Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 3152931 A1 2021/04/08

(21) 3 152 931

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2020/09/29
(87) Date publication PCT/PCT Publication Date: 2021/04/08
(85) Entrée phase nationale/National Entry: 2022/03/29
(86) N° demande PCT/PCT Application No.: US 2020/053304
(87) N° publication PCT/PCT Publication No.: 2021/067291
(30) Priorités/Priorities: 2019/09/30 (US62/908,130); 2019/10/01 (US62/909,078); 2020/07/16 (US63/052,825)

(51) Cl.Int./Int.Cl. *C12N 15/10* (2006.01), *C12N 15/113* (2010.01), *C12N 15/63* (2006.01)

(71) Demandeurs/Applicants:
SIGMA-ALDRICH CO. LLC, US;
WASHINGTON UNIVERSITY, US

(72) Inventeurs/Inventors:
EASTLUND, ERIK, US;
ZHANG, ZHIGANG, US;
DAVIS, GREGORY D., US;
BELLER, ZACHARY WALTER, US;
GORDON, JEFFREY IVAN, US

(74) Agent: BERESKIN & PARR LLP/S.E.N.C.R.L.,S.R.L.

(54) Titre : MODULATION DE COMPOSITIONS DE MICROBIOTE A L'AIDE DE NUCLEASES CIBLEES
(54) Title: MODULATION OF MICROBIOTA COMPOSITIONS USING TARGETED NUCLEASES

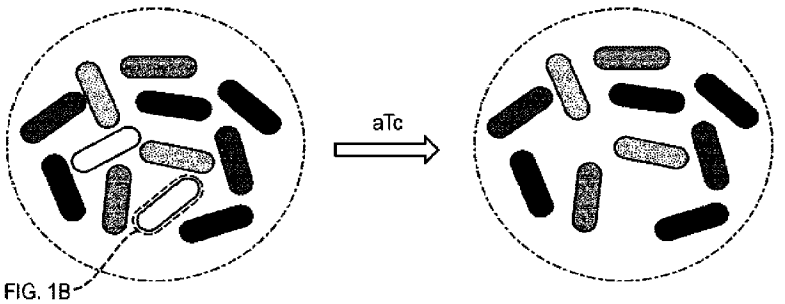


FIG. 1A

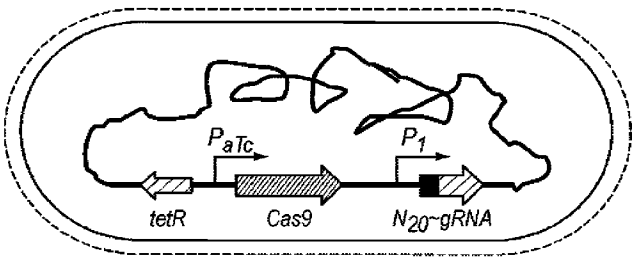


FIG. 1B

(57) Abrégé/Abstract:
Compositions and methods for remodeling complex populations of microbes are provided herein. RNA-guided nuclease systems are engineered to target sites in chromosomal DNA of a targeted prokaryotic, wherein the level of targeted prokaryote can be modulated in a mixed population of prokaryotes.

Date Submitted: 2022/03/29

CA App. No.: 3152931

Abstract:

Compositions and methods for remodeling complex populations of microbes are provided herein. RNA-guided nuclease systems are engineered to target sites in chromosomal DNA of a targeted prokaryotic, wherein the level of targeted prokaryote can be modulated in a mixed population of prokaryotes.

MODULATION OF MICROBIOTA COMPOSITIONS USING TARGETED NUCLEASES

RELATED APPLICATIONS

[0001] The present application claims the benefit of priority of US Provisional Application No. 62/908,130, filed September 30, 2019, and of US Provisional Application No. 62/909,078, filed October 1, 2019, and of US Provisional Application No. 63/052,825, filed July 16, 2020.

SEQUENCE LISTING

[0002] This application contains a Sequence Listing. The ASCII copy, created on September 29, 2020 is named P19-171-_WO-PCT_SL.txt, and is 51,634 bytes in size.

FIELD

[0003] The present disclosure relates to compositions and methods for remodeling the composition of microbiota.

BACKGROUND

[0004] Controlling the composition and expressed functions of microbial populations is a critical aspect of medicine, biotechnology, and environmental cycles. While classic antimicrobial strategies provide some control, what remains elusive is a generalized and programmable strategy that can distinguish between even closely related microorganisms and that allows for fine control over the composition of a microbial population. Recent advances indicate that RNA-guided nuclease systems can be designed to target specific DNA sequences in microbial populations. It would be beneficial to employ similar strategies to target and remove specific species from multi-species bacterial populations.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application

publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0006] **FIGS. 1A-1B** illustrate targeted microbiota modulation using an integrated, inducible CRISPR system. Expression of the CRISPR system (Cas9 endonuclease and guide RNA) leads to chromosomal breaks and ultimately cell death in bacteria. As such, specific *Bacteroides* strains harboring an integrated CRISPR cassette with a targeting guide RNA can be eliminated from a mixed population *in situ* upon anhydrotetracycline (aTc) induction.

[0007] **FIG. 2** presents a schematic of a CRISPR integration vector. The Cas9 protein is expressed from an anhydrotetracycline (aTc)-inducible promoter. The single guide RNA (N20-sgRNA scaffold) is constitutively expressed from P1 promoter, wherein a 20 nucleotide protospacer sequence (N20) specifies the targeted DNA cleavage in the genome when a PAM is present (NGG in the case of *Streptococcus pyogenes* CRISPR/Cas9).

[0008] **FIGS. 3A-3C** illustrate chromosomal integration of a CRISPR System in the human gut-derived bacterium, *Bacteroides thetaiotaomicron* (Bt). **FIG. 3A** diagrams NBU2 integration mechanism. **FIG. 3B** shows CRISPR integration to *Bt* via conjugation. **FIG. 3C** presents colony PCR screening of CRISPR integrants. PCR A: attBT2-1 locus, outside primers; PCR B: attBT2-2 locus, outside primers; PCR C: attBT2-1 locus, left junction; PCR D: attBT2-2 locus, left junction. M1–M4: four *Bt* colonies with non-targeting, control gRNA; T1–T4: four *Bt* colonies with tdk-targeting gRNA; S1–S4: four *Bt* colonies with susC-targeting gRNA.

[0009] **FIGS. 4A-4B** illustrates induced CRISPR killing of individual *Bacteroides* strains using integrated CRISPR system. **FIG. 4A** presents results on blood agar plates. For selected CRISPR integrants (M1 and T1), tube cultures in TYG + Gm 200, Em 25 were diluted and spread (24h tube culture, 10-6 dilution, 100µl spread) on BHI blood agar plate (Gm 200, Em 25) supplemented with anhydrotetracycline (aTc) at concentrations of 0 and 100 ng/ml, respectively. Cells were incubated anaerobically at 37°C for 40h. **FIG. 4B** shows results in TYG liquid medium. Selected CRISPR integrants (M1, M2, T1, T3, S1, S2) were grown from fresh colonies in TYG

medium anaerobically at 37°C for 6h to OD_{600nm} ~0.6, 1:100 dilution to fresh TYG liquid medium (Gm 200, Em 25) supplemented with aTc at final concentrations of 0, 10 and 100 ng/ml, respectively. Growth was assessed during culture under anaerobic conditions at 37°C for 24h.

[0010] **FIGS. 5A-5C** presents targeted, inducible CRISPR killing of specific *Bacteroides* strains in a mixed population *in vitro*. Selected CRISPR integrants (M1, T1, S1) were grown from fresh colonies in TYG medium anaerobically at 37°C for 6h to an OD_{600nm} ~0.6. Equal volumes of cell cultures (1:100 dilutions) were mixed and added to fresh TYG liquid medium (Gm 200, Em 25) supplemented with aTc at final concentrations of 0, 10 and 100 ng/ml, respectively. These cultures were incubated anaerobically at 37°C for 24h. **FIG. 5A:** OD_{600nm} measurement. **FIG. 5B:** PCR amplifying the guide RNA region (primers binding to Cas9 and NBU2 coding sequences, amplicon size of 1.5 kb) was performed for cultures treated with aTc at concentrations of 100 ng/ml, 10 ng/ml and 0 ng/ml followed by Sanger DNA sequencing. Cultures treated with aTc have only non-targeting control gRNA. The DNA sequences appearing in FIG. 5B, in order of appearance from top to bottom, are SEQ ID NO:15 (four instances) and SEQ ID NO. 16 (one instance). **FIG. 5C:** a culture of M1+S1_aTc100 was diluted (10^{-6}) and spread onto a BHI blood agar plate (Gm 200, Em 25) and incubated anaerobically at 37°C for 40h to obtain single colonies. Colony PCR amplifying the guide RNA region was performed for 5 selected single colonies and a scraped mixture from the agar plate, followed by Sanger DNA sequencing, showing all clones that grew harbored only non-targeting, control gRNA. The DNA sequences appearing in FIG 5C, in order of appearance from top to bottom, are each SEQ ID NO:17 (seven instances).

[0011] **FIG. 6** illustrates CRISPR integration on the chromosome of *Bacteroides vulgatus* (Bv). Colonies from each conjugation, Bv.M (labeled VM1, VM2, VM3, VM4, VM5, VM6 and VM7), and susC_Bv (labeled V1, V2, V3, V4, V5), were picked for colony PCR screening. 0, Bv wild-type strain; M, DNA ladder. PCR A (outside primers, 0.5 or 0 kb) were used to screen integration at the attBv.3-1 locus; PCR B (outside primers, 0.5 or 0 kb) were used to screen integration at the attBv.3-2 locus, and PCR C (outside primers, 0.6 or 0 kb) were used to screen integration at the attBv.3-3 locus. PCR D (an

outside primer and an internal primer binding to ermG coding sequence, 0.6 or 0 kb: left junction of attBv.3-1 locus integration) were used to confirm junctions of integrated chromosome and integration plasmid sequences for selected clones. Left panel: Integrated strains with non-targeting, control guide RNA (M); right panel: integrated strains with targeting susC_Bv guide RNA.

[0012] **FIGS. 7A-7C** illustrate the characterization of the growth of *B. thetaiotaomicron* CRISPR-mutants. **FIG. 7A:** Plasmid design for engineering a *B. thetaiotaomicron* VPI-5482 CRISPR mutant. **FIG. 7B:** Bt mutants, containing either scrambled gRNA or a tdk targeting gRNA, cultured on blood agar plates ± 200 ng/mL aTc. **FIG. 7C:** Box plot of time required to achieve OD600=0.2 for Bt CRISPR mutants when grown in LYBHI medium containing 9 ng/mL aTc.

[0013] **FIGS. 8A-8D** illustrate *B. thetaiotaomicron* knockdown. **FIG. 8A:** Experimental design. The arrow designates the time of gavage of the consortium into adult male germ-free C57Bl/6J mice; each recipient mouse received 0.5% ethanol vehicle on days 1-8 when aTc was not administered. **FIGS. 8B,8C:** Bt or *B. cellulosilyticus* relative abundance across time for each treatment condition and aTc exposure shown by horizontal bars. **FIG. 8D:** Heatmap displaying difference of median relative abundance (%) of each consortium member (column) at each time point (row) in the four-day treatment arm relative to the vehicle control arm.

[0014] **FIGS. 9A-9B** illustrate *B. thetaiotaomicron* omission. **FIG. 9A:** Experimental design. The arrow designates the time of introduction of the 13- or 12-member consortia. **FIG. 9B:** Heatmap displaying difference of median relative abundance (%) for each consortium member (column) at each time point (row) in the 12-member community treatment arm relative to the 13-member (12 strains + Bt) community arm.

[0015] **FIG. 10** illustrates targeted microbiota modulation using a stably maintained, inducible CRISPR system. Expression of the CRISPR system (Cas9 endonuclease and guide RNA) leads to chromosomal breaks and ultimately cell death in bacteria. As such, specific *Bacteroides* strains harboring a stably maintained CRISPR cassette with a targeting guide RNA

can be eliminated from a mixed population *in situ* upon anhydrotetracycline (aTc) induction.

[0016] **FIGS. 11A-11D** are photos of blood agar plates. **FIG. 11A** illustrates the 10^{-4} dilution of pRepA-CRISPR targeting *susC* in *Bacteroides thetaiotaomicron* with and without aTc induction on blood BHI plates (no aTc on the left and 100 ng/ml aTc on the right). **FIG. 11B** illustrates the 10^{-6} dilution of pRepA-CRISPR targeting *susC* in *Bacteroides thetaiotaomicron* with and without aTc induction on blood BHI plates (no aTC on the left and 100 ng/ml aTc on the right). **FIG. 11C** illustrates the 10^{-4} dilution of pRepA-CRISPR non-targeting in *Bacteroides thetaiotaomicron* with and without aTc induction on blood BHI plates (no aTC on the left and 100 ng/ml aTc on the right). **FIG. 11D** illustrates the 10^{-6} dilution of pRepA-CRISPR non-targeting in *Bacteroides thetaiotaomicron* with and without aTc induction on blood BHI plates (no aTC on the left and 100 ng/ml aTc on the right).

[0017] **FIG.12** illustrates targeted microbiota modulation using an integrated, inducible CRISPR system. Expression of the CRISPR system (Cas9 endonuclease and guide RNA) leads to chromosomal breaks and ultimately cell death in bacteria. As such, specific *Bacteroides* strains harboring an integrated CRISPR cassette with a targeting guide RNA can be eliminated from a mixed population *in situ* upon anhydrotetracycline (aTc) induction.

[0018] **FIGS. 13A-13B** illustrates that Plasmid pNBU2-CRISPR.susC_BWH2-19 (FIG. 12) integrates only in the attBWH2 site in the t-RNA-Ser gene, BcellWH2_RS22795. The 5' end of the plasmid integration site is shown in **FIG. 13A** and the 3' end of the plasmid integration site is shown in **FIG. 13B**. The DNA sequence listings appearing in FIG. 13A, in order of appearance from top to bottom, are as follows: SEQ ID NOS: 18 (left to right); 19 (right to left); 19 (right to left); 18 (left to right); 19 (right to left); 18 (left to right); 19 (right to left); 18 (left to right); 19 (right to left); 18 (left to right); 19 (right to left); 18 (left to right); 19 (right to left); 20 (left to right); 21 (right to left); 22 (left to right); 23 (right to left); and 24 (left to right). It will be understood, for example, that SEQ ID NO:19 is the complementary strand of SEQ ID NO:18, and so on. The DNA sequence listings appearing in FIG.

13B, in order of appearance from top to bottom, are as follows: SEQ ID NOS: 25 (left to right); 26 (right to left); 25 (left to right); 26 (right to left); 25 (left to right); 26 (right to left); 27 (left to right); 28 (right to left); 29 (left to right); 30 (right to left); 25 (left to right); 26 (right to left); 25 (left to right); 26 (right to left); 25 (left to right); 26 (right to left); 25 (left to right); 26 (right to left); 25 (left to right); 26 (right to left); 31 (left to right); 32 (right to left); 25 (left to right); 26 (right to left); 25 (left to right); 26 (right to left); 33 (left to right); 34 (right to left); 35 (left to right); 36 (right to left); 25 (left to right); 26 (right to left); 37 (left to right); and 38 (right to left). It will be understood, for example, that SEQ ID NO:26 is the complementary strand of SEQ ID NO:25, and so on.

[0019] **FIG. 14** illustrates OD_{600nm} readings taken after 24 hours of growth as described in Example 15.

DETAILED DESCRIPTION

[0020] The present disclosure provides engineered RNA-guided nuclease systems that can be used to remodel complex microbial populations by selective knockdown of the abundance of targeted strains. In particular, the RNA-guided nuclease systems are engineered to target sites in chromosomal DNA of the targeted prokaryotic species, where the term "prokaryotic" refers to members of the domains Bacteria and Archaea. The compositions and methods disclosed herein can be used to manipulate microbial community composition *ex vivo* and within living animals.

(I) Protein-Nucleic Acid Complexes

[0021] One aspect of the present disclosure provides a protein-nucleic acid complex comprising an engineered RNA-guided nuclease system in association with a chromosome of a prokaryote, wherein the engineered RNA-guided nuclease system is targeted to a site in the chromosome, the bacterial chromosome encodes an HU family DNA-binding protein comprising an amino acid sequence having at least 50% sequence identity to the amino acid sequence of SEQ ID NO: 1 (MNKADLISAVAAEAGLSKVDAKKAVEAFVSTVTKALQEGDKVSLIGFGTFSV AERSARTGINPSTKATITIPAKKVTKFKPGAELADAIK), and the chromosome of the bacterial species is associated with HU family DNA-binding proteins

have at least 50% sequence identity to the amino acid sequence of SEQ ID NO: 1. In general, the RNA-guided nuclease system that is targeted to the chromosome DNA of the bacterial species is other than a naturally occurring RNA-guided nuclease (*e.g.*, CRISPR) system that is endogenous to the organism of interest.

[0022] The RNA-guided nuclease system comprises a DNA endonuclease (*e.g.*, CRISPR nuclease) whose cleavage activity is directed by RNA (*e.g.*, guide RNA). The prokaryote expresses the HU family protein, which associates with the chromosomal DNA of the prokaryote. Thus, the protein-nucleic acid complexes disclosed herein comprise ribonucleoprotein complexes (CRISPR nuclease/gRNA) bound to DNA/protein complexes (prokaryotic chromosomal DNA and associated HU family proteins).

(a) RNA-Guided Nuclease Systems

[0023] The protein-nucleic acid complexes disclosed herein comprise an RNA-guided nuclease system, which comprises a DNA endonuclease whose cleavage activity is directed by a guide RNA (gRNA). As detailed below, the gRNA can be engineered to recognize and target a specific sequence in the nucleic acid of interest (*e.g.*, a prokaryotic chromosome).

[0024] In general, the RNA-guided endonuclease is a clustered regularly interspaced short palindromic repeats (CRISPR) nuclease. The CRISPR nuclease can be bacterial or archaeal. In some situations, the CRISPR nuclease can be from a Type I CRISPR system, a type II CRISPR system, a type III CRISPR system, a Type IV CRISPR system, a type V CRISPR system, or a type VI CRISPR system. In specific embodiments, the CRISPR nuclease can be from single-subunit effector systems such as Type II, Type V, or Type VI systems. In various embodiments, the CRISPR nuclease can be a Type II Cas9 nuclease, a Type V Cas12 (formerly called Cpf1) nuclease, a Type VI Cas13 (formerly called C2cd) nuclease, a CasX nuclease, or a CasY nuclease.

[0025] The CRISPR nuclease can be from *Acaryochloris* spp., *Acetohalobium* spp., *Acidaminococcus* spp., *Acidithiobacillus* spp., *Acidothermus* spp., *Akkermansia* spp., *Alicyclobacillus* spp., *Allochromatium* spp., *Ammonifex* spp., *Anabaena* spp., *Arthrospira* spp., *Bacillus* spp.,

Bifidobacterium spp., *Burkholderiales* spp., *Caldicelulosiruptor spp.*, *Campylobacter* spp., *Candidatus* spp., *Clostridium* spp., *Corynebacterium* spp., *Crocosphaera* spp., *Cyanothece* spp., *Deltaproteobacterium* spp., *Exiguobacterium* spp., *Finegoldia* spp., *Francisella* spp., *Ktedonobacter* spp., *Lachnospiraceae* spp., *Lactobacillus* spp., *Leptotrichia* spp., *Lyngbya* spp., *Marinobacter* spp., *Methanohalobium* spp., *Microscilla* spp., *Microcoleus* spp., *Microcystis* spp., *Mycoplasma* spp., *Natranaerobius* spp., *Neisseria* spp., *Nitratifractor* spp., *Nitrosococcus* spp., *Nocardiopsis* spp., *Nodularia* spp., *Nostoc* spp., *Oenococcus* spp., *Oscillatoria* spp., *Parasutterella* spp., *Pelotomaculum* spp., *Petrotoga* spp., *Planctomyces spp.*, *Polaromonas* spp., *Prevotella* spp., *Pseudoalteromonas* spp., *Ralstonia* spp., *Ruminococcus* spp., *Staphylococcus* spp., *Streptococcus* spp., *Streptomyces* spp., *Streptosporangium* spp., *Synechococcus* spp., *Thermosipho* spp., *Verrucomicrobia* spp., or *Wolinella* spp.

[0026] In some aspects, the CRISPR nuclease can be *Streptococcus pyogenes* Cas9, *Francisella novicida* Cas9, *Staphylococcus aureus Cas9*, *Streptococcus thermophilus* Cas9, *Streptococcus pasteurianus* Cas9, *Campylobacter jejuni* Cas9, *Neisseria meningitis* Cas9, *Neisseria cinerea* Cas9, *Francisella novicida* Cas12, *Acidaminococcus* sp. Cas12, *Lachnospiraceae bacterium* ND2006 Cas12a, *Leptotrichia wadeii* Cas13a, *Leptotrichia shahii* Cas13a, *Prevotella* sp. P5-125 Cas13, *Ruminococcus flavefaciens* Cas13d, *Deltaproteobacterium* CasX, *Planctomyces* CasX, or *Candidatus* CasY.

[0027] The CRISPR nuclease can be a wild type or naturally-occurring protein. Wild-type CRISPR nucleases generally comprise two nuclease domains, *e.g.*, Cas9 nucleases comprise RuvC and HNH domains, each of which cleaves one strand of a double-stranded sequence. CRISPR nucleases also comprise domains that interact with the guide RNA (*e.g.*, REC1, REC2) or the RNA/DNA heteroduplex (*e.g.*, REC3), and a domain that interacts with the protospacer-adjacent motif (PAM) (*i.e.*, PAM-interacting domain).

[0028] Alternatively, the CRISPR nuclease can be modified to have improved targeting specificity, improved fidelity, altered PAM specificity, decreased off-target effects, and/or increased stability. For example, the

CRISPR nuclease can be modified to comprise one or more mutations (*i.e.*, substitution, deletion, and/or insertion of at least one amino acid). Non-limiting examples of one or more mutations that improve targeting specificity, improve fidelity, and/or decrease off-target effects include N497A, R661A, Q695A, K810A, K848A, K855A, Q926A, K1003A, R1060A, and/or D1135E (with reference to the numbering system of SpyCas9).

[0029] In various embodiments, the CRISPR nuclease can be a nuclease (*i.e.*, cleave both strands of a double-stranded nucleotide sequence or cleave a single-stranded nucleotide sequence). In other embodiment, CRISPR nuclease can be a nickase, which cleaves one strand of a double-stranded sequence. The nickase can be engineered via inactivation of one of the nuclease domains of the CRISPR nuclease. For example, the RuvC domain of a Cas9 protein can be inactivated by mutations such as D10A, D8A, E762A, and/or D986A, or the HNH of a Cas9 protein domain can be inactivated by mutations such as H840A, H559A, N854A, N856A, and/or N863A (with reference to the numbering system of *Streptococcus pyogenes* Cas9, SpyCas9) to generate a Cas9 nickase (*e.g.*, nCas9). Comparable mutations in other CRISPR nucleases can generate nickases (*e.g.*, nCas12).

[0030] A CRISPR system also comprises a guide RNA. A guide RNA interacts with the CRISPR nuclease and a target sequence in the nucleic acid of interest and guides the CRISPR nuclease to the target sequence. The target sequence has no sequence limitation except that the sequence is adjacent to a protospacer adjacent motif (PAM) sequence. Different CRISPR nucleases recognize different PAM sequences. For example, PAM sequences for Cas9 proteins include 5'-NGG, 5'-NGGNG, 5'-NNAGAAW, 5'-NNNNGATT, and 5-NNNNRYAC, and PAM sequences for Cas12 proteins include 5'-TTN and 5'-TTTV, wherein N is defined as any nucleotide, R is defined as either G or A, W is defined as either A or T, Y is defined an either C or T, and V is defined as A, C, or G. In general, Cas9 PAMs are located 3' of the target sequence, and Cas12 PAMs are located 5' of the target sequence.

[0031] Guide RNAs are engineered to complex with specific CRISPR nucleases. In general, a guide RNA comprises (i) a CRISPR RNA (crRNA) that contains a guide or spacer sequence at the 5' end that hybridizes at the

target site, and (ii) a transacting crRNA (tracrRNA) sequence that interacts with the CRISPR nuclease. The guide or spacer sequence of each guide RNA is different (*i.e.*, is sequence specific). The rest of the guide RNA sequence is generally the same in guide RNAs designed to complex with a specific CRISPR nuclease.

[0032] The crRNA comprises the guide sequence at the 5' end, as well as additional sequence at the 3' end that base-pairs with sequence at the 5' end of the tracrRNA to form a duplex structure, and the tracrRNA comprises additional sequence that forms at least one stem-loop structure, which interacts with the CRISP nuclease. The guide RNA can be a single molecule (*e.g.*, a single guide RNA (sgRNA) or 1-piece sgRNA), wherein the crRNA sequence is linked to the tracrRNA sequence. Alternatively, the guide RNA can be two separate molecules (*e.g.*, 2-piece gRNA) comprising a crRNA and a tracrRNA.

[0033] The crRNA guide sequence is designed to hybridize with the complement of a target sequence (*i.e.*, protospacer) in the nucleic acid of interest. In general, the complementarity between the guide sequence and the target sequence is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99%. In specific embodiments, the complementarity is complete (*i.e.*, 100%). In various embodiments, the length of the crRNA guide sequence can range from about 15 nucleotides to about 25 nucleotides. For example, the crRNA guide sequence can be about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length. In specific embodiments, the guide is about 19, 20, or 21 nucleotides in length. In one embodiment, the crRNA guide sequence has a length of 20 nucleotides. In certain embodiments, the crRNA can comprise additional 3' sequence that interacts with tracrRNA. The additional sequence can comprise from about 10 to about 40 nucleotides. In embodiments in which the guide RNA comprises a single molecule, the crRNA and tracrRNA portions of the gRNA can be linked by sequence that forms a loop. The sequence that form the loop can range in length from about 4 nucleotides to about 10 or more nucleotides.

[0034] As mentioned above, the tracrRNA comprises repeat sequences that form at least one stem loop structure, which interacts with the CRISPR nuclease. The length of each loop and stem can vary. For example, the loop

can range from about 3 to about 10 nucleotides in length, and the stem can range from about 6 to about 20 base pairs in length. The stem can comprise one or more bulges of 1 to about 10 nucleotides. The tracrRNA sequence in the guide RNA generally is based upon the sequence of wild type tracrRNA that interact with the wild-type CRISPR nuclease. The wild-type sequence can be modified to facilitate secondary structure formation, increased secondary structure stability, and the like. For example, one or more nucleotide changes can be introduced into the guide RNA sequence. The tracrRNA sequence can range in length from about 50 nucleotides to about 300 nucleotides. In various embodiments, the tracrRNA can range in length from about 50 to about 90 nucleotides, from about 90 to about 110 nucleotides, from about 110 to about 130 nucleotides, from about 130 to about 150 nucleotides, from about 150 to about 170 nucleotides, from about 170 to about 200 nucleotides, from about 200 to about 250 nucleotides, or from about 250 to about 300 nucleotides. The tracrRNA can comprise an optional extension at the 3' end of the tracrRNA.

[0035] The guide RNA can comprise standard ribonucleotides and/or modified ribonucleotides. In some embodiment, the guide RNA can comprise standard or modified deoxyribonucleotides. In embodiments in which the guide RNA is enzymatically synthesized (*i.e.*, *in vivo* or *in vitro*), the guide RNA generally comprises standard ribonucleotides. In embodiments in which the guide RNA is chemically synthesized, the guide RNA can comprise standard or modified ribonucleotides and/or deoxyribonucleotides. Modified ribonucleotides and/or deoxyribonucleotides include base modifications (*e.g.*, pseudouridine, 2-thiouridine, N6-methyladenosine, and the like) and/or sugar modifications (*e.g.*, 2'-O-methy, 2'-fluoro, 2'-amino, locked nucleic acid (LNA), and so forth). The backbone of the guide RNA can also be modified to comprise phosphorothioate linkages, boranophosphate linkages, or peptide nucleic acids.

[0036] The guide RNA of a CRISPR nuclease system is engineered to target the CRISPR nuclease system to a specific site in prokaryotic chromosomal DNA such that the protein-nucleic acid complexes, as described above, can be formed. In general, the protein-nucleic acid complex is formed within the prokaryote.

[0037] In some embodiments, the engineered CRISPR nuclease system can be integrated into and expressed from the chromosome of the prokaryote. In other embodiments, the engineered CRISPR nuclease system can be carried on and expressed from an extrachromosomal vector. Expression of the engineered CRISPR nuclease system can be regulated. For example, the expression of the engineered CRISPR nuclease system can be regulated by an inducible promoter.

(b) Prokaryotic Chromosome

[0038] The protein-nucleic acid complex disclosed herein further comprises a prokaryotic chromosome, wherein the prokaryotic chromosome encodes HU family DNA-binding protein comprising an amino acid sequence with at least 50% sequence identity to the amino acid sequence of SEQ ID NO:1, and the chromosomal DNA of the prokaryote is associated with said HU family DNA-binding protein. The HU family of DNA-binding proteins comprises small (~ 90 amino acids) basic histone-like proteins that bind double stranded DNA without sequence specificity and bind DNA structures such as forks, three/four way junctions, nicks, overhangs, and bulges. Binding of HU family DNA-binding proteins can stabilize the DNA and protect it from denaturation under extreme environmental conditions.

[0039] The chromosome can be within members of the domain Bacteria or the domain Archaea. In some embodiments, the organism is a bacterial species or different strains of that species. In some embodiments, the HU family DNA-binding protein comprises an amino acid sequence having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:1.

[0040] In specific embodiments, the prokaryote is a member of the genus *Bacteroides*. *Bacteroides* species are prominent anaerobic symbionts of mammalian gut microbiota. They contain a variety of saccharolytic enzymes and are the primary fermenters of polysaccharides in the gut. They maintain complex and generally beneficial relationships with the host when retained in the gut, but can cause significant pathology if they escape this environment. Non-limiting examples of *Bacteroides* species include *B.*

acidifaciens, B. bacterium, B. barnesiaes, B. caccae, B. caecicola, B. caecigallinarum, B. capillosis, B. cellulosilyticus, B. cellulosolvens, B. clarus, B. coagulans, B. coprocola, B. coprophilus, B. coprosuis, B. distasonis, B. dorei, B. eggerthii, B. gracilis, B. faecichinchillae, B. faecis, B. finegoldii, B. fluxus, B. fragilis, B. galacturonicus, B. gallinaceum, B. gallinarum, B. goldsteinii, B. graminisolvens, B. helcogene, B. heparinolyticus, B. intestinalis, B. johnsonii, B. luti, B. massiliensis, B. melaninogenicus, B. neonati, B. nordii, B. oleiciplenus, B. oris, B. ovatus, B. paurosaccharolyticus, B. plebeius, B. polypragmatus, B. propionicifaciens, B. putredinis, B. pyogenes, B. reticulotermitis, B. rodentium, B. salanitronis, B. salyersiae, B. sartorii, B. sediment, B. stercoris, B. stercorirosoris, B. suis, B. tectus, B. thetaiotaomicron, B. timonensis, B. uniformis, B. vulgatus, B. xylanisolvens, B. xylanolyticus., and B. zoogleoformans.

