(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 January 2018 (18.01.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/013967 A1

(51) International Patent Classification:
A61K 8/64 (2006.01) *C07K 14/315* (2006.01)
A61Q 11/00 (2006.01) *C12N 9/04* (2006.01)

(21) International Application Number:
PCT/US2017/042206

(22) International Filing Date:
14 July 2017 (14.07.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

62/362,788	15 July 2016 (15.07.2016)	US
62/362,809	15 July 2016 (15.07.2016)	US
62/420,328	10 November 2016 (10.11.2016)	US

(71) Applicant: **INTREXON CORPORATION** [US/US]; 1750 Kraft Drive, Suite 1400, Blacksburg, VA 24060 (US).

(72) Inventors: **HANDFIELD, Martin**; 9222 SW 41st Lane, Gainesville, FL 32608 (US). **KERS, Johan, Anthony**; 9 Avon Road, Welleskey, MA 02482 (US). **MULEY, Sheela**; 775 Longfellow Drive, Fremont, CA 94539 (US). **MAYO, Melissa**; 41 Rock Harbor Lane, Foster City, CA 94404 (US). **COLBECK, Jeffrey**; 1001 Continentals Way, Apt. 211, Belmont, CA 94002 (US). **SHARP, Robert, Eryl**; 2817 Alhambra Drive, Belmont, CA 94002 (US). **FOSMOE, Albert, G., II**; 7815 SW 43rd Place, Gainesville, FL 32608 (US). **DEFUSCO, Anthony, W.**; 2525 NW 144th Way, Newberry, FL 32669 (US).

(74) Agent: **WOODHOUSE, Scott, M.**; Medler Ferro Woodhouse & Mills PLLC, 8201 Greensboro Drive, Suite 1060, McLean, VA 22102 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2018/013967 A1

(54) Title: LANTIBIOTIC VARIANTS AND USES THEREOF

(57) Abstract: The disclosure relates to lantibiotic structural variants that have improved antimicrobial properties when compared to wild-type lantibiotics, and uses thereof. Embodiments include non- naturally occurring lantibiotics.

LANTIBIOTIC VARIANTS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of U.S. Provisional Patent Application Nos., 62/362,788 filed July 15, 2016, 62/362,809 filed July 15, 2016 and 62/420,328 filed November 10, 2016; each of which is hereby incorporated by reference in its entirety.

REFERENCE TO SEQUENCE LISTING

The present application contains a Sequence Listing which has been filed electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 12, 2017, is named 0100-0017WO1_SL.txt and is 421,465 bytes in size.

FIELD OF THE INVENTION

[0001] The present invention is generally related to the fields of molecular biology, infectious disease and polypeptide-based antibiotics. More specifically, certain aspects of the invention are related to novel lantibiotic (polypeptide) variants and nucleic acid sequences encoding one or more lantibiotic (polypeptide) variants. Certain other aspects of the invention are related to recombinant vectors comprising one or more nucleic acid sequences encoding one or more lantibiotic variants, host cells transformed with said vectors, methods for producing lantibiotic variants and methods of use thereof.

BACKGROUND OF THE INVENTION

[0002] Many strains of disease-causing bacteria have become increasingly resistant to currently available antibiotics. Healthcare-associated infections caused by multi-drug resistant pathogens are particularly vexing. Worldwide, millions suffer from antibiotic-resistant infections, which results in immense cost to the healthcare system. The need for new antibiotics has become a critical, unmet need in the medical community.

[0003] Lantibiotics, a class of antibiotics with potential clinical relevance (reviewed in Smith & Hillman, (2008) Curr. Opin. Microbial. 11:401-408), acquired their name because of the characteristic lanthionine rings that are present. Lantibiotics are also known to have various

unusual amino acids such as 2,3-didehydroalanine (Dha), 2, 3-didehydrobutyrine (Dhb), S-amino vinyi-D-cysteine (AviCys), aminobutyrate (Abu), 2-oxopropionyl, 2-oxobutyryl, and hydroxypropionyl. Hasper *et al.* (2006) Science 313:1636-1637. Mutacin 1140 ("MU1140") is one type of lantibiotic that can be produced by a particular strain of the oral microorganism *Streptococcus mutans.* Smith *et al.* (2000) Eur. J. Biochem. 267:6810-6816.

SUMMARY OF THE INVENTION

[0004] The present disclosure provides non-naturally occurring lantibiotic comprising a single amino acid mutation of MU1140, the lantibiotic having an amino acid sequence encoded by of any one of SEQ ID NOS:2 to 431.

[0005] The present disclosure also provides a non-naturally occurring lantibiotic comprising multisite amino acid mutations of MU1140, the lantibiotic being a variant as described in Figure 7.

[0006] The present disclosure also provides a non-naturally occurring lantibiotic comprising any variant as described in Figure 8: Group 1, Group 2 or Group 3. In some embodiments, the non-naturally occurring lantibiotic comprises any variant as described in Figure 8: Group 1.

[0007] The present disclosure also provides a non-naturally occurring lantibiotic comprising a single amino acid mutation of MU1140 as described in Figure 6.

[0008] The present disclosure also provides a non-naturally occurring lantibiotic comprising an amino acid sequence of MU1140 (e.g., SEQ ID NO:1156) and further comprising a mutation that is: (a) arginine at position 13 changed to asparagine (R13N); (b) phenylalanine at position 17 changed to leucine (F17L) or tyrosine (F17Y); (c) asparagine at position 18 changed to alanine (N18A); (d) tyrosine at position 20 changed to phenylalanine (Y20F); or (e) combinations thereof. In some embodiments, the non-naturally occurring lantibiotic comprises an amino acid sequence of MU1140 (e.g., SEQ ID NO:1156) and further comprises two mutations, wherein one mutation is: (a) arginine at position 13 changed to asparagine (R13N); (b) phenylalanine at position 17 changed to leucine (F17L) or tyrosine (F17Y); (c) asparagine at position 18 changed to alanine (N18A); (d) tyrosine at position 20 changed to phenylalanine (Y20F); or (e) combinations thereof. In some embodiments, the non-naturally occurring lantibiotic comprises

an amino acid sequence of MU1140 (e.g., SEQ ID NO:1156) and further comprises two mutations, wherein one mutation is arginine at position 13 changed to asparagine (R13N).

[0009] In some embodiments, the disclosure provides a non-naturally occurring lantibiotic comprising an amino acid sequence of MU1140 (e.g., SEQ ID NO:1156) and further comprises two mutations, wherein one mutation is: (a) phenylalanine at postion 1 changed to valine (F1V); (b) phenylalanine at position 1 changed to alanine (F1A); (c) phenylalanine at position 1 changed to isoleucine (F1I); (d) phenylalanine at position 1 changed to leucine (F1L); (e) phenylalanine at position 1 changed to threonine (F1T); or (f) phenylalanine at position 1 changed to tyrosine (F1Y). In some embodiments, the disclosure provides a non-naturally occurring lantibiotic comprising an amino acid sequence of MU1140 (e.g., SEQ ID NO:1156) and further comprises two mutations, wherein one mutation is phenylalanine at position 1 changed to valine (FIV).The present disclosure also provides a non-naturally occurring lantibiotic comprising an amino acid sequence of MU1140 (e.g., SEQ ID NO:1156) and further comprising two mutations including: (a) phenylalanine at position 1 changed to alanine in combination with arginine at position 13 changed to: (i) alanine (F1A R13A), (ii) valine (F1A R13V), (iii) asparagine (F1A R13N), or (iv) serine (F1A R13S); (b) phenylalanine at position 1 changed to glycine in combination with arginine at position 13 changed to: (i) glycine (F1G R13G); (c) phenylalanine a position 1 changed to histidine in combination with arginine at position 13 changed to: (i) asparagine (F1H R13N); (d) phenylalanine at position 1 changed to isoleucine in combination with arginine at position 13 changed to: (i) alanine (F1I R13A), (ii) glycine (F1I R13G), (iii) isoleucine (F1I R13I), (iv) asparagine (F1I R13N), (v) proline (F1I R13P), (vi) glutamine (F1I R13Q), (vi) glutamic acid (F1I R13S), (vii) serine (F1I R13V), or (viii) valine (F1I R13E); (e) phenylalanine at position 1 changed to leucine in combination with arginine at position 13 changed to: (i) alanine (F1L R13A), (ii) aspartic acid (F1L R13D), (iii) glycine (F1L R13G), (iv) asparagine (F1L R13N), (v) proline (F1L R13P), or (vi) glutamine (F1L R13Q); (f) phenylalanine at position 1 changed to threonine in combination with arginine at position 13 changed to: (i) alanine (F1T R13A), (ii) asparagine (F1T R13N), or (iii) valine (F1T R13V); (g) phenylalanine at position 1 changed to valine in combination with arginine at position 13 changed to: (i) alanine (F1V R13A), (ii) asparagine (F1V R13N), (iii) glutamine (F1V R13Q), (iv) aspartic acid (F1V R13D), (v) valine (F1V R13V), or (vi) proline (F1V R13P); or (h) phenylalanine at position 1 changed to tyrosine in combination with arginine at position 13 changed to: (i) aspartic acid

(F1Y R13D), or (ii) glycine (F1Y R13G). In some embodiments, the disclosure provides a non-naturally occurring lantibiotic comprising an amino acid sequence of MU1140 (e.g., SEQ ID NO:1156) and further comprising two mutations including phenylalanine at position 1 changed to valine in combination with arginine at position 13 changed to: (i) alanine (F1V R13A), (ii) asparagine (F1V R13N), (iii) glutamine (F1V R13Q), (iv) aspartic acid (F1V R13D), (v) valine (F1V R13V), or (vi) proline (F1V R13P). In some embodiments, the disclosure provides a non-naturally occurring lantibiotic comprising an amino acid sequence of MU1140 (e.g., SEQ ID NO:1156) having two mutations, wherein the two mutations consist of phenylalanine at position 1 changed to valine in combination with arginine at position 13 changed to asparagine (FIV R13N) (e.g., SEQ ID NO:1157).

[00010] The present disclosure also provides a non-naturally occurring lantibiotic comprising an amino acid sequence of MU1140 (e.g., SEQ ID NO:1156) and further comprising three mutations including: (a) phenylalanine at position 1 changed to isoleucine, in combination with arginine at position 13 changed to alanine, in combination with glycine at position 15 changed to alanine (F1I R13A G15A); (b) phenylalanine at position 1 changed to isoleucine, in combination with arginine at position 13 changed to aspartic acid, in combination with glycine at position 15 changed to alanine (F1I R13D G15A); (c) phenylalanine at position 1 changed to isoleucine, in combination with tryptophan at position 4 changed to isoleucine, in combination with arginine at position 13 changed to alanine (F1I W4I R13A); (d) phenylalanine at position 1 changed to isoleucine, in combination with tryptophan at position 4 changed to methionine, in combination with arginine at position 13 changed to aspartic acid (F1I W4M R13D); (e) phenylalanine at position 1 changed to isoleucine, in combination with tryptophan at position 4 changed to methionine, in combination with arginine at position 13 changed to asparagine (F1I W4M R13N); (f) phenylalanine at position 1 changed to isoleucine, in combination with lysine at position 2 changed to alanine, in combination with arginine at position 13 changed to alanine (F1I K2A R13A); (g) phenylalanine at position 1 changed to isoleucine, in combination with leucine at position 6 changed to valine, in combination with arginine at position 13 changed to alanine (F1I L6V R13A); (h) phenylalanine at position 1 changed to isoleucine, in combination with arginine at position 13 changed to alanine, in combination with tyrosine at position 20 changed to phenylalanine (F1I R13A Y20F); (i) phenylalanine at position 1 changed to isoleucine, in combination with arginine at position 13 changed to aspartic acid, in combination

with tyrosine at position 20 changed to phenylalanine (F1I R13D Y20F); (j) phenylalanine at position 1 changed to isoleucine, in combination with arginine at position 13 changed to asparagine, in combination with tyrosine at position 20 changed to phenylalanine (F1I R13N Y20F); (k) phenylalanine at position 1 changed to leucine, in combination with arginine at position 13 changed to asparagine, in combination with tyrosine at position 20 changed to phenylalanine (F1L R13N Y20F); (l) phenylalanine at position 1 changed to leucine, in combination with arginine at position 13 changed to alanine, in combination with tyrosine at position 20 changed to phenylalanine (F1L R13A Y20F); or (m) phenylalanine at position 1 changed to leucine, in combination with arginine at position 13 changed to aspartic acid, in combination with tyrosine at position 20 changed to phenylalanine (F1L R13D Y20F).

[00011] The present disclosure also provides a non-naturally occurring lantibiotic comprising an amino acid sequence of MU1140 (e.g., SEQ ID NO:1156) and further comprising mutations including: (a) phenylalanine at position 1 changed to isoleucine, in combination with lysine at position 2 changed to alanine, in combination with tryptophan at position 4 changed to lysine, in combination with arginine at position 13 changed to alanine (F1I K2A W4K R13A); and (b) phenylalanine at position 1 changed to isoleucine, in combination with lysine at position 2 changed to alanine, in combination with tryptophan at position 4 changed to lysine, in combination with arginine at position 13 changed to aspartic acid (F1I K2A W4K R13D).

[00012] The present disclosure also provides a non-naturally occurring lantibiotic comprising an amino acid sequence of MU1140 (e.g., SEQ ID NO:1156) and further comprising mutations including: phenylalanine at position 1 changed to isoleucine, in combination with lysine at position 2 changed to alanine, in combination with tryptophan at position 4 changed to methionine, in combination with arginine at position 13 changed to alanine, in combination with tyrosine at position 20 changed to phenylalanine (F1I K2A W4K R13A Y20F).

[00013] In some embodiments, the disclosure is directed to an antimicrobial composition comprising a non-naturally occurring lantibiotic as described herein and a pharmaceutically acceptable carrier, pharmaceutically acceptable diluent, other diluent or excipient. In some embodiments, the composition further comprises an antifungal agent, an additional antimicrobial agent, a membrane disrupting agent, or a combination thereof. In some embodiments, the

additional antimicrobial agent has Gram negative bacteriostatic or bacteriocidal activity and the membrane disrupting agent renders Gram negative bacteria susceptible to the variant lantibiotic.

[00014] In some embodiments, the one or more isolated lantibiotics are present in the composition at about 0.001, 0.01, 0.1, 1, 5, 10, 20, 30, 40, 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, or 1,000 mg/kg or mg/L.

[00015] In some embodiments, the disclosure is directed to a method of reducing reproduction of bacteria or reducing numbers of bacteria present in or on a subject, comprising administering to the subject a therapeutically effective amount of the antimicrobial composition as described herein. In some embodiments, the subject is a human.

[00016] In some embodiments, the composition is administered orally, topically, nasally, buccally, sublingually, transmucosally, rectally, transdermally, by inhalation, by injection or intrathecally. In some embodiments, the injection is intramuscular, intravenous, intrapulmonary, intramuscular, intradermal, intraperitoneal, intrathecal, or subcutaneous injection.

[00017] In some embodiments, the disclosure is directed to a preservative comprising an effective amount of the non-naturally occurring lantibiotic as described herein in a physiological solution at a pH of between 3 and 8.

[00018] In some embodiments, the disclosure is directed to a food, beverage, gum, or dentifrice composition comprising an amount of the non-naturally occurring lantibiotic as described herein sufficient to reduce the reproduction of bacteria or numbers of bacteria in the composition.

[00019] In some embodiments, the disclosure is directed to a method of reducing reproduction of bacteria or reducing numbers of bacteria present in or on a composition or object, comprising contacting the antimicrobial composition as described herein with the composition or object for a period effective to reduce reproduction of bacteria or reduce numbers of bacteria in or on the composition or object. In some embodiments, the composition is a food, beverage, gum, or dentifrice.

[00020] In some embodiments, the disclosure is directed to a purified polynucleotide comprising any one of SEQ ID NOs: 2-431 or encoding a variant as described in Figure 7 or combinations thereof. In some embodiments, the disclosure provides a purified polynucleotide comprising any one of SEQ ID NOs: 756, 757, 758, 559, 760, or 761. In some embodiments, the disclosure provides a purified polynucleotide comprising any one of SEQ ID NOs: 709, 714, 716, 735, 747, 751, 754, or 758. In some embodiments, the disclosure ir directed to a purified polynucleotide comprising SEQ ID NO: 758.

[00021] In some embodiments, the disclosure is directed to a composition comprising a solid surface or a textile with the lantibiotic composition as described herein or coated onto, immobilized, linked, or bound to the solid surface or textile.

[00022] In some embodiments, the disclosure is directed to a method of reducing a biofilm or biofouling condition comprising contacting the antimicrobial composition as described herein with the biofilm or biofouling condition for a period effective to reduce reproduction of bacteria or reduce numbers of bacteria in or on the biofilm or biofouling condition.

[00023] In some embodiments, the disclosure is directed to a kit comprising the lantibiotic as described herein and one or more applicators.

[00024] In some embodiments, the disclosure is directed to a method of preventing or treating a subject diagnosed with a bacterial infection, comprising administering the non-naturally occurring lantibiotic as described herein. In some embodiments, the subject is a human. In some embodiments, the subject is infected with a Gram-positive bacteria. In some embodiments, the Gram-positive bacteria is one or more of *Staphylococcus epidermidis*, vancomycin resistant *Enterococci*, vancomycin resistant *Enterococcus faecalis*, *Enterococcus faecalis*, *Enterococcus faecium*, *Propionibacterium acnes*, *Streptococcus salivarius*, *Streptococcus sanguis*, *Streptococcus mitis*, *Streptococcus pyogenes*, *Lactobacillus salivarius*, *Listeria monocytogenes*, *Actinomyces israelii*, *Actinomyces naeslundii*, *Actinomyces viscosus*, *Bacillus anthracis*, *Streptococcus agalactiae, Streptococcus intermedius, Streptococcus pneumoniae, Corynebacterium diphtheria, Clostridium sporogenes Clostridium botulinum, Clostridium perfringens, Clostridium tetani,* and *Clostridium difficile.* In some embodiments, the Gram-positive bacteria is *Clostridium difficile*.

[00025] In some embodiments, the subject is infected with a Gram-negative bacteria. In some embodiments, the Gram-negative bacteria is one or more of *Acinetobacter baumanii*, *Bordatella pertussis, Borrelia burgdotieri, Brucella abortus, Brucella canis, Brucella melitensis, Brucella suis, Campylobacter jejuni, Coxiella burnetii, Escherichia coli, Francisella tularensis, Haemophilus influenza, Helicobacter pylori, Klebsiella pneumoniae, Legionella pneumophila, Leptospira interrogans, Neisseria gonorrhoeae, Neisseria meningitides, Pseudomonas aeruginosa, Rickettsia rickettsii, Salmonella enteritidis, Salmonella typhi, Salmonella typhimurium, Serratia marcescens, Shigella sonnei, Treponema pallidum, Vibrio cholera, Yersinia enterocolitica,* and *Yersinia pestis*. In some embodiments, the non-naturally occurring lantibiotic further comprises one or more additional antimicrobial agents, membrane disrupting agents, or combinations thereof.

[00026] In some embodiments, the disclosure is directed to an isolated recombinant *Streptococcus mutans* strain comprising: (a) a mutation in a polynucleotide involved in lactic acid synthesis such that expression of lactic acid is diminished by about 80% or more as compared to a wildtype *S. mutans* strain; (b) a recombinant alcohol dehydrogenase polynucleotide; and (c) a recombinant polynucleotide encoding a non-naturally occurring lantibiotic as described in Figure 5 or Figure 7.

BRIEF DESCRIPTION OF THE FIGURES

[00027] **Figure 1** shows the primary amino acid sequence, secondary structure and macrocyclic rings of the wild-type (native) mature MU1140 lantibiotic polypeptide (SEQ ID NO:1156) (Figures 1A and 1B), and the primary amino acid sequence, secondary structure and macrocyclic rings of the multisite variant F1V R13N of mature MU1140 lantibiotic polypeptide, (SEQ ID NO: 1157) (Figures 1C and 1D). Abbreviations of amino acids include the following: Ala-S-Ala is lanthionine; Abu-S-Ala is 3-methyl lanthionine; Dha is α, β-didehydroalanine; Dhb is α,β-didehydrobutyrine (Smith *et al.*, Biochemistry, 42(35):10372-84 (2003)).

[00028] **Figure 2** shows a plasmid vector map of pLAN042.

[00029] **Figure 3** shows a plasmid map of pLAN126.

[00030] **Figure 4** is a listing of single amino acid variants of MU1140, including polynucleotides SEQ ID NO:1 (MU1140) and SEQ ID NOS:2-431 (variants of MU1140) which can encode the variants.

[00031] **Figure 5** is a listing of SEQ ID NO: 432 (MU1140) and single amino acid variants of MU1140.

[00032] **Figure 6** is a listing of single amino acid variants of MU1140 that were chromosomally integrated into *S. mutans*.

[00033] **Figure 7** is a listing of SEQ ID NO: 432 (MU1140) featuring single and multiple amino acid variants of MU1140.

[00034] **Figure 8:** Summary of zone clearing results of various mutants. The zone clearing ability is divided into six groups: Group 1: > activity than SM253 control strain (>0.319 cm^2 zone of clearing); Group 2: > activity than SM253 in 2 replicate experiments (1 replicate experiment; Group 3: > activity than SM253 in 1 replicate and ≤ activity in 1 replicate experiment; Group 4: > activity than SM253 control strain (>0.167 cm^2 zone of clearing but < 0.319 cm^2 zone of clearing); Group 5: ≤ activity than SM253 control (≤0.167 cm^2 zone of clearing); and Group 6: undetermined.

[00035] **Figure 9:** Example of sequences of post-translationally modified lantibiotic wild-type (SEQ ID NO: 1159) and lantibiotic variants F1V R13N (SEQ ID NO: 1158) without the secondary structures, e.g., rings, indicated.

[00036] **Figure 10A:** Sequence of full-length 63 amino acid pre-protein of MU1140 (SEQ ID NO:1160, denoting the "core peptide" residues which can be part of the mature, biologically active lantibiotic, and the "cleaved peptide" residues which are cleaved during post-translational modification. **Figure 10B:** Sequence of full-length 63 amino acid pre-protein of F1V R13N (SEQ ID NO:1161), also denoting the "core peptide" residues and the "cleaved peptide" residues.

[00037] **Figure 11A:** Nucleotide sequence of full-length 63 amino acid pre-protein of MU1140 (SEQ ID NO: 1162). **Figure 11B**: Nucleotide sequence of full-length 63 amino acid

pre-protein of variant F1V R13N (SEQ ID NO: 1163). **Figure 11C:** amino acid sequence of the cleaved protein, i.e., full-length 63 amino acid pre-protein minus the core peptide. **Figure 11D:** nucleotide sequence of the cleaved protein.

DETAILED DESCRIPTION OF THE INVENTION

Lantibiotic MU1140 Variants

[00038] Wild-type MU1140 is shown in Figure 1A and Figure 1B. The term "MU1140" refers to (1) the translated, full-length 63 amino acid pre-protein of MU1140 (SEQ ID NO:1160), (2) the translated, cleaved 22 amino acid polypeptide of MU1140 (SEQ ID NO: 432), as well as (3) the post-translationally modified biologically active MU1140 with secondary structure rings indicated (SEQ ID NO: 1156), or without secondary structure rings indicated (SEQ ID NO: 1159). SEQ ID NO: 1160 is a pre-protein form of a lantibiotic which, after proteolytic cleavage and processing by other factors present in a host cell, results in the synthesis of MU1140 (SEQ ID NO:1156 or SEQ ID NO: 1159). SEQ ID NO: 432 is the cleaved 22 amino acid protein ("core peptide") in which the post-translational modifications, e.g., positional mutations and ring formations, are not designated. For clarity, the phrase "lantibiotic comprising an amino acid sequence of MU1140" refers to various forms of the polypeptide that includes (1) the full-length 63 amino acid pre-protein of MU1140, (2) the post translated, cleaved 22 amino acid polypeptide of MU1140, as well as (3) the post-translationally modified biologically active MU1140. MU1140 can include forms of polypeptides as described above that may or may not have "lantibiotic" activity. Thus, a full-length 63 amino acid pre-protein of MU1140 which may lack lantibiotic activity still is considered a "lantibiotic comprising an amino acid sequence of MU1140," because such a pre-protein can be post-translationally modified to obtain lantibiotic acitivity.

[00039] The term "post-translated cleaved polypeptide" refers to the polypeptide cleaved off during post-translational modification that is not part of the lantibiotic (SEQ ID NO: 1164). SEQ ID NO: 1156 refers to the cleaved, post-translationally modified biologically active MU1140. The post-translationally modified biologically active MU1140 has four rings labeled

A, B, C, and D. Two of these rings are formed by lanthionine (Ala-S-Ala) residues, including one in Ring A (Ala_3-S-Ala_7) and one in Ring C (Ala_{16}-S-Ala_{21}); there is a methyl-lanthionine residue (Abu-S-Ala) that forms Ring B comprised of the alpha-aminobutyrate residue in position 8 and the Ala in position 11 (Abu_8-S-Ala_{11}); and the fourth ring, D, is comprised of the Ala in position 19 linked to an aminovinyl group by a thioether linkage (Ala_{19}-S-CH=CH-NH-). The terms "ring" or "rings" may be used interchangeably with "bridge(s)" or "linkage(s)." The term "biologically active" or "biologically functional" refers to polypeptides which can kill or inhibit the growth of gram positive or gram negative bacteria, i.e., they have antimicrobial activity.

[00040] The variants of MU1140 described herein apply to the full-length 63 amino acid pre-protein (SEQ ID NO: 1160), the post-translated, cleaved 22 amino acid polypeptide (SEQ ID NO: 432), and/or the post-translationally modified biologically active MU1140 (SEQ ID NO: 1156). Variants of MU1140 are designated herein by specifying (1) the identity (1 letter designation) of the original amino acid being altered, (2) the location of the amino acid being altered, and (3) the identity of the amino acid in the variant. For clarity, the positional nomenclature used herein refers to the relative position of the 22 amino acid protein. Thus, even when referring to the full-length 63 amino acid pre-protein, "position 1" refers to the first amino acid that would be found in the 22 amino acid post-translated cleaved protein. Figure 10A, for example, depicts the positional nomenclature of amino acids in MU1140 designated 1 through 22. For example, a variant designated "R13T" refers to a MU1140 polypeptide wherein the 13th amino acid of the 22 amino acid polypeptide (which was originally an arginine, "R") is substituted with a threonine, "T"). Likewise, if multiple amino acids of wildtype MU1140 are substituted, each of the substituted amino acid is designated in a similar way. For example, a variant designated "F1T R13T" refers to a MU1140 polypeptide wherein the 1st amino acid of the 22 amino acid polypeptide is changed from a phenylalanine, "F," to a threonine, "T," and the 13th amino acid of the 22 amino acid polypeptide is changed from an arginine, "R," to a threonine, "T." Similar naming conventions apply to variants having three, four, five or more amino acids which differ from wild-type MU1140.

[00041] In embodiments, the disclosure provides a lantibiotic comprising the following mutations of the amino acid sequence of the lantibiotic, MU1140 (e.g., SEQ ID NO:1156):

a. arginine at position 13 changed to asparagine (R13N);
b. phenylalanine at position 17 changed to leucine (F17L) or tyrosine (F17Y);
c. asparagine at position 18 changed to alanine (N18A);
d. tyrosine at position 20 changed to phenylalanine (Y20F); and
e. combinations thereof.

[00042] In some embodiments, the non-naturally occurring lantibiotic comprises an amino acid sequence of MU1140 (e.g., SEQ ID NO:1156) and further comprises two mutations, wherein one mutation is:

a. arginine at position 13 changed to asparagine (R13N);
b. phenylalanine at position 17 changed to leucine (F17L) or tyrosine (F17Y);
c. asparagine at position 18 changed to alanine (N18A);
d. tyrosine at position 20 changed to phenylalanine (Y20F); or
e. combinations thereof.

[00043] In some embodiments, the non-naturally occurring lantibiotic comprises an amino acid sequence of MU1140 (e.g., SEQ ID NO:1156) and further comprises two mutations, wherein one mutation is arginine at position 13 changed to asparagine (R13N).

[00044] In some embodiments, the disclosure provides a non-naturally occurring lantibiotic comprising an amino acid sequence of MU1140 (e.g., SEQ ID NO:1156) and further comprises two mutations, wherein one mutation is:

a. phenylalanine at postion 1 changed to valine (F1V);
b. phenylalanine at position 1 changed to alanine (F1A);
c. phenylalanine at position 1 changed to isoleucine (F1I);
d. phenylalanine at position 1 changed to leucine (F1L);
e. phenylalanine at position 1 changed to threonine (F1T); or
f. phenylalanine at position 1 changed to tyrosine (F1Y).

[00045] In some embodiments, the disclosure provides a non-naturally occurring lantibiotic comprising an amino acid sequence of MU1140 (e.g., SEQ ID NO:1156) and further comprises two mutations, wherein one mutation is phenylalanine at position 1 changed to valine (FIV).

[00046] In some embodiments, the disclosure provides a non-naturally occurring lantibiotic comprising an amino acid sequence of MU1140 (e.g., SEQ ID NO:1156) and further comprises two mutations, wherein one mutation is at position 1 and one mutation is at position 13. In some embodiments, the disclosure provides a non-naturally occurring lantibiotic comprising an amino acid sequence of MU1140 (e.g., SEQ ID NO:1156) and further comprises two mutations, wherein the phenylalanine at position 1 is changed to valine, and the arginine at position 13 is changed to asparagine (F1V R13N). See, e.g., SEQ ID NO: 550.

[00047] In embodiments, the disclosure provides variants of MU1140 (e.g., SEQ ID NO:1156) having two or more of the above mutations.

[00048] In embodiments, the disclosure provides non-naturally occurring lantibiotic comprising the following two mutations of the amino acid sequence of the lantibiotic MU1140 (e.g., SEQ ID NO:1156):

a. phenylalanine at position 1 changed to alanine in combination with arginine at position 13 changed to:
    i. alanine (F1A R13A),
    ii. valine (F1A R13V),
    iii. asparagine (F1A R13N), or
    iv. serine (F1A R13S),
b. phenylalanine at position 1 changed to glycine in combination with arginine at position 13 changed to:
    i. glycine (F1G R13G);
c. phenylalanine a position 1 changed to histidine in combination with arginine at position 13 changed to:
    i. asparagine (F1H R13N);
d. phenylalanine at position 1 changed to isoleucine in combination with arginine at position 13 changed to:
    i. alanine (F1I R13A);
    ii. glycine (F1I R13G);
    iii. isoleucine (F1I R13I);

iv. asparagine (F1I R13N);
v. proline (F1I R13P);
vi. glutamine (F1I R13Q);
vii. glutamic acid (F1I R13S);
viii. serine (F1I R13V); or
ix. valine (F1I R13E);

e. phenylalanine at position 1 changed to leucine in combination with arginine at position 13 changed to:
i. alanine (F1L R13A);
ii. aspartic acid (F1L R13D);
iii. glycine (F1L R13G);
iv. asparagine (F1L R13N);
v. proline (F1L R13P); or
vi. glutamine (F1L R13Q);

f. phenylalanine at position 1 changed to threonine in combination with arginine at position 13 changed to:
i. alanine (F1T R13A);
ii. asparagine (F1T R13N); or
iii. valine (F1T R13V);

g. phenylalanine at position 1 changed to valine in combination with arginine at position 13 changed to:
i. alanine (F1V R13A);
ii. asparagine (F1V R13N);
iii. glutamine (F1V R13Q);
iv. aspartic acid (F1V R13D);
v. valine (F1V R13V); or
vi. proline (F1V R13P);

h. phenylalanine at position 1 changed to tyrosine in combination with arginine at position 13 changed to:
i. aspartic acid (F1Y R13D); or
ii. glycine (F1Y R13G).

[00049] In some embodiments, the disclosure provides non-naturally occurring lantibiotic comprising the amino acid sequence of the lantibiotic MU1140 (e.g., SEQ ID NO:1156), wherein the phenylalanine at position 1 is changed to valine and arginine at position 13 changed to:

i. alanine (F1V R13A);
ii. asparagine (F1V R13N);
iii. glutamine (F1V R13Q);
iv. aspartic acid (F1V R13D);
v. valine (F1V R13V); or
vi. proline (F1V R13P).

[00050] In embodiments, the disclosure provides a non-naturally occurring lantibiotic having the following combination of three mutations of MU1140 (e.g., SEQ ID NO:1156):

a. phenylalanine at position 1 changed to isoleucine, in combination with arginine at position 13 changed to alanine, in combination with glycine at position 15 changed to alanine (F1I R13A G15A);
b. phenylalanine at position 1 changed to isoleucine, in combination with arginine at position 13 changed to aspartic acid, in combination with glycine at position 15 changed to alanine (F1I R13D G15A);
c. phenylalanine at position 1 changed to isoleucine, in combination with tryptophan at position 4 changed to isoleucine, in combination with arginine at position 13 changed to alanine (F1I W4I R13A);
d. phenylalanine at position 1 changed to isoleucine, in combination with tryptophan at position 4 changed to methionine, in combination with arginine at position 13 changed to aspartic acid (F1I W4M R13D);
e. phenylalanine at position 1 changed to isoleucine, in combination with tryptophan at position 4 changed to methionine, in combination with arginine at position 13 changed to asparagine (F1I W4M R13N);
f. phenylalanine at position 1 changed to isoleucine, in combination with lysine at position 2 changed to alanine, in combination with arginine at position 13 changed to alanine (F1I K2A R13A);

g. phenylalanine at position 1 changed to isoleucine, in combination with leucine at position 6 changed to valine, in combination with arginine at position 13 changed to alanine (F1I L6V R13A);
h. phenylalanine at position 1 changed to isoleucine, in combination with arginine at position 13 changed to alanine, in combination with tyrosine at position 20 changed to phenylalanine (F1I R13A Y20F);
i. phenylalanine at position 1 changed to isoleucine, in combination with arginine at position 13 changed to aspartic acid, in combination with tyrosine at position 20 changed to phenylalanine (F1I R13D Y20F);
j. phenylalanine at position 1 changed to isoleucine, in combination with arginine at position 13 changed to asparagine, in combination with tyrosine at position 20 changed to phenylalanine (F1I R13N Y20F);
k. phenylalanine at position 1 changed to leucine, in combination with arginine at position 13 changed to asparagine, in combination with tyrosine at position 20 changed to phenylalanine (F1L R13N Y20F);
l. phenylalanine at position 1 changed to leucine, in combination with arginine at position 13 changed to alanine, in combination with tyrosine at position 20 changed to phenylalanine (F1L R13A Y20F); or
m. phenylalanine at position 1 changed to leucine, in combination with arginine at position 13 changed to aspartic acid, in combination with tyrosine at position 20 changed to phenylalanine (F1L R13D Y20F).

[00051] In embodiments, the disclosure provides variants of MU1140 (e.g., SEQ ID NO:1156) having the following combination of mutations:

a. phenylalanine at position 1 changed to isoleucine, in combination with lysine at position 2 changed to alanine, in combination with tryptophan at position 4 changed to lysine, in combination with arginine at position 13 changed to alanine (F1I K2A W4K R13A); and
b. phenylalanine at position 1 changed to isoleucine, in combination with lysine at position 2 changed to alanine, in combination with tryptophan at position 4 changed

to lysine, in combination with arginine at position 13 changed to aspartic acid (F1I K2A W4K R13D).

[00052] In embodiments, the disclosure provides variants of MU1140 (e.g., SEQ ID NO:1156) having the following combination of mutations including phenylalanine at position 1 changed to isoleucine, in combination with lysine at position 2 changed to alanine, in combination with tryptophan at position 4 changed to methionine, in combination with arginine at position 13 changed to alanine, in combination with tyrosine at position 20 changed to phenylalanine (F1I K2A W4K R13A Y20F).

[00053] In embodiments, the disclosure provides a non-naturally occurring lantibiotic comprising a single amino acid mutation of MU1140, the lantibiotic having an amino acid sequence encoded by a polynucleotide comprising any one of SEQ ID NOS:2 to 431. The disclosure also provides a polynucleotide sequence encoding a full-length 63 amino acid pre-protein comprising an amino acid sequence encoded by of any one of SEQ ID NOS.: 2 to 431 or 708 to 763. For example, a polynucleotide sequence encoding a pre-protein polypeptide of SEQ ID NO. 764 can comprise SEQ ID NO. 1165 and SEQ ID NO. 2. In embodiments, the disclosure provides a non-naturally occurring lantibiotic comprising one or more amino acid mutations of MU1140, the lantibiotic having an amino acid sequence comprising any one of SEQ ID NOS:433-707. In embodiments, the disclosure provides a non-naturally occurring lantibiotic comprising any variant in **Figure 8**: Group 1, Group 2 or Group 3. In embodiments, the disclosure provides a non-naturally occurring lantibiotic comprising any variant in **Figure 8**: Group 1. The disclosure also provides a non-naturally occurring full-length 63 amino acid pre-protein comprising any variant in Figure 8, Group 1, Group 2, or Group 3. The disclosure also provides a non-naturally occurring full-length 63 amino acid pre-protein comprising any variant in Figure 8, Group 1.

[00054] The lantibiotic variants in Figure 5 comprise single amino acid variants. In some embodiments, the disclosure is directed to a non-naturally occurring lantibiotic comprising a single amino acid mutation of MU1140 as found in **Figure 5**. The disclosure also provides for a non-naturally occurring full-length 63 amino acid pre-protein form of the lantibiotic variants described in Figure 5. For example, a pre-protein form of SEQ ID NO: 766 can comprise SEQ

ID NO: 1164 and SEQ ID NO:766, a pre-protein form of SEQ ID NO: 767 can comprise SEQ ID NO: 1164 and SEQ ID NO: 767 and so on.

[00055] In some embodiments, the disclosure provides a lantibiotic variant as found in **Figure** 7. The lantibiotic variants in Figure 7 comprise the "core peptide." The disclosure also provides for a non-naturally occurring full-length 63 amino acid pre-protein form of the "core peptide" variants as described in Figure 7. For example, a pre-protein form of SEQ ID NO: 703 can comprise SEQ ID NO: 1164 and SEQ ID NO:703.

[00056] Lantibiotics of the disclosure comprise lantibiotic variants, which can be non-naturally occurring lantibiotic variants. The terms "lantibiotic variant," "MU1140 variant," "non-naturally occurring lantibiotics," or "variant" are interchangeable and refer to an MU1140 (i.e., wild-type lantibiotic) polypeptide (SEQ ID NO:432), having one or more (e.g., 1, 2, 3, 4, 5, or more amino acid substitutions (including modified amino acid substitutions), deletions, or insertions. The term "modified amino acid substitutions" include a substitution of an amino acid with a modified amino acid. In some embodiments, the term "lantibiotic variant," "MU1140 variant," "non-naturally occurring lantibiotics," or "variant" refers to the translated, full-length 63 amino acid pre-protein of MU1140 (SEQ ID NO:1160) having one or more (e.g., 1, 2, 3, 4, 5, or more amino acid substitutions (including modified amino acid substitutions), deletions, or insertions. In some embodiments, the term "lantibiotic variant," "MU1140 variant," "non-naturally occurring lantibiotics," or "variant" refers to a mature, biologically acitve MU1140 (i.e., wild-type lantibiotic) polypeptide which has been post-translationally modified (SEQ ID NO: 1156) having one or more (e.g., 1, 2, 3, 4, 5, or more amino acid substitutions (including modified amino acid substitutions), deletions, or insertions. Modified amino acids include, for example, 2,3-didehydroalanine (Dha), 2, 3-didehydrobutyrine (Dhb), S-amino vinyi-D-cysteine (AviCys), aminobutyrate (Abu), 2-oxopropionyl, 2-oxobutyryl, and hydroxypropionyl. As used herein, "single-site variant(s)" refers to an MU1140 (i.e., wild-type lantibiotic) polypeptide (e.g., SEQ ID NOs:432 or 1156) having one amino acid substitution (including modified amino acid substitutions), deletion, or insertion and "multi-site variant(s)" refers to an MU1140 (i.e., wild-type lantibiotic) polypeptide (e.g., SEQ ID NOs: 432 or 1156) having more than one (e.g., 2, 3, 4, 5, or more amino acid substitutions (including modified amino acid substitutions), deletions, or insertions. In some embodiments, the "wild-type" lantibiotic MU1140 (i.e., wild-type

lantibiotic) polypeptide (SEQ ID NO:432) or any of the variants described herein can be post-translationally modified (e.g., SEQ ID NO: 1156). Post-translational modifications are described in U.S. Pat. No. 6,964,760, incorporated by reference herein in its entirety. Various post-translational modifications can include, e.g., the presence of dehydrated residues and lanthionine rings. In some embodiments, the modified residues 2,3-didehydroalanine (Dha) and 2,3-didehydrobutyrine (Dhb) can be formed from dehydration of serines and threonines, respectively. In some embodiments, the post-translational modification can include the modified residue S-2-aminobutyric acid (Abu) formed from threonine. In some embodiments, the post-translational modification can include the cyclization between a cysteine and either a Dha or a Dhb to form a lanthionine or a methyllanthionine ring, respectively. In some embodiments, the serine at position 3, 5, 16, and/or 19 are modified to Dha residues, position 8 is modified to Abu, and position 14 is modified to Dhb. In some embodiments, the post-translational modification occurs in any of the host cells described herein, e.g., in some embodiments, the lantibiotic variants as provided herein can be modified by *Streptococcus mutans*.

