Bacteria Engineered to Treat Diseases that Benefit from Reduced Gut Inflammation and/or Tightened Gut Mucosal Barrier

[01] The instant application hereby incorporates by reference U.S. Provisional Application No. 62/127,097, filed 3/2/2015; U.S. Application No. 62/248,814, filed 10/30/2015; U.S. Provisional Application No. 62/256,042, filed 11/16/2015; U.S. Provisional Applicatin No. 62/291,461, filed 2/4/2016; U.S. Provisional Application No. 62/127, 131 filed 3/2/2015; U.S. Provisional Application No. 62/248,825, filed 10/30/2015; U.S. Provisional Application 62/256,044, dated 11/16/2015; U.S. Provisional Application No. 62/291,470, filed 2/4/2016; U.S. Provisional Application 62/184,770, filed 6/25/2015; U.S. Provisional Application No. 62/248,805, filed 10/30/2015; U.S. Provisional Application No. 62/256,048, filed 11/16/2015; U.S. Provisional Application No. 62/291,468, filed 2/4/2016; U.S. Application No. 14/998,376, dated 12/22/2015, the entire contents of each of which are expressly incorporated herein by reference in their respective entireties.

[02] This disclosure relates to compositions and therapeutic methods for inhibiting inflammatory mechanisms in the gut, restoring and tightening gut mucosal barrier function, and/or treating and preventing autoimmune disorders. In certain aspects, the disclosure relates to genetically engineered bacteria that are capable of reducing inflammation in the gut and/or enhancing gut barrier function. In some embodiments, the genetically engineered bacteria are capable of reducing gut inflammation and/or enhancing gut barrier function, thereby ameliorating or preventing an autoimmune disorder. In some aspects, the compositions and methods disclosed herein may be used for treating or preventing autoimmune disorders as well as diseases and conditions associated with gut inflammation and/or compromised gut barrier function, e.g., diarrheal diseases, inflammatory bowel diseases, and related diseases.

[03] Inflammatory bowel diseases (IBDs) are a group of diseases characterized by significant local inflammation in the gastrointestinal tract typically driven by T cells and activated macrophages and by compromised function of the epithelial barrier that separates the luminal contents of the gut from the host circulatory system (Ghishan et

al., 2014). IBD pathogenesis is linked to both genetic and environmental factors and may be caused by altered interactions between gut microbes and the intestinal immune system. Current approaches to treat IBD are focused on therapeutics that modulate the immune system and suppress inflammation. These therapies include steroids, such as prednisone, and tumor necrosis factor (TNF) inhibitors, such as Humira® (Cohen et al., 2014). Drawbacks from this approach are associated with systemic immunosuppression, which includes greater susceptibility to infectious disease and cancer.

[04] Other approaches have focused on treating compromised barrier function by supplying the short-chain fatty acid butyrate via enemas. Recently, several groups have demonstrated the importance of short-chain fatty acid production by commensal bacteria in regulating the immune system in the gut (Smith et al., 2013), showing that butyrate plays a direct role in inducing the differentiation of regulatory T cells and suppressing immune responses associated with inflammation in IBD (Atarashi et al., 2011; Furusawa et al., 2013). Butyrate is normally produced by microbial fermentation of dietary fiber and plays a central role in maintaining colonic epithelial cell homeostasis and barrier function (Hamer et al., 2008). Studies with butyrate enemas have shown some benefit to patients, but this treatment is not practical for long term therapy. More recently, patients with IBD have been treated with fecal transfer from healthy patients with some success (Ianiro et al., 2014). This success illustrates the central role that gut microbes play in disease pathology and suggests that certain microbial functions are associated with ameliorating the IBD disease process. However, this approach raises safety concerns over the transmission of infectious disease from the donor to the recipient. Moreover, the nature of this treatment has a negative stigma and thus is unlikely to be widely accepted.

[05] Compromised gut barrier function also plays a central role in autoimmune diseases pathogenesis (Lerner et al., 2015a; Lerner et al., 2015b; Fasano et al., 2005; Fasano, 2012). A single layer of epithelial cells separates the gut lumen from the immune cells in the body. The epithelium is regulated by intercellular tight junctions and controls the equilibrium between tolerance and immunity to nonself-antigens (Fasano et al., 2005). Disrupting the epithelial layer can lead to pathological exposure of the highly

immunoreactive subepithelium to the vast number of foreign antigens in the lumen (Lerner et al., 2015a) resulting in increased susceptibility to and both intestinal and extraintestinal autoimmune disorders can occur" (Fasano et al., 2005). Some foreign antigens are postulated to resemble self-antigens and can induce epitope-specific cross-reactivity that accelerates the progression of a pre-existing autoimmune disease or initiates an autoimmune disease (Fasano, 2012). Rheumatoid arthritis and celiac disease, for example, are autoimmune disorders that are thought to involve increased intestinal permeability (Lerner et al., 2015b). In individuals who are genetically susceptible to autoimmune disorders, dysregulation of intercellular tight junctions can lead to disease onset (Fasano, 2012). In fact, the loss of protective function of mucosal barriers that interact with the environment is necessary for autoimmunity to develop (Lerner et al., 2015a).

[06] Changes in gut microbes can alter the host immune response (Paun et al., 2015; Sanz et al., 2014; Sanz et al., 2015; Wen et al., 2008). For example, in children with high genetic risk for type 1 diabetes, there are significant differences in the gut microbiome between children who develop autoimmunity for the disease and those who remain healthy (Richardson et al., 2015). Others have shown that gut bacteria are a potential therapeutic target in the prevention of asthma and exhibit strong immunomodulatory capacity… in lung inflammation (Arrieta et al., 2015). Thus, enhancing barrier function and reducing inflammation in the gastrointestinal tract are potential therapeutic mechanisms for the treatment or prevention of autoimmune disorders.

[07] Recently there has been an effort to engineer microbes that produce anti-inflammatory molecules, such as IL-10, and administer them orally to a patient in order to deliver the therapeutic directly to the site of inflammation in the gut. The advantage of this approach is that it avoids systemic administration of immunosuppressive drugs and delivers the therapeutic directly to the gastrointestinal tract. However, while these engineered microbes have shown efficacy in some pre-clinical models, efficacy in patients has not been observed. One reason for the lack of success in treating patients is that the viability and stability of the microbes are compromised due to the constitutive

production of large amounts of non-native proteins, e.g., human interleukin. Thus, there remains a great need for additional therapies to reduce gut inflammation, enhance gut barrier function, and/or treat autoimmune disorders, and that avoid undesirable side effects.

[08] The genetically engineered bacteria disclosed herein are capable of producing therapeutic anti-inflammation and/or gut barrier enhancer molecules. The genetically engineered bacteria are functionally silent until they reach an inducing environment, e.g., a mammalian gut, wherein expression of the therapeutic molecule is induced. In certain embodiments, the genetically engineered bacteria are naturally non-pathogenic and may be introduced into the gut in order to reduce gut inflammation and/or enhance gut barrier function and may thereby further ameliorate or prevent an autoimmune disorder. In certain embodiments, the anti-inflammation and/or gut barrier enhancer molecule is stably produced by the genetically engineered bacteria, and/or the genetically engineered bacteria are stably maintained *in vivo* and/or *in vitro.* The invention also provides pharmaceutical compositions comprising the genetically engineered bacteria, and methods of treating diseases that benefit from reduced gut inflammation and/or tightened gut mucosal barrier function, e.g., an inflammatory bowel disease or an autoimmune disorder.

