-glucose lipid carrier transferase (Stevenson, G., Andrianopoulos, K., Hobbs, M. & Reeves, P. R. J Bacteriol 178, 4885-4893 (1996)) in strain E183. In a wcaJ null background, GDP-fucose accumulates in the *E. coli* cytoplasm (Dumon, C., Priem, B., Martin, S. L., Heyraud, A., et al. Glycoconj J 18, 465-474 (2001)).

A thyA (thymidylate synthase) mutation was introduced into strain E205 to produce strain E214 (FIG. **14**) by P1 transduction. In the absence of exogenous thymidine, thyA strains are unable to make DNA, and die. The defect can be complemented in trans by supplying a wild-type thyA gene on a multicopy plasmid (Belfort, M., Maley, G. F. & Maley, F. Proceedings of the National Academy of Sciences 80, 1858 (1983)). This complementation is used herein as a

means of plasmid maintenance (eliminating the need for a more conventional antibiotic selection scheme to maintain plasmid copy number).

One strategy for GDP-fucose production is to enhance the bacterial cell's natural synthesis capacity. For example, this is enhancement is accomplished by inactivating enzymes involved in GDP-fucose consumption, and/or by overexpressing a positive regulator protein, RcsA, in the colanic acid (a fucose-containing exopolysaccharide) synthesis pathway. Collectively, this metabolic engineering strategy re-directs the flux of GDP-fucose destined for colanic acid synthesis to oligosaccharide synthesis (FIG. **3**). By "GDP-fucose synthesis pathway" is meant a sequence of reactions, usually controlled and catalyzed by enzymes, which results in the synthesis of GDP-fucose. An exemplary GDP-fucose synthesis pathway in *Escherichia coli* as described in FIG. **3** is set forth below. In the GDP-fucose synthesis pathway set forth below, the enzymes for GDP-fucose synthesis include: 1) manA=phosphomannose isomerase (PMI), 2) manB=phosphomannomutase (PMM), 3) manC=mannose-1-phosphate guanylyltransferase (GMP), 4) gmd=GDP-mannose-4,6-dehydratase (GMD), 5) fcl=GDP-fucose synthase (GFS), and 6) ΔwcaJ=mutated UDP-glucose lipid carrier transferase.

Glucose→Glc-6-P→Fru-6-P→[1] Man-6-P→[2] Man-1-P→[3] GDP-Man→[4,5] GDP-Fuc⇢[6] Colanic acid.

Specifically, the magnitude of the cytoplasmic GDP-fucose pool in strain E214 is enhanced by over-expressing the *E. coli* positive transcriptional regulator of colanic acid biosynthesis, RscA (Gottesman, S. & Stout, V. Mol Microbiol 5, 1599-1606 (1991)). This over-expression of RcsA is achieved by incorporating a wild-type rcsA gene, including its promoter region, onto a multicopy plasmid vector and transforming the vector into the *E. coli* host, e.g. into E214. This vector typically also carries additional genes, in particular one or two fucosyltransferase genes under the control of the pL promoter, and thyA and beta-lactamase genes for plasmid selection and maintenance. pG175 (SEQ ID NO: 1 and FIG. **7**), pG176 (SEQ ID NO: 2), pG177 (SEQ ID NO: 3 and FIG. **10**), pG171 (SEQ ID NO: 5) and pG180 (SEQ ID NO: 6) are all examples of fucosyltransferase-expressing vectors that each also carry a copy of the rcsA gene, for the purpose of increasing the intracellular GDP-fucose pool of the *E. coli* hosts transformed with these plasmids. Over-expression of an additional positive regulator of colanic acid biosynthesis, namely RcsB (Gupte G, Woodward C, Stout V. Isolation and characterization of rcsb mutations that affect colanic acid capsule synthesis in *Escherichia coli* K-12. J Bacteriol 1997, July; 179(13):4328-35.), can also be utilized, either instead of or in addition to over-expression of RcsA, to increase intracellular GDP-fucose levels. Over-expression of rcsB is also achieved by including the gene on a multi-copy expression vector. pG186 is such a vector (SEQ ID NO: 8 and FIG. **15**). pG186 expresses rcsB in an operon with futC under pL promoter control. The plasmid also expresses rcsA, driven off its own promoter. pG186 is a derivative of pG175 in which the α(1,2) FT (wbsJ) sequence is replaced by the *H. pylori* futC gene (FutC is MYC-tagged at its C-terminus). In addition, at the XhoI restriction site immediately 3' of the futC CDS, the *E. coli* rcsB gene is inserted, complete with a ribosome binding site at the 5'end of the rcsB CDS, and such that futC and rcsB form an operon.

A third means to increase the intracellular GDP-fucose pool may also be employed. Colanic acid biosynthesis is increased following the introduction of a null mutation into the *E. coli* lon gene. Lon is an ATP-dependant intracellular protease that is responsible for degrading RcsA, mentioned above as a positive transcriptional regulator of colanic acid biosynthesis in *E. coli* (Gottesman, S. & Stout, V. Mol Microbiol 5, 1599-1606 (1991)). In a ion null background, RcsA is stabilized, RcsA levels increase, the genes responsible for GDP-fucose synthesis in *E. coli* are up-regulated, and intracellular GDP-fucose concentrations are enhanced. The lon gene was almost entirely deleted and replaced by an inserted functional, wild-type, but promoter-less *E. coli* $lacZ^+$ gene (Δlon::(kan, $lacZ^+$) in strain E214 to produce strain E390. λ Red recombineering was used to perform the construction. FIG. **12** illustrates the new configuration of genes engineered at the lon locus in E390. FIG. **13**A-E presents the complete DNA sequence of the region, with annotations in GenBank format. Genomic DNA sequence surrounding the lacZ+ insertion into the lon region in *E. coli* strain E390 is set forth below (SEQ ID NO: 7)

The lon mutation in E390 increases intracellular levels of RcsA, and enhances the intracellular GDP-fucose pool. The inserted $lacZ^+$ cassette not only knocks out lon, but also converts the $lacZ^-$ host back to both a $lacZ^+$ genotype and phenotype. The modified strain produces a minimal (albeit still readily detectable) level of β-galactosidase activity (1-2 units), which has very little impact on lactose consumption during production runs, but which is useful in removing residual lactose at the end of runs, is an easily scorable phenotypic marker for moving the lon mutation into other $lacZ^-$ *E. coli* strains by P1 transduction, and can be used as a convenient test for cell lysis (e.g. caused by unwanted bacteriophage contamination) during production runs in the bioreactor.

The production host strain, E390 incorporates all the above genetic modifications and has the following genotype: ampC::(P_{trpB} λcI^+), P_{lacI^q}($\Delta lacI\text{-}lacZ)_{158}$$lacY^+$, ΔwcaJ, $thyA_{748}$::Tn10, Δlon::(kan, $lacZ^+$)

An additional modification of E390 that is useful for increasing the cytoplasmic pool of free lactose (and hence the final yield of 2'-FL) is the incorporation of a lacA mutation. LacA is a lactose acetyltransferase that is only active when high levels of lactose accumulate in the *E. coli* cytoplasm. High intracellular osmolarity (e.g., caused by a high intracellular lactose pool) can inhibit bacterial growth, and *E. coli* has evolved a mechanism for protecting itself from high intra cellular osmolarity caused by lactose by "tagging" excess intracellular lactose with an acetyl group using LacA, and then actively expelling the acetyl-lactose from the cell (Danchin, A. Bioessays 31, 769-773 (2009)). Production of acetyl-lactose in *E. coli* engineered to produce 2'-FL or other human milk oligosaccharides is therefore undesirable: it reduces overall yield. Moreover, acetyl-lactose is a side product that complicates oligosaccharide purification schemes. The incorporation of a lacA mutation resolves these problems. Strain E403 (FIG. **14**) is a derivative of E390 that carries a deletion of the lacA gene and thus is incapable of synthesizing acetyl-lactose.

The production host strain, E403 incorporates all the above genetic modifications and has the following genotype: ampC::($P_{trpB}$$\lambda cI^+$), P_{lacI^q}($\Delta lacI\text{-}lacZ)_{158}$$lacY^+$, ΔwcaJ, $thyA_{748}$::Tn10, Δlon::(kan, $lacZ^+$)ΔlacA

Example 2. 2'-FL Production at Small Scale

Various alternative α(1,2) fucosyltransferases are able to utilize lactose as a sugar acceptor and are available for the purpose of 2'-FL synthesis when expressed under appropriate culture conditions in *E. coli* E214, E390 or E403. For

example the plasmid pG175 (ColE1, thyA+, bla+, P_{L2}-wbsJ, rcsA+) (SEQ ID NO: 1, FIG. **7**) carries the wbsJ α(1,2) fucosyltransferase gene of *E. coli* strain O128:B12 and can direct the production of 2'-FL in. *E. coli* strain E403. In another example plasmid pG171 (ColE1, thyA+, bla+, P_{L2}-futC, rcsA+) (SEQ ID NO: 5), carries the *H. pylori* 26695 futC α(1,2)fucosyltransferase gene (Wang, G., Rasko, D. A., Sherburne, R. & Taylor, D. E. Mol Microbiol 31, 1265-1274 (1999)) and will also direct the production of 2'-FL in strain E403. In a preferred example, the plasmid pG180 (ColE1, thyA+, bla+, P_{L2}-wcfW, rcsA+) (SEQ ID NO: 6) carries the previously uncharacterized *Bacteroides fragilis* NCTC 9343 wcfW α(1,2)fucosyltransferase gene of the current invention and directs the production of 2'-FL in *E. coli* strain E403.

The addition of tryptophan to the lactose-containing growth medium of cultures of any one of the strains E214, E390 or E403, when transformed with any one of the plasmids pG171, pG175 or pG180 leads, for each particular strain/plasmid combination, to activation of the host *E. coli* tryptophan utilization repressor TrpR, subsequent repression of P_{trpB}, and a consequent decrease in cytoplasmic cI levels, which results in a de-repression of P_L, expression of futC, wbsJ or wcfW, respectively, and production of 2'-FL. FIG. **8** is a coomassie blue-stained SDS PAGE gel of lysates of *E. coli* containing pG175 and expressing wbsJ, and of cells containing pG171 and expressing futC. Prominent stained protein bands running at a molecular weight of approximately 35 kDa are seen for both WbsJ and FutC at 4 and 6 h following P_L induction (i.e., after addition of tryptophan). FIG. **16** is a coomassie blue-stained SDS PAGE gel of lysates of *E. coli* containing pG180 and expressing wcfW, and of cells containing pG171 and expressing *H. pylori* futC. Prominent stained bands for both WcfW and FutC are seen at a molecular weight of approximately 40 kDa at 4 and 6 h following P_L induction (i.e., after addition of tryptophan to the growth medium). For 2'-FL production in small scale laboratory cultures (<100 ml) strains were grown at 30 C in a selective medium lacking both thymidine and tryptophan to early exponential phase (e.g. M9 salts, 0.5% glucose, 0.4% casaminoacids). Lactose was then added to a final concentration of 0.5 or 1%, along with tryptophan (200 μM final) to induce expression of the α(1,2) fucosyltransferase, driven from the P_L promoter. At the end of the induction period (~24 h) TLC analysis was performed on aliquots of cell-free culture medium, or of heat extracts of cells (treatments at 98 C for 10 min, to release sugars contained within the cell). FIG. **11** shows a TLC analysis of cytoplasmic extracts of engineered *E. coli* cells transformed with pG175 or pG171. Cells were induced to express wbsJ or futC, respectively, and grown in the presence of lactose. The production of 2'-FL can clearly be seen in heat extracts of cells carrying either plasmid. FIG. **17** shows a TLC analysis of cytoplasmic extracts of engineered *E. coli* cells transformed with pG180 or pG171. Cells were induced to express wcfW or futC, respectively, and grown in the presence of lactose. The production of 2'-FL can clearly be seen with both plasmids. Prior to the present invention the wcfW gene had never been shown to encode a protein with demonstrated α(1,2) fucosyltransferase activity, or to utilize lactose as a sugar acceptor substrate.

The DNA sequence of the *Bacteroides fragilis* strain NCTC 9343 wcfW gene (protein coding sequence) is set forth below (SEQ ID NO: 4).

Example 3. 2'-FL Production in the Bioreactor

2'-FL can be produced in the bioreactor by any one of the host *E. coli* strains E214, E390 or E403, when transformed with any one of the plasmids pG171, pG175 or pG180. Growth of the transformed strain is performed in a minimal medium in a bioreactor, 10 L working volume, with control of dissolved oxygen, pH, lactose substrate, antifoam and nutrient levels. Minimal "FERM" medium is used in the bioreactor, which is detailed below.

Ferm (10 liters): Minimal medium comprising:

- 40 g $(NH_4)_2HPO_4$
- 100 g KH_2PO_4
- 10 g $MgSO_4.7H_2O$
- 40 g NaOH
- Trace Elements:
- 1.3 g NTA
- 0.5 g $FeSO_4.7H_2O$
- 0.09 g $MnCl_2.4H_2O$
- 0.09 g $ZnSO_4.7H_2O$
- 0.01 g $CoCl_2.6H_2O$
- 0.01 g $CuCl_2.2H_2O$
- 0.02 g H_3BO_3
- 0.01 g $Na_2MoO_4.2H_2O$ (pH 6.8)
- Water to 10 liters
- DF204 antifoam (0.1 ml/L)
- 150 g glycerol (initial batch growth), followed by fed batch mode with a 90% glycerol-1% $MgSO_4$-1× trace elements feed, at various rates for various times.

Production cell densities of A_{600}>100 are routinely achieved in these bioreactor runs. Briefly, a small bacterial culture is grown overnight in "FERM"—in the absence of either antibiotic or exogenous thymidine. The overnight culture (@~2 A_{600}) is used to inoculate a bioreactor (10 L working volume, containing "FERM") to an initial cell density of ~0.2 A_{600}. Biomass is built up in batch mode at 30° C. until the glycerol is exhausted (A_{600}~20), and then a fed batch phase is initiated utilizing glycerol as the limiting carbon source. At A_{600}~30, 0.2 g/L tryptophan is added to induce α(1,2) fucosyltransferase synthesis. An initial bolus of lactose is also added at this time. 5 hr later, a continuous slow feed of lactose is started in parallel to the glycerol feed. These conditions are continued for 48 hr (2'-FL production phase). At the end of this period, both the lactose and glycerol feeds are terminated, and the residual glycerol and lactose are consumed over a final fermentation period, prior to harvest. 2'-FL accumulates in the spent fermentation medium at concentrations as much as 30 times higher than in the cytoplasm. The specific yield in the spent medium varies between 10 and 50 g/L, depending on precise growth and induction conditions. FIG. **18** is a TLC of culture medium samples removed from a bioreactor at various times during a 2'-FL production run utilizing plasmid pG171 transformed into strain E403. All of the input lactose was converted to product by the end of the run, and product yield was approximately 25 g/L 2'-FL.

Example 4. 2'-Fucosyllactose Purification

2'-FL purification from *E. coli* fermentation broth is accomplished though five steps:

1. Clarification

Fermentation broth is harvested and cells removed by sedimentation in a preparative centrifuge at 6000×g for 30 min. Each bioreactor run yields about 5-7 L of partially clarified supernatant. Clarified supernatants have a brown/orange coloration attributed to a fraction of caramelized sugars produced during the course of the fermentation, particularly by side-reactions promoted by the ammonium ions present in the fermentation medium.

2. Product Capture on Coarse Carbon

A column packed with coarse carbon (Calgon 12×40 TR) of ~1000 ml volume (dimension 5 cm diameter×60 cm length) is equilibrated with 1 column volume (CV) of water and loaded with clarified culture supernatant at a flow rate of 40 ml/min. This column has a total capacity of about 120 g of sugar (lactose). Following loading and sugar capture, the column is washed with 1.5 CV of water, then eluted with 2.5 CV of 50% ethanol or 25% isopropanol (lower concentrations of ethanol at this step (25-30%) may be sufficient for product elution). This solvent elution step releases about 95% of the total bound sugars on the column and a small portion of the color bodies (caramels). In this first step capture of the maximal amount of sugar is the primary objective. Resolution of contaminants is not an objective. The column can be regenerated with a 5 CV wash with water.

3. Evaporation

A volume of 2.5 L of ethanol or isopropanol eluate from the capture column is rotary-evaporated at 56 C and a sugar syrup in water is generated (this typically is a yellow-brown color). Alternative methods that could be used for this step include lyophilization or spray-drying.