[00057] In some embodiments, the post-translation modification comprises Dha at position 5. In some embodiments, the post-translation modification comprises Abu at position 8. In some embodiments, the post-translation modification comprises Dhb at position 14. In some embodiments, the post-translation modification comprises Dha at position 5, Abu at position 8, and Dhb at position 14. In some embodiments, the post-translation modification comprises a ring formed by lanthionine (Ala-S-Ala) residues between (Ala_3-S-Ala_7) as described in Figure 1 (Ring A). In some embodiments, the post-translation modification comprises a methyl-lanthionine residue (Abu-S-Ala) forming a ring, Ring B, comprising the alpha-aminobutyrate residue in position 8 and the Ala in position 11 (Abu_8-S-Ala_{11}) as described in Figure 1. In some embodiments, the post-translation modification comprises a ring formed by lanthionine (Ala-S-Ala) residues between (Ala_{16}-S-Ala_{21}) as described in Figure 1 (Ring C). In some embodiments, the post-translation modification comprises Ala in position 19 linked to an aminovinyl group by a thioether linkage (Ala_{19}-S-CH=CH-NH-), Ring D, as described in Figure 1. In some embodiments, the post-translation modification comprises Ring A, B, C and D. In some embodiments, the post-translationally modified MU1140 variant comprises Ring A, Ring B, Ring C and Ring D, wherein: (a) two of these rings are formed by lanthionine (Ala-S-Ala) residues, including one in Ring A (Ala_3-S-Ala_7) and one in Ring C (Ala_{16}-S-Ala_{21}); (b) a methyl-

lanthionine residue (Abu-S-Ala) forms Ring B comprising the alpha-aminobutyrate residue in position 8 and the Ala in position 11 (Abu_8-S-Ala_{11}); and (c) the fourth ring, D, is comprised of the Ala in position 19 linked to an aminovinyl group by a thioether linkage (Ala_{19}-S-CH=CH-NH-), and wherein amino acid at position 5 is modified to Dha, the amino acid at position 8 is modified to Abu, and the amino acid at position 14 is modified to Dhb. In some embodiments, the isolated lantibiotic variant is produced in a host cell by expressing a polypeptide from a polynucleotide encoding a lantibiotic variant of Figure 5 or Figure 7, and wherein the expressed polypeptide is post-translationally modified in the host cell. In some embodiments, the host cell is *S. mutans*. In some embodiments, the post-translationally modified lantibiotic variant comprises an amino acid sequence of SEQ ID NO. 1157. In some embodiments, the post-translationally modified lantibiotic variant comprises an amino acid sequence of SEQ ID NO. 1158 (which does not designate secondary structures, e.g., rings). In some embodiments, the post-translationally modified lantibiotic variant comprises an amino acid sequence of SEQ ID NO. 1161.

[00058] For example, in some embodiments, the lantibiotic variant can include the polypeptide of Figure 1, SEQ ID NO: 1157. In some embodiments, the lantibiotic variant can include the polypeptide of Figure 9, SEQ ID NO: 1158. In some embodiments, the lantibiotic variant can include the polypeptide of SEQ ID NO: 1161.

[00059] In some embodiments, the disclosure provides for an isolated lantibiotic variant, wherein the isolated lantibiotic variant is produced in a host cell by expressing a polypeptide from a polynucleotide encoding a lantibiotic of **Figure 5** or **Figure 7**, wherein the expressed polypeptide can be post-translationally modified in the host cell. Thus, for clarity, the disclosure provides for the lantibiotic variants described herein, as well as lantibiotic variants having post-translational modifications. In some embodiments, the host cell is *S. mutans*. In some embodiments, the disclosure provides a post-translationally modified, non-naturally occurring lantibiotic comprising an amino acid sequence of SEQ ID NO. 1157.

[00060] In embodiments, biologically active equivalents of the lantibiotic variants of the disclosure also have one or more conservative amino acid variations or other minor modifications and retain biological activity in addition to the amino acid changes disclosed

above. A biologically active equivalent has substantially equivalent function when compared to the corresponding lantibiotic. In embodiments, a lantibiotic variant of the disclosure has about 1, 2, 3, 4, or 5 conservative amino acid substitutions. A conservative substitution is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and general nature of the polypeptide to be substantially unchanged. Examples of conservative substitutions can be found, e.g., in Yampolsky, et al., Genetics 170(4): 1459–1472 (2005). In some embodiments, conservative substitutions can be characterized are according to their class, e.g.,

Class	**Name of Amino Acids**
Aliphatic	Glycine, Alanine, Valine, Leucine, Isoleucine
Hydroxyl or Sulfur/Selenium-containing	Serine, Cysteine, Selenocysteine, Threonine, Methionine
Cyclic	Proline
Aromatic	Phenylalanine, Tyrosine, Tryptophan
Basic	Histidine, Lysine, Arginine
Acidic and their Amide	Aspartate, Glutamate, Asparagine, Glutamine

[00061] In some embodiments, the conservative substitutions can be made within the following groups:

Group 1: charged positives (R-Arg, H-His and K-Lys)
Group 2: charged negatives (D-Asp and E-Glu)
Group 3: polar uncharged (S-Ser, T-Thr, N-Asn, Q-Gln)
Group 4: hydrophobic (A-Ala, V-Val, I-Ile, L-Leu, M-Met, F-Phe, Y-Tyr, W-Trp)
Group 5: "others" (C-Cys, G-Gly, P-Pro).

For example, a conservative substitution can be a substitution of H for R because both H and R are in Group 1. By way of example, in some embodiments, the disclosure provides for variants of lantibiotics wherein the phenylalanine of position 1 is substituted with an aliphatic class residue, e.g., glycine, alanine, valine, leucine, isoleucine. The disclosure also provides for variants of lantibiotics wherein the arginine of position 13 is substituted with an acidic and their amide class residue, e.g., aspartate, glutamate, asparagine, glutamine.

[00062] In embodiments, the lantibiotics of the disclosure are polypeptides comprising post-translational modifications, i.e., chemical or biochemical modifications after the polypeptide has been translated.

[00063] A purified lantibiotic is a lantibiotic preparation that is substantially free of cellular material, other types of polypeptides, chemical precursors, chemicals used in synthesis of the polypeptide, or combinations thereof. A purified lantibiotic preparation that is substantially free of cellular material, culture medium, chemical precursors, chemicals used in synthesis of the polypeptide, etc., has less than about 30%, 20%, 10%, 5%, 1% of other polypeptides, culture medium, chemical precursors, and/or other chemicals used in synthesis. Therefore, a purified lantibiotic is about 70%, 80%, 90%, 95%, 99% or more pure. A purified lantibiotic does not include unpurified or semi-purified cell extracts or mixtures of polypeptides that are less than 70% pure.

[00064] A lantibiotic of the disclosure can be covalently or non-covalently linked to an amino acid sequence to which the lantibiotic is not normally associated with in nature, i.e., a heterologous amino acid sequence. For example, a heterologous amino acid sequence is from an organism that does not naturally produce a lantibiotic, a synthetic sequence, or a sequence not usually located at the carboxy or amino terminus of a naturally occurring lantibiotic. Additionally, a lantibiotic of the disclosure can be covalently or non-covalently linked to compounds or molecules other than amino acids such as indicator reagents. A lantibiotic of the disclosure can be covalently or non-covalently linked to an amino acid spacer, an amino acid linker, a signal sequence, a stop transfer sequence, transfer-messenger RNA (TMR) stop transfer sequence, a transmembrane domain, a protein purification ligand, or a combination thereof. A polypeptide can also be linked to a moiety (i.e., a functional group that can be a polypeptide or other compound) that facilitates purification (e.g., affinity tags such as a six-histidine tag (SEQ ID NO: 1166), trpE, glutathione-S-transferase, maltose binding protein, staphylococcal Protein A), or a moiety that facilitates polypeptide stability (e.g., polyethylene glycol; amino terminus protecting groups such as acetyl, propyl, succinyl, benzyl, benzyloxycarbonyl or t-butyloxycarbonyl; carboxyl terminus protecting groups such as amide, methylamide, and ethylamide). In one embodiment of the disclosure a protein purification ligand can be one or more amino acid residues at, for example, the amino terminus or carboxy terminus of a polypeptide of the disclosure. An amino acid spacer is a sequence of amino acids that are not associated with a polypeptide of the disclosure in nature. An amino acid spacer can comprise about 1, 5, 10, 20, 100, or 1,000 amino acids.

[00065] In embodiments, a lantibiotic of the disclosure is part of a fusion protein, which can contain heterologous amino acid sequences. Heterologous amino acid sequences can be present at the C or N terminus of a lantibiotic of the disclosure to form a fusion protein. In embodiments, more than one lantibiotic of the disclosure is present in a fusion protein. Fragments of lantibiotics of the disclosure can be present in a fusion protein of the disclosure. A fusion protein of the disclosure can comprise one or more lantibiotic of the disclosure, fragments thereof, or combinations thereof.

[00066] Pharmaceutically acceptable salts, esters, amides, and prodrugs are carboxylate salts, amino acid addition salts, esters, amides, and prodrugs of the lantibiotics are part of the present disclosure. These compounds are suitable for use with subjects and do not cause undue toxicity, irritation, or allergic response, are commensurate with a reasonable benefit/risk ratio, and are effective for their intended use. Salts are the substantially non-toxic, inorganic and organic acid addition salts of lantibiotics of the disclosure. Salts include, for example, hydrobromide, hydrochloride, sulfate, bisulfate, nitrate, acetate, trifluoroacetate, formate, oxalate, valerate, oleate, palmitate, stearate, laurate, borate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate mesylate, glucoheptonate, lactobionate and laurylsulphonate salts, and the like. These may include cations based on the alkali and alkaline earth metals, such as sodium, lithium, potassium, calcium, magnesium, and the like, as well as non-toxic ammonium, quaternary ammonium and amine cations including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like.

[00067] Pharmaceutically acceptable, non-toxic esters of lantibiotics of the disclosure include, for example, C1-C6 alkyl esters wherein the alkyl group is a straight or branched chain. Other esters include C5-C7 cycloalkyl esters as well as arylalkyl esters such as, but not limited to benzyl C1-C4 alkyl esters.

[00068] Pharmaceutically acceptable, non-toxic amides of lantibiotics of the disclosure include amides derived from ammonia, primary C1-C6 alkyl amines and secondary C1-C6 dialkyl amines wherein the alkyl groups are straight or branched chains. In the case of secondary amines, the amine may be in the form of a 5- or 6-membered heterocycle containing one nitrogen

atom. Also included are amides derived from ammonia, C1-C3 alkyl primary amines, and C1-C2 dialkyl secondary amines.

Systems for Producing Lantibiotics

[00069] In some embodiments, a lantibiotic polypeptide of the disclosure can be synthesized using DPOLT methodologies. See e.g., U.S. Pat. No. 7,521,529; U.S. Publ. No. 2009/0215985, each incorporated herein by reference in their entireties. A lantibiotic of the disclosure can be produced recombinantly. In embodiments, a polynucleotide encoding a lantibiotic of the disclosure is introduced into a recombinant expression vector, which is expressed in a suitable expression host cell system using techniques well known in the art. A variety of bacterial, yeast, plant, mammalian, and insect expression systems are available in the art and any such expression system can be used. A lantibiotic of the disclosure can also be purified from *S. mutans* cell culture.

[00070] As noted above, the disclosure provides lantibiotics that are produced recombinantly by expression in a suitable expression host cell system using techniques well known in the art. In embodiments, native lantibiotic-producing bacteria are used to produce the lantibiotics of the disclosure. Such bacteria include, but are not limited to, *Streptococcus mutans*, *Lactococcus lactis, Bacillus subtilis, Streptococcus pyogenes, Staphylococcus epidermis, Staphylococcus gallinarium, Micrococcus varians, Streptococcus salivarius, Lactobacillus sakei, Streptomyces OH-4156, Lactobacillus plantarum, Butyrivibrio fibriosolvens, Streptomyces cinnamoneus, Streptoverticillium hachijoense, Streptoverticillium, Streptomyces griseoluteus, Bacillus sp. strain HIL Y-85, Actinoplanes linguriae, Staphylococcus aureus, Enterococcus faecalis, Ruminococcus gnavus, Carnobacterium piscicola, Streptococcus macedonicus, Streptococcus bovis, Staphylococcus warneri, Streptomyces coelicolor* and *Streptomyces spp.* In some embodiments, the lantibiotic variants described herein can be post-translationally modified by any of the bacteria listed above.

[00071] Systems for producing lantibiotics of the disclosure also include known bacteria or yeast that are genetically tractable hosts and are capable of heterologous production of lantibiotics, i.e., yeast or bacteria that do not natively produce lantibiotics. Such organisms include, but are not limited to, *E. coli* and bacteria of the genus *Pseudomonas*, a Gram-negative

bacteria, and yeasts of the genus *Saccharomyces or Pichia*. In embodiments, *P. fluorescens* is used to produce the lantibiotics of the disclosure.

[00072] In embodiments, the disclosure provides recombinant bacterial strains that produce a lantibiotic of the disclosure. For example, the disclosure provides recombinant bacterial strains that produce lantibiotics, as listed above, that comprise a polynucleotide that expresses a functional variant MU1140. Biological activity of a lantibiotic variant of MU1140 can be assayed using methods known in the art, e.g., zone of inhibition assays.

[00073] In some embodiments, the disclosure is directed to an isolated recombinant *Streptococcus mutans* strain comprising: (a) a mutation in a polynucleotide involved in lactic acid synthesis such that expression of lactic acid is diminished by about 80% or more as compared to a wildtype *S. mutans* strain; (b) a recombinant alcohol dehydrogenase polynucleotide; and (c) a recombinant polynucleotide encoding a non-naturally occurring lantibiotic as described herein, e.g., a lantibiotic of **Figure 5** and **Figure 7**.

Polynucleotides

[00074] Polynucleotides of the disclosure contain less than an entire microbial genome and can be single- or double-stranded nucleic acids. A polynucleotide can be RNA, DNA, cDNA, genomic DNA, chemically synthesized RNA or DNA or combinations thereof. The polynucleotides can be purified free of other components, such as proteins, lipids and other polynucleotides. For example, the polynucleotide can be 50%, 75%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% purified. A nucleic acid molecule existing among hundreds to millions of other nucleic acid molecules within, for example, cDNA or genomic libraries, or gel slices containing a genomic DNA restriction digest are not to be considered an isolated polynucleotide. The polynucleotides of the disclosure encode the polypeptides described above. SEQ ID NO: 1162 encodes the 63 amino acid pre-protein of MU1140. SEQ ID NO: 1 encodes the core protein of wild-type MU1140. SEQ ID NO: 1165 encodes the cleaved peptide as described herein. In embodiments, the disclosure provides purified polynucleotides shown in SEQ ID NOs: 2-431. In embodiments, the disclosure provides purified polynucleotides shown in SEQ ID NOs: 708-763. In some embodiments, the disclosure provides a purified polynucleotide comprising any one of SEQ ID NOs: 756, 757, 758, 759, 760 or 761. In some embodiments, the disclosure provides a purified polynucleotide comprising any one of SEQ ID NOs: 709, 714, 716, 735, 747, 751, 754, or 758. In some embodiments, the disclosure provides a purified polynucleotide comprising SEQ ID NO: 758 (FIV R13N). In some embodiments, the disclosure provides a purified polynucleotide comprising SEQ ID NO: 1163 (FIV R13N).

[00075] The disclosure herein provides for polynucleotides encoding the 63 amino acid pre-protein variants of MU1140. Thus, the disclosure provides for any of the nucleotides described herein encoding the cleaved peptide (SEQ ID NO 1165).

[00076] Polynucleotides of the disclosure can consist of less than about 66, 60, 50, 45, 30, 15 (or any range between about 66 and 15) contiguous nucleotides. The purified polynucleotides can comprise additional heterologous polynucleotides. Polynucleotides of the disclosure can comprise other nucleotide sequences, such as sequences coding for linkers, signal sequences, TMR stop transfer sequences, transmembrane domains, or ligands useful in protein purification such as glutathione-S-transferase, histidine tag, and Staphylococcal protein A. One embodiment

of the disclosure provides a purified polynucleotide comprising at least about 6, 10, 15, 20, 25, 30, 40, 45, 50, 60, 66, or more contiguous nucleotides of SEQ ID NOs: 2-431. One embodiment of the disclosure provides a purified polynucleotide comprising at least about 6, 10, 15, 20, 25, 30, 40, 45, 50, 60, 66, or more contiguous nucleotides of SEQ ID NO: 1163.

[00077] Polynucleotides of the disclosure can be isolated. An isolated polynucleotide is a naturally-occurring polynucleotide that is not immediately contiguous with one or both of the 5' and 3' flanking genomic sequences that it is naturally associated with. An isolated polynucleotide can be, for example, a recombinant DNA molecule of any length. Isolated polynucleotides also include non-naturally occurring nucleic acid molecules. Polynucleotides of the disclosure can encode full-length polypeptides, polypeptide fragments, and variant or fusion polypeptides.

[00078] Degenerate nucleotide sequences encoding polypeptides of the disclosure, as well as homologous nucleotide sequences that are at least about 80, or about: 90, 95, 96, 97, 98, or 99% identical to the polynucleotide sequences of the disclosure and the complements thereof are also polynucleotides of the disclosure. Degenerate nucleotide sequences are polynucleotides that encode a polypeptide of the disclosure or fragments thereof, but differ in nucleic acid sequence from the given polynucleotide sequence, due to the degeneracy of the genetic code. Percent sequence identity has an art recognized meaning and there are a number of methods to measure identity between two polypeptide or polynucleotide sequences. See, e.g., Lesk, Ed., Computational Molecular Biology, Oxford University Press, New York, (1988); Smith, Ed., Biocomputing: Informatics and Genome Projects, Academic Press, New York, (1993); Griffin & Griffin, Eds. Computer Analysis of Sequence Data, Part I, Humana Press, New Jersey, (1994); von Heinje, Sequence Analysis In Molecular Biology, Academic Press, (1987); and Gribskov & Devereux, Eds., Sequence Analysis Primer, M Stockton Press, New York, (1991). Methods for aligning polynucleotides or polypeptides are codified in computer programs, including the GCG program package (Devereux *et al.* (1984) Nuc. Acids Res. 12:387), BLASTP, BLASTN, FASTA (Atschul *et al.* (1990) J. Molec. Biol. 215:403), and Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711) which uses the local homology algorithm of Smith and Waterman ((1981) Adv. App. Math., 2:482-489). For example, the computer program ALIGN

which employs the FAST A algorithm can be used, with an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2.

[00079] When using any of the sequence alignment programs to determine whether a particular sequence is, for instance, about 95% identical to a reference sequence, the parameters are set such that the percentage of identity is calculated over the full length of the reference polynucleotide and that gaps in identity of up to 5% of the total number of nucleotides in the reference polynucleotide are allowed.

[00080] Polynucleotides of the disclosure can be isolated from nucleic acid sequences present in, for example, a bacterial sample. Polynucleotides can also be synthesized in the laboratory, for example, using an automatic synthesizer. An amplification method such as PCR can be used to amplify polynucleotides from either genomic DNA or cDNA encoding the polypeptides.

[00081] Polynucleotides of the disclosure can comprise coding sequences for naturally occurring polypeptides or can encode altered sequences that do not occur in nature. If desired, polynucleotides can be cloned into an expression vector comprising expression control elements, including for example, origins of replication, promoters, enhancers, or other regulatory elements that drive expression of the polynucleotides of the disclosure in host cells. An expression vector can be, for example, a plasmid. Minichromosomes such as MC and MC1, bacteriophages, phagemids, yeast artificial chromosomes, bacterial artificial chromosomes, virus particles, virus-like particles, cosmids (plasmids into which phage lambda cos sites have been inserted) and replicons (genetic elements that are capable of replication under their own control in a cell) can also be used.

[00082] Methods for preparing polynucleotides operably linked to an expression control sequence and expressing them in a host cell are well-known in the art. See, e.g., U.S. Patent No. 4,366,246. A polynucleotide of the disclosure is operably linked when it is positioned adjacent to or close to one or more expression control elements, which direct transcription and/or translation of the polynucleotide.

Compositions

[00083] In embodiments, one or more lantibiotics of the disclosure (e.g., 1, 2, 3, 4, 5, 6, or more) are present in compositions that are antimicrobials, disinfectants, antibiotics, antiseptics, preservatives, antiviral or decontaminating agents. An antimicrobial composition kills microbes or slows the reproduction of microbes, such as bacteria. A disinfectant composition is applied to a non-living object to kill microbes or to slow the reproduction of microbes such as bacteria. An antibiotic kills microbes or slows the reproduction of microbes, such as bacteria, in the body of a subject or in cells or tissues. An antiseptic kills microbes or slows the reproduction of microbes, such as bacteria, on skin, tissue or organs. A preservative composition kills microbes or slows the reproduction of microbes in products such as paints, wood, foods, beverages, biological samples, cell or tissue cultures, or pharmaceutical compositions to prevent decomposition by microbes such as bacteria. A decontaminating agent is a cleaning agent that can be used to kill microbes or to reduce the reproduction of microbes, such as bacteria, in or on a living organism, cells, tissues, or objects.

[00084] In embodiments, the disclosure provides compositions comprising one or more lantibiotics of the disclosure that kill bacteria. In embodiments, the compositions comprising the variant MU1140 lantibiotics (i.e. the lantibiotics of the disclosure) kill about: 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% (or any range between about 5% and 100%) of the bacteria they come in contact with. The difference between whether a lantibiotic acts as bacteriostatic agent or a bacteriocidal agent can be the amount or concentration of lantibiotic delivered to the subject, composition, or object to be treated. In embodiments, the lantibiotics of the disclosure reduce the numbers of bacteria present in a composition, subject, cells, or tissues to be treated. In one embodiment of the disclosure, a composition comprising a lantibiotic of the disclosure reduces the number of bacteria by about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% (or any range between about 5% and 100%).

[00085] In embodiments, variant lantibiotics of the disclosure are present in antimicrobial compositions comprising one or more isolated lantibiotics of the disclosure and one or more pharmaceutically acceptable carriers, diluents or excipients (solids or liquids). In one embodiment of the disclosure, the variant lantibiotic is present in a composition in an amount effective to substantially reduce bacterial reproduction of at least one type of Gram-positive bacteria by about: 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100% (or any range between about 5

and 100%). In embodiments of the disclosure, the variant MU1140 lantibiotic is present in a composition in an amount effective to substantially reduce the numbers of at least one type of Gram-positive bacteria by about: 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100% (or any range between about 5 and 100%). In embodiments, the at least one type of Gram-positive bacteria is, for example, *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, vancomycin resistant *Enterococci*, vancomycin resistant *Enterococcus faecalis*, *Enterococcus faecalis*, *Enterococcus faecium*, *Propionibacterium acnes*, *Streptococcus salivarius*, *Streptococcus sanguis*, *Streptococcus mitis*, *Streptococcus pyogenes*, *Lactobacillus salivarius*, *Listeria monocytogenes*, *Actinomyces israelii*, *Actinomyces naeslundii*, *Actinomyces viscosus*, *Bacillus anthracis*, *Streptococcus agalactiae, Streptococcus intermedius, Streptococcus pneumoniae, Corynebacterium diphtheria, Clostridium sporogenes, Clostridium botulinum, Clostridium perfringens, Clostridium tetani,* and *Clostridium difficile*. All Gram-positive species are susceptible to lantibiotic variants of the disclosure.

[00086] Furthermore, Gram-negative bacteria can be susceptible to lantibiotic variants of the disclosure. Optionally, the outer membrane of Gram-negative bacteria can be disrupted with, for example, a chelating agent such as Tris, Tris-EDTA, or EDTA. Any membrane disrupting compounds can be added to compositions of the disclosure to increase the sensitivity of Gram negative bacteria to the lantibiotic variants of the disclosure, for example, polymixins, membrane disrupting antibiotics, cecropins (e.g., *Musca domestica* cecropin, hyalophora cecropins, cecropin B, cecropin P1), G1 OKHc (see Eckert *et al.,* (2006) Antimicrob. Agents Chemother. 50:1480); alpha and beta defensins, ovine derived cathelicidine (see Anderson *et al.,* (2004) Antimicrob. Agents Chemother. 48:673), squalamine derivatives (e.g., SM-7, see Kikuchi *et al.,* (1997) Antimicrob. Agents Chemother. 41:1433), sodium hexametaphosphate, cellular enzymes of granulocytes (van den Broek, (1989) Rev. Infect. Dis. 11:213), EM49 (Rosenthal *et al.,* (1976) Biochemistry, 15:5783), and sodium lauryl sarcosinate. The combination of lantibiotic variants of the disclosure with a membrane disruption agent and/or other antibiotics or drugs that target Gram-negative species can provide a composition effective against both Gram-positive and Gram-negative species. Therefore, the disclosure includes compositions comprising one or more lantibiotics of the disclosure and one or more additional antimicrobial agents or membrane disrupting agents. The one or more additional antimicrobial agents can have Gram-negative

bacteriostatic or bacteriocidal activity. The membrane disrupting agent can render Gram-negative bacteria susceptible to a lantibiotic of the disclosure (i.e., the membrane disrupting agent in combination with one or more lantibiotic variants of the disclosure are bacteriostatic or bacteriocidal to Gram-negative bacteria). Gram-negative bacteria include, for example, *Acinetobacter baumanii, Bordatella pertussis, Borrelia burgdotieri, Brucella abortus, Brucella canis, Brucella melitensis, Brucella suis, Campylobacter jejuni, Coxiella burnetii, Escherichia coli, Francisella tularensis, Haemophilus influenza, Helicobacter pylori, Klebsiella pneumoniae, Legionella pneumophila, Leptospira interrogans, Neisseria gonorrhoeae, Neisseria meningitides, Pseudomonas aeruginosa, Rickettsia rickettsii, Salmonella enteritidis, Salmonella typhi, Salmonella typhimurium, Serratia marcescens, Shigella sonnei, Treponema pallidum, Vibrio cholera, Yersinia enterocolitica,* and *Yersinia pestis*.

[00087] Gram-variable and Gram-indeterminate bacteria can also be susceptible to lantibiotic variants of the disclosure. Optionally, chelating agents such as EDTA can be added to compositions of the disclosure to disrupt the outer membrane of these organisms. Gram-variable and Gram-indeterminate bacteria include, for example, *Chlamydia pneumoniae, Chlamydia trachomatis, Chlamydia psittaci, Mycobacterium leprae, Mycobacterium tuberculosis, Mycobacterium ulcerans,* and *Mycoplasma pneumoniae*.

[00088] A lantibiotic of the disclosure can be combined in a formulation with one or more pharmaceutically acceptable carriers, other carriers, diluents, adjuvants, excipients or encapsulating substances, which are suitable for administration to an animal, composition, or object. Exemplary pharmaceutically acceptable carriers, other carriers, diluents, adjuvants, excipients or encapsulating substances thereof include sugars, such as lactose, glucose, dextrose, and sucrose; starches, such as cornstarch and potato starch; cellulose and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose, hydropropylmethylcellulose, and methyl cellulose; polysaccharides such as latex functionalized SEPHAROSE® and agarose; powdered tragacanth; glycerol; malt; gelatin; talc; solid lubricants, such as stearic acid and magnesium stearate; calcium sulfate; vegetable oils, such as peanut oil, cottonseed oil, sesame oil, olive oil, and corn oil; polyols such as propylene glycol, glycerine, sorbitol, mannitol, propylene glycol, and polyethylene glycol; proteins such as serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus toxoid; alginic acid; emulsifiers,

such as the TWEEN®s (polysorbate); polylactic acids; polyglycolic acids; polymeric amino acids such as polyglutamic acid, and polylysine; amino acid copolymers; peptoids; lipitoids; inactive avirulent virus particles or bacterial cells; liposomes; hydrogels; cyclodextrins; biodegradable nanocapsules; bioadhesives; wetting agents, such sodium lauryl sulfate; coloring agents; flavoring agents; tableting agents; stabilizers; antioxidants; preservatives; pyrogen-free water; isotonic saline; ethanol; ethyl oleate; pyrrolidone; Ringer's solution, dextrose solution, Hank's solution; sodium alginate; polyvinylpyrrolidone; gum tragacanth; gum acacia; and sterile water and aqueous buffers and solutions such as physiological phosphate-buffered saline. Carriers, such as pharmaceutically acceptable carriers and diluents, for therapeutic use are well known in the art and are described in, for example, Remington's Pharmaceutical Sciences, Mack Publishing Co. (A.R. Gennaro ed. (1985)). Pharmaceutically acceptable salts can also be used in compositions of the disclosure, for example, mineral salts such as hydrochlorides, hydrobromides, phosphates, or sulfates, as well as salts of organic acids such as acetates, proprionates, malonates, or benzoates.

[00089] Various dosage forms can comprise the lantibiotic compositions as described herein. In some embodiments, the dosage form can be adapted for oral, rectal, vaginal, urethral topical, (including transmucosal and transdermal), intramuscular, intravenous, intradermal, subcutaneous, intramuscular, or intraperitoneal delivery of the lantibiotic.

[00090] The variant lantibiotic compositions can be in a formulation in a form suitable for oral delivery, for example, as tablets, troches, lozenges, mouthwashes, dentifrices, buccal tablets, solutions, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Such compositions can contain one or more agents, such as emulsifying agents, wetting agents, pH buffering agents, sweetening agents, flavoring agents, coloring agents and preserving agents. The lantibiotic compositions can be a dry product for reconstitution with water or other suitable liquid before use.

[00091] Lantibiotics of the disclosure can also be administered in the form of suppositories for rectal, vaginal, or urethral administration of the drug. These compositions can be prepared by mixing the variant lantibiotic with a suitable nonirritating carrier that is solid at

ordinary temperatures but liquid at the body temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

[00092] A lantibiotic of the disclosure can also be topically administered in the form of, e.g., lotions, gels, or liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines. Other dosage forms include, for example, injectable, sublingual, enema, and nasal dosage forms. Compositions for inhalation typically can be provided in the form of a solution, suspension or emulsion that can be administered as a dry powder or in the form of an aerosol using a conventional propellant (e.g., dichlorodifluoromethane or trichlorofluoromethane).

[00093] Formulations can contain between about 0.0001% and about 99.9999% by weight of one or more lantibiotic(s) of the disclosure and usually at least about 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, or 100% (weight %) of one or more lantibiotic variants of the present disclosure. Some embodiments contain from about 25% to about 50% or from 5% to 75% of a lantibiotic of disclosure.

[00094] One or more lantibiotics of the disclosure can be combined with one or more antimicrobials, antibiotics, bacteriocins, anti-viral compounds or molecules, virucidal compounds or molecules, or anti-fungal compounds or molecules to form a composition useful in the methods of the disclosure.

[00095] Antibiotics include, for example, penicillins, cephalosporins, polymixins, quinolones, sulfonamides, aminoglycosides, macrolides, tetracyclines, cyclic lipopeptides (e.g., daptomycin), glycylcyclines (e.g., tigecycline), and oxazolidinones (e.g., linezoid).

[00096] Bacteriocins include, for example, acidocin, actagardine, agrocin, alveicin, aureocin, carnocin, carnocyclin, colicin, curvaticin, divercin, duramycin, enterocin, enterolysin, epidermin, erwiniocin, gallidermin, glycinecin, halocin, haloduracin, lactococin, lacticin, leucoccin, macedocin, mersacidin, mesentericin, microbisporicin, mutacin, nisin, paenibacillin, planosporicin, pediocin, pentocin, plantaricin, reutericin, sakacin, salivaricin, subtilin, sulfolobicin, thuricin , trifolitoxin, variacin, vibriocin, warnericin, and warnerin.

[00097] Antifungals include, for example, polyene antifungals (e.g., amphotericin B, natamycin, rimocidin, filipin, nystatin, candicin, hamycin), azole antifungals (e.g., imidazole, triazole, thiazole), imidazoles (e.g., miconazole, ketoconazole, clotrimazole, econazole, omoconazole, bifonazole, butoconazole, fenticonazole, isoconazole, oxiconazole, sertaconazole, sulconazole, tioconazole), triazoles (e.g., fluconazole, itraconazole, isavuconazole, ravuconazole, posaconazole, voriconazole, terconazole, albaconazole), thiazoles (e.g., abagungin), allylamines (e.g., terbinafine, naftifine, butenafine), echinocandins (e.g., anidulafungin, caspofungin, micafungin), polygodial, benzoic acid, ciclopiroxolamine, tolnaftate, undecylenic acid, flucytosine, and griseofulvin.

[00098] Antivirals and virucidal agents include, for example, abacavir, aciclovire, acyclovir, adefovir, amantadine, amprenavir, ampligen, arbidol, atazanavir, atripla, boceprevir, cidofovir, combivir, delavirdine, didanosine, docosanol, efavirenz, emtricitabine, enfuvirtide, entecavir, entry inhibitors, famciclovir, fomivirsen, fosamprenavir, foscarnet, fosfonet, ganciclovir, ibacitabine, imunovir, idoxuridine, imiquimod, indinavir, inosine, integrase inhibitor, interferon types i, ii, or iii, interferon, lamivudine, lopinavir, loviride, maraviroc, moroxydine, methisazone, nelfinavir, nevirapine, nexavir, nucleoside analogues, oseltamivir, peginterferon alpha-2a, penciclovir, peramivir, pleconaril, podophyllotoxin, protease inhibitor, raltegravir, reverse transcriptase inhibitor, ribavirin, rimantadine, ritonavir, pyramidine, saquinavir, stavudine, tenofovir, tenofovir disoproxil, tipranavir, trifluridine, trizivir, tromantadine, truvada, valaciclovir, valganciclovir, vicriviroc, vidarabine, viramidine, zalcitabine, zanamivir, and zidovudine.

Compositions Comprising Recombinant *S. mutans*

[00099] Compositions of the disclosure can be expressed by one or more strains of recombinant *S. mutans* strains as described herein and a pharmaceutically acceptable or nutritionally acceptable carrier. For example, recombinant *S. mutans* strains can be characterized by: 1) a lactic acid deficiency, and 2) production of a recombinant ADH, 3) variant MU1140 production, 4) optionally, an auxotrophy for a specific organic substance (e.g., a D amino acid such as D-alanine), 5) optionally, a deficiency in ComE expression, or combinations thereof.

[000100] The carrier is physiologically compatible with the area of the subject to which it is administered. Carriers can be comprised of solid-based, dry materials for formulation into tablet, capsule, lozenge, or powdered form. A carrier can also be comprised of liquid or gel-based materials.

Uses of Lantibiotics

[000101] In some embodiments, the lantibiotics of the disclosure are used to reduce the growth of bacteria, prevent the growth of bacteria, prevent the reproduction of bacteria, reduce the reproduction of bacteria, or to reduce or eliminate the numbers of bacteria present in or on an object, composition or subject. In one embodiment of the disclosure, the bacteria are at least one type of Gram-positive bacteria, at least one type of Gram-negative bacteria, at least one type of Gram-variable or Gram-indeterminate bacteria, or a combination of at least one type of Gram-positive or at least one type of Gram-negative bacteria or at least one type of Gram-variable or Gram-indeterminate bacteria. In embodiments, the lantibiotics of the disclosure are administered to, added to, or contacted with a composition or subject in need of treatment.

[000102] In embodiments of the disclosure, the bacteriostatic action of a lantibiotic of the disclosure reduces reproduction of the bacteria by about: 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% (or any range between about 5% and 100%). In embodiments, the lantibiotics of the disclosure kill bacteria. In embodiments of the disclosure, the variant MU1140 lantibiotics kill about: 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% (or any range between about 5% and 100%) of the bacteria they come in contact with. The difference between whether a lantibiotic acts as bacteriostatic agent or a bacteriocidal agent can be the amount or concentration of lantibiotic delivered to the subject, composition, or object to be treated. Lantibiotics of the disclosure can reduce the numbers of bacteria present in a composition, subject, cells, or tissues to be treated. In one embodiment of the disclosure, variant MU1140 lantibiotics reduce the number of bacteria by about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% (or any range between about 5% and 100%).

[000103] The disclosure therefore provides methods of treating, ameliorating, or preventing a disease, infection, or colonization. A disease is a pathological condition of a part, organ, or system of an organism resulting from infection and characterized by an identifiable group of signs and symptoms. An infection is invasion by and multiplication of pathogenic

microorganisms, such as bacteria, in a bodily part or tissue, which may produce a subsequent tissue injury and progress to overt disease through a variety of cellular or toxic mechanisms. Colonization is the act or process of a microorganism, such as bacteria, establishing itself on or within a host or object. Colonization may produce a subsequent biofilm or biofouling condition as described below. In embodiments, lantibiotics of the disclosure are used prophylactically to prevent disease, infection or colonization or to prevent the spread of a disease or infection. Examples of diseases, infections and colonizations that can be treated or prevented by the compositions and methods of the disclosure include, for example, septicemia, bacterial meningitis, cystic fibrosis, bovine mastitis, impetigo, bacterial vaginosis, bacterial pneumonia, urinary tract infections, bacterial gastroenteritis, erysipelas, cellulitis, anthrax, whooping cough, brucellosis, enteritis, opportunistic infections, community acquired respiratory infections, upper and lower respiratory infections, diphtheria, nosocomial infections, diarrhea, ulcer, bronchitis, listeriosis, tuberculosis, gonorrhea, pseudomonas infections, salmonellosis, shigellosis, *E. coli* infections, staphylococcal infections, streptococcal infections, recurrent or primary *C. difficile* associated infections, and necrotizing fasciitis.

[000104] In embodiments, the disclosure provides methods of treating a subject having a bacterial infection by administering a lantibiotic of the disclosure. In embodiments, the disclosure provides methods of treating a subject that has been diagnosed with a bacterial infection by a health care provider, such as a doctor, nurse or physician's assistant, by administering a lantibiotic of the disclosure. In embodiments, treating comprises reducing or eliminating the number of bacteria in or on the subject. In embodiments, the disclosure further provides methods of preventing a bacterial infection by administering a lantibiotic of the disclosure. In embodiments, the subject is infected with a Gram-positive bacteria. In embodiments, the Gram-positive bacteria is *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, vancomycin resistant *Enterococci*, vancomycin resistant *Enterococcus faecalis*, *Enterococcus faecalis*, *Enterococcus faecium*, *Propionibacterium acnes*, *Streptococcus salivarius*, *Streptococcus sanguis*, *Streptococcus mitis*, *Streptococcus pyogenes*, *Lactobacillus salivarius*, *Listeria monocytogenes*, *Actinomyces israelii*, *Actinomyces naeslundii*, *Actinomyces viscosus*, *Bacillus anthracis*, *Streptococcus agalactiae, Streptococcus intermedius, Streptococcus pneumoniae, Corynebacterium diphtheria, Clostridium sporogenes Clostridium botulinum, Clostridium*

petiringens, Clostridium tetani, and *Clostridium difficile*. In embodiments, the disclosure provides a method of treating a subject having a *C. difficile* infection, e.g., reducing the number of *C. difficile* bacteria in a subject, by administering to the subject a lantibiotic of the disclosure. In embodiments, the disclosure provides a method of preventing a *C. difficile* infection in a subject by administering to the subject a lantibiotic of the disclosure. In embodiments, the subject is a human. Methods of determining whether a subject has a bacterial infection are well-known in the art.

[000105] In embodiments, the subject is a mammal, such as a mouse, rabbit, guinea pig, macaque, baboon, chimpanzee, human, cow, sheep, pig, horse, dog, cat, or to a non-mammalian animal such as a chicken, duck, or fish. Lantibiotics of the disclosure can also be administered to plants, seeds, or plant media such as soil.

[000106] Administration of the lantibiotics of the disclosure can be by any means known in the art, including injection (e.g., intramuscular, intravenous, intrapulmonary, intramuscular, intradermal, intraperitoneal, intrathecal, or subcutaneous injection), aerosol, intranasal, infusion pump, suppository (rectal, vaginal, urethral), mucosally, topically, buccally, orally, parenterally, infusion techniques, by enemas, by inhalation, enemas, or spray, sublingually, transdermally, as an ophthalmic solution, intraspinal application, or by other means, in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, diluents, excipients, adjuvants, and vehicles. A combination of administration methods can also be used.

[000107] In therapeutic applications, the lantibiotic compositions of the disclosure are administered to subjects to reduce the reproduction of bacteria or reduce the numbers of bacteria, or both. The particular dosages of lantibiotic in a composition will depend on many factors including, but not limited to the species, age, gender, severity of infection, concurrent medication, general condition of the subject to which the composition is administered, and the mode of administration of the composition. An effective amount of the composition of the disclosure can be readily determined using only routine experimentation. A therapeutically effective amount means the administration of that amount to a subject, either in a single dose or as part of a series, which is effective for treatment, amelioration, or prevention of bacterial infection or colonization. A therapeutically effective amount is also an amount effective in

alleviating or reducing the symptoms of an infection or in reducing the reproduction of bacteria in or on a subject or reducing the amount of bacteria in or on a subject.

[000108] The concentration of lantibiotic in a composition can vary, and will be selected primarily based on activity of the lantibiotic, body weight of the subject, overall health of the subject, etc. as described above, in accordance with the particular mode of administration selected and the subject's needs. Concentrations, however, will typically be selected to provide dosages ranging from about: 0.001, 0.01, 0.1, 1, 5, 10, 20, 30, 40, 50, 75,100,150 mg/kg/day (or any range between about 0.001 and 150 mg/kg/day) and sometimes higher. Typical dosages range from about 0.1 mg/kg/day to about 5 mg/kg/day, from about 0.1 mg/kg/day to about 15 mg/kg/day, from about 0.1 mg/kg/day to about 20 mg/kg/day, and from about 0.1 mg/kg/day to about 50 mg/kg/day.

[000109] Lantibiotics of the disclosure are administered over time and several times per day (e.g., 1 day, 3 days, 1 week, 1 month, 2 months, 3 months, 6 months, 1 year or more) or can be administered in maintenance doses for long periods of time to prevent or reduce disease, infection, colonization, biofilms or biofouling conditions.

[000110] Lantibiotics of the disclosure can be administered either to an animal that is not infected or colonized with bacteria or can be administered to a bacterially infected or colonized animal.