[09] In some embodiments, the genetically engineered bacteria of the invention produce one or more therapeutic molecule(s) under the control of one or more promoters induced by an environmental condition, e.g., an environmental condition found in the mammalian gut, such as an inflammatory condition or a low oxygen condition. Thus, in some embodiments, the genetically engineered bacteria of the invention produce one or more therapeutic molecule(s) under the control of an oxygen level-dependent promoter, a reactive oxygen species (ROS)-dependent promoter, or a reactive nitrogen species (RNS)-dependent promoter, and a corresponding transcription factor. In some embodiments, the therapeutic molecule is butyrate; in an inducing environment, the butyrate biosynthetic gene cassette is activated, and butyrate is produced. Local production of butyrate induces the differentiation of regulatory T cells in the gut and/or promotes the barrier function of colonic epithelial cells. The genetically

engineered bacteria of the invention produce their therapeutic effect only in inducing environments such as the gut, thereby lowering the safety issues associated with systemic exposure.

Brief Description of the Figures

[010] **Fig. 1** depicts a schematic of the eight-gene pathway from *C. difficile* for butyrate production. pLogic031 comprises the eight-gene pathway from *C. difficile, bcd2-etfB3-etfA3-thiA1-hbd-crt2-pbt-buk,* synthesized under the control of Tet-inducible promoters (pBR322 backbone). pLogic046 replaces the BCD/EFT complex, a potential rate-limiting step, with single gene from *Treponema denticola*, *ter* (*trans*-enoyl-2-reductase), and comprises *ter-thiA1-hbd-crt2-pbt-buk.*

[011] **Fig. 2** depicts a schematic of a butyrate production pathway in which the circled genes (buk and pbt) may be deleted and replaced with *tesB*, which cleaves the CoA from butyryl-CoA.

[012] **Fig. 3** depicts the gene organization of an exemplary recombinant bacterium of the invention and its derepression in the presence of nitric oxide (NO). In the upper panel, in the absence of NO, the NsrR transcription factor (gray circle, "NsrR") binds to and represses a corresponding regulatory region. Therefore, none of the butyrate biosynthesis enzymes (*bcd2, etfB3, etfA3, thiA1, hbd, crt2, pbt, buk;* black boxes) is expressed. In the lower panel, in the presence of NO, the NsrR transcription factor interacts with NO, and no longer binds to or represses the regulatory sequence. This leads to expression of the butyrate biosynthesis enzymes (indicated by gray arrows and black squiggles) and ultimately to the production of butyrate.

[013] **Fig. 4** depicts the gene organization of another exemplary recombinant bacterium of the invention and its derepression in the presence of NO. In the upper panel, in the absence of NO, the NsrR transcription factor (gray circle, "NsrR") binds to and represses a corresponding regulatory region. Therefore, none of the butyrate biosynthesis enzymes (*ter, thiA1, hbd, crt2, pbt, buk;* black boxes) is expressed. In the lower panel, in the presence of NO, the NsrR transcription factor interacts with NO, and no longer binds to or represses the regulatory sequence. This leads to expression of the

butyrate biosynthesis enzymes (indicated by gray arrows and black squiggles) and ultimately to the production of butyrate.

[014] **Fig. 5** depicts the gene organization of an exemplary recombinant bacterium of the invention and its induction in the presence of H_2O_2. In the upper panel, in the absence of H_2O_2, the OxyR transcription factor (gray circle, "OxyR") binds to, but does not induce, the *oxyS* promoter. Therefore, none of the butyrate biosynthesis enzymes (*bcd2, etfB3, etfA3, thiA1, hbd, crt2, pbt, buk;* black boxes) is expressed. In the lower panel, in the presence of H_2O_2, the OxyR transcription factor interacts with H_2O_2 and is then capable of inducing the *oxyS* promoter. This leads to expression of the butyrate biosynthesis enzymes (indicated by gray arrows and black squiggles) and ultimately to the production of butyrate.

[015] **Fig. 6** depicts the gene organization of another exemplary recombinant bacterium of the invention and its induction in the presence of H_2O_2. In the upper panel, in the absence of H_2O_2, the OxyR transcription factor (gray circle, "OxyR") binds to, but does not induce, the *oxyS* promoter. Therefore, none of the butyrate biosynthesis enzymes (*ter, thiA1, hbd, crt2, pbt, buk;* black boxes) is expressed. In the lower panel, in the presence of H_2O_2, the OxyR transcription factor interacts with H_2O_2 and is then capable of inducing the *oxyS* promoter. This leads to expression of the butyrate biosynthesis enzymes (indicated by gray arrows and black squiggles) and ultimately to the production of butyrate.

[016] **Fig. 7** depicts the gene organization of an exemplary recombinant bacterium of the invention and its induction under low-oxygen conditions. In the upper panel, relatively low butyrate production under aerobic conditions in which oxygen (O_2) prevents (indicated by "X") FNR (grey boxed "FNR") from dimerizing and activating the FNR-responsive promoter ("FNR promoter"). Therefore, none of the butyrate biosynthesis enzymes (*bcd2, etfB3, etfA3, thiA1, hbd, crt2, pbt,* and *buk*; black boxes) is expressed. In the lower panel, increased butyrate production under low-oxygen conditions due to FNR dimerizing (two grey boxed "FNR"s), binding to the FNR-responsive promoter, and inducing expression of the butyrate biosynthesis enzymes, which leads to the production of butyrate.

[017] **Fig. 8** depicts the gene organization of an exemplary recombinant bacterium of the invention and its induction under low-oxygen conditions. In the upper panel, relatively low butyrate production under aerobic conditions in which oxygen (O_2) prevents (indicated by "X") FNR (grey boxed "FNR") from dimerizing and activating the FNR-responsive promoter ("FNR promoter"). Therefore, none of the butyrate biosynthesis enzymes (*ter, thiA1, hbd, crt2, pbt,* and *buk*; black boxes) is expressed. In the lower panel, increased butyrate production under low-oxygen conditions due to FNR dimerizing (two grey boxed "FNR"s), binding to the FNR-responsive promoter, and inducing expression of the butyrate biosynthesis enzymes, which leads to the production of butyrate.

[018] **Fig. 9** depicts the gene organization of an exemplary recombinant bacterium of the invention and its induction under low-oxygen conditions. In the upper panel, relatively low propionate production under aerobic conditions in which oxygen (O_2) prevents (indicated by "X") FNR (grey boxed "FNR") from dimerizing and activating the FNR-responsive promoter ("FNR promoter"). Therefore, none of the propionate biosynthesis enzymes (*pct, lcdA, lcdB, lcdC, etfA, acrB, acrC*; black boxes) is expressed. In the lower panel, increased propionate production under low-oxygen conditions due to FNR dimerizing (two grey boxed "FNR"s), binding to the FNR-responsive promoter, and inducing expression of the propionate biosynthesis enzymes, which leads to the production of propionate.

[019] **Fig. 10** depicts an exemplary propionate biosynthesis gene cassette.