4. Flash Chromatography on Fine Carbon and Ion Exchange Media

A column (GE Healthcare HiScale50/40, 5×40 cm, max pressure 20 bar) connected to a Biotage Isolera One FLASH Chromatography System is packed with 750 ml of a Darco Activated Carbon G60 (100-mesh): Celite 535 (coarse) 1:1 mixture (both column packings obtained from Sigma). The column is equilibrated with 5 CV of water and loaded with sugar from step 3 (10-50 g, depending on the ratio of 2'-FL to contaminating lactose), using either a celite loading cartridge or direct injection. The column is connected to an evaporative light scattering (ELSD) detector to detect peaks of eluting sugars during the chromatography. A four-step gradient of isopropanol, ethanol or methanol is run in order to separate 2'-FL from monosaccharides (if present), lactose and color bodies. e.g., for B=ethanol: Step 1, 2.5 CV 0% B; Step 2, 4 CV 10% B (elutes monosaccharides and lactose contaminants); step 3, 4 CV 25% B (Elutes 2'-FL); step 4, 5 CV 50% B (elutes some of the color bodies and partially regenerates the column). Additional column regeneration is achieved using methanol @ 50% and isopropanol @ 50%. Fractions corresponding to sugar peaks are collected automatically in 120-ml bottles, pooled and directed to step 5. In certain purification runs from longer-than-normal fermentations, passage of the 2'-FL-containing fraction through anion-exchange and cation exchange columns can remove excess protein/DNA/caramel body contaminants. Resins tested successfully for this purpose are Dowex 22 and Toyopearl Mono-Q, for the anion exchanger, and Dowex 88 for the cation exchanger. Mixed bed Dowex resins have proved unsuitable as they tend to adsorb sugars at high affinity via hydrophobic interactions. FIG. **19** illustrates the performance of Darco G60:celite 1:1 in separating lactose from 2'-fucosyllactose when used in Flash chromatography mode.

5. Evaporation/Lyophilization

3.0 L of 25% B solvent fractions is rotary-evaporated at 56 C until dry. Clumps of solid sugar are re-dissolved in a minimum amount of water, the solution frozen, and then lyophilized. A white, crystalline, sweet powder (2'-FL) is obtained at the end of the process. 2'-FL purity obtained lies between 95 and 99%.

Sugars are routinely analyzed for purity by spotting 1 μl aliquots on aluminum-backed silica G60 Thin Layer Chromatography plates (10×20 cm; Macherey-Nagel). A mixture of LDFT (Rf=0.18), 2'-FL (Rf=0.24), lactose (Rf=0.30), trehalose (Rf=0.32), acetyl-lactose (Rf=0.39) and fucose (Rf=0.48) (5 g/L concentration for each sugar) is run alongside as standards. The plates are developed in a 50% butanol:25% acetic acid:25% water solvent until the front is within 1 cm from the top. Improved sugar resolution can be obtained by performing two sequential runs, drying the plate between runs. Sugar spots are visualized by spraying with α-naphthol in a sulfuric acid-ethanol solution (2.4 g α-naphthol in 83% (v/v) ethanol, 10.5% (v/v) sulfuric acid) and heating at 120 C for a few minutes. High molecular weight contaminants (DNA, protein, caramels) remain at the origin, or form smears with Rfs lower than LDFT.

Example 5. 3FL Production

Any one of *E. coli* host strains E214, E390 or E403, when transformed with a plasmid expressing an α(1,3)fucosyltransferase capable of using lactose as the sugar acceptor substrate, will produce the human milk oligosaccharide product, 3-fucosyllactose (3FL). FIG. **9** illustrates the pathways utilized in engineered strains of *E. coli* of this invention to achieve production of 3FL. For example, the plasmid pG176 (ColE1, thyA+, bla+, P_{L2}-futA, rcsA+) (SEQ ID NO: 2), is a derivative of pG175 in which the α(1,2) FT (wbsJ) sequence is replaced by the *Helicobacter pylori* futA gene (Dumon, C., Bosso, C., Utille, J. P., Heyraud, A. & Samain, E. Chembiochem 7, 359-365 (2006)). pG176 will direct the production of 3FL when transformed into any one of the host *E. coli* strains E214, E390 or E403. FIG. **11** shows a TLC analysis of 3FL production from E403 transformed with pG176. Additionally there are several other related bacterial-type α(1,3)-fucosyltransferases identified in *Helicobacter pylori* which could be used to direct synthesis of 3FL, e.g., "11639 FucTa" (Ge, Z., Chan, N. W., Palcic, M. M. & Taylor, D. E. J Biol Chem 272, 21357-21363 (1997); Martin, S. L., Edbrooke, M. R., Hodgman, T. C., van den Eijnden, D. H. & Bird, M. I. J Biol Chem 272, 21349-21356 (1997)) and "UA948 FucTa" (Rasko, D. A., Wang, G., Palcic, M. M. & Taylor, D. E. J Biol Chem 275, 4988-4994 (2000)). In addition to α(1,3)-fucosyltransferases from *H. pylori*, an α(1,3)fucosyltransferase (Hh0072, sequence accession AAP76669) isolated from *Helicobacter hepaticus* exhibits activity towards both non-sialylated and sialylated Type 2 oligosaccharide acceptor substrates (Zhang, L., Lau, K., Cheng, J., Yu, H., et al. Glycobiology (2010)). Furthermore, there are several additional bacterial α(1,3)-fucosyltransferases that may be used to make 3FL according to the methods of this invention. For example, close homologs of Hh0072 are found in H. *H. bilis* (HRAG_01092 gene, sequence accession EEO24035), and in *C. jejuni* (C1336_000250319 gene, sequence accession EFC31050).

3FL biosynthesis is performed as described above for 2'-FL, either at small scale in culture tubes and culture flasks, or in a bioreactor (10 L working volume) utilizing control of dissolved oxygen, pH, lactose substrate, antifoam and carbon:nitrogen balance. Cell densities of A_{600}~100 are reached in the bioreacter, and specific 3FL yields of up to 3 g/L have been achieved. Approximately half of the 3FL produced is found in the culture supernatant, and half inside the cells. Purification of 3FL from *E. coli* culture supernatants is achieved using an almost identical procedure to that described above for 2'-FL. The only substantive difference

being that 3FL elutes from carbon columns at lower alcohol concentrations than does 2'-FL.

Example 6. The Simultaneous Production of Human Milk Oligosaccharides 2'-Fucosyllactose (2'-FL), 3-Fucosyllactose (3FL), and Lactodifucohexaose (LDFT) in *E. coli*

E. coli strains E214, E390 and E403 accumulate cytoplasmic pools of both lactose and GDP-fucose, as discussed above, and when transformed with plasmids expressing either an α(1,2) fucosyltransferase or an α(1,3) fucosyltransferase can synthesize the human milk oligosaccharides 2'-FL or 3FL respectively. The tetrasaccharide lactodifucotetraose (LDFT) is another major fucosylated oligosaccharide found in human milk, and contains both α(1,2)- and α(1,3)-linked fucose residues. pG177 (FIG. **10**, SEQ ID NO: 3) is a derivative of pG175 in which the wbsJ gene is replaced by a two gene operon comprising the *Helicobacter pylori* futA gene and the *Helicobacter pylori* futC gene (i.e., an operon containing both an α(1,3)- and α(1,2)-fucosyltransferase). *E. coli* strains E214, E390 and E403 produce LDFT when transformed with plasmid pG177 and grown, either in small scale or in the bioreactor, as described above. In FIG. **11** (lanes pG177), LDFT made in *E. coli*, directed by pG177, was observed on analysis of cell extracts by thin layer chromatography.

Example 7. 3'-SL Synthesis in the *E. coli* Cytoplasm

The first step in the production of 3'-sialyllactose (3'-SL) in *E. coli* is generation of a host background strain that accumulates cytoplasmic pools of both lactose and CMP-Neu5Ac (CMP-sialic acid). Accumulation of cytoplasmic lactose is achieved through growth on lactose and inactivation of the endogenous *E. coli* β-galactosidase gene (lacZ), being careful to minimize polarity effects on lacY, the lac permease. This accumulation of a lactose pool has already been accomplished and is described above in *E. coli* hosts engineered for 2'-FL, 3FL and LDFT production.

Specifically, a scheme to generate a cytoplasmic CMP-Neu5Ac pool, modified from methods known in the art, (e.g., Ringenberg, M., Lichtensteiger, C. & Vimr, E. Glycobiology 11, 533-539 (2001); Fierfort, N. & Samain, E. J Biotechnol 134, 261-265 (2008)), is shown in FIG. **5**. Under this scheme, the *E. coli* K12 sialic acid catabolic pathway is first ablated through introduction of null mutations in endogenous nanA (N-acetylneuraminate lyase) and nanK (N-acetylmannosamine kinase) genes. By "sialic acid catabolic pathway" is meant a sequence of reactions, usually controlled and catalyzed by enzymes, which results in the degradation of sialic acid. An exemplary sialic acid catabolic pathway in *Escherichia coli* is set forth in FIG. **5**. In the sialic acid catabolic pathway in FIG. **5**, sialic acid (Neu5Ac; N-acetylneuraminic acid) is degraded by the enzymes NanA (N-acetylneuraminic acid lyase) and NanK (N-acetylmannosamine kinase). Other abbreviations for the sialic acid catabolic pathway in FIG. **5** include: (nanT) sialic acid transporter; (ΔnanA) mutated N-acetylneuraminic acid lyase; (ΔnanK) mutated N-acetylmannosamine kinase; (ManNAc-6-P) N-acetylmannosamine-6-phosphate; (GlcNAc-6-P) N-acetylglucosamine-6-phosphate; (GlcN-6-P) Glucosamine-6-phosphate; (Fruc-6-P) Fructose-6-phosphate; (neuA), CMP-N-acetylneuraminic acid synthetase; (CMP-Neu5Ac) CMP-N-acetylneuraminic acid; and (neuB), N-acetylneuraminic acid synthase.

Next, since *E. coli* K12 lacks a de novo sialic acid synthesis pathway, sialic acid synthetic capability is introduced through the provision of three recombinant enzymes; a UDP-GlcNAc 2-epimerase (e.g., neuC), a Neu5Ac synthase (e.g., neuB) and a CMP-Neu5Ac synthetase (e.g., neuA). Equivalent genes from *C. jejuni, E. coli* K1, *H. influenzae* or from *N. meningitides* can be utilized (interchangeably) for this purpose.

The addition of sialic acid to the 3' position of lactose to generate 3'-sialyllactose is then achieved utilizing a bacterial-type α(2,3)sialyltransferase, and numerous candidate genes have been described, including those from *N. meningitidis* and *N. gonorrhoeae* (Gilbert, M., Watson, D. C., Cunningham, A. M., Jennings, M. P., et al. J Biol Chem 271, 28271-28276 (1996); Gilbert, M., Cunningham, A. M., Watson, D. C., Martin, A., et al. Eur J Biochem 249, 187-194 (1997)). The *Neisseria* enzymes are already known to use lactose as an acceptor sugar. The recombinant *N. meningitidis* enzyme generates 3'-sialyllactose in engineered *E. coli* (Fierfort, N. & Samain, E. J Biotechnol 134, 261-265 (2008)). FIG. **20** shows a TLC analysis of culture media taken from a culture of *E. coli* strain E547 (ampC::($P_{trp\beta}$λcI$^+$), P_{lacI^q}(ΔlacI-lacZ)$_{158}$lacY$^+$, ΔlacA, Δnan) and carrying plasmids expressing neuA,B,C and a bacterial-type α(2,3)sialyltransferase. The presence of 3'-sialylactose (3'-SL) in the culture media is clearly seen.

Example 8. The Production of Human Milk Oligosaccharide 3'-Sialyl-3-Fucosyllactose (3'-S3FL) in *E. coli*

Prior to the invention described herein, it was unpredictable that a combination of any particular fucosyltransferase gene and any particular sialyl-transferase gene in the same bacterial strain could produce 3'-S3FL. Described below are results demonstrating that the combination of a fucosyltransferase gene and a sialyl-transferase gene in the same LacZ$^+$ *E. coli* strain resulted in the production of 3'-S3FL. These unexpected results are likely due to the surprisingly relaxed substrate specificity of the particular fucosyltransferase and sialyl-transferase enzymes utilized.

Humans synthesize the sialyl-Lewis X epitope utilizing different combinations of six α(1,3)fucosyl- and six α(2,3) sialyl-transferases encoded in the human genome (de Vries, T., Knegtel, R. M., Holmes, E. H. & Macher, B. A. Glycobiology 11, 119R-128R (2001); Taniguchi, A. Curr Drug Targets 9, 310-316 (2008)). These sugar transferases differ not only in their tissue expression patterns, but also in their acceptor specificities. For example, human myeloid-type α(1,3) fucosyltransferase (FUT IV) will fucosylate Type 2 (Galβ1→4Glc/GlcNAc) chain-based acceptors, but only if they are non-sialylated. In contrast "plasma-type" α(1,3) fucosyltransferase (FUT VI) will utilize Type 2 acceptors whether or not they are sialylated, and the promiscuous "Lewis" α(1,3/4) fucosyltransferase (FUT III), found in breast and kidney, will act on sialylated and non-sialylated Type 1 (Galβ1→3GlcNAc) and Type 2 acceptors (Easton, E. W., Schiphorst, W. E., van Drunen, E., van der Schoot, C. E. & van den Eijnden, D. H. Blood 81, 2978-2986 (1993)). A similar situation exists for the family of human□ α(2,3) sialyl-transferases, with different enzymes exhibiting major differences in acceptor specificity (Legaigneur, P., Breton, C., El Battari, A., Guillemot, J. C., et al. J Biol Chem 276, 21608-21617 (2001); Jeanneau, C., Chazalet, V., Augé, C., Soumpasis, D. M., et al. J Biol Chem 279, 13461-13468 (2004)). This diversity in acceptor specificity highlights a key issue in the synthesis of 3'-sialyl-3-fucosyllactose (3'-

S3FL) in *E. coli*, i.e., to identify a suitable combination of fucosyl- and sialyl-transferases capable of acting cooperatively to synthesize 3'-S3FL (utilizing lactose as the initial acceptor sugar). However, since human and all other eukaryotic fucosyl- and sialyl-transferases are secreted proteins located in the lumen of the golgi, they are poorly suited for the task of 3'-S3FL biosynthesis in the bacterial cytoplasm.

Several bacterial pathogens are known to incorporate fucosylated and/or sialylated sugars into their cell envelopes, typically for reasons of host mimicry and immune evasion. For example; both *Neisseria meningitides* and *Campylobacter jejuni* are able to incorporate sialic acid through 2,3-linkages to galactose moieties in their capsular lipooligosaccharide (LOS) (Tsai, C. M., Kao, G. & Zhu, P. I Infection and Immunity 70, 407 (2002); Gilbert, M., Brisson, J. R., Karwaski, M. F., Michniewicz, J., et al. J Biol Chem 275, 3896-3906 (2000)), and some strains of *E. coli* incorporate α(1,2) fucose groups into lipopolysaccharide (LPS) (Li, M., Liu, X. W., Shao, J., Shen, J., et al. Biochemistry 47, 378-387 (2008); Li, M., Shen, J., Liu, X., Shao, J., et al. Biochemistry 47, 11590-11597 (2008)). Certain strains of *Helicobacter pylori* are able not only to incorporate α(2,3)-sialyl- groups, but also α(1,2)-, α(1,3)-, and α(1,4)-fucosyl- groups into LPS, and thus can display a broad range of human Lewis-type epitopes on their cell surface (Moran, A. P. Carbohydr Res 343, 1952-1965 (2008)). Most bacterial sialyl- and fucosyl-transferases operate in the cytoplasm, i.e., they are better suited to the methods described herein than are eukaryotic golgi-localized sugar transferases.

Strains of *E. coli* engineered to express the transferases described above accumulate a cytoplasmic pool of lactose, as well as an additional pool of either the nucleotide sugar GDP-fucose, or the nucleotide sugar CMP-Neu5Ac (CMP-sialic acid). Addition of these sugars to the lactose acceptor is performed in these engineered hosts using candidate recombinant α(1,3)-fucosyl- or α(2,3)-sialyl-transferases, generating 3-fucosyllactose and 3'-sialyllactose respectively. Finally, the two synthetic capabilities are combined into a single *E. coli* strain to produce 3'-S3FL.

An *E. coli* strain that accumulates cytoplasmic pools of both lactose and GDP-fucose has been developed. This strain, when transformed with a plasmid over-expressing an α(1,2)fucosyltransferase, produces 2'-fucosyllactose (2'-FL) at levels of ~10-50 g/L of bacterial culture medium. A substitution of the α(1,2) fucosyltransferase in this host with an appropriate α(1,3) fucosyltransferase leads to the production of 3-fucosyllactose (3FL). The bacterial α(1,3) fucosyltransferase then works in conjunction with a bacterial α(2,3)sialyltransferase to make the desired product, 3'-S3FL.