[0041] In some embodiments, the prokaryotic chromosome is a chromosome chosen from *Bacteroides thetaiotaomicron, Bacteroides vulgatus, Bacteroides cellulosilyticus, Bacteroides fragilis, Bacteroides helcogenes, Bacteroides ovatus, Bacteroides salanitronis, Bacteroides uniformis, or Bacteroides xylanisolvens*.

[0042] In some embodiments, the chromosome is chosen from *Barnesiella sp.*, *Barnesiella viscericola*, *Capnocytphaga sp.*, *Odoribacter splanchnicus*, *Paludibacter sp.*, *Parabacteroides sp.*, *Porphyromonadaceae bacterium*, and *Schleiferia sp*.

(c) Specific Protein-Nucleic Acid Complexes

[0043] In specific embodiments, the protein-nucleic acid complex can comprise an engineered CRISPR Cas9/gRNA system or an engineered CRISPR Cas12/gRNA system bound to or associated with a *Bacteroides* chromosome.

(II) Methods for Generating the Protein-Nucleic Acid Complexes

[0044] A further aspect of the present disclosure provides methods for generating complexes comprising an engineered RNA-guided (CRISPR) nuclease system and a prokaryotic chromosome encoding an HU family DNA-binding protein as described above. Said methods comprise (a) engineering

the CRISPR nuclease system to target a site in the prokaryote chromosome, and (b) introducing the engineered CRISPR nuclease system into the prokaryote.

[0045] Engineering the CRISPR nuclease system comprises designing a guide RNA whose crRNA guide sequence targets a specific (~19-22 nt) sequence in the prokaryotic chromosome that is adjacent to a PAM sequence (which is recognized by the CRISPR nuclease of interest) and whose tracrRNA sequence is recognized by the CRISPR nuclease of interest, as described above in section (I)(a). The engineered CRISPR system can be introduced into the prokaryote as an encoding nucleic acid. For example, the encoding nucleic acid can be part of a vector. Means for delivering or introducing various vectors into are well known in the art.

[0046] The vector encoding the engineered CRISPR system (*i.e.*, CRISPR nuclease and guide RNA) can be a plasmid vector, phagemid vector, viral vector, bacteriophage vector, bacteriophage-plasmid hybrid vector, or other suitable vector. The vector can be an integrative vector, a conjugation vector, a shuttle vector, an expression vector, an extrachromosomal vector, and so forth.

[0047] The nucleic acid sequence encoding the CRISPR nuclease can be operably linked to a promoter for expression in the prokaryote. In specific embodiments, the promoter operably linked to the engineered CRISPR nuclease can be a regulated promoter. In some aspects, the regulated promoter can be regulated by a promoter inducing chemical. In such embodiments, the promoter can be pTetO, which is based on the *Escherichia coli* Tn10-derived tet regulatory system and consists of a strong tet operator (tetO)-containing mycobacterial promoter and expression cassette for the repressor (TetR) and the promoter inducing chemical can be anhydrotetracycline (aTc). In other embodiments, the promoter can be pBAD or araC-ParaBAD and the promoter inducing chemical can be arabinose. In further embodiments, the promoter can be pLac or tac (trp-lac) and the promoter inducing chemical can be lactose/IPTG. In other embodiments, the promoter can be pPrpB and the promoter inducing chemical can be propionate.

[0048] The nucleic acid sequence encoding the at least one guide RNA can be operably linked to a promoter for expression in the prokaryote of interest. In embodiments in which the prokaryote of interest is *Bacteroides*, the constitutive promoter can be the P1 promoter, which lies upstream of the *B. thetaiotaomicron* 16S rRNA gene BT_r09 (Wegmann et al., *Applied Environ. Microbiol.*, 2013, 79:1980-1989). Other suitable Bacteroides promoters include P2, $P1T_D$, $P1T_P$, $P1T_{DP}$ (Lim et al., *Cell*, 2017, 169:547-558), P_{AM}, P_{cfiA}, P_{cepA}, P_{BT1311} (Mimee et al., Cell Systems, 2015, 1:62-71) or variants of any of the foregoing promoters. In other embodiments, the constitutive promoter can be an *E. coli* σ^{70} promoter or derivative thereof, a *B. subtilis* σ^{A} promoter or derivative thereof, or a *Salmonella* Pspv2 promoter or derivative thereof. Persons skilled in the art are familiar with additional constitutive promoters that are suitable for the prokaryote of interest.

[0049] In some embodiments, the vector can be an integrative vector and can further comprise sequence encoding a recombinase, as well as one or more recombinase recognition sites. In general, the recombinase is an irreversible recombinase. Non-limiting examples of suitable recombinases include the *Bacteroides* intN2 tyrosine integrase (coded by NBU2 gene), *Streptomyces* phage phiC31 (φC31) recombinase, coliphage P4 recombinase, coliphage lambda integrase, *Listeria* A118 phage recombinase, and actinophage R4 Sre recombinase. Recombinases/integrases mediate recombination between two sequence specific recognition (or attachment) sites (*e.g.*, an attP site and an attB site). In some embodiments, the vector can comprise one of the recombinase recognition sites (*e.g.*, attP) and the other recombinase recognition site (*e.g.*, attB) can be located in the chromosome of the prokaryote (*e.g.*, near a tRNA-ser gene). In such situations, the entire vector can be integrated into the chromosome of the prokaryote. In other embodiments, the sequence encoding the engineered CRISPR nuclease system can be flanked by the two recombinase recognition sites, such that only the sequence encoding the engineered CRISPR nuclease system is integrated into the prokaryotic chromosome.

[0050] Any of the vectors described above can further comprise at least one transcriptional termination sequence, as well as at least one origin of

replication and/or at least one selectable marker sequence (*e.g.*, antibiotic resistance genes) for propagation and selection in prokaryotic cells of interest.

[0051] Additional information about vectors and use thereof can be found in "Current Protocols in Molecular Biology" Ausubel *et al.*, John Wiley & Sons, New York, 2003 or "Molecular Cloning: A Laboratory Manual" Sambrook & Russell, Cold Spring Harbor Press, Cold Spring Harbor, NY, 3rd edition, 2001.

[0052] In embodiments in which the vector encoding the engineered CRISPR nuclease system is an integrative vector, the nucleic acid encoding the engineered CRISPR system (or the entire vector) can be stably integrated into the bacterial chromosome after delivery of the vector to the bacterium (and expression of the recombinase/integrase). In embodiments in which the vector encoding the engineered CRISPR nuclease system is not an integrative vector, the vector can remain extrachromosomal after delivery of the vector to the microbe.

[0053] In embodiments in which the sequence encoding the CRISPR nuclease is operably linked to an inducible promoter, expression of the CRISPR nuclease system can be regulated by introducing the promoter inducing chemical into the prokaryote. In specific embodiments, the promoter inducing chemical can be anhydrotetracycline. Upon induction, the CRISPR nuclease is synthesized and complexes with the at least one guide RNA, which targets the CRISPR nuclease system to the target site in the bacterial chromosome, thereby forming the protein-nucleic acid complex as disclosed herein.

(III) Methods for Modulating Microbiota Compositions

[0054] A further aspect of the present disclosure encompasses methods for altering the population and composition of microbiota, by selectively slowing the growth of a target microbe (prokaryote) in a mixed population of microbes. The method comprises expressing an engineered RNA-guided (CRISPR) nuclease system in the target prokaryote, wherein the engineered RNA-guided nuclease system is targeted to a site in a chromosome of the target prokaryote such that at least one double strand break is introduced in the chromosome of the target prokaryote, thereby

slowing the growth or propagation of the target prokaryotes. The growth of the target prokaryote comprising at least one double strand break in chromosomal DNA is slowed or halted because DNA breaks generally are not repaired or are inefficiently repaired in prokaryotes. Slowing the growth of the target prokaryote leads to reduced or eliminated levels of the target prokaryote in the mixed population of prokaryotes.

[0055] Any of the CRISPR nuclease systems described above in section (I)(a) can be engineered as described above in section (II) to target a site in the chromosome of a prokaryote of interest, which are described above in section (I)(b). The engineered CRISPR nuclease system can be introduced as part of a vector into the prokaryote as described above in section (II). In general, the CRISPR nuclease is inducible (*i.e.*, its encoding sequence is operably linked to an inducible promoter). As such, the CRISPR nuclease can be expressed at a defined point in time. In the absence of a promoter inducing chemical, the CRISPR nuclease system cannot be generated. A CRISPR nuclease can be produced by exposing the prokaryote to a promoter inducing chemical, such that the CRISPR nuclease is expressed from the chromosomally integrated encoding sequence or the extrachromosomal encoding sequence as described above in section (II). The CRISPR nuclease complexes with the at least one guide RNA that is constitutively expressed from the chromosomally integrated encoding sequence or the extrachromosomal encoding sequence, thereby forming an active CRISPR nuclease system. The CRISPR nuclease system is targeted to the target site in the prokaryotic chromosome, where it introduces a double strand break in the chromosomal DNA. The double strand break results in slowed growth and/or death of the target prokaryote. As a consequence, the mixed population of prokaryotes has reduced or eliminated levels of the target prokaryote.

[0056] In some embodiments, the target prokaryote can be a *Bacteroides* species, as detailed above in section (I)(b).

[0057] The engineered CRISPR system can be introduced into the target prokaryote within the mixed population of prokaryotes. Alternatively, the engineered CRISPR system can be introduced into the target prokaryote, which is then mixed with the mixed population of prokaryotes.

[0058] In some embodiments, the mixed population of prokaryotes can be harbored in cell culture, wherein exposure to the promoter inducing chemical leads to reduced or eliminated levels of the target prokaryote.

[0059] In other embodiments, the mixed population of prokaryotes can be harbored in a mammal's digestive tract (or gut), wherein administration of the promoter inducing chemical leads to reduced or eliminated levels of the target prokaryote in the gut microbiota. The promoter inducing chemical can be administered orally (*e.g.*, via food, drink, or a pharmaceutical formulation). The mammal can be a mouse, rat, or other research animal. In specific embodiments, the mammal can be a human. Reduction or elimination of the target prokaryote (*e.g., Bacteroides)* can lead to improved gut health.

[0060] The mixed population of prokaryotes (in cell culture or a digestive tract) can comprise a wide diversity of taxa. For example, human gut microbiota can comprise hundreds of different species of bacteria and many strain-level variants of these species.

[0061] In certain embodiments, the mammal (*e.g.*, human) can be undergoing cancer immunotherapy, wherein immunotherapy responders have been shown to have lower levels of *Bacteroides* species in their gut microbiota as compared to non-responders (Gopalakrishnan et al., *Science*, 2018, 359:97-103). Thus, reduction in the levels of *Bacteroides* species in gut microbiota may lead to better human cancer immunotherapy outcomes.

[0062] In certain embodiments, the mammal (*e.g.*, human, canine, feline, porcine, equine, or bovine) can undergo gut surgery for a variety of reasons including, but not limited to, inflammatory bowel disease, Crohn's disease, diverticulitis, bowel blockage, polyp removal, cancerous tissue removal, ulcerative colitis, bowel resection, proctectomy, complete colectomy, or partial colectomy wherein attenuation of *Bacteroides fragilis* species within the mammalian gut pre-surgery by an inducible CRISPR system may reduce the risk of post-surgery infections by *B. fragilis* at locations outside the gut, but within the mammalian body. Locations outside the gut include the external surface of the gut. The inducible CRISPR systems within *B. fragilis* can be targeted to cut or modify a location similar, but not limited to, a pathogenicity island, toxins (*i.e.*, *B. fragilis* toxin or BFT) or other unique sequence associated with infectious strains of *B. fragilis* or other native gut prokaryotes

known to cause post-surgical infections. For example, levels of nontoxigenic *B. fragilis* (NTBF) and enterotoxigenic *B. fragilis* (ETBF) maybe be selectively modulated using engineered inducible CRISPR systems placed within ETBF strains, but not NTBF strains. Other bacterial taxa that cause infections after gut surgery may include *Bacteroides capillosis, Escherchia coli, Enterococcus faecalis, Gamella haemolysan, and Morganella morganii*. Delivery of the inducible CRISPR system to the gut microbiota may occur as part of a probiotic treatment before, during, or after surgery. Delivery of the inducible CRISPR system to the target prokaryote may occur outside the mammalian body or within the mammalian body. Delivery of the inducble CRISPR system to the target prokaryote may occur via nucleic acid vectors such as plasmids or bacteriophage. Delivery of plasmids may occur via electroporation, chemical transformation, or bacteria-to-bacteria conjugation.

[0063] In various embodiments, the level of the target prokaryote can be reduced by at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 99% relative to that before expression of the CRISPR nuclease. In certain embodiments, the target prokaryote can be reduced to undetectable levels in the mixed population of prokaryotes after expression of the CRISPR nuclease.

(IV) CRISPR Integrated Prokaryotes as Probiotics

[0064] Yet another aspect of the present disclosure encompasses engineered prokaryotes for use as probiotics. The engineered prokaryotes comprise any of engineered CRISPR nuclease systems described in section (I) integrated into the prokaryotic chromosome or maintained as episomal vectors within the prokaryotic cell. In some embodiments, the engineered prokaryote is an engineered *Bacteroides* comprising an inducible CRISPR nuclease system. Administration of the engineered *Bacteroides* to a mammalian subject followed by induction of the CRISPR system can be used to reduce the relative abundance of *Bacteroides* strains in gut microbiota. In other embodiments, the *Bacteroides* strains can be engineered to out-compete wildtype strains of *Bacteroides* in gut microbiota. In these and other embodiments, engineered *Bacteroides* strains providing a therapeutic benefit

for the mammalian subject can then be removed from the mammalian subject by induction of the inducible CRISPR nuclease system.

DEFINITIONS

[0065] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton *et al.*, Dictionary of Microbiology and Molecular Biology (2nd Ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger *et al.* (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

[0066] When introducing elements of the present disclosure or the preferred embodiments(s) thereof, the articles "a", "an", "the" and "said" are intended to mean that there are one or more of the elements. The terms "comprising", "including" and "having" are intended to be inclusive and mean that there may be additional elements other than the listed elements.

[0067] The term "about" when used in relation to a numerical value, x, for example means x ± 5%.

[0068] As used herein, the terms "complementary" or "complementarity" refer to the association of double-stranded nucleic acids by base pairing through specific hydrogen bonds. The base paring may be standard Watson-Crick base pairing (*e.g.*, 5'-A G T C-3' pairs with the complementary sequence 3'-T C A G-5'). The base pairing also may be Hoogsteen or reversed Hoogsteen hydrogen bonding. Complementarity is typically measured with respect to a duplex region and thus, excludes overhangs, for example. Complementarity between two strands of the duplex region may be partial and expressed as a percentage (*e.g.*, 70%), if only some (*e.g.*, 70%) of the bases are complementary. The bases that are not complementary are "mismatched." Complementarity may also be complete (*i.e.*, 100%), if all the bases in the duplex region are complementary.

[0069] The term "expression" with respect to a gene or polynucleotide refers to transcription of the gene or polynucleotide and, as appropriate, translation of an mRNA transcript to a protein or polypeptide. Thus, as will be clear from the context, expression of a protein or polypeptide results from transcription and/or translation of the open reading frame.

[0070] A "gene," as used herein, refers to a DNA region (including exons and introns) encoding a gene product, as well as all DNA regions which regulate the production of the gene product, whether or not such regulatory sequences are adjacent to coding and/or transcribed sequences. Accordingly, a gene includes, but is not necessarily limited to, promoter sequences, terminators, translational regulatory sequences such as ribosome binding sites and internal ribosome entry sites, enhancers, silencers, insulators, boundary elements, replication origins, matrix attachment sites, and locus control regions.

[0071] The term "heterologous" refers to an entity that is not endogenous or native to the cell of interest. For example, a heterologous protein refers to a protein that is derived from or was originally derived from an exogenous source, such as an exogenously introduced nucleic acid sequence. In some instances, the heterologous protein is not normally produced by the cell of interest.

[0072] The term "nuclease," which is used interchangeably with the term "endonuclease," refers to an enzyme that cleaves both strands of a double-stranded nucleic acid sequence or cleaves a single-stranded nucleic acid sequence.

[0073] The terms "nucleic acid" and "polynucleotide" refer to a deoxyribonucleotide or ribonucleotide polymer, in linear or circular conformation, and in either single- or double-stranded form. For the purposes of the present disclosure, these terms are not to be construed as limiting with respect to the length of a polymer. The terms can encompass known analogs of natural nucleotides, as well as nucleotides that are modified in the base, sugar and/or phosphate moieties (*e.g.*, phosphorothioate backbones). In general, an analog of a particular nucleotide has the same base-pairing specificity; *i.e.*, an analog of A will base-pair with T.

[0074] The term "nucleotide" refers to deoxyribonucleotides or ribonucleotides. The nucleotides may be standard nucleotides (*i.e.*, adenosine, guanosine, cytidine, thymidine, and uridine), nucleotide isomers, or nucleotide analogs. A nucleotide analog refers to a nucleotide having a modified purine or pyrimidine base or a modified ribose moiety. A nucleotide analog may be a naturally occurring nucleotide (*e.g.*, inosine, pseudouridine, etc.) or a non-naturally occurring nucleotide. Non-limiting examples of modifications on the sugar or base moieties of a nucleotide include the addition (or removal) of acetyl groups, amino groups, carboxyl groups, carboxymethyl groups, hydroxyl groups, methyl groups, phosphoryl groups, and thiol groups, as well as the substitution of the carbon and nitrogen atoms of the bases with other atoms (*e.g.*, 7-deaza purines). Nucleotide analogs also include dideoxy nucleotides, 2'-O-methyl nucleotides, locked nucleic acids (LNA), peptide nucleic acids (PNA), and morpholinos.

[0075] The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues.

[0076] The terms "target sequence," and "target site" are used interchangeably to refer to the specific sequence in the nucleic acid of interest (*e.g.*, chromosomal DNA or cellular RNA) to which the CRISPR system is targeted, and the site at which the CRISPR system modifies the nucleic acid or protein(s) associated with the nucleic acid.

[0077] Techniques for determining nucleic acid and amino acid sequence identity are known in the art. Typically, such techniques include determining the nucleotide sequence of the mRNA for a gene and/or determining the amino acid sequence encoded thereby, and comparing these sequences to a second nucleotide or amino acid sequence. Genomic sequences can also be determined and compared in this fashion. In general, identity refers to an exact nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Two or more sequences (polynucleotide or amino acid) can be compared by determining their percent identity. The percent identity of two sequences, whether nucleic acid or amino acid sequences, is the number of exact matches between two aligned sequences divided by the length of the shorter sequences and multiplied by 100. An approximate alignment for

nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2:482-489 (1981). This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff, Atlas of Protein Sequences and Structure, M. O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA, and normalized by Gribskov, Nucl. Acids Res. 14(6):6745-6763 (1986). An exemplary implementation of this algorithm to determine percent identity of a sequence is provided by the Genetics Computer Group (Madison, Wis.) in the "BestFit" utility application. Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, for example, another alignment program is BLAST, used with default parameters. For example, BLASTN and BLASTP can be used using the following default parameters: genetic code=standard; filter=none; strand=both; cutoff=60; expect=10; Matrix=BLOSUM62; Descriptions=50 sequences; sort by=HIGH SCORE; Databases=non-redundant, GenBank+EMBL+DDBJ +PDB+GenBank CDS translations+Swiss protein+Spupdate+PIR. Details of these programs can be found on the GenBank website.

[0078] As various changes could be made in the above-described cells and methods without departing from the scope of the invention, it is intended that all matter contained in the above description and in the examples given below, shall be interpreted as illustrative and not in a limiting sense.

EXAMPLES

[0079] The following examples illustrate certain aspects of the disclosure.

Example 1. Vector construction

[0080] The CRISPR integration pNBU2-CRISPR plasmids were constructed using Gibson cloning (NEBuild HIFI DNA Assembly Master Mix, New England Biolabs) of plasmid backbone (RP4-oriT, R6K ori, bla, ermG) from pExchange-tdk, NBU2 integrase from pNBU2-tetQb, and anhydrotetracycline (aTc) inducible CRISPR cassettes (P2-A21-tetR, P1TDP-GH023-SpCas9, P1-N20 sgRNA scaffold) assembled from synthetic DNAs or

PCR of genomic DNA of *Streptococcus pyogenes* strain SF370. **FIG. 2** illustrates the plasmid design.

[0081] The plasmid backbone harbors R6K origin of replication and *bla* sequence for ampicillin selection in *E. coli,* RP4-oriT sequence for conjugation and *ermG* sequence for erythromycin (Em) selection in *Bacteroides*. NBU2 encodes the intN2 tyrosine integrase, which mediates sequence-specific recombination between the attN2 site on pNBU2-CRISPR plasmid and one of the attB sites located on the chromosome of *Bacteroides* cells. The attN2 and attB have the same 13 bp recognition nucleotide sequence (5'-3'): CCTGTCTCTCCGC (SEQ ID NO: 2).

[0082] The inducible CRISPR cassettes include aTc inducible SpCas9 under the control of TetR regulator (P2-A21-tetR, P1TDP-GH023-SpCas9), and constitutively expressed guide RNA under P1 promoter (P1-N20 sgRNA scaffold). The promoters and ribosomal binding sites are derived and engineered from regulatory sequences of *Bacteroides thetaiotaomicron* 16S rRNA genes, as described in Lim et al., *Cell*, 2017, 169:547-558. The guide RNA is a nucleotide sequence that is homologous to a coding DNA sequence, or non-coding DNA sequence, or a non-targeting scramble nucleotide sequence. This sequence can be of any form as long as it is compatible with protospacer adjacent motif (PAM) requirements of different Cas9 homologs. The guide RNA can be either in separate transcriptional units of tracrRNA and crRNA or fused into a hybrid chimeric tracr/crRNA single guide(sgRNA).

[0083] The DNA sequence for the above plasmid is presented in SEQ ID NO: 3:

Plasmid (pNBU2.CRISPR-susC_Bt) DNA sequence (10,396 bp)
GGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACT
CATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT
GTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACC
ATGATTACGCCCTTAAGACCCACTTTCACATTTAAGTTGTTTTTCTAATC
CGCATATGATCAATTCAAGGCCGAATAAGAAGGCTGGCTCTGCACCTT
GGTGATCAAATAATTCGATAGCTTGTCGTAATAATGGCGGCATACTATC
AGTAGTAGGTGTTTCCCTTTCTTCTTTAGCGACTTGATGCTCTTGATCT

TCCAATACGCAACCTAAAGTAAAATGCCCCACAGCGCTGAGTGCATAT
AATGCATTCTCTAGTGAAAAACCTTGTTGGCATAAAAAGGCTAATTGAT
TTTCGAGAGTTTCATACTGTTTTTCTGTAGGCCGTGTACCTAAATGTAC
TTTTGCTCCATCGCGATGACTTAGTAAAGCACATCTAAAACTTTTAGCG
TTATTACGTAAAAAATCTTGCCAGCTTTCCCCTTCTAAAGGGCAAAAGT
GAGTATGGTGCCTATCTAACATCTCAATGGCTAAGGCGTCGAGCAAAG
CCCGCTTATTTTTTACATGCCAATACAATGTAGGCTGCTCTACACCTAG
CTTCTGGGCGAGTTTACGGGTTGTTAAACCTTCGATTCCGACCTCATTA
AGCAGCTCTAATGCGCTGTTAATCACTTTACTTTTATCTAATCTAGACAT
ATTCGTTTAATATCATAAATAATTTATTTTATTTTAAAATGCGCGGGTGC
AAAGGTAAGAGGTTTTATTTTAACTACCAAATGTTTTCGGAAGTTTTTTC
GCTTTTCTTTTTCTATCGTTTCTCAGACTCTCTTAGCGAAAGGGAAAGA
AGGTAAAGAAGAAAAACAAAACGCCTTTTCTTTTTTGCACCCGCTTTCC
AAGAGAAGAAAGCCTTGTTAAATTGACTTAGTGTAAAAGCGCAGTACT
GCTTGACCATAAGAACAAAAAAATCTCTATCACTGATAGGGATAAAGTT
TGGAAGATAAAGCTAAAAGTTCTTATCTTTGCAGTCTCCCTATCAGTGA
TAGAGACGAAATAAAGACATATAAAAGAAAAGACACCATGGATAAGAAA
TACTCAATAGGCTTAGATATCGGCACAAATAGCGTCGGATGGGCGGTG
ATCACTGATGAATATAAGGTTCCGTCTAAAAAGTTCAAGGTTCTGGGAA
ATACAGACCGCCACAGTATCAAAAAAAATCTTATAGGGGCTCTTTTATT
TGACAGTGGAGAGACAGCGGAAGCGACTCGTCTCAAACGGACAGCTC
GTAGAAGGTATACACGTCGGAAGAATCGTATTTGTTATCTACAGGAGA
TTTTTTCAAATGAGATGGCGAAAGTAGATGATAGTTTCTTTCATCGACTT
GAAGAGTCTTTTTTGGTGGAAGAAGACAAGAAGCATGAACGTCATCCT
ATTTTTGGAAATATAGTAGATGAAGTTGCTTATCATGAGAAATATCCAA
CTATCTATCATCTGCGAAAAAAATTGGTAGATTCTACTGATAAAGCGGA
TTTGCGCTTAATCTATTTGGCCTTAGCGCATATGATTAAGTTTCGTGGT
CATTTTTTGATTGAGGGAGATTTAAATCCTGATAATAGTGATGTGGACA
AACTATTTATCCAGTTGGTACAAACCTACAATCAATTATTTGAAGAAAAC
CCTATTAACGCAAGTGGAGTAGATGCTAAAGCGATTCTTTCTGCACGA
TTGAGTAAATCAAGACGATTAGAAAATCTCATTGCTCAGCTCCCCGGT
GAGAAGAAAAATGGCTTATTTGGGAATCTCATTGCTTTGTCATTGGGTT
TGACCCCTAATTTTAAATCAAATTTTGATTTGGCAGAAGATGCTAAATTA

CAGCTTTCAAAAGATACTTACGATGATGATTTAGATAATTTATTGGCGC
AAATTGGAGATCAATATGCTGATTTGTTTTTGGCAGCTAAGAATTTATC
AGATGCTATTTTACTTTCAGATATCCTAAGAGTAAATACTGAAATAACTA
AGGCTCCCCTATCAGCTTCAATGATTAAACGCTACGATGAACATCATCA
AGACTTGACTCTTTTAAAAGCTTTAGTTCGACAACAACTTCCAGAAAAG
TATAAAGAAATCTTTTTTGATCAATCAAAAAACGGATATGCAGGTTATAT
TGATGGGGGAGCTAGCCAAGAAGAATTTTATAAATTTATCAAACCAATT
TTAGAAAAAATGGATGGTACTGAGGAATTATTGGTGAAACTAAATCGTG
AAGATTTGCTGCGCAAGCAACGGACCTTTGACAACGGCTCTATTCCCC
ATCAAATTCACTTGGGTGAGCTGCATGCTATTTTGAGAAGACAAGAAG
ACTTTTATCCATTTTTAAAAGACAATCGTGAGAAGATTGAAAAAATCTTG
ACTTTTCGAATTCCTTATTATGTTGGTCCATTGGCGCGTGGCAATAGTC
GTTTTGCATGGATGACTCGGAAGTCTGAAGAAACAATTACCCCATGGA
ATTTTGAAGAAGTTGTCGATAAAGGTGCTTCAGCTCAATCATTTATTGA
ACGCATGACAAACTTTGATAAAAATCTTCCAAATGAAAAAGTACTACCA
AAACATAGTTTGCTTTATGAGTATTTTACGGTTTATAACGAATTGACAAA
GGTCAAATATGTTACTGAAGGAATGCGAAAACCAGCATTTCTTTCAGGT
GAACAGAAGAAAGCCATTGTTGATTTACTCTTCAAAACAAATCGAAAAG
TAACCGTTAAGCAATTAAAAGAAGATTATTTCAAAAAAATAGAATGTTTT
GATAGTGTTGAAATTTCAGGAGTTGAAGATAGATTTAATGCTTCATTAG
GTACCTACCATGATTTGCTAAAAATTATTAAAGATAAAGATTTTTTGGAT
AATGAAGAAAATGAAGATATCTTAGAGGATATTGTTTTAACATTGACCTT
ATTTGAAGATAGGGAGATGATTGAGGAAAGACTTAAAACATATGCTCAC
CTCTTTGATGATAAGGTGATGAAACAGCTTAAACGTCGCCGTTATACTG
GTTGGGGACGTTTGTCTCGAAAATTGATTAATGGTATTAGGGATAAGC
AATCTGGCAAAACAATATTAGATTTTTTGAAATCAGATGGTTTTGCCAAT
CGCAATTTTATGCAGCTGATCCATGATGATAGTTTGACATTTAAAGAAG
ACATTCAAAAAGCACAAGTGTCTGGACAAGGCGATAGTTTACATGAAC
ATATTGCAAATTTAGCTGGTAGCCCTGCTATTAAAAAAGGTATTTTACA
GACTGTAAAAGTTGTTGATGAATTGGTCAAAGTAATGGGGCGGCATAA
GCCAGAAAATATCGTTATTGAAATGGCACGTGAAAATCAGACAACTCAA
AAGGGCCAGAAAAATTCGCGAGAGCGTATGAAACGAATCGAAGAAGG
TATCAAAGAATTAGGAAGTCAGATTCTTAAAGAGCATCCTGTTGAAAAT