[020] **Fig. 11** depicts the gene organization of an exemplary recombinant bacterium of the invention and its induction under low-oxygen conditions. In the upper panel, relatively low propionate production under aerobic conditions in which oxygen (O_2) prevents (indicated by "X") FNR (grey boxed "FNR") from dimerizing and activating the FNR-responsive promoter ("FNR promoter"). Therefore, none of the propionate biosynthesis enzymes (*thrA, thrB, thrC, ilvA, aceE, aceF, lpd*; black boxes) is expressed. In the lower panel, increased propionate production under low-oxygen conditions due to FNR dimerizing (two grey boxed "FNR"s), binding to the FNR-responsive promoter, and

inducing expression of the propionate biosynthesis enzymes, which leads to the production of propionate.

[021] **Fig. 12** depicts an exemplary propionate biosynthesis gene cassette.

[022] **Fig. 13** depicts the gene organization of an exemplary recombinant bacterium of the invention and its induction under low-oxygen conditions. In the upper panel, relatively low propionate production under aerobic conditions in which oxygen (O_2) prevents (indicated by "X") FNR (grey boxed "FNR") from dimerizing and activating the FNR-responsive promoter ("FNR promoter"). Therefore, none of the propionate biosynthesis enzymes (*thrA, thrB, thrC, ilvA, aceE, aceF, lpd, tesB*; black boxes) is expressed. In the lower panel, increased propionate production under low-oxygen conditions due to FNR dimerizing (two grey boxed "FNR"s), binding to the FNR-responsive promoter, and inducing expression of the propionate biosynthesis enzymes, which leads to the production of propionate.

[023] **Fig. 14** depicts an exemplary propionate biosynthesis gene cassette.

[024] **Fig. 15** depicts a schematic of a butyrate gene cassette, pLogic031 comprising the eight-gene butyrate cassette.

[025] **Fig. 16** depicts a schematic of a butyrate gene cassette, pLogic046 comprising the *ter* substitution (oval).

[026] **Fig. 17** depicts a linear schematic of a butyrate gene cassette, pLogic046.

[027] **Fig. 18** depicts a graph of butyrate production. pLOGIC031 (bcd)/+O2 is Nissle containing plasmid pLOGIC031 grown aerobically. pLOGIC046 (ter)/+O2 is Nissle containing plasmid pLOGIC046 grown aerobically. pLOGIC031 (bcd)/-O2 is Nissle containing plasmid pLOGIC031 grown anaerobically. pLOGIC046 (ter)/-O2 is Nissle containing plasmid pLOGIC046 grown anaerobically. The *ter* construct results in higher butyrate production.

[028] **Fig. 19** depicts a graph of butyrate production using *E. coli* BW25113 butyrate-producing circuits comprising a *nuoB* gene deletion, which results in greater levels of butyrate production as compared to a wild-type parent control. *nuoB* is a main protein complex involved in the oxidation of NADH during respiratory growth. In some

embodiments, preventing the coupling of NADH oxidation to electron transport increases the amount of NADH being used to support butyrate production.

[029] **Fig. 20** depicts a schematic of pLogic046-tesB, in which buk and pbt are deleted and tesB substituted.

[030] **Fig. 21** depicts a linear schematic of a butyrate gene cassette, pLogic046-delta.ptb-buk-tesB+.

[031] **Fig. 22** depicts butyrate production using pLOGIC046 (a Nissle strain comprising plasmid pLOGIC046, an ATC-inducible ter-comprising butyrate construct) and pLOGIC046-delta.pbt-buk/tesB+ (a Nissle strain comprising plasmid pLOGIC046-delta pbt.buk/tesB+, an ATC-inducible ter-comprising butyrate construct with a deletion in the pbt-buk genes and their replacement with the tesB gene). The *tesB* construct results in greater butyrate production.

[032] **Fig. 23** depicts a schematic of a butyrate gene cassette, ydfZ-butyrate, comprising the *ter* substitution.

[033] **Fig. 24** depicts SYN363 in the presence and absence of glucose and oxygen *in vitro*. SYN363 comprises a butyrate gene cassette comprising the *ter-thiA1-hbd-crt2-tesB* genes under the control of a *ydfZ* promoter.

[034] **Fig. 25** depicts a graph measuring gut-barrier function in dextran sodium sulfate (DSS)-induced mouse models of IBD. The amount of FITC dextran found in the plasma of mice administered different concentrations of DSS was measured as an indicator of gut barrier function.

[035] **Fig. 26** depicts serum levels of FITC-dextran analyzed by spectrophotometry. FITC-dextran is a readout for gut barrier function in the DSS-induced mouse model of IBD.

[036] **Fig. 27** depicts levels of mouse lipocalin 2 and calprotectin quantified by ELISA using the fecal samples in an *in vivo* model of IBD. SYN363 reduces inflammation and/or protects gut barrier function as compared to control SYN94.

[037] **Fig. 28** depicts ATC or nitric oxide-inducible reporter constructs. These constructs, when induced by their cognate inducer, lead to expression of GFP. Nissle cells harboring plasmids with either the control, ATC-inducible P_{tet}-GFP reporter

construct or the nitric oxide inducible P_{nsrR}-GFP reporter construct induced across a range of concentrations. Promoter activity is expressed as relative florescence units.

[038] **Fig. 29** depicts a dot blot of bacteria harboring a plasmid expressing NsrR under control of a constitutive promoter and the reporter gene *gfp* (green fluorescent protein) under control of an NsrR-inducible promoter. IBD is induced in mice by supplementing drinking water with 2-3% dextran sodium sulfate (DSS). Chemiluminescent is shown for NsrR-regulated promoters induced in DSS-treated mice.

[039] **Fig. 30** depicts the construction and gene organization of an exemplary plasmid comprising a gene encoding NsrR, a regulatory sequence from *norB,* and a butyrogenic gene cassette (pLogic031-nsrR-norB-butyrate construct).

[040] **Fig. 31** depicts the construction and gene organization of another exemplary plasmid comprising a gene encoding NsrR, a regulatory sequence from *norB,* and a butyrogenic gene cassette (pLogic046- nsrR-norB-butyrogenic gene cassette).

[041] **Fig. 32** depicts butyrate production using SYN001 + tet (control wild-type Nissle comprising no plasmid), SYN067 + tet (Nissle comprising the pLOGIC031 ATC-inducible butyrate plasmid), and SYN080 + tet (Nissle comprising the pLOGIC046 ATC-inducible butyrate plasmid).

[042] **Fig. 33** depicts butyrate production by genetically engineered Nissle comprising the pLogic031-nsrR-norB-butyrate construct (SYN133) or the pLogic046-nsrR-norB-butyrate construct (SYN145), which produce more butyrate as compared to wild-type Nissle (SYN001).

[043] **Fig. 34** depicts the construction and gene organization of an exemplary plasmid comprising an *oxyS* promoter and butyrogenic gene cassette (pLogic031-oxyS-butyrogenic gene cassette).

[044] **Fig. 35** depicts the construction and gene organization of another exemplary plasmid comprising an *oxyS* promoter and butyrogenic gene cassette (pLogic046-oxyS- butyrogenic gene cassette).