An α(1,3)fucosyltransferase (Hh0072) isolated from *Helicobacter hepaticus* exhibits activity towards both non-sialylated and sialylated Type 2 oligosaccharide acceptor substrates (Zhang, L., Lau, K., Cheng, J., Yu, H., et al. Glycobiology (2010)). This enzyme is cloned, expressed, and evaluated to measure utilization of a lactose acceptor and to evaluate production of 3FL in the context of the current GDP-fucose-producing *E. coli* host. Hh0072 is also tested in concert with various bacterial α(2,3)sialyltransferases for its competence in 3'-S3FL synthesis. As alternatives to Hh0072, there are two characterized homologous bacterial-type 3-fucosyltransferases identified in *Helicobacter pylori*, "11639 FucTa" (Ge, Z., Chan, N. W., Palcic, M. M. & Taylor, D. E. J Biol Chem 272, 21357-21363 (1997); Martin, S. L., Edbrooke, M. R., Hodgman, T. C., van den Eijnden, D. H. & Bird, M. I. J Biol Chem 272, 21349-21356 (1997)) and "UA948 FucTa" (Rasko, D. A., Wang, G., Palcic, M. M. & Taylor, D. E. J Biol Chem 275, 4988-4994 (2000)). These two paralogs exhibit differing acceptor specificities, "11639 FucTa" utilizes only Type 2 acceptors and is a strict α(1,3)-fucosyltransferase, whereas "UA948 FucTa" has relaxed acceptor specificity (utilizing both Type1 and Type 2 acceptors) and is able to generate both α(1,3)- and α(1,4)-fucosyl linkages. The precise molecular basis of this difference in specificity was determined (Ma, B., Lau, L. H., Palcic, M. M., Hazes, B. & Taylor, D. E. J Biol Chem 280, 36848-36856 (2005)), and characterization of several additional α(1,3)-fucosyltransferase paralogs from a variety of additional *H. pylori* strains revealed significant strain-to-strain acceptor specificity diversity.

In addition to the enzymes from *H. pylori* and *H. hepaticus*, other bacterial α(1,3)-fucosyltransferases are optionally used. For example, close homologs of Hh0072 are found in *H. bilis* (HRAG_01092 gene, sequence accession EEO24035), and in *C. jejuni* (C1336_000250319 gene, sequence accession EFC31050).

Described below is 3'-S3FL synthesis in *E. coli*. The first step towards this is to combine into a single *E. coli* strain the 3-fucosyllactose synthetic ability, outlined above, with the ability to make 3'-sialyllactose, also outlined above. All of the chromosomal genetic modifications discussed above are introduced into a new host strain, which will then simultaneously accumulate cytoplasmic pools of the 3 specific precursors; lactose, GDP-fucose and CMP-Neu5Ac. This "combined" strain background is then used to host simultaneous production of an α(1,3)fucosyltransferase with an α(2,3)sialyltransferase, with gene expression driven either off two compatible multicopy plasmids or with both enzyme genes positioned on the same plasmid as an artificial operon. Acceptor specificities for some of the bacterial α(1,3)fucosyltransferases and α(2,3)sialyltransferases, particularly with respect to fucosylation of 3'-sialyllactose and sialylation of 3-fucosyllactose and different combinations of α(1,3)fucosyltransferase and α(2,3)sialyltransferase enzymes are evaluated. Production levels and ratios of 3'-SL, 3FL and 3'-S3FL are monitored, e.g., by TLC, with confirmation of identity by NMR and accurate quantitation either by calibrated mass spectrometry utilizing specific ion monitoring, or by capillary electrophoresis (Bao, Y., Zhu, L. & Newburg, D. S. Simultaneous quantification of sialyloligosaccharides from human milk by capillary electrophoresis. Anal Biochem 370, 206-214 (2007)).

The sequences corresponding to the SEQ ID NOs described herein are provided below. The sequence of PG175 is set forth below (SEQ ID NO: 1):

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TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCG
GAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCG
TCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATG
CGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATATGCGGTGTGAAA
TACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCTCCTCAAC
CTGTATATTCGTAAACCACGCCCAATGGGAGCTGTCTCAGGTTTGTTCCT
GATTGGTTACGGCGCGTTTCGCATCATTGTTGAGTTTTTCCGCCAGCCCG
ACGCGCAGTTTACCGGTGCCTGGGTGCAGTACATCAGCATGGGGCAAATT
CTTTCCATCCCGATGATTGTCGCGGGTGTGATCATGATGGTCTGGGCATA
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TCGTCGCAGCCCACAGCAACACGTTTCCTGAGGAACCATGAAACAGTATT

TAGAACTGATGCAAAAAGTGCTCGACGAAGGCACACAGAAAAACGACCGT

ACCGGAACCGGAACGCTTTCCATTTTTGGTCATCAGATGCGTTTTAACCT

GCAAGATGGATTCCCGCTGGTGACAACTAAACGTTGCCACCTGCGTTCCA

TCATCCATGAACTGCTGTGGTTTCTGCAGGGCGACACTAACATTGCTTAT

CTACACGAAAACAATGTCACCATCTGGGACGAATGGGCCGATGAAAACGG

CGACCTCGGGCCAGTGTATGGTAAACAGTGGCGCGCCTGGCCAACGCCAG

ATGGTCGTCATATTGACCAGATCACTACGGTACTGAACCAGCTGAAAAAC

GACCCGGATTCGCGCCGCATTATTGTTTCAGCGTGGAACGTAGGCGAACT

GGATAAAATGGCGCTGGCACCGTGCCATGCATTCTTCCAGTTCTATGTGG

CAGACGGCAAACTCTCTTGCCAGCTTTATCAGCGCTCCTGTGACGTCTTC

CTCGGCCTGCCGTTCAACATTGCCAGCTACGCGTTATTGGTGCATATGAT

GGCGCAGCAGTGCGATCTGGAAGTGGGTGATTTTGTCTGGACCGGTGGCG

ACACGCATCTGTACAGCAACCATATGGATCAAACTCATCTGCAATTAAGC

CGCGAACCGCGTCCGCTGCCGAAGTTGATTATCAAACGTAAACCCGAATC

CATCTTCGACTACCGTTTCGAAGACTTTGAGATTGAAGGCTACGATCCGC

ATCCGGGCATTAAAGCGCCGGTGGCTATCTAATTACGAAACATCCTGCCA

GAGCCGACGCCAGTGTGCGTCGGTTTTTTTACCCTCCGTTAAATTCTTCG

AGACGCCTTCCCGAAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGG

GAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGG

GGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGT

CACGACGTTGTAAAACGACGGCCAGTGCCAAGCTTTCTTTAATGAAGCAG

GGCATCAGGACGGTATCTTTGTGGAGAAAGCAGAGTAATCTTATTCAGCC

TGACTGGTGGGAAACCACCAGTCAGAATGTGTTAGCGCATGTTGACAAAA

ATACCATTAGTCACATTATCCGTCAGTCGGACGACATGGTAGATAACCTG

TTTATTATGCGTTTTGATCTTACGTTTAATATTACCTTTATGCGATGAAA

CGGTCTTGGCTTTGATATTCATTTGGTCAGAGATTTGAATGGTTCCCTGA

CCTGCCATCCACATTCGCAACATACTCGATTCGGTTCGGCTCAATGATAA

CGTCGGCATATTTAAAAACGAGGTTATCGTTGTCTCTTTTTTCAGAATAT

CGCCAAGGATATCGTCGAGAGATTCCGGTTTAATCGATTTAGAACTGATC

AATAAATTTTTTCTGACCAATAGATATTCATCAAAATGAACATTGGCAAT

TGCCATAAAAACGATAAATAACGTATTGGGATGTTGATTAATGATGAGCT

TGATACGCTGACTGTTAGAAGCATCGTGGATGAAACAGTCCTCATTAATA

AACACCACTGAAGGGCGCTGTGAATCACAAGCTATGGCAAGGTCATCAAC

GGTTTCAATGTCGTTGATTTCTCTTTTTTTAACCCCTCTACTCAACAGAT

ACCCGGTTAAACCTAGTCGGGTGTAACTACATAAATCCATAATAATCGTT

GACATGGCATACCCTCACTCAATGCGTAACGATAATTCCCCTTACCTGAA

TATTTCATCATGACTAAACGGAACAACATGGGTCACCTAATGCGCCACTC

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CCTGGAATTGTCTTAAAGTAAAGTAAATGTTGCGATATGTGAGTGAGCTT

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TACTACCCTGTACGATTACTGCAGCTCGAGTTATTATAATTTTACCCACG

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TTACTGGAAACATAATCACCTCTTCTAATATGTAATGAACAAGTATCATT

TTCTTTAATTAAATTAAGCAATTCATTTTGATAACTATTAAACTTGGTTT

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TCAGTTTGAAAATAACCGACGATTTTTTTATTTATACTTTTGGTATCAAT

ATCTGGATCATACTCTAAACTTTTCTCAACGTAATGCTTTCTGAACATTC

CTTTTTTCATGAAATGTGGGATTTTTTCGGAAAATAAGTATTTTTCAAAT

GGCCATGCTTTTTTTTACAAATTCTGAACTACAAGATAATTCAACTAATCT

TAATGGATGAGTTTTATATTTTACTGCATCAGATATATCAACAGTCAAAT

TTTGATGAGTTCTTTTTGCAATAGCAAATGCAGTTGCATACTGAAACATT

TGATTACCAAGACCACCAATAATTTTAACTTCCATATGTATATCTCCTTC

TTCTAGAATTCTAAAAATTGATTGAATGTATGCAAATAAATGCATACACC

ATAGGTGTGGTTTAATTTGATGCCCTTTTTCAGGGCTGGAATGTGTAAGA

GCGGGGTTATTTATGCTGTTGTTTTTTTGTTACTCGGGAAGGGCTTTACC

TCTTCCGCATAAACGCTTCCATCAGCGTTTATAGTTAAAAAAATCTTTCG

GAACTGGTTTTGCGCTTACCCCAACCAACAGGGGATTTGCTGCTTTCCAT

TGAGCCTGTTTCTCTGCGCGACGTTCGCGGCGGCGTGTTTGTGCATCCAT

CTGGATTCTCCTGTCAGTTAGCTTTGGTGGTGTGTGGCAGTTGTAGTCCT

GAACGAAAACCCCCCGCGATTGGCACATTGGCAGCTAATCCGGAATCGCA

CTTACGGCCAATGCTTCGTTTCGTATCACACACCCCAAAGCCTTCTGCTT

TGAATGCTGCCCTTCTTCAGGGCTTAATTTTTAAGAGCGTCACCTTCATG

GTGGTCAGTGCGTCCTGCTGATGTGCTCAGTATCACCGCCAGTGGTATTT

ATGTCAACACCGCCAGAGATAATTTATCACCGCAGATGGTTATCTGTATG

TTTTTTATATGAATTTATTTTTTGCAGGGGGGCATTGTTTGGTAGGTGAG

AGATCAATTCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTT

GCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGG

TCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGG

TTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGG

CCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCC

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GTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCC
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CTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTA
TCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCT
TGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAA
GCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCT
TTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATT
TTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTA
AAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTG
ACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTA
TTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGAT
ACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACC
CACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGG
GCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTAT
TAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGC
GCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTT
GGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATG
ATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCG
TTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCA
CTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGAC
TGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGA
GTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGA
ACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTC
AAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCAC
CCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCA
AAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAA
ATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATC
AGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAAT
AAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGT
CTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCA
CGAGGCCCTTTCGTC

The sequence of pG176 is set forth below (SEQ ID NO: 2):

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GAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCG
TCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATG

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CGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATATGCGGTGTGAAA
TACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCATGAAACA
GTATTTAGAACTGATGCAAAAAGTGCTCGACGAAGGCACACAGAAAAACG
ACCGTACCGGAACCGGAACGCTTTCCATTTTTGGTCATCAGATGCGTTTT
AACCTGCAAGATGGATTCCCGCTGGTGACAACTAAACGTTGCCACCTGCG
TTCCATCATCCATGAACTGCTGTGGTTTCTGCAGGGCGACACTAACATTG
CTTATCTACACGAAAACAATGTCACCATCTGGGACGAATGGGCCGATGAA
AACGGCGACCTCGGGCCAGTGTATGGTAAACAGTGGCGCGCCTGGCCAAC
GCCAGATGGTCGTCATATTGACCAGATCACTACGGTACTGAACCAGCTGA
AAAACGACCCGGATTCGCGCCGCATTATTGTTTCAGCGTGGAACGTAGGC
GAACTGGATAAAATGGCGCTGGCACCGTGCCATGCATTCTTCCAGTTCTA
TGTGGCAGACGGCAAACTCTCTTGCCAGCTTTATCAGCGCTCCTGTGACG
TCTTCCTCGGCCTGCCGTTCAACATTGCCAGCTACGCGTTATTGGTGCAT
ATGATGGCGCAGCAGTGCGATCTGGAAGTGGGTGATTTTGTCTGGACCGG
TGGCGACACGCATCTGTACAGCAACCATATGGATCAAACTCATCTGCAAT
TAAGCCGCGAACCGCGTCCGCTGCCGAAGTTGATTATCAAACGTAAACCC
GAATCCATCTTCGACTACCGTTTCGAAGACTTTGAGATTGAAGGCTACGA
TCCGCATCCGGGCATTAAAGCGCCGGTGGCTATCTAAGGCGCCATTCGCC
ATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGC
TATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGG
GTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGC
CAAGCTTTCTTTAATGAAGCAGGGCATCAGGACGGTATCTTTGTGGAGAA
AGCAGAGTAATCTTATTCAGCCTGACTGGTGGGAAACCACCAGTCAGAAT
GTGTTAGCGCATGTTGACAAAAATACCATTAGTCACATTATCCGTCAGTC
GGACGACATGGTAGATAACCTGTTTATTATGCGTTTTGATCTTACGTTTA
ATATTACCTTTATGCGATGAAACGGTCTTGGCTTTGATATTCATTTGGTC
AGAGATTTGAATGGTTCCCTGACCTGCCATCCACATTCGCAACATACTCG
ATTCGGTTCGGCTCAATGATAACGTCGGCATATTTAAAAACGAGGTTATC
GTTGTCTCTTTTTTCAGAATATCGCCAAGGATATCGTCGAGAGATTCCGG
TTTAATCGATTTAGAACTGATCAATAAATTTTTTCTGACCAATAGATATT
CATCAAAATGAACATTGGCAATTGCCATAAAAACGATAAATAACGTATTG
GGATGTTGATTAATGATGAGCTTGATACGCTGACTGTTAGAAGCATCGTG
GATGAAACAGTCCTCATTAATAAACACCACTGAAGGGCGCTGTGAATCAC
AAGCTATGGCAAGGTCATCAACGGTTTCAATGTCGTTGATTTCTCTTTTT
TTAACCCCTCTACTCAACAGATACCCGGTTAAACCTAGTCGGGTGTAACT
ACATAAATCCATAATAATCGTTGACATGGCATACCCTCACTCAATGCGTA
ACGATAATTCCCCTTACCTGAATATTTCATCATGACTAAACGGAACAACA
TGGGTCACCTAATGCGCCACTCTCGCGATTTTTCAGGCGGACTTACTATC
CCGTAAAGTGTTGTATAATTTGCCTGGAATTGTCTTAAAGTAAAGTAAAT