ACTCAATTGCAAAATGAAAAGCTCTATCTCTATTATCTCCAAAATGGAA
GAGACATGTATGTGGACCAAGAATTAGATATTAATCGTTTAAGTGATTA
TGATGTCGATCACATTGTTCCACAAAGTTTCCTTAAAGACGATTCAATA
GACAATAAGGTCTTAACGCGTTCTGATAAAAATCGTGGTAAATCGGATA
ACGTTCCAAGTGAAGAAGTAGTCAAAAAGATGAAAAACTATTGGAGAC
AACTTCTAAACGCCAAGTTAATCACTCAACGTAAGTTTGATAATTTAAC
GAAAGCTGAACGTGGAGGTTTGAGTGAACTTGATAAAGCTGGTTTTAT
CAAACGCCAATTGGTTGAAACTCGCCAAATCACTAAGCATGTGGCACA
AATTTTGGATAGTCGCATGAATACTAAATACGATGAAAATGATAAACTT
ATTCGAGAGGTTAAAGTGATTACCTTAAAATCTAAATTAGTTTCTGACTT
CCGAAAAGATTTCCAATTCTATAAAGTACGTGAGATTAACAATTACCAT
CATGCCCATGATGCGTATCTAAATGCCGTCGTTGGAACTGCTTTGATTA
AGAAATATCCAAAACTTGAATCGGAGTTTGTCTATGGTGATTATAAAGT
TTATGATGTTCGTAAAATGATTGCTAAGTCTGAGCAAGAAATAGGCAAA
GCAACCGCAAAATATTTCTTTTACTCTAATATCATGAACTTCTTCAAAAC
AGAAATTACACTTGCAAATGGAGAGATTCGCAAACGCCCTCTAATCGA
AACTAATGGGGAAACTGGAGAAATTGTCTGGGATAAAGGGCGAGATTT
TGCCACAGTGCGCAAAGTATTGTCCATGCCCCAAGTCAATATTGTCAA
GAAAACAGAAGTACAGACAGGCGGATTCTCCAAGGAGTCAATTTTACC
AAAAAGAAATTCGGACAAGCTTATTGCTCGTAAAAAAGACTGGGATCC
AAAAAAATATGGTGGTTTTGATAGTCCAACGGTAGCTTATTCAGTCCTA
GTGGTTGCTAAGGTGGAAAAAGGGAAATCGAAGAAGTTAAAATCCGTT
AAAGAGTTACTAGGGATCACAATTATGGAAAGAAGTTCCTTTGAAAAAA
ATCCGATTGACTTTTTAGAAGCTAAAGGATATAAGGAAGTTAAAAAAGA
CTTAATCATTAAACTACCTAAATATAGTCTTTTTGAGTTAGAAAACGGTC
GTAAACGGATGCTGGCTAGTGCCGGAGAATTACAAAAAGGAAATGAGC
TGGCTCTGCCAAGCAAATATGTGAATTTTTTATATTTAGCTAGTCATTAT
GAAAAGTTGAAGGGTAGTCCAGAAGATAACGAACAAAAACAATTGTTT
GTGGAGCAGCATAAGCATTATTTAGATGAGATTATTGAGCAAATCAGTG
AATTTTCTAAGCGTGTTATTTTAGCAGATGCCAATTTAGATAAAGTTCTT
AGTGCATATAACAAACATAGAGACAAACCAATACGTGAACAAGCAGAA
AATATTATTCATTTATTTACGTTGACGAATCTTGGAGCTCCCGCTGCTTT
TAAATATTTTGATACAACAATTGATCGTAAACGATATACGTCTACAAAAG

AAGTTTTAGATGCCACTCTTATCCATCAATCCATCACTGGTCTTTATGAA
ACACGCATTGATTTGAGTCAGCTAGGAGGTGACTGAATTAATGCGGCT
GCAATTTTTTTGGGCGGGGCCGCCCAAAAAAATCCTAGCACCCTGCAG
CAGTACTGCTTGACCATAAGAACAAAAAAACTTCCGATAAAGTTTGGAA
GATAAAGCTAAAAGTTCTTATCTTTGCAGTATGACGGGAATGTACCCCA
GGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCA
ACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGATCTGTCGAC
TCTAGAGGATCCCCGGGTACCGAGCTCGAATTCACTGGCCGTCGTTTT
ACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGTAC
TTGTGCCTGTTCTATTTCCGAACCGACCGCTTGTATGAATCCATCAAAA
TTCGTTTTCTCTATGTTGGATTCCTTGTTGCTCATATTGTGATGATAATT
TCTACAAATATAGTCATTGGTAACTATCTATGAAACTGTTTGATACTTTT
ATAGTTGATTAAACTTGTTCATGGCATTTGCCTTAATATCATCCGCTATG
TCAATGTAGGGTTTCATAGCTTTGTAGTCGCTGTGTCCCGTCCATTTCA
TGACCACCTGTGCCGGGATTCCGAGAGCCAGCGCATTGCAGATGAAT
GTCCTTTTTCCTGCATGGGTACTGAGCAAAGCGTATTTGGGTGTGACT
TCATCAATACGTTCATTTCCCTTGTAGTAGGTTTCCCGTACAGGCTCGT
TGATTTCTGCCAGTTCGCCCAGCTCTTTCAGGTAATCGTTCATCTTCTG
GTTGCTGATGACGGGCAGAGCCATGTAATTCTCGAAATGGATGTCCTT
GTATTTGTCCAGTATGGCTTTGCTGTATTTGTTCAGTTCAATCGTCAGG
CTGTCGGCAGTCTTGACTGTGGTTATTTCGATGTGGTCGGACTTCACA
TCGCTTCTTTTCAGATTGCGAACATCCGAATACCGCAAACTCGTAAAGC
AGCAGAACAGGAAAACATCACGCACACGTTCCAGGTATTGCTTATCCT
TGGGTATCTGGTAGTCTTTCAGCTTGTTCAGTTCATCCCAAGTCAGGAA
GATTACTTTTTTCGAGGTGGTTTTCAGTTTCGGTTTGAACGTATCGTAT
GCAATGTTCTGATGATGTCCTTTCTTGAAGCTCCAGCGCAGGAACCAT
TTGAGGAATCCCATTTGCTTGCCGATGGTGCTGTTTCTCATATCCTTGG
TGTCACGCAGGAAGTTGACGTATTCGTTCAATCCAAACTCGTTGAAATA
GTTGAACGTTGCATCCTCCTTGAACTCTTTGAGGTGGTTCCTCACTGCT
GCAAATTTTTCATAGGTGGATGCCGTCCAGTTATTCTGGTTACCGCACT
CTTTTACAAACTCATCGAACACCTCCCAAAAGCTGACAGGGGCTTCTT
CCGGCTGTTCTTCACTGGTGTCTTTCATTCTCATGTTGAAAGCTTCCTT
CAACTGTTGGGTCGTTGGCATGACCTCCTGCACCTCAAATTCCTTGAA

AATATTCTGGATTTCGGCATAGTATTTCAGCAAGTCCGTATTGATTTCG
GCTGCACTTTGCTTTAGCTTGTTGGTACATCCGTTCTTTACCCGCTGCT
TATCTGCATCCCATTTGGCTACGTCAATCCGGTAGCCCGTTGTAAACTC
GATACGTTGGCTGGCAAAGATGACACGCATACGGATGGGTACGTTCTC
TACGATTGGCACACCGTTCTTTTTCCGGCTCTCCAATGCAAAAATGATG
TTGCGCTTGATATTCATAATTGGGTGCGTTTGAAATTCTACACCCAAAT
ATACACCCAATTATTGAGATAGCAAAAGACATTTAGAAACATTTACTTTT
ACTCTATATTGTAATTTACACTTGATTATCAGTCGTTTGCAGTCTTATGA
TATTCTGTGAAAGTATAAGTTCGAGAGCCTGTCTCTCCGCAAAAAACG
CTGAAAATCAGCAGATTGCAAAACAAACACCCTGTTTTACACCCAAGAA
TGTAAAGTCGGCTGTTTTTGTTTTATTTAAGATAATACAACCACTACATA
ATAAAAGAGTAGCGATATTAAAAGAATCCGATGAGAAAAGACTAATATT
TATCTATCCATTCAGTTTGATTTTTCAGGACTTTACATCGTCCTGAAAGT
ATTTGTTGGTACCGGTACCGAGGACGCGTAAACATTTACAGTTGCATG
TGGCCTATTGTTTTTAGCCGTTAAATATTTTATAACTATTAAATAGCGAT
ACAAATTGTTCGAAACTAATATTGTTTATATCATATATTCTCGCATGTTTT
AAAGCTTTATTAAATTGATTTTTTGTAAACAGTTTTTCGTACTCTTTGTTA
ACCCATTTCATTACAAAAGTTTCATATTTTTTTCTCTCTTTAAATGCCATT
TTTGCTGGCTTTCTTTTTAATACAATTAATGTGCTATCCACTTTAGGTTT
TGGATGGAAATAATACCTAGGAATTTTTGCTAATATAGAAATATCTACCT
CTGCCATTAACAGCAATGCTAGTGATCTGTTTGTATCTAATAACATTTTA
GCAAAACCATATTCCACTATTAAATAACTTATTGTGGCTGAACTTTCAAA
AACAATTTTTCGAATTATATTTGTGCTTATGTTGTAAGGTATGCTGCCAA
ATATTTTATATGGATTGTGGCTAGGAAATGTAAATTTCAGTATATCATCA
TTTACTATTTGATAGTTAGGATAATTTAAGAGCTTATTACGAGTTACCTC
ACATAATTTAGAATCAATTTCTATCGCCGTTACAAAATTACATCTCTTTA
CCAATCCAGCAGTAAAATGACCTTTCCCTGCACCTATTTCAAAGATGTT
ATCTTTTTCATCTAAACTTATGCAATTCATTATTTTTTCTATGTGATATTT
TGAAGTAATAAAATTTTGACTATCTTTTATATTTACTTTGTTCATTATAAC
CTCTCCTTAATTTATTGCATCTCTTTTCGAATATTTATGTTTTTTGAGAAA
AGAACGTACTCATGGTTCATCCCGATATGCGTATCGGTCTGTATATCAG
CAACTTTCTATGTGTTTCAACTACAATAGTCATCTATTCTCATCTTTCTG
AGTCCACCCCCTGCAAAGCCCCTCTTTACGACATAAAAATTCGGTCGG

AAAAGGTATGCAAAAGATGTTTCTCTCTTTAAGAGAAACTCTTCGGGAT
GCAAAAATATGAAAATAACTCCAATTCACCAAATTATATAGCGACTTTTT
TACAAAATGCTAAAATTTGTTGATTTCCGTCAAGCAATTGTTGAGCAAA
AATGTCTTTTACGATAAAATGATACCTCAATATCAACTGTTTAGCAAAAC
GATATTTCTCTTAAAGAGAGAAACACCTTTTTGTTCACCAATCCCCGAC
TTTTAATCCCGCGGCCATGATTGAAAAAGGAAGAGTATGAGTATTCAAC
ATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGT
TTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCA
GTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTA
AGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCA
CTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCG
GGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGG
TTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAG
TAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGG
CCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTT
TTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAAC
CGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATG
CCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTA
CTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGAT
AAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTT
ATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATT
GCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTAC
ACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGC
TGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGT
TTACTCATAACGCGTCAATTCGAGGGGGATCAATTCCGTGATAGGTGG
GCTGCCCTTCCTGGTTGGCTTGGTTTCATCAGCCATCCGCTTGCCCTC
ATCTGTTACGCCGGCGGTAGCCGGCCAGCCTCGCAGAGCAGGATTCC
CGTTGAGCACCGCCAGGTGCGAATAAGGGACAGTGAAGAAGGAACAC
CCGCTCGCGGGTGGGCCTACTTCACCTATCCTGCCCGGCTGACGCCG
TTGGATACACCAAGGAAAGTCTACACGAACCCTTTGGCAAAATCCTGT
ATATCGTGCGAAAAAGGATGGATATACCGAAAAAATCGCTATAATGAC
CCCGAAGCAGGGTTATGCAGCGGAAAACGGAATTGATCCGGCCACGA
TGCGTCCGGCGTAGAGGATCTGAAGATCAGCAGTTCAACCTGTTGATA

GTACGTACTAAGCTCTCATGTTTCACGTACTAAGCTCTCATGTTTAACG
TACTAAGCTCTCATGTTTAACGAACTAAACCCTCATGGCTAACGTACTA
AGCTCTCATGGCTAACGTACTAAGCTCTCATGTTTCACGTACTAAGCTC
TCATGTTTGAACAATAAAATTAATATAAATCAGCAACTTAAATAGCCTCT
AAGGTTTTAAGTTTTATAAGAAAAAAAAGAATATATAAGGCTTTTAAAGC
TTTTAAGGTTTAACGGTTGTGGACAACAAGCCAGGGATGTAACGCACT
GAGAAGCCCTTAGAGCCTCTCAAAGCAATTTTGAGTGACACAGGAACA
CTTAACGGCTGACATGGGAATTCCCCTCCACCGCGGTGG (SEQ ID NO:
3).

Example 2. CRISPR integration on the chromosome of *Bacteroides thetaiotaomicron*

[0084] The pNBU2.CRISPR plasmids were transformed to *E. coli* S-17 lambda-*pir*, followed by delivery to *Bacteroides* cells via conjugation. In this specific example, the pNBU2-CRISPR plasmid encodes the intN2 tyrosine integrase, which mediates sequence-specific recombination between the attN2 site on pNBU2-CRISPR plasmid and one of two attBT sites located in the 3' ends of the two tRNA-Ser genes, BT_t70 (attBT2-1) and BT_t71 (attBT2-2), on the chromosome *of B. thetaiotaomicron VPI-5482* (*Bt* in short). Insertion of the pNBU2-CRISPR plasmid inactivates one of the two tRNA-Ser genes, and simultaneous insertion into both BT_t70 and BT_t71 is unlikely because of the essentiality of tRNA-Ser.

[0085] In this specific example, three plasmids were constructed which express a non-targeting control guide RNA (termed 'M'), a guide RNA targeting *tdk*_Bt (BT_2275) and *susC*_Bt (BT_3702) coding sequences in the *Bt* genome. The *tdk* gene encodes thymidine kinase, and the *susC* gene encodes outer membrane protein involved in starch binding in *B. thetaiotaomicron.* The protospacer sequence for *tdk*_Bt is 5'-AATTGAGGCATCGGTCCGAA-3' (SEQ ID NO: 4), and that for *susC*_Bt is 5'-ATGACGGGAATGTACCCCAG-3' (SEQ ID NO: 5). *In silico* analyses of the non-targeting control protospacer sequence (5'-TGATGGAGAGGTGCAAGTAG-3'; SEQ ID NO: 6) against *Bacteroides* genomes did not result in any significant sequence matches, so no 'off-target'

activity is expected. The sgRNA scaffold sequence was 5'-GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTT-3" (SEQ ID NO:7). The resulting plasmids are called pNBU2-CRISPR.M, pNBU2-CRISPR.*tdk*_Bt, and pNBU2-CRISPR.*susC*_Bt, respectively.

[0086] The pNBU2-CRISPR plasmids were conjugated to *Bt* cells with erythromycin selection, resulting in 500-1000 colonies per conjugation (**FIGS. 3A, 3B**). Due to a lack of origin of replication for *Bacteroides*, these plasmids cannot be maintained in *Bacteroides* cells. The erythromycin resistant colonies were likely chromosomal integrants. Four colonies from each conjugation, labeled as M (M1, M2, M3, M4), *tdk*_Bt (T1, T2, T3, T4) and *susC*_Bt (S1, S2, S3, S4), were picked for colony PCR screening of CRISPR integration at either one of the two attBT loci (**FIG. 3C**). Outside primers for each locus were used to identify PCR amplicon sizes: either wild-type or with the plasmid integrated. Since the whole plasmid is about 10 kb, it is unlikely to obtain a PCR amplicon for its integration using colony PCR, while it is possible using purified genomic DNA. For each locus, PCR using outside primers was carried out. If no integration occurred, a PCR amplicon about 0.5 kb (attBT2-1 locus) or 0.65 kb (attBT2-2 locus) is expected on a gel; otherwise, no PCR product is expected. In addition, PCR amplifying left junction of integration was performed using an outside primer binding to chromosomal sequence and an internal primer binding to ermG coding sequence from integration plasmid. If an integration occurred, a PCR product should be seen on gel; otherwise, no PCR product is expected. The PCR amplification was carried out with Q5 Hot-start 2X Master Mix (New England Biolabs), using the following cycling conditions: 98°C for 30 seconds for initial denaturation; 25 cycles of 98°C for 20 seconds, 58°C for 20 seconds, and 72°C for 45 seconds; and a final extension at 72°C for 5 minutes. The PCR products were resolved on a 1% agarose gel. As shown in **FIG. 3C**, based on the sizes of PCR A (attBT2-1 locus) and PCR B (attBT2-2 locus) using the outside primers, it is deduced that clones M1-M4, T1, T2, T4, S1, S3, S4 all harbor CRISPR cassettes integrated at the attBT2-1 locus, while clones T3 and S2 integrated at the attBT2-2 locus on the *Bt* chromosome. The junctions between chromosome and plasmid sequences were further confirmed correct

by PCR (PCR C and D) and Sanger DNA sequencing for selected clones M1, M2, T1, T3, S1 and S2.

Example 3. Inducible CRISPR killing of individual *B. thetaiotaomicron* strains

[0087] For selected *B. thetaiotaomicron* CRISPR integrants M1, T1 and S1, all with an inducible CRISPR cassette integrated at the attBT2-1 locus, the inducible CRISPR/Cas9 mediated cell killing was investigated either on BHI blood agar plate or in TYG liquid medium (**FIG. 4A** and **FIG. 4B**, respectively). Single colonies of M1 and T1 strains were grown anaerobically in a coy chamber (Coy Laboratory Products Inc.) overnight in falcon tube cultures containing 5 ml TYG liquid medium supplemented with 200 µg/ml gentamicin (Gm) and 25 µg/ml erythromycin (Em). The cultures were diluted (10^{-6}), and 100µl were spread onto BHI blood agar plates (Gm 200 µg/ml and Em 25 µg/ml) supplemented with anhydrotetracycline (aTc) at concentrations of 0 and 100 ng/ml, respectively. The agar plates were incubated anaerobically at 37°C for 2-3 days. About 10^3-10^4 CFU (colony forming units) were obtained on blood agar plates without aTc present (0 ng/ml) for all strains. No CFU formation was observed on blood agar plates with aTc present (100 ng/ml) for the T1strain, while 10^3-10^4 CFU were still obtained for the M1 strain (**FIG. 4A**).

[0088] Similarly, except M1, no cell growth was observed in liquid tube cultures containing TYG medium supplemented with aTc at 100 ng/ml, even after 4 days of anaerobic incubation at 37°C. However, slight growth was observed for clones T1 and S2 at aTc concentration of 10 ng/ml after 24h of anaerobic incubation, suggesting higher aTc concentration is desired for complete depletion (**FIG. 4B**). The data shows a chromosomally integrated CRISPR/Cas9 system is activated by exogenously provided inducer aTc to generate lethal genomic DNA cleavages guided by a targeting RNA (*tdk_Bt* or *susC_Bt*), resulting in loss of cell viability.

Example 4. Targeted, inducible CRISPR killing of *B. thetaiotaomicron* cells in a mixed population *in vitro*

[0089] A mixed culture of CRISPR integrated *Bt* strains expressing either a non-targeting (M) or targeting (*tdk*_Bt or *susC*_Bt) guide RNA was employed to demonstrate targeted CRISPR killing of a specific strain in a mixed population *in vitro*. Equal amounts of exponential growth phase cultures were mixed and incubated anaerobically in 5 ml TYG liquid medium supplemented with aTc at final concentrations of 0, 10 or 100 ng/ml, respectively. After 24h, all cultures grew up to about 1.3 OD_{600nm} (**FIG. 5A**). For one set of the aTc treated cultures (M1 + T1, supplemented with aTc at 0, 10 and 100 ng/ml, respectively), PCR and DNA sequencing were performed on the region of guide RNA (P1-N20 sgRNA scaffold). From the DNA sequencing chromatograms, aTc treated cultures (aTc 10 and aTc 100) were only those cells harboring non-targeting, control guide RNA (M), while the culture without aTc treatment (aTc 0) is a mixed population of cells harboring both non-targeting guide RNA (M) and *tdk*_Bt targeting guide RNA (**FIG. 5B**).

[0090] One of the aTc-treated cultures (M1 + S1-aTc100) was diluted and spread on BHI blood agar without aTc supplementation to obtain single colonies. Individual colonies as well as a scrape of colonies on the agar plate were analyzed by PCR of the gRNA region followed by Sanger DNA sequencing. It was found that all the individual colonies and the colony mixture only harbored non-targeting, control gRNA, suggesting that the *susC_Bt* gRNA harboring integrants were successfully depleted by aTc inducible CRISPR killing, and not growth inhibition due to induced Cas9 protein expression *per se* in the tube culture (**FIG. 5C**).

[0091] A similar experiment was performed for mixed culture of M1+T1, resulting in the same observations. These data demonstrated targeted, inducible CRISPR killing of *B. thetaiotaomicron* cells in a mixed population *in vitro*.

Example 5. Long-term growth and targeted CRISPR killing of *B. thetaiotaomicron* strains without antibiotic selection

[0092] Serial limiting dilution was used for testing long-term growth and targeted killing in liquid cultures without any antibiotic selection. The CRISPR integrated *Bt* strains M1, T1 and S1 were inoculated in TYG medium from glycerol stocks and grew anaerobically at 37°C in a coy chamber for 24 h.

The culture was re-inoculated into fresh TYG medium at a dilution of 1:100 and grown anaerobically for another 24h. The same procedures were repeated 4 times, resulting in about 5 days, 40 generations of growth in liquid medium. The cultures were then spread onto BHI blood agar plates forming single colonies. The antibiotic resistances of about 50 colonies each were tested on BHI blood agar plates supplemented with either Gm (200 μg/ml) or Em (25 μg/ml). All colonies tested were resistant to both antibiotics, suggesting the long-term maintenance of the CRISPR cassettes in integrated *Bt* strains.

Example 6. CRISPR integration on the chromosome of *Bacteroides vulgatus*

[0093] The inducible CRISPR cassettes were also integrated on the chromosome of *Bacteroides vulgatus* ATCC 8482 strain (*Bv* in short). The pNBU2.CRISPR plasmid used for chromosomal integration on *Bv* was constructed as in Example 1, except that a guide RNA targeting *susC*_Bv (BVU_RS05095) on *Bv* genome was cloned. The 20 bp protospacer sequence for expressing *susC*_Bv guide RNA is 5'-ATTCGGCAGTGAATTCCAGA-3' (SEQ ID NO: 8).

[0094] The pNBU2-CRISPR plasmids expressing either non-targeting, control guide RNA (M) or *susC*_Bv targeting guide RNA were transformed to *E. coli* S17 lambda-pir, and conjugated to *Bv* cells. About 10,000 Em resistant colonies were obtained for each conjugation. Seven colonies (labeled VM1, VM2, VM3, VM4, VM5, VM6 and VM7) were picked from the non-targeting control conjugation plate, and five colonies (labeled V1, V2, V3, V4, V5) were picked from the *susC*_Bv targeting conjugation plate, respectively, for chromosomal CRISPR integration screening by colony PCR. There are three potential NBU2 integrase recognition loci on *Bv* chromosome, attBv.3-1 (tRNA-Ser, BVU_RS10595), attBv.3-2 (BVU_RS21625) and attBv.3-3 (intergenic region, nucleotide coordinates from 3,171,462 to 3,171,474). For each locus, PCR using outside primers was carried out. If no integration occurred, a PCR amplicon about 0.5 kb is expected on a gel; otherwise, no PCR product is expected. For attBv.3-1 locus, PCR amplifying left junction of integration was performed using an outside primer binding to chromosomal

sequence and an internal primer binding to *ermG* coding sequence from integration plasmid. If an integration occurred at the attBv.3-1 locus, about 0.6 kb PCR product should be seen on a gel; otherwise, no PCR product is expected. As shown in **FIG. 6**, for non-targeting, control guide RNA integrants (VM), all seven clones harbored CRISPR integration at attBv.3-1 locus; for *susC*_Bv targeting guide RNA integrants (V), clone V1 may harbor CRISPR integration at both attBv.3-1 and attBv.3-2 loci; clone V2 may harbor CRISPR integration at both attBv.3-1 and attBv.3-3 loci, and for clones V3, V4 and V5, they all harbored CRISPR cassette integration at the attBv.3-1 locus only. This data set shows that the NBU2 based CRISPR integration system works well in *Bacteroides vulgatus* strains.

Example 7. Targeted, inducible CRISPR killing of *Bacteroides vulgatus*

[0095] As in Example 3, individual CRISPR integrated Bv strains VM1 (expressing non-targeting guide RNA) and V1, V2, V3, V4, V5 (all expressing susC_Bv guide RNA) were anaerobically grown in TYG liquid medium overnight. Then the cultures were re-inoculated (1:100 dilution) to fresh TYG medium supplemented with 100 ng/ml aTc, followed by anerobic growth at 37°C for 24h. Only VM1 culture grew to high turbidity, while other cultures expressing targeting guide RNA exhibited no growth.

[0096] As in Example 4, a mixed culture of VM1 (non-targeting guide RNA) and V3 (expressing *susC*_Bv guide RNA) were treated with aTc at 100 ng/ml, followed by anaerobic incubation in TYG liquid medium for 24h. The culture grew up to high turbidity. PCR and DNA sequencing of the guide RNA region of the mixed culture indicates that the treated culture contained only cells expressing non-targeting, control guide RNA. This demonstrates targeted CRISPR killing of specific *B. vulgatus* strain in a mixed cell population upon the addition of an inducer.

Example 8. CRISPR integration on the chromosome of other *Bacteroides* strains

[0097] The NBU2 integrase recombination tRNA-ser sites (13 bp) are conserved and exist in many other Bacteroides strains as well, including *Bacteroides cellulosilyticus, Bacteroides fragilis, Bacteroides helcogenes,*

Bacteroides ovatus, Bacteroides salanitronis, Bacteroides uniformis and Bacteroides xylanisolvens, based on published genome sequences. The inducible CRISPR cassette expressing a targeting guide RNA can be integrated on the chromosome of these *Bacteroides* strains (as described in Examples 3 and 6), and targeted CRISPR killing of a specific strain expressing a targeting guide RNA can be achieved by treatment with aTc inducer (as described in Examples 4, 5 and 7).

[0098] In situations in which there are no NBU2 integrase sites on the chromosome of a specific species, these 13 base-pair DNA sequences can be readily inserted on the chromosome via recombination (*e.g.*, Cre/loxP) or allelic exchange as described in the art to enable chromosomal CRISPR integration and targeted strain killing.

Example 9. CRISPR integrated *Bacteroides* strains delivered as a human probiotic

[0099] CRISPR integrated *Bacteroides* strains can be used as a method to reduce the relative abundance of wildtype *Bacteroides* strains in the human gut. Anti-PD-1 immunotherapy in human melanoma patients has been shown to differ depending on the presence of *Bacteroides* strains in gut microbiota (Gopalakrishnan et al., *Science*, 2018, 359:97-103). Non-responders have increased relative amounts of *Bacteroides* strains in their microbiota when compared with immunotherapy responders. Before immunotherapy is started, *Bacteroides* strain elimination, via the induced integrated CRISPR system, could be performed to improve the outcome of human cancer immunotherapy.

Example 10. CRISPR-targeted reduction in the representation of a *Bacteroides* member of a model human gut microbiota

[0100] The gut microbiota is an important determinant of many aspects of human health as well as various diseases. The rapidly growing appreciation of its myriad effects on host biology has stimulated efforts to develop microbiota-directed therapeutics. Many of these nascent therapies have shown striking dependencies on the initial configuration of the gut microbiota. As such, tools for the delineation of ecological relationships between

members of a microbiota (e.g., niche partitioning including the underpinnings of competition/cooperation for nutrients), will play a vital role in the advancement of effective microbiota-directed therapeutics (e.g. Patnode et al., 2019).

[0101] In order to study interactions among gut microbial community members, we developed a genetic system with the capability of independently perturbing the representation of specific gut bacterial strains in a defined model human gut microbiota. This system was implemented using *Bacteroides thetaiotaomicron*, a prominent and common member of the adult human gut microbiota in healthy individuals.

[0102] We generated a set of mutants in *Bacteroides thetaiotaomicron* strain VPI-5482 (Bt). Mutants contained (i) an anhydrotetracycline-inducible (aTc) spCas9 gene, (ii) an erythromycin resistance cassette, and (iii) a constitutively active guide RNA that targets either random, non-genomic sequence (negative control), or one of two Bt genes (tdk and SusC).These cassettes were integrated at one of two genomic locations. Using these mutants, we documented potent aTc-inducible killing in plate assays and in liquid cultures (**FIGS. 7A-7C**).

[0103] We colonized germ-free mice with a consortium of 13 cultured human gut bacterial strains whose genomes had been sequenced (see below); this consortium included a Bt-CRISPR mutant with a tdk-targeting gRNA. Mice were singly-housed and fed a human diet high in saturated fats and low in fruits and vegetables. The low fiber, 'HiSF/LoFV' diet was supplemented with 10% (w/w) pea fiber: this formulation was previously shown to maintain the relative abundance of Bt at 15-20% in this community/diet context (Patnode et al., 2019). In treatment arms, one- or four-days post-gavage, drinking water was supplemented with aTc at 10 µg/mL (**FIG. 8A**); otherwise after day one post-gavage mice received drinking water containing 0.5% ethanol alone (i.e. the vehicle used to maintain aTc solubility). Short-read shotgun sequencing of fecal DNA was used to define the relative abundances of community members at strain level resolution. In addition, we quantified the absolute abundance of organisms by including two 'spike-in' bacterial taxa not found in the mammalian gut (see below for details).

[0104] The results revealed that a 35-fold reduction in the relative and in absolute abundances of Bt was achieved 2 days after initiation of aTc treatment in the 4-day treatment arm and had similar effects in the early treatment condition (50-fold relative abundance reduction). Although Bt abundance began increasing after reaching this nadir, it never reached the level observed in the control group (**FIG. 8B**).

[0105] The depletion of Bt was accompanied by significant changes in the relative abundances of several other *Bacteroides*: *B. cellulosilyticus*, *B. ovatus*, and *B. caccae* (**FIG. 8C,D**; Linear Mixed Model Marginal Means $P<0.05$). The pattern of change in absolute abundances of Bt and these other Bacteroides paralleled the relative abundance measurements.

[0106] In a related experiment, mice were colonized with the 13-member community, including a WT Bt strain, or a 12-member community, excluding Bt. These mice were singly-housed and fed the HiSF/LoFV+10% pea fiber diet ad libitum for 20 days post-gavage. COPRO-Seq analysis of DNA isolated from serially collected fecal samples disclosed that omission of WT Bt prior to installation of the consortium resulted in changes in the relative/absolute abundances of these other Bacteroides that were largely consistent with the effects of CRISPR-Bt knockdown (**FIGS. 9A-9B**).