[045] **Fig. 36** depicts a schematic of an *E. coli* that is genetically engineered to express the essential gene *tnaB*, 5-methyltetrahydrofolate-homocysteine

methyltransferase (*mtr*), tryptophan transporter, and the enzymes IDO and TDO to convert tryptophan into kynurenine.

[046] **Fig. 37** depicts a schematic of an *E. coli* that is genetically engineered to express interleukin under the control of a FNR-responsive promoter and further comprising a TAT secretion system.

[047] **Fig. 38** depicts a schematic of an *E. coli* that is genetically engineered to express SOD under the control of a FNR-responsive promoter and further comprising a TAT secretion system.

[048] **Fig. 39** depicts a schematic of an *E. coli* that is genetically engineered to express GLP-2 under the control of a FNR-responsive promoter and further comprising a TAT secretion system.

[049] **Fig. 40** depicts a schematic of an *E. coli* that is genetically engineered to express a propionate gene cassette under the control of a FNR-responsive promoter.

[050] **Fig. 41** depicts a schematic of an *E. coli* that is genetically engineered to express butyrate under the control of a FNR-responsive promoter.

[051] **Fig. 42** depicts a schematic of an *E. coli* that is genetically engineered to express kynurenine, interleukin, SOD, GLP-2, a propionate gene cassette, and a butyrate gene cassette under the control of a FNR-responsive promoter and further comprising a TAT secretion system.

[052] **Fig. 43** depicts a schematic of an *E. coli* that is genetically engineered to express interleukin, OSD, GLP-2, a propionate gene cassette, and a butyrate gene cassette under the control of a FNR-responsive promoter and further comprising a TAT secretion system.

[053] **Fig. 44** depicts a schematic of an *E. coli* that is genetically engineered to express SOD, a propionate gene cassette, and a butyrate gene cassette under the control of a FNR-responsive promoter and further comprising a TAT secretion system.

[054] **Fig. 45** depicts a schematic of an *E. coli* that is genetically engineered to express interleukin, a propionate gene cassette, and a butyrate gene cassette under the control of a FNR-responsive promoter and further comprising a TAT secretion system.

[055] **Fig. 46** depicts a schematic of an *E. coli* that is genetically engineered to express interleukin-10 (IL-10), a propionate gene cassette, and a butyrate gene cassette under the control of a FNR-responsive promoter and further comprising a TAT secretion system.

[056] **Fig. 47** depicts a schematic of an *E. coli* that is genetically engineered to express IL-2, IL-10, a propionate gene cassette, and a butyrate gene cassette under the control of a FNR-responsive promoter and further comprising a TAT secretion system.

[057] **Fig. 48** depicts a schematic of an *E. coli* that is genetically engineered to express IL-2, IL-10, a propionate gene cassette, a butyrate gene cassette, and SOD under the control of a FNR-responsive promoter and further comprising a TAT secretion system.

[058] **Fig. 49** depicts a schematic of an *E. coli* that is genetically engineered to express IL-2, IL-10, a propionate gene cassette, a butyrate gene cassette, SOD, and GLP-2 under the control of a FNR-responsive promoter and further comprising a TAT secretion system.

[059] **Fig. 50** depicts a map of exemplary integration sites within the *E. coli* 1917 Nissle chromosome. These sites indicate regions where circuit components may be inserted into the chromosome without interfering with essential gene expression. Backslashes (/) are used to show that the insertion will occur between divergently or convergently expressed genes. Insertions within biosynthetic genes, such as *thyA*, can be useful for creating nutrient auxotrophies. In some embodiments, an individual circuit component is inserted into more than one of the indicated sites.

[060] **Fig. 51** depicts an exemplary schematic of the *E. coli* 1917 Nissle chromosome comprising multiple mechanisms of action (MoAs).

[061] **Fig. 52** depicts an exemplary schematic of the *E. coli* 1917 Nissle chromosome comprising multiple mechanisms of action for producing IL-2, IL-10, IL-22, IL-27, propionate, and butyrate.

[062] **Fig. 53** depicts an exemplary schematic of the *E. coli* 1917 Nissle chromosome comprising multiple mechanisms of action for producing IL-10, IL-27, GLP-2, and butyrate.

[063] **Fig. 54** depicts an exemplary schematic of the *E. coli* 1917 Nissle chromosome comprising multiple mechanisms of action for producing GLP-2, IL-10, IL-22, SOD, butyrate, and propionate.

[064] **Fig. 55** depicts an exemplary schematic of the *E. coli* 1917 Nissle chromosome comprising multiple mechanisms of action for producing GLP-2, IL-2, IL-10, IL-22, IL-27, SOD, butyrate, and propionate.

[065] **Fig. 56** depicts a table illustrating the survival of various amino acid auxotrophs in the mouse gut, as detected 24 hours and 48 hours post-gavage. These auxotrophs were generated using BW25113, a non-Nissle strain of *E. coli*.

[066] **Fig. 57** depicts a schematic of a repression-based kill switch. In a toxin-based system, the AraC transcription factor is activated in the presence of arabinose and induces expression of TetR and an anti-toxin. TetR prevents the expression of the toxin. When arabinose is removed, TetR and the anti-toxin do not get made and the toxin is produced which kills the cell. In an essential gene-based system, the AraC transcription factor is activated in the presence of arabinose and induces expression of an essential gene.

[067] **Fig. 58** depicts another non-limiting embodiment of the disclosure, wherein the expression of a heterologous gene is activated by an exogenous environmental signal, e.g., low-oxygen conditions. In the absence of arabinose, the AraC transcription factor adopts a conformation that represses transcription. In the presence of arabinose, the AraC transcription factor undergoes a conformational change that allows it to bind to and activate the araBAD promoter, which induces expression of TetR (tet repressor) and an anti-toxin. The anti-toxin builds up in the recombinant bacterial cell, while TetR prevents expression of a toxin (which is under the control of a promoter having a TetR binding site). However, when arabinose is not present, both the anti-toxin and TetR are not expressed. Since TetR is not present to repress expression of the toxin, the toxin is expressed and kills the cell. **Fig. 58** also depicts another non-limiting embodiment of the disclosure, wherein the expression of an essential gene not found in the recombinant bacteria is activated by an exogenous environmental signal. In the absence of arabinose, the AraC transcription factor adopts a conformation that represses

transcription of the essential gene under the control of the araBAD promoter and the bacterial cell cannot survive. In the presence of arabinose, the AraC transcription factor undergoes a conformational change that allows it to bind to and activate the araBAD promoter, which induces expression of the essential gene and maintains viability of the bacterial cell.

[068] **Fig. 59** depicts a non-limiting embodiment of the disclosure, where an anti-toxin is expressed from a constitutive promoter, and expression of a heterologous gene is activated by an exogenous environmental signal. In the absence of arabinose, the AraC transcription factor adopts a conformation that represses transcription. In the presence of arabinose, the AraC transcription factor undergoes a conformational change that allows it to bind to and activate the araBAD promoter, which induces expression of TetR, thus preventing expression of a toxin. However, when arabinose is not present, TetR is not expressed, and the toxin is expressed, eventually overcoming the anti-toxin and killing the cell. The constitutive promoter regulating expression of the anti-toxin should be a weaker promoter than the promoter driving expression of the toxin. The *araC* gene is under the control of a constitutive promoter in this circuit.