[000111] One embodiment of the disclosure provides a method for decontaminating or reducing bacterial growth on or in an inanimate object comprising contacting the object with a lantibiotic of the disclosure for a period effective to substantially inhibit bacterial growth of at least one type of bacteria. The contacting can be for 1, 15, 30, or 60 minutes, or 2, 3, 10, 12, 24, 36 or 48 hours (or any range between about 1 minute and 48 hours). An object can be, for example, a food preparation surface, food preparation equipment, industrial equipment, pipes, or a medical device such as catheter, scalpel, knife, scissors, spatula, expander, clip, tweezers, speculum, retractor, suture, surgical mesh, chisel, drill, level, rasp, saw, splint, caliper, clamp, forceps, hook, lancet, needle, cannula, curette, depressor, dilator, elevator, articulator, extractor, probe, staple, artificial joint, wound dressing, catheter, stent, tubing, bowl, tray, sponge, snare, spoon, syringe, pacemaker, screw, plate, pin, wire, guide wire, pacemaker lead, implant, sensor,

glucose sensor, blood bypass tubing, i.v. bag, ventricular assist device components, ophthalmic lens, and balloon.

[000112] Other objects that can be decontaminated include textiles such as a woven (woven from natural or non-natural materials or a blend of natural and synthetic materials) or nonwoven material (e.g., elastic or non-elastic thermoplastic polymers). The textiles can be used for, e.g., a protective article worn by patients, healthcare workers, or other persons who may come in contact with potentially infectious agents or microbes, such as a gown, robe, face mask, head cover, shoe cover, or glove. Other protective textiles can include surgical drapes, surgical covers, drapes, sheets, bedclothes or linens, padding, gauze dressing, wipe, sponge, and other antimicrobial articles for household, institutional, health care, and industrial applications.

[000113] In embodiments of the disclosure, a lantibiotic is coated onto, immobilized, linked, or bound to a solid surface such as a food preparation surface, food preparation equipment, industrial equipment, pipes, or a medical device such as catheter, scalpel, knife, scissors, spatula, expander, clip, tweezers, speculum, retractor, suture, surgical mesh, chisel, drill, level, rasp, saw, splint, caliper, clamp, forceps, hook, lancet, needle, cannula, curette, depressor, dilator, elevator, articulator, extractor, probe, staple, artificial joint, wound dressing, catheter, stent, tubing, bowl, tray, sponge, snare, spoon, syringe, pacemaker, screw, plate, pin, wire, guide wire, pacemaker lead, implant, sensor, glucose sensor, blood bypass tubing, i.v. bag, ventricular assist device components, ophthalmic lens, balloon, and textiles as described above.

[000114] In another embodiment of the disclosure, lantibiotic compositions of the disclosure are present in a transdermal formulation. A transdermal formulation can be designed so the lantibiotic composition acts locally at the point of administration or systemically by entering an animal or human's blood circulation. Therefore, delivery can occur by direct topical application of the lantibiotic composition in the form of an ointment or lotion, or by adhesion of a patch embedded with the lantibiotic composition or with a reservoir that holds the lantibiotic composition and releases it to the skin all at once or in a time-controlled fashion.

[000115] Optionally, lantibiotic compositions can be contained within vesicles such as microparticles, microspheres, liposomes, lipid vesicles, or transfersomes for transdermal or topical delivery. Ultrasound devices to generate shock waves to enlarge pores, use of electric

current to drive substances across skin, and the use of microneedles to pierce skin and deliver lantibiotic compositions into the bloodstream can also be used with transdermal or topical administration. Methods of coating, binding, or immobilizing peptides, such as the lantibiotics of the disclosure onto surfaces are well-known in the art. See e.g., Modern Methods of Protein Immobilization, William H. Scouten, First Ed. (2001) CRC Press; Protein Immobilization (Biotechnology and Bioprocessing), Richard F. Taylor (1991) CRC Press.

[000116] Methods of the disclosure can also be used to ameliorate, reduce, remove, or prevent biofouling or biofilms. Biofouling is the undesirable accumulation of microorganisms, such as bacteria on structures exposed to solvent. Biofouling can occur, for example on the hulls of ships, in membrane systems, such as membrane bioreactors and reverse osmosis spiral wound membranes, water cooling systems of large industrial equipment and power stations, and oil pipelines carrying, e.g., used oils, cutting oils, soluble oils or hydraulic oils.

[000117] A biofilm can cause biofouling and is an aggregate of organisms wherein the organisms are adhered to each other, to a surface, or a combination thereof. A biofilm can comprise one or more species of bacteria, fungi, filamentous fungi, yeasts, algae, cyanobacteria, viruses, and protozoa and combinations thereof. Microorganisms present in a biofilm can be embedded within a self-produced matrix of extracellular polymeric substances. When a microorganism switches to a biofilm mode of growth, it can undergo a phenotypic shift in behavior wherein large suites of genes are differentially regulated. Nearly every species of microorganism can form biofilms. Biofilms can be found on or in living organisms or in or on non-living structures. Biofilms can be present on structures contained in naturally occurring bodies of water or man-made bodies of water, on the surface of water, surfaces exposed to moisture, interiors of pipes, cooling water systems, marine systems, boat hulls, on teeth, on plant surfaces, inside plants, on human and animal body surfaces, inside humans and animals, on contact lenses, on catheters, prosthetic cardiac valves, other prosthesis, intrauterine devices, and other structures/devices.

[000118] Biofilms can cause corrosion of metal surfaces, inhibit vessel speed, cause plant diseases, and can cause human and animal diseases. Biofilms are involved in human and animal infections, including, for example, urinary tract infections, catheter infections, middle-ear

infections, dental plaque, gingivitis, dental caries, periodontal diseases, endocarditis, infections in cystic fibrosis, chronic sinusitis, and infections of permanent indwelling devices such as joint prostheses and heart valves. Biofilms can also impair cutaneous wound healing and reduce topical antibacterial efficiency in healing or treating infected skin wounds.

[000119] Some microorganisms that can form biofilms, cause biofouling and/or cause disease in humans and animals include, for example, bacteria, fungi, yeast, algae, protozoa, and viruses as described above. Biofilms can be treated in living organisms as described above. Biofilms and biofouling conditions on non-living surfaces can be treated by applying the lantibiotics of the disclosure onto the nonliving surface or to the area surrounding the surface. Lantibiotic of the disclosure can also be added to the water, oil, or other fluid surrounding and in contact with the nonliving surface.

[000120] The disclosure provides methods of ameliorating or preventing a biofouling condition or a biofilm condition, caused by one or more microorganisms, such as bacteria. The methods comprise administering one or more of the variant lantibiotics to the biofouling condition or biofilm condition, wherein the biofouling condition or biofilm condition is ameliorated.

[000121] The one or more lantibiotics can be administered to a surface that has a biofilm or biofouling condition or can be administered to a surface as a prophylactic measure. The lantibiotics can be in a dried form (e.g., lyophilized or tablet form) or a liquid solution or suspension form. The dried or liquid forms can be swabbed, poured, sprayed, flushed through the surface (e.g., pipes or membranes) or otherwise applied to the surface. Lantibiotics of the disclosure can be present in a composition with a carrier or diluent in an amount from about 0.001, 0.01, 0.1, 1, 5, 10, 20, 30, 40, 50, 75, 100, 150 mg/m^2 (or any range between about 0.001 and about 30 150 mg/m^2) and sometimes higher.

[000122] Where the biofilm is present or potentially present on an artificial surface within a human or animal (e.g., a catheter or medical device), the artificial surface can be contacted with the one or more lantibiotics prior to insertion into the human or animal. Optionally, the lantibiotics can be delivered to the surface after the artificial surface is inserted into the human or animal.

[000123] In one embodiment of the disclosure, a variant lantibiotic can be used for decontaminating or reducing bacterial reproduction or bacterial numbers in a biological tissue or cell culture. The lantibiotic can be present in a pharmaceutically acceptable carrier, diluent or excipient at the dosage rates as for pharmaceutical compositions described above. The lantibiotic or lantibiotic composition can be contacted with the tissue or cell culture for a period effective to substantially inhibit bacterial growth of at least one type of bacteria. The lantibiotic can be provided in an amount effective to maintain the physiological characteristics of the biological tissue or cells and/or in an amount effective to substantially maintain the viability of the biological tissue or cells.

[000124] One embodiment of the disclosure provides a method for preparing isograft organs, tissues or cells, autograft tissues or cells, allograft organs, tissues or cells, xenograft organs, tissues or cells, or other cells or tissue for transplantation. The method comprises contacting the organs, cells or tissues with a lantibiotic composition of the disclosure for a period effective to inhibit or reduce bacterial growth or bacterial numbers of at least one type of Gram-positive bacteria. The cells, organs or tissues can be, for example, a heart valve, a blood vessel, pericardium or musculoskeletal tissue, ligaments such as anterior cruciate ligaments, knee joints, hip joints, ankle joints, meniscal tissue, skin, cornea, heart, lung, small bowel, intestine, liver, kidney, bone marrow, bone, and tendons.

[000125] The contacting step can be performed at a temperature from about 2°C to about 42°C for about: 0.5, 1, 2, 3, 5, 10, 24, 36, or 48 hours. The lantibiotic composition can further comprise a physiological solution further comprising one or more broad spectrum antimicrobials and/or one or more antifungal agents, such as, for example vancomycin, imipenem, amikacin, and amphotericin B.

[000126] Lantibiotic compositions of the disclosure can also be used as a preservative for allograft and xenograft process solutions, and cell culture and tissue solutions. The solutions can comprise an effective amount of one or more lantibiotics in a physiological solution at a pH of between 3 and 8.

[000127] One or more lantibiotics of the disclosure can be added to foods or beverages as a preservative. Examples of foods include, processed cheese products, pasteurized dairy products,

canned vegetables, high moisture, hot baked flour products, pasteurized liquid egg, and natural cheese products. Lantibiotics of the disclosure can also be used to control *Listeria* in foods, to control spoilage by lactic acid bacteria in, e.g., beer, wine, alcohol production and low pH foods such as salad dressings. Lantibiotics of the disclosure can be used as an adjunct in food processing technologies such as higher pressure sterilization and electroporation. Lantibiotics can be present in a food or beverages in an amount from about: 0.001, 0.01, 0.1, 1, 5, 10, 20, 30, 40, 50, 75, 100, 150, 250, 300, 400, 500, 600, 700, 800, 900, 1,000 or more mg/kg or mg/L (or any range between about 0.001 and about 1,000 mg/kg or 10 mg/1 and sometimes higher.

[000128] Lantibiotics of the disclosure can also be used as molecular wires, molecular switches, or molecular based memory systems. Therefore, variant lantibiotics and wild-type lantibiotics have potential use for building nano-circuitry, as well as other nano-based applications. Molecular wires (also known as molecular nanowires) are molecular-scale substances that conduct electrical current, which are the fundamental building blocks for molecular electronic devices. The typical diameter of molecular wires is less than three nanometers, while the length can extend to centimeters or more. A molecular wire allows the flow of electrons from one end of the wire to the other end of the wire. Molecular wires can comprise at least two terminals for contacting additional components of a nano-electronic device.

[000129] A molecular switch (also known as a controllable wire) is a molecular structure where the electron flow can be turned on and off on demand. A molecular based memory system is one or more molecule wires or switches that have the ability to alter its conductivity by storing electrons. A molecular wire, switch, or molecular based memory system can be present on or anchored to substrates such as silicon wafers, synthetic polymer supports, glass, agarose, nitrocellulose, nylon, Au, Cu, Pd, Pt, Ni, Al, Al_2O_3, nickel grids or disks, carbon supports, aminosilane-treated silica, polylysine coated glass, mica, and semiconductors.

[000130] In embodiments, the disclosure provides recombinant *S. mutans* strains that produce one or more lantibiotic variants of the disclosure. Examples of such strains include JH1000 or JH1140. Recombinant *S. mutans* strains can further contain an erythromycin gene in the *mutA'* – *mutB* intergenic region. Examples of such strains include SM152. Recombinant *S.*

mutans strains can also be strains that produce for example, 2X, 3X, 4X, 5X, 6X, 7X, 8X, 9X, 10X, 100X, 1000X more lantibiotic than wild type recombinant *S. mutans* strains.

[000131] In embodiments, the disclosure provides recombinant *S. mutans* strains that produce one or more lantibiotic variants of the disclosure to outcompete and substantially eliminate wild-type, cariogenic *S. mutans* from the oral cavity of a host (e.g., reduce the number of wild-type *S. mutans* by about: 5, 10, 25, 50, 75, 90, 95, 99, or 100% (or any range between about 5 % and about 100%)). Production of lantibiotic variants of the invention with enhanced biological activity as compared to a wild-type MU1140 lantibiotic can therefore provide an *S. mutans* with a selective advantage over MU1140 producing, non-MU1140 lantibiotic producing, or non-lantibiotic producing *S. mutans* strains present in the oral cavity of a host. The lantibiotic variants of the disclosure, when expressed by a recombinant *S. mutans* strain of the disclosure, eliminates the resident, lantibiotic-susceptible *S. mutans* strains, thus interfering with colonization of lantibiotic-susceptible strains and promoting recombinant *S. mutans* colonization of the oral cavity. Since the wild-type, native *S. mutans* is displaced from the oral cavity, the incidence and/or severity of dental caries is reduced.

[000132] In one embodiment of the disclosure, the recombinant strain can additionally express lanB, lanC, lanD, lanE, lanF, lanG, lanK, lanM, lanP, lanR, lanT, lanI, mutR, mutAA', mutBCDPT, mutFEG or combinations of two or more of these *S. mutans* polypeptides.

[000133] In embodiments, the recombinant *S. mutans* strains of the disclosure are lactic acid deficient, meaning that a recombinant *S. mutans* strain produces substantially decreased amounts of lactic acid relative to wild-type *S. mutans*. Substantially decreased amounts of lactic acid are about 40, 50, 60, 70, 80, 90, 95, or 100% (or any range between about 40% and about 100%) less lactic acid than is produced by a wild-type *S. mutans* strain (e.g. *S. mutans* strain UA159 (ATCC 700610)) or other species belonging to the Streptococcus genus including *Streptococcus sobrinus (e.g. S. sobrinus* strain SL1 (ATCC 33478)), *Streptococcus rattus* (e.g., *S. rattus* strain FA1 (ATCC 19645)), *Streptococcus cricetus* (S. *crecitus* strain HS6 (ATCC 19642)), and *Streptococcus ferus* (S. *ferus* strain 8S1)). In one embodiment of the disclosure, a lactic acid deficient *S. mutans* effector strain produces no detectable lactic acid. Lactic acid expression can be detected as described in, e.g., Hillman *et al.,* Infect. Immun. 62:60 (1994);

Hillman *et al.*, Infect. Immun. 64:4319 (1996); Hillman *et al.*, 1990, Infect. Immun., 58:1290-1295.

[000134] Recombinant *S. mutans* strains of the disclosure can be lactic acid deficient as a result of a non-functional, inactivated, partially functional, or partially inactivated regulatory region, translational signal, transcriptional signal, or structural sequence in the lactic acid synthesis pathway as disclosed in International Application No. PCT/US2013/027340 or U.S. Patent No. 5,607,672, each of which is incorporated by reference herein in its entirety.

[000135] Because defects in lactic acid synthesis are lethal for *S. mutans*, the defect in the recombinant, lactic acid-deficient *S. mutans* strains must be complemented by the production of a recombinant alcohol dehydrogenase (ADH). *See e.g.*, Hillman *et al.*, Infect. Immun. 64:4319 (1996). Production of the recombinant ADH prevents accumulation of metabolites, *e.g.*, pyruvate, that otherwise causes the death of lactic acid-deficient *S. mutans*.

[000136] A *S. mutans* strain can be genetically engineered to express a recombinant alcohol dehydrogenase as disclosed in International Application No. PCT/US2013/027340, incorporated herein by reference in its entirety. Recombinant *S. mutans* strains of the disclosure can optionally be genetically engineered to be auxotrophic for an organic substance not normally present in the oral cavity or diet of a host so that the oral cavity colonization by the recombinant *S. mutans* strains can be controlled. That is, the recombinant *S. mutans* strains can optionally be genetically engineered so that they are unable to synthesize a particular organic compound required for growth, as disclosed in International Application No. PCT/US2013/027340.

[000137] Optionally, a recombinant *S. mutans* strain of the disclosure can comprise an inactivated or non-functional *comE* gene. "ComE deficient" or "deficiency in ComE production" means that a recombinant *S. mutans* strain produces substantially decreased amounts of ComE protein relative to wild-type *S. mutans*. Substantially decreased amounts of ComE are about 40, 50, 60, 70, 80, 90, 95, or 100% (or any range between about 40% and about 100%) less ComE protein than is produced by a wild-type *S. mutans* strain. In one embodiment of the disclosure, a ComE deficient recombinant *S. mutans* strain produces no detectable ComE protein. ComE expression can be assayed as described in, e.g., Chen & Gotschlich, J. Bact. 183:3160 (2001). Recombinant *S. mutans* strains of the disclosure can be ComE deficient as a result of a non-

functional, inactivated, partially functional, or partially inactivated regulatory region, translational signal, transcriptional signal, or structural sequence in ComE synthesis, as disclosed in International Application No. PCT/US2013/027340.

[000138] In embodiments, the recombinant *S. mutans* of the disclosure are present in a composition of the disclosure in a therapeutically effective amount. Therapeutically effective means effective to prevent or reduce the number or incidence (e.g., 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100% fewer cavities than controls that did not receive the composition) and/or reduce the severity (e.g., 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100% less severe cavities than controls that did not receive the composition) of cavities.

[000139] A therapeutically effective amount or dosage is an amount or dosage of a composition of the disclosure at high enough levels to prevent caries and/or reduce caries number and/or caries severity, but low enough to avoid serious side effects (at a reasonable benefit/risk ratio), within the scope of sound medical/dental judgment. The compositions of the disclosure can be applied in a therapeutically effective amount to the oral cavity of a host for the treatment and/or prevention of cavities using methods known in the art.

[000140] In one embodiment of the disclosure a composition can comprise one or more isolated recombinant strains of the disclosure along with one or more isolated *Streptococcus oralis* strains, one or more isolated *S. rattus* strains, and/or one or more isolated *Streptococcus uberis* strains using methods known in the art.

[000141] One embodiment of the disclosure provides a method for treating dental caries comprising administering a composition comprising one or more recombinant *S. mutans* strains of the disclosure to the oral cavity of a subject in need thereof using methods known in the art.

[000142] The disclosure also provides a method of reducing the amount of bacteria that can cause dental caries in a subject. Another embodiment of the disclosure provides a method of preventing dental caries in a subject, for example, compositions comprising the recombinant *S. mutans* strains of the disclosure are administered to the oral cavity of a host or subject such as an animal, including a mammal, for example, a human, a non-human primate, a dog, a cat, a horse,

a bovine, a goat, or a rabbit using methods known in the art. The compositions of the disclosure can be orally administered using methods known in the art.

Kits

[000143] Compositions of the disclosure can be present in a kit comprising a container of one or more lantibiotics of the disclosure. The lantibiotics can be lyophilized and in the form of a lyophilized powder or tablet or can be in a solution or suspension optionally with buffers, excipients, diluents, adjuvants, or pharmaceutically acceptable carriers. A kit can also comprise one or more applicators for the one or more lantibiotics to a body part or tissue or surface. The applicator can be, for example, a swab, a syringe (with or without a needle), a dropper, a sprayer, a surgical dressing, wound packing, or a bandage. Optionally, the kit can comprise one or more buffers, diluents, adjuvants, therapeutically acceptable carriers, or pharmaceutically acceptable carriers for reconstituting, diluting, or preparing the one or more variant MU1140 lantibiotics.

[000144] A kit of the disclosure can contain a single dose, a one week, one month, two month, three month, four month, five month, six month, or 12 month supply of a composition of the disclosure. A composition of the disclosure can be packaged and, in turn, a plurality of the packaged compositions can be provided in a storage container or outer package or carton. Where the one or more strains of *S. mutans* are auxotrophic, the kit can include a bacterial auxotroph-maintaining amount of an organic substance, e.g., a composition comprising a D-amino acid such as D-alanine.

[000145] All patents, patent applications, and other scientific or technical writings referred to anywhere herein are incorporated by reference herein in their entirety. The disclosure illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms, while retaining their ordinary meanings. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the disclosure claimed. Thus, it should be

understood that although the present disclosure has been specifically disclosed by embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this disclosure as defined by the description and the appended claims.

[000146] The following are provided for exemplification purposes only and are not intended to limit the scope of the disclosure described in broad terms above.

EXAMPLES

[000147] The present disclosure is further defined in the following Examples. The Examples set forth herein, represent certain features, elements and embodiments of the disclosure, and is not intended, nor should be construed, to limit the scope of the disclosure.

[000148] MU1140 variants (*i.e.*, MU1140 polypeptide variants including non-naturally occurring variants) with improved antimicrobial/antibiotic activity and/or stability were produced by mutating select single and multiple combinations of amino acids within the (mature) 22 amino acid native (wild-type) MU1140 polypeptide sequence (SEQ ID NO:1156, FIG. 1). The *mutA* variants were constructed using pLAN042, from which a linear amplicon was used to substitute the native *mutA* sequence with a *mutA* sequence containing codon substitutions. Following transformation of the *mutA* variant library into SM152 (JH1140 *lan*A'B::erm) and verification of codon substitution in *mutA*, *S. mutans* variant clones were picked, expressed and analyzed for antimicrobial activity (*see*, **Table 1** and **Table 2**, below) and stability. Data was interpreted by qualitative comparison of the size of the zone of clearing for each lantibiotic variant producing strain with the *mutA* invariant control SM152 and the wild-type parent strain JH1140.

EXAMPLE 1

MU1140 SINGLE AND MULTI-SITE VARIANTS

Construction of mutA variant strains

[000149] A plasmid for construction of MU1140 variants was constructed using mutA, the primary structural gene encoding the MU1140 propeptide in *S. mutans*. The episomal plasmid was constructed using plasmid pMK4 (GenBank Accession No.: EU549778.1), which is a plasmid with a ColE1 origin of replication, ampicillin resistance in *E. coli*, and chloramphenicol resistance in *S. mutans*. A DNA fragment of S. mutans JH1140 (ATCC Deposit: 55676) containing the mutAA' locus was ligated into pMK4 and designated pLAN042 (**Figure 2**). Plasmid pLAN042 was used as a template to build mutA variants using PCR mutagenesis (Raman and Martin, *Nature Methods*, 11, 2014), using the codon substitutions listed in **Table 1A**.

[000150] A chromosomal integration strategy using homologous recombination was employed to produce MU1140 variants in *S. mutans* JH1140 by replacement of the native chromosomal mutA gene with mutA variants encoding codon substitutions. An integration arm upstream of mutAA', an integration arm downstream of mutAA', and an erythromycin resistance gene were amplified by PCR and assembled using splicing overlap extension PCR (SOE PCR). The SOE product was ligated to pMK4, transformed into *E. coli*, verified by DNA sequencing, and designated pLAN126 (**Figure 3**).

[000151] MutA variant libraries were constructed by PCR amplification of upstream and downstream recombination arms from the pLAN126 template and subsequent assembly with PCR amplified mutA variants from the pLAN042 mutA variant library **(Table 1A)** or an oligonucleotide library encoding mutA codon variants using SOE PCR (**Table 1B**). A final PCR reaction was used to amplify the single SOE product containing 750 bp recombination arms.

[000152] DNA vectors were transformed into *S. mutans* JH1140 using standard techniques (Biswas *et al.*, Biotechniques 42.4:487 (2007)). Transformants were plated onto TSYEX agar (Ghobrial 2008, Pharmacokinetics and pharmacodynamics of the lantibiotic MU1140. Ph.D. dissertation, University of Florida) containing 3 μg/mL erythromycin, and incubated in a candle

jar for 3 days at 37°C. PCR and Sanger DNA sequencing was utilized to confirm replacement of the chromosomal copy of mutA with the mutA variant encoded on the integration vector.

Table 1A: Nucleotide identities of mutA variants.

Mutation	Core peptide sequence																					
MU1140 (Wild-type) SEQ ID NO: 1	TTC	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AGG	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I SEQ ID NO: 19	ATT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AGG	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1L SEQ ID NO: 4	CTT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AGG	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
R13N SEQ ID NO: 241	TTC	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AAT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F17L SEQ ID NO: 316	TTC	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AGG	ACA	GGT	AGT	TTA	AAT	AGT	TAC	TGT	TGC
F17Y SEQ ID NO: 320	TTC	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AGG	ACA	GGT	AGT	TAT	AAT	AGT	TAC	TGT	TGC
N18A SEQ ID NO: 335	TTC	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AGG	ACA	GGT	AGT	TTC	GCT	AGT	TAC	TGT	TGC
Y20F SEQ ID NO: 384	TTC	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AGG	ACA	GGT	AGT	TTC	AAT	AGT	TTT	TGT	TGC

Table 1B: Nucleotide identities of multisite mutA variants and control strains (single site variants).

Mutation	Core peptide sequence																					
MU1140 (Wild-type) SEQ ID NO: 1	TTC	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AGG	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I SEQ ID NO: 19	ATT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AGG	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1L SEQ ID NO: 4	CTT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AGG	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
R13N SEQ ID NO: 241	TTC	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AAT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F17L SEQ ID NO: 316	TTC	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AGG	ACA	GGT	AGT	TTA	AAT	AGT	TAC	TGT	TGC
F17Y SEQ ID NO: 320	TTC	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AGG	ACA	GGT	AGT	TAT	AAT	AGT	TAC	TGT	TGC

N18A SEQ ID NO: 335	TTC	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AGG	ACA	GGT	AGT	TTC	GCT	AGT	TAC	TGT	TGC
Y20F SEQ ID NO: 384	TTC	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AGG	ACA	GGT	AGT	TTC	AAT	AGT	TTT	TGT	TGC
F1A R13A SEQ ID NO: 1167	GCT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GCT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1A R13G SEQ ID NO: 708	GCT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GGT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1A R13N SEQ ID NO: 709	GCT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AAT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1A R13S SEQ ID NO: 710	GCT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AGT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1A R13V SEQ ID NO: 711	GCT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GTT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1G R13A SEQ ID NO: 712	GGT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GCT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1G R13G SEQ ID NO: 713	GGT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GGT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1G R13N SEQ ID NO: 714	GGT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AAT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1G R13V SEQ ID NO: 715	GGT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GTT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1H R13N SEQ ID NO: 716	CAT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AAT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I R13A G15A SEQ ID NO: 717	ATT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GCT	ACA	GCT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I R13D G15A SEQ ID NO: 718	ATT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GAT	ACA	GCT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I R13N G15A SEQ ID NO: 719	ATT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AAT	ACA	GCT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I W4I R13A SEQ ID NO: 720	ATT	AAA	AGT	ATT	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GCT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I W4I R13D SEQ ID NO: 721	ATT	AAA	AGT	ATT	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GAT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I W4M R13A SEQ ID NO: 722	ATT	AAA	AGT	ATG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GCT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I W4M R13N SEQ ID NO: 723	ATT	AAA	AGT	ATG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AAT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I K2A R13A SEQ ID NO: 724	ATT	GCT	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GCT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I K2A W4K	ATT	GCT	AGT	AAA	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GCT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC

R13A SEQ ID NO: 725																					
F1I K2A W4K R13D SEQ ID NO: 726	ATT	GCT	AGT	AAA	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GAT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I K2A W4K R13A Y20F SEQ ID NO: 727	ATT	GCT	AGT	AAA	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GCT	ACA	GGT	AGT	TTC	AAT	AGT	TTT	TGT	TGC
F1I L6V R13A SEQ ID NO: 728	ATT	AAA	AGT	TGG	AGC	GTT	TGT	ACG	CCT	GGT	TGT	GCA	GCT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I R13A SEQ ID NO: 729	ATT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GCT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I R13A Y20F SEQ ID NO: 730	ATT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GCT	ACA	GGT	AGT	TTC	AAT	AGT	TTT	TGT	TGC
F1I R13D SEQ ID NO: 731	ATT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GAT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I R13D Y20F SEQ ID NO: 732	ATT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GAT	ACA	GGT	AGT	TTC	AAT	AGT	TTT	TGT	TGC
F1I R13G SEQ ID NO: 733	ATT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GGT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I R13I SEQ ID NO: 734	ATT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	ATT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I R13N SEQ ID NO: 735	ATT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AAT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I R13N Y20F SEQ ID NO: 736	ATT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AAT	ACA	GGT	AGT	TTC	AAT	AGT	TTT	TGT	TGC
F1I R13P SEQ ID NO: 737	ATT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	CCT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I R13Q SEQ ID NO: 738	ATT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	CAA	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I R13S SEQ ID NO: 739	ATT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AGT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I R13V SEQ ID NO: 740	ATT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GTT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1I R13E SEQ ID NO: 741	ATT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GAA	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1L R13A SEQ ID NO: 742	CTT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GCT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1L R13A Y20F SEQ ID NO: 743	CTT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GCT	ACA	GGT	AGT	TTC	AAT	AGT	TTT	TGT	TGC
F1L R13D	CTT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GAT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC

SEQ ID NO: 744																			
F1L R13D Y20F SEQ ID NO: 745	CTT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GAT	ACA	GGT	AGT	TTC	AAT	AGT	TTT	TGT	TGC
F1L R13G SEQ ID NO: 746	CTT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GGT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1L R13N SEQ ID NO: 747	CTT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AAT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1L R13P SEQ ID NO: 748	CTT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	CCT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
FIL R13Q SEQ ID NO: 749	CTT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	CAA	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1L R13N Y20F SEQ ID NO: 750	CTT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AAT	ACA	GGT	AGT	TTC	AAT	AGT	TTT	TGT	TGC
F1S R13N SEQ ID NO: 751	AGT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AAT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1T R13A SEQ ID NO: 752	ACA	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GCT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1T R13G SEQ ID NO: 753	ACA	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GGT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1T R13N SEQ ID NO: 754	ACA	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AAT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1T R13V SEQ ID NO: 755	ACA	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GTT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1V R13A SEQ ID NO: 756	GTT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GCT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1V R13D SEQ ID NO: 757	GTT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GAT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1V R13N SEQ ID NO: 758	GTT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	AAT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1V R13Q SEQ ID NO: 759	GTT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	CAA	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1V R13V SEQ ID NO: 760	GTT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GTT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1V R13P SEQ ID NO: 761	GTT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	CCT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1Y R13D SEQ ID NO: 762	TAT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GAT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC
F1Y R13G SEQ ID NO: 763	TAT	AAA	AGT	TGG	AGC	CTT	TGT	ACG	CCT	GGT	TGT	GCA	GGT	ACA	GGT	AGT	TTC	AAT	AGT	TAC	TGT	TGC

[000153] In this manner, a plasmid library of 418 mutA variants was thus constructed using pLAN042 as the base vector. The mutA variant library was constructed in an addressable fashion, with each of the 418 vector designs containing a single codon mutation to confer a designated amino acid change. Confirmation of the codon change for each of the 418 variants was confirmed by DNA sequencing.

[000154] Multi-site *mutA* variants were also constructed using a similar genomic integration strategy to improve levels and consistency of MU1140 variant production.

EXAMPLE 2

Non-Normalized Testing of MU1140 Single and Multi-Site Variants

[000155] In order to determine if engineered *S. mutans mutA* variant strains produce MU1140 variants with antimicrobial activity, *mutA* variant strain culture supernatants were initially tested for their ability to induce formation of zones of clearing against *Micrococcus luteus* 272, an MU1140 sensitive antibiotic indicator strain.

High-Throughput (HTP) Growth

[000156] *S. mutans* colonies were picked into 2.2 mL 96 deep well plates containing 1 mL of TSYEX (3% tryptic soy broth and 0.3% yeast extract) and 3 µg/mL erythromycin. The plates were grown micro-aerobically overnight at 37°C without shaking. One hundred (100) µL of overnight culture was transferred to 2.2 mL 96 well deep well plates containing 900 µL Ghobrial expression media (5% Yeast Extract, 4% Glucose, 0.1% CaCl2, 100 mM bis-tris Buffer pH 6.7; Ghobrial, 2008). The expression plates were sealed with porous seals and shaken for 20 hours at 37°C and 900 rpm. The cell cultures were centrifuged for 10 minutes at 3220 x g and the supernatant was removed for testing in the *Micrococcus luteus* bioassay. Freezer stocks were constructed by diluting overnight TSYEX cultures of *S. mutans* strains with 25% glycerol (final volume) and incubation at -80°C.

Micrococcus luteus HTP Bioassay

[000157] *M. luteus* (ATCC Deposit No.: 272) was streaked onto an LB agar plate and grown at 30°C for 48 hours, then 5 mL of LB broth was transferred to a 14 mL culture tube. *M.*

luteus cells were inoculated from plate cultures and grown overnight at 30°C in a shaker at 250 rpm. Cells were diluted 1:100 using in a volume of 50 mL LB broth in a 250 mL Erlenmeyer flask and grown for 2 hours at 30°C. Subsequently, *M. luteus* cells were centrifuged for 5 minutes at 3220 x g, re-suspended in 5 mL of fresh LB, inoculated into 45°C molten 125 mL soft nutrient agar (5 g/L Bacto agar, 8 g/L Difco nutrient broth) at OD600 0.1 and poured into a QTray (Genetix). The QTray was dried with the lid removed in a laminar flow hood for 30 minutes. A QPIX™ 450 gridding robot equipped with a 96-metal pin head was used to replicate spot each variant 10 times from a 96-well source plate (shallow Greiner U-bottom) containing 150 µL medium to the QTray. The QTrays were incubated overnight at 30°C and imaged using an EPSON® Expression 10,000 XL optical scanner. Zones of clearing were scored as less than or equal activity to SM152 (indicated as "1"), and greater than SM152 activity (indicated as "2").

Results of MU1140 Single Site Variants (expressed on episomal plasmid)

[000158] MU1140 variant producing strains were assessed for improvements in antimicrobial activity using the *M. luteus* zone of clearing assay using non-normalized culture supernatants obtained from overnight growth of *S. mutans* strains (**Table 2**). Consistent (well-to-well and plate-to-plate) zones of clearing were observed for both *S. mutans* strains JH1140 and SM152. Strain *S. mutans* SM152 (mutA invariant) and variant producing mutA variant strains contain an erythromycin resistance gene in the mutA' mutB intergenic region from the pLAN126-based integration vector (**Figure 3**). During validation of the SM152 mutA invariant strain, it was found that insertion of the erythromycin resistance cassette caused a consistent gain of function improvement in the size of the zone of activity observed compared to the size of the zone of activity observed in the parent strain JH1140 (**Table 2**). The increase in antimicrobial activity observed may be due to deregulation of the regulatory region leading to higher levels of expression of the upstream mutAA' or downstream mutBCDPT operons. Changes in the size of the zone of clearing in this assay could be due to increases in specific antimicrobial activity, stability, titer, and/or diffusibility, or a combination of such factors.

[000159] Multi-site MU1140 variant producing strains were subsequently tested to determine if mutation of more than one amino acid position would confer improved performance in the *M. luteus* zone of clearing assay (**Table 3**). Multi-site variants tested exhibited larger

zones of clearing than SM152 and the single amino acid variant strains F1I and F1L, indicating possible increases in specific antimicrobial activity, stability, titer, and/or diffusibility, or a combination of such factors.

TABLE 2: Non-normalized size of zones of clearing detected in *M. luteus* zone of clearing assay. A score of one (1) indicates size of zone of clearing produced by parental strain JH1140. A score of two (2) indicates zone of clearing greater in size than JH1140. Strains were replicated in a minimum of two independent experiments.

Variant	**Zone of clearing 1**	**Zone of clearing 2**
JH1140 (wt)	1	1
SM152	2	2
F1I	2	2
F1L	2	2
R13N	2	2
F17I	2	2
F17L	2	2
N18A	2	2
Y20F	2	2

TABLE 3: Non-normalized size of zones of clearing detected in *M. luteus* zone of clearing assay. A score of one (1) indicates size of zone of clearing produced by control strain SM152. A score of two (2) indicates zone of clearing greater in size than SM152. Strains were replicated in a minimum of two independent replicate experiments.

Variant	zone of clearing 1	zone of clearing 2
SM152	1	1
F1I	1	1
F1L	1	1
F1A R13A	2	2
F1A R13V	2	2
F1A R13G	2	2
F1A R13N	2	2
F1A R13S	2	2
F1G R13A	2	2
F1G R13G	2	2
F1G R13N	2	2
F1G R13V	2	2
F1H R13N	2	2
F1I R13A G15A	2	2

F1I R13D G15A	2	2
F1I R13N G15A	2	2
F1I W4I R13A	2	2
F1I W4I R13D	2	2
F1I W4M R13A	2	2
F1I W4M R13N	2	2
F1I K2A R13A	2	2
F1I K2A W4K R13A	2	2
F1I K2A W4K R13D	2	2
F1I K2A W4K R13A Y20F	2	2
F1I L6V R13A	2	2
F1I R13A	2	2
F1I R13A Y20F	2	2
F1I R13D	2	2
F1I R13D Y20F	2	2
F1I R13G	2	2
F1I R13I	2	2
F1I R13N	2	2
F1I R13N Y20F	2	2
F1I R13P	2	2
F1I R13Q	2	2
F1I R13S	2	2
F1I R13V	2	2
F1I R13E	1	1
F1L R13A	2	2
F1L R13A Y20F	2	2
F1L R13D	2	2
F1L R13G	2	2
F1L R13N	2	2
F1L R13P	2	2
F1L R13Q	2	2
F1L R13N Y20F	2	2
F1L R13D Y20F	2	2
F1S R13N	2	2
F1T R13A	2	2
F1T R13G	2	2
F1T R13N	2	2
F1T R13V	2	2
F1V R13A	2	2
F1V R13N	2	2
F1V R13Q	2	2
F1V R13D	2	2
F1V R13P	2	2

F1V R13V	2	2
F1Y R13D	2	2
F1Y R13G	2	2

[000160] Following transformation of the addressable 418 *mutA* variant library, two *S. mutans* SM126 ΔmutAA' pLAN042 variant clones per mutA variant were picked, expressed and analyzed for antimicrobial activity (*see*, **Table 4, plates 1-6** below). Data was interpreted by qualitative comparison of the size of the zone of clearing for each variant producing strain with the positive control SM126 ΔmutAA' pLAN042 and the negative control SM126 ΔmutAA' pMK4 (empty vector). Forty one (41) variants exhibited greater antimicrobial activity than the positive control for at least one of the two clones (**Table 4**). Seventy three (73) variants exhibited antimicrobial activity equal to the control for at least one of the two clones. Eighty three (83) variants exhibited less activity than the positive control, but still generated a detectable zone of clearing. Two hundred twenty one (221) variants did not generate detectable zones of clearing for either clone tested.

[000161] Variation was observed from clone to clone and experiment to experiment using this plasmid-borne complementation system, as controls included in each 96-well plate experiment demonstrated significant variation, from no detectable antimicrobial activity to greater antimicrobial activity than control (**Table 5**). Individual clones were spot-sequenced to exclude DNA mutation of the pLAN042 plasmid as a source of experimental error.

TABLE 4 (Plate 1): ZONES OF CLEARING GENERATED BY MU1140 VARIANTS

Well	Mutation	*M luteus* Zone of Clearing Colony 1	*M luteus* Zone of Clearing Colony 2	Well	Mutation	*M luteus* Zone of Clearing Colony 1	*M luteus* Zone of Clearing Colony 2
A01	F1K	1	1	E01	F1L	3	2
A02	F1N	2	3	E02	F1Q	1	1
A03	F1R	2	2	E04	K2N	2	2
A04	K2A	2	2	E05	K2R	0	0
A05	K2P	0	0	E06	S3W	0	0
A06	K2T	0	0	E07	S3D	0	0
A07	S3M	0	0	E08	S3T	1	0
A08	S3G	0	0	E09	W4Y	2	1
A10	W4Q	1	1	E10	W4I	0	3
A11	S5V	0	0	E11	S5C	0	0
A12	S5D	0	0	E12	S5G	1	0
B01	F1V	3	3	F01	WT	2	2
B02	F1Y	2	1	F02	WT	2	2
B03	F1H	3	2	F03	WT	2	2
B04	K2L	2	1	F04	WT	2	2
B05	K2Q	2	1	F05	WT	2	2
B06	K2I	2	2	F06	WT	2	2
B07	S3C	0	0	F07	pMK4	0	0
B08	S3R	0	0	F08	pMK4	0	0
B09	W4M	3	3	F09	pMK4	0	0
B10	W4F	3	3	F10	pMK4	0	0
B11	S5W	0	2	F11	pMK4	0	0
B12	S5P	0	0	F12	pMK4	0	0
C01	F1W	0	1	G01	F1M	2	3
C02	F1D	0	0	G03	K2V	0	2
C03	F1S	3	3	G04	K2Y	0	1
C04	K2M	2	2	G05	K2H	1	2
C05	K2F	2	2	G06	S3A	0	0
C06	S3K	0	0	G07	S3P	0	0
C07	S3N	0	0	G08	W4K	1	1
C08	S3H	0	0	G09	W4P	0	0
C09	W4C	0	0	G10	S5K	0	0
C10	W4R	0	1	G11	S5N	1	0
C11	S5A	0	2	G12	S5R	0	0
C12	S5Q	1	2	H01	F1C	1	0
D01	F1A	3	1	H02	F1G	2	3
D02	F1P	3	2	H03	K2W	1	0
D03	F1T	2	3	H04	K2D	1	0
D04	K2C	1	2	H05	K2S	1	2
D05	K2G	1	0	H06	S3L	1	0
D06	S3V	0	0	H07	S3F	0	0

D07	S3Y	0	0	H08	W4V	0	2
D09	W4N	1	0	H09	EMPTY		
D10	W4H	3	2	H10	EMPTY		
D11	S5L	1	2	H11	EMPTY		
D12	S5F	1	0	H12	EMPTY		

Zones were scored as 0 (no zone of clearing), 1 (zone of clearing smaller than positive control), 2 (zone of clearing same as positive control), or 3 (zone of clearing larger than positive control).