A. In vitro growth assays

[0107] All growth assays were performed in a soft-sided anaerobic growth chamber (Coy Laboratory Products) under an atmosphere of 3% hydrogen, 20% CO_2, and 77% N_2. Stock solutions of anhydrotetracycline hydrochloride (37919, Millipore Sigma) were prepared at 2 mg/mL in ethanol and filter-sterilized (Millipore Sigma SLGV033RS). Stocks of Bt mutants were serially diluted and plated on blood agar plates ± 200 ng/mL anhydrotetracycline. After two days of growth, plates were imaged. Glycerol stocks of Bt mutants were colony purified and inoculated into 5 mL LYBHI containing 25 µg/mL erythromycin. After overnight incubation, cultures were diluted 1:50 in LYBHI medium containing 9 ng/mL aTc and a 200 µL aliquot was pipetted into wells of a 96-well full-area plate (Costar; Cat. No.; CLS3925). Plates were sealed with an optically clear membrane (Axygen; Cat. No.; UC500) and growth monitored via optical density (600 nm) every 15 minutes at 37°C (Biotek Eon with a BioStack 4).

B. Gnotobiotic mice

[0108] **Husbandry** - All experiments involving mice were carried out in accordance with protocols approved by the Animal Studies Committee of Washington University in St. Louis. Germ-free male C57/B6 mice (18-22 weeks-old) were singly-housed in cages located within thin-film, flexible plastic isolators and fed an autoclavable mouse chow (Envigo; Cat. No.: 2018S). Cages contained paper houses for environmental enrichment. Animals were maintained on a strict light cycle (lights on at 0600 h, off at 1900 h).

[0109] The HiSF/LoFV+10% pea fiber was produced using human foods, selected based on consumption patterns from the National Health and Nutrition Examination Survey (NHANES) database1. The diet was milled to powder (D90 particle size, 980 mm), and mixed with pea fiber at 10% (w/w) fiber (Rattenmaier; Cat. No.: Pea Fiber EF 100). This mixture was then extruded into pellets. The pellets were packaged, vacuum sealed, and sterilized by gamma irradiation (20-50 kilogreys). Sterility was confirmed by culturing the diet under aerobic and anaerobic conditions (atmosphere, 75% N_2, 20% CO_2, 5%H_2) at 37°C in TYG medium, and by feeding the diets to germ-free mice followed by short read shotgun sequencing (Community PROfiling by sequencing, COPRO-Seq) analysis of their fecal DNA.

[0110] Fresh sterile drinking water containing 0.5% ethanol or 10 µg/mL aTC was prepared every other day in gnotobiotic isolators.

[0111] **Colonization** - *Bacteroides caccae* TSDC17.2-1.2, *Bacteroides finegoldii* TSDC17.2-1.1, *Bacteroides massiliensis* TSDC17.2-1.1, *Collinsella aerofaciens* TSDC17.2-1.1, *Escherichia coli* TSDC17.2-1.2, *Odoribacter splanchnicus* TSDC17.2-1.2, *Parabacteroides distasonis* TSDC17.2-1.1, *Ruminococcaceae sp.* TSDC17.2-1.2, and *Subdoligranulum variabile* TSDC17.2-1.1. were cultured from a fecal sample collected from a lean co-twin in an obesity-discordant twin-pair [Twin Pair 1 in Ridaura et al. (2013)]. The annotated genome sequences of these isolates and of *Bacteroides ovatus* ATCC 8483, *Bacteroides vulgatus* ATCC 8482, *Bacteroides thetaiotaomicron* VPI-5482, *and Bacteroides cellulosilyticus* WH2 are described in Patnode et al 2019. *Bacteroides thetaiotaomicron* VPI-5482 mutants were generated according to the protocol defined above. Each of

these 13 strains were colony purified, grown to early stationary phase in TYGs or LYBHI medium (Goodman et al., 2019). Aliquots of the monocultures were stored at -80°C in 15% glycerol. Equivalent numbers of organisms were pooled (based on OD600 measurements) and aliquots maintained in 15% glycerol at 80°C until use. On experimental day 0, aliquots were thawed and introduced into gnotobiotic isolators. The bacterial consortium was administered to germ-free mice through a plastic tipped oral gavage needle (total volume, 300 µL per mouse).

[0112] Mice were switched to unsupplemented HiSF/LoFV diet ad libitum for four days prior to colonization. After colonization mice were started on HiSF/LoFV supplemented with 10% pea fiber. One day following gavage, all mice were started on drinking water containing 0.5% ethanol or anhydrotetracycline (10 µg/mL). After colonization and after aTc withdrawal, bedding (Aspen Woodchips; Northeastern Products) was replaced. Fresh fecal samples were collected, within seconds of being produced, from each animal on experimental days 0-8 and immediately frozen at -80°C.

C. COPRO-Seq analysis of relative and absolute abundances of community members

[0113] Frozen fecal samples, and cecal contents (collected at the time of euthanasia) were weighed in a 1.8 mL screw-top tube. Two bacteria strains not found in mammalian microbiota were 'spiked in' to each sample tube at a known concentration [30 µL of both $2.22x10^8$ cell/mL *Alicyclobacillus acidiphilus* DSM 14558 and 9.93×10^8 cell/mL of *Agrobacterium radiobacter* DSM 30147]. DNA extraction began by bead-beating samples with 250 µL 0.1 mm zirconia/silica beads and one 3.97 mm steel ball in 500 µL of 2x buffer A (200 mM Tris, 200 mM NaCl, 20 mM EDTA), 210 µL 20 % (wt:wt) sodium dodecyl sulfate, and 500 µL of phenol:chloroform:amyl alcohol (pH 7.9; 25:24:1) for four minutes (Biospec Minibeadbeater-96). After centrifuging at 3,220 g for 4 minutes, 420 µL of the aqueous phase was removed and purified (QIAquick 96 PCR purification kit; Qiagen) according to the manufacturer's protocol.

[0114] Sequencing libraries were prepared from purified DNA using the Nextera DNA Library Prep Kit (Illumina; Cat. No.: 15028211) and

combinations of custom barcoded primers (Adey). Libraries were sequenced using an Illumina NextSeq instrument [read length, 75 nt; sequencing depth, 1.02 x 106 ± 2.2 x 104 reads/sample (mean ± SD)]. Reads were mapped onto bacterial genomes with Bowtie II and relative abundances were calculated using read counts scaled by informative genome size (Hibberd et al., 2017). Samples with less than 100,000 reads were omitted from further analysis. We defined the absolute abundance of given community members using the relationship:

$$\rho_{species} = \frac{\alpha(1 - P_\alpha - P_r)}{P_\alpha W} \cdot P_{species}$$

(where P_i is fractional abundance of some species in the 13-member community $1 = \sum_{i=1}^{13} P_i$, lower case letters denote cell number, $P_{\alpha/r}$ is the fractional abundance of spike-in bacteria in the total $1 = P_\alpha + P_r + P_{community}$, α denotes *A. acidiphilus*, r denotes *A. radiobacter*, ρ is bacterial density (cells/mg feces), and W is fecal pellet mass (mg)).

D. Linear Modeling

[0115] Relative abundance data from the vehicle control arm and the four-day treatment arm for days post gavage 4-7 was modelled using linear models (R core team, 2018) in the form:

[0116] lm(log10(percent) ~ Condition*DPG, data = data)

[0117] Using estimated marginal means (Lenth, 2019), effects of condition by each day were tested for significance on the response scale.

Example 11. Vector construction

[0118] The stably maintained RepA CRISPR plasmids were constructed using Gibson cloning (NEBuild HIFI DNA Assembly Master Mix, New England Biolabs) of plasmid backbone (RP4-oriT, R6K ori, bla, ermG) from pExchangetdk, RepA from pBI143 (Smith et al., Plasmid, 1995, 34:211-222), and anhydrotetracycline (aTc) inducible CRISPR cassettes (P2-A21-tetR, P1TDP-GH023-SpCas9, P1-N20 sgRNA scaffold) assembled from synthetic DNAs or PCR of genomic DNA of *Streptococcus pyogenes* strain SF370. **FIG. 10** illustrates the plasmid design.

[0119] The plasmid backbone harbors R6K origin of replication and *bla* sequence for ampicillin selection in *E. coli, repA* sequence for replication in

Bacteroides, RP4-oriT sequence for conjugation and *ermG* sequence for erythromycin (Em) selection in *Bacteroides*.

[0120] The inducible CRISPR cassettes include aTc inducible SpCas9 under the control of TetR regulator (P2-A21-tetR, P1TDP-GH023-SpCas9), and constitutively expressed guide RNA under P1 promoter (P1-N20 sgRNA scaffold). The promoters and ribosomal binding sites are derived and engineered from regulatory sequences of *Bacteroides thetaiotaomicron* 16S rRNA genes, as described in Lim et al., *Cell*, 2017, 169:547-558. The guide RNA is a nucleotide sequence that is homologous to a coding DNA sequence, or non-coding DNA sequence, or a non-targeting scramble nucleotide sequence. This sequence can be of any form as long as it is compatible with protospacer adjacent motif (PAM) requirements of different Cas9 homologs. The guide RNA can be either in separate transcriptional units of tracrRNA and crRNA or fused into a hybrid chimeric tracr/crRNA single guide(sgRNA).

[0121] The DNA sequence for the above plasmid is presented in SEQ ID NO: 9:

Plasmid (pRepA-CRISPR.susC_Bt) DNA sequence (9,321 bp)
CTCGAGTCCCCGACCGATGATTTTTAAGTGACTGATTTTGTGCT
GTTTTGGGGGTATATTAAGAATAAAAGAAATAGAATAAGTTAAGT
ACTTGATACACAATATAGGGCATTTTCCATATTGGAAATTCTCAT
TTTCCAATCTAGAAAATACCGATTTCCTAATATGATACTAAATAG
GAAAATAATATTTCCCTTAATATTGTTTTTATGGAAAATAATAGTT
TACTTTGTGGAGAATAATATTTCCCAAAAACATATCAAAATGGAA
AATAAAAAAGCAGTTAAGTTAACCGATTTTCAAAAGAAGCAAGAA
AATCCTTTTATGAAAACAAGCTATAGAGGTATTGAAAATCATGTT
GTTAAAAAGTATAAGAGTAATAGTGGTGGCGATAAGAGAGCTGT
AGTAGCTTTAGCCGACACTGAAACTGGAGAAGTGTTTAAGACTT
CGTTTATCCGTCAAATAGAAGTAGATGAAGAACAATTCACTAAAT
TGTATCTTTCTAACTTTGCTGCATTCTTTGACCTATCACAAGCAG
CTATTCGGGTTTTTGGTTACTTTATGACCTGCATGAAACCCAAAA
ATGATTTAATCATTTTCAATAGAAAAAAATGCCTAGAATATACCA
AATACAAAACAGACAAAGCCGTTTATAAAGGACTTGCAGAACTT
GTAAAAGCTGAAATCATAGCCCGAGGACCAGCCGATAATCTTTG

GTTTATTAATCCTCTGATAGTATTCAATGGTGACCGAGTGACATT
TGCTAAAACATACGTTTCGGAAAAAGACTTTAGCTGCCCAAAAG
AAAGAAGAAGCAGAGAAACGACAATTATCACTTGGCTTTGATGA
ACAGTAACACTCCATTGAGTGAAGCTGCCGTTTGGTCGCTCCCC
TTTGGGCGGGGGGGGGATAGATAAAGTTCCTCTATGTAAAGTTAT
AATGGGGGATGAAGGCAAGGTCGCTAACCTTACCGAGGACGCG
TAAACATTTACAGTTGCATGTGGCCTATTGTTTTTAGCCGTTAAA
TATTTTATAACTATTAAATAGCGATACAAATTGTTCGAAACTAATA
TTGTTTATATCATATATTCTCGCATGTTTTAAAGCTTTATTAAATT
GATTTTTTGTAAACAGTTTTTCGTACTCTTTGTTAACCCATTTCAT
TACAAAAGTTTCATATTTTTTTCTCTCTTTAAATGCCATTTTTGCT
GGCTTTCTTTTTAATACAATTAATGTGCTATCCACTTTAGGTTTTG
GATGGAAATAATACCTAGGAATTTTTGCTAATATAGAAATATCTA
CCTCTGCCATTAACAGCAATGCTAGTGATCTGTTTGTATCTAATA
ACATTTTAGCAAAACCATATTCCACTATTAAATAACTTATTGTGG
CTGAACTTTCAAAAACAATTTTTCGAATTATATTTGTGCTTATGTT
GTAAGGTATGCTGCCAAATATTTTATATGGATTGTGGCTAGGAA
ATGTAAATTTCAGTATATCATCATTTACTATTTGATAGTTAGGATA
ATTTAAGAGCTTATTACGAGTTACCTCACATAATTTAGAATCAAT
TTCTATCGCCGTTACAAAATTACATCTCTTTACCAATCCAGCAGT
AAAATGACCTTTCCCTGCACCTATTTCAAAGATGTTATCTTTTTC
ATCTAAACTTATGCAATTCATTATTTTTTCTATGTGATATTTTGAA
GTAATAAAATTTTGACTATCTTTTATATTTACTTTGTTCATTATAAC
CTCTCCTTAATTTATTGCATCTCTTTTCGAATATTTATGTTTTTTG
AGAAAAGAACGTACTCATGGTTCATCCCGATATGCGTATCGGTC
TGTATATCAGCAACTTTCTATGTGTTTCAACTACAATAGTCATCT
ATTCTCATCTTTCTGAGTCCACCCCCTGCAAAGCCCCTCTTTAC
GACATAAAAATTCGGTCGGAAAAGGTATGCAAAAGATGTTTCTC
TCTTTAAGAGAAACTCTTCGGGATGCAAAAATATGAAAATAACTC
CAATTCACCAAATTATATAGCGACTTTTTTACAAAATGCTAAAATT
TGTTGATTTCCGTCAAGCAATTGTTGAGCAAAAATGTCTTTTACG
ATAAAATGATACCTCAATATCAACTGTTTAGCAAAACGATATTTC
TCTTAAAGAGAGAAACACCTTTTTGTTCACCAATCCCCGACTTTT

AATCCCGCGGCCATGATTGAAAAAGGAAGAGTATGAGTATTCAA
CATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTT
CCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGC
TGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGAT
CTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAAC
GTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCG
GTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCC
GCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTC
ACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATG
CAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTAC
TTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTG
CACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAAC
CGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCAC
GATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTG
GCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGG
ATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCC
TTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAG
CGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTA
AGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGC
AACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCT
CACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATAAC
GCGTCAATTCGAGGGGGATCAATTCCGTGATAGGTGGGCTGCC
CTTCCTGGTTGGCTTGGTTTCATCAGCCATCCGCTTGCCCTCAT
CTGTTACGCCGGCGGTAGCCGGCCAGCCTCGCAGAGCAGGAT
TCCCGTTGAGCACCGCCAGGTGCGAATAAGGGACAGTGAAGAA
GGAACACCCGCTCGCGGGTGGGCCTACTTCACCTATCCTGCCC
GGCTGACGCCGTTGGATACACCAAGGAAAGTCTACACGAACCC
TTTGGCAAAATCCTGTATATCGTGCGAAAAAGGATGGATATACC
GAAAAAATCGCTATAATGACCCCGAAGCAGGGTTATGCAGCGG
AAAACGGAATTGATCCGGCCACGATGCGTCCGGCGTAGAGGAT
CTGAAGATCAGCAGTTCAACCTGTTGATAGTACGTACTAAGCTC
TCATGTTTCACGTACTAAGCTCTCATGTTTAACGTACTAAGCTCT
CATGTTTAACGAACTAAACCCTCATGGCTAACGTACTAAGCTCT

CATGGCTAACGTACTAAGCTCTCATGTTTCACGTACTAAGCTCT
CATGTTTGAACAATAAAATTAATATAAATCAGCAACTTAAATAGC
CTCTAAGGTTTTAAGTTTTATAAGAAAAAAAAGAATATATAAGGC
TTTTAAAGCTTTTAAGGTTTAACGGTTGTGGACAACAAGCCAGG
GATGTAACGCACTGAGAAGCCCTTAGAGCCTCTCAAAGCAATTT
TGAGTGACACAGGAACACTTAACGGCTGACATGGGAATTCCCC
TCCACCGCGGTGGCTTAAGACCCACTTTCACATTTAAGTTGTTTT
TCTAATCCGCATATGATCAATTCAAGGCCGAATAAGAAGGCTGG
CTCTGCACCTTGGTGATCAAATAATTCGATAGCTTGTCGTAATAA
TGGCGGCATACTATCAGTAGTAGGTGTTTCCCTTTCTTCTTTAGC
GACTTGATGCTCTTGATCTTCCAATACGCAACCTAAAGTAAAATG
CCCCACAGCGCTGAGTGCATATAATGCATTCTCTAGTGAAAAAC
CTTGTTGGCATAAAAAGGCTAATTGATTTTCGAGAGTTTCATACT
GTTTTTCTGTAGGCCGTGTACCTAAATGTACTTTTGCTCCATCGC
GATGACTTAGTAAAGCACATCTAAAACTTTTAGCGTTATTACGTA
AAAAATCTTGCCAGCTTTCCCCTTCTAAAGGGCAAAAGTGAGTA
TGGTGCCTATCTAACATCTCAATGGCTAAGGCGTCGAGCAAAGC
CCGCTTATTTTTTACATGCCAATACAATGTAGGCTGCTCTACACC
TAGCTTCTGGGCGAGTTTACGGGTTGTTAAACCTTCGATTCCGA
CCTCATTAAGCAGCTCTAATGCGCTGTTAATCACTTTACTTTTAT
CTAATCTAGACATATTCGTTTAATATCATAAATAATTTATTTTATTT
TAAAATGCGCGGGTGCAAAGGTAAGAGGTTTTATTTTAACTACC
AAATGTTTTCGGAAGTTTTTTCGCTTTTCTTTTTCTATCGTTTCTC
AGACTCTCTTAGCGAAAGGGAAAGAAGGTAAAGAAGAAAAACAA
AACGCCTTTTCTTTTTTGCACCCGCTTTCCAAGAGAAGAAAGCC
TTGTTAAATTGACTTAGTGTAAAAGCGCAGTACTGCTTGACCATA
AGAACAAAAAAATCTCTATCACTGATAGGGATAAAGTTTGGAAG
ATAAAGCTAAAAGTTCTTATCTTTGCAGTCTCCCTATCAGTGATA
GAGACGAAATAAAGACATATAAAAGAAAAGACACCATGGATAAG
AAATACTCAATAGGCTTAGATATCGGCACAAATAGCGTCGGATG
GGCGGTGATCACTGATGAATATAAGGTTCCGTCTAAAAAGTTCA
AGGTTCTGGGAAATACAGACCGCCACAGTATCAAAAAAAATCTT
ATAGGGGCTCTTTTATTTGACAGTGGAGAGACAGCGGAAGCGA

CTCGTCTCAAACGGACAGCTCGTAGAAGGTATACACGTCGGAA
GAATCGTATTTGTTATCTACAGGAGATTTTTTCAAATGAGATGGC
GAAAGTAGATGATAGTTTCTTTCATCGACTTGAAGAGTCTTTTTT
GGTGGAAGAAGACAAGAAGCATGAACGTCATCCTATTTTTGGAA
ATATAGTAGATGAAGTTGCTTATCATGAGAAATATCCAACTATCT
ATCATCTGCGAAAAAAATTGGTAGATTCTACTGATAAAGCGGATT
TGCGCTTAATCTATTTGGCCTTAGCGCATATGATTAAGTTTCGTG
GTCATTTTTTGATTGAGGGAGATTTAAATCCTGATAATAGTGATG
TGGACAAACTATTTATCCAGTTGGTACAAACCTACAATCAATTAT
TTGAAGAAAACCCTATTAACGCAAGTGGAGTAGATGCTAAAGCG
ATTCTTTCTGCACGATTGAGTAAATCAAGACGATTAGAAAATCTC
ATTGCTCAGCTCCCCGGTGAGAAGAAAAATGGCTTATTTGGGAA
TCTCATTGCTTTGTCATTGGGTTTGACCCCTAATTTTAAATCAAA
TTTTGATTTGGCAGAAGATGCTAAATTACAGCTTTCAAAAGATAC
TTACGATGATGATTTAGATAATTTATTGGCGCAAATTGGAGATCA
ATATGCTGATTTGTTTTTGGCAGCTAAGAATTTATCAGATGCTAT
TTTACTTTCAGATATCCTAAGAGTAAATACTGAAATAACTAAGGC
TCCCCTATCAGCTTCAATGATTAAACGCTACGATGAACATCATCA
AGACTTGACTCTTTTAAAAGCTTTAGTTCGACAACAACTTCCAGA
AAAGTATAAAGAAATCTTTTTTGATCAATCAAAAAACGGATATGC
AGGTTATATTGATGGGGGAGCTAGCCAAGAAGAATTTTATAAAT
TTATCAAACCAATTTTAGAAAAAATGGATGGTACTGAGGAATTAT
TGGTGAAACTAAATCGTGAAGATTTGCTGCGCAAGCAACGGAC
CTTTGACAACGGCTCTATTCCCCATCAAATTCACTTGGGTGAGC
TGCATGCTATTTTGAGAAGACAAGAAGACTTTTATCCATTTTTAA
AAGACAATCGTGAGAAGATTGAAAAAATCTTGACTTTTCGAATTC
CTTATTATGTTGGTCCATTGGCGCGTGGCAATAGTCGTTTTGCA
TGGATGACTCGGAAGTCTGAAGAAACAATTACCCCATGGAATTT
TGAAGAAGTTGTCGATAAAGGTGCTTCAGCTCAATCATTTATTGA
ACGCATGACAAACTTTGATAAAAATCTTCCAAATGAAAAAGTACT
ACCAAAACATAGTTTGCTTTATGAGTATTTTACGGTTTATAACGA
ATTGACAAAGGTCAAATATGTTACTGAAGGAATGCGAAAACCAG
CATTTCTTTCAGGTGAACAGAAGAAAGCCATTGTTGATTTACTCT

TCAAAACAAATCGAAAAGTAACCGTTAAGCAATTAAAAGAAGATT
ATTTCAAAAAAATAGAATGTTTTGATAGTGTTGAAATTTCAGGAG
TTGAAGATAGATTTAATGCTTCATTAGGTACCTACCATGATTTGC
TAAAAATTATTAAAGATAAAGATTTTTTGGATAATGAAGAAAATGA
AGATATCTTAGAGGATATTGTTTTAACATTGACCTTATTTGAAGA
TAGGGAGATGATTGAGGAAAGACTTAAAACATATGCTCACCTCT
TTGATGATAAGGTGATGAAACAGCTTAAACGTCGCCGTTATACT
GGTTGGGGACGTTTGTCTCGAAAATTGATTAATGGTATTAGGGA
TAAGCAATCTGGCAAAACAATATTAGATTTTTTGAAATCAGATGG
TTTTGCCAATCGCAATTTTATGCAGCTGATCCATGATGATAGTTT
GACATTTAAAGAAGACATTCAAAAAGCACAAGTGTCTGGACAAG
GCGATAGTTTACATGAACATATTGCAAATTTAGCTGGTAGCCCT
GCTATTAAAAAAGGTATTTTACAGACTGTAAAAGTTGTTGATGAA
TTGGTCAAAGTAATGGGGCGGCATAAGCCAGAAAATATCGTTAT
TGAAATGGCACGTGAAAATCAGACAACTCAAAAGGGCCAGAAAA
ATTCGCGAGAGCGTATGAAACGAATCGAAGAAGGTATCAAAGAA
TTAGGAAGTCAGATTCTTAAAGAGCATCCTGTTGAAAATACTCAA
TTGCAAAATGAAAAGCTCTATCTCTATTATCTCCAAAATGGAAGA
GACATGTATGTGGACCAAGAATTAGATATTAATCGTTTAAGTGAT
TATGATGTCGATCACATTGTTCCACAAAGTTTCCTTAAAGACGAT
TCAATAGACAATAAGGTCTTAACGCGTTCTGATAAAAATCGTGG
TAAATCGGATAACGTTCCAAGTGAAGAAGTAGTCAAAAAGATGA
AAAACTATTGGAGACAACTTCTAAACGCCAAGTTAATCACTCAAC
GTAAGTTTGATAATTTAACGAAAGCTGAACGTGGAGGTTTGAGT
GAACTTGATAAAGCTGGTTTTATCAAACGCCAATTGGTTGAAACT
CGCCAAATCACTAAGCATGTGGCACAAATTTTGGATAGTCGCAT
GAATACTAAATACGATGAAAATGATAAACTTATTCGAGAGGTTAA
AGTGATTACCTTAAAATCTAAATTAGTTTCTGACTTCCGAAAAGA
TTTCCAATTCTATAAAGTACGTGAGATTAACAATTACCATCATGC
CCATGATGCGTATCTAAATGCCGTCGTTGGAACTGCTTTGATTA
AGAAATATCCAAAACTTGAATCGGAGTTTGTCTATGGTGATTATA
AAGTTTATGATGTTCGTAAAATGATTGCTAAGTCTGAGCAAGAAA
TAGGCAAAGCAACCGCAAAATATTTCTTTTACTCTAATATCATGA

ACTTCTTCAAAACAGAAATTACACTTGCAAATGGAGAGATTCGC
AAACGCCCTCTAATCGAAACTAATGGGGAAACTGGAGAAATTGT
CTGGGATAAAGGGCGAGATTTTGCCACAGTGCGCAAAGTATTG
TCCATGCCCCAAGTCAATATTGTCAAGAAAACAGAAGTACAGAC
AGGCGGATTCTCCAAGGAGTCAATTTTACCAAAAAGAAATTCGG
ACAAGCTTATTGCTCGTAAAAAAGACTGGGATCCAAAAAAATAT
GGTGGTTTTGATAGTCCAACGGTAGCTTATTCAGTCCTAGTGGT
TGCTAAGGTGGAAAAAGGGAAATCGAAGAAGTTAAAATCCGTTA
AAGAGTTACTAGGGATCACAATTATGGAAAGAAGTTCCTTTGAA
AAAAATCCGATTGACTTTTTAGAAGCTAAAGGATATAAGGAAGTT
AAAAAAGACTTAATCATTAAACTACCTAAATATAGTCTTTTTGAGT
TAGAAAACGGTCGTAAACGGATGCTGGCTAGTGCCGGAGAATT
ACAAAAAGGAAATGAGCTGGCTCTGCCAAGCAAATATGTGAATT
TTTTATATTTAGCTAGTCATTATGAAAAGTTGAAGGGTAGTCCAG
AAGATAACGAACAAAAACAATTGTTTGTGGAGCAGCATAAGCAT
TATTTAGATGAGATTATTGAGCAAATCAGTGAATTTTCTAAGCGT
GTTATTTTAGCAGATGCCAATTTAGATAAAGTTCTTAGTGCATAT
AACAAACATAGAGACAAACCAATACGTGAACAAGCAGAAAATAT
TATTCATTTATTTACGTTGACGAATCTTGGAGCTCCCGCTGCTTT
TAAATATTTTGATACAACAATTGATCGTAAACGATATACGTCTAC
AAAAGAAGTTTTAGATGCCACTCTTATCCATCAATCCATCACTGG
TCTTTATGAAACACGCATTGATTTGAGTCAGCTAGGAGGTGACT
GAATTAATGCGGCTGCAATTTTTTTGGGCGGGGCCGCCCAAAA
AAATCCTAGCACCCTGCAGCAGTACTGCTTGACCATAAGAACAA
AAAAACTTCCGATAAAGTTTGGAAGATAAAGCTAAAAGTTCTTAT
CTTTGCAGTATGACGGGAATGTACCCCAGGTTTTAGAGCTAGAA
ATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGT
GGCACCGAGTCGGTGCTTTTTTTGAGATCTGTCCATACCCATGG
GACGTCTGATGATTAAGGATCTTGC (SEQ ID NO:9)

Example 12. Inducible CRISPR killing of individual *B. thetaiotaomicron* strains

[0122] Two stably maintained plasmids were conjugated into *B. thetaiotaomicron* on Brain Heart Infusion (BHI) blood agar plates with no

antibiotic selection. One plasmid was a negative control (termed 'M') with a scrambled non-targeting protospacer sequence (5'-TGATGGAGAGGTGCAAGTAG-3'; SEQ ID NO 6). *In silico* analyses of this non-targeting control protospacer sequence against *Bacteroides* genomes did not result in any significant sequence matches, so no 'off-target' activity is expected. The other plasmid has a protospacer sequence (5'-ATGACGGGAATGTACCCCAG-3'; SEQ ID NO:5) that targets the *susC*_Bt (BT_3702) coding sequence on the *Bt* genome. *susC* gene encodes an outer membrane protein involved in starch binding in *B. thetaiotaomicron.* The sgRNA scaffold sequence was 5'-GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAA AGTGGCACCGAGTCGGTGCTTTTTT-3' (SEQ ID NO:7). The resulting two plasmids are called pRepA-CRISPR.Mand pRepA-CRISPR.*susC*_Bt. Colonies were picked and re-streaked on BHI blood agar plates with 200 µg/ml gentamin (Gm) and 50 µg/ml erythromycin (Em) and grown anaerobically in a coy chamber (Coy Laboratory Products Inc.). From this re-streaked plate, a single colony was picked and grown in 10 ml of TYG liquid medium at 200 µg/ml Gm and 50 µg/ml Em. OD_{600nm} readings were taken so the concentrations could be adjusted to an OD of 1. One to ten dilutions were then made in one ml volumes. One hundred microliters of two dilutions (10^{-4} and 10^{-6}) were spread on BHI blood agar plates with 200 µg/ml gentamin (Gm) and 50 µg/ml erythromycin (Em) supplemented with anhydrotetracycline (aTc) at concentrations of 0 and 100 ng/ml, respectively. The agar plates were incubated anaerobically at 37 °C for 2-3 days. Colony forming units (CFU) were obtained on blood agar plates without aTc present (0 ng/ml) for all strains. No CFU formation was observed on blood agar plates with aTc present (100 ng/ml) for the *susC* targeting plasmid, while CFUs were still obtained for the M strain (**FIGS. 11 A-D**).