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GTTGCGATATGTGAGTGAGCTTAAAACAAATATTTCGCTGCAGGAGTATC

CTGGAAGATGTTCGTAGAAGCTTACTGCTCACAAGAAAAAAGGCACGTCA

TCTGACGTGCCTTTTTTATTTGTACTACCCTGTACGATTACTGCAGCTCG

AGTTAATTCAAATCTTCTTCAGAAATCAATTTTTGTTCCAAACCCAATTT

TTTAACCAACTTTCTCACCGCGCGCAACAAAGGCAAGGATTTTTGATAAG

CTTTGCGATAGATTTTAAAAGTGGTGTTTTGAGAGAGTTCTAATAAAGGC

GAAGCGTTTTGTAAAAGCCGGTCATAATTAACCCTCAAATCATCATAATT

AACCCTCAAATCATCAATGGATACTAACGGCTTATGCAGATCGTACTCCC

ACATGAAAGATGTTGAGAATTTGTGATAAATCGTATCGTTTTCTAAAATC

GTTTTAAAAAAATCTAGGATTTTTTTAAAACTCAAATCTTGGTAAAAGTA

AGCTTTCCCATCAAGGGTGTTTAAAGGGTTTTCATAGAGCATGTCTAAAT

AAGCGTTTGGGTGCGTGTGCAGGTATTTGATATAATCAATCGCTTCATCA

AAGTTGTTGAAATCATGCACATTCACAAAACTTTTAGGGTTAAAATCTTT

CGCCACGCTGGGACTCCCCCAATAAATAGGAATGGTATGGCTAAAATACG

CATCAAGGATTTTTTCGGTTACATAGCCATAACCTTGCGAGTTTTCAAAA

CAGAGATTGAACTTGTATTGGCTTAAAAACTCGCTTTTGTTTCCAACCTT

ATAGCCTAAAGTGTTTCTCACACTTCCTCCCCCAGTAACTGGCTCTATGG

AATTTAGAGCGTCATAAAAAGCGTTCCTCATAGGAGCGTTAGCGTTGCTC

GCTACAAAACTGGCAAACCCTCTTTTTAAAAGATCGCTCTCATCATTCAC

TACTGCGCACAAATTAGGGTGGTTTTCTTTAAAATGATGAGAGGGTTTTT

TTAAAGCATAAAGGCTGTTGTCTTTGAGTTTGTAGGGCGCAGTGGTGTCA

TTAACAAGCTCGGCTTTATAGTGCAAATGGGCATAATACAAAGGCATTCT

CAAATAACGATCATTAAAATCCAATTCATCAAAGCCTATGGCGTAATCAA

AGAGGTTGAAATTAGGTGATTCGTTTTCACCGGTGTAAAACACTCGTTTA

GTGTTTTGATAAGATAAAATCTTTCTAGCCGCTCCAAGAGGATTGCTAAA

AACTAGATCTGAAAATTCATTGGGGTTTTGGTGGAGGGTGATTGCGTAGC

GTTGGCTTAGGATAAAATAAAGAACGCTCTTTTTAAATTCTTTAATTTCT

TCATCTCCCCACCAATTCGCCACAGCGATTTTTAGGGGGGGGGGGGGAGA

TTTAGAGGCCATTTTTTCAATGGAAGCGCTTTCTATAAAGGCGTCTAATA

GGGGTTGGAACATATGTATATCTCCTTCTTGAATTCTAAAAATTGATTGA

ATGTATGCAAATAAATGCATACACCATAGGTGTGGTTTAATTTGATGCCC

TTTTTCAGGGCTGGAATGTGTAAGAGCGGGGTTATTTATGCTGTTGTTTT

TTTGTTACTCGGGAAGGGCTTTACCTCTTCCGCATAAACGCTTCCATCAG

CGTTTATAGTTAAAAAAATCTTTCGGAACTGGTTTTGCGCTTACCCCAAC

CAACAGGGGATTTGCTGCTTTCCATTGAGCCTGTTTCTCTGCGCGACGTT

CGCGGCGGCGTGTTTGTGCATCCATCTGGATTCTCCTGTCAGTTAGCTTT

GGTGGTGTGTGGCAGTTGTAGTCCTGAACGAAAACCCCCCGCGATTGGCA

CATTGGCAGCTAATCCGGAATCGCACTTACGGCCAATGCTTCGTTTCGTA

TCACACACCCCAAAGCCTTCTGCTTTGAATGCTGCCCTTCTTCAGGGCTT

AATTTTTAAGAGCGTCACCTTCATGGTGGTCAGTGCGTCCTGCTGATGTG

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CTCAGTATCACCGCCAGTGGTATTTATGTCAACACCGCCAGAGATAATTT

ATCACCGCAGATGGTTATCTGTATGTTTTTTATATGAATTTATTTTTTGC

AGGGGGGCATTGTTTGGTAGGTGAGAGATCAATTCTGCATTAATGAATCG

GCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCC

TCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATC

AGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACG

CAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAA

AAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCA

TCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTAT

AAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTT

CCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAG

CGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGG

TCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGAC

CGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACA

CGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGA

GGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGC

TACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTAC

CTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTG

GTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAA

GGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTG

GAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGA

TCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAA

AGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGA

GGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGAC

TCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCC

AGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATC

AGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAA

CTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTA

AGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGG

CATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTT

CCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCG

GTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGT

GTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGC

CATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTC

TGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACG

GGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAA

AACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCC

AGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTAC

TTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAA

AAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTT

TTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATA

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CATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACAT

TTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACA

TTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTC

The sequence of pG177 is set forth below (SEQ ID NO: 3):

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCG

GAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCG

TCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATG

CGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATATGCGGTGTGAAA

TACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCATGAAACA

GTATTTAGAACTGATGCAAAAAGTGCTCGACGAAGGCACACAGAAAAACG

ACCGTACCGGAACCGGAACGCTTTCCATTTTTGGTCATCAGATGCGTTTT

AACCTGCAAGATGGATTCCCGCTGGTGACAACTAAACGTTGCCACCTGCG

TTCCATCATCCATGAACTGCTGTGGTTTCTGCAGGGCGACACTAACATTG

CTTATCTACACGAAAACAATGTCACCATCTGGGACGAATGGGCCGATGAA

AACGGCGACCTCGGGCCAGTGTATGGTAAACAGTGGCGCGCCTGGCCAAC

GCCAGATGGTCGTCATATTGACCAGATCACTACGGTACTGAACCAGCTGA

AAAACGACCCGGATTCGCGCCGCATTATTGTTTCAGCGTGGAACGTAGGC

GAACTGGATAAAATGGCGCTGGCACCGTGCCATGCATTCTTCCAGTTCTA

TGTGGCAGACGGCAAACTCTCTTGCCAGCTTTATCAGCGCTCCTGTGACG

TCTTCCTCGGCCTGCCGTTCAACATTGCCAGCTACGCGTTATTGGTGCAT

ATGATGGCGCAGCAGTGCGATCTGGAAGTGGGTGATTTTGTCTGGACCGG

TGGCGACACGCATCTGTACAGCAACCATATGGATCAAACTCATCTGCAAT

TAAGCCGCGAACCGCGTCCGCTGCCGAAGTTGATTATCAAACGTAAACCC

GAATCCATCTTCGACTACCGTTTCGAAGACTTTGAGATTGAAGGCTACGA

TCCGCATCCGGGCATTAAAGCGCCGGTGGCTATCTAAGGCGCCATTCGCC

ATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGC

TATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGG

GTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGC

CAAGCTTTCTTTAATGAAGCAGGGCATCAGGACGGTATCTTTGTGGAGAA

AGCAGAGTAATCTTATTCAGCCTGACTGGTGGGAAACCACCAGTCAGAAT

GTGTTAGCGCATGTTGACAAAAATACCATTAGTCACATTATCCGTCAGTC

GGACGACATGGTAGATAACCTGTTTATTATGCGTTTTGATCTTACGTTTA

ATATTACCTTTATGCGATGAAACGGTCTTGGCTTTGATATTCATTTGGTC

AGAGATTTGAATGGTTCCCTGACCTGCCATCCACATTCGCAACATACTCG

ATTCGGTTCGGCTCAATGATAACGTCGGCATATTTAAAAACGAGGTTATC

GTTGTCTCTTTTTTCAGAATATCGCCAAGGATATCGTCGAGAGATTCCGG

TTTAATCGATTTAGAACTGATCAATAAATTTTTTCTGACCAATAGATATT

CATCAAAATGAACATTGGCAATTGCCATAAAAACGATAAATAACGTATTG

GGATGTTGATTAATGATGAGCTTGATACGCTGACTGTTAGAAGCATCGTG

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GATGAAACAGTCCTCATTAATAAACACCACTGAAGGGCGCTGTGAATCAC

AAGCTATGGCAAGGTCATCAACGGTTTCAATGTCGTTGATTTCTCTTTTT

TTAACCCCTCTACTCAACAGATACCCGGTTAAACCTAGTCGGGTGTAACT

ACATAAATCCATAATAATCGTTGACATGGCATACCCTCACTCAATGCGTA

ACGATAATTCCCCTTACCTGAATATTTCATCATGACTAAACGGAACAACA

TGGGTCACCTAATGCGCCACTCTCGCGATTTTTCAGGCGGACTTACTATC

CCGTAAAGTGTTGTATAATTTGCCTGGAATTGTCTTAAAGTAAAGTAAAT

GTTGCGATATGTGAGTGAGCTTAAAACAAATATTTCGCTGCAGGAGTATC

CTGGAAGATGTTCGTAGAAGCTTACTGCTCACAAGAAAAAAGGCACGTCA

TCTGACGTGCCTTTTTTATTTGTACTACCCTGTACGATTACTGCAGCTCG

AGTTAATTCAAATCTTCTTCAGAAATCAATTTTTGTTCAGCGTTATACTT

TTGGGATTTTACCTCAAAATGGGATTCTATTTTCACCCACTCCTTACAAA

GGATATTCTCATGCCCAAAAAGCCAGTGTTTGGGGCCAATAATGATTTTT

TCTGGATTTTCTATCAAATAGGCCGCCCACCAGCTATAAGTGCTATTAGC

GATAATGCCATGCTGACAAGATTGCATGAGCAGCATGTCCCAATACGCCT

CTTCTTCTTTATCCCTAGTGGTCATGTCCATAAAAGGGTAGCCAAGATCA

AGATTTTGCGTGAATTCTAAGTCTTCGCAAAACACAAAAAGCTCCATGTT

TGGCACGCGCTTTGCCATATACTCAAGCGCCTTTTTTTGATAGTCAATAC

CAAGCTGACAGCCAATCCCCACATAATCCCCTCTTCTTATATGCACAAAC

ACGCTGTTTTTAGCGGCTAAAATCAAAGAAAGCTTGCACTGATATTCTTC

CTCTTTTTTATTATTATTCTTATTATTTTCGGGTGGTGGTGGTAGAGTGA

AGGTTTGCTTGATTAAAGGGGATATAGCATCAAAGTATCGTGGATCTTGG

AAATAGCCAAAAAAATAAGTCAAGCGGCTTGGCTTTAGCAATTTAGGCTC

GTATTCAAAAACGATTTCTTGACTCACCCTATCAAATCCCATGCATTTGA

GCGCGTCTCTTACTAGCTTGGGGAGGTGTTGCATTTTAGCTATAGCGATT

TCTTTCGCGCTCGCATAGGGCAAATCAATAGGGAAAAGTTCTAATTGCAT

TTTCCTATCGCTCCAATCAAAAGAAGTGATATCTAACAGCACAGGCGTAT

TAGAGTGTTTTTGCAAACTTTTAGCGAAAGCGTATTGAAACATTTGATTC

CCAAGCCCTCCGCAAATTTGCACCACCTTAAAAGCCATATGTATATCTCC

TTCTTGCTCGAGTTAATTCAAATCTTCTTCAGAAATCAATTTTTGTTCCA

AACCCAATTTTTTAACCAACTTTCTCACCGCGCGCAACAAAGGCAAGGAT

TTTTGATAAGCTTTGCGATAGATTTTAAAAGTGGTGTTTTGAGAGAGTTC

TAATAAAGGCGAAGCGTTTTGTAAAAGCCGGTCATAATTAACCCTCAAAT

CATCATAATTAACCCTCAAATCATCAATGGATACTAACGGCTTATGCAGA

TCGTACTCCCACATGAAAGATGTTGAGAATTTGTGATAAATCGTATCGTT

TTCTAAAATCGTTTTAAAAAAATCTAGGATTTTTTTAAAACTCAAATCTT

GGTAAAAGTAAGCTTTCCCATCAAGGGTGTTTAAAGGGTTTTCATAGAGC

ATGTCTAAATAAGCGTTTGGGTGCGTGTGCAGGTATTTGATATAATCAAT

CGCTTCATCAAAGTTGTTGAAATCATGCACATTCACAAAACTTTTAGGGT

TAAAATCTTTCGCCACGCTGGGACTCCCCCAATAAATAGGAATGGTATGG

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CTAAAATACGCATCAAGGATTTTTTCGGTTACATAGCCATAACCTTGCGA

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TTCCAACCTTATAGCCTAAAGTGTTTCTCACACTTCCTCCCCCAGTAACT

GGCTCTATGGAATTTAGAGCGTCATAAAAAGCGTTCCTCATAGGAGCGTT

AGCGTTGCTCGCTACAAAACTGGCAAACCCTCTTTTTAAAAGATCGCTCT

CATCATTCACTACTGCGCACAAATTAGGGTGGTTTTCTTTAAAATGATGA

GAGGGTTTTTTTAAAGCATAAAGGCTGTTGTCTTTGAGTTTGTAGGGCGC

AGTGGTGTCATTAACAAGCTCGGCTTTATAGTGCAAATGGGCATAATACA

AAGGCATTCTCAAATAACGATCATTAAAATCCAATTCATCAAAGCCTATG

GCGTAATCAAAGAGGTTGAAATTAGGTGATTCGTTTTCACCGGTGTAAAA

CACTCGTTTAGTGTTTTGATAAGATAAAATCTTTCTAGCCGCTCCAAGAG

GATTGCTAAAAACTAGATCTGAAAATTCATTGGGGTTTTGGTGGAGGGTG

ATTGCGTAGCGTTGGCTTAGGATAAAATAAAGAACGCTCTTTTTAAATTC

TTTAATTTCTTCATCTCCCCACCAATTCGCCACAGCGATTTTTAGGGGGG

GGGGGGGAGATTTAGAGGCCATTTTTTCAATGGAAGCGCTTTCTATAAAG

GCGTCTAATAGGGGTTGGAACATATGTATATCTCCTTCTTGAATTCTAAA

AATTGATTGAATGTATGCAAATAAATGCATACACCATAGGTGTGGTTTAA

TTTGATGCCCTTTTTCAGGGCTGGAATGTGTAAGAGCGGGGTTATTTATG

CTGTTGTTTTTTTGTTACTCGGGAAGGGCTTTACCTCTTCCGCATAAACG

CTTCCATCAGCGTTTATAGTTAAAAAAATCTTTCGGAACTGGTTTTGCGC

TTACCCCAACCAACAGGGGATTTGCTGCTTTCCATTGAGCCTGTTTCTCT

GCGCGACGTTCGCGGCGGCGTGTTTGTGCATCCATCTGGATTCTCCTGTC

AGTTAGCTTTGGTGGTGTGTGGCAGTTGTAGTCCTGAACGAAAACCCCCC

GCGATTGGCACATTGGCAGCTAATCCGGAATCGCACTTACGGCCAATGCT

TCGTTTCGTATCACACACCCCAAAGCCTTCTGCTTTGAATGCTGCCCTTC

TTCAGGGCTTAATTTTTAAGAGCGTCACCTTCATGGTGGTCAGTGCGTCC

TGCTGATGTGCTCAGTATCACCGCCAGTGGTATTTATGTCAACACCGCCA

GAGATAATTTATCACCGCAGATGGTTATCTGTATGTTTTTTATATGAATT

TATTTTTTGCAGGGGGGCATTGTTTGGTAGGTGAGAGATCAATTCTGCAT

TAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTC

TTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGC

GAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCA

GGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAG

GAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCC

CTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCG

ACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCG

CTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCC

CTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGT

TCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGT

TCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACC

CGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATT

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AGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCC

TAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGA

AGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAA

ACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCG

CAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTG

ACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTA

TCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAA

ATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCT

TAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATA

GTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACC

ATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTC

CAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGT

GGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGA

AGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCA

TTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTC

AGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTG

CAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGT

TGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTT

ACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAAC

CAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGG

CGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTC

ATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCT

GTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAG

CATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAA

AATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCAT

ACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCA

TGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTT

CCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTAT

TATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTC

The sequence of *Bacteroides fragilis* NCTC 9343 wcfW CDS DNA is set for the below (SEQ ID NO: 4):

ATGATTGTATCATCTTTGCGAGGAGGATTGGGGAATCAAATGTTTATTTA

CGCTATGGTGAAGGCCATGGCATTAAGAAACAATGTACCATTCGCTTTTA

ATTTGACTACTGATTTTGCAAATGATGAAGTTTATAAAAGGAAACTTTTA

TTATCATATTTTGCATTAGACTTGCCTGAAAATAAAAAATTAACATTTGA

TTTTTCATATGGGAATTATTATAGAAGGCTAAGTCGTAATTTAGGTTGTC

ATATACTTCATCCATCATATCGTTATATTTGCGAAGAGCGCCCTCCCCAC

TTTGAATCAAGGTTAATTAGTTCTAAGATTACAAATGCTTTTCTGGAAGG

ATATTGGCAGTCAGAAAAATATTTTCTTGATTATAAACAAGAGATAAAAG

AGGACTTTGTAATACAAAAAAAATTAGAATACACATCGTATTTGGAATTG

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GAAGAAATAAAATTGCTAGATAAGAATGCCATAATGATTGGGGTTAGACG
GTATCAGGAAAGTGATGTAGCTCCTGGTGGAGTGTTAGAAGATGATTACT
ATAAATGTGCTATGGATATTATGGCATCAAAAGTTACTTCTCCTGTTTTC
TTTTGTTTTTCACAAGATTTAGAATGGGTTGAAAAACATCTAGCGGGAAA
ATATCCTGTTCGTTTGATAAGTAAAAAGGAGGATGATAGTGGTACTATAG
ATGATATGTTTCTAATGATGCATTTTCGTAATTATATAATATCGAATAGC
TCTTTTTACTGGTGGGGAGCATGGCTTTCGAAATATGATGATAAGCTGGT
GATTGCTCCAGGTAATTTTATAAATAAGGATTCTGTACCAGAATCTTGGT
TTAAATTGAATGTAAGATAA

The sequence of pG171 is set forth below (SEQ ID NO: 5):