[069] **Fig. 60** depicts a schematic of a repression-based kill switch in which the AraC transcription factor is activated in the presence of arabinose and induces expression of TetR and an anti-toxin. TetR prevents the expression of the toxin. When arabinose is removed, TetR and the anti-toxin do not get made and the toxin is produced which kills the cell.

[070] **Fig. 61** depicts another non-limiting embodiment of the disclosure, wherein the expression of a heterologous gene is activated by an exogenous environmental signal. In the absence of arabinose, the AraC transcription factor adopts a conformation that represses transcription. In the presence of arabinose, the AraC transcription factor undergoes a conformational change that allows it to bind to and activate the araBAD promoter, which induces expression of TetR (tet repressor) and an anti-toxin. The anti-toxin builds up in the recombinant bacterial cell, while TetR prevents expression of a toxin (which is under the control of a promoter having a TetR binding site). However, when arabinose is not present, both the anti-toxin and TetR are not

expressed. Since TetR is not present to repress expression of the toxin, the toxin is expressed and kills the cell. The *araC* gene is under the control of a constitutive promoter in this circuit.

[071] **Fig. 62** depicts one non-limiting embodiment of the disclosure, where an exogenous environmental condition, e.g., low-oxygen conditions, or one or more environmental signals activates expression of a heterologous gene and at least one recombinase from an inducible promoter or inducible promoters. The recombinase then flips a toxin gene into an activated conformation, and the natural kinetics of the recombinase create a time delay in expression of the toxin, allowing the heterologous gene to be fully expressed. Once the toxin is expressed, it kills the cell.

[072] **Fig. 63** depicts another non-limiting embodiment of the disclosure, where an exogenous environmental condition, e.g., low-oxygen conditions, or one or more environmental signals activates expression of a heterologous gene, an anti-toxin, and at least one recombinase from an inducible promoter or inducible promoters. The recombinase then flips a toxin gene into an activated conformation, but the presence of the accumulated anti-toxin suppresses the activity of the toxin. Once the exogenous environmental condition or cue(s) is no longer present, expression of the anti-toxin is turned off. The toxin is constitutively expressed, continues to accumulate, and kills the bacterial cell.

[073] **Fig. 64** depicts another non-limiting embodiment of the disclosure, where an exogenous environmental condition, e.g., low-oxygen conditions, or one or more environmental signals activates expression of a heterologous gene and at least one recombinase from an inducible promoter or inducible promoters. The recombinase then flips at least one excision enzyme into an activated conformation. The at least one excision enzyme then excises one or more essential genes, leading to senescence, and eventual cell death. The natural kinetics of the recombinase and excision genes cause a time delay, the kinetics of which can be altered and optimized depending on the number and choice of essential genes to be excised, allowing cell death to occur within a matter of hours or days. The presence of multiple nested recombinases can be used to further control the timing of cell death.

[074] **Fig. 65** depicts a schematic of an activation-based kill switch, in which P_i is any inducible promoter, e.g., a FNR-responsive promoter. When the therapeutic is induced, the anti-toxin and recombinases are turned on, which results in the toxin being 'flipped' to the ON position after 4-6 hours, which results in a build-up of anti-toxin before the toxin is expressed. In absence of the inducing signal, only toxin is made and the cell dies.

[075] **Fig. 66** depicts a one non-limiting embodiment of the disclosure, in which the genetically engineered bacteria produces equal amount of a Hok toxin and a short-lived Sok anti-toxin. When the cell loses the plasmid, the anti-toxin decays, and the cell dies. In the upper panel, the cell produces equal amounts of toxin and anti-toxin and is stable. In the center panel, the cell loses the plasmid and anti-toxin begins to decay. In the lower panel, the anti-toxin decays completely, and the cell dies.

[076] **Fig. 67** depicts the use of GeneGuards as an engineered safety component. All engineered DNA is present on a plasmid which can be conditionally destroyed. *See, e.g.*, Wright et al., 2015.

[077] **Fig. 68** depicts a modified type 3 secretion system (T3SS) to allow the bacteria to inject secreted therapeutic proteins into the gut lumen. An inducible promoter (small arrow, top), e.g. a FNR-responsive promoter, drives expression of the T3 secretion system gene cassette (3 large arrows, top) that produces the apparatus that secretes tagged peptides out of the cell. An inducible promoter (small arrow, bottom), e.g. a FNR-responsive promoter, drives expression of a regulatory factor, e.g. T7 polymerase, that then activates the expression of the tagged therapeutic peptide (hexagons).

[078] **Fig. 69** depicts a schematic of a secretion system based on the flagellar type III secretion in which an incomplete flagellum is used to secrete a therapeutic peptide of interest (star) by recombinantly fusing the peptide to an N-terminal flagellar secretion signal of a native flagellar component so that the intracellularly expressed chimeric peptide can be mobilized across the inner and outer membranes into the surrounding host environment.

[079] **Fig. 70** depicts a schematic of a type V secretion system for the extracellular production of recombinant proteins in which a therapeutic peptide (star) can be fused to an N-terminal secretion signal, a linker and the beta-domain of an autotransporter. In this system, the N-terminal signal sequence directs the protein to the SecA-YEG machinery which moves the protein across the inner membrane into the periplasm, followed by subsequent cleavage of the signal sequence. The beta-domain is recruited to the Bam complex where the beta-domain is folded and inserted into the outer membrane as a beta-barrel structure. The therapeutic peptide is then thread through the hollow pore of the beta-barrel structure ahead of the linker sequence. The therapeutic peptide is freed from the linker system by an autocatalytic cleavage or by targeting of a membrane-associated peptidase (scissors) to a complementary protease cut site in the linker.

[080] **Fig. 71** depicts a schematic of a type I secretion system, which translocates a passenger peptide directly from the cytoplasm to the extracellular space using HlyB (an ATP-binding cassette transporter); HlyD (a membrane fusion protein); and TolC (an outer membrane protein) which form a channel through both the inner and outer membranes. The secretion signal-containing C-terminal portion of HlyA is fused to the C-terminal portion of a therapeutic peptide (star) to mediate secretion of this peptide.

[081] **Fig. 72** depicts a schematic diagram of a wild-type clbA construct (upper panel) and a schematic diagram of a clbA knockout construct (lower panel).

[082] **Fig. 73** depicts exemplary sequences of a wild-type clbA construct and a clbA knockout construct.

[083] **Fig. 74** depicts a schematic for inflammatory bowel disease (IBD) therapies that target pro-inflammatory neutrophils and macrophages and regulatory T cells (Treg), restore epithelial barrier integrity, and maintain mucosal barrier function. Decreasing the pro-inflammatory action of neutrophils and macrophages and increasing Treg restores epithelial barrier integrity and the mucosal barrier.