TABLE 4 (Plate 2): ZONES OF CLEARING GENERATED BY MU1140 VARIANTS

Well	Mutation	*M luteus* Zone of Clearing Colony 1	*M luteus* Zone of Clearing Colony 2	Well	Mutation	*M luteus* Zone of Clearing Colony 1	*M luteus* Zone of Clearing Colony 2
A01	S5H	3	2	E01	L6W	3	1
A02	L6C	0	1	E02	L6P	0	1
A03	L6G	0	1	E03	L6I	0	2
A04	C7W	0	1	E04	C7D	0	0
A05	C7F	0	0	E05	C7S	0	0
A06	T8K	0	0	E06	T8L	0	0
A07	T8N	0	0	E07	T8Q	0	0
A08	T8R	0	0	E08	T8I	0	0
A09	P9A	0	0	E09	P9D	1	1
A10	P9F	0	0	E10	P9T	2	0
A11	G10K	0	0	E11	G10C	1	1
A12	G10Y	0	0	E12	G10F	1	1
B01	S5T	2	0	F01	WT	2	2
B02	L6N	2	2	F02	WT	2	2
B03	L6R	2	0	F03	WT	2	2
B04	C7A	1	0	F04	WT	2	2
B05	C7G	0	0	F05	WT	2	2
B06	T8V	0	0	F06	WT	2	2
B07	T8Y	0	1	F07	pMK4	0	0
B08	T8H	0	1	F08	pMK4	0	0
B09	P9L	0	1	F09	pMK4	0	0
B10	P9R	0	1	F10	pMK4	0	0
B11	G10V	0	0	F11	pMK4	0	0
B12	G10D	0	1	F12	pMK4	0	0
C01	S5I	2	0	G01	L6A	2	2
C02	L6Y	2	0	G02	L6Q	3	0
C03	L6H	2	2	G03	C7K	1	0
C04	C7L	0	1	G04	C7P	2	0
C05	C7R	0	2	G05	C7T	0	0
C06	T8W	0	1	G06	T8M	0	2
C07	T8D	0	1	G07	T8F	0	0
C08	T8S	2	2	G08	P9V	1	0

C09	P9M	1	2	G09	P9Q	0	1
C10	P9H	2	2	G10	P9I	0	1
C11	G10L	1	1	G11	G10N	0	1
C12	G10P	1	1	G12	G10R	0	1
D01	L6V	2	0	H02	L6F	1	1
D02	L6D	1	0	H03	C7V	1	0
D03	L6T	2	3	H04	C7Q	1	0
D04	C7M	0	2	H05	C7I	0	0
D05	C7H	0	1	H06	T8C	0	0
D06	T8A	0	2	H07	T8G	0	0
D07	T8P	0	1	H08	P9W	0	0
D09	P9N	1	2	H09	EMPTY		
D10	P9S	0	1	H10	EMPTY		
D11	G10M	0	1	H11	EMPTY		
D12	G10Q	0	1	H12	EMPTY		

Zones were scored as 0 (no zone of clearing), 1 (zone of clearing smaller than positive control), 2 (zone of clearing same as positive control), or 3 (zone of clearing larger than positive control.

TABLE 4 (Plate 3): ZONES OF CLEARING GENERATED BY MU1140 VARIANTS

Well	Mutation	*M Luteus* Zone of Clearing: Colony 1	*M Luteus* Zone of Clearing: Colony 2	Well	Mutation	*M Luteus* Zone of Clearing: Colony 1	*M Luteus* Zone of Clearing: Colony 2
A01	C21N	0	0	E01	C21Q	0	1
A02	C21R	1	0	E02	C21I	2	0
A03	C22W	0	0	E04	C22I	0	0
A04	C22F	0	0	E05	C7E	0	0
A05	S3E	0	0	E06	G15E	0	1
A06	G10E	0	0	E07	C22E	0	1
A07	N18E	0	0	E08	S5Y	2	0
A08	W4D	0	0	E09	P9C	2	1
A09	L6M	2	2	E10	G10I	2	1
A10	P9G	0	0	E11	R13V	1	1
A11	A12C	0	0	E12	G15A	1	3
B01	C21Y	0	0	F01	WT	2	2
B02	C21H	0	0	F02	WT	2	2
B03	C22A	0	0	F03	WT	2	2
B04	C22R	0	0	F04	WT	2	2
B05	W4E	0	0	F05	WT	2	2
B06	C11E	2	3	F06	WT	2	2
B07	S19E	0	0	F07	pMK4	0	0
B08	W4G	0	0	F08	pMK4	0	0
B09	L6S	1	1	F09	pMK4	0	0
B10	G10W	1	0	F10	pMK4	0	0
B11	A12P	1	1	F11	pMK4	0	0

B12	R13T	1	2	F12	pMK4	0	0
C01	C21D	0	1	G01	C21F	0	0
C02	C21S	0	1	G02	C22K	0	0
C03	C22L	1	0	G03	C22D	0	1
C04	C22S	0	0	G04	F1E	0	1
C05	S5E	0	0	G05	T8E	0	1
C06	R13E	0	3	G06	S16E	0	0
C07	Y20E	1	1	G07	S3Q	0	0
C08	W4S	0	1	G09	P9Y	1	0
C09	C7N	0	0	G10	C11A	2	3
C10	G10A	1	1	G11	R13N	2	1
C11	A12G	2	1	G12	G15D	1	0
C12	G15K	1	0	H01	C21G	0	2
D01	C21P	1	0	H02	C22V	0	0
D02	C21T	0	2	H03	C22P	0	0
D03	C22M	2	0	H04	K2E	2	3
D04	C22T	0	0	H05	P9E	1	2
D05	L6E	0	1	H06	F17E	1	0
D06	T14E	0	2	H07	W4L	1	3
D07	C21E	0	1	H08	L6K	0	0
D08	S5M	2	2	H09	EMPTY		
D09	P9K	1	2	H10	EMPTY		
D10	G10S	2	1	H11	EMPTY		
D11	A12T	1	3	H12	EMPTY		
D12	G15W	1	0				

Zones were scored as 0 (no zone of clearing), 1 (zone of clearing smaller than positive control), 2 (zone of clearing same as positive control), or 3 (zone of clearing larger than positive control).

TABLE 4 (Plate 4): ZONES OF CLEARING GENERATED BY MU1140 VARIANTS

Well	Mutation	*M Luteus* Zone of Clearing: Colony 1	*M Luteus* Zone of Clearing: Colony 2	Well	Mutation	*M Luteus* Zone of Clearing: Colony 1	*M Luteus* Zone of Clearing: Colony 2
A01	G10H	0	0	E01	C11W	0	0
A02	C11N	0	0	E02	C11Q	2	0
A03	C11R	0	0	E03	C11I	0	0
A04	A12W	0	0	E04	A12N	0	0
A05	A12Q	0	0	E05	A12S	0	0
A06	R13W	1	0	E06	R13C	0	0
A07	R13Q	0	0	E07	R13S	2	3
A08	T14A	0	0	E08	T14N	0	0
A09	T14P	0	0	E09	T14R	0	0
A10	T14S	0	0	E10	G15C	0	0
A11	G15Y	0	0	E11	G15H	0	0

A12	G15T	0	0	E12	S16A	0	0
B01	G10T	0	0	F01	WT	2	2
B02	C11Y	0	0	F02	WT	2	2
B03	C11H	0	0	F03	WT	2	2
B05	A12F	0	0	F04	WT	2	2
B06	R13A	2	0	F05	WT	2	2
B07	R13F	0	3	F06	WT	2	2
B08	T14L	0	0	F07	pMK4	0	0
B09	T14Q	2	0	F08	pMK4	0	0
B10	G15V	0	0	F09	pMK4	0	0
B11	G15P	0	0	F10	pMK4	0	0
B12	S16K	0	0	F11	pMK4	0	0
C01	C11K	0	0	F12	pMK4	0	0
C02	C11D	0	0	G01	C11L	0	0
C03	C11S	0	0	G02	C11F	0	0
C04	A12L	0	0	G03	A12K	0	0
C05	A12R	0	0	G04	A12Y	0	0
C06	R13L	0	0	G05	A12I	0	0
C08	T14M	0	0	G06	R13Y	0	0
C09	T14F	0	0	G07	R13I	0	0
C10	G15L	0	0	G08	T14Y	0	0
C11	G15Q	0	0	G09	T14H	0	0
C12	S16V	0	0	G10	G15N	2	3
D01	C11V	0	0	G11	G15S	2	3
D02	C11P	0	0	G12	S16L	0	0
D03	C11T	0	0	H01	C11M	0	0
D04	A12M	0	0	H02	C11G	2	0
D05	A12H	0	0	H03	A12V	2	0
D06	R13M	0	0	H04	A12D	2	0
D07	R13H	0	0	H05	R13K	0	0
D08	T14C	0	0	H06	R13P	0	0
D09	T14G	0	0	H07	T14K	0	0
D10	G15M	0	0	H08	T14D	0	0
D11	G15F	0	0	H09	EMPTY		
D12	S16W	0	0	H10	EMPTY		
				H11	EMPTY		
				H12	EMPTY		

Zones were scored as 0 (no zone of clearing), 1 (zone of clearing smaller than positive control), 2 (zone of clearing same as positive control), or 3 (zone of clearing larger than positive control.

TABLE 4 (Plate 5): ZONES OF CLEARING GENERATED BY MU1140 VARIANTS

Well	Mutation	*M luteus* Zone of Clearing Colony 1	*M luteus* Zone of Clearing Colony 2	Well	Mutation	*M luteus* Zone of Clearing Colony 1	*M luteus* Zone of Clearing Colony 2
A01	S16M	0	0	E01	S16D	0	0
A02	S16G	0	0	E02	S16I	0	0
A03	F17W	0	0	E03	F17Y	2	3
A04	F17Q	2	0	E04	F17S	0	0
A05	N18V	0	0	E05	N18M	0	0
A06	N18D	0	0	E06	N18G	0	0
A07	N18T	0	0	E07	S19C	0	0
A08	S19D	0	0	E08	S19G	0	0
A09	S19S	1	2	E09	Y20W	0	0
A10	Y20M	1	2	E10	Y20D	0	0
A11	Y20F	3	3	E11	Y20S	0	0
A12	Y20I	1	0	E12	C21L	1	0
B01	S16C	0	0	F01	WT	2	2
B02	S16R	0	0	F02	WT	2	2
B03	F17L	2	0	F03	WT	2	2
B05	N18W	0	0	F04	WT	2	2
B06	N18P	0	0	F05	WT	2	2
B07	S19V	0	0	F06	WT	2	2
B08	S19P	0	0	F07	pMK4	0	0
B09	S19T	0	0	F08	pMK4	0	0
B10	Y20C	0	0	F09	pMK4	0	0
B11	Y20G	0	0	F10	pMK4	0	0
B12	C21V	2	0	F11	pMK4	0	0
C01	S16N	0	0	F12	pMK4	0	0
C02	S16H	0	0	G01	S16P	0	0
C03	F17C	0	0	G02	F17K	0	0
C04	F17G	0	0	G03	F17D	0	0
C05	N18A	2	0	G04	F17T	2	0
C06	N18Q	0	0	G05	N18C	0	0
C07	S19A	0	0	G06	N18H	0	0
C08	S19Q	0	0	G07	S19N	0	0
C09	Y20K	0	0	G08	S19R	0	0
C10	Y20N	0	0	G09	Y20L	2	0
C11	Y20R	0	0	G10	Y20Q	0	0
C12	C21W	0	0	G11	Y20T	0	0
D01	S16Y	2	0	G12	C21M	0	0
D02	S16T	0	0	H01	S16Q	0	0
D03	F17N	0	0	H02	F17V	0	0
D04	F17H	2	3	H03	F17P	0	0
D05	N18L	0	0	H04	F17I	2	0

D06	N18F	0	0	H05	N18Y	0	0
D07	S19M	0	0	H06	N18S	0	0
D08	S19F	0	0	H07	S19Y	0	0
D09	Y20V	0	0	H08	S19H	0	0
D11	Y20H	0	0	H09	EMPTY		
D12	C21A	0	0	H10	EMPTY		
				H11	EMPTY		
				H12	EMPTY		

Zones were scored as 0 (no zone of clearing), 1 (zone of clearing smaller than positive control), 2 (zone of clearing same as positive control), or 3 (zone of clearing larger than positive control).

TABLE 4 (Plate 6): ZONES OF CLEARING GENERATED BY MU1140 VARIANTS

Well	Mutation	*M luteus* Zone of Clearing Colony 1	*M luteus* Zone of Clearing Colony 2	Well	Mutation	*M luteus* Zone of Clearing Colony 1	*M luteus* Zone of Clearing Colony 2
A01	G15R	0	0	E01	F17A	3	0
A02	N18K	0	0	E02	S19W	0	0
A03	Y20A	1	0	E03	C22Q	0	0
A04	A12E	0	2	E04	R13G	0	0
A05	T14I	0	0	E05	EMPTY	0	0
A06	R13Q	1	3	E06	EMPTY	0	0
A07	T14P	0	3	E07	EMPTY	0	0
A08	T14S	2	0	E08	EMPTY	0	0
A09	G15Y	0	0	E09	EMPTY	0	0
A10	G15P	0	0	E10	EMPTY	0	0
A11	S16K	0	0	E11	EMPTY	0	0
A12	R13L	3	3	E12	EMPTY	0	0
B01	G15I	2	2	F01	WT	2	2
B02	N18R	0	0	F02	WT	2	2
B03	C21K	0	0	F03	WT	2	2
B04	S3I	0	0	F04	WT	2	2
B05	Y20P	0	0	F05	WT	2	2
B06	C11V	0	0	F06	WT	2	2
B07	R13M	0	0	F07	pMK4	0	0
B08	A12I	0	0	F08	pMK4	0	0
B09	T14H	2	1	F09	pMK4	0	0
B11	C21M	0	1	F10	pMK4	0	0
B12	EMPTY	0	0	F11	pMK4	0	0
C01	S16F	0	0	F12	pMK4	0	0
C02	N18I	0	1	G01	F17M	3	0
C03	C22N	0	1	G02	S19L	0	0
C04	W4T	1	0	G03	C22G	0	0
C05	EMPTY	0	0	G04	T14V	0	0

C06	EMPTY	0	0	G05	EMPTY	0	0
C07	EMPTY	0	0	G06	EMPTY	0	0
C08	EMPTY	0	0	G07	EMPTY	0	0
C09	EMPTY	0	0	G08	EMPTY	0	0
C10	EMPTY	0	0	G09	EMPTY	0	0
C11	EMPTY	0	0	G10	EMPTY	0	0
C12	EMPTY	0	0	G11	EMPTY	0	0
D02	S19K	0	0	G12	EMPTY	0	0
D03	C22Y	0	0	H01	F17R	3	2
D04	C7Y	0	0	H02	S19I	0	0
D05	EMPTY	0	0	H03	C22H	0	0
D06	EMPTY	0	0	H04	T14W	0	0
D07	EMPTY	0	0	H05	EMPTY	0	0
D08	EMPTY	0	0	H06	EMPTY	0	0
D09	EMPTY	0	0	H07	EMPTY	0	0
D10	EMPTY	0	0	H08	EMPTY	0	0
D11	EMPTY	0	0	H09	EMPTY	0	0
D12	EMPTY	0	0	H10	EMPTY	0	0
				H11	EMPTY	0	0
				H12	EMPTY	0	0

Zones were scored as 0 (no zone of clearing), 1 (zone of clearing smaller than positive control), 2 (zone of clearing same as positive control), or 3 (zone of clearing larger than positive control).

TABLE 5: INCONSISTENCY OBSERVED IN *M. LUTEUS* ZONE OF CLEARING SIZES BY IDENTICAL GENOTYPES GROWN IN DIFFERENT 96-WELL PLATES AND WELLS

plate 1	G02	F1F	1	2
plate 1	D08	S3S	2	0
plate 3	G08	S5S	3	2
plate 2	H01	L6L	1	1
plate 2	D08	T8T	0	1
plate 4	B04	A12A	0	0
plate 4	C07	R13R	0	0
Plate 6	D01	S16S	1	0
plate 5	B04	F17F	0	3
plate 5	A09	S19S	1	2
plate 5	D10	Y20Y	0	0
plate 3	E03	C22C	1	0

[000162] Construction and testing of the saturated scanning mutagenesis library containing 418 mutA variants enables unbiased sampling of the MU1140 amino acid sequence for screening of variants that confer improved antimicrobial activity against a sensitive indicator microbe (*i.e.*, *M. luteus*). The mutA variant plasmid expression system used in this study may not be

sufficiently robust for the determination of differential changes in antimicrobial activity, as experimental variation was observed using this plasmid-based system. Antimicrobial activity levels from plasmid-based mutA expression were low, inconsistent and not normalized against levels of protein production (*e.g., see* **Table 5**). Without wishing to be bound by any particular theory, Applicants contemplate that certain MU1140 variants set forth in **Table 1** may have such high levels of antibiotic/antimicrobial activity that these variants may severely inhibit or kill the *S. mutans* SM126 host cell.

[000163] In total, 41 mutA variants generated larger zones of clearing relative to the positive control strain (*i.e.*, SM126 ΔmutAA') for at least one of the two clones tested, representing 12 of the 22 possible amino acid positions of the MU1140 polypeptide. As set forth in Example 2 below, genomic integration of mutA variants was selected as an alternative expression strategy to improve levels and consistency of MU1140 variant production.

Results of MU1140 Single Site Variants (Stably Integrated into Chromosome)

[000164] A first generation library of 418 MU1140 variants was constructed and tested in *S. mutans* (*see*, Example 1), resulting in detection of 114 variants that demonstrated equal or improved antimicrobial activity against *M. luteus* (*i.e.*, relative to native (wild-type) MU1140). A 58 member subset of the 114 MU1140 variant producing strains were reconstructed using vectors designed to integrate site specifically at the mutA locus on the *S. mutans* chromosome, replacing the native copy of mutA. The 58 MU1140 variants were selected based on improved antimicrobial activity against *M. luteus*. See **Figure 6**.

[000165] The 58 MU1140 variant producing strains were assessed for improvements in antimicrobial activity using the *M. luteus* zone of clearing assay using non-normalized culture supernatants obtained from overnight growth of *S. mutans* strains. Consistent (well-to-well and plate-to-plate) zones of clearing were observed for both *S. mutans* strains JH1140 and SM152. Strains expressing twenty six (26) different variants that generated zones of clearing larger than the positive control (strain SM152) were identified for further analysis. Strains expressing an additional 15 variants (with zones of clearing of similar or smaller than the control strain SM152) were identified representing novel and/or underrepresented amino acid positions. In total, 41 variants were identified for further analysis based on antimicrobial activity and

positional diversity criteria (**Table 6**).

TABLE 6: STRAINS SELECTED FOR FURTHER ANALYSIS BASED ON THE SIZE OF THE NON-NORMALIZED ZONE OF CLEARING AND/OR POSITIONAL DIVERSITY

Zone of clearing		
Larger than SM152	Similar to SM152	Smaller than SM152
F1A	F1N	W4V
F1I	F1L	S5M
F1S	K2I	P9H
F1T	K2M	C11E
F1Y	S5A	
K2A	L6H	
K2E	L6N	
K2N	L6V	
K2Q	C11A	
W4A	R13S	
W4F	Y20F	
W4Y		
L6A		
L6T		
C11G		
R13A		
R13D		
R13L		
R13N		
R13Q		
R13T		
G15N		
G15S		
F17L		
F17Y		
N18A		

EXAMPLE 3

MASS SPECTRA OF NATIVE MU1140 and MU1140 VARIANTS

[000166] Mass Spectrometry was used to confirm identity of the MU1140 variants that were produced using codon substitutions of the mutA gene in *S. mutans* JH1140. The theoretical molecular weight of each variant was calculated based on the published 2,266 Dalton (Da) molecular weight of MU1140 (Chen *et al.*, *Microbiology*, *79*(13), 4015–23, 2013), by subtraction of the molecular weight of the specific amino acid residue, addition of the molecular weight of the replacement amino acid residue, and adjustment for ring hydrolysis (18 Da) or 2, 3-didehydroalanine (DHA) substitution (2 Da) as appropriate.

Results

[000167] The multi-site codon MU1140 variants were independently resolved by LC-MS using standard methods. The results for the single site variants and the multi-site variants are provided in **Table 7** and **Table 8**, respectively.

Table 7: Mass spectral analysis of single site MU1140 variants

Mutation	**Retention time for LC-MS**	**Calculated MW**	**MW by MS analysis**
F1I	2.26	2232	2231.1
F1L	2.29	2232	2230.5
R13N	2.63	2223.9	2223
F17L	2.35	2232	2230.8
F17Y	2.16	2282	2280.6
N18A	2.42	2223	2220
Y20F	2.53	2268	2248.2

Table 8: Mass spectral analysis of multi-site MU1140 variants

Analogue	Calculated MW	Measured masses (monoisotopic)	Retention time LC-MS [min][1]
F1I	2232	2231	16.06
F1A R13A	2104.8	2102.8	16.94
F1I R13D	2190.9	2189.9	17.58
F1T R13G	2120.8	2119.8	16.78
F1L R13D	2190.9	2188.9	17.73
F1I R13N	2189.9	2187.9	17.23
F1I R13N G15A	2203.9	2201.9	17.51
F1I W4M R13A	2091.9	2089.9	16.51
F1I R13A	2146.9	2144.9	17.58
F1L R13N	2189.9	2187.9	17.35
F1L R13A	2146.9	2144.9	17.68
F1S R13N	2163.8	2161.9	16.45
F1V R13N	2175.8	2173.9	16.93
F1G R13N	2133.8	2131.8	16.42
F1I R13N Y20F	2173.9	2171.9	17.91

[000168] Variant molecular weights were found to agree with the calculated molecular weight within 2.0 daltons. The agreement between the experimental and calculated molecular weights confirms production of the variants as engineered in *S. mutans*.

EXAMPLE 4

THERAPEUTIC PROPERTIES OF MU1140 VARIANTS

Scale-up and purification

[000169] *S. mutans* strains were grown in 1 L shake flasks or 1 L fermenters. Clarified culture supernatants were recovered using chloroform extraction (Chen *et al. Microbiology*, *79*(13), 4015–23, 2013), and purified using flash chromatography to greater than 90% purity based on absorbance at 280 nm using standard techniques. Variants were lyophilized to dryness, quantified by dry weight and UPLC, and then assayed for activity using *C. difficile* MIC and simulated intestinal fluid stability assays.

[000170] Follow-up testing of MU1140 variant performance in an animal model required more than 200 mg pure variant per treatment. Select variants were scaled to 5 L fermenters and purified using two step chromatographic purification to greater than 90% purity based on absorbance at 280 nm.

***C. difficile* MIC assay**

[000171] A minimum inhibitory concentration (MIC) assessment was performed using the anaerobic human gastrointestinal pathogen *Clostridium difficile*. A modification of the CLSI M11-A8 broth dilution method for *Bacteroides fragilis* susceptibility testing was used for *C. difficile*. (Methods for Dilution In Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Eighth Edition. CLSI document M07-A8 (ISBN 1-56238-689-1). Vol. 29 No. 2. Clinical and Laboratory Standards Institute, USA, 2009). The testing determined the MIC of vancomycin and MU1140 variants, defined as the lowest concentration of an antimicrobial agent that completely prevents visible growth of the bacteria in broth medium. *C. difficile* was streaked and incubated on blood agar plates under anaerobic conditions. Vancomycin and MU1140 variants were dissolved and diluted with 100% DMSO in 2-fold dilutions. *C. difficile* strains (ATCC 9689, BAA-1805, ATCC 70057, ATCC 43596, ATCC 43255, BI1 and BAA-1875 for selected single and/or multi-site variants and UNT103-1, UNT107-1, ATCC BAA 1874, ATCC 43597 & ATCC BAA-1808 for selected muti-site variants) were each tested at 11 concentrations by 2-fold serial dilutions from 64 to 0.0625 μg/mL of vanomycin or MU1140 variant. A 4 μL aliquot of each dilution was added to 196 μL of Reinforced Clostridial Medium (RCM), with test strains in wells of a 96 well plate. The final volume was 200 μL in each well. The assay plates were loosely wrapped in a plastic bag and incubated 46-48 hours at 36°C for *C. difficile* under anaerobic conditions and then visually examined and scored positive (+) for inhibition of growth or turbidity or negative (-) for no effect upon growth or turbidity.

MIC assay against other Gram Positive and Acid-fast Micro-organisms

A minimum inhibitory concentration (MIC) assessment against a number of other Gram positive microorganisms and *Mycobacterium phlei* (an acid-fast organism) was done using the broth dilution assay as described by the CLSI (CLSI document M07-A8). Single site variants were

tested against three Vancomycin resistant Enterococci (VRE; two *Enterococcus faecalis* strains (ATCC 51575 and ATCC 51299), one *Enterococcus faecium* strain (ATCC 700221)), five *Staphylococcus aureus* strains (ATCC 29213, 19636, 33591, 700699 and BAA-1717), *Streptococcus pneumoniae* (ATCC 700675), and *Mycobacterium phlei* (ATCC 11758). Multi-site variants were tested against three VRE strains (two *Enterococcus faecalis* strains (CCUG 47775 and ATCC 700221), one *Enterococcus faecium* strain (ATCC 700221)), *Clostridium scindens* (ATCC 35704), *Bifidobacterium breve* (ATCC 15700), *Lactobacillus acidophilus* (CCUG 4356), *Peptostreptococcus anaerobius* (ATCC 27337) and *Eggerthella lenta* (ATCC 27337). Samples and a standard reference agent for each strain (Linezolid, Ampicillin, Gentamicin and Vancomycin, as applicable) were dissolved and diluted in 100% DMSO and 4 μL was added per well to 96-well plate with 196 μL of broth seeded with the test organism. Samples were tested at 11 concentrations from 64 to 0.0625 μg/mL. The assay plates were loosely wrapped in a plastic bag and incubated 20-24 hours at 36°C under aerobic conditions for Enterococcus Spp., *S. aureus, M. phlei* and *P. aeruginosa* and under 5% CO2 for *S. pneumoniae, C. scindens, B. breve, L. acidophilus, P. anaerobius* and *E. lenta*, and then visually examined and scored positive (+) for inhibition of growth or turbidity or negative (-) for no effect upon growth or turbidity.

MIC against Gram Negatives (Including *H. pylori*)

[000172] A minimum inhibitory concentration (MIC) assessment against *Pseudomonas aerigunosa* (a Gram negative bacterium) was performed for single site variants in the broth dilution assay as described by the CLSI (CLSI document M07-A8). Samples and Ofloxacin control were dissolved and serially diluted in 100% DMSO and 4 μL was added per well to 96-well plate with 196 μL of Cation Adjusted Mueller Hinton II Broth seeded with *P. aeruginosa*. The compounds were tested at 11 concentrations from 64 to 0.0625 μg/mL. The assay plates were loosely wrapped in a plastic bag and incubated 20-24 hours at 36°C (aerobic conditions) and then visually examined and scored positive (+) for inhibition of growth or turbidity or negative (-) for no effect upon growth or turbidity.

A minimum inhibitory concentration (MIC) assessment against *Heliobacter pylori* (a Gram negative) was performed for multi-site variants in the broth dilution assay as described by the

CLSI (CLSI document M07-A8) with and without the addition of 0.05M EDTA (Ethylenediaminetetraacetic acid, a cell membrane sensitizing agent). Samples and Tetracycline control were dissolved and serially diluted in 100% DMSO and 4 µL was added per well to 96-well plate with 196 µL of Brucella Broth – 7% FBS seeded with *H. pylori* ± 0.05 M EDTA. The compounds were tested at 11 concentrations from 64 to 0.0625 µg/mL. The assay plates were loosely wrapped in a plastic bag and incubated 20-24 hours at 36°C under 5% CO_2 (anaerobic conditions) and then visually examined and scored positive (+) for inhibition of growth or turbidity or negative (-) for no effect upon growth or turbidity.

Simulated intestinal fluid assay

[000173] An *in vitro* simulated intestinal fluid stability assay was performed to simulate the stability of MU1140 variants in the gastrointestinal tract in the presence of simulated intestinal fluid and proteases. Assay incubation conditions included 475 µL simulated intestinal fluid (FaSSIF-v2 (Ekarat *et al.*, Pharm Res. Vol.25, No.7, July 2008) containing 1% porcine pancreatin, 3 µg/ml bovine trypsin, and 3 µg/ml bovine chymotrypsin mixed with 25 µl of 1 mg/mL variant for a final concentration of 50 µg/ml variant in a 96 well format. Samples were incubated at 37°C and analyzed using LC-MS/MS for loss of substrate. The percent remaining, elimination rate constant (Ke), and *in vitro* protein half-lives were calculated.

Simulated Gastric Fluid Assay

[000174] An *in vitro* simulated gastric fluid stability assay was performed to simulate the stability of nine MU1140 variants in the gastric chamber in the presence of gastric fluid and proteases. Assay incubation conditions included 242.5 µL simulated gastric fluid (SGF, USP 39 Test Solution; 2 g/L NaCl, 7 mL/L glacial HCl, pH 1.4) containing 1.75 mg/mL Pepsin (3200 – 4500 U/mg) mixed with 7.5 µL of 10 mg/mL variant (or positive control, NH_2-Met-Arg-Phe-Ala-OH, MRFA, CAS# 67368-29-0) for a final concentration of 300 µg/ml variant in a microfuge tube. Samples were incubated at 37°C and analyzed using HPLC or LC-MS for loss of substrate. The percent remaining, elimination rate constant (Ke), and *in vitro* protein half-lives were calculated. A control of SGF without enzyme was also run and analyzed in the same manner for each variant.

Animal study

[000175] A cannulated Syrian hamster *C. difficile* model was used to assess efficacy of MU1140 variants in the treatment of *C. difficile* infection. Following ileal cannulation surgery and recovery, male Golden Syrian hamsters were inoculated with *Clostridium difficile* UNT103-1 (VA11, non epidemic (cdtB-, REA group J) Curtis Donskey, Cleveland VA Hospital, Cleveland, OH). On Day 1, at 24 hrs after infection, all animals received a single subcutaneous injection of clindamycin (10 mg/kg). Test article formulations were administered three times per day (TID), starting on Day 2 at 18 hours after Clindamycin injection, for 5 consecutive days (Days 2 through 6). Vancomycin (positive control) was administered once a day at 20 mg/kg on Days 2 through 6, and followed until Day 22 to assess recurrence. The cecal contents from all hamsters that died on study or euthanized at the end (Day 21) were tested for *C. difficile* Toxin A and Toxin B (tgc BIOMICS ELISA Kit, cat# TGC-E002-1) and for *C. difficile* Spore counts. Spore titer was determined by incubating the cecal contents in 50% final concentration of ethanol for 60 minutes at room temperature, centrifuging and resuspending the pellet in nano-pure water. The resuspension is incubated at 65°C for 15 minutes, centrifuged again and the pellet resuspended again in nano-pure water. The sample is diluted and spotted onto Colombia agar plates with 7% laked house blood, 0.5 mg/mL cylcoserine, 15 μg/mL cefoxitin and 1 mg/mL taurocholate. After 48 hours of anaerobic incubation, spore counts are determined as colony forming units (CFUs).

[000176] A follow up non-cannulated Syrian hamster *C. difficile* infection model was used to assess efficacy of seven MU1140 variants in the treatment of *C. difficile* infection following an oral gavage regimen. Following the recovery phase, male Golden Syrian hamsters were inoculated with *Clostridium difficile* UNT103-1 (VA11, non epidemic (cdtB-, REA group J) Curtis Donskey, Cleveland VA Hospital, Cleveland, OH). On Day 1, at 24 hrs after infection, all animals received a single subcutaneous injection of clindamycin (10 mg/kg). Test article formulations were administered three times per day (TID), starting on Day 2 at 18 hours after Clindamycin injection, for 5 consecutive days (Days 2 through 6), and followed until Day 22 to assess recurrence. Vancomycin (positive control) was administered once a day at 20 mg/kg on Days 2 through 6. Cecal spore counts and Toxins A and B were determined as described above

[000177] A second non-cannulated Syrian hamster *C. difficile* infection model was used to better define the optimal therapeutic regimen of two MU1140 variants in the treatment of *C. difficile* infection following an oral gavage regimen. Following the recovery phase, male Golden Syrian hamsters were inoculated with *Clostridium difficile* UNT103-1 (VA11, non epidemic (cdtB-, REA group J) Curtis Donskey, Cleveland VA Hospital, Cleveland, OH). On Day 1, at 24 hrs after infection, all animals received a single subcutaneous injection of clindamycin (10 mg/kg). Test article formulated at 3 to 5 different doses were administered three times per day (TID), starting on Day 2 at 18 hours after Clindamycin injection, for 5 consecutive days (Days 2 through 6), and followed until Day 22 to assess recurrence. Vancomycin (positive control) was administered once a day at 20 mg/kg on Days 2 through 6. Cecal spore counts and Toxins A and B were determined as described above.

Results

[000178] Variants were tested for specific antimicrobial activity against the anaerobic Gram-positive pathogen *C. difficile* (**Table 9**) on a weight/volume basis. Three individual *C. difficile* strains were evaluated to assess the spectrum of activity of MU1140 variants against *C. difficile*. Although improvements in antimicrobial activity were observed in non-normalized culture supernatants against *M. luteus*, in a normalized assay the variants did not demonstrate improvements in antimicrobial activity against *C. difficile* relative to MU1140. Nevertheless, variants tested demonstrated specific antimicrobial activity that was better than vancomycin, a current standard of care for *C. difficile* infection.

[000179] Multisite variants were subsequently tested for specific antimicrobial activity in a *C. difficile* MIC assay (**Table 10**) (Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria Approved Standard-Seventh Edition. CLSI document M11-A8 (ISBN 1-56238-790-1). Vol. 32 No. 5. Clinical and Laboratory Standards Institute, USA, 2012).

Table 9: MIC assay single site variants to *C. difficile*

Variant/ Vancomycin	***C. difficile* MIC (µg/ml)**						
	ATCC 9689	**BAA-1805**	**BAA-1875**	**ATCC 43596**	**ATCC 43255**	**ATCC 70057**	**BI1**
MU1140	1, 1	0.25	0.5	0.5	0.25	0.06, 0.1	0.11
F1I	4	N/A	N/A	N/A	N/A	0.06	0.49
F1L	4, 4	0.5	0.5	0.5	0.5	0.07, 0.5	0.07
R13N	4, 4	0.5	0.5	0.5	0.5	1.6, 0.5	0.81
F17L	4	N/A	N/A	N/A	N/A	0.07	0.07
F17Y	2	N/A	N/A	N/A	N/A	0.07	2.4
N18A	2, 4	0.5	2	0.5	0.5	0.2, 2	0.19
Y20F	2, 0.5	0.25	0.25	0.25	0.25	N/A, 0.25	N/A
Vancomycin	4, 8	1	1	1	1	0.5, 1	8

Note, two values provided (x, y) for variants that were tested at two independent times

Table 10: MIC assay multi-site variants to *C. difficile*

Variant	*C. difficile MIC* (ug/ml)							
	ATCC 9689	**ATCC BAA-1805**	**ATCC BAA-1875**	**103-1**	**107-1**	**ATCC BAA-1874**	**ATCC 43597**	**ATCC BAA-1808**
Vancomycin	0.5	1	0.5	1	4	0.5	0.5	0.5
F11 standard	0.5	0.25	0.25	0.5	0.5	0.25	0.25	0.125
F1I	0.5	0.5	0.25	0.06249	0.25	0.25	0.25	0.25
F1A R13A	2	2	2	0.5	4	4	2	1
F1I R13D	2	1	1	0.5	2	0.5	1	1
F1T R13G	8	8	8	4	16	8	8	8
F1L R13D	1	1	1	0.5	2	1	1	1
F1I R13N	0.5	0.25	0.5	0.125	1	0.5	0.25	0.5
F1I R13N G15A	8	8	8	2	8	8	8	8
F1I W4M R13A	8	4	4	1	4	4	4	8
F1I R13A	1	0.25	0.5	0.25	1	0.5	0.5	0.25
F1L R13N	0.5	0.25	0.5	0.125	0.5	0.25	0.25	0.25
F1L R13A	0.5	0.25	0.5	0.25	0.5	0.5	0.5	0.5
F1S R13N	16	8	16	2	16	16	8	8
F1V R13N	0.5	0.25	0.5	0.25	0.5	0.5	0.25	0.25
F1G R13N	8	8	8	2	16	16	8	8
F1I R13N Y20F	0.5	0.5	1	0.25	0.5	0.5	0.5	0.25
F1A R13G	8	8	8	2	8	8	4	8

[000180] Single site variants were tested for specific antimicrobial activity against a host of gram positive and an acid fast microorganisms (**Table 11**) on a weight/volume basis. Three individual *Enterococci* strains were evaluated to assess the spectrum of activity of MU1140 variants against VRE, five strains of *S. aureus*, *S. pneumonia* and the acid-fast strain *M. phlei*. While variants tested did not show significant differences than MU1140, all demonstrated specific antimicrobial activity that were in the tested range ($\leq$ 64 μg/mL) against all the organisms tested, though many were less susceptible than to the control.

[000181] Multisite variants were subsequently tested for specific antimicrobial activity against a host of gram positive microorganisms (**Table 12**) and a weight/volume basis. Individual *Enterococci* strains were evaluated to assess the spectrum of activity of MU1140 variants against VRE, and strains of *C. scindens, B. breve, L. acidophilus, P. anaerobius, E. lenta* were also assessed. Multi-site variants demonstrated MICs against most of the organisms

tested, though many were less susceptible than the control. A few of the multisite variants did not demonstrate any activity against some of the VRE strains tested.

Table 11: Other Gram Positives - MIC assay single site variants

Variant/ Control	**Gram Positive / Acid-Fast Microorganism MIC (µg/mL)**									
	VRE ATCC 51575	**VRE ATCC 51299**	**VRE ATCC 700221**	***S. aureus* ATCC 29213**	***S. aureus* ATCC 19636**	***SS. aureus* ATCC 33591**	***S. aureus* BAA-1717**	***S. aureus* ATCC 700699**	***S. pneum-onia***	***pM. phlei***
MU1140	4	4	2	2	1	1	2	2	8	2
F1I	8	8	4	4	4	0.5	2	4	16	2
F1L	8	4	2	4	2	0.5	2	2	16	2
R13N	8	16	4	2	2	0.5	2	4	32	8
F17L	8	16	2	4	2	1	2	2	64	2
F17Y	8	8	4	2	1	1	2	2	32	2
N18A	16	8	2	4	4	2	2	2	32	2
Y20F	64	4	1	2	1	1	2	2	8	2
Control	L	L	L	G	G	G	G	V	A	V
MIC	2	2	2	0.25	0.125	1	0.25	2	1	0.25

Controls: L = Linezoid, G = Gentamicin, V = Vancomycin, A = Ampicillin

Table 12: Other Gram Positives – MIC assay multi-site variants

Variant / Control	Gram Positive Microorganism MIC (µg/mL)							
	VRE CCUG 47775	**VRE ATCC 51575**	**VRE ATCC 700221**	***C. scindens***	***B. breve***	***L. acidophilus***	***P. anaerobius***	***E. lenta***
F11 standard	8	4	4	2	0.5	0.25	0.25	0.25
F1I	8	4	4	2	0.5	0.5	0.25	0.5
F1A R13A	32	32	16	16	4	8	2	4
F1I R13D	32	32	32	8	2	2	1	2
F1T R13G	64	64	32	64	16	8	8	8
F1L R13D	32	64	32	8	2	8	2	1
F1I R13N	16	16	32	2	1	0.5	0.25	0.5
F1I R13N G15A	> 64	32	32	16	8	8	4	8
F1I W4M R13A	> 64	> 64	> 64	32	8	32	8	16
F1I R13A	16	16	8	4	1	0.5	1	2
F1L R13N	16	16	16	4	1	0.5	0.25	0.25
F1L R13A	16	32	16	4	1	0.5	0.5	0.5
F1S R13N	> 64	> 64	64	32	16	16	8	16
F1V R13N	32	32	16	4	1	0.5	0.25	0.5
F1G R13N	> 64	> 64	32	16	16	8	4	16
F1I R13N Y20F	16	8	8	2	0.5	0.5	0.125	0.125
F1A R13G	> 64	> 64	32	32	8	16	4	16
Control MIC	L 2	L 2	L 4	V 1	V 0.5	V 1	V 1	L 1

Controls: L = Linezoid, V = Vancomycin

[000182] Single site variants were tested for specific antimicrobial activity against *P. aeruginosa* (a Gram negative microorganism) on a weigh/volume basis (**Table 13**). All single-site variants demonstrated no inhibition of growth at all the dilutions tested (MIC > 64 µg/mL), consistent with the specificity of MU1140 for activity against Gram positive organisms.

[000183] The multi-site variants were tested for specific antimicrobial activity against *H. pylori* (a Gram negative microorganism) with and without 0.05 M EDTA (a membrane sensitizing agent) on a weigh/volume basis (**Table 14**). Inhibition of growth was observed for some of the variants, but at levels significantly higher than the Tetracyline control. The addition of 0.05M EDTA had a notable positive effect on the MIC values of several variants but not all.