Example 13. Vector construction

[0123] The CRISPR integration pNBU2.CRISPR plasmids were constructed using Gibson cloning (NEBuild HIFI DNA Assembly Master Mix, New England Biolabs) of plasmid backbone (RP4-oriT, R6K ori, bla, ermG)

from pExchangetdk, NBU2 integrase from pNBU2-tetQb, and an anhydrotetracycline (aTc) inducible CRISPR cassettes (P2-A21-tetR, P1TDP-GH023-SpCas9, P1-N20 sgRNA scaffold) assembled from synthetic DNAs or PCR of genomic DNA of *Streptococcus pyogenes* strain SF370. The erythromycin (*ermG*) antibiotic resistance gene was replaced by the cefoxitin antibiotic resistance gene (*cfxA*) using synthetic DNA and traditional restriction enzyme cloning. **FIG. 12** illustrates the plasmid design.

[0124] The plasmid backbone harbors R6K origin of replication and *bla* sequence for ampicillin selection in *E. coli,* RP4-oriT sequence for conjugation and *cfxA* sequence for cefoxitin (FOX) selection in *Bacteroides* (Parker and Smith, Antimicrobial agents and Chemotherapy, 1993, 37: 1028-1036). NBU2 encodes the intN2 tyrosine integrase, which mediates sequence-specific recombination between the attN2 site on pNBU2-CRISPR plasmid and one of the attB sites located on the chromosome of *Bacteroides* cells. The attN2 and attB have the same 13 bp recognition nucleotide sequence (5'-3'): CCTGTCTCTCCGC (SEQ ID NO: 2).

[0125] The inducible CRISPR cassettes include aTc inducible SpCas9 under the control of TetR regulator (P2-A21-tetR, P1TDP-GH023-SpCas9), and constitutively expressed guide RNA under P1 promoter (P1-N20 sgRNA scaffold). The promoters and ribosomal binding sites are derived and engineered from regulatory sequences of *Bacteroides thetaiotaomicron* 16S rRNA genes, as described in Lim et al., *Cell*, 2017, 169:547-558. The guide RNA is a nucleotide sequence that is homologous to a coding DNA sequence, or non-coding DNA sequence, or a non-targeting scramble nucleotide sequence. This sequence can be of any form as long as it is compatible with protospacer adjacent motif (PAM) requirements of different Cas9 homologs. The guide RNA can be either in separate transcriptional units of tracrRNA and crRNA or fused into a hybrid chimeric tracr/crRNA single guide(sgRNA).

[0126] The DNA sequence for the above plasmid (FIG. 12) is presented in SEQ ID NO:10:

Plasmid (pNBU2-CRISPR.non-targeting M_BWH2) DNA sequence (10,627 bp)

GGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACT
CATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT
GTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACC
ATGATTACGCCCTTAAGACCCACTTTCACATTTAAGTTGTTTTTCTAATC
CGCATATGATCAATTCAAGGCCGAATAAGAAGGCTGGCTCTGCACCTT
GGTGATCAAATAATTCGATAGCTTGTCGTAATAATGGCGGCATACTATC
AGTAGTAGGTGTTTCCCTTTCTTCTTTAGCGACTTGATGCTCTTGATCT
TCCAATACGCAACCTAAAGTAAAATGCCCCACAGCGCTGAGTGCATAT
AATGCATTCTCTAGTGAAAAACCTTGTTGGCATAAAAAGGCTAATTGAT
TTTCGAGAGTTTCATACTGTTTTTCTGTAGGCCGTGTACCTAAATGTAC
TTTTGCTCCATCGCGATGACTTAGTAAAGCACATCTAAAACTTTTAGCG
TTATTACGTAAAAAATCTTGCCAGCTTTCCCCTTCTAAAGGGCAAAAGT
GAGTATGGTGCCTATCTAACATCTCAATGGCTAAGGCGTCGAGCAAAG
CCCGCTTATTTTTTACATGCCAATACAATGTAGGCTGCTCTACACCTAG
CTTCTGGGCGAGTTTACGGGTTGTTAAACCTTCGATTCCGACCTCATTA
AGCAGCTCTAATGCGCTGTTAATCACTTTACTTTTATCTAATCTAGACAT
ATTCGTTTAATATCATAAATAATTTATTTTATTTTAAAATGCGCGGGTGC
AAAGGTAAGAGGTTTTATTTTAACTACCAAATGTTTTCGGAAGTTTTTTC
GCTTTTCTTTTTCTATCGTTTCTCAGACTCTCTTAGCGAAAGGGAAAGA
AGGTAAAGAAGAAAAACAAAACGCCTTTTCTTTTTTGCACCCGCTTTCC
AAGAGAAGAAAGCCTTGTTAAATTGACTTAGTGTAAAAGCGCAGTACT
GCTTGACCATAAGAACAAAAAAATCTCTATCACTGATAGGGATAAAGTT
TGGAAGATAAAGCTAAAAGTTCTTATCTTTGCAGTCTCCCTATCAGTGA
TAGAGACGAAATAAAGACATATAAAAGAAAAGACACCATGGATAAGAAA
TACTCAATAGGCTTAGATATCGGCACAAATAGCGTCGGATGGGCGGTG
ATCACTGATGAATATAAGGTTCCGTCTAAAAAGTTCAAGGTTCTGGGAA
ATACAGACCGCCACAGTATCAAAAAAAATCTTATAGGGGCTCTTTTATT
TGACAGTGGAGAGACAGCGGAAGCGACTCGTCTCAAACGGACAGCTC
GTAGAAGGTATACACGTCGGAAGAATCGTATTTGTTATCTACAGGAGA
TTTTTTCAAATGAGATGGCGAAAGTAGATGATAGTTTCTTTCATCGACTT
GAAGAGTCTTTTTTGGTGGAAGAAGACAAGAAGCATGAACGTCATCCT
ATTTTTGGAAATATAGTAGATGAAGTTGCTTATCATGAGAAATATCCAA
CTATCTATCATCTGCGAAAAAAATTGGTAGATTCTACTGATAAAGCGGA

TTTGCGCTTAATCTATTTGGCCTTAGCGCATATGATTAAGTTTCGTGGT
CATTTTTTGATTGAGGGAGATTTAAATCCTGATAATAGTGATGTGGACA
AACTATTTATCCAGTTGGTACAAACCTACAATCAATTATTTGAAGAAAAC
CCTATTAACGCAAGTGGAGTAGATGCTAAAGCGATTCTTTCTGCACGA
TTGAGTAAATCAAGACGATTAGAAAATCTCATTGCTCAGCTCCCCGGT
GAGAAGAAAAATGGCTTATTTGGGAATCTCATTGCTTTGTCATTGGGTT
TGACCCCTAATTTTAAATCAAATTTTGATTTGGCAGAAGATGCTAAATTA
CAGCTTTCAAAAGATACTTACGATGATGATTTAGATAATTTATTGGCGC
AAATTGGAGATCAATATGCTGATTTGTTTTTGGCAGCTAAGAATTTATC
AGATGCTATTTTACTTTCAGATATCCTAAGAGTAAATACTGAAATAACTA
AGGCTCCCCTATCAGCTTCAATGATTAAACGCTACGATGAACATCATCA
AGACTTGACTCTTTTAAAAGCTTTAGTTCGACAACAACTTCCAGAAAAG
TATAAAGAAATCTTTTTTGATCAATCAAAAAACGGATATGCAGGTTATAT
TGATGGGGGAGCTAGCCAAGAAGAATTTTATAAATTTATCAAACCAATT
TTAGAAAAAATGGATGGTACTGAGGAATTATTGGTGAAACTAAATCGTG
AAGATTTGCTGCGCAAGCAACGGACCTTTGACAACGGCTCTATTCCCC
ATCAAATTCACTTGGGTGAGCTGCATGCTATTTTGAGAAGACAAGAAG
ACTTTTATCCATTTTTAAAAGACAATCGTGAGAAGATTGAAAAAATCTTG
ACTTTTCGAATTCCTTATTATGTTGGTCCATTGGCGCGTGGCAATAGTC
GTTTTGCATGGATGACTCGGAAGTCTGAAGAAACAATTACCCCATGGA
ATTTTGAAGAAGTTGTCGATAAAGGTGCTTCAGCTCAATCATTTATTGA
ACGCATGACAAACTTTGATAAAAATCTTCCAAATGAAAAAGTACTACCA
AAACATAGTTTGCTTTATGAGTATTTTACGGTTTATAACGAATTGACAAA
GGTCAAATATGTTACTGAAGGAATGCGAAAACCAGCATTTCTTTCAGGT
GAACAGAAGAAAGCCATTGTTGATTTACTCTTCAAAACAAATCGAAAAG
TAACCGTTAAGCAATTAAAAGAAGATTATTTCAAAAAAATAGAATGTTTT
GATAGTGTTGAAATTTCAGGAGTTGAAGATAGATTTAATGCTTCATTAG
GTACCTACCATGATTTGCTAAAAATTATTAAAGATAAAGATTTTTTGGAT
AATGAAGAAAATGAAGATATCTTAGAGGATATTGTTTTAACATTGACCTT
ATTTGAAGATAGGGAGATGATTGAGGAAAGACTTAAAACATATGCTCAC
CTCTTTGATGATAAGGTGATGAAACAGCTTAAACGTCGCCGTTATACTG
GTTGGGGACGTTTGTCTCGAAAATTGATTAATGGTATTAGGGATAAGC
AATCTGGCAAAACAATATTAGATTTTTTGAAATCAGATGGTTTTGCCAAT

CGCAATTTTATGCAGCTGATCCATGATGATAGTTTGACATTTAAAGAAG
ACATTCAAAAAGCACAAGTGTCTGGACAAGGCGATAGTTTACATGAAC
ATATTGCAAATTTAGCTGGTAGCCCTGCTATTAAAAAAGGTATTTTACA
GACTGTAAAAGTTGTTGATGAATTGGTCAAAGTAATGGGGCGGCATAA
GCCAGAAAATATCGTTATTGAAATGGCACGTGAAAATCAGACAACTCAA
AAGGGCCAGAAAAATTCGCGAGAGCGTATGAAACGAATCGAAGAAGG
TATCAAAGAATTAGGAAGTCAGATTCTTAAAGAGCATCCTGTTGAAAAT
ACTCAATTGCAAAATGAAAAGCTCTATCTCTATTATCTCCAAAATGGAA
GAGACATGTATGTGGACCAAGAATTAGATATTAATCGTTTAAGTGATTA
TGATGTCGATCACATTGTTCCACAAAGTTTCCTTAAAGACGATTCAATA
GACAATAAGGTCTTAACGCGTTCTGATAAAAATCGTGGTAAATCGGATA
ACGTTCCAAGTGAAGAAGTAGTCAAAAAGATGAAAAACTATTGGAGAC
AACTTCTAAACGCCAAGTTAATCACTCAACGTAAGTTTGATAATTTAAC
GAAAGCTGAACGTGGAGGTTTGAGTGAACTTGATAAAGCTGGTTTTAT
CAAACGCCAATTGGTTGAAACTCGCCAAATCACTAAGCATGTGGCACA
AATTTTGGATAGTCGCATGAATACTAAATACGATGAAAATGATAAACTT
ATTCGAGAGGTTAAAGTGATTACCTTAAAATCTAAATTAGTTTCTGACTT
CCGAAAAGATTTCCAATTCTATAAAGTACGTGAGATTAACAATTACCAT
CATGCCCATGATGCGTATCTAAATGCCGTCGTTGGAACTGCTTTGATTA
AGAAATATCCAAAACTTGAATCGGAGTTTGTCTATGGTGATTATAAAGT
TTATGATGTTCGTAAAATGATTGCTAAGTCTGAGCAAGAAATAGGCAAA
GCAACCGCAAAATATTTCTTTTACTCTAATATCATGAACTTCTTCAAAAC
AGAAATTACACTTGCAAATGGAGAGATTCGCAAACGCCCTCTAATCGA
AACTAATGGGGAAACTGGAGAAATTGTCTGGGATAAAGGGCGAGATTT
TGCCACAGTGCGCAAAGTATTGTCCATGCCCCAAGTCAATATTGTCAA
GAAAACAGAAGTACAGACAGGCGGATTCTCCAAGGAGTCAATTTTACC
AAAAAGAAATTCGGACAAGCTTATTGCTCGTAAAAAAGACTGGGATCC
AAAAAAATATGGTGGTTTTGATAGTCCAACGGTAGCTTATTCAGTCCTA
GTGGTTGCTAAGGTGGAAAAAGGGAAATCGAAGAAGTTAAAATCCGTT
AAAGAGTTACTAGGGATCACAATTATGGAAAGAAGTTCCTTTGAAAAAA
ATCCGATTGACTTTTTAGAAGCTAAAGGATATAAGGAAGTTAAAAAAGA
CTTAATCATTAAACTACCTAAATATAGTCTTTTTGAGTTAGAAAACGGTC
GTAAACGGATGCTGGCTAGTGCCGGAGAATTACAAAAAGGAAATGAGC

TGGCTCTGCCAAGCAAATATGTGAATTTTTTATATTTAGCTAGTCATTAT
GAAAAGTTGAAGGGTAGTCCAGAAGATAACGAACAAAAACAATTGTTT
GTGGAGCAGCATAAGCATTATTTAGATGAGATTATTGAGCAAATCAGTG
AATTTTCTAAGCGTGTTATTTTAGCAGATGCCAATTTAGATAAAGTTCTT
AGTGCATATAACAAACATAGAGACAAACCAATACGTGAACAAGCAGAA
AATATTATTCATTTATTTACGTTGACGAATCTTGGAGCTCCCGCTGCTTT
TAAATATTTTGATACAACAATTGATCGTAAACGATATACGTCTACAAAAG
AAGTTTTAGATGCCACTCTTATCCATCAATCCATCACTGGTCTTTATGAA
ACACGCATTGATTTGAGTCAGCTAGGAGGTGACTGAATTAATGCGGCT
GCAATTTTTTTGGGCGGGGCCGCCCAAAAAAATCCTAGCACCCTGCAG
CAGTACTGCTTGACCATAAGAACAAAAAAACTTCCGATAAAGTTTGGAA
GATAAAGCTAAAAGTTCTTATCTTTGCAGTTGATGGAGAGGTGCAAGTA
GGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCA
ACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGATCTGTCGAC
TCTAGAGGATCCCCGGGTACCGAGCTCGAATTCACTGGCCGTCGTTTT
ACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGTAC
TTGTGCCTGTTCTATTTCCGAACCGACCGCTTGTATGAATCCATCAAAA
TTCGTTTTCTCTATGTTGGATTCCTTGTTGCTCATATTGTGATGATAATT
TCTACAAATATAGTCATTGGTAACTATCTATGAAACTGTTTGATACTTTT
ATAGTTGATTAAACTTGTTCATGGCATTTGCCTTAATATCATCCGCTATG
TCAATGTAGGGTTTCATAGCTTTGTAGTCGCTGTGTCCCGTCCATTTCA
TGACCACCTGTGCCGGGATTCCGAGAGCCAGCGCATTGCAGATGAAT
GTCCTTCTTCCTGCATGGGTACTGAGCAAAGCGTATTTGGGTGTGACT
TCATCAATACGTTCATTTCCCTTGTAGTAGGTTTCCCGTACAGGCTCGT
TGATTTCTGCCAGTTCGCCCAGCTCTTTCAGGTAATCGTTCATCTTCTG
GTTGCTGATGACGGGCAGAGCCATGTAATTCTCGAAATGGATGTCCTT
GTATTTGTCCAGTATGGCTTTGCTGTATTTGTTCAGTTCAATCGTCAGG
CTGTCGGCAGTCTTGACTGTGGTTATTTCGATGTGGTCGGACTTCACA
TCGCTTCTTTTCAGATTGCGAACATCCGAATACCGCAAACTCGTAAAGC
AGCAGAACAGGAAAACATCACGCACACGTTCCAGGTATTGCTTATCCT
TGGGTATCTGGTAGTCTTTCAGCTTGTTCAGTTCATCCCAAGTCAGGAA
GATTACTTTTTTCGAGGTGGTTTTCAGTTTCGGTTTGAACGTATCGTAT
GCAATGTTCTGATGATGTCCTTTCTTGAAGCTCCAGCGCAGGAACCAT

TTGAGGAATCCCATTTGCTTGCCGATGGTGCTGTTTCTCATATCCTTGG
TGTCACGCAGGAAGTTGACGTATTCGTTCAATCCAAACTCGTTGAAATA
GTTGAACGTTGCATCCTCCTTGAACTCTTTGAGGTGGTTCCTCACTGCT
GCAAATTTTTCATAGGTGGATGCCGTCCAGTTATTCTGGTTACCGCACT
CTTTTACAAACTCATCGAACACCTCCCAAAAGCTGACAGGGGCTTCTT
CCGGCTGTTCTTCGCTGGTGTCTTTCATTCTCATGTTGAAAGCTTCCTT
CAACTGTTGGGTCGTTGGCATGACCTCCTGCACCTCAAATTCCTTGAA
AATATTCTGGATTTCGGCATAGTATTTCAGCAAGTCCGTATTGATTTCG
GCTGCACTTTGCTTTAGCTTGTTGGTACATCCGCTCTTTACCCGCTGCT
TATCTGCATCCCATTTGGCTACGTCAATCCGGTAGCCCGTTGTAAACTC
GATGCGTTGGCTGGCAAAGATGACACGCATACGGATGGGTACGTTCT
CTACGATTGGCACACCGTTCTTTTTCCGGCTCTCCAATGCAAAAATGAT
GTTGCGCTTGATATTCATAATTGGGTGCGTTTGAAATTCTACACCCAAA
TATACACCCAATTATTGAGATAGCAAAAGACATTTAGAAACATTTACTTT
TACTCTATATTGTAATTTACACTTGATTATCAGTCGTTTGCAGTCTTATG
ATATTCTGTGAAAGTATAAGTTCGAGAGCCTGTCTCTCCGCAAAAAACG
CTGAAAATCAGCAGATTGCAAAACAAACACCCTGTTTTACACCCAAGAA
TGTAAAGTCGGCTGTTTTTGTTTTATTTAAGATAATACAACCACTACATA
ATAAAAGAGTAGCGATATTAAAAGAATCCGATGAGAAAAGACTAATATT
TATCTATCCATTCAGTTTGATTTTTCAGGACTTTACATCGTCCTGAAAGT
ATTTGTTGGTACCGGTACCGAGGACGCGTAAACATTTACAGTTGCATG
TGGCCTATTGTTTTTAAGATTTTACTGAAGTTTGCATTAATAAAGAATAT
ACTACAGCTGATATATGCGCAACATATTGTGACGCTTGTGATTTATTTC
CCTTGAAATCCTTAACAAATACCGCTAAGGTATAACTGATATTATTAGG
CAGACATATATAGGCAACATCATTGTGAGCTGCAAGAACACCATTTTCA
TTAACATAACCTGAACCTGTCTTATGCGCTATAACAACCCCTTCTTTATC
AAGAAGTGGAGCTGCTATCCTATCTACACCTGTTTTGCATTCTTTTAAC
GTATTCTTAATGAAACTTTGTTTCTCATCATCGATAAGACCTTCAGTAAA
CAAACGATTCATCAACATTGCAGCACCAAGAGGAGATGTATAGTTAGA
GTAAGCCTTGTTATGGTCAGCCGACATTTCCTCTTCCGTATAAGCTATC
TGAAAACTTGAACGAGGAATGAGTGTGGCTATAAAACTATCTGTTTGAG
CGACATTAACCATATCCTTAAACATAAGGTTGCTTGCATTGTTGTCACT
CTGAGTAAGAGTATAACGCAGCAAATCTCTCACTGTCAATGATATGACT

GGCCCTGAATAATCTTTCAGCATAGGACTCCAAGTCTTTGGGTCAAGTT
TATCCCTATTTATATTTACTAAGGTATCAAGTGAAATTCCTTTATTGTCA
AAGTCATTACAAAGAGCTAATGCCTGATGAACCTTAAACACACTCATCA
TAGGATAAACACTCTTATTATTGACCTTAACCGTATCTCTGTTATTAACA
ATAACCGCCACACCAATTTCGCCAGGACAAGCTGAGACAATTTGAGAA
ATGCTATCAGTCAAAACATTTGTTAAAGGAGGATTTGCGCTATCTTTTG
TCGCTGATTTATGGAACAATGAAAATACCAAGATGAAAATGCAAACTAA
AGCTATACTCAAAACTACGATTTGTTTTTTTCTGTTTTTTTCCATTATAAC
CTCTCCTTAATTTATTGCATCTCTTTTCGAATATTTATGTTTTTTGAGAAA
AGAACGTACTCATGGTTCATCCCGATATGCGTATCGGTCTGTATATCAG
CAACTTTCTATGTGTTTCAACTACAATAGTCATCTATTCTCATCTTTCTG
AGTCCACCCCCTGCAAAGCCCCTCTTTACGACATAAAAATTCGGTCGG
AAAAGGTATGCAAAAGATGTTTCTCTCTTTAAGAGAAACTCTTCGGGAT
GCAAAAATATGAAAATAACTCCAATTCACCAAATTATATAGCGACTTTTT
TACAAAATGCTAAAATTTGTTGATTTCCGTCAAGCAATTGTTGAGCAAA
AATGTCTTTTACGATAAAATGATACCTCAATATCAACTGTTTAGCAAAAC
GATATTTCTCTTAAAGAGAGAAACACCTTTTTGTTCACCAATCCCCGAC
TTTTAATCCCGCGGCCATGATTGAAAAAGGAAGAGTATGAGTATTCAAC
ATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGT
TTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCA
GTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTA
AGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCA
CTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCG
GGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGG
TTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAG
TAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGG
CCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTT
TTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAAC
CGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATG
CCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTA
CTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGAT
AAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTT
ATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATT

GCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTAC
ACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGC
TGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGT
TTACTCATAACGCGTCAATTCGAGGGGGATCAATTCCGTGATAGGTGG
GCTGCCCTTCCTGGTTGGCTTGGTTTCATCAGCCATCCGCTTGCCCTC
ATCTGTTACGCCGGCGGTAGCCGGCCAGCCTCGCAGAGCAGGATTCC
CGTTGAGCACCGCCAGGTGCGAATAAGGGACAGTGAAGAAGGAACAC
CCGCTCGCGGGTGGGCCTACTTCACCTATCCTGCCCGGCTGACGCCG
TTGGATACACCAAGGAAAGTCTACACGAACCCTTTGGCAAAATCCTGT
ATATCGTGCGAAAAAGGATGGATATACCGAAAAAATCGCTATAATGAC
CCCGAAGCAGGGTTATGCAGCGGAAAACGGAATTGATCCGGCCACGA
TGCGTCCGGCGTAGAGGATCTGAAGATCAGCAGTTCAACCTGTTGATA
GTACGTACTAAGCTCTCATGTTTCACGTACTAAGCTCTCATGTTTAACG
TACTAAGCTCTCATGTTTAACGAACTAAACCCTCATGGCTAACGTACTA
AGCTCTCATGGCTAACGTACTAAGCTCTCATGTTTCACGTACTAAGCTC
TCATGTTTGAACAATAAAATTAATATAAATCAGCAACTTAAATAGCCTCT
AAGGTTTTAAGTTTTATAAGAAAAAAAAGAATATATAAGGCTTTTAAAGC
TTTTAAGGTTTAACGGTTGTGGACAACAAGCCAGGGATGTAACGCACT
GAGAAGCCCTTAGAGCCTCTCAAAGCAATTTTGAGTGACACAGGAACA
CTTAACGGCTGACATGGGAATTCCCCTCCACCGCGGTGG (SEQ ID
NO:10)

Example 14. CRISPR integration on the chromosome of *Bacteroides cellulosilyticus* WH2

[0127] The pNBU2-CRISPR plasmids were transformed to *E. coli* S-17 lambda-*pir*, followed by delivery to *Bacteroides cellulosilyticus* WH2 cells via conjugation. In this specific example, the pNBU2-CRISPR plasmid encodes the intN2 tyrosine integrase, which mediates sequence-specific recombination between the attN2 site on pNBU2-CRISPR plasmid and one of three attBWH2 sites located in the 3' ends of the two tRNA-Ser genes, BcellWH2_RS22795 or BcellWH2_RS23000, or a non-coding region (nucleotide coordinates 6,071,791-6,071,803) on the chromosome of *Bacteroides cellulosilyticus* WH2 (BWH2 in short). Insertion of the pNBU2-CRISPR plasmid may inactivate one of the two tRNA-Ser genes (will not inactivate a tRNA-Ser gene if inserted in

the non-coding region), and simultaneous insertion into both tRNA-Ser genes is unlikely because of the essentiality of tRNA-Ser.

[0128] Five plasmids were constructed which express a non-targeting control guide (termed 'M'), two guide RNAs targeting tdk_BWH2 (BcellWH2_RS17975) (termed 'T2' and 'T3') and two guide RNAs targeting susC_BWH2 (BcellWH2_RS26295) (termed 'S6' and 'S19'). The *tdk* gene encodes thymidine kinase, and the *susC* gene encodes the SusC/RagA family Ton-B-linked outer membrane protein involved in starch binding in *Bacteroides cellulosilyticus* WH2. The two protospacer sequences for tdk_BWH2 are T2 (5'-ATACAGGAAACCAATCGTAG-3'; SEQ ID NO:11) and T3, (5'-GGAAGAATCGAAGTTATATG-3'; SEQ ID NO:12) and for susC_BWH2 are S6 (5'-AATCCACTGGATGCCATCCG-3'; SEQ ID NO:13) and S19 (5'-GCTTATGTCTATCTATCCGG-3'; SEQ ID NO:14). In silico analysis of the non-targeting control protospacer sequence M (5'-TGATGGAGAGGTGCAAGTAG-3'; SEQ ID NO 6) against *Bacteroides* genomes did not result in any significant sequence matches, so no "off-target" activity is expected. The sgRNA scaffold sequence was 5'-GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTT-3' (SEQ ID NO:7). The resulting plasmids are called pNBU2-CRISPR.M_BWH2, pNBU2-CRISPR.tdk_BWH2-2, pNBU2-CRISPR.tdk_BWH2-3, pNBU2-CRISPR.susC_BWH2-6 and pNBU2-CRISPR.susC_BWH2-19.

[0129] An example of a targeted insertion between the attN2 site on a pNBU2-CRISPR plasmid with cefoxitin resistance and one of three attBWH2 sites in the *Bacteroides cellulosilyticus* WH2 genome is shown in **FIGS. 13A-B**. Plasmid pNBU2-CRISPR.susC_BWH2-19 integrates only in the attBWH2 site in the t-RNA-Ser gene, BcellWH2_RS22795. The 5' end of the plasmid integration site is shown in **FIG. 13A** and the 3' end of the plasmid integration site is shown in **FIG. 13B**. Sequencing was performed using Illumina Next Gen Sequencing technology, and the analysis was done with Geneious align/assemble software.

Example 15. Inducible CRISPR killing of individual B. cellulosilyticus WH2 strains

[0130] For selected *Bacteroides cellulosilyticus* WH2 integrants (M1, M2, T2, T3, S6 and S19), inducible CRISPR Cas9 mediated cell killing was investigated in TYG liquid medium. Single colonies of M1, M2, T2, T3, S6 and S19 (M1 and M2 were separate colonies from the same M non-targeting conjugation plate) were grown anaerobically in a coy chamber (coy Laboratory Products Inc.) overnight in falcon tubes cultures containing 5 ml of TYG liquid medium supplemented with 200 µg/ml gentamicin (Gm) and 10 µg/ml cefoxitin (FOX). Overnight cultures were then normalized to an OD_{600nm} of 1 with TYG medium. These normalized cultures were diluted at a 1:100 ratio (250 µl into 24.75 ml of TYG) to make a seed culture. This 25 ml seed culture was aliquoted into 5 ml cultures in fresh 15 ml falcon tubes. Anhydrotetracycline (aTc) was added to the 5 ml cultures at either 0 ng/ml. 10 ng/ml or 100 ng/ml and incubated anaerobically at 37 °C for 24 hours. OD_{600nm} readings taken after 24 hours of growth are shown in **FIG. 14**. The data shows a chromosomally integrated CRISPR/Cas9 system is activated by an exogenously provided inducer (aTc) to generate lethal genomic DNA cleavage guided by a targeting RNA (tdk_BWH2 or susC_BWH2), resulting in loss of cell viability.

REFERENCES

[0131] Goodman, A. L. et al. Identifying genetic determinants needed to establish a human gut symbiont in its habitat. Cell Host Microbe 6, 279–289 (2009).

[0132] Hibberd, M. C. et al. The effects of micronutrient deficiencies on bacterial species from the human gut microbiota. Sci. Transl. Med. 9, eaal4069 (2017).

[0133] Patnode, M. et al. Interspecies competition impacts targeted manipulation of human gut bacteria by fiber-derived glycans. Cell, 179(1), 59-73.

[0134] Lenth, R. (2019). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.4.1. https://CRAN.R-project.org/package=emmeans.

[0135] Ridaura, V. K. et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 341, 1241214.

[0136] R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

[0137] Smith, C. J. et al. Nucleotide Sequence Determination and Genetic Analysis of the *Bacteroides* Plasmid, pBI143. Plasmid, 1995, 34:211-222

[0138] Lim et al. Engineered Regulatory Systems Modulate Gene Expression of Human Commensals in the Gut. Cell, 2017, 169:547-558

WHAT IS CLAIMED IS:

1. A protein-nucleic acid complex comprising an engineered RNA-guided nuclease system in association with a chromosome of a bacterial or archaeal species, wherein the engineered RNA-guided nuclease system is targeted to a site in the chromosome of the microorganism, and the chromosome of the microorganism encodes an HU family DNA-binding protein comprising an amino acid sequence with at least 50% sequence identity to SEQ ID NO:1.
2. The protein-nucleic acid complex of claim 1, wherein the engineered RNA-guided nuclease system is a CRISPR system chosen from a Type I CRISPR system, a type II CRISPR system, a type III CRISPR system, a Type IV CRISPR system, a type V CRISPR system, or a type VI CRISPR system.
3. The protein-nucleic acid complex of claim 2, wherein the CRISPR system comprises a CRISPR nuclease and a guide RNA.
4. The protein-nucleic acid complex of claim 3, wherein the CRISPR nuclease is Cas9, Cas12, Cas13, or CasX.
5. The protein-nucleic acid complex of any one of claims 1 to 4, wherein the engineered RNA-guided nuclease system is expressed from a nucleic acid that encodes the engineered RNA-guided nuclease system and is integrated into the bacterial or archaeal chromosome.
6. The protein-nucleic acid complex of any one of claims 1 to 4, wherein the engineered RNA-guided nuclease system is expressed from a nucleic acid that encodes the engineered RNA-guided nuclease system and is carried on an extrachromosomal vector.
7. The protein-nucleic acid complex of any one of claims 1 to 6, wherein the prokaryotic chromosome is within a *Bacteroides* species.
8. The protein-nucleic acid complex of claim 7, wherein the *Bacteroides* species is chosen from *B. thetaiotaomicron*, *B. vulgatus*, *B. cellulosilyticus*, *B. fragilis*, *B. helcogenes*, *B. ovatus*, *B. salanitronis*, *B. uniformis*, or *B. xylanisolvens*.
9. A method for slowing growth of a target prokaryote in a mixed population of prokaryotes, the method comprising expressing an engineered RNA-guided nuclease system in the target prokaryote,

wherein the engineered RNA-guided nuclease system is targeted to a site in a chromosome of the target prokaryote such that at least one double stranded break is introduced in the chromosome of the target prokaryote, thereby slowing growth of the target prokaryote.