[084] **Fig. 75** depicts a schematic of non-limiting processes for designing and producing the genetically engineered bacteria of the present disclosure: identifying diverse candidate approaches based on microbial physiology and disease

biology, using bioinformatics to determine candidate metabolic pathways, prospective tools to determine performance targets required of optimized engineered synthetic biotics (**A**); cutting-edge DNA assembly to enable combinatorial testing of pathway organization, mathematical models to predict pathway efficiency, internal stable of proprietary switches and parts to permit control and tuning of engineered circuits (**B**); building core structures ("chassies"), stably integrating engineered circuits into optimal chromosomal locations for efficient expression, employing unique functional assays to assess genetic circuit fidelity and activity (**C**); chromosomal markers enabling monitoring of synthetic biotic localization and transit times in animal models, expert microbiome network and bioinformatics support expanding understanding of how specific disease states affect GI microbial flora and the behaviors of synthetic biotics in that environment, activating process development research and optimization in-house during the discovery phase enables rapid and seamless transition of development candidates to pre-clinical progression, extensive experience in specialized disease animal model refinement supports prudent, high quality testing of candidate synthetic biotics (**D**).

[085] **Fig. 76** depicts a schematic of non-limiting manufacturing processes for upstream and downstream production of the genetically engineered bacteria of the present disclosure. **A** depicts the parameters for starter culture 1 (SC1): loop full – glycerol stock, duration overnight, temperature 37° C, shaking at 250 rpm. **B** depicts the parameters for starter culture 2 (SC2): 1/100 dilution from SC1, duration 1.5 hours, temperature 37° C, shaking at 250 rpm. **C** depicts the parameters for the production bioreactor: inoculum – SC2, temperature 37° C, pH set point 7.00, pH dead band 0.05, dissolved oxygen set point 50%, dissolved oxygen cascade agitation/gas FLO, agitation limits 300-1200 rpm, gas FLO limits 0.5-20 standard liters per minute, duration 24 hours. **D** depicts the parameters for harvest: centrifugation at speed 4000 rpm and duration 30 minutes, wash 1X 10% glycerol/PBS, centrifugation, re-suspension 10% glycerol/PBS. **E** depicts the parameters for vial fill/storage: 1-2 mL aliquots, -80° C.

Description of Embodiments

[086] The present disclosure includes genetically engineered bacteria, pharmaceutical compositions thereof, and methods of reducing gut inflammation, enhancing gut barrier function, and/or treating or preventing autoimmune disorders. In some embodiments, the genetically engineered bacteria comprise at least one non-native gene and/or gene cassette for producing a non-native anti-inflammation and/or gut barrier function enhancer molecule(s). The at least one gene and/or gene cassette is further operably linked to a regulatory region that is controlled by a transcription factor that is capable of sensing an inducing condition, e.g., a low-oxygen environment, the presence of ROS, or the presence of RNS. The genetically engineered bacteria are capable of producing the anti-inflammation and/or gut barrier function enhancer molecule(s) in inducing environments, e.g., in the gut. Thus, the genetically engineered bacteria and pharmaceutical compositions comprising those bacteria may be used to treat or prevent autoimmune disorders and/or diseases or conditions associated with gut inflammation and/or compromised gut barrier function, including IBD.

[087] In order that the disclosure may be more readily understood, certain terms are first defined. These definitions should be read in light of the remainder of

the disclosure and as understood by a person of ordinary skill in the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art. Additional definitions are set forth throughout the detailed description.

[088] As used herein, "diseases and conditions associated with gut inflammation and/or compromised gut barrier function" include, but are not limited to, inflammatory bowel diseases, diarrheal diseases, and related diseases. "Inflammatory bowel diseases" and "IBD" are used interchangeably herein to refer to a group of diseases associated with gut inflammation, which include, but are not limited to, Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, diversion colitis, Behcet's disease, and

indeterminate colitis. As used herein, "diarrheal diseases" include, but are not limited to, acute watery diarrhea, e.g., cholera; acute bloody diarrhea, e.g., dysentery; and persistent diarrhea. As used herein, related diseases include, but are not limited to, short bowel syndrome, ulcerative proctitis, proctosigmoiditis, left-sided colitis, pancolitis, and fulminant colitis.

[089] Symptoms associated with the aforementioned diseases and conditions include, but are not limited to, one or more of diarrhea, bloody stool, mouth sores, perianal disease, abdominal pain, abdominal cramping, fever, fatigue, weight loss, iron deficiency, anemia, appetite loss, weight loss, anorexia, delayed growth, delayed pubertal development, inflammation of the skin, inflammation of the eyes, inflammation of the joints, inflammation of the liver, and inflammation of the bile ducts.

[090] A disease or condition associated with gut inflammation and/or compromised gut barrier function may be an autoimmune disorder. A disease or condition associated with gut inflammation and/or compromised gut barrier function may be co-morbid with an autoimmune disorder. As used herein, "autoimmune disorders" include, but are not limited to, acute disseminated encephalomyelitis (ADEM), acute necrotizing hemorrhagic leukoencephalitis, Addison's disease, agammaglobulinemia, alopecia areata, amyloidosis, ankylosing spondylitis, anti-GBM/anti-TBM nephritis, antiphospholipid syndrome (APS), autoimmune angioedema, autoimmune aplastic anemia, autoimmune dysautonomia, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune hyperlipidemia, autoimmune immunodeficiency, autoimmune inner ear disease (AIED), autoimmune myocarditis, autoimmune oophoritis, autoimmune pancreatitis, autoimmune retinopathy, autoimmune thrombocytopenic purpura (ATP), autoimmune thyroid disease, autoimmune urticarial, axonal & neuronal neuropathies, Balo disease, Behcet's disease, bullous pemphigoid, cardiomyopathy, Castleman disease, celiac disease, Chagas disease, chronic inflammatory demyelinating polyneuropathy (CIDP), chronic recurrent multifocal ostomyelitis (CRMO), Churg-Strauss syndrome, cicatricial pemphigoid/benign mucosal pemphigoid, Crohn's disease, Cogan's syndrome, cold agglutinin disease, congenital heart block, Coxsackie myocarditis, CREST disease, essential mixed cryoglobulinemia,

demyelinating neuropathies, dermatitis herpetiformis, dermatomyositis, Devic's disease (neuromyelitis optica), discoid lupus, Dressler's syndrome, endometriosis, eosinophilic esophagitis, eosinophilic fasciitis, erythema nodosum, experimental allergic encephalomyelitis, Evans syndrome, fibrosing alveolitis, giant cell arteritis (temporal arteritis), giant cell myocarditis, glomerulonephritis, Goodpasture's syndrome, granulomatosis with polyangiitis (GPA), Graves' disease, Guillain-Barre syndrome, Hashimoto's encephalitis, Hashimoto's thyroiditis, hemolytic anemia, Henoch-Schonlein purpura, herpes gestationis, hypogammaglobulinemia, idiopathic thrombocytopenic purpura (ITP), IgA nephropathy, IgG4-related sclerosing disease, immunoregulatory lipoproteins, inclusion body myositis, interstitial cystitis, juvenile arthritis, juvenile idiopathic arthritis, juvenile myositis, Kawasaki syndrome, Lambert-Eaton syndrome, leukocytoclastic vasculitis, lichen planus, lichen sclerosus, ligneous conjunctivitis, linear IgA disease (LAD), lupus (systemic lupus erythematosus), chronic Lyme disease, Meniere's disease, microscopic polyangiitis, mixed connective tissue disease (MCTD), Mooren's ulcer, Mucha-Habermann disease, multiple sclerosis, myasthenia gravis, myositis, narcolepsy, neuromyelitis optica (Devic's), neutropenia, ocular cicatricial pemphigoid, optic neuritis, palindromic rheumatism, PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcus), paraneoplastic cerebellar degeneration, paroxysmal nocturnal hemoglobinuria (PNH), Parry Romberg syndrome, Parsonnage-Turner syndrome, pars planitis (peripheral uveitis), pemphigus, peripheral neuropathy, perivenous encephalomyelitis, pernicious anemia, POEMS syndrome, polyarteritis nodosa, type I, II, & III autoimmune polyglandular syndromes, polymyalgia rheumatic, polymyositis, postmyocardial infarction syndrome, postpericardiotomy syndrome, progesterone dermatitis, primary biliary cirrhosis, primary sclerosing cholangitis, psoriasis, psoriatic arthritis, idiopathic pulmonary fibrosis, pyoderma gangrenosum, pure red cell aplasia, Raynaud's phenomenon, reactive arthritis, reflex sympathetic dystrophy, Reiter's syndrome, relapsing polychondritis, restless legs syndrome, retroperitoneal fibrosis, rheumatic fever, rheumatoid arthritis, sarcoidosis, Schmidt syndrome, scleritis, scleroderma, Sjogren's syndrome, sperm & testicular autoimmunity, stiff person syndrome, subacute bacterial endocarditis (SBE), Susac's