Table 13: MIC Assay of Single Site Variants to a Gram Negative

Variant/Control	MIC to *P. aeruginosa*
MU1140	> 64 µg/mL
F1I	> 64 µg/mL
F1L	> 64 µg/mL
R13N	> 64 µg/mL
F17L	> 64 µg/mL
F17Y	> 64 µg/mL
N18A	> 64 µg/mL
Y20F	> 64 µg/mL
Ofloxacin Control	1 µg/mL

Table 14: MIC Assay Multi-Site Variants to *H. pylori*

Variant/Control	**MIC to *H. Pylori* without EDTA (µg/mL)**	**MIC to *H. Pylori* with 0.05M EDTA (µg/mL)**
F11 standard	16	8
F1I	16	8
F1A R13A	> 64	64
F1I R13D	8	8
F1T R13G	> 64	> 64
F1L R13D	8	4
F1I R13N	8	8
F1I R13N G15A	> 64	64
F1I W4M R13A	64	64
F1I R13A	8	8
F1L R13N	8	8
F1L R13A	8	8
F1S R13N	> 64	> 64
F1V R13N	8	8
F1G R13N	> 64	> 64
F1I R13N Y20F	8	4
F1A R13G	> 64	> 64
Tetracycline Control	0.5	0.25

[000184] MU1140 variants were tested in simulated intestinal fluid assay to determine stability of variants in the presence of pancreatin, trypsin, and chymotrypsin (**Table 15**). A half-life of 53.2 to 72.4 min was observed for MU1140, while substitution at position 1 (a putative pepsin cleavage site) prolonged half-lives of variants F1I and F1L to 95 and 77.3 minutes, respectively. Mutation at position 13, a putative trypsin cleavage site, prolonged the half-life of variant R13N to > 240 min. Mutation at position 20, a putative chymotrypsin site, prolonged the half-life of variant Y20F to 148 min. Mutation of position 18 to N18A however, rendered the molecule susceptible to proteolytic degradation and shortened the half-life to 24 min.

Table 15: Simulated Intestinal Fluid Assay

Variant	**Half-life in FaSSIF+ (min)**
MU1140	53.2
SM152	72.4
F1I	95
F1L	77.3
R13N	>240
N18A	24
Y20F	148

[000185] Next, the hypothesis that independent mutations increase variant stability in an additive manner was tested. Multi-site variants were tested in simulated intestinal fluid containing pancreatin, trypsin, and chymotrypsin to determine if the half-lives of multi-site variants demonstrate improved survival (**Table 16**). Ten of the fifteen multi-site variants tested exhibited a half-life of more than 720 minutes, compared to a half-life of 48 and 58 minutes observed for the SM253 reference standard and the positive control F1I that was purified using the same conditions as the multi-site variants. The improved stability of multi-site variants relative to the single-site variant SM253 indicates that the putative protease cleavage sites in native MU1140 are susceptible to protease-mediated cleavage under physiological conditions despite the extensive post-translational modifications of the lantibiotic.

Table 16: Simulated Intestinal Fluid Assay

Variant	**FaSSIF only half-life (min)**	**FaSSIF+1%pancreatin+3µg/ml trypsin+ 3µg/ml chymotrypsin half-life (min)**
F1I reference standard	>720	48 & 58 (2 tests)
F1A R13A	>720	130
F1I R13D	>720	>720
F1T R13G	>720	114
F1L R13D	>720	>720
F1I R13N	>720	>720
F1I R13N G15A	>720	>720
F1I W4M R13A	>720	349
F1I R13A	>720	>720
F1L R13N	>720	>720
F1L R13A	>720	>720
F1S R13N	>720	>720
F1V R13N	>720	>720
F1G R13N	>720	225
F1I R13N Y20F	>720	>720
F1A R13G	299	124

[000186] Next, the hypothesis that lantibiotics are not degraded in gastric fluid with pepsin (due to the lack of a c-terminal Amino Acid) and that key independent mutations did not effect that stability was tested. Multi-site variants that demonstrated improved stability in FaSSIF and that had equal or better MICs than FI1 (SM253) were tested in simulated gastric fluid (pH 1.5) containing pepsin to determine if the half-lives of multi-site variants demonstrate differences in degradation (**Table 17**). All eight of the variants tested demonstrated a half-life of more than 1440 minutes (24 hours) which was comparable to F1I. The prolonged stability of the multi-site variants indicates that the mutations within the peptide that lead to improved performance do not lead to reduced stability during upper gastric transit.

Table 17: Simulated Gastric Fluid Assay

Variant	**SGF only half-life (min)**	**SGF + 1.75 mg/ml Pepsin half-life (min)**
F1I reference standard	>1440	>1440
F1I R13D	>1440	>1440
F1L R13D	>1440	>1440
F1I R13N	>1440	>1440
F1I R13A	>1440	>1440
F1L R13N	>1440	>1440
F1L R13A	>1440	>1440
F1V R13N	>1440	>1440
F1I R13N Y20F	>1440	>1440
Positive Control	>1440	906

[000187] Six variants were evaluated *in vivo* using a *C. difficile* cannulated Syrian Hamster Model to determine if improvements in the efficacy of *C. difficile* treatment could be observed relative to a current clinical standard of care, vancomycin (Surawicz *et al. Am J Gastroenterol.*, 108(4):478-98, 2013). The survival results for *C. difficile* infected hamsters treated with vehicle, vancomycin, or MU1140 variants are presented in **Table 18**. By the end of the study at Day 21, variants F1I, R13N, and Y20F exhibited superiority to vancomycin, as up to 100% survival was observed in variant treated hamsters compared to the 33% survival observed for the vancomycin control. The spore counts and toxin levels in animals at the end of the study were consistent with the clinical survival picture.

Table 18: Efficacy study in Ileal Cannulated Hamster *C. difficile* Infection Model

Treatment	Percent Survival at Day-22	Mean LOG_{10} CFU/mL of feces homogenates (cecal spore counts) at Day-22	Toxins (A/B) levels at Day-22 (ng/mL)
Vehicle	0	4.09	1955.0/1368.7
Vancomycin	33	3.24	2435.9/1209.1
F17L	33	3.33	1490.4/1149.9
F1I	100	<1.62*	<1.6/<1.6**
Y20F	67	2.21	369.5/331.3
F1L	17	4.31	269.5/328.3
N18A	33	3.52	684.2/614.6
R13N	67	2.24	1944.0/956.3

* Limit of Detection (LOD) = 1.62 LOG10 CFU/gram of sample
** LOD = 1.6 ng/mL toxin

[000188] Eight variants were evaluated *in vivo* using a *C. difficile* Syrian Hamster Model where the peptides were delivered by oral gavage, to determine if improvements in the efficacy of *C. difficile* treatment could be observed relative to a current clinical standard of care, vancomycin. The survival results for *C. difficile* infected hamsters treated with vehicle, vancomycin, or MU1140 variants are presented in **Table 19**. By the end of the study at Day 22, variants F1V R13N and F1I R13N Y20F exhibited equal or superior efficacy, as compared to Vancomycin-treated animals. The spore counts and toxin levels in animals at the end of the study were consistent with the clinical survival picture.

Table 19: Efficacy study in Hamster *C. difficile* Infection Model by Oral Gavage

Treatment	**Percent Survival at Day-22**	**Mean LOG_{10} CFU/mL of feces homogenates (cecal spore counts) at Day-*22***	***Toxins* (A/B) levels at Day-22 (ng/mL)**
Vehicle	0	7.23	6630.9/2351.1
Vancomycin	83	2.96	84.2/8
F1I	0	7.21	4313.7/1488.3
F1I R13D	50	4.8	648.5/216.6
F1I R13N	66	3.64	138.1/120.2
F1I R13A	50	4.67	147/205.5
F1L R13N	0	7.36	5477.8/2189.1
F1L R13A	0	7.28	4532.5/1795.5
F1V R13N	100	2.4*	0.8/0.8**
F1I R13N Y20F	83	2.65	5.1/6

* Limit of detection = 2.40
** Limit of detection = 0.8

[000189] Two selected variants were evaluated *in vivo* using a *C. difficile* Syrian Hamster Model where the peptides were delivered by oral gavage, and following an ascending dose regimen, to determine if improvements in the efficacy of *C. difficile* treatment could be observed relative to a current clinical standard of care, vancomycin. The survival results for *C. difficile* infected hamsters treated with vehicle, vancomycin, or MU1140 variants are presented in **Table 20**. By the end of the study at Day 21, variants F1V R13N exhibited superior efficacy, as compared to Vancomycin-treated animals at the same dose, and followed a dose-dependent response. The spore counts and toxin levels in animals at the end of the study were consistent with the clinical survival picture.

Table 20: Efficacy study and Dose Range-Finding in Hamster *C. difficile* Infection Model by Oral Gavage.

Treatment (dose)	Percent Survival at Day-22	Mean LOG_{10} CFU/mL of feces homogenates (cecal spore counts) at Day-22	Toxins (A/B) levels at Day-22 (ng/mL)
Vehicle	0	5.15	2857.1/1852.8
Vancomycin	50	6.66	292.6/71.1
F1V R13N (2 mg/Kg)	0	6.13	578.5/462.7
F1V R13N (6 mg/Kg)	50	4.27	266.2/238.1
F1V R13N (20 mg/Kg)	66	3.88	74.2/57.8
F1V R13N (35 mg/Kg)	100	2.40*	0.8/0.8**
F1V R13N (60 mg/Kg)	83	2.51	7.3/5.5
F1I R13N Y20F (6 mg/Kg)	0	5.47	215.5/242.2
F1I R13N Y20F (20 mg/Kg)	33	5.37	165.5/272.3
F1I R13N Y20F (60 mg/Kg)	83	3.18	47.6/108.7

*Limit of detection = 2.40
** Limit of detection = 0.8

EXAMPLE 5

Multiple Amino Acid Substitutions

[000190] As a follow-up to production of the variants comprising single amino acid substitutions, 305 variants were designed to combine up to eight amino acid substitutions per variant, at positions 1,2,4,5,6,7,12,13,15, and 20 of the 22 amino acid mature peptide sequence. 270 variant strains were successfully built, as verified by colony PCR and DNA sequence verification of the mutA locus. Although several attempts at vector construction and transformation were attempted, we cannot determine whether strain construction failed due to technical reasons or biological reasons (the variant was toxic to the host).

[000191] Variant producing strains were assessed for improvements in antimicrobial activity using the *M. luteus* zone of clearing assay using non-normalized culture supernatants

obtained from overnight growth of *S. mutans* strains. It is not known if the larger zones of clearing are due to production of variants with higher specific antimicrobial activity, or if higher titers are responsible for the larger zones of clearing. Consistent production of zones of clearing was observed for both *S. mutans* strains JH1140 and SM253 from well-to-well and plate-to-plate. Strains encoding F1N, F1P, F1E, S5F, L6I, and A12T mutations resulted in either low levels of antimicrobial activity or activity below the level of detection. The majority of the variant library demonstrated equal or greater antimicrobial activity than the current lead candidate and control strain F1I.

[000192] Variant stability is a pharmacological property that is essential for successful development of MU1140 as a successful therapeutic for gastrointestinal applications. Native MU1140 contains a trypsin cleavage site at position R13, although this site may be protected from efficient protease cleavage (Chen *et al.*, *Microbiology*, *79*(13), 4015–23, 2013). Amino acid substitutions to F1 and R13 have each resulted in extended half-lives of purified MU1140 variants in simulated intestinal fluid.

[000193] Mutation of F1 and K2 residues may impact maturation of the MU1140 pro-peptide by the protease mutP as well as cleavage by pepsin and chymotrypsin. Strains containing F1E and F1P substitutions each underperformed the SM253 control strain (F1I). However, F1A, F1G, F1I, and F1V substitutions generally yielded higher activity than SM253.

[000194] Thirteen different amino acid substitutions were made at position 13. All were functional and performed ≥ SM253 in at least one variant. R13A mutations were over-represented in the high performing group of variants that generated zones of clearing > 0.319 cm^2.

[000195] A summary of the amino acid substitutions is found in **Figure 8**, separated into 6 different groups:

Group 1: > activity than SM253 control strain (>0.319 cm^2 zone of clearing)

Group 2: > activity than SM253 in 2 replicate experiments (1 replicate experiment

Group 3: > activity than SM253 in 1 replicate and ≤ activity in 1 replicate experiment

Group 4: > activity than SM253 control strain (>0.167 cm^2 zone of clearing but <0.319 cm^2 zone of clearing)

Group 5: ≤ activity than SM253 control (≤0.167 cm^2 zone of clearing)

Group 6: Undetermined

CLAIMS

What is claimed is:

1. A non-naturally occurring lantibiotic comprising an amino acid sequence of MU1140 and further comprising a mutation including:
   a. arginine at position 13 changed to asparagine (R13N);
   b. phenylalanine at position 17 changed to leucine (F17L) or tyrosine (F17Y);
   c. asparagine at position 18 changed to alanine (N18A);
   d. tyrosine at position 20 changed to phenylalanine (Y20F); or
   e. combinations thereof.

2. A non-naturally occurring lantibiotic comprising an amino acid sequence of MU1140 and further comprising two mutations, wherein one mutation includes:
   a. arginine at position 13 changed to asparagine (R13N);
   b. phenylalanine at position 17 changed to leucine (F17L) or tyrosine (F17Y);
   c. asparagine at position 18 changed to alanine (N18A);
   d. tyrosine at position 20 changed to phenylalanine (Y20F); or
   e. combinations thereof.

3. The non-naturally occurring lantibiotic of claim 2, wherein one mutation is arginine at position 13 changed to asparagine (R13N).

4. The non-naturally occurring lantibiotic of claims 2 or 3, wherein one mutation is phenylalanine at position 1 changed to valine (FIV).

5. A non-naturally occurring lantibiotic comprising an amino acid sequence of MU1140 and further comprising two mutations, wherein one mutation includes:
   a. phenylalanine at postion 1 changed to valine (F1V);
   b. phenylalanine at position 1 changed to alanine (F1A);
   c. phenylalanine at position 1 changed to isoleucine (F1I);
   d. phenylalanine at position 1 changed to leucine (F1L);
   e. phenylalanine at position 1 changed to threonine (F1T); or
   f. phenylalanine at position 1 changed to tyrosine (F1Y).

6. The non-naturally occurring lantibiotic of claim 5, wherein one mutation is phenylalanine at position 1 changed to valine (F1V).

7. The non-naturally occurring lantibiotic of claims 5 or 6, wherein one mutation is arginine at position 13 changed to asparagine (R13N).

8. A non-naturally occurring lantibiotic comprising an amino acid sequence of MU1140 and further comprising two mutations including:
   a. phenylalanine at position 1 changed to alanine in combination with arginine at position 13 changed to:
      i. alanine (F1A R13A),
      ii. valine (F1A R13V),
      iii. asparagine (F1A R13N), or
      iv. serine (F1A R13S);
   b. phenylalanine at position 1 changed to glycine in combination with arginine at position 13 changed to:
      i. glycine (F1G R13G);
   c. phenylalanine a position 1 changed to histidine in combination with arginine at position 13 changed to:
      i. asparagine (F1H R13N);
   d. phenylalanine at position 1 changed to isoleucine in combination with arginine at position 13 changed to:
      i. alanine (F1I R13A),
      ii. glycine (F1I R13G),
      iii. isoleucine (F1I R13I),
      iv. asparagine (F1I R13N),
      v. proline (F1I R13P),
      vi. glutamine (F1I R13Q),
      vii. glutamic acid (F1I R13S),
      viii. serine (F1I R13V), or
      ix. valine (F1I R13E);

e. phenylalanine at position 1 changed to leucine in combination with arginine at position 13 changed to:
   i. alanine (F1L R13A),
   ii. aspartic acid (F1L R13D),
   iii. glycine (F1L R13G),
   iv. asparagine (F1L R13N),
   v. proline (F1L R13P), or
   vi. glutamine (F1L R13Q);
f. phenylalanine at position 1 changed to threonine in combination with arginine at position 13 changed to:
   i. alanine (F1T R13A),
   ii. asparagine (F1T R13N), or
   iii. valine (F1T R13V);
g. phenylalanine at position 1 changed to valine in combination with arginine at position 13 changed to:
   i. alanine (F1V R13A),
   ii. asparagine (F1V R13N),
   iii. glutamine (F1V R13Q),
   iv. aspartic acid (F1V R13D),
   v. valine (F1V R13V), or
   vi. proline (F1V R13P); or
h. phenylalanine at position 1 changed to tyrosine in combination with arginine at position 13 changed to:
   i. aspartic acid (F1Y R13D), or
   ii. glycine (F1Y R13G).

9. The non-naturally occurring lantibiotic of claim 8, comprising two mutations including phenylalanine at position 1 changed to valine in combination with arginine at position 13 changed to:
   i. alanine (F1V R13A),
   ii. asparagine (F1V R13N),
   iii. glutamine (F1V R13Q),

iv. aspartic acid (F1V R13D),
v. valine (F1V R13V), or
vi. proline (F1V R13P).

10. The non-naturally occurring lantibiotic of claim 9, wherein the two mutations consist of phenylalanine at position 1 changed to valine in combination with arginine at position 13 changed to asparagine (FIV R13N).

11. A non-naturally occurring lantibiotic comprising an amino acid sequence of MU1140 and further comprising three mutations including:
a. phenylalanine at position 1 changed to isoleucine, in combination with arginine at position 13 changed to alanine, in combination with glycine at position 15 changed to alanine (F1I R13A G15A);
b. phenylalanine at position 1 changed to isoleucine, in combination with arginine at position 13 changed to aspartic acid, in combination with glycine at position 15 changed to alanine (F1I R13D G15A);
c. phenylalanine at position 1 changed to isoleucine, in combination with tryptophan at position 4 changed to isoleucine, in combination with arginine at position 13 changed to alanine (F1I W4I R13A);
d. phenylalanine at position 1 changed to isoleucine, in combination with tryptophan at position 4 changed to methionine, in combination with arginine at position 13 changed to aspartic acid (F1I W4M R13D);
e. phenylalanine at position 1 changed to isoleucine, in combination with tryptophan at position 4 changed to methionine, in combination with arginine at position 13 changed to asparagine (F1I W4M R13N);
f. phenylalanine at position 1 changed to isoleucine, in combination with lysine at position 2 changed to alanine, in combination with arginine at position 13 changed to alanine (F1I K2A R13A);
g. phenylalanine at position 1 changed to isoleucine, in combination with leucine at position 6 changed to valine, in combination with arginine at position 13 changed to alanine (F1I L6V R13A);

h. phenylalanine at position 1 changed to isoleucine, in combination with arginine at position 13 changed to alanine, in combination with tyrosine at position 20 changed to phenylalanine (F1I R13A Y20F);
i. phenylalanine at position 1 changed to isoleucine, in combination with arginine at position 13 changed to aspartic acid, in combination with tyrosine at position 20 changed to phenylalanine (F1I R13D Y20F);
j. phenylalanine at position 1 changed to isoleucine, in combination with arginine at position 13 changed to asparagine, in combination with tyrosine at position 20 changed to phenylalanine (F1I R13N Y20F);
k. phenylalanine at position 1 changed to leucine, in combination with arginine at position 13 changed to asparagine, in combination with tyrosine at position 20 changed to phenylalanine (F1L R13N Y20F);
l. phenylalanine at position 1 changed to leucine, in combination with arginine at position 13 changed to alanine, in combination with tyrosine at position 20 changed to phenylalanine (F1L R13A Y20F); or
m. phenylalanine at position 1 changed to leucine, in combination with arginine at position 13 changed to aspartic acid, in combination with tyrosine at position 20 changed to phenylalanine (F1L R13D Y20F).

12. A non-naturally occurring lantibiotic comprising an amino acid sequence of MU1140 and further comprising mutations wherein:
a. phenylalanine at position 1 changed to isoleucine, in combination with lysine at position 2 changed to alanine, in combination with tryptophan at position 4 changed to lysine, in combination with arginine at position 13 changed to alanine (F1I K2A W4K R13A); or
b. phenylalanine at position 1 changed to isoleucine, in combination with lysine at position 2 changed to alanine, in combination with tryptophan at position 4 changed to lysine, in combination with arginine at position 13 changed to aspartic acid (F1I K2A W4K R13D).

13. A non-naturally occurring lantibiotic comprising an amino acid sequence of MU1140 and further comprising mutations wherein phenylalanine at position 1 changed to

isoleucine, in combination with lysine at position 2 changed to alanine, in combination with tryptophan at position 4 changed to methionine, in combination with arginine at position 13 changed to alanine, in combination with tyrosine at position 20 changed to phenylalanine (F1I K2A W4K R13A Y20F).

14. A non-naturally occurring, post-translationally modified lantibiotic comprising an amino acid sequence of MU1140 (SEQ ID NO:1156) and further comprising a mutation including:
    a. arginine at position 13 changed to asparagine (R13N);
    b. phenylalanine at position 17 changed to leucine (F17L) or tyrosine (F17Y);
    c. asparagine at position 18 changed to alanine (N18A);
    d. tyrosine at position 20 changed to phenylalanine (Y20F); or
    e. combinations thereof.

15. A non-naturally occurring, post-translationally modified lantibiotic comprising an amino acid sequence of MU1140 (SEQ ID NO:1156) and further comprising two mutations, wherein one mutation includes:
    a. arginine at position 13 changed to asparagine (R13N);
    b. phenylalanine at position 17 changed to leucine (F17L) or tyrosine (F17Y);
    c. asparagine at position 18 changed to alanine (N18A);
    d. tyrosine at position 20 changed to phenylalanine (Y20F); or
    e. combinations thereof.

16. The non-naturally occurring, post-translationally modified lantibiotic of claim 15, wherein one mutation is arginine at position 13 changed to asparagine (R13N).

17. The non-naturally occurring, post-translationally modified lantibiotic of claims 15 or 16, wherein one mutation is phenylalanine at position 1 changed to valine (FIV).

18. A non-naturally occurring, post-translationally modified lantibiotic comprising an amino acid sequence of MU1140 (SEQ ID NO:1156) and further comprising two mutations, wherein one mutation includes:
    a. phenylalanine at postion 1 changed to valine (F1V);

b. phenylalanine at position 1 changed to alanine (F1A);
c. phenylalanine at position 1 changed to isoleucine (F1I);
d. phenylalanine at position 1 changed to leucine (F1L);
e. phenylalanine at position 1 changed to threonine (F1T); or
f. phenylalanine at position 1 changed to tyrosine (F1Y).

19. The non-naturally occurring, post-translationally modified lantibiotic of claim 18, wherein one mutation is phenylalanine at position 1 changed to valine (FIV).

20. The non-naturally occurring, post-translationally modified lantibiotic of claims 18 or 19, wherein one mutation is arginine at position 13 changed to asparagine (R13N).

21. A non-naturally occurring, post-translationally modified lantibiotic comprising an amino acid sequence of MU1140 (SEQ ID NO:1156) and further comprising two mutations including:
a. phenylalanine at position 1 changed to alanine in combination with arginine at position 13 changed to:
i. alanine (F1A R13A),
ii. valine (F1A R13V),
iii. asparagine (F1A R13N), or
iv. serine (F1A R13S);
b. phenylalanine at position 1 changed to glycine in combination with arginine at position 13 changed to:
i. glycine (F1G R13G);
c. phenylalanine a position 1 changed to histidine in combination with arginine at position 13 changed to:
i. asparagine (F1H R13N);
d. phenylalanine at position 1 changed to isoleucine in combination with arginine at position 13 changed to:
i. alanine (F1I R13A),
ii. glycine (F1I R13G),
iii. isoleucine (F1I R13I),

iv. asparagine (F1I R13N),
v. proline (F1I R13P),
vi. glutamine (F1I R13Q),
vii. glutamic acid (F1I R13S),
viii. serine (F1I R13V), or
ix. valine (F1I R13E);

e. phenylalanine at position 1 changed to leucine in combination with arginine at position 13 changed to:
i. alanine (F1L R13A),
ii. aspartic acid (F1L R13D),
iii. glycine (F1L R13G),
iv. asparagine (F1L R13N),
v. proline (F1L R13P), or
vi. glutamine (F1L R13Q);

f. phenylalanine at position 1 changed to threonine in combination with arginine at position 13 changed to:
i. alanine (F1T R13A),
ii. asparagine (F1T R13N), or
iii. valine (F1T R13V);

g. phenylalanine at position 1 changed to valine in combination with arginine at position 13 changed to:
i. alanine (F1V R13A),
ii. asparagine (F1V R13N),
iii. glutamine (F1V R13Q),
iv. aspartic acid (F1V R13D),
v. valine (F1V R13V), or
vi. proline (F1V R13P); or

h. phenylalanine at position 1 changed to tyrosine in combination with arginine at position 13 changed to:
i. aspartic acid (F1Y R13D), or
ii. glycine (F1Y R13G).

22. The non-naturally occurring, post-translationally modified lantibiotic of claim 21, comprising two mutations including phenylalanine at position 1 changed to valine in combination with arginine at position 13 changed to:

i. alanine (F1V R13A),
ii. asparagine (F1V R13N),
iii. glutamine (F1V R13Q),
iv. aspartic acid (F1V R13D),
v. valine (F1V R13V), or
vi. proline (F1V R13P).

23. The non-naturally occurring, post-translationally modified lantibiotic of claim 22, wherein the two mutations consist of phenylalanine at position 1 changed to valine in combination with arginine at position 13 changed to asparagine (FIV R13N) (SEQ ID NO: 550).

24. A non-naturally occurring, post-translationally modified lantibiotic comprising an amino acid sequence of MU1140 (SEQ ID NO:1156) and further comprising three mutations including:

a. phenylalanine at position 1 changed to isoleucine, in combination with arginine at position 13 changed to alanine, in combination with glycine at position 15 changed to alanine (F1I R13A G15A);
b. phenylalanine at position 1 changed to isoleucine, in combination with arginine at position 13 changed to aspartic acid, in combination with glycine at position 15 changed to alanine (F1I R13D G15A);
c. phenylalanine at position 1 changed to isoleucine, in combination with tryptophan at position 4 changed to isoleucine, in combination with arginine at position 13 changed to alanine (F1I W4I R13A);
d. phenylalanine at position 1 changed to isoleucine, in combination with tryptophan at position 4 changed to methionine, in combination with arginine at position 13 changed to aspartic acid (F1I W4M R13D);

e. phenylalanine at position 1 changed to isoleucine, in combination with tryptophan at position 4 changed to methionine, in combination with arginine at position 13 changed to asparagine (F1I W4M R13N);
f. phenylalanine at position 1 changed to isoleucine, in combination with lysine at position 2 changed to alanine, in combination with arginine at position 13 changed to alanine (F1I K2A R13A);
g. phenylalanine at position 1 changed to isoleucine, in combination with leucine at position 6 changed to valine, in combination with arginine at position 13 changed to alanine (F1I L6V R13A);
h. phenylalanine at position 1 changed to isoleucine, in combination with arginine at position 13 changed to alanine, in combination with tyrosine at position 20 changed to phenylalanine (F1I R13A Y20F);
i. phenylalanine at position 1 changed to isoleucine, in combination with arginine at position 13 changed to aspartic acid, in combination with tyrosine at position 20 changed to phenylalanine (F1I R13D Y20F);
j. phenylalanine at position 1 changed to isoleucine, in combination with arginine at position 13 changed to asparagine, in combination with tyrosine at position 20 changed to phenylalanine (F1I R13N Y20F);
k. phenylalanine at position 1 changed to leucine, in combination with arginine at position 13 changed to asparagine, in combination with tyrosine at position 20 changed to phenylalanine (F1L R13N Y20F);
l. phenylalanine at position 1 changed to leucine, in combination with arginine at position 13 changed to alanine, in combination with tyrosine at position 20 changed to phenylalanine (F1L R13A Y20F); or
m. phenylalanine at position 1 changed to leucine, in combination with arginine at position 13 changed to aspartic acid, in combination with tyrosine at position 20 changed to phenylalanine (F1L R13D Y20F).

25. A non-naturally occurring, post-translationally modified lantibiotic comprising an amino acid sequence of MU1140 (SEQ ID NO:1156) and further comprising mutations wherein:

a. phenylalanine at position 1 changed to isoleucine, in combination with lysine at position 2 changed to alanine, in combination with tryptophan at position 4 changed to lysine, in combination with arginine at position 13 changed to alanine (F1I K2A W4K R13A); or
b. phenylalanine at position 1 changed to isoleucine, in combination with lysine at position 2 changed to alanine, in combination with tryptophan at position 4 changed to lysine, in combination with arginine at position 13 changed to aspartic acid (F1I K2A W4K R13D).

26. A non-naturally occurring, post-translationally modified lantibiotic comprising an amino acid sequence of MU1140 (SEQ ID NO:1156) and further comprising mutations wherein phenylalanine at position 1 changed to isoleucine, in combination with lysine at position 2 changed to alanine, in combination with tryptophan at position 4 changed to methionine, in combination with arginine at position 13 changed to alanine, in combination with tyrosine at position 20 changed to phenylalanine (F1I K2A W4K R13A Y20F).

27. A non-naturally occurring lantibiotic comprising a single amino acid mutation of MU1140, the lantibiotic having an amino acid sequence encoded by of any one of SEQ ID NOS:2 to 431.

28. A non-naturally occurring lantibiotic comprising multisite amino acid mutations of MU1140, the lantibiotic being a variant as described in Figure 7.

29. A non-naturally occurring lantibiotic comprising any variant as described in Figure 8: Group 1, Group 2 or Group 3.

30. A non-naturally occurring lantibiotic comprising any variant as described in Figure 8: Group 1.

31. A non-naturally occurring lantibiotic comprising a single amino acid mutation of MU1140 as described in Figure 6.

32. A non-naturally occurring lantibiotic comprising an amino acid sequence of SEQ ID NO. 550.

33. The non-naturally occurring lantibiotic of any one of claim 27 to 32, wherein the lantibiotic is post-translationally modified.

34. The non-naturally occurring lantibiotic of claim 33, wherein the post-translation modification comprises Dha at position 5.

35. The non-naturally occurring lantibiotic of claim 33 or claim 34, wherein the post-translation modification comprises Abu at position 8.

36. The non-naturally occurring lantibiotic of any one of claims 33 to claim 35, wherein the post-translation modification comprises Dhb at position 14.

37. The non-naturally occurring lantibiotic of any one of claims 33 to claim 36, wherein the post-translation modification comprises Dha at position 5, Abu at position 8, and Dhb at position 14.

38. The non-naturally occurring lantibiotic of any one of claims 33 to claim 37, wherein the post-translation modification comprises a ring formed by lanthionine (Ala-S-Ala) residues between (Ala_3-S-Ala_7) as described in Figure 1 (Ring A).

39. The non-naturally occurring lantibiotic of any one of claims 33 to claim 38, wherein the post-translation modification comprises a methyl-lanthionine residue (Abu-S-Ala) forming a ring, Ring B, comprising the alpha-aminobutyrate residue in position 8 and the Ala in position 11 (Abu_8-S-Ala_{11}) as described in Figure 1.

40. The non-naturally occurring lantibiotic of any one of claims 33 to claim 39, wherein the post-translation modification comprises a ring formed by lanthionine (Ala-S-Ala) residues between (Ala_{16}-S-Ala_{21}) as described in Figure 1 (Ring C).

41. The non-naturally occurring lantibiotic of any one of claims 33 to claim 40, wherein the post-translation modification comprises Ala in position 19 linked to an aminovinyl group by a thioether linkage (Ala_{19}-S-CH=CH-NH-), Ring D, as described in Figure 1.

42. The non-naturally occurring lantibiotic of any one of claims 33 to claim 41, wherein the post-translation modification comprises Ring A, B, C and D.

43. The non-naturally occurring lantiobiotic of any one of claims 33 to 42, wherein the post-translationally modified MU1140 variant comprises Ring A, Ring B, Ring C and Ring D, wherein:

a. two of these rings are formed by lanthionine (Ala-S-Ala) residues, including one in Ring A (Ala_3-S-Ala_7) and one in Ring C (Ala_{16}-S-Ala_{21});

b. a methyl-lanthionine residue (Abu-S-Ala) forms Ring B comprising the alpha-aminobutyrate residue in position 8 and the Ala in position 11 (Abu_8-S-Ala_{11}); and

c. the fourth ring, D, is comprised of the Ala in position 19 linked to an aminovinyl group by a thioether linkage (Ala_{19}-S-CH=CH-NH-).

and wherein amino acid at position 5 is modified to Dha, the amino acid at position 8 is modified to Abu, and the amino acid at position 14 is modified to Dhb.

44. An isolated lantibiotic variant, wherein the isolated lantibiotic variant is produced in a host cell by expressing a polypeptide from a polynucleotide encoding a lantibiotic variant of Figure 5 or Figure 7, and wherein the expressed polypeptide is post-translationally modified in the host cell.

45. The isolated lantibiotic variant of claim 44, wherein the host cell is *S. mutans*.

46. A post-translationally modified, non-naturally occurring lantibiotic comprising an amino acid sequence of SEQ ID NO. 1157.

47. An antimicrobial composition comprising a non-naturally occurring lantibiotic of any one of claims 1 to 26 or 33 to 46 and a pharmaceutically acceptable carrier, pharmaceutically acceptable diluent, other diluent or excipient.

48. The antimicrobial composition of claim 47, further comprising an antifungal agent, an additional antimicrobial agent, a membrane disrupting agent, or a combination thereof.

49. The antimicrobial composition of claim 48, wherein the additional antimicrobial agent has Gram negative bacteriostatic or bacteriocidal activity and the membrane disrupting agent renders Gram negative bacteria susceptible to the variant lantibiotic.

50. The antimicrobial composition of any one of claims 47 to 49, wherein the non-naturally occuring lantibiotics is present in the composition at about 0.001, 0.01, 0.1, 1, 5, 10, 20, 30, 40, 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, or 1,000 mg/kg or mg/L.

51. A method of reducing reproduction of bacteria or reducing numbers of bacteria present in or on a subject, comprising administering to the subject a therapeutically effective amount of the antimicrobial composition of any one of claims 47 to 50.

52. The method of claim 51, wherein the subject is a human.

53. The method of claim 47 or 52, wherein the composition is administered orally, topically, nasally, buccally, sublingually, transmucosally, rectally, transdermally, by inhalation, by injection or intrathecally.

54. The method of claim 53, wherein the injection is intramuscular, intravenous, intrapulmonary, intramuscular, intradermal, intraperitoneal, intrathecal, or subcutaneous injection.

55. A preservative comprising an effective amount of the non-naturally occurring lantibiotic of any one of claims 1 to 26 or 33 to 46 in a physiological solution at a pH of between 3 and 8.

56. A food, beverage, gum, or dentifrice composition comprising an amount of the non-naturally occurring lantibiotic of any one of claims 1 to 26 or 33 to 46 sufficient to reduce the reproduction of bacteria or numbers of bacteria in the composition.

57. A method of reducing reproduction of bacteria or reducing numbers of bacteria present in or on a composition or object, comprising contacting the antimicrobial composition of any one of claims 1 to 26 or 33 to 46 with the composition or object for a period effective to reduce reproduction of bacteria or reduce numbers of bacteria in or on the composition or object.

58. The method of claim 57, wherein the composition is a food, beverage, gum, or dentifrice.

59. A composition comprising a solid surface or a textile with the lantibiotic composition of any one of claims claims 1 to 26 or 33 to 46 or coated onto, immobilized, linked, or bound to the solid surface or textile.

60. A method of reducing a biofilm or biofouling condition comprising contacting the antimicrobial composition of any one of claims 47 to 50 with the biofilm or biofouling condition for a period effective to reduce reproduction of bacteria or reduce numbers of bacteria in or on the biofilm or biofouling condition.

61. A kit comprising the lantibiotic of any one of claims 1 to 26 or 33 to 46 and one or more applicators.

62. A method of preventing or treating a subject diagnosed with a bacterial infection, comprising administering the non-naturally occurring lantibiotic of any one of claims 1 to 26 or 33 to 46.

63. The method of claim 62, wherein the subject is a human.

64. The method of claim 62 or 63, wherein the subject is infected with a Gram-positive bacteria.

65. The method of claim 64, wherein the Gram-positive bacteria is one or more of *Staphylococcus epidermidis*, vancomycin resistant *Enterococci*, vancomycin resistant *Enterococcus faecalis*, *Enterococcus faecalis*, *Enterococcus faecium*, *Propionibacterium acnes*, *Streptococcus salivarius*, *Streptococcus sanguis*, *Streptococcus mitis*, *Streptococcus pyogenes*, *Lactobacillus salivarius*, *Listeria monocytogenes*, *Actinomyces israelii*, *Actinomyces naeslundii*, *Actinomyces viscosus*, *Bacillus anthracis*, *Streptococcus agalactiae, Streptococcus intermedius, Streptococcus pneumoniae, Corynebacterium diphtheria, Clostridium sporogenes Clostridium botulinum, Clostridium perfringens, Clostridium tetani*, or *Clostridium difficile.*

66. The method of claim 65, wherein the Gram-positive bacteria is *Clostridium difficile*.

67. The method of claim 62 or 63, wherein the subject is infected with a Gram-negative bacteria.

68. The method of claim 67, wherein the Gram-negative bacteria is one or more of *Acinetobacter baumanii, Bordatella pertussis, Borrelia burgdotieri, Brucella abortus, Brucella canis, Brucella melitensis, Brucella suis, Campylobacter jejuni, Coxiella burnetii, Escherichia coli, Francisella tularensis, Haemophilus influenza, Helicobacter pylori, Klebsiella pneumoniae, Legionella pneumophila, Leptospira interrogans, Neisseria gonorrhoeae, Neisseria meningitides, Pseudomonas aeruginosa, Rickettsia rickettsii, Salmonella enteritidis, Salmonella typhi, Salmonella typhimurium, Serratia marcescens, Shigella sonnei, Treponema pallidum, Vibrio cholera, Yersinia enterocolitica,* or *Yersinia pestis*.

69. The method of any one of claims 62 to 68, wherein the non-naturally occurring lantibiotic further comprises one or more additional antimicrobial agents, membrane disrupting agents, or combinations thereof.

70. An antimicrobial composition, comprising the non-naturally occurring lantibiotic of claim 46, and a pharmaceutically acceptable carrier, pharmceutically acceptable diluent, other diluent, excipient, or combinations thereof.

71. The antimicrobial composition of claim 47, further comprising an antifungal agent, an additional antimicrobial agent, a membrane disrupting agent, or a combination thereof.

72. The antimicrobial composition of claim 48, wherein the additional antimicrobial agent has Gram negative bacteriostatic or bacteriocidal activity and the membrane disrupting agent renders Gram negative bacteria susceptible to the variant lantibiotic.

73. The antimicrobial composition of any one of claims 70 to 72, wherein the non-naturally occurring lantibiotic is present in the composition at about 0.001, 0.01, 0.1, 1, 5, 10, 20, 30, 40, 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, or 1,000 mg/kg or mg/L.

74. A method of reducing reproduction of bacteria or reducing numbers of bacteria present in or on a subject, comprising administering to the subject a therapeutically effective amount of the antimicrobial composition of any one of claims 70 to 73.

75. The method of claim 74, wherein the subject is a human.

76. The method of claim 73 or 74, wherein the composition is administered orally, topically, nasally, buccally, sublingually, transmucosally, rectally, transdermally, by inhalation, by injection, or intrathecally.

77. The method of claim 75, wherein the injection is intramuscular, intravenous, intrapulmonary, intramuscular, intradermal, intraperitoneal, intrathecal, or subcutaneous injection.

78. A preservative comprising an effective amount of the non-naturally occurring lantibiotic of claim 46 in a physiological solution at a pH of between 3 and 8.

79. A food, beverage, gum, or dentifrice composition comprising an amount of the non-naturally occurring lantibiotic of claim 46 sufficient to reduce the reproduction of bacteria or numbers of bacteria in the composition.

80. A method of reducing reproduction of bacteria or reducing numbers of bacteria present in or on a composition or object, comprising contacting the non-naturally occurring lantibiotic of claim 46 with the composition or object for a period effective to reduce reproduction of bacteria or reduce numbers of bacteria in or on the composition or object.

81. The method of claim 80, wherein the composition is a food, beverage, gum, or dentifrice.

82. A composition comprising a solid surface or a textile with the non-natrually occurring lantibiotic of claim 46 or coated onto, immobilized, linked, or bound to the solid surface or textile.

83. A method of reducing a biofilm or biofouling condition comprising contacting the non-naturally occurring lantibiotic of claim 46 with the biofilm or biofouling condition for a period effective to reduce reproduction of bacteria or reduce numbers of bacteria in or on the biofilm or biofouling condition.

84. A kit comprising the lantibiotic of claim 46 and one or more applicators.

85. A method of preventing or treating a subject diagnosed with a bacterial infection, comprising administering the non-naturally occurring lantibiotic of claim 46.

86. The method of claim 85, wherein the subject is a human.

87. The method of claim 85 or 86, wherein the subject is infected with a Gram-positive bacteria.

88. The method of claim 87, wherein the Gram-positive bacteria is one or more of *Staphylococcus epidermidis*, vancomycin resistant *Enterococci*, vancomycin resistant *Enterococcus faecalis*, *Enterococcus faecalis*, *Enterococcus faecium*, *Propionibacterium acnes*, *Streptococcus salivarius*, *Streptococcus sanguis*, *Streptococcus mitis*, *Streptococcus pyogenes*, *Lactobacillus salivarius*, *Listeria monocytogenes*, *Actinomyces israelii*, *Actinomyces naeslundii*, *Actinomyces viscosus*, *Bacillus anthracis*, *Streptococcus agalactiae, Streptococcus intermedius, Streptococcus pneumoniae, Corynebacterium diphtheria, Clostridium sporogenes Clostridium botulinum, Clostridium perfringens, Clostridium tetani,* or *Clostridium difficile.*

89. The method of claim 88, wherein the Gram-positive bacteria is *Clostridium difficile*.

90. The method of claim 85 or 86, wherein the subject is infected with a Gram-negative bacteria.

91. The method of claim 90, wherein the Gram-negative bacteria is one or more of *Acinetobacter baumanii, Bordatella pertussis, Borrelia burgdotieri, Brucella abortus, Brucella canis, Brucella melitensis, Brucella suis, Campylobacter jejuni, Coxiella burnetii, Escherichia coli, Francisella tularensis, Haemophilus influenza, Helicobacter pylori, Klebsiella pneumoniae, Legionella pneumophila, Leptospira interrogans, Neisseria gonorrhoeae, Neisseria meningitides, Pseudomonas aeruginosa, Rickettsia rickettsii, Salmonella enteritidis, Salmonella typhi, Salmonella typhimurium, Serratia marcescens, Shigella sonnei, Treponema pallidum, Vibrio cholera, Yersinia enterocolitica,* or *Yersinia pestis.*

92. The method of any one of claims 85 to 91, wherein the non-naturally occurring lantibiotic further comprises one or more additional antimicrobial agents, membrane disrupting agents, or combinations thereof.