10. The method of claim 9, wherein slowing growth of the target prokaryote leads to reduced or eliminated levels of the target prokaryote in the mixed population of prokaryotes.
11. The method of claim 9 or 10, wherein expression of the RNA-guided nuclease system is inducible.
12. The method of any one of claims 9 to 11, wherein the engineered RNA-guided nuclease system is a CRISPR system chosen from a Type I CRISPR system, a type II CRISPR system, a type III CRISPR system, a Type IV CRISPR system, a type V CRISPR system, or a type VI CRISPR system.
13. The method of claim 12, wherein the CRISPR system comprises a CRISPR nuclease and a guide RNA.
14. The method of claim 13, wherein the CRISPR nuclease is a Cas9, a Cas12, a Cas13, or a CasX nuclease.
15. The method of claim 13, wherein the CRISPR nuclease and guide RNA are expressed from at least one nucleic acid integrated into the chromosome of the target prokaryote.
16. The method of claim 13, wherein the CRISPR nuclease and guide RNA are expressed from at least one nucleic acid carried on an extrachromosomal vector.
17. The method of claim 15 or 16, wherein the nucleic acid encoding the CRISPR nuclease is operably linked to an inducible promoter.
18. The method of claim 17, wherein the expressing step comprises contacting the mixed population of prokaryotes with a promoter inducing chemical.
19. The method of any one of claims 9 to 18, wherein the mixed population of prokaryotes is harbored in cell culture.
20. The method of any one of claims 9 to 18, wherein the mixed population of prokaryotes is harbored in a mammal's digestive tract.

21. The method of claim 20, wherein the engineered RNA-guided nuclease system is an engineered CRISPR nuclease system, at least one nucleic acid encoding the engineered CRISPR nuclease system is introduced into the target prokaryote, the nucleic acid encoding the CRISPR nuclease is operably linked to an inducible promoter, and the expressing step comprises administering a promoter inducing chemical to the mammal.
22. The method of claim 21, wherein the administering comprises orally administering the promoter inducing chemical.
23. The method of any one of claims 18, 21, or 22, wherein the promoter inducing chemical is anhydrotetracycline.
24. The method of any one of claims 20 to 23, wherein the mammal is a human.
25. The method of claim 24, wherein the human is undergoing treatment for cancer, and reduction or elimination of the target prokaryote from the mixed population of prokaryotes in the gastrointestinal tract of the human improves the response of the human to the treatment for cancer.
26. The method of claim 25, wherein the treatment for cancer comprises immunotherapy.
27. The method of any one of claims 9 to 26, wherein the target prokaryote is a *Bacteroides* species.
28. The method of claim 27, wherein the *Bacteroides* species is chosen from *B. thetaiotaomicron*, *B. vulgatus*, *B. cellulosilyticus*, *B. fragilis*, *B. helcogenes*, *B. ovatus*, *B. salanitronis*, *B. uniformis*, or *B. xylanisolvens*.

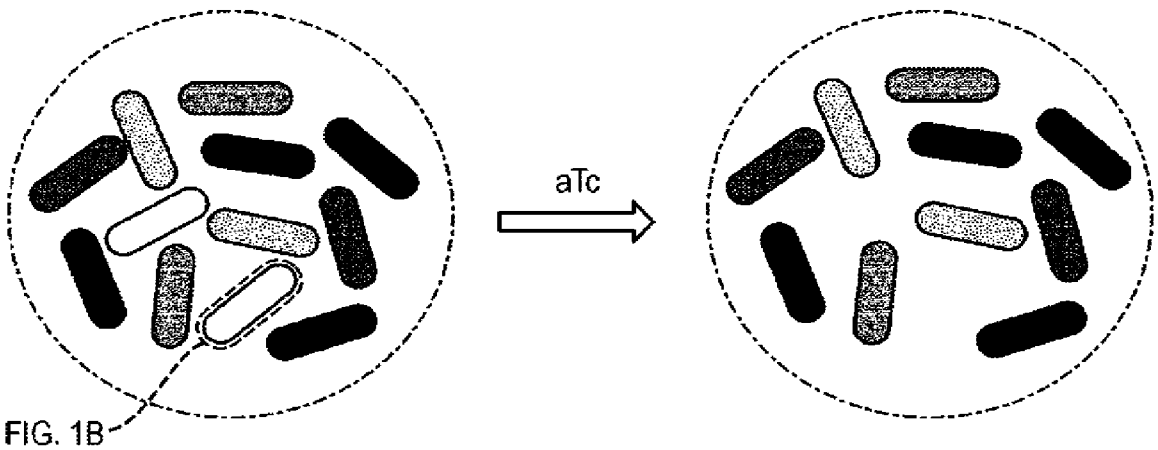


FIG. 1A

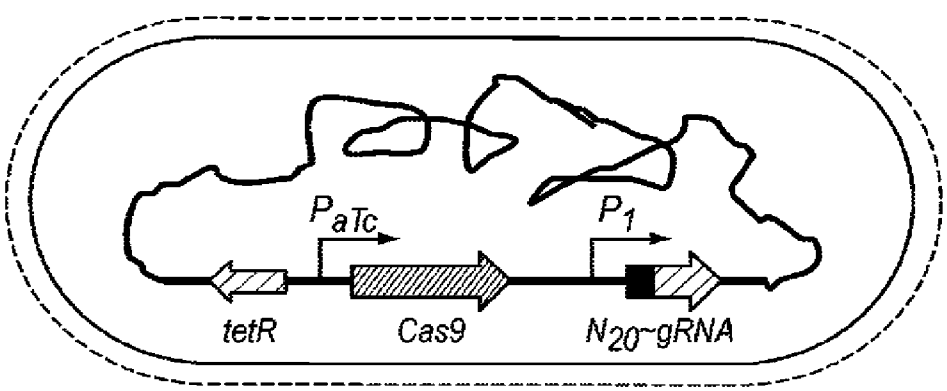


FIG. 1B

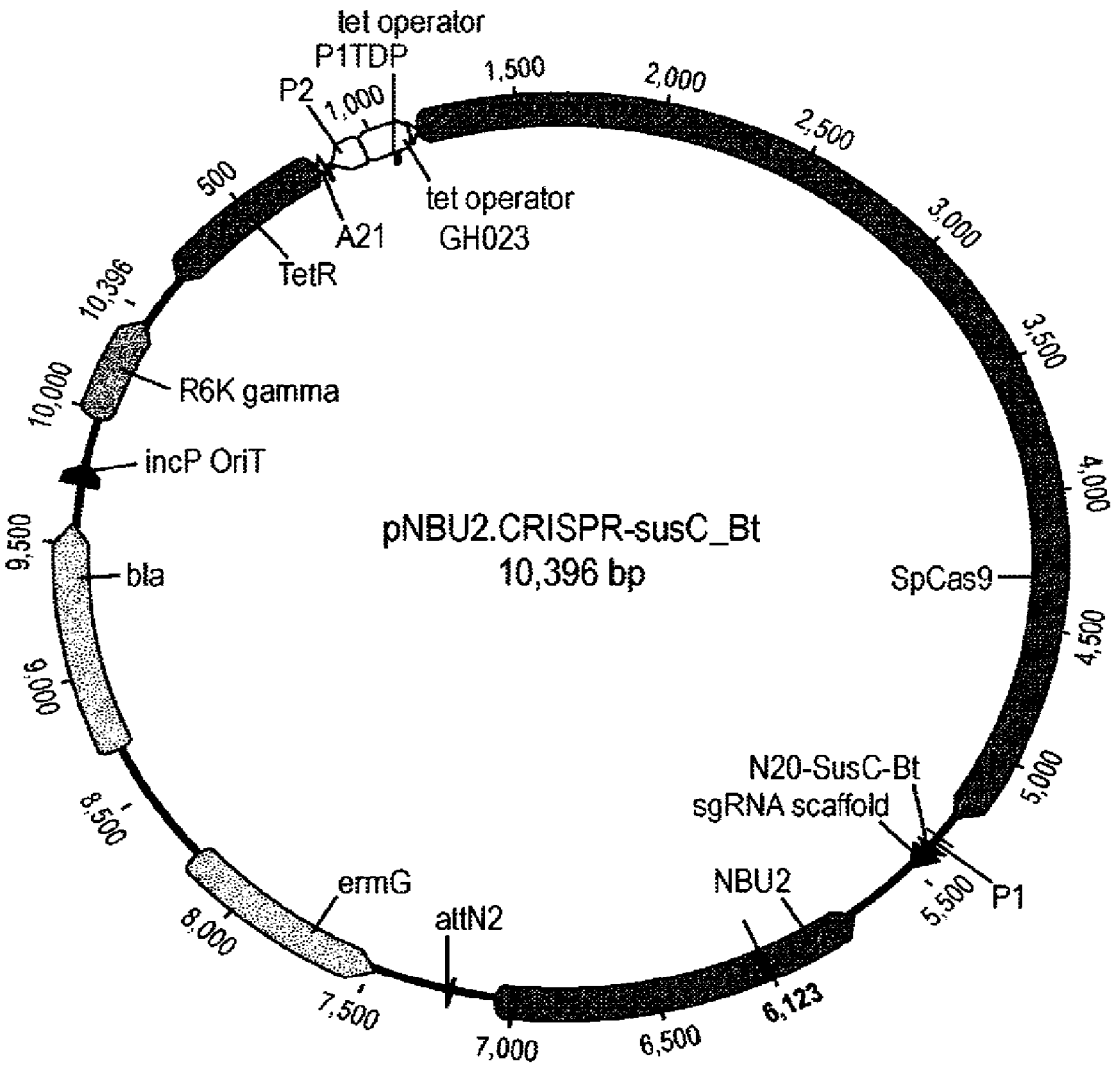
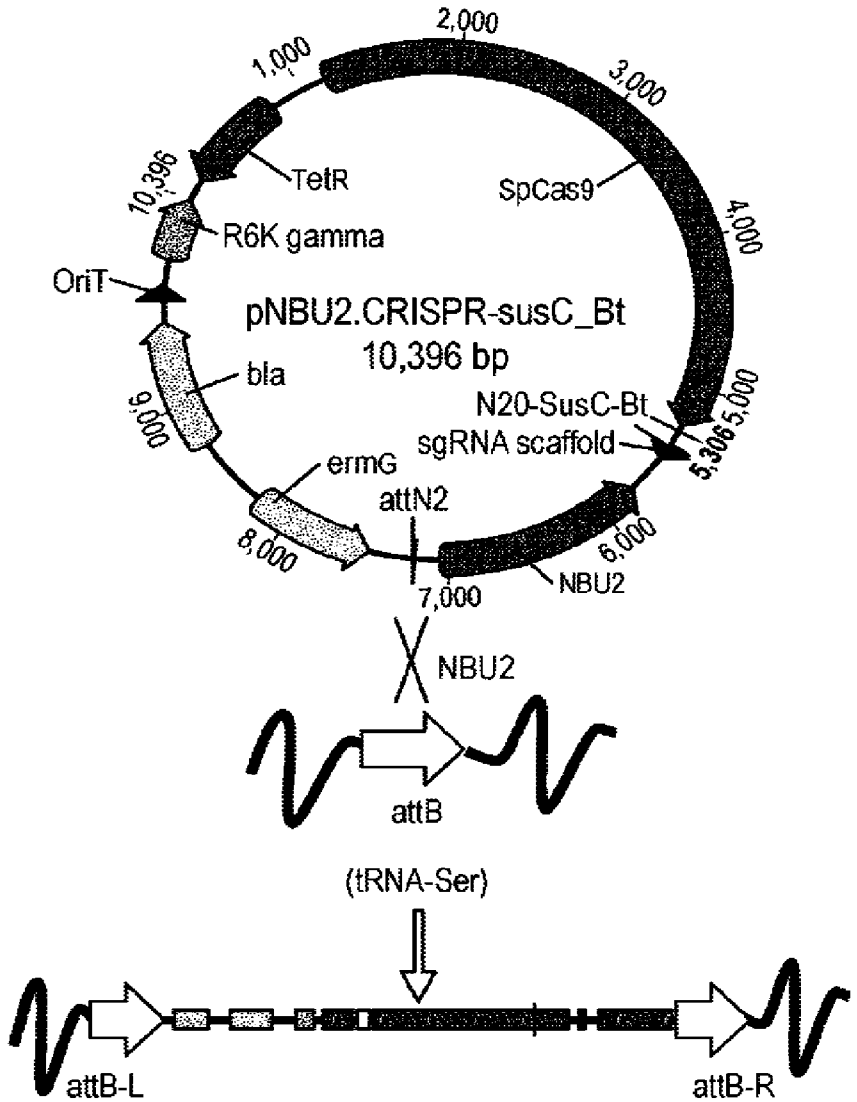

tet operator
P1TDP
P2
1,000
1,500
2,000
2,500
3,000
3,500
4,000
4,500
5,000
5,500
6,123
6,500
7,000
7,500
8,000
8,500
9,000
9,500
10,000
10,396
500
A21
tet operator
GH023
TetR
R6K gamma
incP OriT
bla
pNBU2.CRISPR-susC_Bt
10,396 bp
SpCas9
N20-SusC-Bt
sgRNA scaffold
P1
NBU2
attN2
ermG

FIG. 2



CRISPR integration on the chromosome

FIG. 3A

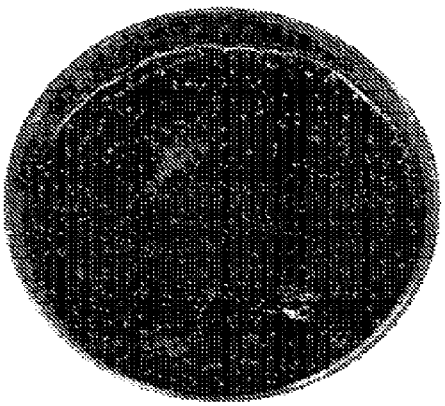


FIG. 3B

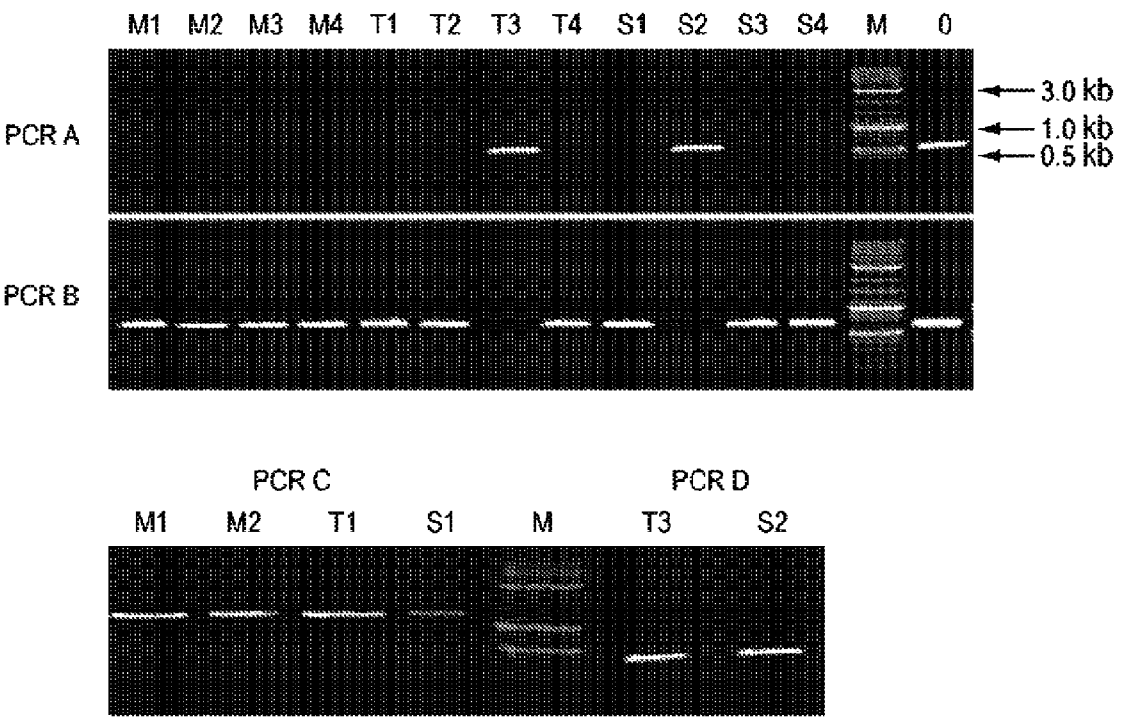

M1 M2 M3 M4 T1 T2 T3 T4 S1 S2 S3 S4 M 0
PCR A
PCR B
3.0 kb
1.0 kb
0.5 kb
PCR C
PCR D
M1 M2 T1 S1 M T3 S2

FIG. 3C

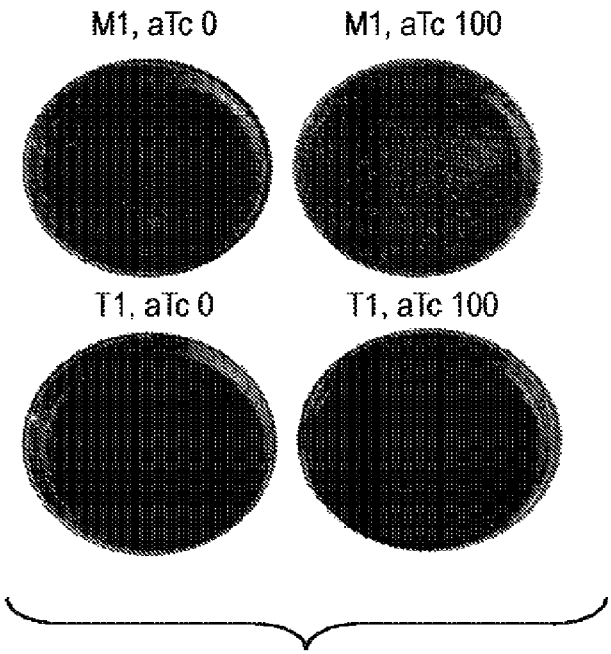


FIG. 4A

Strain	gRNA	OD600nm in TYG + aTc (ng/ml)		
		0	10	100
M1	Non-targeting	+++	+++	+++
M2		+++	+++	+++
T1	*tdk*_Bt	+++	+	-
T3		+++	-	-
S1	*susC*_Bt	+++	-	-
S2		+++	+	-

OD_{600nm}: +++, ~1.3; +, ~0.1; -, 0

FIG. 4B

Strain	gRNAs	OD600nm at aTc (ng/ml)		
		0	10	100
M1 + T1	*Non-targeting* + *tdk*_Bt	+++	+++	+++
M1 + S1	*Non-targeting* + *susC*_Bt	+++	+++	+++

OD_{600nm}: +++, ~1.3

FIG. 5A

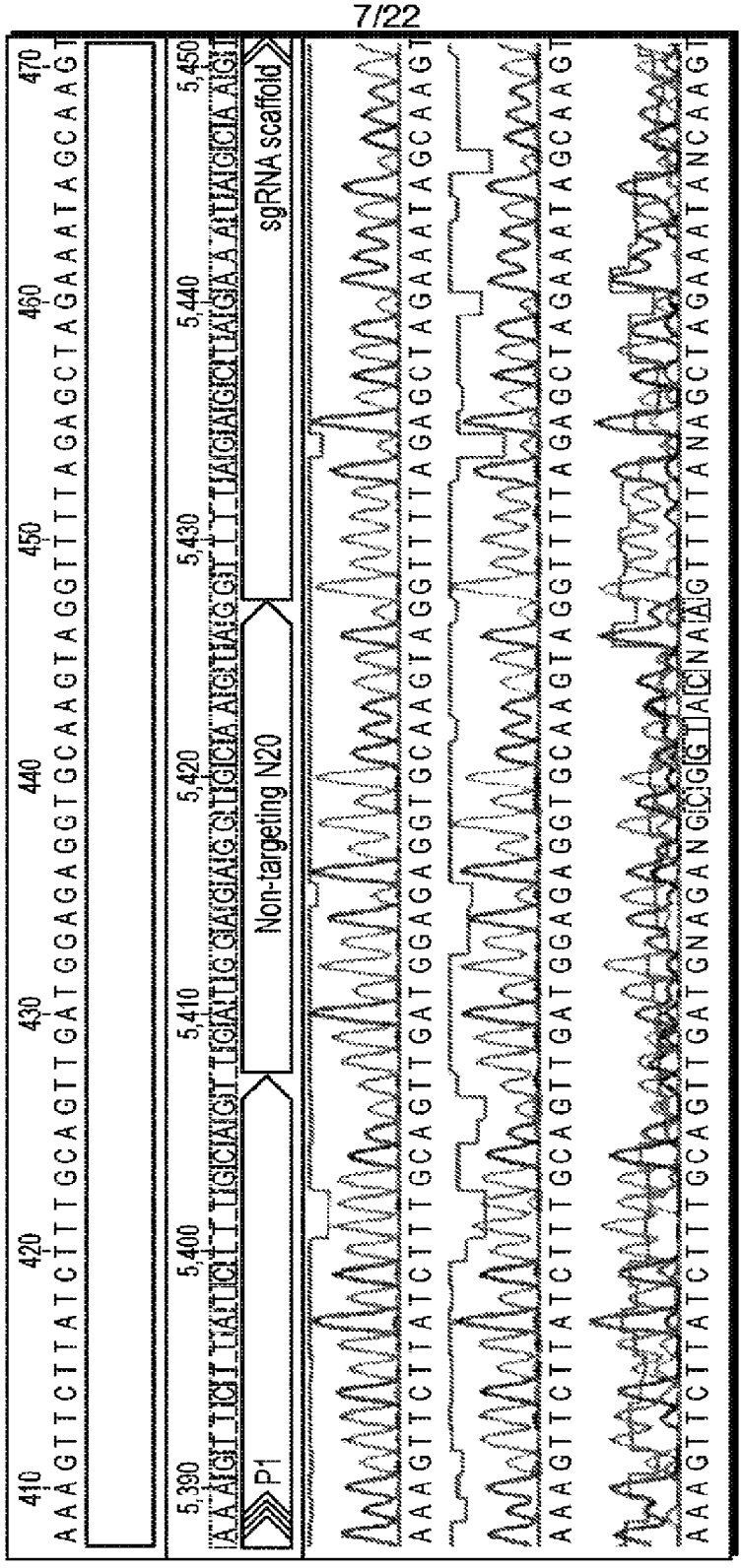

410
420
430
440
450
460
470
5,390
5,400
5,410
5,420
5,430
5,440
5,450
P1
Non-targeting N20
sgRNA scaffold
M1+T1_aTc100
M1+T1_aTc10
M1+T1_aTc0

FIG. 5B

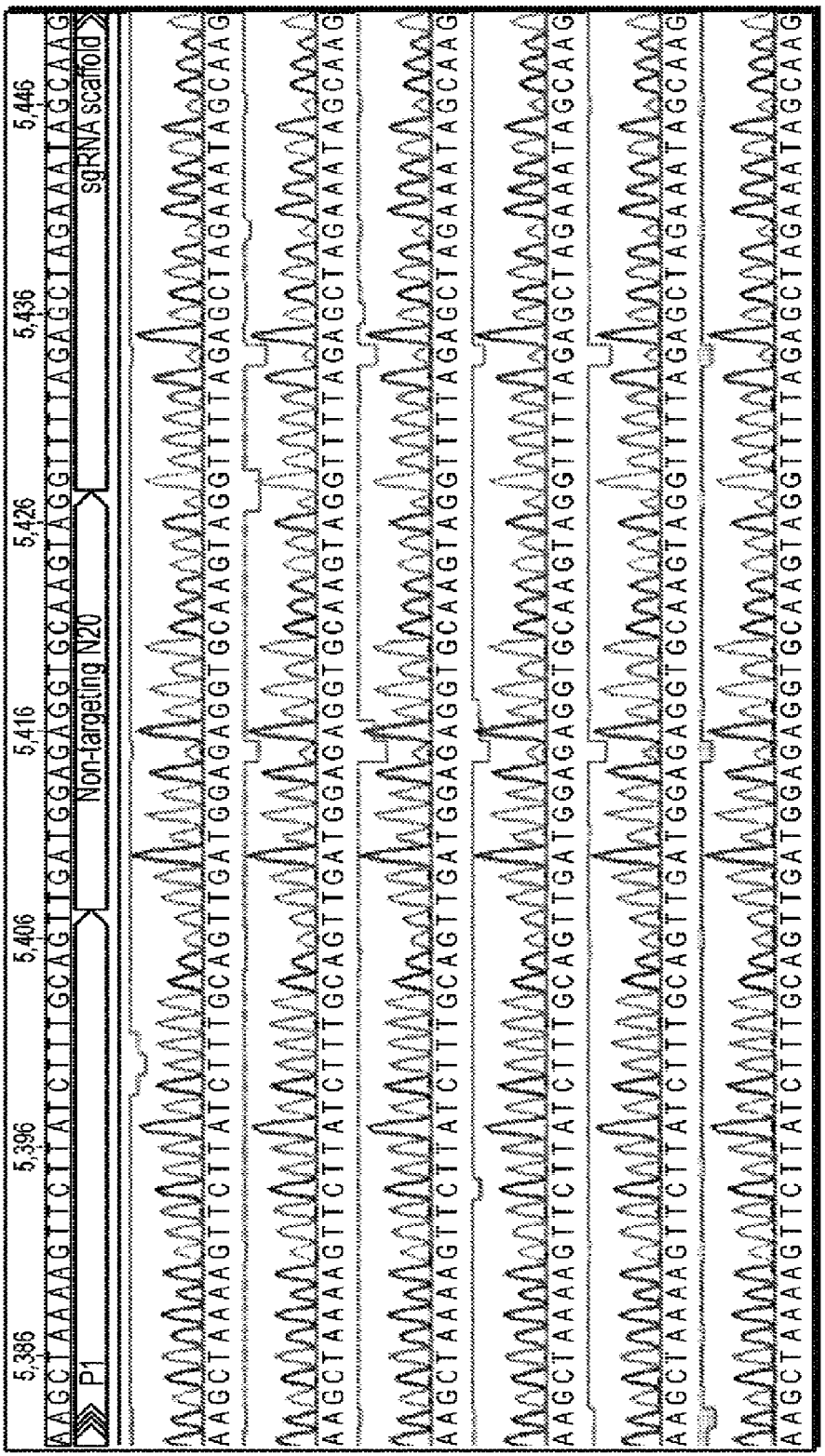

5,386
5,396
5,406
5,416
5,426
5,436
5,446
P1
Non-targeting N20
sgRNA scaffold
Colony 1
Colony 2
Colony 3
Colony 4
Colony 5
Mixed colonies