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCG
GAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCG
TCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATG
CGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATATGCGGTGTGAAA
TACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCTCCTCAAC
CTGTATATTCGTAAACCACGCCCAATGGGAGCTGTCTCAGGTTTGTTCCT
GATTGGTTACGGCGCGTTTCGCATCATTGTTGAGTTTTTCCGCCAGCCCG
ACGCGCAGTTTACCGGTGCCTGGGTGCAGTACATCAGCATGGGGCAAATT
CTTTCCATCCCGATGATTGTCGCGGGTGTGATCATGATGGTCTGGGCATA
TCGTCGCAGCCCACAGCAACACGTTTCCTGAGGAACCATGAAACAGTATT
TAGAACTGATGCAAAAAGTGCTCGACGAAGGCACACAGAAAAACGACCGT
ACCGGAACCGGAACGCTTTCCATTTTTGGTCATCAGATGCGTTTTAACCT
GCAAGATGGATTCCCGCTGGTGACAACTAAACGTTGCCACCTGCGTTCCA
TCATCCATGAACTGCTGTGGTTTCTGCAGGGCGACACTAACATTGCTTAT
CTACACGAAAACAATGTCACCATCTGGGACGAATGGGCCGATGAAAACGG
CGACCTCGGGCCAGTGTATGGTAAACAGTGGCGCGCCTGGCCAACGCCAG
ATGGTCGTCATATTGACCAGATCACTACGGTACTGAACCAGCTGAAAAAC
GACCCGGATTCGCGCCGCATTATTGTTTCAGCGTGGAACGTAGGCGAACT
GGATAAAATGGCGCTGGCACCGTGCCATGCATTCTTCCAGTTCTATGTGG
CAGACGGCAAACTCTCTTGCCAGCTTTATCAGCGCTCCTGTGACGTCTTC
CTCGGCCTGCCGTTCAACATTGCCAGCTACGCGTTATTGGTGCATATGAT
GGCGCAGCAGTGCGATCTGGAAGTGGGTGATTTTGTCTGGACCGGTGGCG
ACACGCATCTGTACAGCAACCATATGGATCAAACTCATCTGCAATTAAGC
CGCGAACCGCGTCCGCTGCCGAAGTTGATTATCAAACGTAAACCCGAATC
CATCTTCGACTACCGTTTCGAAGACTTTGAGATTGAAGGCTACGATCCGC
ATCCGGGCATTAAAGCGCCGGTGGCTATCTAATTACGAAACATCCTGCCA
GAGCCGACGCCAGTGTGCGTCGGTTTTTTTACCCTCCGTTAAATTCTTCG
AGACGCCTTCCCGAAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGG
GAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGG
GGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGT
CACGACGTTGTAAAACGACGGCCAGTGCCAAGCTTTCTTTAATGAAGCAG
GGCATCAGGACGGTATCTTTGTGGAGAAAGCAGAGTAATCTTATTCAGCC
TGACTGGTGGGAAACCACCAGTCAGAATGTGTTAGCGCATGTTGACAAAA
ATACCATTAGTCACATTATCCGTCAGTCGGACGACATGGTAGATAACCTG
TTTATTATGCGTTTTGATCTTACGTTTAATATTACCTTTATGCGATGAAA
CGGTCTTGGCTTTGATATTCATTTGGTCAGAGATTTGAATGGTTCCCTGA
CCTGCCATCCACATTCGCAACATACTCGATTCGGTTCGGCTCAATGATAA
CGTCGGCATATTTAAAAACGAGGTTATCGTTGTCTCTTTTTTCAGAATAT
CGCCAAGGATATCGTCGAGAGATTCCGGTTTAATCGATTTAGAACTGATC
AATAAATTTTTTCTGACCAATAGATATTCATCAAAATGAACATTGGCAAT
TGCCATAAAAACGATAAATAACGTATTGGGATGTTGATTAATGATGAGCT
TGATACGCTGACTGTTAGAAGCATCGTGGATGAAACAGTCCTCATTAATA
AACACCACTGAAGGGCGCTGTGAATCACAAGCTATGGCAAGGTCATCAAC
GGTTTCAATGTCGTTGATTTCTCTTTTTTTAACCCCTCTACTCAACAGAT
ACCCGGTTAAACCTAGTCGGGTGTAACTACATAAATCCATAATAATCGTT
GACATGGCATACCCTCACTCAATGCGTAACGATAATTCCCCTTACCTGAA
TATTTCATCATGACTAAACGGAACAACATGGGTCACCTAATGCGCCACTC
TCGCGATTTTTCAGGCGGACTTACTATCCCGTAAAGTGTTGTATAATTTG
CCTGGAATTGTCTTAAAGTAAAGTAAATGTTGCGATATGTGAGTGAGCTT
AAAACAAATATTTCGCTGCAGGAGTATCCTGGAAGATGTTCGTAGAAGCT
TACTGCTCACAAGAAAAAAGGCACGTCATCTGACGTGCCTTTTTTATTTG
TACTACCCTGTACGATTACTGCAGCTCGAGTTTAATTCAAATCTTCTTCA
GAAATCAATTTTTGTTCAGCGTTATACTTTTGGGATTTTACCTCAAAATG
GGATTCTATTTTCACCCACTCCTTACAAAGGATATTCTCATGCCCAAAAA
GCCAGTGTTTGGGGCCAATAATGATTTTTTCTGGATTTTCTATCAAATAG
GCCGCCCACCAGCTATAAGTGCTATTAGCGATAATGCCATGCTGACAAGA
TTGCATGAGCAGCATGTCCCAATACGCCTCTTCTTCTTTATCCCTAGTGG
TCATGTCCATAAAAGGGTAGCCAAGATCAAGATTTTGCGTGAATTCTAAG
TCTTCGCAAAACACAAAAAGCTCCATGTTTGGCACGCGCTTTGCCATATA
CTCAAGCGCCTTTTTTTGATAGTCAATACCAAGCTGACAGCCAATCCCCA
CATAATCCCCTCTTCTTATATGCACAAACACGCTGTTTTTAGCGGCTAAA
ATCAAAGAAAGCTTGCACTGATATTCTTCCTCTTTTTTATTATTATTCTT
ATTATTTTCGGGTGGTGGTGGTAGAGTGAAGGTTTGCTTGATTAAAGGGG
ATATAGCATCAAAGTATCGTGGATCTTGGAAATAGCCAAAAAAATAAGTC
AAGCGGCTTGGCTTTAGCAATTTAGGCTCGTATTCAAAAACGATTTCTTG
ACTCACCCTATCAAATCCCATGCATTTGAGCGCGTCTCTTACTAGCTTGG
GGAGGTGTTGCATTTTAGCTATAGCGATTTCTTTCGCGCTCGCATAGGGC
AAATCAATAGGGAAAAGTTCTAATTGCATTTTCCTATCGCTCCAATCAAA
AGAAGTGATATCTAACAGCACAGGCGTATTAGAGTGTTTTTGCAAACTTT

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TAGCGAAAGCGTATTGAAACATTTGATTCCCAAGCCCTCCGCAAATTTGC

ACCACCTTAAAAGCCATATGTATATCTCCTTCTTGAATTCTAAAAATTGA

TTGAATGTATGCAAATAAATGCATACACCATAGGTGTGGTTTAATTTGAT

GCCCTTTTTCAGGGCTGGAATGTGTAAGAGCGGGGTTATTTATGCTGTTG

TTTTTTTGTTACTCGGGAAGGGCTTTACCTCTTCCGCATAAACGCTTCCA

TCAGCGTTTATAGTTAAAAAAATCTTTCGGAACTGGTTTTGCGCTTACCC

CAACCAACAGGGGATTTGCTGCTTTCCATTGAGCCTGTTTCTCTGCGCGA

CGTTCGCGGCGGCGTGTTTGTGCATCCATCTGGATTCTCCTGTCAGTTAG

CTTTGGTGGTGTGTGGCAGTTGTAGTCCTGAACGAAAACCCCCCGCGATT

GGCACATTGGCAGCTAATCCGGAATCGCACTTACGGCCAATGCTTCGTTT

CGTATCACACACCCCAAAGCCTTCTGCTTTGAATGCTGCCCTTCTTCAGG

GCTTAATTTTTAAGAGCGTCACCTTCATGGTGGTCAGTGCGTCCTGCTGA

TGTGCTCAGTATCACCGCCAGTGGTATTTATGTCAACACCGCCAGAGATA

ATTTATCACCGCAGATGGTTATCTGTATGTTTTTTATATGAATTTATTTT

TTGCAGGGGGGCATTGTTTGGTAGGTGAGAGATCAATTCTGCATTAATGA

ATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGC

TTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGG

TATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGAT

AACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCG

TAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACG

AGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGA

CTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCC

TGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGG

GAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTG

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GACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGA

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CGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAG

TTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACC

GCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAA

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AGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAA

AGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAAT

CTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCA

GTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCC

TGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGG

CCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATT

TATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCT

GCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAG

AGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTA

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CAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCC

GGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAA

AGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCG

CAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTC

ATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTC

ATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAA

TACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATT

GGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAG

ATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTT

TTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCC

GCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTT

CCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCG

GATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGC

ACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCAT

GACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTC

The sequence of pG180 is set forth below (SEQ ID NO: 6):

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCG

GAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCG

TCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATG

CGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATATGCGGTGTGAAA

TACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCTCCTCAAC

CTGTATATTCGTAAACCACGCCCAATGGGAGCTGTCTCAGGTTTGTTCCT

GATTGGTTACGGCGCGTTTCGCATCATTGTTGAGTTTTTCCGCCAGCCCG

ACGCGCAGTTTACCGGTGCCTGGGTGCAGTACATCAGCATGGGGCAAATT

CTTTCCATCCCGATGATTGTCGCGGGTGTGATCATGATGGTCTGGGCATA

TCGTCGCAGCCCACAGCAACACGTTTCCTGAGGAACCATGAAACAGTATT

TAGAACTGATGCAAAAAGTGCTCGACGAAGGCACACAGAAAAACGACCGT

ACCGGAACCGGAACGCTTTCCATTTTTGGTCATCAGATGCGTTTTAACCT

GCAAGATGGATTCCCGCTGGTGACAACTAAACGTTGCCACCTGCGTTCCA

TCATCCATGAACTGCTGTGGTTTCTGCAGGGCGACACTAACATTGCTTAT

CTACACGAAAACAATGTCACCATCTGGGACGAATGGGCCGATGAAAACGG

CGACCTCGGGCCAGTGTATGGTAAACAGTGGCGCGCCTGGCCAACGCCAG

ATGGTCGTCATATTGACCAGATCACTACGGTACTGAACCAGCTGAAAAAC

GACCCGGATTCGCGCCGCATTATTGTTTCAGCGTGGAACGTAGGCGAACT

GGATAAAATGGCGCTGGCACCGTGCCATGCATTCTTCCAGTTCTATGTGG

CAGACGGCAAACTCTCTTGCCAGCTTTATCAGCGCTCCTGTGACGTCTTC

CTCGGCCTGCCGTTCAACATTGCCAGCTACGCGTTATTGGTGCATATGAT

GGCGCAGCAGTGCGATCTGGAAGTGGGTGATTTTGTCTGGACCGGTGGCG

ACACGCATCTGTACAGCAACCATATGGATCAAACTCATCTGCAATTAAGC

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CGCGAACCGCGTCCGCTGCCGAAGTTGATTATCAAACGTAAACCCGAATC

CATCTTCGACTACCGTTTCGAAGACTTTGAGATTGAAGGCTACGATCCGC

ATCCGGGCATTAAAGCGCCGGTGGCTATCTAATTACGAAACATCCTGCCA

GAGCCGACGCCAGTGTGCGTCGGTTTTTTTACCCTCCGTTAAATTCTTCG

AGACGCCTTCCCGAAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGG

GAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGG

GGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGT

CACGACGTTGTAAAACGACGGCCAGTGCCAAGCTTTCTTTAATGAAGCAG

GGCATCAGGACGGTATCTTTGTGGAGAAAGCAGAGTAATCTTATTCAGCC

TGACTGGTGGGAAACCACCAGTCAGAATGTGTTAGCGCATGTTGACAAAA

ATACCATTAGTCACATTATCCGTCAGTCGGACGACATGGTAGATAACCTG

TTTATTATGCGTTTTGATCTTACGTTTAATATTACCTTTATGCGATGAAA

CGGTCTTGGCTTTGATATTCATTTGGTCAGAGATTTGAATGGTTCCCTGA

CCTGCCATCCACATTCGCAACATACTCGATTCGGTTCGGCTCAATGATAA

CGTCGGCATATTTAAAAACGAGGTTATCGTTGTCTCTTTTTTCAGAATAT

CGCCAAGGATATCGTCGAGAGATTCCGGTTTAATCGATTTAGAACTGATC

AATAAATTTTTTCTGACCAATAGATATTCATCAAAATGAACATTGGCAAT

TGCCATAAAAACGATAAATAACGTATTGGGATGTTGATTAATGATGAGCT

TGATACGCTGACTGTTAGAAGCATCGTGGATGAAACAGTCCTCATTAATA

AACACCACTGAAGGGCGCTGTGAATCACAAGCTATGGCAAGGTCATCAAC

GGTTTCAATGTCGTTGATTTCTCTTTTTTTAACCCCTCTACTCAACAGAT

ACCCGGTTAAACCTAGTCGGGTGTAACTACATAAATCCATAATAATCGTT

GACATGGCATACCCTCACTCAATGCGTAACGATAATTCCCCTTACCTGAA

TATTTCATCATGACTAAACGGAACAACATGGGTCACCTAATGCGCCACTC

TCGCGATTTTTCAGGCGGACTTACTATCCCGTAAAGTGTTGTATAATTTG

CCTGGAATTGTCTTAAAGTAAAGTAAATGTTGCGATATGTGAGTGAGCTT

AAAACAAATATTTCGCTGCAGGAGTATCCTGGAAGATGTTCGTAGAAGCT

TACTGCTCACAAGAAAAAAGGCACGTCATCTGACGTGCCTTTTTTATTTG

TACTACCCTGTACGATTACTGCAGCTCGAGTTTAATTCAAATCTTCTTCA

GAAATCAATTTTTGTTCTCTTACATTCAATTTAAACCAAGATTCTGGTAC

AGAATCCTTATTTATAAAATTACCTGGAGCAATCACCAGCTTATCATCAT

ATTTCGAAAGCCATGCTCCCCACCAGTAAAAAGAGCTATTCGATATTATA

TAATTACGAAAATGCATCATTAGAAACATATCATCTATAGTACCACTATC

ATCCTCCTTTTTACTTATCAAACGAACAGGATATTTTCCCGCTAGATGTT

TTTCAACCCATTCTAAATCTTGTGAAAAACAAAAGAAAACAGGAGAAGTA

ACTTTTGATGCCATAATATCCATAGCACATTTATAGTAATCATCTTCTAA

CACTCCACCAGGAGCTACATCACTTTCCTGATACCGTCTAACCCCAATCA

TTATGGCATTCTTATCTAGCAATTTTATTTCTTCCAATTCCAAATACGAT

GTGTATTCTAATTTTTTTTTGTATTACAAAGTCCTCTTTTATCTCTTGTTT

ATAATCAAGAAAATATTTTTCTGACTGCCAATATCCTTCCAGAAAAGCAT

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TCGCAAATATAACGATATGATGGATGAAGTATATGACAACCTAAATTACG

ACTTAGCCTTCTATAATAATTCCCATATGAAAAATCAAATGTTAATTTTT

TATTTTCAGGCAAGTCTAATGCAAAATATGATAATAAAAGTTTCCTTTTA

TAAACTTCATCATTTGCAAAATCAGTAGTCAAATTAAAAGCGAATGGTAC

ATTGTTTCTTAATGCCATGGCCTTCACCATAGCGTAAATAAACATTTGAT

TCCCCAATCCTCCTCGCAAAGATGATACAATCATATGTATATCTCCTTCT

TGTCTAGAATTCTAAAAATTGATTGAATGTATGCAAATAAATGCATACAC

CATAGGTGTGGTTTAATTTGATGCCCTTTTTCAGGGCTGGAATGTGTAAG

AGCGGGGTTATTTATGCTGTTGTTTTTTTGTTACTCGGGAAGGGCTTTAC

CTCTTCCGCATAAACGCTTCCATCAGCGTTTATAGTTAAAAAAATCTTTC

GGAACTGGTTTTGCGCTTACCCCAACCAACAGGGGATTTGCTGCTTTCCA

TTGAGCCTGTTTCTCTGCGCGACGTTCGCGGCGGCGTGTTTGTGCATCCA

TCTGGATTCTCCTGTCAGTTAGCTTTGGTGGTGTGTGGCAGTTGTAGTCC

TGAACGAAAACCCCCCGCGATTGGCACATTGGCAGCTAATCCGGAATCGC

ACTTACGGCCAATGCTTCGTTTCGTATCACACACCCCAAAGCCTTCTGCT

TTGAATGCTGCCCTTCTTCAGGGCTTAATTTTTAAGAGCGTCACCTTCAT

GGTGGTCAGTGCGTCCTGCTGATGTGCTCAGTATCACCGCCAGTGGTATT

TATGTCAACACCGCCAGAGATAATTTATCACCGCAGATGGTTATCTGTAT

GTTTTTTATATGAATTTATTTTTTGCAGGGGGGCATTGTTTGGTAGGTGA

GAGATCAATTCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTT

TGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCG

GTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACG

GTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAG

GCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTC

CATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCA

GAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTG

GAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATAC

CTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACG

CTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTG

TGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTAT

CGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGC

CACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGT

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ATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTC

TTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCA

AGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATC

TTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGAT

TTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATT

AAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCT

GACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCT

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TACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGAC

CCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAG

GGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTA

TTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTG

CGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTT

TGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACAT

GATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATC

GTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGC

ACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGA

CTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCG

AGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAG

AACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCT

CAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCA

CCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGC

AAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGA

AATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTAT

CAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAA

TAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACG

TCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATC

ACGAGGCCCTTTCGTC

The sequence of W3110 deltalon::Kan::lacZ with RBS *Escherichia coli* str. K-12 substr. W3110 is set forth below (SEQ ID NO: 7):

GTCCATGGAAGACGTCGAAAAAGTGGTTATCGACGAGTCGGTAATTGATG

GTCAAAGCAAACCGTTGCTGATTTATGGCAAGCCGGAAGCGCAACAGGCA

TCTGGTGAATAATTAACCATTCCCATACAATTAGTTAACCAAAAAGGGGG

GATTTTATCTCCCCTTTAATTTTTCCTCTATTCTCGGCGTTGAATGTGGG

GGAAACATCCCCATATACTGACGTACATGTTAATAGATGGCGTGAAGCAC

AGTCGTGTCATCTGATTACCTGGCGGAAATTAAACTAAGAGAGAGCTCTA

TGATTCCGGGGATCCGTCGACCTGCAGTTCGAAGTTCCTATTCTCTAGAA

AGTATAGGAACTTCAGAGCGCTTTTGAAGCTCACGCTGCCGCAAGCACTC

AGGGCGCAAGGGCTGCTAAAGGAAGCGGAACACGTAGAAAGCCAGTCCGC

AGAAACGGTGCTGACCCCGGATGAATGTCAGCTACTGGGCTATCTGGACA

AGGGAAAACGCAAGCGCAAAGAGAAAGCAGGTAGCTTGCAGTGGGCTTAC

ATGGCGATAGCTAGACTGGGCGGTTTTATGGACAGCAAGCGAACCGGAAT

TGCCAGCTGGGGCGCCCTCTGGTAAGGTTGGGAAGCCCTGCAAAGTAAAC

TGGATGGCTTTCTTGCCGCCAAGGATCTGATGGCGCAGGGGATCAAGATC

TGATCAAGAGACAGGATGAGGATCGTTTCGCATGATTGAACAAGATGGAT

TGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGAC

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TGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTC

AGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCC

TGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACG

GGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGA

CTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACC

TTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTG

CATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCG

CATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATG

ATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGG

CTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGA

TGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCA

TCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTG

GCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTT

CCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCT

ATCGCCTTCTTGACGAGTTCTTCTAATAAGGGGATCTTGAAGTTCCTATT

CCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGAAGCAGCTCCAGC

CTACATAAAGCGGCCGCTTATTTTTGACACCAGACCAACTGGTAATGGTA

GCGACCGGCGCTCAGCTGGAATTCCGCCGATACTGACGGGCTCCAGGAGT

CGTCGCCACCAATCCCCATATGGAAACCGTCGATATTCAGCCATGTGCCT

TCTTCCGCGTGCAGCAGATGGCGATGGCTGGTTTCCATCAGTTGCTGTTG

ACTGTAGCGGCTGATGTTGAACTGGAAGTCGCCGCGCCACTGGTGTGGGC

CATAATTCAATTCGCGCGTCCCGCAGCGCAGACCGTTTTCGCTCGGGAAG

ACGTACGGGGTATACATGTCTGACAATGGCAGATCCCAGCGGTCAAAACA

GGCGGCAGTAAGGCGGTCGGGATAGTTTTCTTGCGGCCCTAATCCGAGCC

AGTTTACCCGCTCTGCTACCTGCGCCAGCTGGCAGTTCAGGCCAATCCGC

GCCGGATGCGGTGTATCGCTCGCCACTTCAACATCAACGGTAATCGCCAT

TTGACCACTACCATCAATCCGGTAGGTTTTCCGGCTGATAAATAAGGTTT

TCCCCTGATGCTGCCACGCGTGAGCGGTCGTAATCAGCACCGCATCAGCA

AGTGTATCTGCCGTGCACTGCAACAACGCTGCTTCGGCCTGGTAATGGCC

CGCCGCCTTCCAGCGTTCGACCCAGGCGTTAGGGTCAATGCGGGTCGCTT

CACTTACGCCAATGTCGTTATCCAGCGGTGCACGGGTGAACTGATCGCGC

AGCGGCGTCAGCAGTTGTTTTTTATCGCCAATCCACATCTGTGAAAGAAA

GCCTGACTGGCGGTTAAATTGCCAACGCTTATTACCCAGCTCGATGCAAA

AATCCATTTCGCTGGTGGTCAGATGCGGGATGGCGTGGGACGCGGCGGGG

AGCGTCACACTGAGGTTTTCCGCCAGACGCCACTGCTGCCAGGCGCTGAT

GTGCCCGGCTTCTGACCATGCGGTCGCGTTCGGTTGCACTACGCGTACTG

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CAGCGGCTTACCATCCAGCGCCACCATCCAGTGCAGGAGCTCGTTATCGC

TATGACGGAACAGGTATTCGCTGGTCACTTCGATGGTTTGCCCGGATAAA

CGGAACTGGAAAAACTGCTGCTGGTGTTTTGCTTCCGTCAGCGCTGGATG

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TCGGCGTATCGCCAAAATCACCGCCGTAAGCCGACCACGGGTTGCCGTTT

TCATCATATTTAATCAGCGACTGATCCACCCAGTCCCAGACGAAGCCGCC

CTGTAAACGGGGATACTGACGAAACGCCTGCCAGTATTTAGCGAAACCGC

CAAGACTGTTACCCATCGCGTGGGCGTATTCGCAAAGGATCAGCGGGCGC

GTCTCTCCAGGTAGCGAAAGCCATTTTTTGATGGACCATTTCGGCACAGC

CGGGAAGGGCTGGTCTTCATCCACGCGCGCGTACATCGGGCAAATAATAT

CGGTGGCCGTGGTGTCGGCTCCGCCGCCTTCATACTGCACCGGGCGGGAA

GGATCGACAGATTTGATCCAGCGATACAGCGCGTCGTGATTAGCGCCGTG

GCCTGATTCATTCCCCAGCGACCAGATGATCACACTCGGGTGATTACGAT

CGCGCTGCACCATTCGCGTTACGCGTTCGCTCATCGCCGGTAGCCAGCGC

GGATCATCGGTCAGACGATTCATTGGCACCATGCCGTGGGTTTCAATATT

GGCTTCATCCACCACATACAGGCCGTAGCGGTCGCACAGCGTGTACCACA

GCGGATGGTTCGGATAATGCGAACAGCGCACGGCGTTAAAGTTGTTCTGC

TTCATCAGCAGGATATCCTGCACCATCGTCTGCTCATCCATGACCTGACC

ATGCAGAGGATGATGCTCGTGACGGTTAACGCCTCGAATCAGCAACGGCT

TGCCGTTCAGCAGCAGCAGACCATTTTCAATCCGCACCTCGCGGAAACCG

ACATCGCAGGCTTCTGCTTCAATCAGCGTGCCGTCGGCGGTGTGCAGTTC

AACCACCGCACGATAGAGATTCGGGATTTCGGCGCTCCACAGTTTCGGGT

TTTCGACGTTCAGACGTAGTGTGACGCGATCGGCATAACCACCACGCTCA

TCGATAATTTCACCGCCGAAAGGCGCGGTGCCGCTGGCGACCTGCGTTTC

ACCCTGCCATAAAGAAACTGTTACCCGTAGGTAGTCACGCAACTCGCCGC

ACATCTGAACTTCAGCCTCCAGTACAGCGCGGCTGAAATCATCATTAAAG

CGAGTGGCAACATGGAAATCGCTGATTTGTGTAGTCGGTTTATGCAGCAA

CGAGACGTCACGGAAAATGCCGCTCATCCGCCACATATCCTGATCTTCCA

GATAACTGCCGTCACTCCAGCGCAGCACCATCACCGCGAGGCGGTTTTCT

CCGGCGCGTAAAAATGCGCTCAGGTCAAATTCAGACGGCAAACGACTGTC

CTGGCCGTAACCGACCCAGCGCCCGTTGCACCACAGATGAAACGCCGAGT

TAACGCCATCAAAAATAATTCGCGTCTGGCCTTCCTGTAGCCAGCTTTCA

TCAACATTAAATGTGAGCGAGTAACAACCCGTCGGATTCTCCGTGGGAAC

AAACGGCGGATTGACCGTAATGGGATAGGTCACGTTGGTGTAGATGGGCG

CATCGTAACCGTGCATCTGCCAGTTTGAGGGGACGACGACAGTATCGGCC

TCAGGAAGATCGCACTCCAGCCAGCTTTCCGGCACCGCTTCTGGTGCCGG

AAACCAGGCAAAGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAG

GGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGA

TGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACG

ACGTTGTAAAACGACGGCCAGTGAATCCGTAATCATGGTCATAGTAGGTT

TCCTCAGGTTGTGACTGCAAAATAGTGACCTCGCGCAAAATGCACTAATA

AAAACAGGGCTGGCAGGCTAATTCGGGCTTGCCAGCCTTTTTTTGTCTCG

CTAAGTTAGATGGCGGATCGGGCTTGCCCTTATTAAGGGGTGTTGTAAGG

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GGATGGCTGGCCTGATATAACTGCTGCGCGTTCGTACCTTGAAGGATTCA

AGTGCGATATAAATTATAAAGAGGAAGAGAAGAGTGAATAAATCTCAATT

GATCGACAAGATTGCTGCAGGGGCTGATATCTCTAAAGCTGCGGCTGGCC

GTGCGTTAGATGCTATTATTGCTTCCGTAACTGAATCTCTGAAAGAAGG

The sequence of pG186 is set forth below (SEQ ID NO: 8):

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCG

GAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCG

TCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATG

CGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATATGCGGTGTGAAA

TACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCTCCTCAAC

CTGTATATTCGTAAACCACGCCCAATGGGAGCTGTCTCAGGTTTGTTCCT

GATTGGTTACGGCGCGTTTCGCATCATTGTTGAGTTTTTCCGCCAGCCCG

ACGCGCAGTTTACCGGTGCCTGGGTGCAGTACATCAGCATGGGGCAAATT

CTTTCCATCCCGATGATTGTCGCGGGTGTGATCATGATGGTCTGGGCATA

TCGTCGCAGCCCACAGCAACACGTTTCCTGAGGAACCATGAAACAGTATT

TAGAACTGATGCAAAAAGTGCTCGACGAAGGCACACAGAAAAACGACCGT

ACCGGAACCGGAACGCTTTCCATTTTTGGTCATCAGATGCGTTTTAACCT

GCAAGATGGATTCCCGCTGGTGACAACTAAACGTTGCCACCTGCGTTCCA

TCATCCATGAACTGCTGTGGTTTCTGCAGGGCGACACTAACATTGCTTAT

CTACACGAAAACAATGTCACCATCTGGGACGAATGGGCCGATGAAAACGG

CGACCTCGGGCCAGTGTATGGTAAACAGTGGCGCGCCTGGCCAACGCCAG

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Other Embodiments

While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. All United States patents and published or unpublished United States patent applications cited herein are incorporated by reference. All published foreign patents and patent applications cited herein are hereby incorporated by reference. Genbank and NCBI submissions indicated by accession number cited herein are hereby incorporated by reference. All other published references, documents, manuscripts and scientific literature cited herein are hereby incorporated by reference.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

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<211> LENGTH: 424
<212> TYPE: PRT
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<400> SEQUENCE: 9

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Ser Val Tyr Ile Cys Asp Glu Cys Val Asp Leu Cys Asn Asp Ile Ile
        35                  40                  45

Arg Glu Glu Ile Lys Glu Val Ala Pro His Arg Glu Arg Ser Ala Leu
    50                  55                  60

Pro Thr Pro His Glu Ile Arg Asn His Leu Asp Asp Tyr Val Ile Gly
65                  70                  75                  80

Gln Glu Gln Ala Lys Lys Val Leu Ala Val Ala Val Tyr Asn His Tyr
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Lys Arg Leu Arg Asn Gly Asp Thr Ser Asn Gly Val Glu Leu Gly Lys
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Ser Asn Ile Leu Leu Ile Gly Pro Thr Gly Ser Gly Lys Thr Leu Leu
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Gln Arg Gly Ile Val Tyr Ile Asp Glu Ile Asp Lys Ile Ser Arg Lys
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Ser Asp Asn Pro Ser Ile Thr Arg Asp Val Ser Gly Glu Gly Val Gln
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Val Ile Ser His Arg Val Glu Thr Gly Ser Gly Ile Gly Phe Gly Ala
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Thr Val Lys Ala Lys Ser Asp Lys Ala Ser Glu Gly Glu Leu Leu Ala
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Gln Val Glu Pro Glu Asp Leu Ile Lys Phe Gly Leu Ile Pro Glu Phe
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Ile Gly Arg Leu Pro Val Val Ala Thr Leu Asn Glu Leu Ser Glu Glu
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Glu Ala Leu Asp Ala Ile Ala Lys Lys Ala Met Ala Arg Lys Thr Gly
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Ala Arg Gly Leu Arg Ser Ile Val Glu Ala Ala Leu Leu Asp Thr Met
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Lys Ile Met Leu Val Ala Gln Lys Glu Ala Ser Thr Asp Glu Pro Gly
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Leu Lys Leu Pro Asp Gly Thr Val Lys Val Leu Val Glu Gly Leu Gln
                85                  90                  95

Arg Ala Arg Ile Ser Ala Leu Ser Asp Asn Gly Glu His Phe Ser Ala
            100                 105                 110

Lys Ala Glu Tyr Leu Glu Ser Pro Thr Ile Asp Glu Arg Glu Gln Glu
        115                 120                 125

Val Leu Val Arg Thr Ala Ile Ser Gln Phe Glu Gly Tyr Ile Lys Leu
    130                 135                 140

Asn Lys Lys Ile Pro Pro Glu Val Leu Thr Ser Leu Asn Ser Ile Asp
145                 150                 155                 160

Asp Pro Ala Arg Leu Ala Asp Thr Ile Ala Ala His Met Pro Leu Lys
                165                 170                 175

Leu Ala Asp Lys Gln Ser Val Leu Glu Met Ser Asp Val Asn Glu Arg
            180                 185                 190

Leu Glu Tyr Leu Met Ala Met Met Glu Ser Glu Ile Asp Leu Leu Gln
        195                 200                 205

Val Glu Lys Arg Ile Arg Asn Arg Val Lys Lys Gln Met Glu Lys Ser
    210                 215                 220

Gln Arg Glu Tyr Tyr Leu Asn Glu Gln Met Lys Ala Ile Gln Lys Glu
225                 230                 235                 240

Leu Gly Glu Met Asp Asp Ala Pro Asp Glu Asn Glu Ala Leu Lys Arg
                245                 250                 255

Lys Ile Asp Ala Ala Lys Met Pro Lys Glu Ala Lys Glu Lys Ala Glu
            260                 265                 270

Ala Glu Leu Gln Lys Leu Lys Met Met Ser Pro Met Ser Ala Glu Ala
        275                 280                 285

Thr Val Val Arg Gly Tyr Ile Asp Trp Met Val Gln Val Pro Trp Asn
    290                 295                 300

Ala Arg Ser Lys Val Lys Lys Asp Leu Arg Gln Ala Gln Glu Ile Leu
305                 310                 315                 320

Asp Thr Asp His Tyr Gly Leu Glu Arg Val Lys Asp Arg Ile Leu Glu
                325                 330                 335

Tyr Leu Ala Val Gln Ser Arg Val Asn Lys Ile Lys Gly Pro Ile Leu
            340                 345                 350

Cys Leu Val Gly Pro Pro Gly Val Gly Lys Thr Ser Leu Gly Gln Ser
        355                 360                 365

Ile Ala Lys Ala Thr Gly Arg Lys Tyr Val Arg Met Ala Leu Gly Gly
    370                 375                 380

Val Arg Asp Glu Ala Glu Ile Arg Gly His Arg Arg Thr Tyr Ile Gly
385                 390                 395                 400

Ser Met Pro Gly Lys Leu Ile Gln Lys Met Ala Lys Val Gly Val Lys
                405                 410                 415

Asn Pro Leu Phe Leu Leu Asp Glu Ile Asp Lys Met Ser Ser Asp Met
            420                 425                 430

Arg Gly Asp Pro Ala Ser Ala Leu Leu Glu Val Leu Asp Pro Glu Gln
        435                 440                 445

Asn Val Ala Phe Ser Asp His Tyr Leu Glu Val Asp Tyr Asp Leu Ser
    450                 455                 460