93. A purified polynucleotide comprising any one of SEQ ID NOs: 2-431 or encoding a variant as described in Figure 7 or combinations thereof.

94. A purified polynucleotide comprising any one of SEQ ID NOs: 756, 757, 758, 759, or 760.

95. A purified polynucleotide comprising any one of SEQ ID NOs: 709, 714, 716, 735, 747, 751, 754, or 758.

96. A purified polynucleotide comprising SEQ ID NO: 758.

97. A purified polynucleotide comprising SEQ ID NO: 1163.

98. A host cell comprising a polynucleotide of SEQ ID NO: 758.

99. A host cell comprising a polynucleotide of SEQ ID NO: 1163.

100. An isolated recombinant *Streptococcus mutans* strain comprising a polynucleotide of SEQ ID NO: 758.

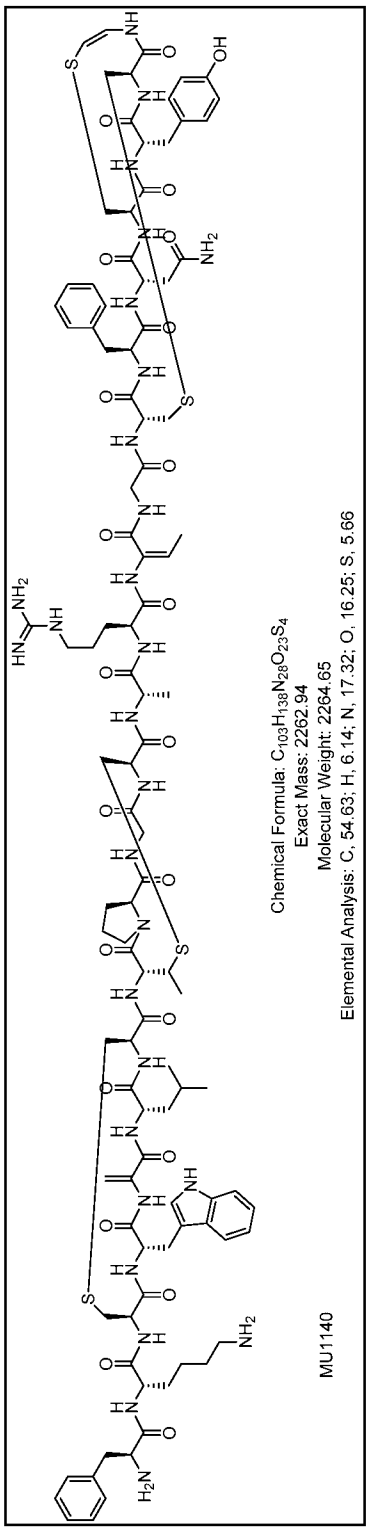
101. An isolated recombinant *Streptococcus mutans* strain comprising a polynucleotide of SEQ ID NO: 1163.

102. A non-naturally occurring polypeptide comprising an amino acid sequence of SEQ ID NO. 1161.

103. An isolated recombinant *Streptococcus mutans* strain comprising: (a) a mutation in a polynucleotide involved in lactic acid synthesis such that expression of lactic acid is diminished by about 80% or more as compared to a wildtype *S. mutans* strain; (b) a recombinant alcohol dehydrogenase polynucleotide; and (c) a recombinant polynucleotide encoding the non-naturally occurring lantibiotic of any one of claims 1 to 26 or 33 to 46

FIGURE 1

A


Chemical Formula: $C_{103}H_{138}N_{28}O_{23}S_4$
Exact Mass: 2262.94
Molecular Weight: 2264.65
Elemental Analysis: C, 54.63; H, 6.14; N, 17.32; O, 16.25; S, 5.66
MU1140

B

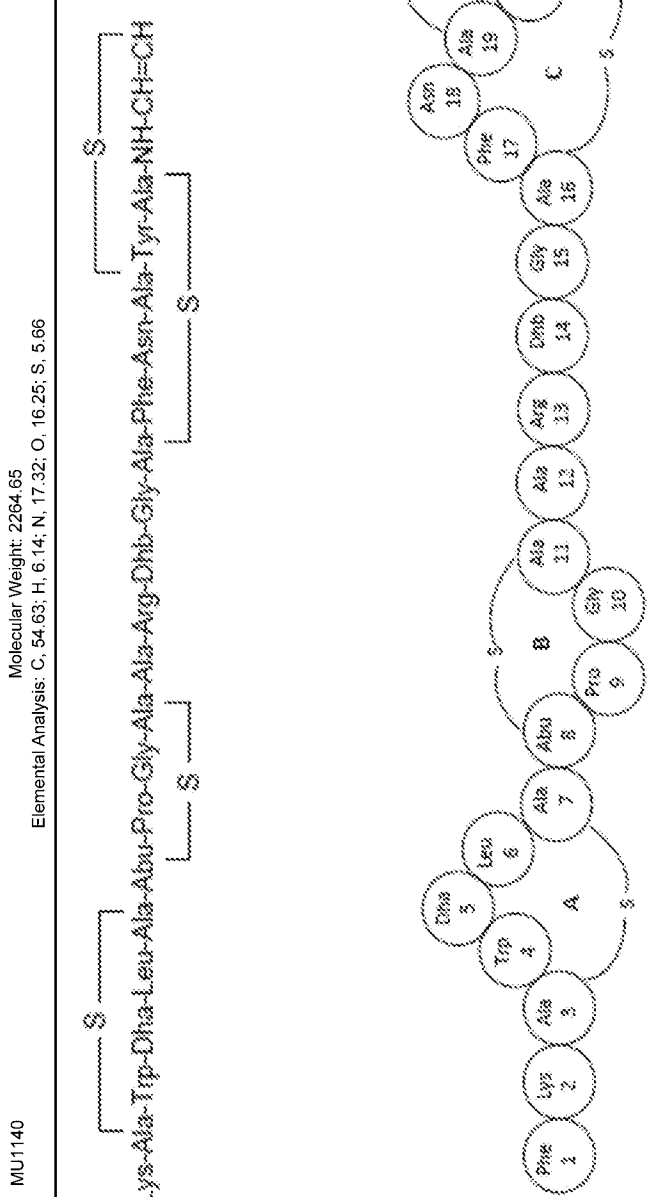
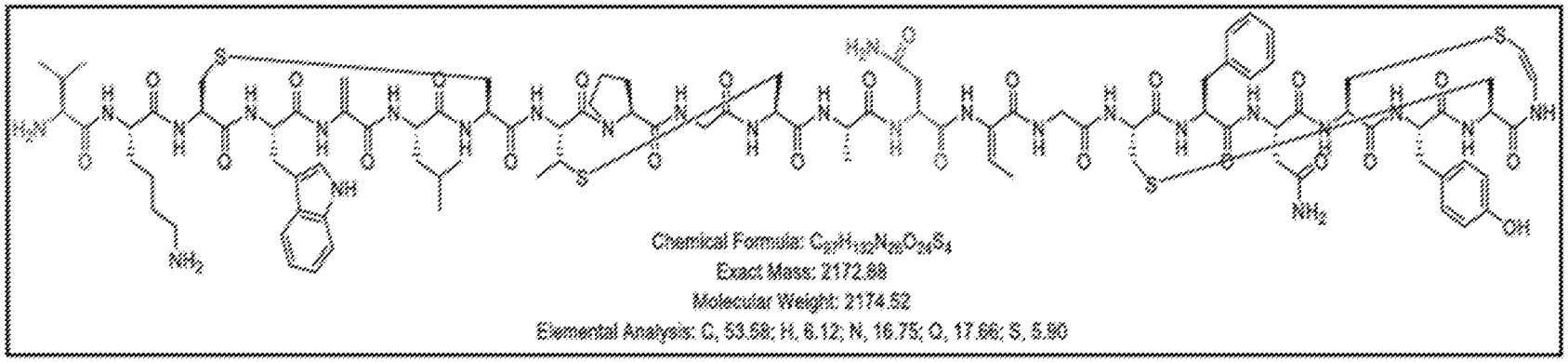
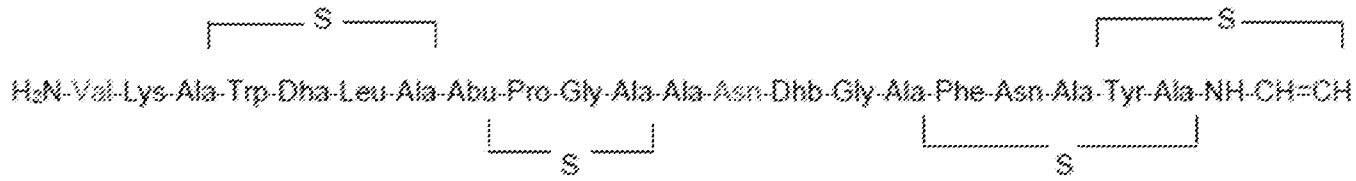

H2N-Phe-Lys-Ala-Trp-Dha-Leu-Ala-Abu-Pro-Gly-Ala-Ala-Arg-Dhb-Gly-Ala-Phe-Asn-Ala-Tyr-Ala-NH-CH=CH
SEQ ID NO: 1156

FIGURE 1 (continued)

C.



D.



SEQ ID NO: 1157

FIGURE 2

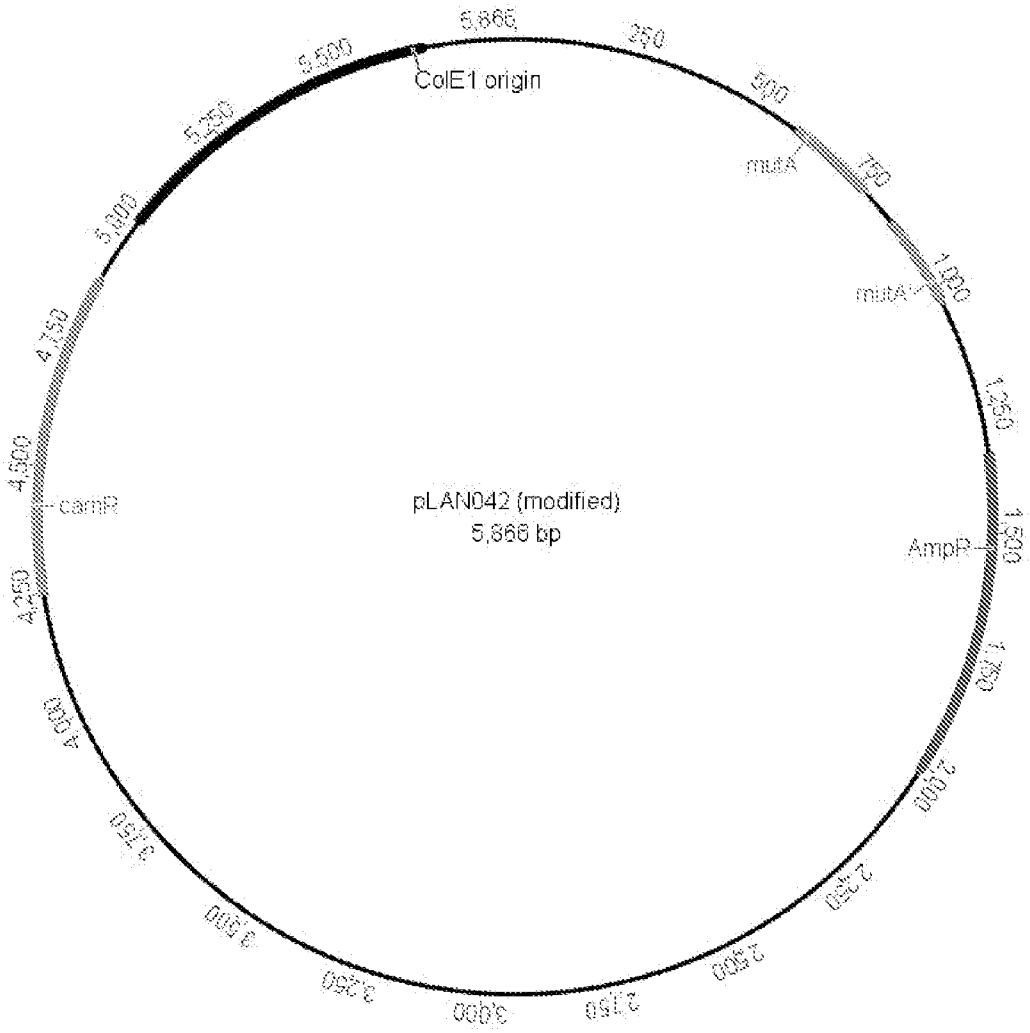

5,866
250
500
750
1,000
1,250
1,500
1,750
2,000
2,250
2,500
2,750
3,000
3,250
3,500
3,750
4,000
4,250
4,500
4,750
5,000
5,250
5,500
ColE1 origin
mutA
mutA
AmpR
camR
pLAN042 (modified)
5,866 bp

FIGURE 3

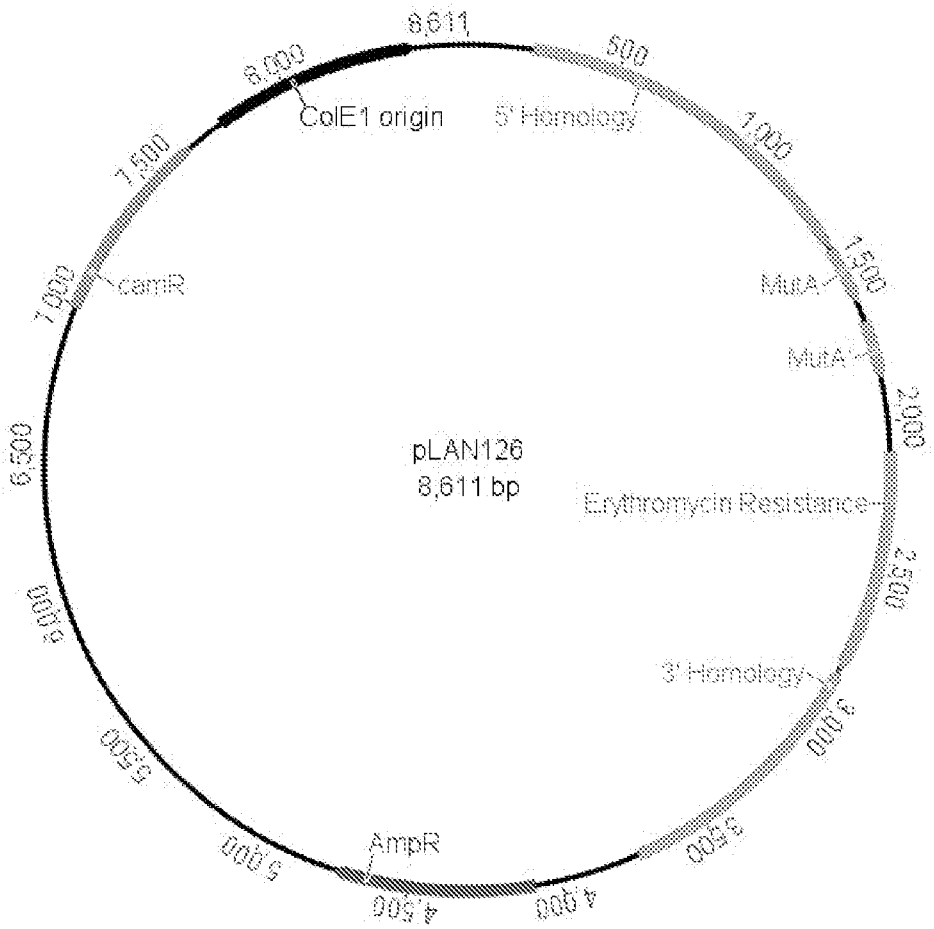

8,611
500
1,000
1,500
2,000
2,500
3,000
3,500
4,000
4,500
5,000
5,500
6,000
6,500
7,000
7,500
8,000
ColE1 origin
5' Homology
MutA
MutA
Erythromycin Resistance
3' Homology
AmpR
camR
pLAN126
8,611 bp

FIGURE 4

SEQ ID	Variant	Nucleotide Sequence
1	MU1140	TTC AAA AGT TGG AGC CTT TGT ACG CCT GGT TGT GCA AGG ACA GGT AGT TTC AAT AGT TAC TGT TGC
2	F1W	tgg aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
3	F1A	gct aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
1168	F1L	tta aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
5	F1M	atg aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
6	F1C	tgt aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
7	F1N	aat aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
8	F1Y	tat aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
9	F1D	gat aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
10	F1E	gaa aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
11	F1P	cct aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
12	F1Q	caa aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
13	F1F	ttt aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
14	F1G	ggt aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
15	F1R	cgt aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
16	F1H	cat aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
17	F1S	tct aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
18	F1T	aca aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
19	F1I	att aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
20	K2V	ttc gtt agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
21	K2W	ttc tgg agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
22	K2A	ttc gct agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
23	K2L	ttc tta agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
24	K2M	ttc atg agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
25	K2C	ttc tgt agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc

26	K2N	ttc aat agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
27	K2Y	ttc tat agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
28	K2D	ttc gat agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
29	K2E	ttc gaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
30	K2P	ttc cct agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
31	K2Q	ttc caa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
32	K2F	ttc ttt agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
33	K2G	ttc ggt agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
34	K2R	ttc cgt agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
35	K2H	ttc cat agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
36	K2S	ttc tct agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
37	K2T	ttc aca agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
38	K2I	ttc att agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
39	S3K	ttc aaa aaa tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
40	S3V	ttc aaa gtt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
41	S3W	ttc aaa tgg tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
42	S3A	ttc aaa gct tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
43	S3L	ttc aaa tta tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
44	S3M	ttc aaa atg tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
45	S3C	ttc aaa tgt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
46	S3N	ttc aaa aat tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
47	S3Y	ttc aaa tat tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
48	S3D	ttc aaa gat tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
49	S3E	ttc aaa gaa tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
50	S3P	ttc aaa cct tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
51	S3Q	ttc aaa caa tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
52	S3F	ttc aaa ttt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
53	S3G	ttc aaa ggt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
54	S3R	ttc aaa cgt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc

55	S3H	ttc aaa cat tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
56	S3S	ttc aaa tct tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
57	S3T	ttc aaa aca tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
58	S3I	ttc aaa att tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
59	W4K	ttc aaa agt aaa agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
60	W4V	ttc aaa agt gtt agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
61	W4A	ttc aaa agt gct agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
62	W4L	ttc aaa agt tta agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
63	W4M	ttc aaa agt atg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
64	W4C	ttc aaa agt tgt agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
65	W4N	ttc aaa agt aat agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
66	W4Y	ttc aaa agt tat agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
67	W4D	ttc aaa agt gat agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
68	W4E	ttc aaa agt gaa agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
69	W4P	ttc aaa agt cct agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
70	W4Q	ttc aaa agt caa agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
71	W4F	ttc aaa agt ttt agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
72	W4G	ttc aaa agt ggt agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
73	W4R	ttc aaa agt cgt agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
74	W4H	ttc aaa agt cat agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
75	W4S	ttc aaa agt tct agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
76	W4T	ttc aaa agt aca agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
77	W4I	ttc aaa agt att agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
78	S5K	ttc aaa agt tgg aaa ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
79	S5V	ttc aaa agt tgg gtt ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
80	S5W	ttc aaa agt tgg tgg ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
81	S5A	ttc aaa agt tgg gct ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
82	S5L	ttc aaa agt tgg tta ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
83	S5M	ttc aaa agt tgg atg ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc

84	S5C	ttc aaa agt tgg tgt ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
85	S5N	ttc aaa agt tgg aat ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
86	S5Y	ttc aaa agt tgg tat ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
87	S5D	ttc aaa agt tgg gat ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
88	S5E	ttc aaa agt tgg gaa ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
89	S5P	ttc aaa agt tgg cct ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
90	S5Q	ttc aaa agt tgg caa ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
91	S5F	ttc aaa agt tgg ttt ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
92	S5G	ttc aaa agt tgg ggt ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
93	S5R	ttc aaa agt tgg cgt ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
94	S5H	ttc aaa agt tgg cat ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
95	S5S	ttc aaa agt tgg tct ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
96	S5T	ttc aaa agt tgg aca ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
97	S5I	ttc aaa agt tgg att ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
98	L6K	ttc aaa agt tgg agc aaa tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
99	L6V	ttc aaa agt tgg agc gtt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
100	L6W	ttc aaa agt tgg agc tgg tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
101	L6A	ttc aaa agt tgg agc gct tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
102	L6L	ttc aaa agt tgg agc tta tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
103	L6M	ttc aaa agt tgg agc atg tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
104	L6C	ttc aaa agt tgg agc tgt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
105	L6N	ttc aaa agt tgg agc aat tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
106	L6Y	ttc aaa agt tgg agc tat tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
107	L6D	ttc aaa agt tgg agc gat tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
108	L6E	ttc aaa agt tgg agc gaa tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
109	L6P	ttc aaa agt tgg agc cct tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
110	L6Q	ttc aaa agt tgg agc caa tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
111	L6F	ttc aaa agt tgg agc ttt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
112	L6G	ttc aaa agt tgg agc ggt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc

113	L6R	ttc aaa agt tgg agc cgt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
114	L6H	ttc aaa agt tgg agc cat tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
115	L6S	ttc aaa agt tgg agc tct tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
116	L6T	ttc aaa agt tgg agc aca tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
117	L6I	ttc aaa agt tgg agc att tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
118	C7K	ttc aaa agt tgg agc ctt aaa acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
119	C7V	ttc aaa agt tgg agc ctt gtt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
120	C7W	ttc aaa agt tgg agc ctt tgg acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
121	C7A	ttc aaa agt tgg agc ctt gct acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
122	C7L	ttc aaa agt tgg agc ctt tta acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
123	C7M	ttc aaa agt tgg agc ctt atg acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
124	C7N	ttc aaa agt tgg agc ctt aat acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
125	C7Y	ttc aaa agt tgg agc ctt tat acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
126	C7D	ttc aaa agt tgg agc ctt gat acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
127	C7E	ttc aaa agt tgg agc ctt gaa acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
128	C7P	ttc aaa agt tgg agc ctt cct acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
129	C7Q	ttc aaa agt tgg agc ctt caa acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
130	C7F	ttc aaa agt tgg agc ctt ttt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
131	C7G	ttc aaa agt tgg agc ctt ggt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
132	C7R	ttc aaa agt tgg agc ctt cgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
133	C7H	ttc aaa agt tgg agc ctt cat acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
134	C7S	ttc aaa agt tgg agc ctt tct acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
135	C7T	ttc aaa agt tgg agc ctt aca acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
136	C7I	ttc aaa agt tgg agc ctt att acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
137	T8K	ttc aaa agt tgg agc ctt tgt aaa cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
138	T8V	ttc aaa agt tgg agc ctt tgt gtt cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
139	T8W	ttc aaa agt tgg agc ctt tgt tgg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
140	T8A	ttc aaa agt tgg agc ctt tgt gct cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
141	T8L	ttc aaa agt tgg agc ctt tgt tta cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc

142	T8M	ttc aaa agt tgg agc ctt tgt atg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
143	T8C	ttc aaa agt tgg agc ctt tgt tgt cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
144	T8N	ttc aaa agt tgg agc ctt tgt aat cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
145	T8Y	ttc aaa agt tgg agc ctt tgt tat cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
146	T8D	ttc aaa agt tgg agc ctt tgt gat cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
147	T8E	ttc aaa agt tgg agc ctt tgt gaa cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
148	T8P	ttc aaa agt tgg agc ctt tgt cct cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
149	T8Q	ttc aaa agt tgg agc ctt tgt caa cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
150	T8F	ttc aaa agt tgg agc ctt tgt ttt cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
151	T8G	ttc aaa agt tgg agc ctt tgt ggt cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
152	T8R	ttc aaa agt tgg agc ctt tgt cgt cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
153	T8H	ttc aaa agt tgg agc ctt tgt cat cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
154	T8S	ttc aaa agt tgg agc ctt tgt tct cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
155	T8T	ttc aaa agt tgg agc ctt tgt aca cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
156	T8I	ttc aaa agt tgg agc ctt tgt att cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
157	P9K	ttc aaa agt tgg agc ctt tgt acg aaa ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
158	P9V	ttc aaa agt tgg agc ctt tgt acg gtt ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
159	P9W	ttc aaa agt tgg agc ctt tgt acg tgg ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
160	P9A	ttc aaa agt tgg agc ctt tgt acg gct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
161	P9L	ttc aaa agt tgg agc ctt tgt acg tta ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
162	P9M	ttc aaa agt tgg agc ctt tgt acg atg ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
163	P9C	ttc aaa agt tgg agc ctt tgt acg tgt ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
164	P9N	ttc aaa agt tgg agc ctt tgt acg aat ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
165	P9Y	ttc aaa agt tgg agc ctt tgt acg tat ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
166	P9D	ttc aaa agt tgg agc ctt tgt acg gat ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
167	P9E	ttc aaa agt tgg agc ctt tgt acg gaa ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
168	P9Q	ttc aaa agt tgg agc ctt tgt acg caa ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
169	P9F	ttc aaa agt tgg agc ctt tgt acg ttt ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
170	P9G	ttc aaa agt tgg agc ctt tgt acg ggt ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc

171	P9R	ttc aaa agt tgg agc ctt tgt acg cgt ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
172	P9H	ttc aaa agt tgg agc ctt tgt acg cat ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
173	P9S	ttc aaa agt tgg agc ctt tgt acg tct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
174	P9T	ttc aaa agt tgg agc ctt tgt acg aca ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
175	P9I	ttc aaa agt tgg agc ctt tgt acg att ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
176	G10K	ttc aaa agt tgg agc ctt tgt acg cct aaa tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
177	G10V	ttc aaa agt tgg agc ctt tgt acg cct gtt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
178	G10W	ttc aaa agt tgg agc ctt tgt acg cct tgg tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
179	G10A	ttc aaa agt tgg agc ctt tgt acg cct gct tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
180	G10L	ttc aaa agt tgg agc ctt tgt acg cct tta tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
181	G10M	ttc aaa agt tgg agc ctt tgt acg cct atg tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
182	G10C	ttc aaa agt tgg agc ctt tgt acg cct tgt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
183	G10N	ttc aaa agt tgg agc ctt tgt acg cct aat tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
184	G10Y	ttc aaa agt tgg agc ctt tgt acg cct tat tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
185	G10D	ttc aaa agt tgg agc ctt tgt acg cct gat tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
186	G10E	ttc aaa agt tgg agc ctt tgt acg cct gaa tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
187	G10P	ttc aaa agt tgg agc ctt tgt acg cct cct tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
188	G10Q	ttc aaa agt tgg agc ctt tgt acg cct caa tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
189	G10F	ttc aaa agt tgg agc ctt tgt acg cct ttt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
190	G10R	ttc aaa agt tgg agc ctt tgt acg cct cgt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
191	G10H	ttc aaa agt tgg agc ctt tgt acg cct cat tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
192	G10S	ttc aaa agt tgg agc ctt tgt acg cct tct tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
193	G10T	ttc aaa agt tgg agc ctt tgt acg cct aca tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
194	G10I	ttc aaa agt tgg agc ctt tgt acg cct att tgt gca agg aca ggt agt ttc aat agt tac tgt tgc
195	C11K	ttc aaa agt tgg agc ctt tgt acg cct ggt aaa gca agg aca ggt agt ttc aat agt tac tgt tgc
196	C11V	ttc aaa agt tgg agc ctt tgt acg cct ggt gtt gca agg aca ggt agt ttc aat agt tac tgt tgc
197	C11W	ttc aaa agt tgg agc ctt tgt acg cct ggt tgg gca agg aca ggt agt ttc aat agt tac tgt tgc
198	C11A	ttc aaa agt tgg agc ctt tgt acg cct ggt gct gca agg aca ggt agt ttc aat agt tac tgt tgc
199	C11L	ttc aaa agt tgg agc ctt tgt acg cct ggt tta gca agg aca ggt agt ttc aat agt tac tgt tgc

200	C11M	ttc aaa agt tgg agc ctt tgt acg cct ggt atg gca agg aca ggt agt ttc aat agt tac tgt tgc
201	C11N	ttc aaa agt tgg agc ctt tgt acg cct ggt aat gca agg aca ggt agt ttc aat agt tac tgt tgc
202	C11Y	ttc aaa agt tgg agc ctt tgt acg cct ggt tat gca agg aca ggt agt ttc aat agt tac tgt tgc
203	C11D	ttc aaa agt tgg agc ctt tgt acg cct ggt gat gca agg aca ggt agt ttc aat agt tac tgt tgc
204	C11E	ttc aaa agt tgg agc ctt tgt acg cct ggt gaa gca agg aca ggt agt ttc aat agt tac tgt tgc
205	C11P	ttc aaa agt tgg agc ctt tgt acg cct ggt cct gca agg aca ggt agt ttc aat agt tac tgt tgc
206	C11Q	ttc aaa agt tgg agc ctt tgt acg cct ggt caa gca agg aca ggt agt ttc aat agt tac tgt tgc
207	C11F	ttc aaa agt tgg agc ctt tgt acg cct ggt ttt gca agg aca ggt agt ttc aat agt tac tgt tgc
208	C11G	ttc aaa agt tgg agc ctt tgt acg cct ggt ggt gca agg aca ggt agt ttc aat agt tac tgt tgc
209	C11R	ttc aaa agt tgg agc ctt tgt acg cct ggt cgt gca agg aca ggt agt ttc aat agt tac tgt tgc
210	C11H	ttc aaa agt tgg agc ctt tgt acg cct ggt cat gca agg aca ggt agt ttc aat agt tac tgt tgc
211	C11S	ttc aaa agt tgg agc ctt tgt acg cct ggt tct gca agg aca ggt agt ttc aat agt tac tgt tgc
212	C11T	ttc aaa agt tgg agc ctt tgt acg cct ggt aca gca agg aca ggt agt ttc aat agt tac tgt tgc
213	C11I	ttc aaa agt tgg agc ctt tgt acg cct ggt att gca agg aca ggt agt ttc aat agt tac tgt tgc
214	A12K	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt aaa agg aca ggt agt ttc aat agt tac tgt tgc
215	A12V	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gtt agg aca ggt agt ttc aat agt tac tgt tgc
216	A12W	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt tgg agg aca ggt agt ttc aat agt tac tgt tgc
217	A12A	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gct agg aca ggt agt ttc aat agt tac tgt tgc
218	A12L	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt tta agg aca ggt agt ttc aat agt tac tgt tgc
219	A12M	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt atg agg aca ggt agt ttc aat agt tac tgt tgc
220	A12C	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt tgt agg aca ggt agt ttc aat agt tac tgt tgc
221	A12N	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt aat agg aca ggt agt ttc aat agt tac tgt tgc
222	A12Y	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt tat agg aca ggt agt ttc aat agt tac tgt tgc
223	A12D	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gat agg aca ggt agt ttc aat agt tac tgt tgc
224	A12E	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gaa agg aca ggt agt ttc aat agt tac tgt tgc
225	A12P	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt cct agg aca ggt agt ttc aat agt tac tgt tgc
226	A12Q	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt caa agg aca ggt agt ttc aat agt tac tgt tgc
227	A12F	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt ttt agg aca ggt agt ttc aat agt tac tgt tgc
228	A12G	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt ggt agg aca ggt agt ttc aat agt tac tgt tgc

229	A12R	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt cgt agg aca ggt agt ttc aat agt tac tgt tgc
230	A12H	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt cat agg aca ggt agt ttc aat agt tac tgt tgc
231	A12S	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt tct agg aca ggt agt ttc aat agt tac tgt tgc
232	A12T	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt aca agg aca ggt agt ttc aat agt tac tgt tgc
233	A12I	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt att agg aca ggt agt ttc aat agt tac tgt tgc
234	R13K	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca aaa aca ggt agt ttc aat agt tac tgt tgc
235	R13V	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca gtt aca ggt agt ttc aat agt tac tgt tgc
236	R13W	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca tgg aca ggt agt ttc aat agt tac tgt tgc
237	R13A	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca gct aca ggt agt ttc aat agt tac tgt tgc
238	R13L	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca tta aca ggt agt ttc aat agt tac tgt tgc
239	R13M	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca atg aca ggt agt ttc aat agt tac tgt tgc
240	R13C	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca tgt aca ggt agt ttc aat agt tac tgt tgc
241	R13N	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca aat aca ggt agt ttc aat agt tac tgt tgc
242	R13Y	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca tat aca ggt agt ttc aat agt tac tgt tgc
243	R13D	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca gat aca ggt agt ttc aat agt tac tgt tgc
244	R13E	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca gaa aca ggt agt ttc aat agt tac tgt tgc
245	R13P	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca cct aca ggt agt ttc aat agt tac tgt tgc
246	R13Q	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca caa aca ggt agt ttc aat agt tac tgt tgc
247	R13F	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca ttt aca ggt agt ttc aat agt tac tgt tgc
248	R13G	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca ggt aca ggt agt ttc aat agt tac tgt tgc
249	R13R	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca cgt aca ggt agt ttc aat agt tac tgt tgc
250	R13H	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca cat aca ggt agt ttc aat agt tac tgt tgc
251	R13S	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca tct aca ggt agt ttc aat agt tac tgt tgc
252	R13T	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca aca aca ggt agt ttc aat agt tac tgt tgc
253	R13I	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca att aca ggt agt ttc aat agt tac tgt tgc
254	T14K	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aaa ggt agt ttc aat agt tac tgt tgc
255	T14V	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg gtt ggt agt ttc aat agt tac tgt tgc
256	T14W	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg tgg ggt agt ttc aat agt tac tgt tgc
257	T14A	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg gct ggt agt ttc aat agt tac tgt tgc

258	T14L	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg tta ggt agt ttc aat agt tac tgt tgc
259	T14M	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg atg ggt agt ttc aat agt tac tgt tgc
260	T14C	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg tgt ggt agt ttc aat agt tac tgt tgc
261	T14N	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aat ggt agt ttc aat agt tac tgt tgc
262	T14Y	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg tat ggt agt ttc aat agt tac tgt tgc
263	T14D	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg gat ggt agt ttc aat agt tac tgt tgc
264	T14E	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg gaa ggt agt ttc aat agt tac tgt tgc
265	T14P	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg cct ggt agt ttc aat agt tac tgt tgc
266	T14Q	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg caa ggt agt ttc aat agt tac tgt tgc
267	T14F	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg ttt ggt agt ttc aat agt tac tgt tgc
268	T14G	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg ggt ggt agt ttc aat agt tac tgt tgc
269	T14R	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg cgt ggt agt ttc aat agt tac tgt tgc
270	T14H	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg cat ggt agt ttc aat agt tac tgt tgc
271	T14S	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg tct ggt agt ttc aat agt tac tgt tgc
272	T14I	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg att ggt agt ttc aat agt tac tgt tgc
273	G15K	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca aaa agt ttc aat agt tac tgt tgc
274	G15V	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca gtt agt ttc aat agt tac tgt tgc
275	G15W	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca tgg agt ttc aat agt tac tgt tgc
276	G15A	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca gct agt ttc aat agt tac tgt tgc
277	G15L	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca tta agt ttc aat agt tac tgt tgc
278	G15M	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca atg agt ttc aat agt tac tgt tgc
279	G15C	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca tgt agt ttc aat agt tac tgt tgc
280	G15N	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca aat agt ttc aat agt tac tgt tgc
281	G15Y	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca tat agt ttc aat agt tac tgt tgc
282	G15D	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca gat agt ttc aat agt tac tgt tgc
283	G15E	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca gaa agt ttc aat agt tac tgt tgc
284	G15P	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca cct agt ttc aat agt tac tgt tgc
285	G15Q	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca caa agt ttc aat agt tac tgt tgc
286	G15F	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ttt agt ttc aat agt tac tgt tgc

287	G15R	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca cgt agt ttc aat agt tac tgt tgc
288	G15H	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca cat agt ttc aat agt tac tgt tgc
289	G15S	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca tct agt ttc aat agt tac tgt tgc
290	G15T	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca aca agt ttc aat agt tac tgt tgc
291	G15I	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca att agt ttc aat agt tac tgt tgc
292	S16K	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt aaa ttc aat agt tac tgt tgc
293	S16V	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt gtt ttc aat agt tac tgt tgc
294	S16W	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt tgg ttc aat agt tac tgt tgc
295	S16A	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt gct ttc aat agt tac tgt tgc
296	S16L	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt tta ttc aat agt tac tgt tgc
297	S16M	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt atg ttc aat agt tac tgt tgc
298	S16C	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt tgt ttc aat agt tac tgt tgc
299	S16N	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt aat ttc aat agt tac tgt tgc
300	S16Y	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt tat ttc aat agt tac tgt tgc
301	S16D	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt gat ttc aat agt tac tgt tgc
302	S16E	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt gaa ttc aat agt tac tgt tgc
303	S16P	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt cct ttc aat agt tac tgt tgc
304	S16Q	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt caa ttc aat agt tac tgt tgc
305	S16F	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt ttt ttc aat agt tac tgt tgc
306	S16G	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt ggt ttc aat agt tac tgt tgc
307	S16R	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt cgt ttc aat agt tac tgt tgc
308	S16H	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt cat ttc aat agt tac tgt tgc
309	S16S	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt tct ttc aat agt tac tgt tgc
310	S16T	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt aca ttc aat agt tac tgt tgc
311	S16I	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt att ttc aat agt tac tgt tgc
312	F17K	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt aaa aat agt tac tgt tgc
313	F17V	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt gtt aat agt tac tgt tgc
314	F17W	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt tgg aat agt tac tgt tgc
315	F17A	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt gct aat agt tac tgt tgc

316	F17L	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt tta aat agt tac tgt tgc
317	F17M	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt atg aat agt tac tgt tgc
318	F17C	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt tgt aat agt tac tgt tgc
319	F17N	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt aat aat agt tac tgt tgc
320	F17Y	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt tat aat agt tac tgt tgc
321	F17D	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt gat aat agt tac tgt tgc
322	F17E	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt gaa aat agt tac tgt tgc
323	F17P	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt cct aat agt tac tgt tgc
324	F17Q	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt caa aat agt tac tgt tgc
325	F17F	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttt aat agt tac tgt tgc
326	F17G	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ggt aat agt tac tgt tgc
327	F17R	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt cgt aat agt tac tgt tgc
328	F17H	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt cat aat agt tac tgt tgc
329	F17S	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt tct aat agt tac tgt tgc
330	F17T	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt aca aat agt tac tgt tgc
331	F17I	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt att aat agt tac tgt tgc
332	N18K	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aaa agt tac tgt tgc
333	N18V	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc gtt agt tac tgt tgc
334	N18W	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc tgg agt tac tgt tgc
335	N18A	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc gct agt tac tgt tgc
336	N18L	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc tta agt tac tgt tgc
337	N18M	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc atg agt tac tgt tgc
338	N18C	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc tgt agt tac tgt tgc
339	N18Y	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc tat agt tac tgt tgc
340	N18D	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc gat agt tac tgt tgc
341	N18E	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc gaa agt tac tgt tgc
342	N18P	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc cct agt tac tgt tgc
343	N18Q	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc caa agt tac tgt tgc
344	N18F	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc ttt agt tac tgt tgc

345	N18G	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc ggt agt tac tgt tgc
346	N18R	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc cgt agt tac tgt tgc
347	N18H	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc cat agt tac tgt tgc
348	N18S	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc tct agt tac tgt tgc
349	N18T	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aca agt tac tgt tgc
350	N18I	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc att agt tac tgt tgc
351	S19K	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat aaa tac tgt tgc
352	S19V	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat gtt tac tgt tgc
353	S19W	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat tgg tac tgt tgc
354	S19A	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat gct tac tgt tgc
355	S19L	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat tta tac tgt tgc
356	S19M	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat atg tac tgt tgc
357	S19C	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat tgt tac tgt tgc
358	S19N	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat aat tac tgt tgc
359	S19Y	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat tat tac tgt tgc
360	S19D	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat gat tac tgt tgc
361	S19E	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat gaa tac tgt tgc
362	S19P	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat cct tac tgt tgc
363	S19Q	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat caa tac tgt tgc
364	S19F	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat ttt tac tgt tgc
365	S19G	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat ggt tac tgt tgc
366	S19R	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat cgt tac tgt tgc
367	S19H	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat cat tac tgt tgc
368	S19S	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat tct tac tgt tgc
369	S19T	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat aca tac tgt tgc
370	S19I	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat att tac tgt tgc
371	Y20K	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt aaa tgt tgc
372	Y20V	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt gtt tgt tgc
373	Y20W	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tgg tgt tgc