FIG. 5C

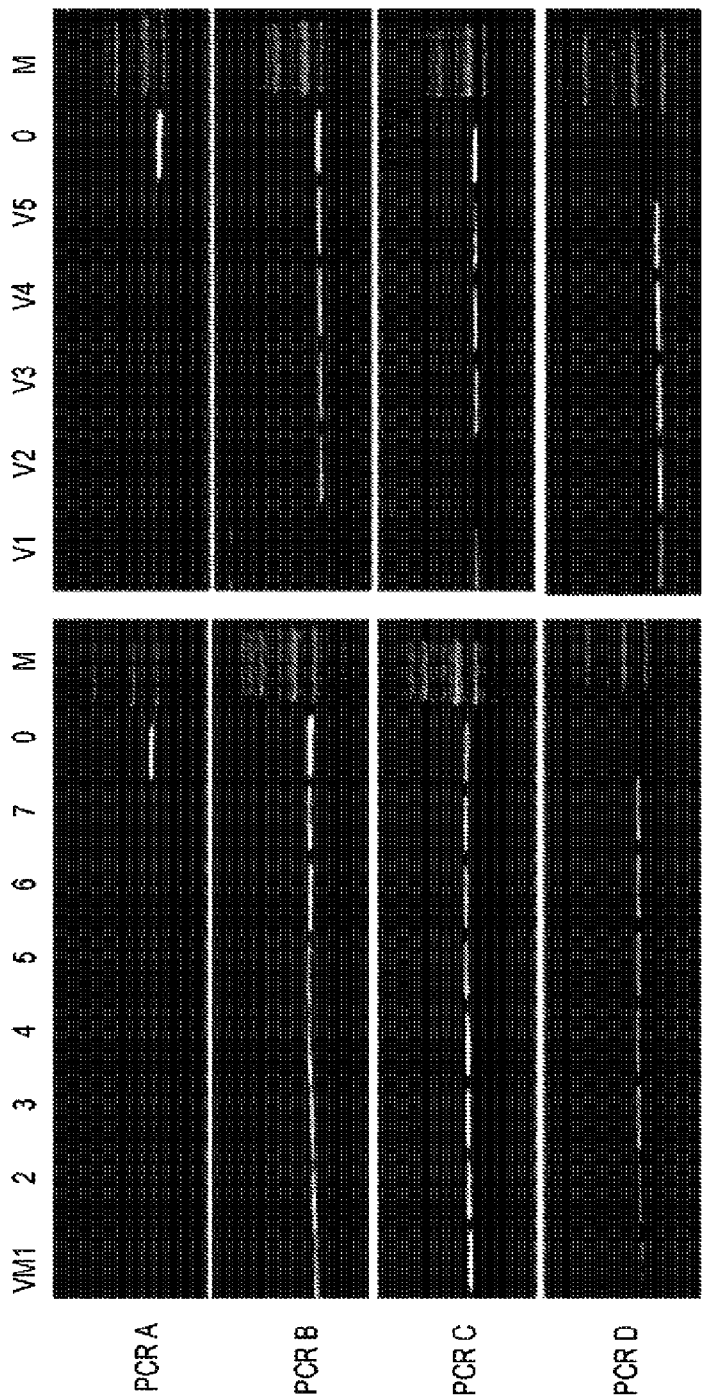


FIG. 6

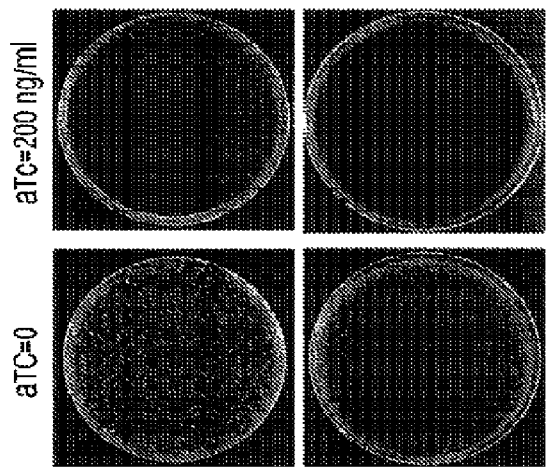


FIG. 7B

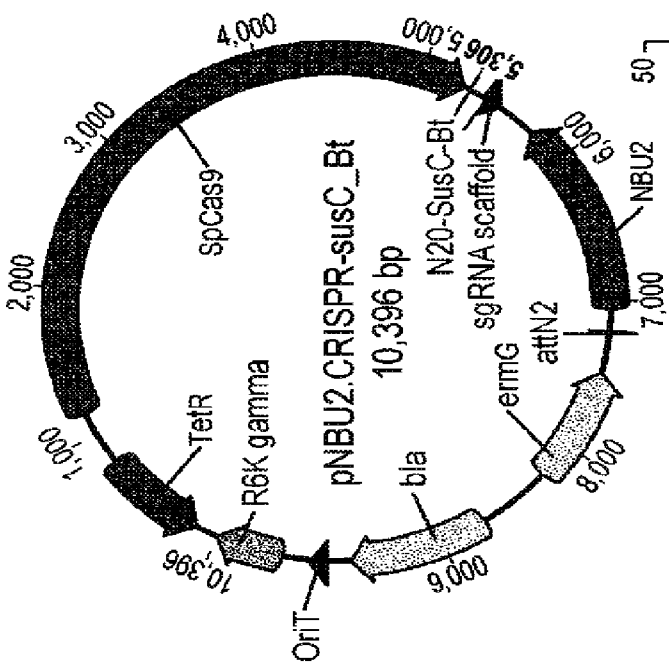


FIG. 7A

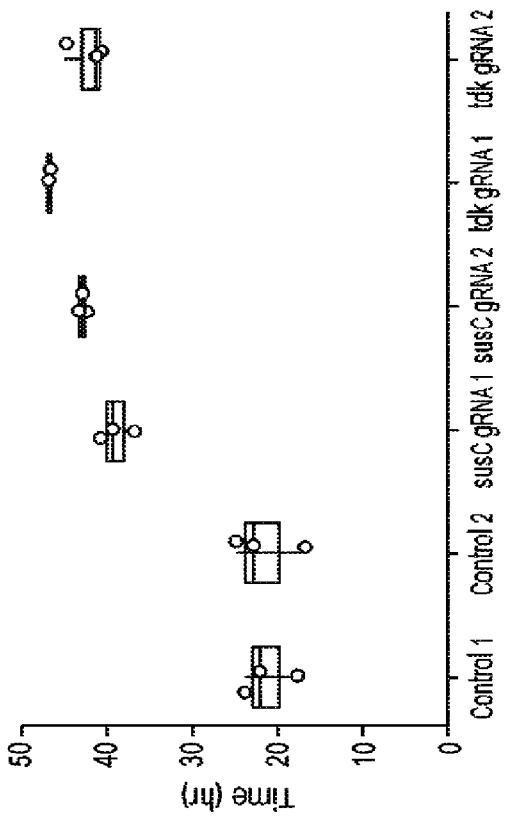


FIG. 7C

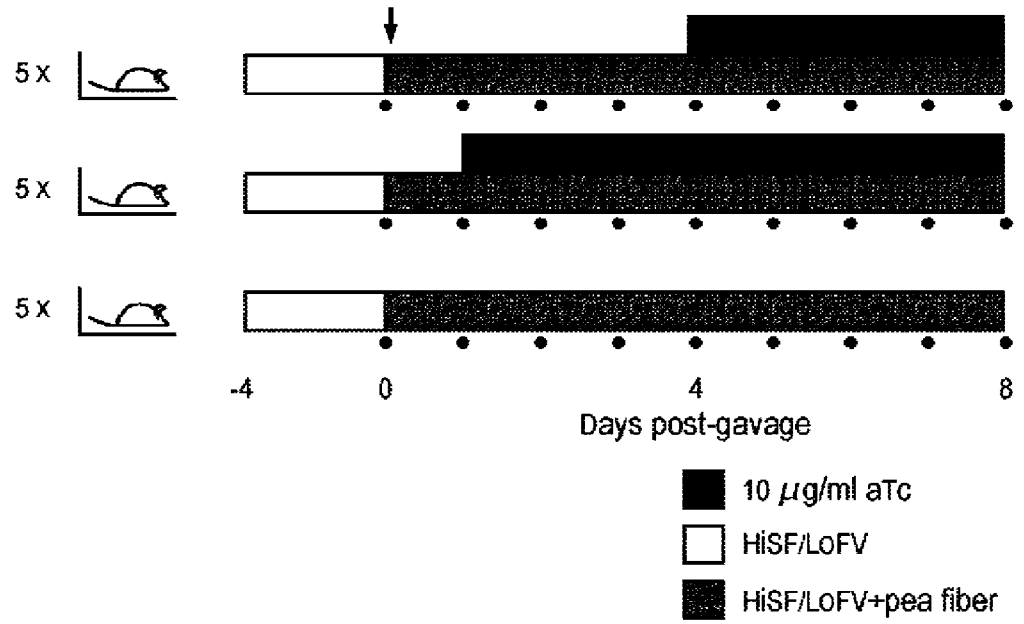

5 X
5 X
5 X
-4
0
4
8
Days post-gavage
10 μg/ml aTc
HiSF/LoFV
HiSF/LoFV+pea fiber

FIG. 8A

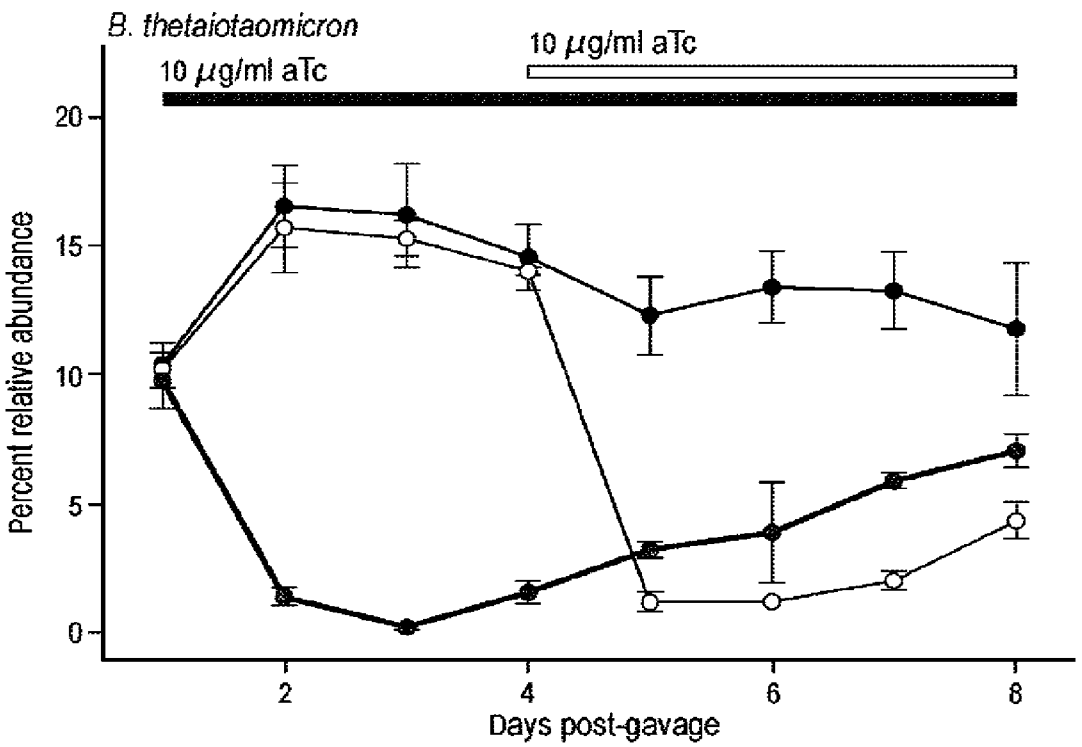

B. thetaiotaomicron
10 μg/ml aTc
10 μg/ml aTc
Percent relative abundance
20
15
10
5
0
2
4
6
8
Days post-gavage

FIG. 8B

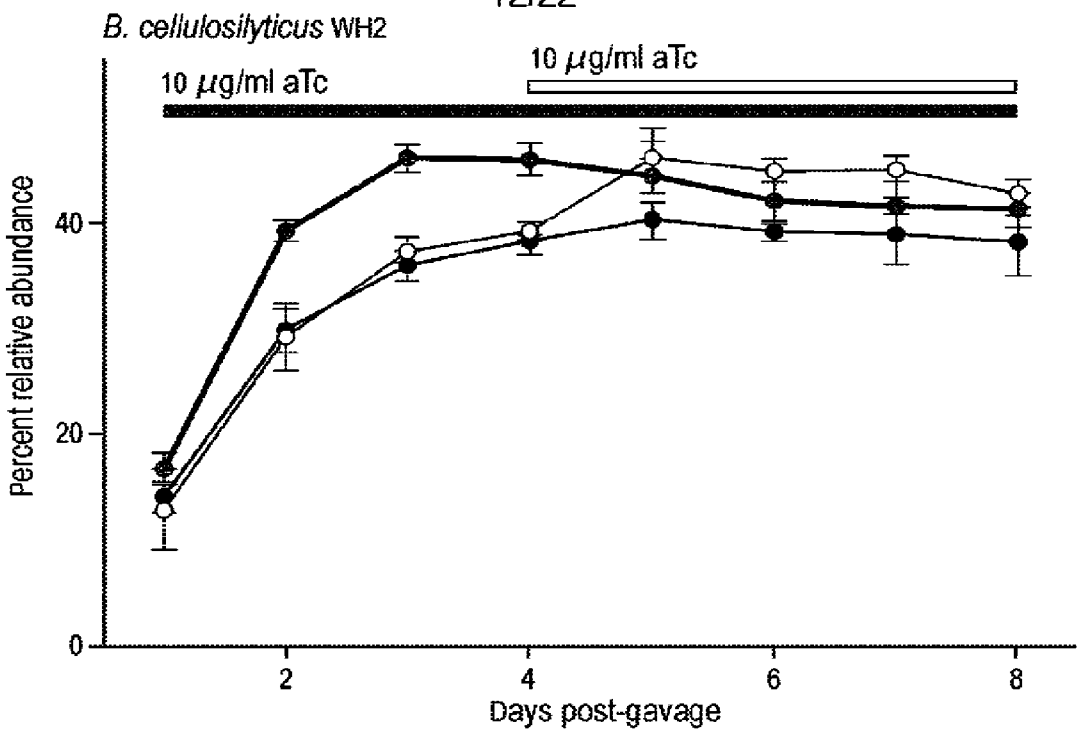

B. cellulosilyticus WH2
10 μg/ml aTc
10 μg/ml aTc
Percent relative abundance
0
20
40
2
4
6
8
Days post-gavage

FIG. 8C

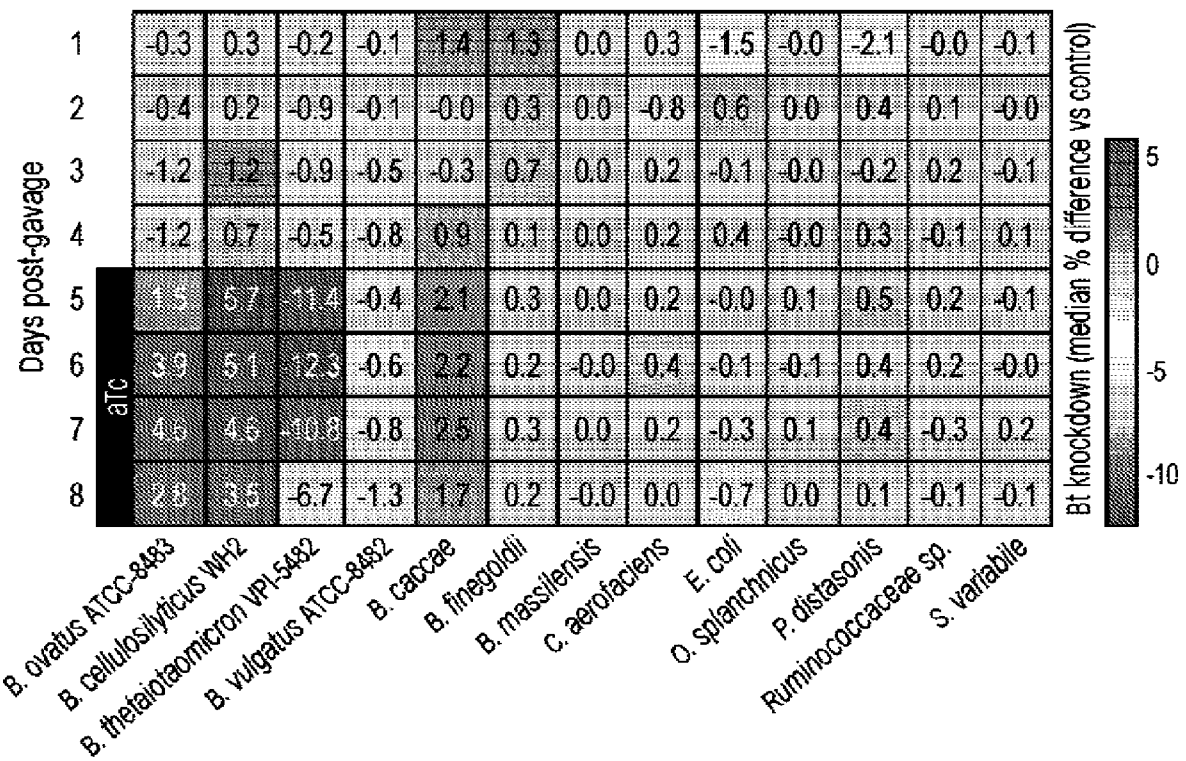

Days post-gavage
aTc
1 -0.3 0.3 -0.2 -0.1 1.4 1.3 0.0 0.3 -1.5 -0.0 -2.1 -0.0 -0.1
2 -0.4 0.2 -0.9 -0.1 -0.0 0.3 0.0 -0.8 0.6 0.0 0.4 0.1 -0.0
3 -1.2 -1.2 -0.9 -0.5 -0.3 0.7 0.0 0.2 -0.1 -0.0 -0.2 0.2 -0.1
4 -1.2 0.7 -0.5 -0.8 0.9 0.1 0.0 0.2 0.4 -0.0 0.3 -0.1 0.1
5 1.5 5.7 -11.4 -0.4 2.1 0.3 0.0 0.2 -0.0 0.1 0.5 0.2 -0.1
6 3.9 5.1 -12.3 -0.6 2.2 0.2 -0.0 0.4 -0.1 -0.1 0.4 0.2 -0.0
7 4.6 4.6 -10.8 -0.8 2.5 0.3 0.0 0.2 -0.3 0.1 0.4 -0.3 0.2
8 2.8 3.5 -6.7 -1.3 1.7 0.2 -0.0 0.0 -0.7 0.0 0.1 -0.1 -0.1
B. ovatus ATCC-8483
B. cellulosilyticus WH2
B. thetaiotaomicron VPI-5482
B. vulgatus ATCC-8482
B. caccae
B. finegoldii
B. massiliensis
C. aerofaciens
E. coli
O. splanchnicus
P. distasonis
Ruminococcaceae sp.
S. variabile
Bt knockdown (median % difference vs control)
5
0
-5
-10

FIG. 8D

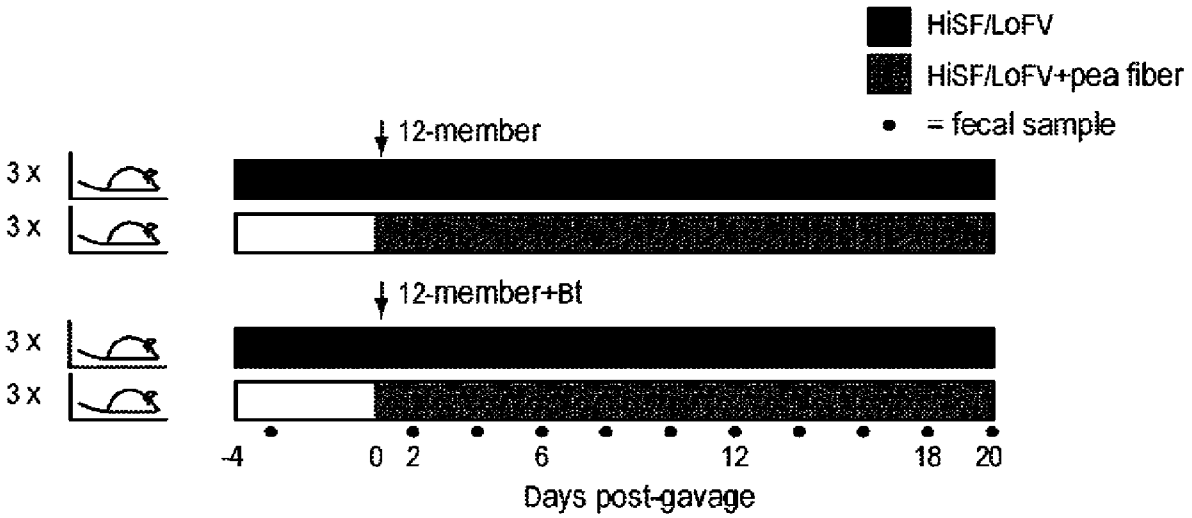

HiSF/LoFV
HiSF/LoFV+pea fiber
= fecal sample
12-member
3 x
3 x
12-member+Bt
3 x
3 x
-4
0
2
6
12
18
20
Days post-gavage

FIG. 9A

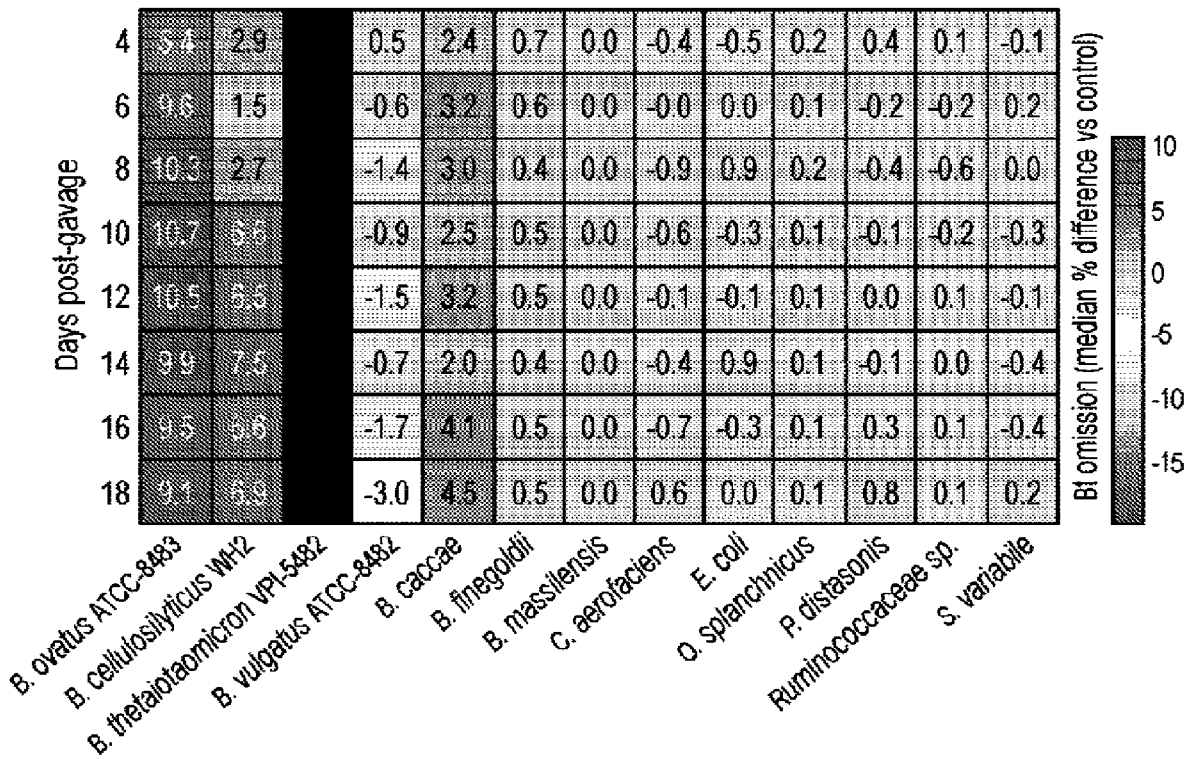

Days post-gavage
4 5.4 2.9 0.5 2.4 0.7 0.0 -0.4 -0.5 0.2 0.4 0.1 -0.1
6 9.6 1.5 -0.6 3.2 0.6 0.0 -0.0 0.0 0.1 -0.2 -0.2 0.2
8 10.3 2.7 -1.4 3.0 0.4 0.0 -0.9 0.9 0.2 -0.4 -0.6 0.0
10 10.7 5.6 -0.9 2.5 0.5 0.0 -0.6 -0.3 0.1 -0.1 -0.2 -0.3
12 10.5 6.6 -1.5 3.2 0.5 0.0 -0.1 -0.1 0.1 0.0 0.1 -0.1
14 9.9 7.5 -0.7 2.0 0.4 0.0 -0.4 0.9 0.1 -0.1 0.0 -0.4
16 9.5 8.6 -1.7 4.1 0.5 0.0 -0.7 -0.3 0.1 0.3 0.1 -0.4
18 9.1 8.9 -3.0 4.5 0.5 0.0 0.6 0.0 0.1 0.8 0.1 0.2
Bt omission (median % difference vs control)
10
5
0
-5
-10
-15
B. ovatus ATCC-8483
B. cellulosilyticus WH2
B. thetaiotaomicron VPI-5482
B. vulgatus ATCC-8482
B. caccae
B. finegoldii
B. massiliensis
C. aerofaciens
E. coli
O. splanchnicus
P. distasonis
Ruminococcaceae sp.
S. variabile

FIG. 9B

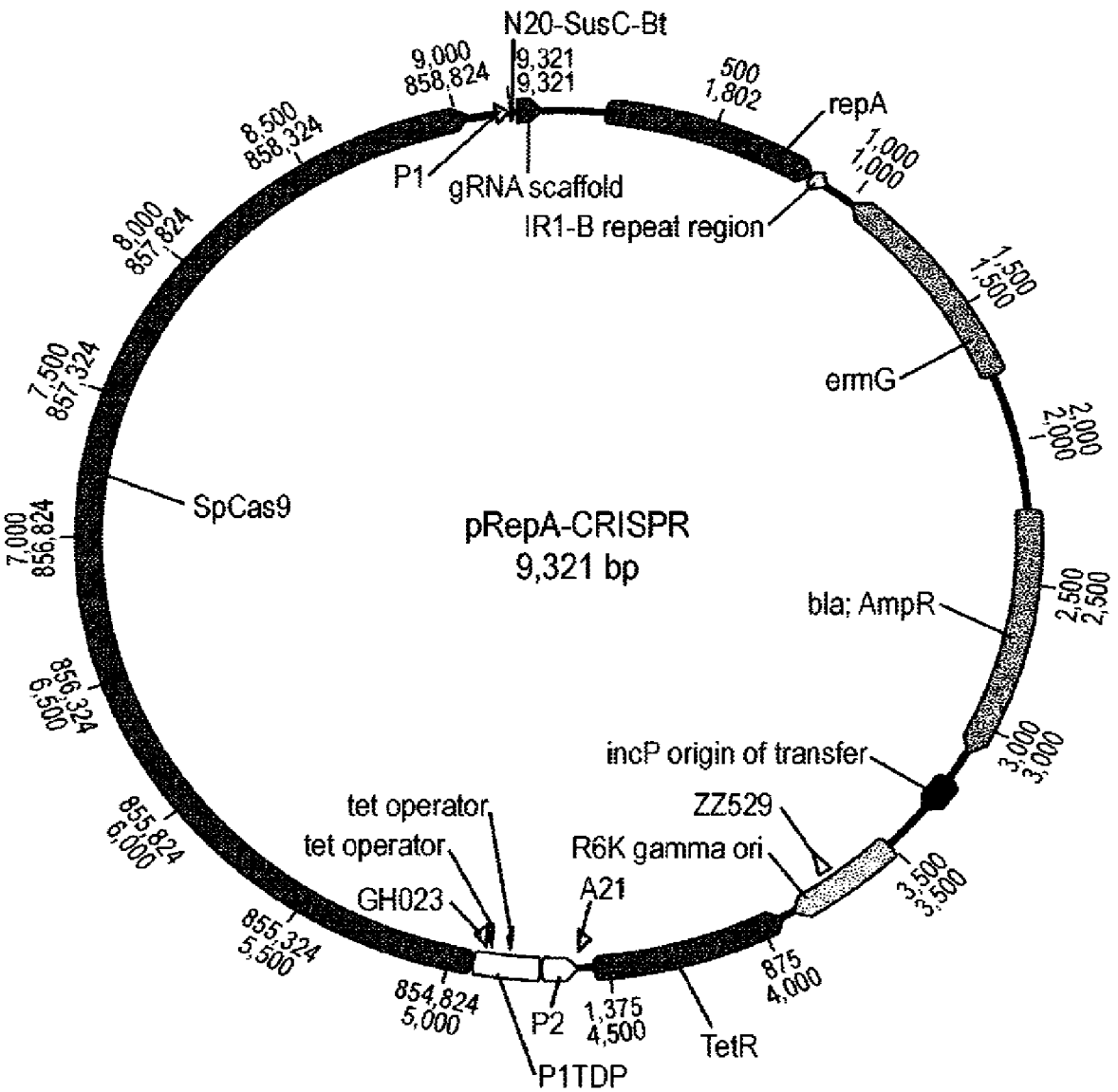

N20-SusC-Bt
9,321
9,321
9,000
858,824
500
1,802
repA
1,000
1,000
P1
gRNA scaffold
IR1-B repeat region
8,500
858,324
8,000
857,824
1,500
1,500
ermG
7,500
857,324
2,000
2,000
SpCas9
pRepA-CRISPR
9,321 bp
7,000
856,824
2,500
2,500
bla; AmpR
6,500
856,324
3,000
3,000
incP origin of transfer
tet operator
ZZ529
tet operator
R6K gamma ori
6,000
855,824
GH023
A21
3,500
3,500
5,500
855,324
4,000
875
854,824
5,000
P2
1,375
4,500
TetR
P1TDP