Asp Val Met Phe Val Ala Thr Ser Asn Ser Met Asn Ile Pro Ala Pro
465                 470                 475                 480

Leu Leu Asp Arg Met Glu Val Ile Arg Leu Ser Gly Tyr Thr Glu Asp
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                485                 490                 495

Glu Lys Leu Asn Ile Ala Lys Arg His Leu Leu Pro Lys Gln Ile Glu
            500                 505                 510

Arg Asn Ala Leu Lys Lys Gly Glu Leu Thr Val Asp Asp Ser Ala Ile
        515                 520                 525

Ile Gly Ile Ile Arg Tyr Tyr Thr Arg Glu Ala Gly Val Arg Gly Leu
    530                 535                 540

Glu Arg Glu Ile Ser Lys Leu Cys Arg Lys Ala Val Lys Gln Leu Leu
545                 550                 555                 560

Leu Asp Lys Ser Leu Lys His Ile Glu Ile Asn Gly Asp Asn Leu His
                565                 570                 575

Asp Tyr Leu Gly Val Gln Arg Phe Asp Tyr Gly Arg Ala Asp Asn Glu
            580                 585                 590

Asn Arg Val Gly Gln Val Thr Gly Leu Ala Trp Thr Glu Val Gly Gly
        595                 600                 605

Asp Leu Leu Thr Ile Glu Thr Ala Cys Val Pro Gly Lys Gly Lys Leu
    610                 615                 620

Thr Tyr Thr Gly Ser Leu Gly Glu Val Met Gln Glu Ser Ile Gln Ala
625                 630                 635                 640

Ala Leu Thr Val Val Arg Ala Arg Ala Glu Lys Leu Gly Ile Asn Pro
                645                 650                 655

Asp Phe Tyr Glu Lys Arg Asp Ile His Val His Val Pro Glu Gly Ala
            660                 665                 670

Thr Pro Lys Asp Gly Pro Ser Ala Gly Ile Ala Met Cys Thr Ala Leu
        675                 680                 685

Val Ser Cys Leu Thr Gly Asn Pro Val Arg Ala Asp Val Ala Met Thr
    690                 695                 700

Gly Glu Ile Thr Leu Arg Gly Gln Val Leu Pro Ile Gly Gly Leu Lys
705                 710                 715                 720

Glu Lys Leu Leu Ala Ala His Arg Gly Gly Ile Lys Thr Val Leu Ile
                725                 730                 735

Pro Phe Glu Asn Lys Arg Asp Leu Glu Glu Ile Pro Asp Asn Val Ile
            740                 745                 750

Ala Asp Leu Asp Ile His Pro Val Lys Arg Ile Glu Glu Val Leu Thr
        755                 760                 765

Leu Ala Leu Gln Asn Glu Pro Ser Gly Met Gln Val Val Thr Ala Lys
    770                 775                 780


<210> SEQ ID NO 11
<211> LENGTH: 264
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 11

Met Ile Glu Gln Asp Gly Leu His Ala Gly Ser Pro Ala Ala Trp Val
1               5                   10                  15

Glu Arg Leu Phe Gly Tyr Asp Trp Ala Gln Gln Thr Ile Gly Cys Ser
            20                  25                  30

Asp Ala Ala Val Phe Arg Leu Ser Ala Gln Gly Arg Pro Val Leu Phe
        35                  40                  45

Val Lys Thr Asp Leu Ser Gly Ala Leu Asn Glu Leu Gln Asp Glu Ala
    50                  55                  60

Ala Arg Leu Ser Trp Leu Ala Thr Thr Gly Val Pro Cys Ala Ala Val
65                  70                  75                  80
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Leu Asp Val Val Thr Glu Ala Gly Arg Asp Trp Leu Leu Leu Gly Glu
                85                  90                  95

Val Pro Gly Gln Asp Leu Leu Ser Ser His Leu Ala Pro Ala Glu Lys
            100                 105                 110

Val Ser Ile Met Ala Asp Ala Met Arg Arg Leu His Thr Leu Asp Pro
        115                 120                 125

Ala Thr Cys Pro Phe Asp His Gln Ala Lys His Arg Ile Glu Arg Ala
    130                 135                 140

Arg Thr Arg Met Glu Ala Gly Leu Val Asp Gln Asp Asp Leu Asp Glu
145                 150                 155                 160

Glu His Gln Gly Leu Ala Pro Ala Glu Leu Phe Ala Arg Leu Lys Ala
                165                 170                 175

Arg Met Pro Asp Gly Glu Asp Leu Val Val Thr His Gly Asp Ala Cys
            180                 185                 190

Leu Pro Asn Ile Met Val Glu Asn Gly Arg Phe Ser Gly Phe Ile Asp
        195                 200                 205

Cys Gly Arg Leu Gly Val Ala Asp Arg Tyr Gln Asp Ile Ala Leu Ala
    210                 215                 220

Thr Arg Asp Ile Ala Glu Glu Leu Gly Gly Glu Trp Ala Asp Arg Phe
225                 230                 235                 240

Leu Val Leu Tyr Gly Ile Ala Ala Pro Asp Ser Gln Arg Ile Ala Phe
                245                 250                 255

Tyr Arg Leu Leu Asp Glu Phe Phe
            260


<210> SEQ ID NO 12
<211> LENGTH: 1024
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 12

Met Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg Arg Asp
1               5                   10                  15

Trp Glu Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro
            20                  25                  30

Pro Phe Ala Ser Trp Arg Asn Ser Glu Glu Ala Arg Thr Asp Arg Pro
        35                  40                  45

Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe
    50                  55                  60

Pro Ala Pro Glu Ala Val Pro Glu Ser Trp Leu Glu Cys Asp Leu Pro
65                  70                  75                  80

Glu Ala Asp Thr Val Val Val Pro Ser Asn Trp Gln Met His Gly Tyr
                85                  90                  95

Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro
            100                 105                 110

Pro Phe Val Pro Thr Glu Asn Pro Thr Gly Cys Tyr Ser Leu Thr Phe
        115                 120                 125

Asn Val Asp Glu Ser Trp Leu Gln Glu Gly Gln Thr Arg Ile Ile Phe
    130                 135                 140

Asp Gly Val Asn Ser Ala Phe His Leu Trp Cys Asn Gly Arg Trp Val
145                 150                 155                 160

Gly Tyr Gly Gln Asp Ser Arg Leu Pro Ser Glu Phe Asp Leu Ser Ala
                165                 170                 175

Phe Leu Arg Ala Gly Glu Asn Arg Leu Ala Val Met Val Leu Arg Trp
            180                 185                 190
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Ser Asp Gly Ser Tyr Leu Glu Asp Gln Asp Met Trp Arg Met Ser Gly
        195                 200                 205

Ile Phe Arg Asp Val Ser Leu Leu His Lys Pro Thr Thr Gln Ile Ser
    210                 215                 220

Asp Phe His Val Ala Thr Arg Phe Asn Asp Asp Phe Ser Arg Ala Val
225                 230                 235                 240

Leu Glu Ala Glu Val Gln Met Cys Gly Glu Leu Arg Asp Tyr Leu Arg
                245                 250                 255

Val Thr Val Ser Leu Trp Gln Gly Glu Thr Gln Val Ala Ser Gly Thr
            260                 265                 270

Ala Pro Phe Gly Gly Glu Ile Ile Asp Glu Arg Gly Gly Tyr Ala Asp
        275                 280                 285

Arg Val Thr Leu Arg Leu Asn Val Glu Asn Pro Lys Leu Trp Ser Ala
    290                 295                 300

Glu Ile Pro Asn Leu Tyr Arg Ala Val Val Glu Leu His Thr Ala Asp
305                 310                 315                 320

Gly Thr Leu Ile Glu Ala Glu Ala Cys Asp Val Gly Phe Arg Glu Val
                325                 330                 335

Arg Ile Glu Asn Gly Leu Leu Leu Leu Asn Gly Lys Pro Leu Leu Ile
            340                 345                 350

Arg Gly Val Asn Arg His Glu His His Pro Leu His Gly Gln Val Met
        355                 360                 365

Asp Glu Gln Thr Met Val Gln Asp Ile Leu Leu Met Lys Gln Asn Asn
    370                 375                 380

Phe Asn Ala Val Arg Cys Ser His Tyr Pro Asn His Pro Leu Trp Tyr
385                 390                 395                 400

Thr Leu Cys Asp Arg Tyr Gly Leu Tyr Val Val Asp Glu Ala Asn Ile
                405                 410                 415

Glu Thr His Gly Met Val Pro Met Asn Arg Leu Thr Asp Asp Pro Arg
            420                 425                 430

Trp Leu Pro Ala Met Ser Glu Arg Val Thr Arg Met Val Gln Arg Asp
        435                 440                 445

Arg Asn His Pro Ser Val Ile Ile Trp Ser Leu Gly Asn Glu Ser Gly
    450                 455                 460

His Gly Ala Asn His Asp Ala Leu Tyr Arg Trp Ile Lys Ser Val Asp
465                 470                 475                 480

Pro Ser Arg Pro Val Gln Tyr Glu Gly Gly Gly Ala Asp Thr Thr Ala
                485                 490                 495

Thr Asp Ile Ile Cys Pro Met Tyr Ala Arg Val Asp Glu Asp Gln Pro
            500                 505                 510

Phe Pro Ala Val Pro Lys Trp Ser Ile Lys Lys Trp Leu Ser Leu Pro
        515                 520                 525

Gly Glu Thr Arg Pro Leu Ile Leu Cys Glu Tyr Ala His Ala Met Gly
    530                 535                 540

Asn Ser Leu Gly Gly Phe Ala Lys Tyr Trp Gln Ala Phe Arg Gln Tyr
545                 550                 555                 560

Pro Arg Leu Gln Gly Gly Phe Val Trp Asp Trp Val Asp Gln Ser Leu
                565                 570                 575

Ile Lys Tyr Asp Glu Asn Gly Asn Pro Trp Ser Ala Tyr Gly Gly Asp
            580                 585                 590

Phe Gly Asp Thr Pro Asn Asp Arg Gln Phe Cys Met Asn Gly Leu Val
        595                 600                 605
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Phe Ala Asp Arg Thr Pro His Pro Ala Leu Thr Glu Ala Lys His Gln
    610                 615                 620

Gln Gln Phe Phe Gln Phe Arg Leu Ser Gly Gln Thr Ile Glu Val Thr
625                 630                 635                 640

Ser Glu Tyr Leu Phe Arg His Ser Asp Asn Glu Leu Leu His Trp Met
                645                 650                 655

Val Ala Leu Asp Gly Lys Pro Leu Ala Ser Gly Glu Val Pro Leu Asp
            660                 665                 670

Val Ala Pro Gln Gly Lys Gln Leu Ile Glu Leu Pro Glu Leu Pro Gln
        675                 680                 685

Pro Glu Ser Ala Gly Gln Leu Trp Leu Thr Val Arg Val Val Gln Pro
    690                 695                 700

Asn Ala Thr Ala Trp Ser Glu Ala Gly His Ile Ser Ala Trp Gln Gln
705                 710                 715                 720

Trp Arg Leu Ala Glu Asn Leu Ser Val Thr Leu Pro Ala Ala Ser His
                725                 730                 735

Ala Ile Pro His Leu Thr Thr Ser Glu Met Asp Phe Cys Ile Glu Leu
            740                 745                 750

Gly Asn Lys Arg Trp Gln Phe Asn Arg Gln Ser Gly Phe Leu Ser Gln
        755                 760                 765

Met Trp Ile Gly Asp Lys Lys Gln Leu Leu Thr Pro Leu Arg Asp Gln
    770                 775                 780

Phe Thr Arg Ala Pro Leu Asp Asn Asp Ile Gly Val Ser Glu Ala Thr
785                 790                 795                 800

Arg Ile Asp Pro Asn Ala Trp Val Glu Arg Trp Lys Ala Ala Gly His
                805                 810                 815

Tyr Gln Ala Glu Ala Ala Leu Leu Gln Cys Thr Ala Asp Thr Leu Ala
            820                 825                 830

Asp Ala Val Leu Ile Thr Thr Ala His Ala Trp Gln His Gln Gly Lys
        835                 840                 845

Thr Leu Phe Ile Ser Arg Lys Thr Tyr Arg Ile Asp Gly Ser Gly Gln
    850                 855                 860

Met Ala Ile Thr Val Asp Val Glu Val Ala Ser Asp Thr Pro His Pro
865                 870                 875                 880

Ala Arg Ile Gly Leu Asn Cys Gln Leu Ala Gln Val Ala Glu Arg Val
                885                 890                 895

Asn Trp Leu Gly Leu Gly Pro Gln Glu Asn Tyr Pro Asp Arg Leu Thr
            900                 905                 910

Ala Ala Cys Phe Asp Arg Trp Asp Leu Pro Leu Ser Asp Met Tyr Thr
        915                 920                 925

Pro Tyr Val Phe Pro Ser Glu Asn Gly Leu Arg Cys Gly Thr Arg Glu
    930                 935                 940

Leu Asn Tyr Gly Pro His Gln Trp Arg Gly Asp Phe Gln Phe Asn Ile
945                 950                 955                 960

Ser Arg Tyr Ser Gln Gln Gln Leu Met Glu Thr Ser His Arg His Leu
                965                 970                 975

Leu His Ala Glu Glu Gly Thr Trp Leu Asn Ile Asp Gly Phe His Met
            980                 985                 990

Gly Ile Gly Gly Asp Asp Ser Trp  Ser Pro Ser Val Ser  Ala Glu Phe
        995                 1000                1005

Gln Leu  Ser Ala Gly Arg Tyr  His Tyr Gln Leu Val  Trp Cys Gln
    1010                1015                1020

Lys
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<210> SEQ ID NO 13
<211> LENGTH: 784
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 13

Met Asn Pro Glu Arg Ser Glu Arg Ile Glu Ile Pro Val Leu Pro Leu
1 5 10 15

Arg Asp Val Val Val Tyr Pro His Met Val Ile Pro Leu Phe Val Gly
20 25 30

Arg Glu Lys Ser Ile Arg Cys Leu Glu Ala Ala Met Asp His Asp Lys
35 40 45

Lys Ile Met Leu Val Ala Gln Lys Glu Ala Ser Thr Asp Glu Pro Gly
50 55 60

Val Asn Asp Leu Phe Thr Val Gly Thr Val Ala Ser Ile Leu Gln Met
65 70 75 80

Leu Lys Leu Pro Asp Gly Thr Val Lys Val Leu Val Glu Gly Leu Gln
85 90 95

Arg Ala Arg Ile Ser Ala Leu Ser Asp Asn Gly Glu His Phe Ser Ala
100 105 110

Lys Ala Glu Tyr Leu Glu Ser Pro Thr Ile Asp Glu Arg Glu Gln Glu
115 120 125

Val Leu Val Arg Thr Ala Ile Ser Gln Phe Glu Gly Tyr Ile Lys Leu
130 135 140

Asn Lys Lys Ile Pro Pro Glu Val Leu Thr Ser Leu Asn Ser Ile Asp
145 150 155 160

Asp Pro Ala Arg Leu Ala Asp Thr Ile Ala Ala His Met Pro Leu Lys
165 170 175

Leu Ala Asp Lys Gln Ser Val Leu Glu Met Ser Asp Val Asn Glu Arg
180 185 190

Leu Glu Tyr Leu Met Ala Met Met Glu Ser Glu Ile Asp Leu Leu Gln
195 200 205

Val Glu Lys Arg Ile Arg Asn Arg Val Lys Lys Gln Met Glu Lys Ser
210 215 220

Gln Arg Glu Tyr Tyr Leu Asn Glu Gln Met Lys Ala Ile Gln Lys Glu
225 230 235 240

Leu Gly Glu Met Asp Asp Ala Pro Asp Glu Asn Glu Ala Leu Lys Arg
245 250 255

Lys Ile Asp Ala Ala Lys Met Pro Lys Glu Ala Lys Glu Lys Ala Glu
260 265 270

Ala Glu Leu Gln Lys Leu Lys Met Met Ser Pro Met Ser Ala Glu Ala
275 280 285

Thr Val Val Arg Gly Tyr Ile Asp Trp Met Val Gln Val Pro Trp Asn
290 295 300

Ala Arg Ser Lys Val Lys Lys Asp Leu Arg Gln Ala Gln Glu Ile Leu
305 310 315 320

Asp Thr Asp His Tyr Gly Leu Glu Arg Val Lys Asp Arg Ile Leu Glu
325 330 335

Tyr Leu Ala Val Gln Ser Arg Val Asn Lys Ile Lys Gly Pro Ile Leu
340 345 350

Cys Leu Val Gly Pro Pro Gly Val Gly Lys Thr Ser Leu Gly Gln Ser
355 360 365

Ile Ala Lys Ala Thr Gly Arg Lys Tyr Val Arg Met Ala Leu Gly Gly

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    370                 375                 380

Val Arg Asp Glu Ala Glu Ile Arg Gly His Arg Arg Thr Tyr Ile Gly
385                 390                 395                 400