374	Y20A	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt gct tgt tgc
375	Y20L	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tta tgt tgc
376	Y20M	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt atg tgt tgc
377	Y20C	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tgt tgt tgc
378	Y20N	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt aat tgt tgc
379	Y20Y	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tat tgt tgc
380	Y20D	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt gat tgt tgc
381	Y20E	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt gaa tgt tgc
382	Y20P	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt cct tgt tgc
383	Y20Q	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt caa tgt tgc
384	Y20F	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt ttt tgt tgc
385	Y20G	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt ggt tgt tgc
386	Y20R	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt cgt tgt tgc
387	Y20H	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt cat tgt tgc
388	Y20S	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tct tgt tgc
389	Y20T	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt aca tgt tgc
390	Y20I	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt att tgt tgc
391	C21K	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac aaa tgc
392	C21V	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac gtt tgc
393	C21W	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgg tgc
394	C21A	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac gct tgc
395	C21L	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tta tgc
396	C21M	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac atg tgc
397	C21N	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac aat tgc
398	C21Y	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tat tgc
399	C21D	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac gat tgc
400	C21E	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac gaa tgc
401	C21P	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac cct tgc
402	C21Q	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac caa tgc

403	C21F	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac ttt tgc
404	C21G	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac ggt tgc
405	C21R	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac cgt tgc
406	C21H	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac cat tgc
407	C21S	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tct tgc
408	C21T	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac aca tgc
409	C21I	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac att tgc
410	C22K	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt aaa
411	C22V	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt gtt
412	C22W	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgg
413	C22A	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt gct
414	C22L	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tta
415	C22M	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt atg
416	C22C	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgt
417	C22N	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt aat
418	C22Y	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tat
419	C22D	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt gat
420	C22E	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt gaa
421	C22P	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt cct
422	C22Q	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt caa
423	C22F	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt ttt
424	C22G	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt ggt
425	C22R	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt cgt
426	C22H	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt cat
427	C22S	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tct
428	C22T	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt aca
429	C22I	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt att
430	C21E	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac gaa tgc
431	C22E	ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt gaa

FIGURE 5

SEQ ID	Variant	Sequence
432	M01140	F K S W S L C T P G C A R T G S F N S Y C C
764	F1W	W K S W S L C T P G C A R T G S F N S Y C C
765	F1A	A K S W S L C T P G C A R T G S F N S Y C C
766	F1L	L K S W S L C T P G C A R T G S F N S Y C C
767	F1M	M K S W S L C T P G C A R T G S F N S Y C C
768	F1C	C K S W S L C T P G C A R T G S F N S Y C C
769	F1N	N K S W S L C T P G C A R T G S F N S Y C C
770	F1Y	Y K S W S L C T P G C A R T G S F N S Y C C
771	F1D	D K S W S L C T P G C A R T G S F N S Y C C
772	F1E	E K S W S L C T P G C A R T G S F N S Y C C
773	F1P	P K S W S L C T P G C A R T G S F N S Y C C
774	F1Q	Q K S W S L C T P G C A R T G S F N S Y C C
775	F1G	G K S W S L C T P G C A R T G S F N S Y C C
776	F1R	R K S W S L C T P G C A R T G S F N S Y C C
777	F1H	H K S W S L C T P G C A R T G S F N S Y C C
778	F1S	S K S W S L C T P G C A R T G S F N S Y C C
779	F1T	T K S W S L C T P G C A R T G S F N S Y C C
780	F1I	I K S W S L C T P G C A R T G S F N S Y C C
781	K2V	F V S W S L C T P G C A R T G S F N S Y C C
782	K2W	F W S W S L C T P G C A R T G S F N S Y C C
783	K2A	F A S W S L C T P G C A R T G S F N S Y C C
784	K2L	F L S W S L C T P G C A R T G S F N S Y C C
785	K2M	F M S W S L C T P G C A R T G S F N S Y C C
786	K2C	F C S W S L C T P G C A R T G S F N S Y C C
787	K2N	F N S W S L C T P G C A R T G S F N S Y C C
788	K2Y	F Y S W S L C T P G C A R T G S F N S Y C C

789	K1D	FDSSWSLCTPGGCARTGGSFNSSYCC
790	K2E	FESSWSLCTPGGCARTGGSFNSSYCC
791	K2P	FPSSWSLCTPGGCARRFGGSFNSSYCC
792	K1Q	FQSSWSLCTPGGCARTGGSFNSSYCC
793	K2F	FFSSWSLCTPGGCARTGGSFNSSYCC
794	K2G	FGSSWSLCTPGGCAEFGGSFNSSYCC
795	K2R	FRSSWSLCTPGGCARTGGSFNSSYCC
796	K2H	FHSSWSLCTPGGCARTGGSFNSSYCC
797	K2S	FSSSWSLCTPGGCARTGGSFNSSYCC
798	K2T	FTSSWSLCTPGGCARRFGGSFNSSYCC
799	K1I	FISSWSLCTPGGCARTGGSFNSSYCC
1169	S3K	FKKWSLCEPGGCARTGGSFNSSYCC
1170	S3V	FKVWSLCTPGGCARRFGGSFNSSYCC
1171	S3W	FKWWSLCTPGGCARTGGSFNSSYCC
1172	S3A	FKAWSLCEPGGCARTGGSFNSSYCC
1173	S3L	FKLWSLCTPGGCARTGGSFNSSYCC
1174	S3M	FKMWSLCTPGGCARRFGGSFNSSYCC
1175	S3C	FKCWSLCEPGGCARTGGSFNSSYCC
1176	S3N	FKNWSLCTPGGCARTGGSFNSSYCC
1177	S3Y	FKYWSLCTPGGCARRFGGSFNSSYCC
1178	S3D	FKDWSLCTPGGCARTGGSFNSSYCC
1179	S3E	FKEWSLCTPGGCARTGGSFNSSYCC
1180	S3P	FKRWSLCTPGGCARRFGGSFNSSYCC
1181	S3Q	FKQWSLCTPGGCARRFGGSFNSSYCC
1182	S3F	FKFWSLCTPGGCARTGGSFNSSYCC
1183	S3G	FKGWSLCTPGGCARTGGSFNSSYCC
1184	S3R	FKRWSLCTPGGCARRFGGSFNSSYCC
1185	S3H	FKHWSLCTPGGCARTGGSFNSSYCC
1186	S3T	FKTWSLCTPGGCARTGGSFNSSYCC
1187	S3I	FKIWSLCEPGGCARTGGSFNSSYCC
1188	W4K	FKSSKSLCTPGGCARTGGSFNSSYCC
800	W4V	FKSSVSLCTPGGCARTGGSFNSSYCC
801	W4A	FKSSASLCTPGGCARTGGSFNSSYCC

802	W4L	FKSSLSLCTPGCARTGSSFSNSSYC	C
803	W4M	FKSSMSLCTPGCARTGSSFSNSSYC	C
804	W4C	FKSSCSLCTPGCARTGSSFSNSSYC	C
805	W4N	FKSSNSLCTPGCARTGSSFSNSSYC	C
806	W4Y	FKSSYSLCTPGCARTGSSFSNSSYC	C
807	W4D	FKSSDSLCTPGCARTGSSFSNSSYC	C
808	W4E	FKSSESLCTPGCARTGSSFSNSSYC	C
809	W4P	FKSSPSLCTPGCARTGSSFSNSSYC	C
810	W4Q	FKSSQSLCTPGCARTGSSFSNSSYC	C
811	W4F	FKSSFSLCTPGCARTGSSFSNSSYC	C
812	W4G	FKSSGSLCTPGCARTGSSFSNSSYC	C
813	W4R	FKSSRSLCTPGCARTGSSFSNSSYC	C
814	W4H	FKSSHSLCTPGCARTGSSFSNSSYC	C
815	W4S	FKSSSSLCTPGCARTGSSFSNSSYC	C
816	W4T	FKSSTSLCTPGCARTGSSFSNSSYC	C
817	W4I	FKSSISLCTPGCARTGSSFSNSSYC	C
818	W4K	FKSSKSLCTPGCARTGSSFSNSSYC	C
819	S5V	FKSSVSLCTPGCARTGSSFSNSSYC	C
820	S5W	FKSSWSLCTPGCARTGSSFSNSSYC	C
821	S5A	FKSSASLCTPGCARTGSSFSNSSYC	C
822	S5L	FKSSLSLCTPGCARTGSSFSNSSYC	C
823	S5M	FKSSMSLCTPGCARTGSSFSNSSYC	C
824	S5C	FKSSCSLCTPGCARTGSSFSNSSYC	C
825	S5N	FKSSNSLCTPGCARTGSSFSNSSYC	C
826	S5Y	FKSSYSLCTPGCARTGSSFSNSSYC	C
827	S5D	FKSSDSLCTPGCARTGSSFSNSSYC	C
828	S5E	FKSSESLCTPGCARTGSSFSNSSYC	C
829	S5P	FKSSPSLCTPGCARTGSSFSNSSYC	C
830	S5Q	FKSSQSLCTPGCARTGSSFSNSSYC	C
831	S5F	FKSSFSLCTPGCARTGSSFSNSSYC	C
832	S5G	FKSSGSLCTPGCARTGSSFSNSSYC	C
833	S5R	FKSSRSLCTPGCARTGSSFSNSSYC	C
834	S5H	FKSSHSLCTPGCARTGSSFSNSSYC	C

835	S5T	F K S W T L C E P G C A R T G S F N S Y C
836	S5I	F K S W I L C E P G C A R T G S F N S Y C
837	L6K	F K S S M S C T P G C A R E G S F N S Y C
838	L6V	F K S S M S V C T P G C A R T G S F N S Y C
839	M6T	F K S S M S W C T P G C A R T G S F N S Y C
840	L6A	F K S S M S A C T P G C A E E G S F N S Y C
841	L6M	F K S S M S M C T P G C A R T G S F N S Y C
842	L6C	F K S S M S C C T P G C A R T G S F N S Y C
843	L6N	F K S S M S N C T P G C A R T G S F N S Y C
844	L6Y	F K S S M S Y C T P G C A R T G S F N S Y C
845	L6D	F K S S M S D C T P G C A R T G S F N S Y C
846	L6E	F K S S M S E C T P G C A R T G S F N S Y C
847	L6P	F K S S M S P C T P G C A R T G S F N S Y C
848	L6Q	F K S S M S Q C T P G C A R T G S F N S Y C
849	L6F	F K S S M S F C T P G C A R T G S F N S Y C
850	L6S	F K S S M S G C T P G C A R T G S F N S Y C
851	L6R	F K S S M S R C T P G C A R T G S F N S Y C
852	L6H	F K S S M S H C T P G C A R T G S F N S Y C
853	L6S	F K S S M S S C T P G C A R T G S F N S Y C
854	L6T	F K S S M S T C T P G C A R E G S F N S Y C
855	L6I	F K S S M S I C T P G C A R T G S F N S Y C
856	C7K	F K S S M S L K T P G C A R T G S F N S Y C
857	C7V	F K S S M S L V T P G C A R E G S F N S Y C
858	C7W	F K S S M S L W T P G C A R E G S F N S Y C
859	C7A	F K S S M S L A T P G C A R T G S F N S Y C
860	C7L	F K S S M S L L T P G C A R T G S F N S Y C
861	C7M	F K S S M S L M T P G C A R E G S F N S Y C
862	C7N	F K S S M S L N T P G C A R T G S F N S Y C
863	C7Y	F K S S M S L Y T P G C A R T G S F N S Y C
864	C7D	F K S S M S L D T P G C A E E G S F N S Y C
865	C7E	F K S S M S L E T P G C A R T G S F N S Y C
866	C7P	F K S S M S L P T P G C A R T G S F N S Y C
867	C7Q	F K S S M S L Q T P G C A R T G S F N S Y C

868	C77F	FKKSWSSLFFPGGCARTGSFNPCCXSSNFG
869	C77G	FKKSWSSLFGPGGCARTGSFNPCCYSSNFG
870	C77R	FKKSWSSLFRPGGCARTGSFNPCCXSSNFG
871	C77H	FKKSWSSLFHPGGCARTGSFNPCCXSSNFG
872	C77S	FKKSWSSLFSPGGCARTGSFNPCCYSSNFG
873	C77E	FKKSWSSLFEPGGCARTGSFNPCCXSSNFG
874	C77I	FKKSWSSLFIPGGCARTGSFNPCCXSSNFG
875	M78T	FKKSWSSLTKRGGCARTGSFNPCCXSSNFG
876	A78V	FKKSWSSLTCAGGCARTGSFNPCCXSSNFG
877	M78L	FKKSWSSLTMPGGCARTGSFNPCCXSSNFG
878	T78A	FKKSWSSLTCAGGCARTGSFNPCCXSSNFG
879	T78L	FKKSWSSLTCLGGCARTGSFNPCCYSSNFG
880	M78L	FKKSWSSLTMPGGCARTGSFNPCCYSSNFG
881	C78Z	FKKSWSSLTCCGGCARTGSFNPCCXSSNFG
882	M78L	FKKSWSSLTMCGGCARTGSFNPCCXSSNFG
883	T78K	FKKSWSSLTCAGGCARTGSFNPCCYSSNFG
884	T78D	FKKSWSSLTCDGGCARTGSFNPCCXSSNFG
885	T78E	FKKSWSSLTCEGGCARTGSFNPCCXSSNFG
886	T78L	FKKSWSSLTCLGGCARTGSFNPCCXSSNFG
887	Q78L	FKKSWSSLTCQGGCARTGSFNPCCXSSNFG
888	T78F	FKKSWSSLTCFGGCARTGSFNPCCXSSNFG
889	G78L	FKKSWSSLTCLGGCARTGSFNPCCXSSNFG
890	R78W	FKKSWSSLTCWGGCARTGSFNPCCXSSNFG
891	T78C	FKKSWSSLTCCGGCARTGSFNPCCXSSNFG
892	S78L	FKKSWSSLTCLGGCARTGSFNPCCXSSNFG
893	I78L	FKKSWSSLTCLGGCARTGSFNPCCXSSNFG
894	K78C	FKKSWSSLTCCGGCARTGSFNPCCXSSNFG
895	A78L	FKKSWSSLTCLGGCARTGSFNPCCXSSNFG
896	M78L	FKKSWSSLTCLGGCARTGSFNPCCXSSNFG
897	V78A	FKKSWSSLTCAGGCARTGSFNPCCXSSNFG
898	T78L	FKKSWSSLTCLGGCARTGSFNPCCXSSNFG
899	M78L	FKKSWSSLTCLGGCARTGSFNPCCXSSNFG
900	C78Q	FKKSWSSLTCQGGCARTGSFNPCCXSSNFG

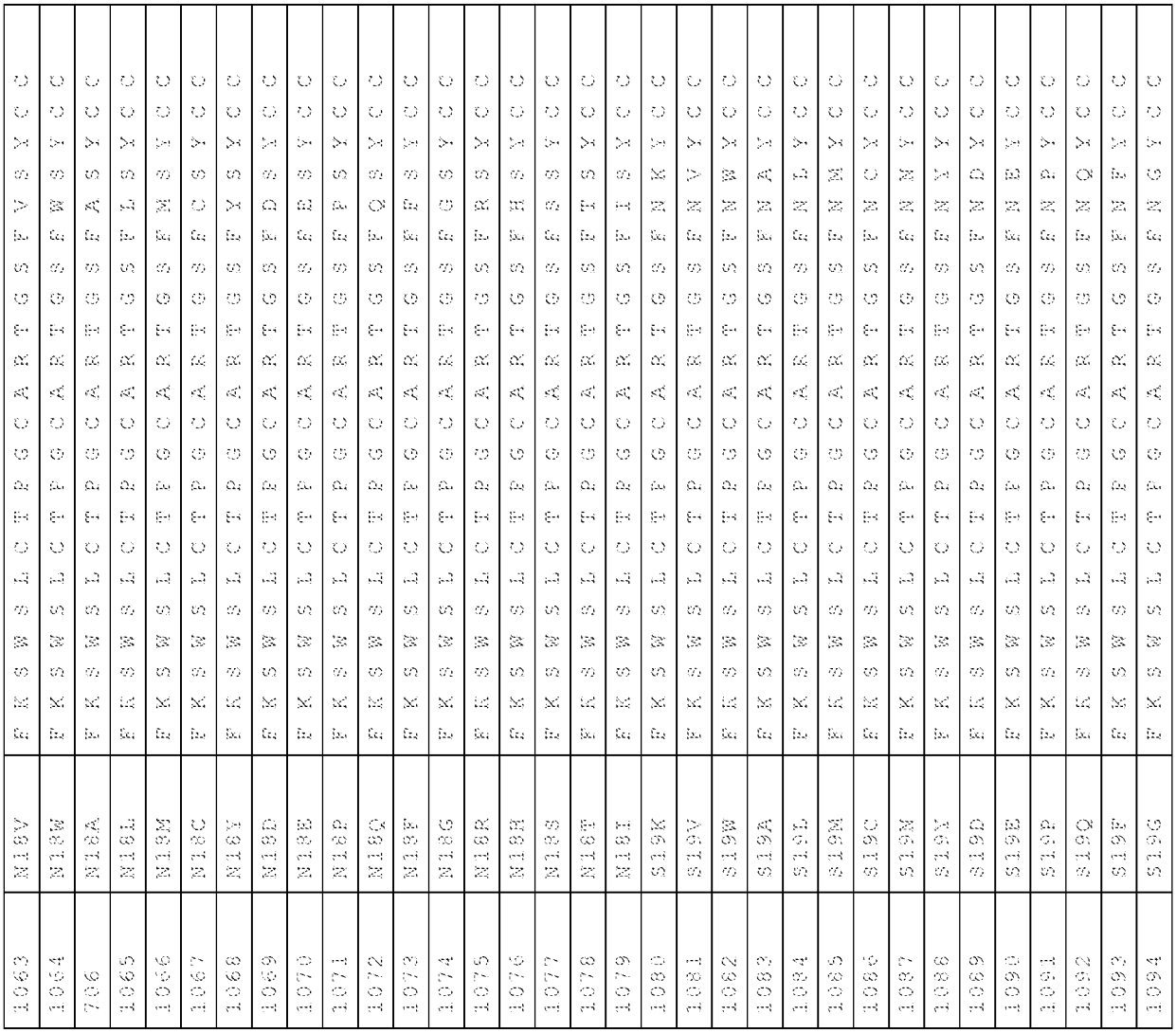
901	P68M	FKSSWSSLCEEMGGCARTGSSFSNSSXC
902	P68Y	FKSSWSSLCETXGGCARTGSSFSNSSYC
903	P68D	FKSSWSSLCETDGGCARTGSSFSNSSXC
904	P68E	FKSSWSSLCETEGGCARTGSSFSNSSXC
905	P68Q	FKSSWSSLCETQGGCARTGSSFSNSSYC
906	P68F	FKSSWSSLCEEFGGCARTGSSFSNSSYC
907	P68G	FKSSWSSLCETGGGCARTGSSFSNSSXC
908	P68R	FKSSWSSLCETRGGCARTGSSFSNSSYC
909	P68M	FKSSWSSLCEEMGGCARTGSSFSNSSYC
910	P68S	FKSSWSSLCETSGGCARTGSSFSNSSXC
911	P68T	FKSSWSSLCETTGGCARTGSSFSNSSYC
912	P68I	FKSSWSSLCEEIGGCARTGSSFSNSSYC
913	G10K	FKSSWSSLCETPKCARTGSSFSNSSYC
914	G10V	FKSSWSSLCETPVCARTGSSFSNSSXC
915	G10W	FKSSWSSLCEEPWCARTGSSFSNSSYC
916	G10A	FKSSWSSLCETPACARTGSSFSNSSYC
917	G10L	FKSSWSSLCETPLCARTGSSFSNSSXC
918	G10M	FKSSWSSLCEEPMCARTGSSFSNSSXC
919	G10C	FKSSWSSLCETPCCARTGSSFSNSSYC
920	G10N	FKSSWSSLCETPNCARTGSSFSNSSYC
921	G10Y	FKSSWSSLCETPYCARTGSSFSNSSXC
922	G10D	FKSSWSSLCEEPDCARTGSSFSNSSYC
923	G10E	FKSSWSSLCETPECARTGSSFSNSSYC
924	G10P	FKSSWSSLCETPPCARTGSSFSNSSVCC
925	G10C	FKSSWSSLCETPQCARTGSSFSNSSXC
926	G10F	FKSSWSSLCETPFCARTGSSFSNSSYC
927	G10R	FKSSWSSLCETPRCARTGSSFSNSSVCC
928	G10H	FKSSWSSLCETPHCARTGSSFSNSSXC
929	G10S	FKSSWSSLCETPSCARTGSSFSNSSYC
930	G10F	FKSSWSSLCEEPFCARTGSSFSNSSYC
931	G10I	FKSSWSSLCETPICARTGSSFSNSSXC
932	C11K	FKSSWSSLCEEPGKARTGSSFSNSSYC
933	C11V	FKSSWSSLCEEPGVARTGSSFSNSSYC

934	C1IW	FKSSWSSLCTPGGWARTGSSFSNSSYC
935	C1IA	FKSSWSSLCTPGGAARTGSSFSNSSYC
936	C1IF	FKSSWSSLCTPGGLARREGSSFSNSSAC
937	C1IM	FRSSWSSLCTPGGMARRTGSSFSNSSYC
938	C1IN	FKSSWSSLCTPGGNARTTGSSFSNSSYC
939	C1IY	FKSSWSSLCTPGGYARREGSSFSNSSAC
940	C1ID	FKSSWSSLCTPGGDARRTGSSFSNSSYC
941	C1IE	FKSSWSSLCTPGGEARTTGSSFSNSSYC
942	C1IP	FKSSWSSLCTPGGPARRTGSSFSNSSYC
943	C1IQ	FKSSWSSLCTPGGQARREGSSFSNSSYC
944	C1IF	FKSSWSSLCTPGGFARTTGSSFSNSSYC
945	C1IG	FKSSWSSLCTPGGGARTTGSSFSNSSYC
946	C1IR	FKSSWSSLCTPGGRARREGSSFSNSSYC
947	C1IH	FKSSWSSLCTPGGHARRTGSSFSNSSYC
948	C1IS	FKSSWSSLCTPGGSARTTGSSFSNSSYC
949	C1IT	FKSSWSSLCTPGGTARRTGSSFSNSSYC
950	C1II	FKSSWSSLCTPGGIARRTGSSFSNSSYC
951	A12K	FKSSWSSLCTPGGKMRTTGSSFSNSSYC
952	A12V	FKSSWSSLCTPGGVRRTTGSSFSNSSYC
953	A12W	FKSSWSSLCTPGGWRRTTGSSFSNSSYC
954	A12L	FRSSWSSLCTPGGLLRRTTGSSFSNSSYC
955	A12M	FKSSWSSLCTPGGMRRTTGSSFSNSSYC
956	A12C	FKSSWSSLCTPGGCRRTTGSSFSNSSYC
957	A12N	FRSSWSSLCTPGGNRRTTGSSFSNSSAC
958	A12Y	FRSSWSSLCTPGGYRRTTGSSFSNSSYC
959	A12D	FKSSWSSLCTPGGDRRTTGSSFSNSSYC
960	A12E	FKSSWSSLCTPGGERRTTGSSFSNSSAC
961	A12P	FRSSWSSLCTPGGPRRTTGSSFSNSSYC
962	A12Q	FKSSWSSLCTPGGQRRTTGSSFSNSSYC
963	A12F	FKSSWSSLCTPGGFRRTTGSSFSNSSAC
964	A12G	FRSSWSSLCTPGGGRRTTGSSFSNSSYC
965	A12R	FKSSWSSLCTPGGRRRTTGSSFSNSSYC
966	A12W	FKSSWSSLCTPGGWRRTTGSSFSNSSAC

967	A12S	FKSWSSLCTPGCSRTGSSFNSSYC
968	A12T	FKSWSSLCTPGCTRTGSSFNSSYC
969	A12I	FKSWSSLCTPGCIRTGSSFNSSYC
970	R13K	FKSWSSLCTPGCAKTGSSFNSSYC
971	R13V	FKSWSSLCTPGCAVTGSSFNSSYC
972	R13W	FKSWSSLCTPGCAWTGSSFNSSYC
973	R13A	FKSWSSLCTPGCAATGSSFNSSYC
974	R13L	FKSWSSLCTPGCALTGSSFNSSYC
975	R13M	FKSWSSLCTPGCAMTGSSFNSSYC
976	R13C	FKSWSSLCTPGCACTGSSFNSSYC
703	R13N	FKSWSSLCTPGCANTGSSFNSSYC
977	R13Y	FKSWSSLCTPGCAYTGSSFNSSYC
978	R13D	FKSWSSLCTPGCADTGSSFNSSYC
979	R13E	FKSWSSLCTPGCAETGSSFNSSYC
980	R13F	FKSWSSLCTPGCAFTGSSFNSSYC
981	R13Q	FKSWSSLCTPGCAQTGSSFNSSYC
982	R13P	FKSWSSLCTPGCAPTGSSFNSSYC
983	R13G	FKSWSSLCTPGCAGTGSSFNSSYC
984	R13H	FKSWSSLCTPGCAHTGSSFNSSYC
985	R13S	FKSWSSLCTPGCASTGSSFNSSYC
986	R13T	FKSWSSLCTPGCATTGSSFNSSYC
987	R13I	FKSWSSLCTPGCAITGSSFNSSYC
988	T14K	FKSWSSLCTPGCARKGSSFNSSYC
989	T14V	FKSWSSLCTPGCARVGSSFNSSYC
990	T14W	FKSWSSLCTPGCARWGSSFNSSYC
991	T14A	FKSWSSLCTPGCARAGSSFNSSYC
992	T14L	FKSWSSLCTPGCARLGSSFNSSYC
993	T14M	FKSWSSLCTPGCARMGSSFNSSYC
994	T14C	FKSWSSLCTPGCARCGSSFNSSYC
995	T14N	FKSWSSLCTPGCANGGSSFNSSYC
996	T14Y	FKSWSSLCTPGCAYGGSSFNSSYC
997	T14D	FKSWSSLCTPGCADGGSSFNSSYC
998	T14E	FKSWSSLCTPGCAEGGSSFNSSYC

999	T14E	FKSSWSSLCTEPGGCARESGGSFNSSYCC
1000	T14Q	FKSSWSSLCTEPGGCARQSGGSFNSSYCC
1001	T14F	FKSSWSSLCTEPGGCARFSGGSFNSSYCC
1002	T14G	FKSSWSSLCTEPGGCARGSGGSFNSSYCC
1003	T14R	FKSSWSSLCTEPGGCARRSGGSFNSSYCC
1004	T14W	FKSSWSSLCTEPGGCAWGSGGSFNSSYCC
1005	T14S	FKSSWSSLCTEPGGCARSSGGSFNSSYCC
1006	T14I	FKSSWSSLCTEPGGCARISGGSFNSSYCC
1007	G15K	FKSSWSSLCTEPGGCARTKSGGSFNSSYCC
1008	G15V	FKSSWSSLCTEPGGCARTEVSGGSFNSSYCC
1009	G15W	FKSSWSSLCTEPGGCARTWSGGSFNSSYCC
1010	G15A	FKSSWSSLCTEPGGCARTASGGSFNSSYCC
1011	G15L	FKSSWSSLCTEPGGCARTLSGGSFNSSYCC
1012	G15M	FKSSWSSLCTEPGGCARTMSGGSFNSSYCC
1013	G15C	FKSSWSSLCTEPGGCARTCSGGSFNSSYCC
1014	G15M	FKSSWSSLCTEPGGCARTTNSGGSFNSSYCC
1015	G15Y	FKSSWSSLCTEPGGCARTEYSGGSFNSSYCC
1016	G15D	FKSSWSSLCTEPGGCARTDSGGSFNSSYCC
1017	G15E	FKSSWSSLCTEPGGCARTESGGSFNSSYCC
1018	G15P	FKSSWSSLCTEPGGCARTPSGGSFNSSYCC
1019	G15Q	FKSSWSSLCTEPGGCARTQSGGSFNSSYCC
1020	G15F	FKSSWSSLCTEPGGCARTFSGGSFNSSYCC
1021	G15R	FKSSWSSLCTEPGGCARTRSGGSFNSSYCC
1022	G15H	FKSSWSSLCTEPGGCARTHSGGSFNSSYCC
1023	G15S	FKSSWSSLCTEPGGCARTSSGGSFNSSYCC
1024	G15T	FKSSWSSLCTEPGGCARTTSGGSFNSSYCC
1025	G15I	FKSSWSSLCTEPGGCARTISGGSFNSSYCC
1026	S16K	FKSSWSSLCTEPGGCARTGKGGSFNSSYCC
1027	S16V	FKSSWSSLCTEPGGCARTGVGGSFNSSYCC
1028	S16W	FKSSWSSLCTEPGGCARTGWGGSFNSSYCC
1029	S16A	FKSSWSSLCTEPGGCARTGAGGSFNSSYCC
1030	S16L	FKSSWSSLCTEPGGCARTGLGGSFNSSYCC
1031	S16M	FKSSWSSLCTEPGGCARTGMGGSFNSSYCC

1032	S16C	FKSSWSSLCTPGCARTGSCFSNSSCCXC
1033	S16M	FKSSWSSLCTPGCARTGSNFSSSYCCC
1034	S16Y	FKSSWSSLCTPGCARFGSYFSNSSCCC
1035	S16D	FKSSWSSLCTPGCARTGSDFSNSSCCC
1036	S16E	FKSSWSSLCTPGCARTGSEFSNSSCCC
1037	S16P	FKSSWSSLCTPGCARFGSAFSNSSCCC
1038	S16Q	FKSSWSSLCTPGCARTGSQFSNSSCCC
1039	S16F	FKSSWSSLCTPGCARTGSFFSNSSCCC
1040	S16G	FKSSWSSLCTPGCARTGSGFSNSSCCC
1041	S16R	FKSSWSSLCTPGCARTGSRFSNSSCCC
1042	S16H	FKSSWSSLCTPGCARTGSHFSNSSCCC
1043	S16T	FKSSWSSLCTPGCARTGTFFSNSSCCC
1044	S16I	FKSSWSSLCTPGCARTGSIFSNSSCCC
1045	F17K	FKSSWSSLCTPGCARTGSKWSSSYCCC
1046	F17V	FKSSWSSLCTPGCARTGSSVSNSSCCC
1047	F17W	FKSSWSSLCTPGCARTGSSWSNSSCCC
1048	F17A	FKSSWSSLCTPGCARTGSSAWSSYCCC
705	F17L	FKSSWSSLCTPGCARTGSSLWSSYCCC
1049	F17M	FKSSWSSLCTPGCARTGSSMWSSYCCC
1050	F17C	FKSSWSSLCTPGCARTGSSCWSSYCCC
1051	F17N	FKSSWSSLCTPGCARTGSNWSSSYCCC
704	F17Y	FKSSWSSLCTPGCARTGSSYWSNSSCCC
1052	F17D	FKSSWSSLCTPGCARTGSSDWSNSSCCC
1053	F17E	FKSSWSSLCTPGCARTGSSEWSSSYCCC
1054	F17S	FKSSWSSLCTPGCARTGSSFWSNSSCCC
1055	F17Q	FKSSWSSLCTPGCARTGSSQWSNSSCCC
1056	F17G	FKSSWSSLCTPGCARTGSSGWSSSYCCC
1057	F17R	FKSSWSSLCTPGCARTGSSRWSNSSCCC
1058	F17H	FKSSWSSLCTPGCARTGSSHWSNSSCCC
1059	F17S	FKSSWSSLCTPGCARTGSSSWSSSYCCC
1060	F17T	FKSSWSSLCTPGCARTGSSTWSNSSCCC
1061	F17I	FKSSWSSLCTPGCARTGSSIWSNSSCCC
1062	M18K	FKSSWSSLCTPGCARTGSSKMSNSSCCC



1063	N18V	FKSWSSLCTPGCARTGSSVFSCCXSS
1064	N18W	FKSWSSLCTPGCARTGSSWFSCCYSS
706	N18A	FKSWSSLCTPGCARTGSSAFSCCXSS
1065	N18L	FKSWSSLCTPGCARTGSSLFSCCXSS
1066	N18M	FKSWSSLCTPGCARTGSSMFSCCYSS
1067	N18C	FKSWSSLCTPGCARTGSSCFSCCXSS
1068	N18Y	FKSWSSLCTPGCARTGSSYFSCCXSS
1069	N18D	FKSWSSLCTPGCARTGSSDFSCCXSS
1070	N18E	FKSWSSLCTPGCARTGSSEFSCCYSS
1071	N18P	FKSWSSLCTPGCARTGSSPFSCCXSS
1072	N18Q	FKSWSSLCTPGCARTGSSQFSCCXSS
1073	N18F	FKSWSSLCTPGCARTGSSFFSCCYSS
1074	N18G	FKSWSSLCTPGCARTGSSGFSCCYSS
1075	N18R	FKSWSSLCTPGCARTGSSRFSCCXSS
1076	N18H	FKSWSSLCTPGCARTGSSHFSCCYSS
1077	N18S	FKSWSSLCTPGCARTGSSSFSCCYSS
1078	N18T	FKSWSSLCTPGCARTGSSTFSCCXSS
1079	N18I	FKSWSSLCTPGCARTGSSIFSCCXSS
1080	S19K	FKSWSSLCTPGCARTGSSKFSCCXSS
1081	S19V	FKSWSSLCTPGCARTGSSVFSCCXSS
1082	S19W	FKSWSSLCTPGCARTGSSWFSCCXSS
1083	S19A	FKSWSSLCTPGCARTGSSAFSCCXSS
1084	S19L	FKSWSSLCTPGCARTGSSLFSCCYSS
1085	S19M	FKSWSSLCTPGCARTGSSMFSCCXSS
1086	S19C	FKSWSSLCTPGCARTGSSCFSCCXSS
1087	S19M	FKSWSSLCTPGCARTGSSNFSCCYSS
1088	S19Y	FKSWSSLCTPGCARTGSSYFSCCXSS
1089	S19D	FKSWSSLCTPGCARTGSSDFSCCXSS
1090	S19E	FKSWSSLCTPGCARTGSSEFSCCYSS
1091	S19P	FKSWSSLCTPGCARTGSSPFSCCXSS
1092	S19Q	FKSWSSLCTPGCARTGSSQFSCCXSS
1093	S19F	FKSWSSLCTPGCARTGSSFFSCCYSS
1094	S19G	FKSWSSLCTPGCARTGSSGFSCCYSS

1095	S19R	FKSSWSSLCEPGGCARTGSSFNRXC
1096	S19M	FKSSWSSLCEPGGCARTGSSFNMYC
1097	S19F	FKSSWSSLCEPGGCARTGSSFNFVC
1098	S19I	FKSSWSSLCEPGGCARTGSSFNIXC
1099	Y20K	FKSSWSSLCEPGGCARTGSSFNKYC
1100	Y20V	FKSSWSSLCEPGGCARTGSSFNVAC
1101	Y20W	FKSSWSSLCEPGGCARTGSSFNWMC
1102	Y20A	FKSSWSSLCEPGGCARTGSSFNAVC
1103	Y20L	FKSSWSSLCEPGGCARTGSSFNLTC
1104	Y20M	FKSSWSSLCEPGGCARTGSSFNMMC
1105	Y20C	FKSSWSSLCEPGGCARTGSSFNCSC
1106	N20M	FKSSWSSLCEPGGCARTGSSFNMMC
1107	N20D	FKSSWSSLCEPGGCARTGSSFNDDC
1108	N20E	FKSSWSSLCEPGGCARTGSSFNEEC
1109	N20H	FKSSWSSLCEPGGCARTGSSFNHAC
1110	N20Q	FKSSWSSLCEPGGCARTGSSFNQQC
707	N20F	FKSSWSSLCEPGGCARTGSSFNFPC
1111	N20G	FKSSWSSLCEPGGCARTGSSFNGGC
1112	N20R	FKSSWSSLCEPGGCARTGSSFNRRC
1113	N20H	FKSSWSSLCEPGGCARTGSSFNHRC
1114	N20S	FKSSWSSLCEPGGCARTGSSFNSSC
1115	N20T	FKSSWSSLCEPGGCARTGSSFNTEC
1116	N20I	FKSSWSSLCEPGGCARTGSSFNIFC
1117	C21M	FKSSWSSLCEPGGCARTGSSFNMXC
1118	C21V	FKSSWSSLCEPGGCARTGSSFNVXC
1119	C21W	FKSSWSSLCEPGGCARTGSSFNWYC
1120	C21A	FKSSWSSLCEPGGCARTGSSFNAVC
1121	C21L	FKSSWSSLCEPGGCARTGSSFNLTC
1122	C21M	FKSSWSSLCEPGGCARTGSSFNMYC
1123	M22D	FKSSWSSLCEPGGCARTGSSFNDAC
1124	M22Y	FKSSWSSLCEPGGCARTGSSFNYXC
1125	M22D	FKSSWSSLCEPGGCARTGSSFNDYC
1126	M22E	FKSSWSSLCEPGGCARTGSSFNEYC

1127	C21E	FKSSWSSLCTTPGGCARTGSSFNSSYAC
1128	C21Q	FKSSWSSLCTTPGGCARTGSSFNSSYQC
1129	C21F	FKSSWSSLCTTPGGCARRFGGSSFNSSYAFC
1130	C21G	FKSSWSSLCTTPGGCARTGSSFNSSYGC
1131	C21R	FKSSWSSLCTTPGGCARTGSSFNSSYRRC
1132	C21W	FKSSWSSLCTTPGGCARRFGGSSFNSSYAWC
1133	C21S	FKSSWSSLCTTPGGCARRTGGSSFNSSYSSC
1134	C21T	FKSSWSSLCTTPGGCARTGGSSFNSSYTTC
1135	C21I	FKSSWSSLCTTPGGCARTGGSSFNSSYIIC
1136	C21K	FKSSWSSLCTTPGGCARRTGGSSFNSSYKKR
1137	C21V	FKSSWSSLCTRPGGCARTGSSFNSSYCVC
1138	C22W	FKSSWSSLCTTPGGCARTGSSFNSSYCWC
1139	C22A	FKSSWSSLCTTPGGCARRTGSSFNSSYCCA
1140	C22L	FKSSWSSLCTTPGGCARTGSSFNSSYCCL
1141	C22M	FKSSWSSLCTTPGGCARTGSSFNSSYCCM
1142	C22N	FKSSWSSLCTTPGGCARTGSSFNSSYCCN
1143	C22Y	FKSSWSSLCTTPGGCARTGSSFNSSYCCY
1144	C22D	FKSSWSSLCTTPGGCARTGGSSFNSSYCCD
1145	C22E	FKSSWSSLCTTPGGCARTGGSSFNSSYCCE
1146	C22P	FKSSWSSLCTTPGGCARRTGGSSFNSSYCCP
1147	C22Q	FKSSWSSLCTTPGGCARTGSSFNSSYCCQ
1148	C22F	FKSSWSSLCTTPGGCARTGSSFNSSYCCF
1149	C22G	FKSSWSSLCTTPGGCARRTGSSFNSSYCCG
1150	C22R	FKSSWSSLCTTPGGCARRTGGSSFNSSYCCR
1151	C22H	FKSSWSSLCTTPGGCARTGSSFNSSYCCH
1152	C22S	FKSSWSSLCTTPGGCARTGGSSFNSSYCCS
1153	C22T	FKSSWSSLCTTPGGCARRTGGSSFNSSYCCT
1154	C22I	FKSSWSSLCTTPGGCARTGSSFNSSYCCI

FIGURE 6

F1A
F1H
F1T
F1L
F1M
F1N
F1P
F1R
F1S
F1T
F1V
F1Y
K2A
K2E
K2F
K2I
K2M

K2N
K2Q
W4A
W4F
W4H
W4I
W4V
W4Y
S5A
S5M
L6A
L6H
L6T
L6V
P9H
C11A
C11E

C11G
R13A
R13D
R13L
R13N
R13Q
R13S
R13T
G15T
G15N
G15S
F17L
F17R
F17Y
N18A
Y20F

FIGURE 7

	Variant	Sequence
SEQ ID NO:432	MU1140	F K S W S L C T P G C A R T G S F N S Y C C
SEQ ID NO:703	R13N	F K S W S L C T P G C A N T G S F N S Y C C
SEQ ID NO:704	F17Y	F K S W S L C T P G C A R T G S Y N S Y C C
SEQ ID NO:705	F17L	F K S W S L C T P G C A R T G S L N S Y C C
SEQ ID NO:706	N18A	F K S W S L C T P G C A R T G S F A S Y C C
SEQ ID NO:707	Y20F	F K S W S L C T P G C A R T G S F N S F C C
SEQ ID NO:433	F1I R13N	I K S W S L C T P G C A N T G S F N S Y C C
SEQ ID NO:434	F1I R13D	I K S W S L C T P G C A D T G S F N S Y C C
SEQ ID NO:435	F1I R13A	I K S W S L C T P G C A A T G S F N S Y C C
SEQ ID NO:436	F1I R13T	I K S W S L C T P G C A T T G S F N S Y C C
SEQ ID NO:437	F1I R13K	I K S W S L C T P G C A K T G S F N S Y C C
SEQ ID NO:438	F1I R13S	I K S W S L C T P G C A S T G S F N S Y C C
SEQ ID NO:439	F1I R13G	I K S W S L C T P G C A G T G S F N S Y C C
SEQ ID NO:440	F1I R13V	I K S W S L C T P G C A V T G S F N S Y C C
SEQ ID NO:441	F1I R13I	I K S W S L C T P G C A I T G S F N S Y C C
SEQ ID NO:442	F1I R13P	I K S W S L C T P G C A P T G S F N S Y C C
SEQ ID NO:443	F1I R13Q	I K S W S L C T P G C A Q T G S F N S Y C C
SEQ ID NO:444	F1I R13E	I K S W S L C T P G C A E T G S F N S Y C C
SEQ ID NO:445	F1I R13H	I K S W S L C T P G C A H T G S F N S Y C C
SEQ ID NO:446	F1L R13N	L K S W S L C T P G C A N T G S F N S Y C C
SEQ ID NO:447	F1L R13D	L K S W S L C T P G C A D T G S F N S Y C C
SEQ ID NO:448	F1L R13A	L K S W S L C T P G C A A T G S F N S Y C C
SEQ ID NO:449	F1L R13T	L K S W S L C T P G C A T T G S F N S Y C C
SEQ ID NO:450	F1L R13K	L K S W S L C T P G C A K T G S F N S Y C C