syndrome, sympathetic ophthalmia, Takayasu's arteritis, temporal arteritis/giant cell arteritis, thrombocytopenic purpura (TTP), Tolosa-Hunt syndrome, transverse myelitis, type 1 diabetes, asthma, ulcerative colitis, undifferentiated connective tissue disease (UCTD), uveitis, vasculitis, vesiculobullous dermatosis, vitiligo, and Wegener's granulomatosis.

[091] As used herein, "anti-inflammation molecules" and/or "gut barrier function enhancer molecules" include, but are not limited to, short-chain fatty acids, butyrate, propionate, acetate, IL-2, IL-22, superoxide dismutase (SOD), kynurenine, GLP-2, GLP-1, IL-10, IL-27, TGF-β1, TGF-β2, N-acylphosphatidylethanolamines (NAPEs), elafin (also called peptidase inhibitor 3 and SKALP), trefoil factor, melatonin, PGD_2, and kynurenic acid, as well as other molecules disclosed herein. Such molecules may also include compounds that inhibit pro-inflammatory molecules, e.g., a single-chain variable fragment (scFv), antisense RNA, siRNA, or shRNA that neutralizes TNF-α, IFN-γ, IL-1β, IL-6, IL-8, IL-17, and/or chemokines, e.g., CXCL-8 and CCL2. A molecule may be primarily anti-inflammatory, e.g., IL-10, or primarily gut barrier function enhancing, e.g., GLP-2. A molecule may be both anti-inflammatory and gut barrier function enhancing. An anti-inflammation and/or gut barrier function enhancer molecule may be encoded by a single gene, e.g., elafin is encoded by the *PI3* gene. Alternatively, an anti-inflammation and/or gut barrier function enhancer molecule may be synthesized by a biosynthetic pathway requiring multiple genes, e.g., butyrate. These molecules may also be referred to as therapeutic molecules.

[092] As used herein, the term "gene" or "gene sequene" is meant to refer to a nucleic acid sequence encoding any of the anti-inflammatory and gut barrier function enhancing molecules described herein, e.g., IL-2, IL-22, superoxide dismutase (SOD), kynurenine, GLP-2, GLP-1, IL-10, IL-27, TGF-β1, TGF-β2, N-acylphosphatidylethanolamines (NAPEs), elafin, and trefoil factor, as well as others. The nucleic acid sequence may comprise the entire gene sequence or a partial gene sequence encoding a functional molecule. The nucleic acid sequence may be a natural sequence or a synthetic sequence. The nucleic acid sequence may comprise a native or wild-type sequence or may comprise

a modified sequence having one or more insertions, deletions, substitutions, or other modifications, for example, the nucleic acid sequence may be codon-optimized.

[093] As used herein, a "gene cassette" or "operon" encoding a biosynthetic pathway refers to the two or more genes that are required to produce an anti-inflammation and/or gut barrier function enhancer molecule, e.g., butyrate, propionate, and acetate. In addition to encoding a set of genes capable of producing said molecule, the gene cassette or operon may also comprise additional transcription and translation elements, e.g., a ribosome binding site.

[094] As used herein, "butyrogenic gene cassette" and "butyrate biosynthesis gene cassette" are used interchangeably to refer to a set of genes capable of producing butyrate in a biosynthetic pathway. Unmodified bacteria that are capable of producing butyrate via an endogenous butyrate biosynthesis pathway include, but are not limited to, *Clostridium, Peptoclostridium, Fusobacterium, Butyrivibrio, Eubacterium,* and *Treponema*, and these endogenous butyrate biosynthesis pathways may be a source of genes for the genetically engineered bacteria of the invention. The genetically engineered bacteria of the invention may comprise butyrate biosynthesis genes from a different species, strain, or substrain of bacteria, or a combination of butyrate biosynthesis genes from different species, strains, and/or substrains of bacteria. A butyrogenic gene cassette may comprise, for example, the eight genes of the butyrate production pathway from *Peptoclostridium difficile* (also called *Clostridium difficile*): *bcd2, etfB3, etfA3, thiA1, hbd, crt2, pbt,* and *buk,* which encode butyryl-CoA dehydrogenase subunit, electron transfer flavoprotein subunit beta, electron transfer flavoprotein subunit alpha, acetyl-CoA C-acetyltransferase, 3-hydroxybutyryl-CoA dehydrogenase, crotonase, phosphate butyryltransferase, and butyrate kinase, respectively (Aboulnaga et al., 2013). One or more of the butyrate biosynthesis genes may be functionally replaced or modified, e.g., codon optimized. *Peptoclostridium difficile* strain 630 and strain 1296 are both capable of producing butyrate, but comprise different nucleic acid sequences for *etfA3, thiA1, hbd, crt2, pbt,* and *buk*. A butyrogenic gene cassette may comprise *bcd2, etfB3, etfA3,* and *thiA1* from *Peptoclostridium difficile* strain 630, and *hbd, crt2, pbt,* and *buk* from *Peptoclostridium difficile* strain 1296.

Alternatively, a single gene from *Treponema denticola* (*ter,* encoding *trans*-2-enoynl-CoA reductase) is capable of functionally replacing all three of the *bcd2, etfB3,* and *etfA3* genes from *Peptoclostridium difficile*. Thus, a butyrogenic gene cassette may comprise *thiA1, hbd, crt2, pbt,* and *buk* from *Peptoclostridium difficile* and *ter* from *Treponema denticola*. Alternatively, addition of the tesB gene from *Escherichia Coli* is capable of functionally replacing *pbt* and *buk* genes from *Peptoclostridium difficile*. Thus, a butyrogenic gene cassette may comprise *thiA1, hbd, and crt2* from *Peptoclostridium difficile, ter* from *Treponema denticola*, and t*esB* from *Escherichia Coli*, for example, *thiA1* from *Peptoclostridium difficile* strain 630, *hbd and crt2* from *Peptoclostridium difficile* strain 1296, *ter* from *Treponema denticola* and *tesB* from *Escherichia Coli*. The butyrogenic gene cassette may comprise genes for the aerobic biosynthesis of butyrate and/or genes for the anaerobic or microaerobic biosynthesis of butyrate. One or more of the butyrate biosynthesis genes may be functionally replaced or modified, e.g., codon optimized. Exemplary butyrate gene cassettes are shown in Figs. 1, 3, 4, 5, 6, 7, and 8.