Ser Met Pro Gly Lys Leu Ile Gln Lys Met Ala Lys Val Gly Val Lys
                405                 410                 415

Asn Pro Leu Phe Leu Leu Asp Glu Ile Asp Lys Met Ser Ser Asp Met
            420                 425                 430

Arg Gly Asp Pro Ala Ser Ala Leu Leu Glu Val Leu Asp Pro Glu Gln
        435                 440                 445

Asn Val Ala Phe Ser Asp His Tyr Leu Glu Val Asp Tyr Asp Leu Ser
    450                 455                 460

Asp Val Met Phe Val Ala Thr Ser Asn Ser Met Asn Ile Pro Ala Pro
465                 470                 475                 480

Leu Leu Asp Arg Met Glu Val Ile Arg Leu Ser Gly Tyr Thr Glu Asp
                485                 490                 495

Glu Lys Leu Asn Ile Ala Lys Arg His Leu Leu Pro Lys Gln Ile Glu
            500                 505                 510

Arg Asn Ala Leu Lys Lys Gly Glu Leu Thr Val Asp Asp Ser Ala Ile
        515                 520                 525

Ile Gly Ile Ile Arg Tyr Tyr Thr Arg Glu Ala Gly Val Arg Gly Leu
    530                 535                 540

Glu Arg Glu Ile Ser Lys Leu Cys Arg Lys Ala Val Lys Gln Leu Leu
545                 550                 555                 560

Leu Asp Lys Ser Leu Lys His Ile Glu Ile Asn Gly Asp Asn Leu His
                565                 570                 575

Asp Tyr Leu Gly Val Gln Arg Phe Asp Tyr Gly Arg Ala Asp Asn Glu
            580                 585                 590

Asn Arg Val Gly Gln Val Thr Gly Leu Ala Trp Thr Glu Val Gly Gly
        595                 600                 605

Asp Leu Leu Thr Ile Glu Thr Ala Cys Val Pro Gly Lys Gly Lys Leu
    610                 615                 620

Thr Tyr Thr Gly Ser Leu Gly Glu Val Met Gln Glu Ser Ile Gln Ala
625                 630                 635                 640

Ala Leu Thr Val Val Arg Ala Arg Ala Glu Lys Leu Gly Ile Asn Pro
                645                 650                 655

Asp Phe Tyr Glu Lys Arg Asp Ile His Val His Val Pro Glu Gly Ala
            660                 665                 670

Thr Pro Lys Asp Gly Pro Ser Ala Gly Ile Ala Met Cys Thr Ala Leu
        675                 680                 685

Val Ser Cys Leu Thr Gly Asn Pro Val Arg Ala Asp Val Ala Met Thr
    690                 695                 700

Gly Glu Ile Thr Leu Arg Gly Gln Val Leu Pro Ile Gly Gly Leu Lys
705                 710                 715                 720

Glu Lys Leu Leu Ala Ala His Arg Gly Gly Ile Lys Thr Val Leu Ile
                725                 730                 735

Pro Phe Glu Asn Lys Arg Asp Leu Glu Glu Ile Pro Asp Asn Val Ile
            740                 745                 750

Ala Asp Leu Asp Ile His Pro Val Lys Arg Ile Glu Glu Val Leu Thr
        755                 760                 765

Leu Ala Leu Gln Asn Glu Pro Ser Gly Met Gln Val Val Thr Ala Lys
    770                 775                 780
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<210> SEQ ID NO 14

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<211> LENGTH: 90
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 14

Met Asn Lys Ser Gln Leu Ile Asp Lys Ile Ala Ala Gly Ala Asp Ile
1 5 10 15

Ser Lys Ala Ala Ala Gly Arg Ala Leu Asp Ala Ile Ile Ala Ser Val
20 25 30

Thr Glu Ser Leu Lys Glu Gly Asp Asp Val Ala Leu Val Gly Phe Gly
35 40 45

Thr Phe Ala Val Lys Glu Arg Ala Ala Arg Thr Gly Arg Asn Pro Gln
50 55 60

Thr Gly Lys Glu Ile Thr Ile Ala Ala Ala Lys Val Pro Ser Phe Arg
65 70 75 80

Ala Gly Lys Ala Leu Lys Asp Ala Val Asn
85 90

<210> SEQ ID NO 15
<211> LENGTH: 5049
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 15

gtccatggaa gacgtcgaaa aagtggttat cgacgagtcg gtaattgatg gtcaaagcaa 60

accgttgctg atttatggca agccggaagc gcaacaggca tctggtgaat aattaaccat 120

tcccatacaa ttagttaacc aaaaaggggg gattttatct ccccttaat ttttcctcta 180

ttctcggcgt tgaatgtggg ggaaacatcc ccatatactg acgtacatgt taatagatgg 240

cgtgaagcac agtcgtgtca tctgattacc tggcggaaat taaactaaga gagagctcta 300

tgattccggg gatccgtcga cctgcagttc gaagttccta ttctctagaa agtataggaa 360

cttcagagcg cttttgaagc tcacgctgcc gcaagcactc agggcgcaag ggctgctaaa 420

ggaagcggaa cacgtagaaa gccagtccgc agaaacggtg ctgaccccgg atgaatgtca 480

gctactgggc tatctggaca agggaaaacg caagcgcaaa gagaaagcag gtagcttgca 540

gtgggcttac atggcgatag ctagactggg cggttttatg gacagcaagc gaaccggaat 600

tgccagctgg ggcgccctct ggtaaggttg ggaagccctg caaagtaaac tggatggctt 660

tcttgccgcc aaggatctga tggcgcaggg gatcaagatc tgatcaagag acaggatgag 720

gatcgtttcg catgattgaa caagatggat tgcacgcagg ttctccggcc gcttgggtgg 780

agaggctatt cggctatgac tgggcacaac agacaatcgg ctgctctgat gccgccgtgt 840

tccggctgtc agcgcagggg cgcccggttc tttttgtcaa gaccgacctg tccggtgccc 900

tgaatgaact gcaggacgag gcagcgcggc tatcgtggct ggccacgacg ggcgttcctt 960

gcgcagctgt gctcgacgtt gtcactgaag cgggaaggga ctggctgcta ttgggcgaag 1020

tgccggggca ggatctcctg tcatctcacc ttgctcctgc cgagaaagta tccatcatgg 1080

ctgatgcaat gcggcggctg catacgcttg atccggctac ctgcccattc gaccaccaag 1140

cgaaacatcg catcgagcga gcacgtactc ggatggaagc cggtcttgtc gatcaggatg 1200

atctggacga agagcatcag gggctcgcgc cagccgaact gttcgccagg ctcaaggcgc 1260

gcatgcccga cggcgaggat ctcgtcgtga cccatggcga tgcctgcttg ccgaatatca 1320

tggtggaaaa tggccgcttt tctggattca tcgactgtgg ccggctgggt gtggcggacc 1380

gctatcagga catagcgttg gctacccgtg atattgctga agagcttggc ggcgaatggg 1440

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atcgccttct tgacgagttc ttctaataag gggatcttga agttcctatt ccgaagttcc 1560

tattctctag aaagtatagg aacttcgaag cagctccagc ctacataaag cggccgctta 1620

tttttgacac cagaccaact ggtaatggta gcgaccggcg ctcagctgga attccgccga 1680

tactgacggg ctccaggagt cgtcgccacc aatccccata tggaaaccgt cgatattcag 1740

ccatgtgcct tcttccgcgt gcagcagatg gcgatggctg gtttccatca gttgctgttg 1800

actgtagcgg ctgatgttga actggaagtc gccgcgccac tggtgtgggc cataattcaa 1860

ttcgcgcgtc ccgcagcgca gaccgttttc gctcgggaag acgtacgggg tatacatgtc 1920

tgacaatggc agatcccagc ggtcaaaaca ggcggcagta aggcggtcgg gatagttttc 1980

ttgcggccct aatccgagcc agtttacccg ctctgctacc tgcgccagct ggcagttcag 2040

gccaatccgc gccggatgcg gtgtatcgct cgccacttca acatcaacgg taatcgccat 2100

ttgaccacta ccatcaatcc ggtaggtttt ccggctgata aataaggttt tcccctgatg 2160

ctgccacgcg tgagcggtcg taatcagcac cgcatcagca agtgtatctg ccgtgcactg 2220

caacaacgct gcttcggcct ggtaatggcc cgccgccttc cagcgttcga cccaggcgtt 2280

agggtcaatg cgggtcgctt cacttacgcc aatgtcgtta tccagcggtg cacgggtgaa 2340

ctgatcgcgc agcggcgtca gcagttgttt tttatcgcca atccacatct gtgaaagaaa 2400

gcctgactgg cggttaaatt gccaacgctt attacccagc tcgatgcaaa aatccatttc 2460

gctggtggtc agatgcggga tggcgtggga cgcggcgggg agcgtcacac tgaggttttc 2520

cgccagacgc cactgctgcc aggcgctgat gtgcccggct tctgaccatg cggtcgcgtt 2580

cggttgcact acgcgtactg tgagccagag ttgcccggcg ctctccggct gcggtagttc 2640

aggcagttca atcaactgtt taccttgtgg agcgacatcc agaggcactt caccgcttgc 2700

cagcggctta ccatccagcg ccaccatcca gtgcaggagc tcgttatcgc tatgacggaa 2760

caggtattcg ctggtcactt cgatggtttg cccggataaa cggaactgga aaaactgctg 2820

ctggtgtttt gcttccgtca gcgctggatg cggcgtgcgg tcggcaaaga ccagaccgtt 2880

catacagaac tggcgatcgt tcggcgtatc gccaaaatca ccgccgtaag ccgaccacgg 2940

gttgccgttt tcatcatatt taatcagcga ctgatccacc cagtcccaga cgaagccgcc 3000

ctgtaaacgg ggatactgac gaaacgcctg ccagtattta gcgaaaccgc caagactgtt 3060

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ccatttttttg atggaccatt tcggcacagc cgggaagggc tggtcttcat ccacgcgcgc 3180

gtacatcggg caaataatat cggtggccgt ggtgtcggct ccgccgcctt catactgcac 3240

cgggcgggaa ggatcgacag atttgatcca gcgatacagc gcgtcgtgat tagcgccgtg 3300

gcctgattca ttccccagcg accagatgat cacactcggg tgattacgat cgcgctgcac 3360

cattcgcgtt acgcgttcgc tcatcgccgg tagccagcgc ggatcatcgg tcagacgatt 3420

cattggcacc atgccgtggg tttcaatatt ggcttcatcc accacataca ggccgtagcg 3480

gtcgcacagc gtgtaccaca gcggatggtt cggataatgc gaacagcgca cggcgttaaa 3540

gttgttctgc ttcatcagca ggatatcctg caccatcgtc tgctcatcca tgacctgacc 3600

atgcagagga tgatgctcgt gacggttaac gcctcgaatc agcaacggct tgccgttcag 3660

cagcagcaga ccattttcaa tccgcacctc gcggaaaccg acatcgcagg cttctgcttc 3720

aatcagcgtg ccgtcggcgg tgtgcagttc aaccaccgca cgatagagat tcgggatttc 3780

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ggcgctccac agtttcgggt tttcgacgtt cagacgtagt gtgacgcgat cggcataacc	3840
accacgctca tcgataattt caccgccgaa aggcgcggtg ccgctggcga cctgcgtttc	3900
accctgccat aaagaaactg ttacccgtag gtagtcacgc aactcgccgc acatctgaac	3960
ttcagcctcc agtacagcgc ggctgaaatc atcattaaag cgagtggcaa catggaaatc	4020
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ccacatatcc tgatcttcca gataactgcc gtcactccag cgcagcacca tcaccgcgag	4140
gcggttttct ccggcgcgta aaaatgcgct caggtcaaat tcagacggca aacgactgtc	4200
ctggccgtaa ccgacccagc gcccgttgca ccacagatga aacgccgagt taacgccatc	4260
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cacgttggtg tagatgggcg catcgtaacc gtgcatctgc cagtttgagg ggacgacgac	4440
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gcgggcctct tcgctattac gccagctggc gaaaggggga tgtgctgcaa ggcgattaag	4620
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ctctaaagct gcggctggcc gtgcgttaga tgctattatt gcttccgtaa ctgaatctct	5040
gaaagaagg	5049

What is claimed is:

1. A method for producing a fucosylated oligosaccharide in a bacterium, comprising

providing an isolated *E. coli* bacterium comprising,

(i) a deletion or functional inactivation of an endogenous β-galactosidase gene;

(ii) an exogenous functional β-galactosidase gene comprising a detectable level of β-galactosidase activity that is reduced compared to that of a wild-type *E. coli* bacterium, wherein the level of β-galactosidase activity comprises between 0.05 and 200 units;

(iii) an inactivating mutation in a colanic acid synthesis gene; and

(iv) an exogenous lactose-accepting fucosyltransferase gene;

culturing said bacterium in the presence of lactose; and

retrieving a fucosylated oligosaccharide from said bacterium or from a culture supernatant of said bacterium.

2. The method of claim **1**, wherein said colanic acid synthesis gene comprises an *E. coli* wcaJ, wzxC, wcaD, wza, wzb, or wzc gene.

3. The method of claim **2**, wherein said colanic acid synthesis gene comprises a wcaJ gene.

4. The method of claim **1**, wherein the bacterium comprises an increased intracellular guanosine diphosphate (GDP)-fucose level, wherein the increased intracellular GDP-fucose level is at least 10% more than the level of GDP-fucose in a wild-type bacterium.

5. The method of claim **1**, wherein said exogenous lactose-accepting fucosyltransferase gene encodes α(1,2) fucosyltransferase and/or α(1,3) fucosyltransferase.

6. The method of claim **5**, wherein said α(1,2) fucosyltransferase gene comprises a *Bacteroides fragilis* wcfW gene.

7. The method of claim **5**, wherein said α(1,3) fucosyltransferase gene comprises a *Helicobacter pylori* 26695 futA gene.

8. The method of claim **1**, wherein said exogenous functional β-galactosidase gene comprises an *E. coli* lacZ gene.

9. The method of claim **8**, wherein the lacZ gene is inserted into an endogenous lon gene.

10. The method of claim **1**, wherein said bacterium further comprises a functional lactose permease gene.

11. The method of claim **10**, wherein said lactose permease gene is an endogenous lactose permease gene.

12. The method of claim **10**, wherein said lactose permease gene comprises an *E. coli* lacY gene.

13. The method of claim **1**, wherein said bacterium further comprises an exogenous *E. coli* rcsA or *E. coli* rcsB gene.

14. The method of claim **1**, wherein said bacterium further comprises an inactivating mutation in a lacA gene.

15. The method of claim **1**, wherein said bacterium further comprises an exogenous sialyltransferase gene.

16. The method of claim **15**, wherein said exogenous sialyltransferase gene encodes an α(2,3)sialyl transferase.

17. The method of claim 1, wherein said bacterium further comprises a deficient sialic acid catabolic pathway comprising a null mutation in an endogenous N-acetylneuraminate lyase gene or a null mutation in an endogenous N-acetylmannosamine kinase gene.

18. The method of claim 1, wherein the level of β-galactosidase activity comprises between 0.05 and 5 units.

19. The method of claim 1, wherein said bacterium further comprises an inactivating mutation in a lon gene.

20. The method of claim 1, wherein said bacterium comprises an increased intracellular lactose level, wherein the increased intracellular lactose level is at least 10% more than the level in a wild-type bacterium.

21. The method of claim 1, wherein said exogenous functional β-galactosidase gene is an *E. coli* lacZ gene lacking an operably linked promoter, and said colanic acid synthesis gene comprises an *E. coli* wcaJ, wzxC, wcaD, wza, wzb, or wzc gene.

22. The method of claim 1, wherein said bacterium comprises the genotype of

(a) ampC::(P_{trpB}λcI$^+$), P_{lacI^q}(ΔlacI-lacZ)lacY$^+$, ΔwcaJ, thyA::Tn10, Δlon::(kan, lacZ$^+$); or

(b) ampC::(P_{trpB}λcI$^+$), P_{lacI^q}(ΔlacI-lacZ)lacY$^+$, ΔwcaJ, thyA::Tn10, Δlon::(kan, lacZ$^+$), ΔlacA.

23. The method of claim 1, wherein said exogenous functional β-galactosidase gene is inserted into an endogenous gene.

24. The method of claim 1, wherein said exogenous functional β-galactosidase gene comprises a recombinant β-galactosidase gene engineered to produce a detectable level of β-galactosidase activity that is reduced compared to the level of β-galactosidase activity in a wild-type *E. coli* bacterium.

25. The method of claim 24, wherein the level of β-galactosidase activity comprises between 0.05 and 5 units.

26. The method of claim 1, wherein the level of β-galactosidase activity comprises between 0.05 and 4 units.

27. The method of claim 1, wherein the level of β-galactosidase activity comprises between 0.05 and 3 units.

28. The method of claim 1, wherein the level of β-galactosidase activity comprises between 0.05 and 2 units.

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