SEQ ID NO:451	F1L R13S	LKSWSLCTPGCASTGSFNSYCC
SEQ ID NO:452	F1L R13G	LKSWSLCTPGCAGTGSFNSYCC
SEQ ID NO:453	F1L R13V	LKSWSLCTPGCAVTGSFNSYCC
SEQ ID NO:454	F1L R13I	LKSWSLCTPGCAITGSFNSYCC
SEQ ID NO:455	F1L R13P	LKSWSLCTPGCAPTGSFNSYCC
SEQ ID NO:456	F1L R13Q	LKSWSLCTPGCAQTGSFNSYCC
SEQ ID NO:457	F1L R13E	LKSWSLCTPGCAETGSFNSYCC
SEQ ID NO:458	F1L R13H	LKSWSLCTPGCAHTGSFNSYCC
SEQ ID NO:459	F1A R13N	AKSWSLCTPGCANTGSFNSYCC
SEQ ID NO:460	F1A R13D	AKSWSLCTPGCADTGSFNSYCC
SEQ ID NO:461	F1A R13A	AKSWSLCTPGCAATGSFNSYCC
SEQ ID NO:462	F1A R13T	AKSWSLCTPGCATTGSFNSYCC
SEQ ID NO:463	F1A R13K	AKSWSLCTPGCAKTGSFNSYCC
SEQ ID NO:464	F1A R13S	AKSWSLCTPGCASTGSFNSYCC
SEQ ID NO:465	F1A R13G	AKSWSLCTPGCAGTGSFNSYCC
SEQ ID NO:466	F1A R13V	AKSWSLCTPGCAVTGSFNSYCC
SEQ ID NO:467	F1A R13I	AKSWSLCTPGCAITGSFNSYCC
SEQ ID NO:468	F1A R13P	AKSWSLCTPGCAPTGSFNSYCC
SEQ ID NO:469	F1A R13Q	AKSWSLCTPGCAQTGSFNSYCC
SEQ ID NO:470	F1A R13E	AKSWSLCTPGCAETGSFNSYCC
SEQ ID NO:471	F1A R13H	AKSWSLCTPGCAHTGSFNSYCC
SEQ ID NO:472	F1N R13N	NKSWSLCTPGCANTGSFNSYCC
SEQ ID NO:473	F1N R13D	NKSWSLCTPGCADTGSFNSYCC
SEQ ID NO:474	F1N R13A	NKSWSLCTPGCAATGSFNSYCC
SEQ ID NO:475	F1N R13T	NKSWSLCTPGCATTGSFNSYCC
SEQ ID NO:476	F1N R13K	NKSWSLCTPGCAKTGSFNSYCC
SEQ ID NO:477	F1N R13S	NKSWSLCTPGCASTGSFNSYCC
SEQ ID NO:478	F1N R13G	NKSWSLCTPGCAGTGSFNSYCC

SEQ ID NO:479	F1N R13V	N K S S W S S L C T P G C A V T G S F N S Y C C
SEQ ID NO:480	F1N R13I	N K S S W S S L C T P G C A I T G S F N S Y C C
SEQ ID NO:481	F1N R13P	N K S S W S S L C T P G C A P T G S F N S Y C C
SEQ ID NO:482	F1N R13Q	N K S S W S S L C T P G C A Q T G S F N S Y C C
SEQ ID NO:483	F1N R13E	N K S S W S S L C T P G C A E T G S F N S Y C C
SEQ ID NO:484	F1N R13H	N K S S W S S L C T P G C A H T G S F N S Y C C
SEQ ID NO:485	F1S R13N	S K S S W S S L C T P G C A N T G S F N S Y C C
SEQ ID NO:486	F1S R13D	S K S S W S S L C T P G C A D T G S F N S Y C C
SEQ ID NO:487	F1S R13A	S K S S W S S L C T P G C A A T G S F N S Y C C
SEQ ID NO:488	F1S R13T	S K S S W S S L C T P G C A T T G S F N S Y C C
SEQ ID NO:489	F1S R13K	S K S S W S S L C T P G C A K T G S F N S Y C C
SEQ ID NO:490	F1S R13S	S K S S W S S L C T P G C A S T G S F N S Y C C
SEQ ID NO:491	F1S R13G	S K S S W S S L C T P G C A G T G S F N S Y C C
SEQ ID NO:492	F1S R13V	S K S S W S S L C T P G C A V T G S F N S Y C C
SEQ ID NO:493	F1S R13I	S K S S W S S L C T P G C A I T G S F N S Y C C
SEQ ID NO:494	F1S R13P	S K S S W S S L C T P G C A P T G S F N S Y C C
SEQ ID NO:495	F1S R13Q	S K S S W S S L C T P G C A Q T G S F N S Y C C
SEQ ID NO:496	F1S R13E	S K S S W S S L C T P G C A E T G S F N S Y C C
SEQ ID NO:497	F1S R13H	S K S S W S S L C T P G C A H T G S F N S Y C C
SEQ ID NO:498	F1T R13N	T K S S W S S L C T P G C A N T G S F N S Y C C
SEQ ID NO:499	F1T R13D	T K S S W S S L C T P G C A D T G S F N S Y C C
SEQ ID NO:500	F1T R13A	T K S S W S S L C T P G C A A T G S F N S Y C C
SEQ ID NO:501	F1T R13T	T K S S W S S L C T P G C A T T G S F N S Y C C
SEQ ID NO:502	F1T R13K	T K S S W S S L C T P G C A K T G S F N S Y C C
SEQ ID NO:503	F1T R13S	T K S S W S S L C T P G C A S T G S F N S Y C C
SEQ ID NO:504	F1T R13G	T K S S W S S L C T P G C A G T G S F N S Y C C
SEQ ID NO:505	F1T R13V	T K S S W S S L C T P G C A V T G S F N S Y C C
SEQ ID NO:506	F1T R13I	T K S S W S S L C T P G C A I T G S F N S Y C C

SEQ ID NO:507	F1T R13P	TKSWSLCTPGCAPTGSFNSYCC
SEQ ID NO:508	F1T R13Q	TKSWSLCTPGCAQTGSFNSYCC
SEQ ID NO:509	F1T R13E	TKSWSLCTPGCAETGSFNSYCC
SEQ ID NO:510	F1T R13H	TKSWSLCTPGCAHTGSFNSYCC
SEQ ID NO:511	F1Y R13N	YKSWSLCTPGCANTGSFNSYCC
SEQ ID NO:512	F1Y R13D	YKSWSLCTPGCADTGSFNSYCC
SEQ ID NO:513	F1Y R13A	YKSWSLCTPGCAATGSFNSYCC
SEQ ID NO:514	F1Y R13T	YKSWSLCTPGCATTGSFNSYCC
SEQ ID NO:515	F1Y R13K	YKSWSLCTPGCAKTGSFNSYCC
SEQ ID NO:516	F1Y R13S	YKSWSLCTPGCASTGSFNSYCC
SEQ ID NO:517	F1Y R13G	YKSWSLCTPGCAGTGSFNSYCC
SEQ ID NO:518	F1Y R13V	YKSWSLCTPGCAVTGSFNSYCC
SEQ ID NO:519	F1Y R13I	YKSWSLCTPGCAITGSFNSYCC
SEQ ID NO:520	F1Y R13P	YKSWSLCTPGCAPTGSFNSYCC
SEQ ID NO:521	F1Y R13Q	YKSWSLCTPGCAQTGSFNSYCC
SEQ ID NO:522	F1Y R13E	YKSWSLCTPGCAETGSFNSYCC
SEQ ID NO:523	F1Y R13H	YKSWSLCTPGCAHTGSFNSYCC
SEQ ID NO:524	F1H R13N	HKSWSLCTPGCANTGSFNSYCC
SEQ ID NO:525	F1H R13D	HKSWSLCTPGCADTGSFNSYCC
SEQ ID NO:526	F1H R13A	HKSWSLCTPGCAATGSFNSYCC
SEQ ID NO:527	F1H R13T	HKSWSLCTPGCATTGSFNSYCC
SEQ ID NO:528	F1H R13K	HKSWSLCTPGCAKTGSFNSYCC
SEQ ID NO:529	F1H R13S	HKSWSLCTPGCASTGSFNSYCC
SEQ ID NO:530	F1H R13G	HKSWSLCTPGCAGTGSFNSYCC
SEQ ID NO:531	F1H R13V	HKSWSLCTPGCAVTGSFNSYCC
SEQ ID NO:532	F1H R13I	HKSWSLCTPGCAITGSFNSYCC
SEQ ID NO:533	F1H R13P	HKSWSLCTPGCAPTGSFNSYCC
SEQ ID NO:534	F1H R13Q	HKSWSLCTPGCAQTGSFNSYCC

SEQ ID NO:535	F1H R13E	H K S W S L C T P G C A E T G S F N S Y C C
SEQ ID NO:536	F1H R13H	H K S W S L C T P G C A H T G S F N S Y C C
SEQ ID NO:537	F1P R13N	P K S W S L C T P G C A N T G S F N S Y C C
SEQ ID NO:538	F1P R13D	P K S W S L C T P G C A D T G S F N S Y C C
SEQ ID NO:539	F1P R13A	P K S W S L C T P G C A A T G S F N S Y C C
SEQ ID NO:540	F1P R13T	P K S W S L C T P G C A T T G S F N S Y C C
SEQ ID NO:541	F1P R13K	P K S W S L C T P G C A K T G S F N S Y C C
SEQ ID NO:542	F1P R13S	P K S W S L C T P G C A S T G S F N S Y C C
SEQ ID NO:543	F1P R13G	P K S W S L C T P G C A G T G S F N S Y C C
SEQ ID NO:544	F1P R13V	P K S W S L C T P G C A V T G S F N S Y C C
SEQ ID NO:545	F1P R13I	P K S W S L C T P G C A I T G S F N S Y C C
SEQ ID NO:546	F1P R13P	P K S W S L C T P G C A P T G S F N S Y C C
SEQ ID NO:547	F1P R13Q	P K S W S L C T P G C A Q T G S F N S Y C C
SEQ ID NO:548	F1P R13E	P K S W S L C T P G C A E T G S F N S Y C C
SEQ ID NO:549	F1P R13H	P K S W S L C T P G C A H T G S F N S Y C C
SEQ ID NO:550	F1V R13N	V K S W S L C T P G C A N T G S F N S Y C C
SEQ ID NO:551	F1V R13D	V K S W S L C T P G C A D T G S F N S Y C C
SEQ ID NO:552	F1V R13A	V K S W S L C T P G C A A T G S F N S Y C C
SEQ ID NO:553	F1V R13T	V K S W S L C T P G C A T T G S F N S Y C C
SEQ ID NO:554	F1V R13K	V K S W S L C T P G C A K T G S F N S Y C C
SEQ ID NO:555	F1V R13S	V K S W S L C T P G C A S T G S F N S Y C C
SEQ ID NO:556	F1V R13G	V K S W S L C T P G C A G T G S F N S Y C C
SEQ ID NO:557	F1V R13V	V K S W S L C T P G C A V T G S F N S Y C C
SEQ ID NO:558	F1V R13I	V K S W S L C T P G C A I T G S F N S Y C C
SEQ ID NO:559	F1V R13P	V K S W S L C T P G C A P T G S F N S Y C C
SEQ ID NO:560	F1V R13Q	V K S W S L C T P G C A Q T G S F N S Y C C
SEQ ID NO:561	F1V R13E	V K S W S L C T P G C A E T G S F N S Y C C
SEQ ID NO:562	F1V R13H	V K S W S L C T P G C A H T G S F N S Y C C

SEQ ID NO:563	F1G R13N	GKSWSLCTPGCANTGSFNSYCC
SEQ ID NO:564	F1G R13D	GKSWSLCTPGCADTGSFNSYCC
SEQ ID NO:565	F1G R13A	GKSWSLCTPGCAATGSFNSYCC
SEQ ID NO:566	F1G R13T	GKSWSLCTPGCATTGSFNSYCC
SEQ ID NO:567	F1G R13K	GKSWSLCTPGCAKTGSFNSYCC
SEQ ID NO:568	F1G R13S	GKSWSLCTPGCASTGSFNSYCC
SEQ ID NO:569	F1G R13G	GKSWSLCTPGCAGTGSFNSYCC
SEQ ID NO:570	F1G R13V	GKSWSLCTPGCAVTGSFNSYCC
SEQ ID NO:571	F1G R13I	GKSWSLCTPGCAITGSFNSYCC
SEQ ID NO:572	F1G R13P	GKSWSLCTPGCAPTGSFNSYCC
SEQ ID NO:573	F1G R13Q	GKSWSLCTPGCAQTGSFNSYCC
SEQ ID NO:574	F1G R13E	GKSWSLCTPGCAETGSFNSYCC
SEQ ID NO:575	F1G R13H	GKSWSLCTPGCAHTGSFNSYCC
SEQ ID NO:576	F1E R13N	EKSWSLCTPGCANTGSFNSYCC
SEQ ID NO:577	F1E R13D	EKSWSLCTPGCADTGSFNSYCC
SEQ ID NO:578	F1E R13A	EKSWSLCTPGCAATGSFNSYCC
SEQ ID NO:579	F1E R13T	EKSWSLCTPGCATTGSFNSYCC
SEQ ID NO:580	F1E R13K	EKSWSLCTPGCAKTGSFNSYCC
SEQ ID NO:581	F1E R13S	EKSWSLCTPGCASTGSFNSYCC
SEQ ID NO:582	F1E R13G	EKSWSLCTPGCAGTGSFNSYCC
SEQ ID NO:583	F1E R13V	EKSWSLCTPGCAVTGSFNSYCC
SEQ ID NO:584	F1E R13I	EKSWSLCTPGCAITGSFNSYCC
SEQ ID NO:585	F1E R13P	EKSWSLCTPGCAPTGSFNSYCC
SEQ ID NO:586	F1E R13Q	EKSWSLCTPGCAQTGSFNSYCC
SEQ ID NO:587	F1E R13E	EKSWSLCTPGCAETGSFNSYCC
SEQ ID NO:588	F1E R13H	EKSWSLCTPGCAHTGSFNSYCC
SEQ ID NO:589	F1L R13N Y20F	LKSWSLCTPGCANTGSFNSFCC

SEQ ID NO:590	F1L R13D Y20F	L K S W S L C T P G C A D T G S F N S F C C
SEQ ID NO:591	F1L R13A Y20F	L K S W S L C T P G C A A T G S F N S F C C
SEQ ID NO:592	F1I R13N Y20F	I K S W S L C T P G C A N T G S F N S F C C
SEQ ID NO:593	F1I R13D Y20F	I K S W S L C T P G C A D T G S F N S F C C
SEQ ID NO:594	F1I R13A Y20F	I K S W S L C T P G C A A T G S F N S F C C
SEQ ID NO:595	F1I K2A R13N	I A S W S L C T P G C A N T G S F N S Y C C
SEQ ID NO:596	F1I K2A R13D	I A S W S L C T P G C A D T G S F N S Y C C
SEQ ID NO:597	F1I K2A R13A	I A S W S L C T P G C A A T G S F N S Y C C
SEQ ID NO:598	F1I K2A R13T	I A S W S L C T P G C A T T G S F N S Y C C
SEQ ID NO:599	F1I K2A R13K	I A S W S L C T P G C A K T G S F N S Y C C
SEQ ID NO:600	F1I K2A R13S	I A S W S L C T P G C A S T G S F N S Y C C
SEQ ID NO:601	F1I K2A R13G	I A S W S L C T P G C A G T G S F N S Y C C
SEQ ID NO:602	F1I K2A R13V	I A S W S L C T P G C A V T G S F N S Y C C
SEQ ID NO:603	F1I K2A R13I	I A S W S L C T P G C A I T G S F N S Y C C
SEQ ID NO:604	F1I K2A R13P	I A S W S L C T P G C A P T G S F N S Y C C
SEQ ID NO:605	F1I K2A R13Q	I A S W S L C T P G C A Q T G S F N S Y C C
SEQ ID NO:606	F1I K2A R13E	I A S W S L C T P G C A E T G S F N S Y C C
SEQ ID NO:607	F1I K2A R13H	I A S W S L C T P G C A H T G S F N S Y C C
SEQ ID NO:608	F1I K2A R13N Y20F	I A S W S L C T P G C A N T G S F N S F C C
SEQ ID NO:609	F1I K2A R13D Y20F	I A S W S L C T P G C A D T G S F N S F C C
SEQ ID NO:610	F1I K2A R13A Y20F	I A S W S L C T P G C A A T G S F N S F C C
SEQ ID NO:611	F1I K2A R13T Y20F	I A S W S L C T P G C A T T G S F N S F C C
SEQ ID NO:612	F1I K2A R13K Y20F	I A S W S L C T P G C A K T G S F N S F C C
SEQ ID NO:613	F1I K2A R13S Y20F	I A S W S L C T P G C A S T G S F N S F C C
SEQ ID NO:614	F1I K2A R13G Y20F	I A S W S L C T P G C A G T G S F N S F C C
SEQ ID NO:615	F1I K2A R13V Y20F	I A S W S L C T P G C A V T G S F N S F C C
SEQ ID NO:616	F1I K2A R13I Y20F	I A S W S L C T P G C A I T G S F N S F C C

SEQ ID NO:617	F1I K2A R13P Y20F	I A S W S L C T P G C A P T G S F N S F C C
SEQ ID NO:618	F1I K2A R13Q Y20F	I A S W S L C T P G C A Q T G S F N S F C C
SEQ ID NO:619	F1I K2A R13E Y20F	I A S W S L C T P G C A E T G S F N S F C C
SEQ ID NO:620	F1I K2A R13H Y20F	I A S W S L C T P G C A H T G S F N S F C C
SEQ ID NO:621	F1I K2A W4K R13N	I A S K S L C T P G C A N T G S F N S Y C C
SEQ ID NO:622	F1I K2A W4K R13D	I A S K S L C T P G C A D T G S F N S Y C C
SEQ ID NO:623	F1I K2A W4K R13A	I A S K S L C T P G C A A T G S F N S Y C C
SEQ ID NO:624	F1I K2A W4K R13N Y20F	I A S K S L C T P G C A N T G S F N S F C C
SEQ ID NO:625	F1I K2A W4K R13D Y20F	I A S K S L C T P G C A D T G S F N S F C C
SEQ ID NO:626	F1I K2A W4K R13A Y20F	I A S K S L C T P G C A A T G S F N S F C C
SEQ ID NO:627	F1I K2A W4K S5F R13N	I A S K F L C T P G C A N T G S F N S Y C C
SEQ ID NO:628	F1I K2A W4K S5F R13D	I A S K F L C T P G C A D T G S F N S Y C C
SEQ ID NO:629	F1I K2A W4K S5F R13A	I A S K F L C T P G C A A T G S F N S Y C C
SEQ ID NO:630	F1I K2A W4K S5F R13N Y20F	I A S K F L C T P G C A N T G S F N S F C C
SEQ ID NO:631	F1I K2A W4K S5F R13D Y20F	I A S K F L C T P G C A D T G S F N S F C C
SEQ ID NO:632	F1I K2A W4K S5F R13A Y20F	I A S K F L C T P G C A A T G S F N S F C C
SEQ ID NO:633	F1I K2T R13N	I T S W S L C T P G C A N T G S F N S Y C C
SEQ ID NO:634	F1I K2T R13D	I T S W S L C T P G C A D T G S F N S Y C C
SEQ ID NO:635	F1I K2T R13A	I T S W S L C T P G C A A T G S F N S Y C C
SEQ ID NO:636	F1I K2T R13T	I T S W S L C T P G C A T T G S F N S Y C C
SEQ ID NO:637	F1I K2T R13K	I T S W S L C T P G C A K T G S F N S Y C C
SEQ ID NO:638	F1I K2T R13S	I T S W S L C T P G C A S T G S F N S Y C C
SEQ ID NO:639	F1I K2T R13G	I T S W S L C T P G C A G T G S F N S Y C C
SEQ ID NO:640	F1I K2T R13V	I T S W S L C T P G C A V T G S F N S Y C C
SEQ ID NO:641	F1I K2T R13I	I T S W S L C T P G C A I T G S F N S Y C C

SEQ ID NO:642	F1I K2T R13P	ITSWSLCTPGCAPTGSFNSYCC
SEQ ID NO:643	F1I K2T R13Q	ITSWSLCTPGCAQTGSFNSYCC
SEQ ID NO:644	F1I K2T R13E	ITSWSLCTPGCAETGSFNSYCC
SEQ ID NO:645	F1I K2T R13H	ITSWSLCTPGCAHTGSFNSYCC
SEQ ID NO:646	F1I K2T R13N Y20F	ITSWSLCTPGCANTGSFNSFCC
SEQ ID NO:647	F1I K2T R13D Y20F	ITSWSLCTPGCADTGSFNSFCC
SEQ ID NO:648	F1I K2T R13A Y20F	ITSWSLCTPGCAATGSFNSFCC
SEQ ID NO:649	F1I K2T R13T Y20F	ITSWSLCTPGCATTGSFNSFCC
SEQ ID NO:650	F1I K2T R13K Y20F	ITSWSLCTPGCAKTGSFNSFCC
SEQ ID NO:651	F1I K2T R13S Y20F	ITSWSLCTPGCASTGSFNSFCC
SEQ ID NO:652	F1I K2T R13G Y20F	ITSWSLCTPGCAGTGSFNSFCC
SEQ ID NO:653	F1I K2T R13V Y20F	ITSWSLCTPGCAVTGSFNSFCC
SEQ ID NO:654	F1I K2T R13I Y20F	ITSWSLCTPGCAITGSFNSFCC
SEQ ID NO:655	F1I K2T R13P Y20F	ITSWSLCTPGCAPTGSFNSFCC
SEQ ID NO:656	F1I K2T R13Q Y20F	ITSWSLCTPGCAQTGSFNSFCC
SEQ ID NO:657	F1I K2T R13E Y20F	ITSWSLCTPGCAETGSFNSFCC
SEQ ID NO:658	F1I K2T R13H Y20F	ITSWSLCTPGCAHTGSFNSFCC
SEQ ID NO:659	F1I K2T S5F L6I C7G R13N Y20F	ITSWFIGTPGCANTGSFNSFCC
SEQ ID NO:660	F1I K2T S5F L6I C7G R13D Y20F	ITSWFIGTPGCADTGSFNSFCC
SEQ ID NO:661	F1I K2T S5F L6I C7G R13A Y20F	ITSWFIGTPGCAATGSFNSFCC
SEQ ID NO:662	F1I K2T S5F L6I C7G A12G R13N Y20F	ITSWFIGTPGCGNTGSFNSFCC
SEQ ID NO:663	F1I K2T S5F L6I C7G A12G R13D Y20F	ITSWFIGTPGCGDTGSFNSFCC
SEQ ID NO:664	F1I K2T S5F L6I C7G A12G R13A Y20F	ITSWFIGTPGCGATGSFNSFCC
SEQ ID NO:665	F1I K2T W4K R13N	ITSKSLCTPGCANTGSFNSYCC
SEQ ID NO:666	F1I K2T W4K R13D	ITSKSLCTPGCADTGSFNSYCC
SEQ ID NO:667	F1I K2T W4K R13A	ITSKSLCTPGCAATGSFNSYCC

SEQ ID NO:668	F1I K2T W4K R13N Y20F	I T S K S L C T P G C A N T G S F N S F C C
SEQ ID NO:669	F1I K2T W4K R13D Y20F	I T S K S L C T P G C A D T G S F N S F C C
SEQ ID NO:670	F1I K2T W4K R13A Y20F	I T S K S L C T P G C A A T G S F N S F C C
SEQ ID NO:671	F1V K2T R13N	V T S W S L C T P G C A N T G S F N S Y C C
SEQ ID NO:672	F1V K2T R13D	V T S W S L C T P G C A D T G S F N S Y C C
SEQ ID NO:673	F1V K2T R13A	V T S W S L C T P G C A A T G S F N S Y C C
SEQ ID NO:674	F1V K2T R13N Y20F	V T S W S L C T P G C A N T G S F N S F C C
SEQ ID NO:675	F1V K2T R13D Y20F	V T S W S L C T P G C A D T G S F N S F C C
SEQ ID NO:676	F1V K2T R13A Y20F	V T S W S L C T P G C A A T G S F N S F C C
SEQ ID NO:677	F1V K2T W4K R13N	V T S K S L C T P G C A N T G S F N S Y C C
SEQ ID NO:678	F1V K2T W4K R13D	V T S K S L C T P G C A D T G S F N S Y C C
SEQ ID NO:679	F1V K2T W4K R13A	V T S K S L C T P G C A A T G S F N S Y C C
SEQ ID NO:680	F1V K2T W4K R13N Y20F	V T S K S L C T P G C A N T G S F N S F C C
SEQ ID NO:681	F1V K2T W4K R13D Y20F	V T S K S L C T P G C A D T G S F N S F C C
SEQ ID NO:682	F1V K2T W4K R13A Y20F	V T S K S L C T P G C A A T G S F N S F C C
SEQ ID NO:683	F1I W4M R13N	I K S M S L C T P G C A N T G S F N S Y C C
SEQ ID NO:684	F1I W4M R13D	I K S M S L C T P G C A D T G S F N S Y C C
SEQ ID NO:685	F1I W4M R13A	I K S M S L C T P G C A A T G S F N S Y C C
SEQ ID NO:686	F1I W4I R13N	I K S I S L C T P G C A N T G S F N S Y C C
SEQ ID NO:687	F1I W4I R13D	I K S I S L C T P G C A D T G S F N S Y C C
SEQ ID NO:688	F1I W4I R13A	I K S I S L C T P G C A A T G S F N S Y C C
SEQ ID NO:689	F1I L6V R13N	I K S W S V C T P G C A N T G S F N S Y C C

SEQ ID NO:690	F1I L6V R13D	I K S W S V C T P G C A D T G S F N S Y C C
SEQ ID NO:691	F1I L6V R13A	I K S W S V C T P G C A A T G S F N S Y C C
SEQ ID NO:692	F1I A12T R13N	I K S W S L C T P G C T N T G S F N S Y C C
SEQ ID NO:693	F1I A12T R13D	I K S W S L C T P G C T D T G S F N S Y C C
SEQ ID NO:694	F1I A12T R13A	I K S W S L C T P G C T A T G S F N S Y C C
SEQ ID NO:695	F1I R13N G15A	I K S W S L C T P G C A N T A S F N S Y C C
SEQ ID NO:696	F1I R13D G15A	I K S W S L C T P G C A D T A S F N S Y C C
SEQ ID NO:697	F1I R13A G15A	I K S W S L C T P G C A A T A S F N S Y C C
SEQ ID NO:698	F1I K2T W4V R13N	I T S V S L C T P G C A N T G S F N S Y C C
SEQ ID NO:699	F1I K2T W4V R13D	I T S V S L C T P G C A D T G S F N S Y C C
SEQ ID NO:700	F1I K2T W4V R13A	I T S V S L C T P G C A A T G S F N S Y C C
SEQ ID NO:701	F1I K2T W4M R13N	I T S M S L C T P G C A N T G S F N S Y C C
SEQ ID NO:702	F1I K2T W4M R13D	I T S M S L C T P G C A D T G S F N S Y C C
SEQ ID NO:1155	F1I K2T W4M R13A	I T S M S L C T P G C A A T G S F N S Y C C

FIGURE 8

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
F1L R13G	F1I K2A R13P Y20F	F1I R13H	F1I R13N	MU1140	F1L R13I
F1A R13N	F1I K2A W4M R13N	F1N R13I	F1I R13D	F1I R13K	F1A R13I
F1A R13A	F1I K2A W4M R13A	F1S R13G	F1I R13A	F1I R13E	F1N R13N
F1A R13S	F1I K2A L6V R13A	F1I K2T R13N	F1I R13T	F1L R13K	F1N R13D
F1A R13G		F1I K2T R13N G15A	F1I R13S	F1L R13V	F1N R13V
F1A R13V			F1I R13G	F1L R13H	F1N R13H
F1A R13P			F1I R13V	F1N R13A	F1S R13S
F1T R13A			F1I R13I	F1N R13T	F1S R13H
F1T R13G			F1I R13P	F1N R13S	F1T R13K
F1T R13V			F1I R13Q	F1N R13G	F1T R13P
F1V R13N			F1L R13N	F1N R13P	F1T R13E
F1V R13A			F1L R13D	F1N R13Q	F1Y R13N
F1V R13T			F1L R13A	F1N R13E	F1P R13N
F1V R13P			F1L R13T	F1S R13D	F1P R13D
F1V R13Q			F1L R13S	F1S R13P	F1P R13G
F1G R13N			F1L R13P	F1T R13D	F1P R13Q
F1G R13V			F1L R13Q	F1T R13T	F1V R13S
F1I K2A R13A			F1L R13E	F1Y R13A	F1V R13G
F1I K2A R13T			F1A R13D	F1Y R13K	F1G R13K
F1I K2A R13V			F1A R13T	F1H R13D	F1G R13I
F1I K2A R13A Y20F			F1A R13K	F1H R13G	F1G R13P
F1I K2A R13G Y20F			F1A R13Q	F1H R13P	F1E R13Q
F1I K2A R13Q Y20F			F1A R13E	F1H R13E	F1I K2A R13N

F1I K2A W4K R13A		F1A R13H	F1P R13A	F1I K2A R13D
F1I K2A W4K R13N Y20F		F1N R13K	F1P R13T	F1I K2A R13G
F1I K2A W4K R13A Y20F		F1S R13N	F1P R13K	F1I K2A R13D Y20F
F1I K2T W4K R13A Y20F		F1S R13A	F1P R13S	F1I K2A R13S Y20F
F1I W4M R13N		F1S R13T	F1P R13V	F1I K2A W4K S5F R13A Y20F
F1I W4M R13A		F1S R13K	F1P R13I	F1I K2T R13H Y20F
F1I W4I R13A		F1S R13V	F1P R13P	F1I L6V R13N
F1I R13N G15A		F1S R13I	F1P R13E	F1I A12T R13A
F1I R13A G15A		F1S R13Q	F1P R13H	F1I K2T R13A G15A
		F1S R13E	F1G R13D	F1I K2A W4I R13N
		F1T R13N	F1G R13E	F1I K2A W4I R13D
		F1T R13S	F1E R13N	Y19F
		F1T R13I	F1E R13D	
		F1T R13Q	F1E R13A	
		F1T R13H	F1E R13T	
		F1Y R13D	F1E R13K	
		F1Y R13T	F1E R13S	
		F1Y R13S	F1E R13G	
		F1Y R13G	F1E R13V	
		F1Y R13V	F1E R13I	
		F1Y R13I	F1E R13P	
		F1Y R13P	F1E R13E	
		F1Y R13Q	F1E R13H	
		F1Y R13E	F1I K2A R13E	
		F1Y R13H	F1I K2A W4K S5F R13N	
		F1H R13N	F1I K2A W4K S5F R13D	
		F1H R13A	F1I K2A W4K S5F R13A	
		F1H R13T	F1I K2A W4K S5F R13N Y20F	
		F1H R13K	F1I K2A W4K S5F R13D Y20F	
		F1H R13S	F1I K2T R13D	
		F1H R13V	F1I K2T R13A	
		F1H R13I	F1I K2T R13T	
		F1H R13Q	F1I K2T R13K	
		F1H R13H	F1I K2T R13S	

			F1V R13D	F1I K2T R13G	
			F1V R13K	F1I K2T R13V	
			F1V R13V	F1I K2T R13I	
			F1V R13I	F1I K2T R13P	
			F1V R13E	F1I K2T R13Q	
			F1V R13H	F1I K2T R13E	
			F1G R13A	F1I K2T R13H	
			F1G R13T	F1I K2T R13N Y20F	
			F1G R13S	F1I K2T R13D Y20F	
			F1G R13G	F1I K2T R13A Y20F	
			F1G R13Q	F1I K2T R13S Y20F	
			F1G R13H	F1I K2T R13G Y20F	
			F1L R13N Y20F	F1I K2T R13V Y20F	
			F1L R13D Y20F	F1I K2T R13I Y20F	
			F1L R13A Y20F	F1I K2T R13P Y20F	
			F1I R13N Y20F	F1I K2T R13Q Y20F	
			F1I R13D Y20F	F1I K2T R13E Y20F	
			F1I R13A Y20F	F1I K2T S5F L6I C7G R13N Y20F	
			F1I K2A R13K	F1I K2T S5F L6I C7G R13D Y20F	
			F1I K2A R13S	F1I K2T S5F L6I C7G R13A Y20F	
			F1I K2A R13I	F1I K2T S5F L6I C7G A12G R13N Y20F	
			F1I K2A R13P	F1I K2T S5F L6I C7G A12G R13D Y20F	
			F1I K2A R13Q	F1I K2T S5F L6I C7G A12G R13A Y20F	
			F1I K2A R13H	F1I K2T W4K R13D	
			F1I K2A R13N Y20F	F1V K2T R13D	
			F1I K2A R13T Y20F	F1V K2T R13A	
			F1I K2A R13K Y20F	F1V K2T R13N Y20F	
			F1I K2A R13I Y20F	F1V K2T R13D Y20F	
			F1I K2A R13E Y20F	F1V K2T R13A Y20F	
			F1I K2A R13H Y20F	F1V K2T W4K R13N	
			F1I K2A W4K R13N	F1V K2T W4K R13D	
			F1I K2A W4K R13D	F1V K2T W4K R13D Y20F	
			F1I K2A W4K R13D Y20F	F1V K2T W4K R13A Y20F	
			F1I K2T R13T Y20F	F1I W4I R13N	
			F1I K2T R13K Y20F	F1I A12T R13D	
			F1I K2T W4K R13N	F1I K2T W4V R13D	
			F1I K2T W4K R13A	F1I K2T W4M R13D	
			F1I K2T W4K R13N Y20F	F1I K2T W4I R13N	

			F1I K2T W4K R13D Y20F	**F1I K2T W4I R13D**	
			F1V K2T R13N	**F1I K2T W4I R13A**	
			F1V K2T W4K R13A	**F1I K2T L6V R13N**	
			F1V K2T W4K R13N Y20F	**F1I K2T L6V R13D**	
			F1I W4M R13D	**F1I K2T A12T R13N**	
			F1I W4I R13D	**F1I K2T A12T R13D**	
			F1I L6V R13D	**F1I K2T A12T R13A**	
			F1I L6V R13A	**F1I K2T R13D G15A**	
			F1I A12T R13N	**F1I K2A W4V R13N**	
			F1I R13D G15A	**F1I K2A W4V R13D**	
			F1I K2T W4V R13N	**F1I K2A W4M R13D**	
			F1I K2T W4M R13N	**F1I K2A L6V R13D**	
			F1I K2T W4M R13A	**F1I K2A A12T R13N**	
			F1I K2T L6V R13A	**F1I K2A A12T R13D**	
			F1I K2A W4V R13A	**F1I K2A A12T R13A**	
			F1I K2A W4I R13A	**F1I K2A R13N G15A**	
			F1I K2A L6V R13N	**F1I K2A R13D G15A**	
			R13N	**F1I K2A R13A G15A**	
			F1I K2A R13V Y20F	**F1I**	
			F1I K2T W4V R13A	**F1L**	

FIGURE 9

	Variant	**Sequence**
SEQ ID NO:432	MU1140	Phe Lys Ser Trp Ser Leu Cys Thr Pro Gly Cys Ala Arg Thr Gly Ser Phe Asn Ser Tyr Cys Cys
SEQ ID NO:1159	MU1140 (with post translational modifications but no secondary structures)	Phe Lys Ala Trp Dha Leu Ala Abu Pro Gly Ala Ala Arg Dhb Gly Ala Phe Asn Ala Tyr Ala Cys
SEQ ID NO:1158	F1V R13N (with post translational modifications but no secondary structures)	Val Lys Ala Trp Dha Leu Ala Abu Pro Gly Ala Ala Asn Dhb Gly Ala Phe Asn Ala Tyr Ala Cys

FIGURE 10

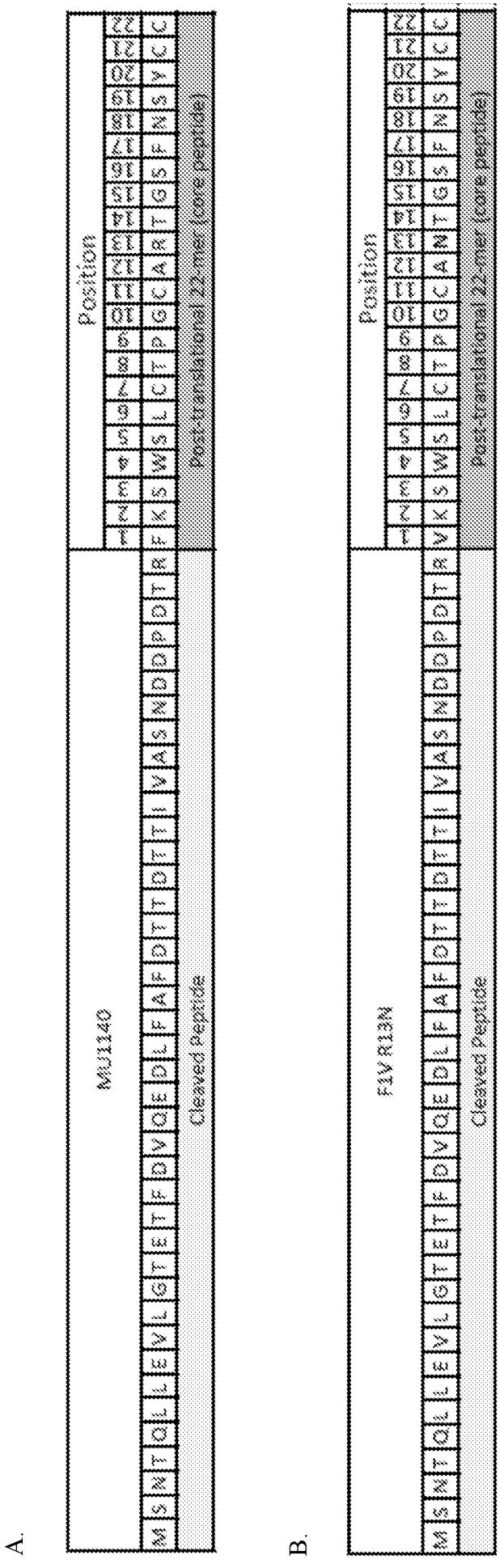

A.
MU1140
Position
Cleaved Peptide
Post-translational 22-mer (core peptide)
B.
F1V R13N
Position
Cleaved Peptide
Post-translational 22-mer (core peptide)

FIGURE 11

A. SEQ ID NO: 1162

atg tca aac aca caa tta tta gaa gtc ctt ggt act gaa act ttt gat gtt caa gaa gat ctc ttt gct ttt gat aca aca gat act act att gtg gca agc aac gac gat cca gat act cgt ttc aaa agt tgg agc ctt tgt acg cct ggt tgt gca agg aca ggt agt ttc aat agt tac tgt tgc

B. SEQ ID NO: 1163

atg tca aac aca caa tta tta gaa gtc ctt ggt act gaa act ttt gat gtt caa gaa gat ctc ttt gct ttt gat aca aca gat act act att gtg gca agc aac gac gat cca gat act cgt **gtt** aaa agt tgg agc ctt tgt acg cct ggt tgt gca **aat** aca ggt agt ttc aat agt tac tgt tgc

C. SEQ ID NO: 1164

MSNTQ LLEVL GTETF DVQED LFAFD TTDTT IVASN DDPDT R

D. SEQ ID NO: 1165

atg tca aac aca caa tta tta gaa gtc ctt ggt act gaa act ttt gat gtt caa gaa gat ctc ttt gct ttt gat aca aca gat act act att gtg gca agc aac gac gat cca gat act cgt

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/042206

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K8/64 A61Q11/00 C07K14/315 C12N9/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61Q C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/130349 A1 (ORAGENICS INC [US]; TEXAS A & M UNIVESITY SYSTEM [US]) 6 September 2013 (2013-09-06)	5
Y	example 3 claims 1-23	1-103

X	SHAORONG CHEN ET AL: "Site-Directed Mutations in the Lanthipeptide Mutacin 1140", APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 79, no. 13, 19 April 2013 (2013-04-19), pages 4015-4023, XP055405976, ISSN: 0099-2240, DOI: 10.1128/AEM.00704-13	5
Y	the whole document	1-103

	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 September 2017	27/09/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bayer, Martin

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/042206

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ELVIS LEGALA ONGEY ET AL: "Lanthipeptides: chemical synthesis versus in vivo biosynthesis as tools for pharmaceutical production", MICROBIAL CELL FACTORIES, vol. 15, no. 1, 7 June 2016 (2016-06-07), XP055406099, DOI: 10.1186/s12934-016-0502-y the whole document -----	1-103

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2017/042206

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013130349 A1	06-09-2013	AU 2013226402 A1	21-08-2014
		AU 2013226404 A1	21-08-2014
		CA 2865395 A1	06-09-2013
		CA 2865405 A1	06-09-2013
		CN 104302307 A	21-01-2015
		CN 104321338 A	28-01-2015
		EP 2820036 A1	07-01-2015
		EP 2833902 A1	11-02-2015
		EP 3219325 A1	20-09-2017
		HK 1205458 A1	18-12-2015
		HK 1205519 A1	18-12-2015
		JP 2015509715 A	02-04-2015
		JP 2015509945 A	02-04-2015
		US 2015044146 A1	12-02-2015
		US 2015125503 A1	07-05-2015
		WO 2013130349 A1	06-09-2013
		WO 2013130351 A1	06-09-2013