FIG. 10

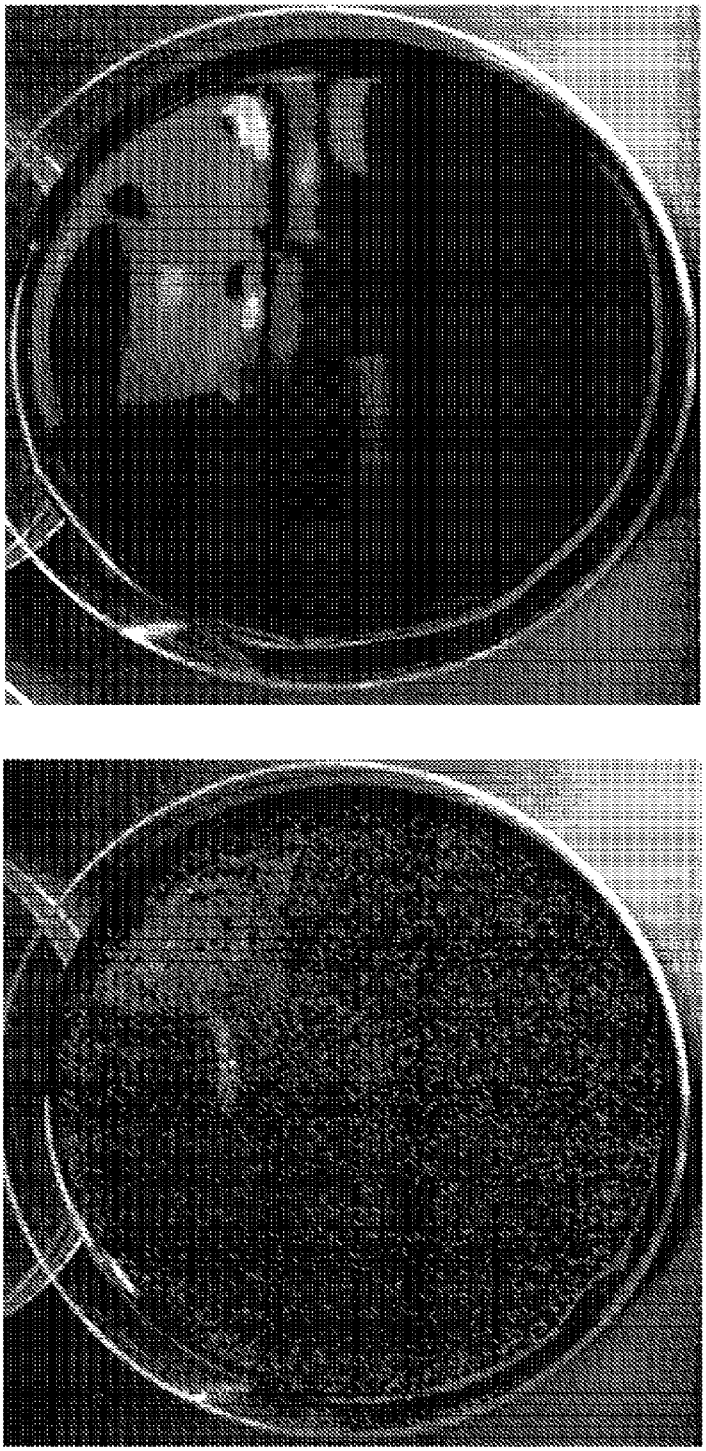


FIG. 11A

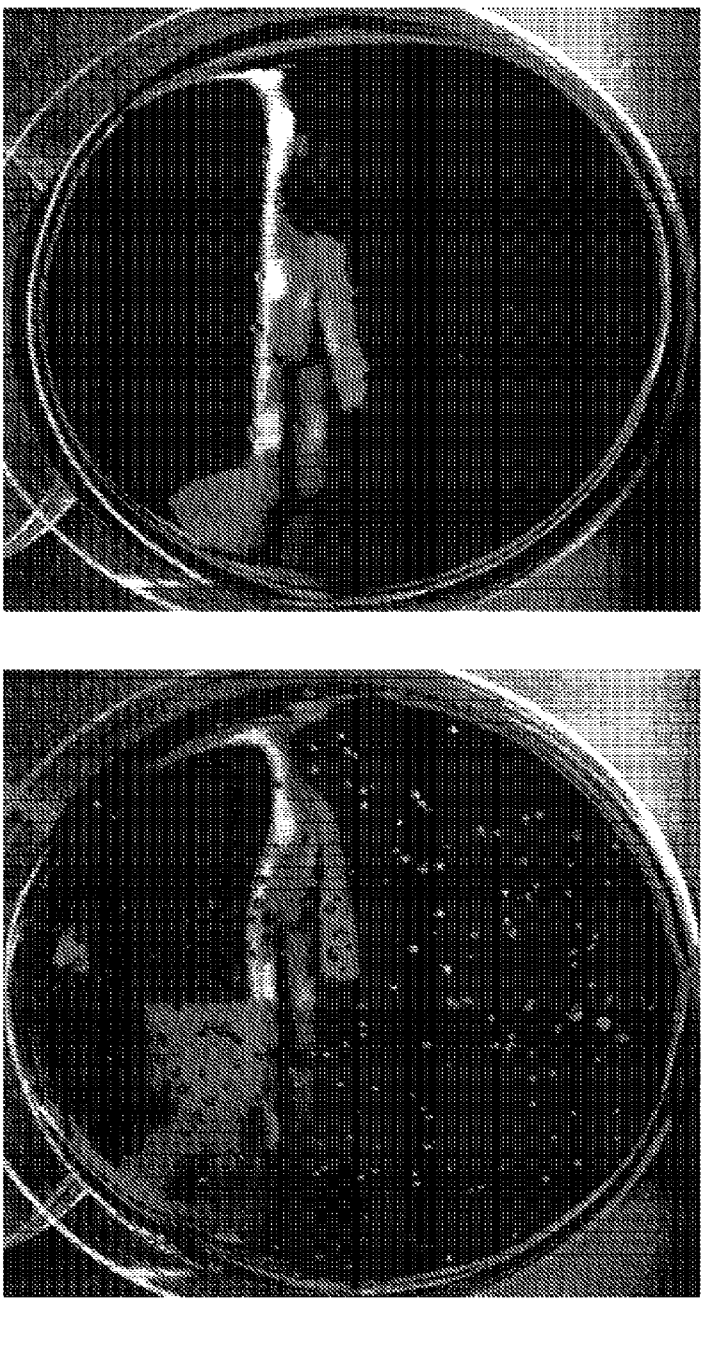


FIG. 11B

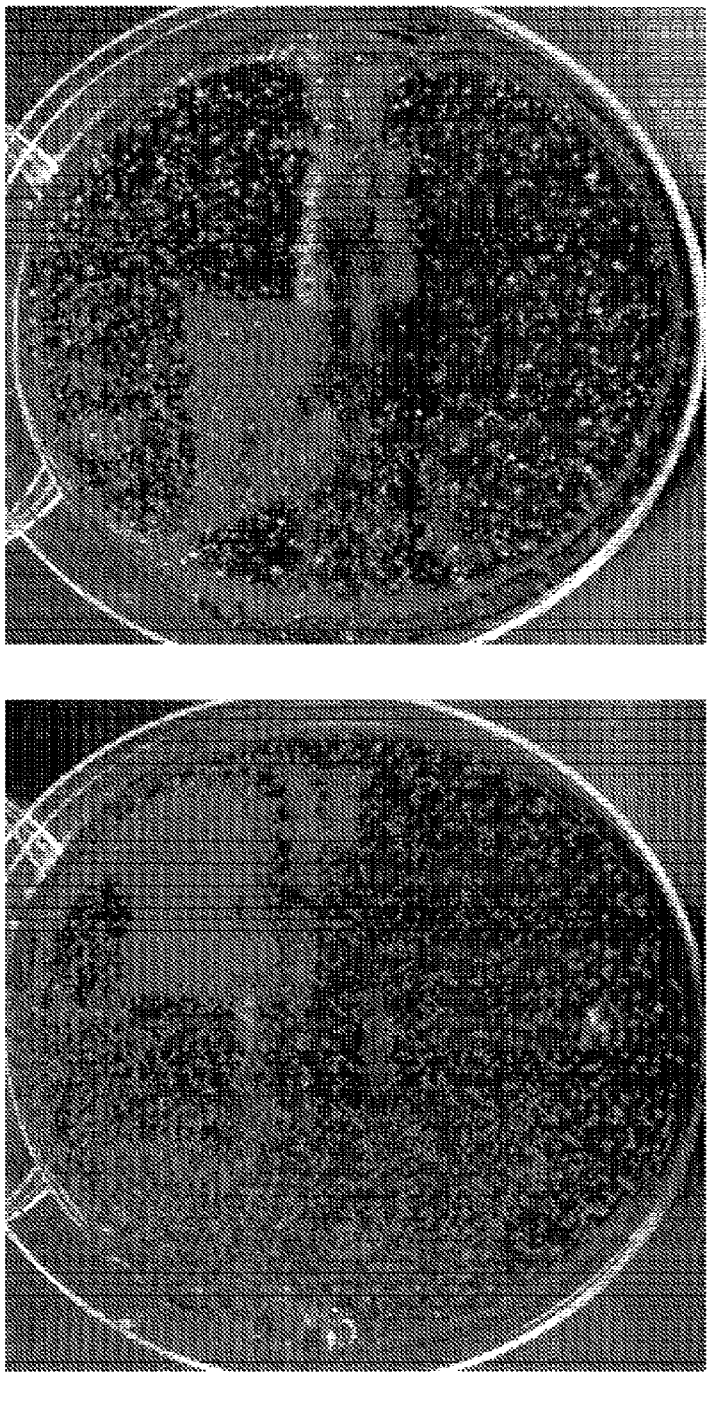


FIG. 11C

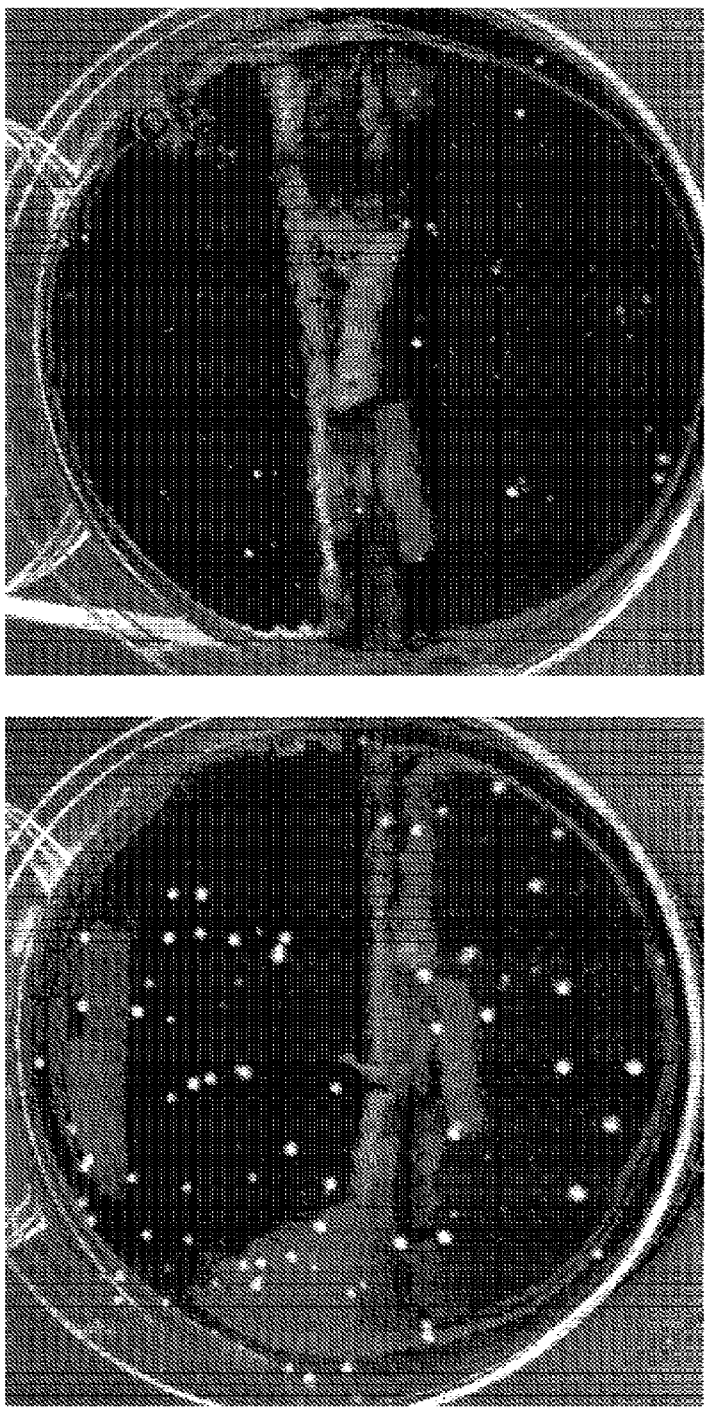


FIG. 11D

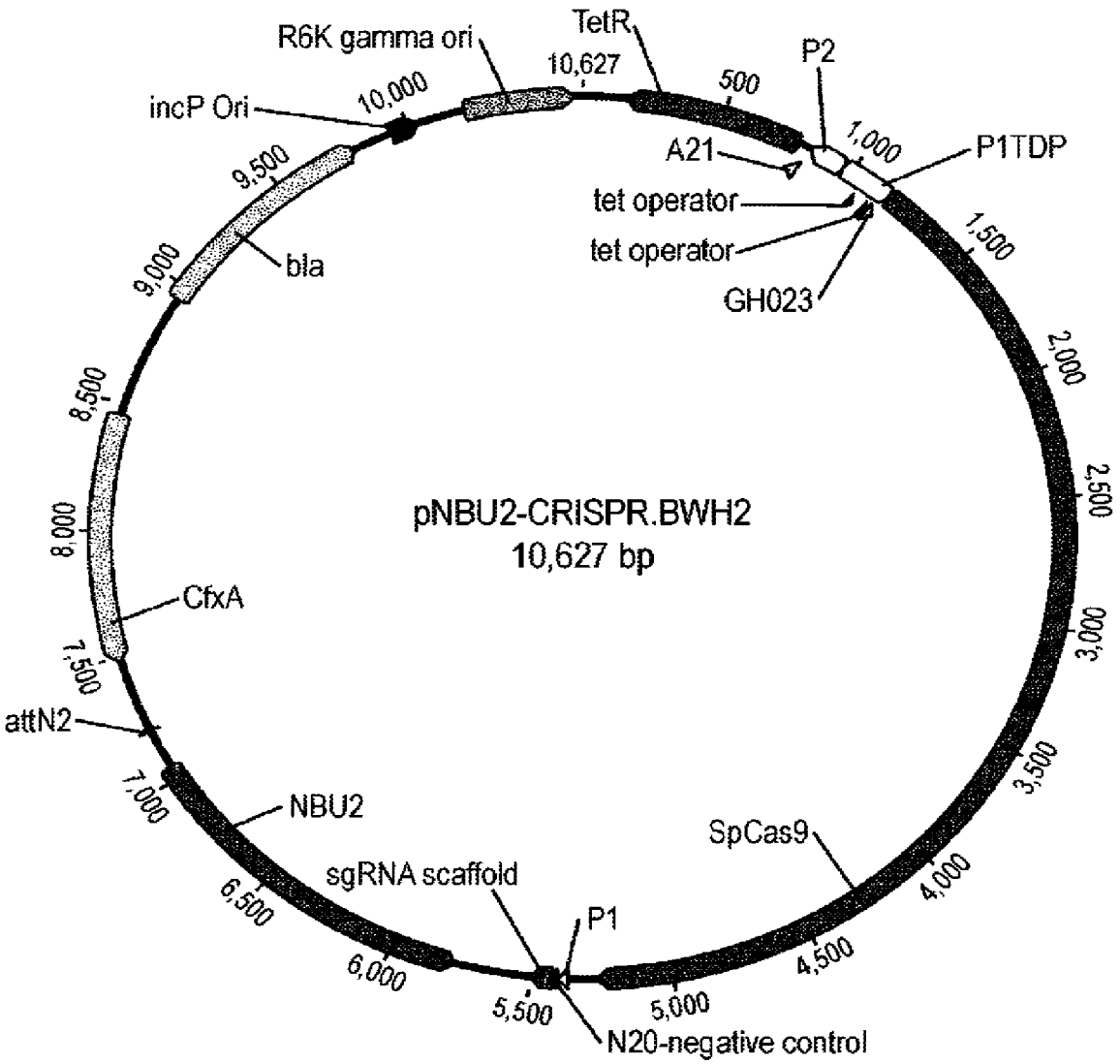

pNBU2-CRISPR.BWH2
10,627 bp
R6K gamma ori
TetR
P2
incP Ori
A21
P1TDP
tet operator
tet operator
GH023
bla
CfxA
attN2
NBU2
SpCas9
sgRNA scaffold
P1
N20-negative control
10,627
500
1,000
1,500
2,000
2,500
3,000
3,500
4,000
4,500
5,000
5,500
6,000
6,500
7,000
7,500
8,000
8,500
9,000
9,500
10,000

FIG. 12

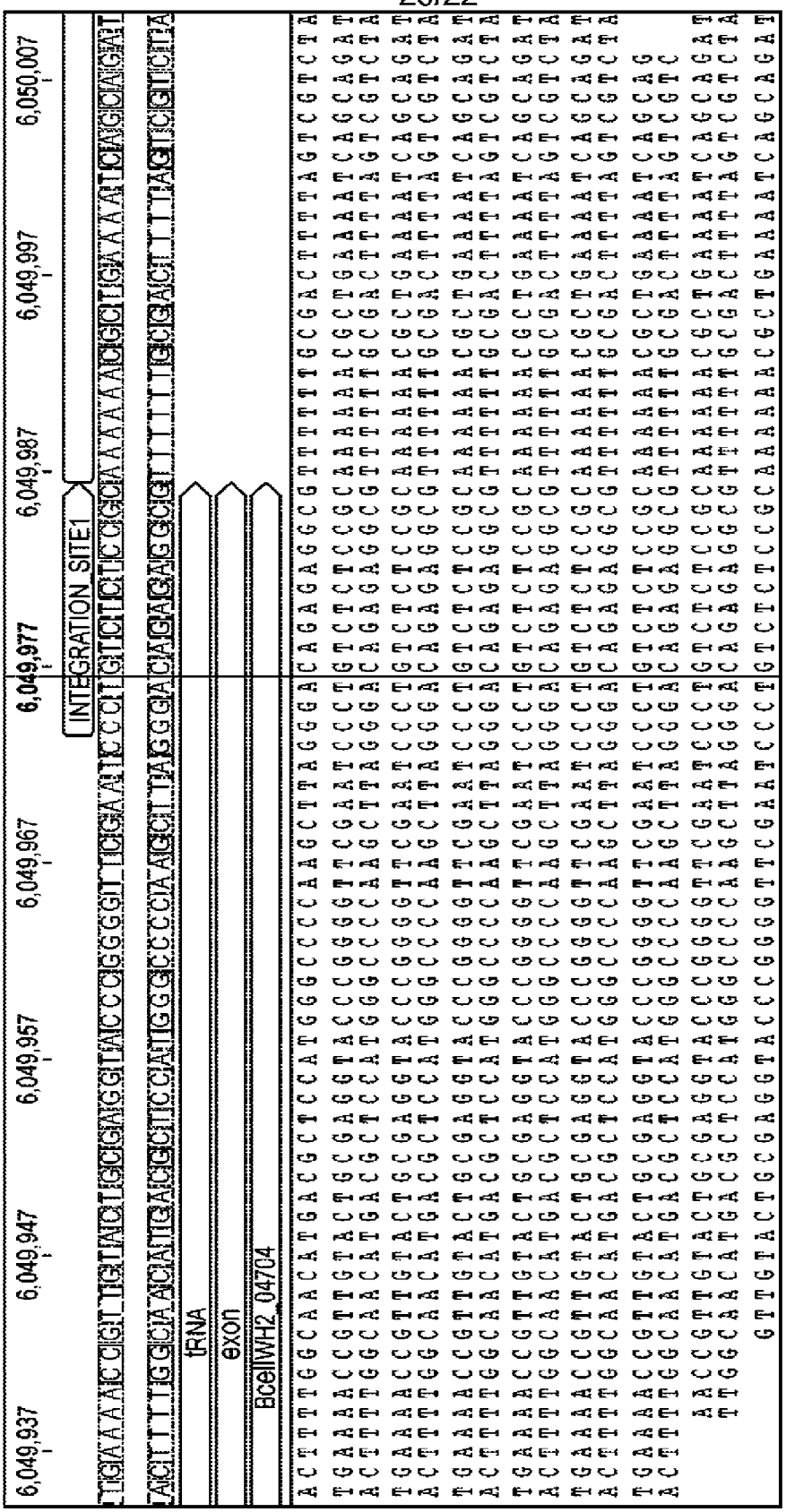

6,049,937
6,049,947
6,049,957
6,049,967
6,049,977
6,049,987
6,049,997
6,050,007
INTEGRATION_SITE1
tRNA
exon
BcellWH2_04704

FIG. 13A

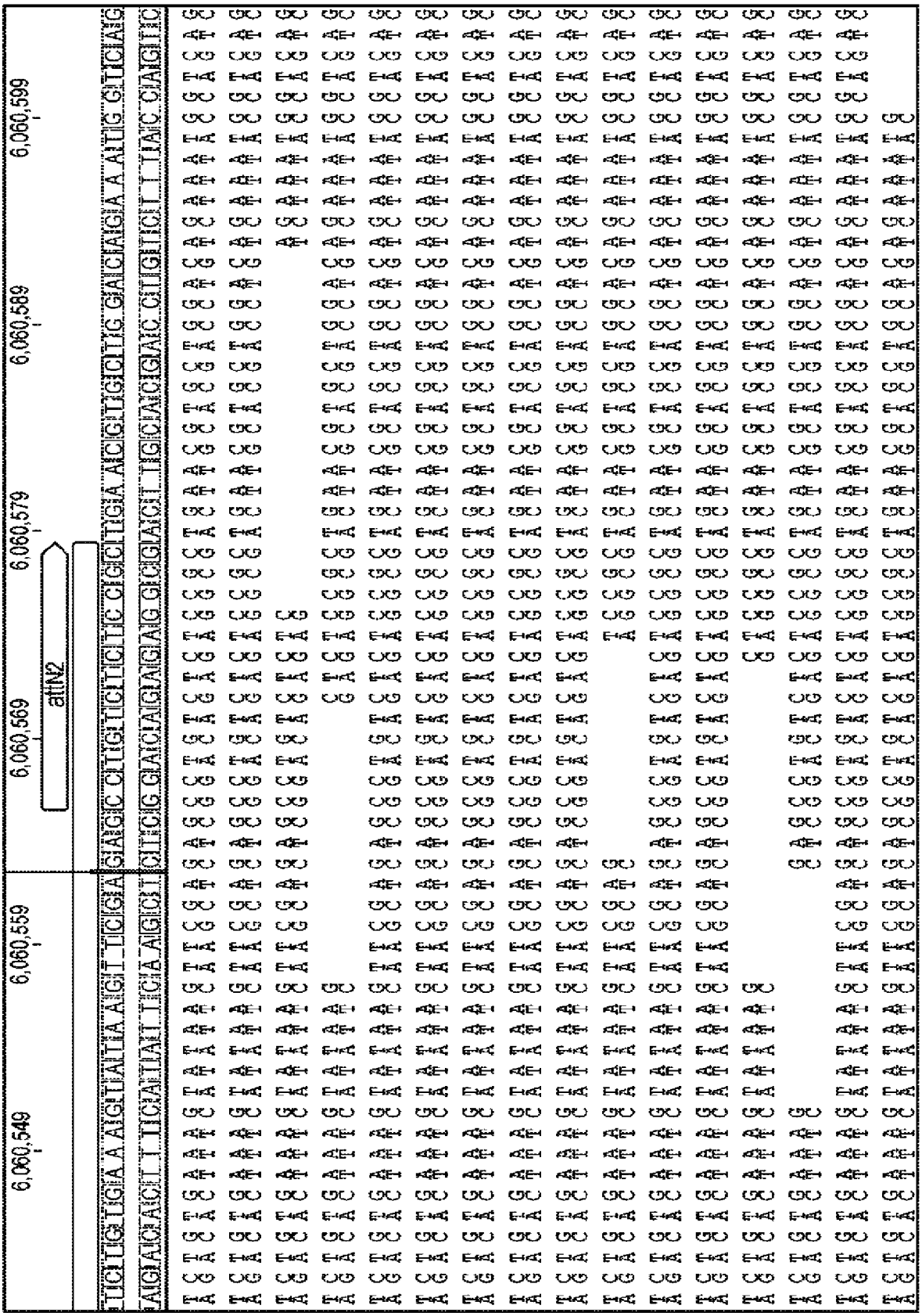


FIG. 13B

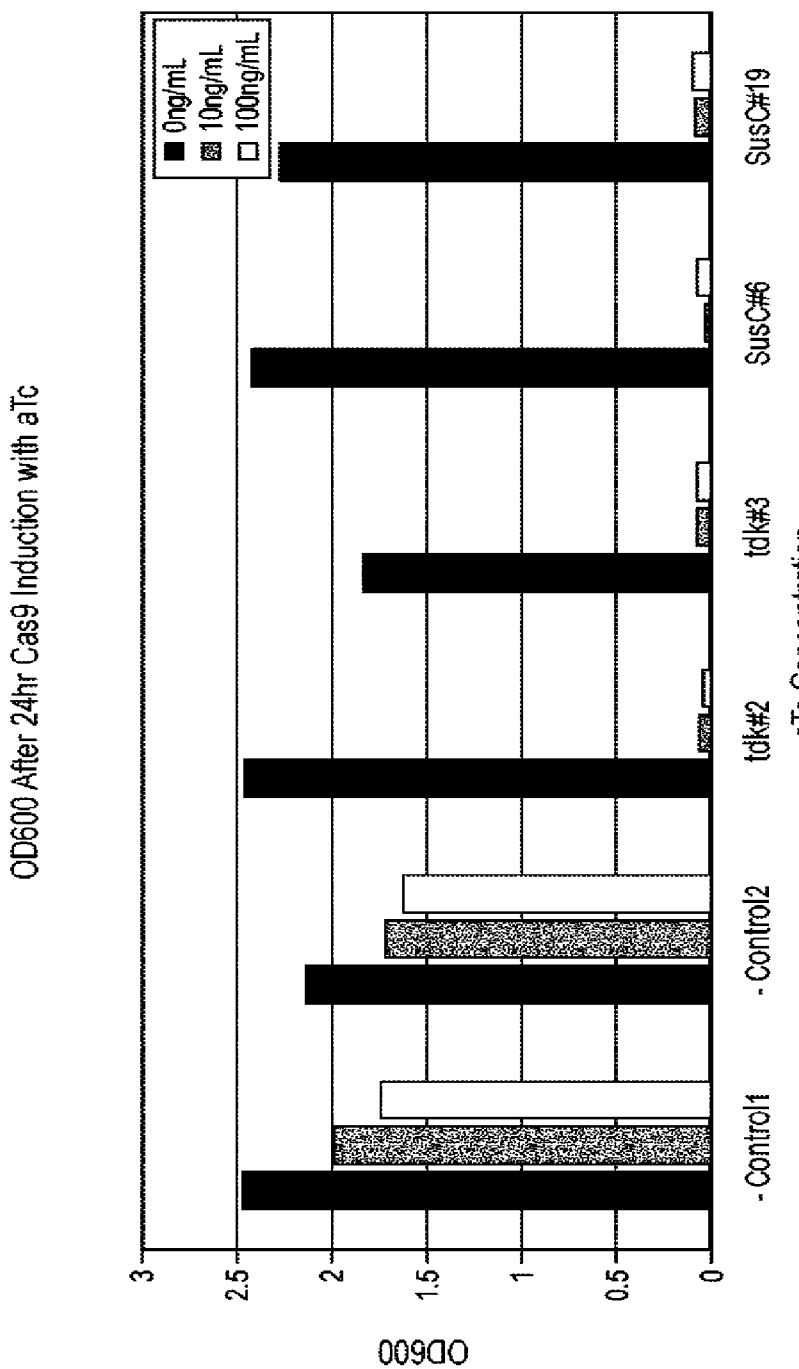

OD600 After 24hr Cas9 Induction with aTc
0ng/mL
10ng/mL
100ng/mL
OD600
3
2.5
2
1.5
1
0.5
0
-Control1
-Control2
tdk#2
tdk#3
SusC#6
SusC#19
aTc Concentration

FIG. 14

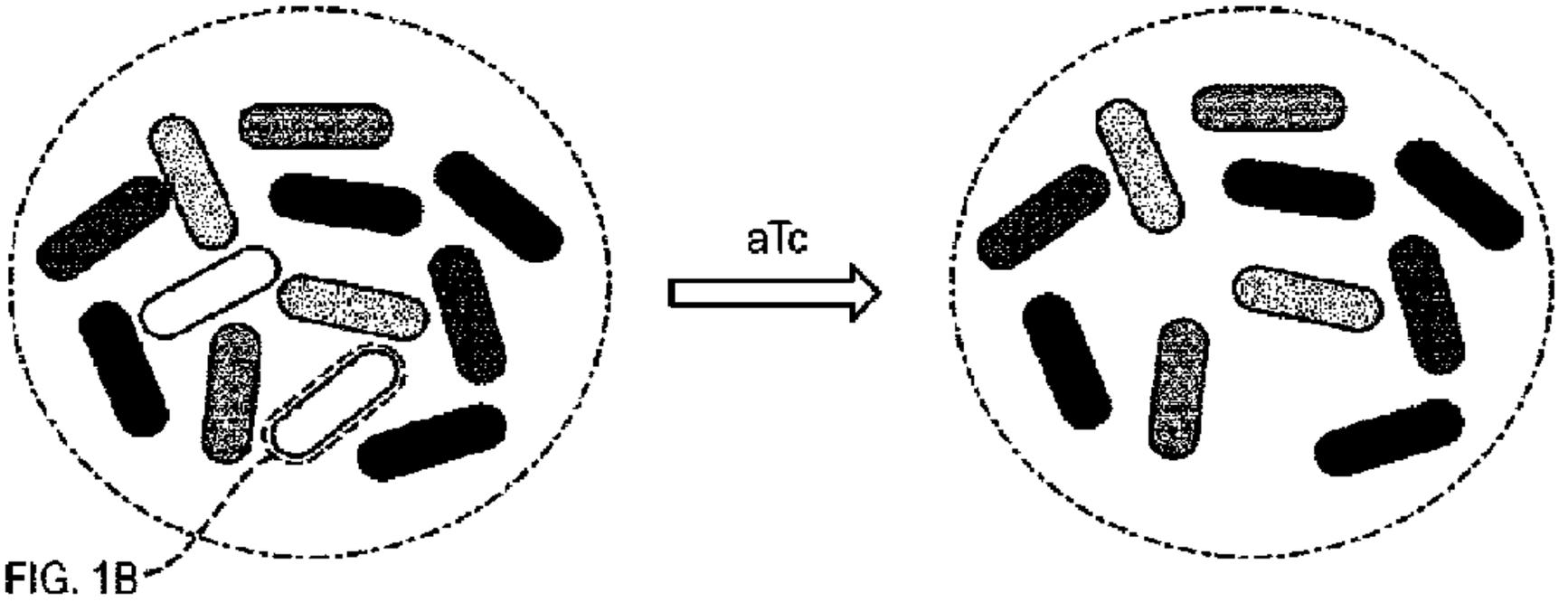


FIG. 1B

FIG. 1A

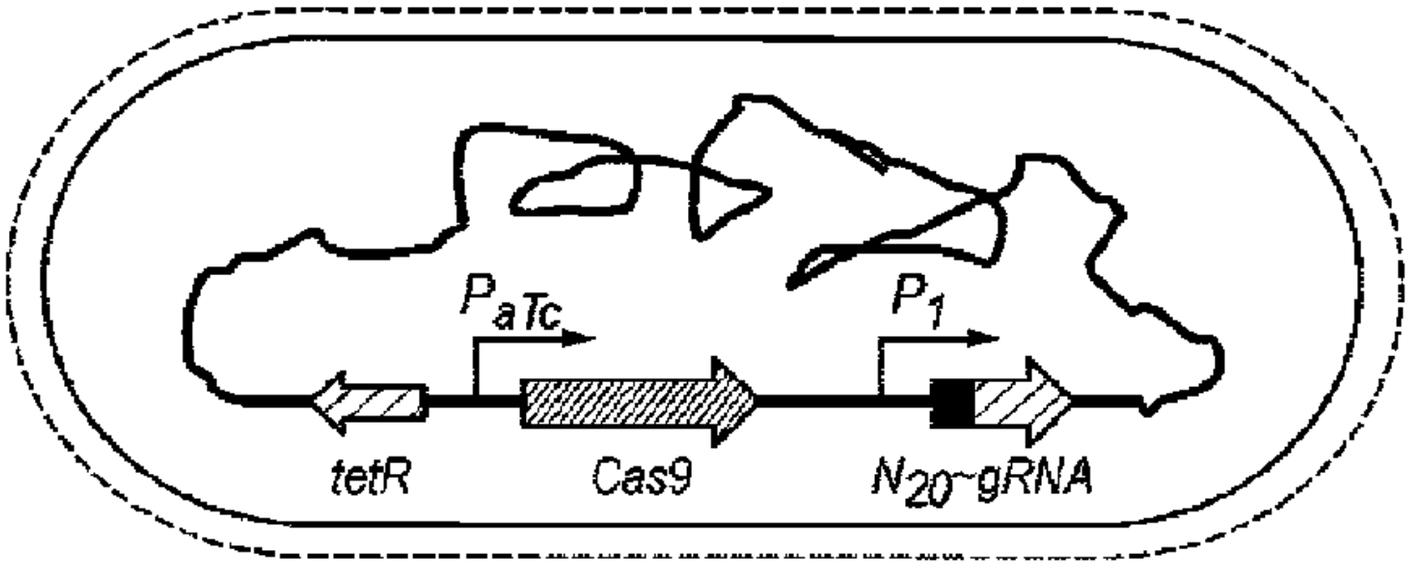


FIG. 1B