[095] As used herein, "propionate gene cassette" and "propionate biosynthesis gene cassette" refer to a set of genes capable of producing propionate in a biosynthetic pathway. Unmodified bacteria that are capable of producing propionate via an endogenous propionate biosynthesis pathway include, but are not limited to, *Clostridium propionicum*, *Megasphaera elsdenii*, and *Prevotella ruminicola*, and these endogenous propionate biosynthesis pathways may be a source of genes for the genetically engineered bacteria of the invention. The genetically engineered bacteria of the invention may comprise propionate biosynthesis genes from a different species, strain, or substrain of bacteria, or a combination of propionate biosynthesis genes from different species, strains, and/or substrains of bacteria. In some embodiments, the propionate gene cassette comprises acrylate pathway propionate biosynthesis genes, e.g., *pct*, *lcdA*, *lcdB*, *lcdC*, *etfA*, *acrB*, and *acrC,* which encode propionate CoA-transferase, lactoyl-CoA dehydratase A, lactoyl-CoA dehydratase B, lactoyl-CoA dehydratase C, electron transfer flavoprotein subunit A, acryloyl-CoA reductase B, and acryloyl-CoA reductase C, respectively (Hetzel et al., 2003, Selmer et al., 2002). In alternate embodiments, the propionate gene cassette comprises pyruvate pathway propionate biosynthesis genes

(*see, e.g.,* Tseng et al., 2012), e.g., *thrA*^{fbr}, *thrB*, *thrC*, *ilvA*^{fbr}, *aceE*, *aceF*, and *lpd*, which encode homoserine dehydrogenase 1, homoserine kinase, L-threonine synthase, L-threonine dehydratase, pyruvate dehydrogenase, dihydrolipoamide acetyltransferase, and dihydrolipoyl dehydrogenase, respectively. In some embodiments, the propionate gene cassette further comprises *tesB*, which encodes acyl-CoA thioesterase. The propionate gene cassette may comprise genes for the aerobic biosynthesis of propionate and/or genes for the anaerobic or microaerobic biosynthesis of propionate. One or more of the proprionate biosynthesis genes may be functionally replaced or modified, e.g., codon optimized. Exemplary propionic gene cassettes are shown in Figs. 9, 11, and 13.

[096] As used herein, "acetate gene cassette" and "acetate biosynthesis gene cassette" refers to a set of genes capable of producing acetate in a biosynthetic pathway. Bacteria synthesize acetate from a number of carbon and energy sources, including a variety of substrates such as cellulose, lignin, and inorganic gases, and utilize different biosynthetic mechanisms and genes, which are known in the art (Ragsdale, 2008). Unmodified bacteria that are capable of producing acetate via an endogenous acetate biosynthesis pathway may be a source of acetate biosynthesis genes for the genetically engineered bacteria of the invention. The genetically engineered bacteria of the invention may comprise acetate biosynthesis genes from a different species, strain, or substrain of bacteria, or a combination of acetate biosynthesis genes from different species, strains, and/or substrains of bacteria. *Escherichia coli* are capable of consuming glucose and oxygen to produce acetate and carbon dioxide during aerobic growth (Kleman et al., 1994). Several bacteria, such as *Acetitomaculum, Acetoanaerobium, Acetohalobium, Acetonema, Balutia, Butyribacterium, Clostridium, Moorella, Oxobacter, Sporomusa,* and *Thermoacetogenium,* are acetogenic anaerobes that are capable of converting CO or CO_2 + H_2 into acetate, e.g., using the Wood-Ljungdahl pathway (Schiel-Bengelsdorf et al, 2012). Genes in the Wood-Ljungdahl pathway for various bacterial species are known in the art. The acetate gene cassette may comprise genes for the aerobic biosynthesis of acetate and/or genes for the anaerobic or microaerobic biosynthesis of acetate. One or more of the acetate biosynthesis genes may be

functionally replaced or modified, e.g., codon optimized. Examples of acetate gene cassettes are described herein.

[097] Each gene sequence and/or gene cassette may be present on a plasmid or bacterial chromosome. In embodiments in which the engineered bacteria comprise one or more gene sequence(s) and one or more gene cassettes, the gene sequence(s)may be present on one or more plasmids and the gene cassette(s) may be present in the bacterial chromosome, and vice versa. In addition, multiple copies of any gene, gene cassette, or regulatory region may be present in the bacterium, wherein one or more copies of the gene, gene cassette, or regulatory region may be mutated or otherwise altered as described herein. In some embodiments, the genetically engineered bacteria are engineered to comprise multiple copies of the same gene, gene cassette, or regulatory region in order to enhance copy number. In some embodiments, the genetically engineered bacteria are engineered to comprise multiple different components of a gene cassette performing multiple different functions. In some embodiments, the genetically engineered bacteria are engineered to comprise one or more copies of different genes, gene cassettes, or regulatory regions to produce engineered bacteria that express more than one therapeutic molecule and/or perform more than one function.

[098] Each gene or gene cassette may be operably linked to an inducible promoter, e.g., an FNR-responsive promoter, an ROS-responsive promoter, and/or an RNS-responsive promoter. An "inducible promoter" refers to a regulatory region that is operably linked to one or more genes, wherein expression of the gene(s) is increased in the presence of an inducer of said regulatory region.

[099] As used herein, a "directly inducible promoter" refers to a regulatory region, wherein the regulatory region is operably linked to a gene or a gene cassette encoding a biosynthetic pathway for producing an anti-inflammation and/or gut barrier function enhancer molecule, e.g. butyrate. In the presence of an inducer of said regulatory region, an anti-inflammation and/or gut barrier function enhancer molecule is expressed. An "indirectly inducible promoter" refers to a regulatory system comprising two or more regulatory regions, for example, a first regulatory region that is operably

linked to a gene encoding a first molecule, e.g., a transcription factor, which is capable of regulating a second regulatory region that is operably linked to a gene or a gene cassette encoding a biosynthetic pathway for producing an anti-inflammation and/or gut barrier function enhancer molecule, e.g. butyrate (or other anti-inflammation and/or gut barrier function enhancer molecule). In the presence of an inducer of the first regulatory region, the second regulatory region may be activated or repressed, thereby activating or repressing production of butyrate (or other anti-inflammation and/or gut barrier function enhancer molecule). Both a directly inducible promoter and an indirectly inducible promoter are encompassed by "inducible promoter."

[0100] As used herein, "operably linked" refers a nucleic acid sequence, e.g., a gene or gene cassette for producing an anti-inflammation and/or gut barrier enhancer molecule, that is joined to a regulatory region sequence in a manner which allows expression of the nucleic acid sequence, e.g., acts in *cis*. A regulatory region is a nucleic acid that can direct transcription of a gene of interest and may comprise promoter sequences, enhancer sequences, response elements, protein recognition sites, inducible elements, promoter control elements, protein binding sequences, 5′ and 3′ untranslated regions, transcriptional start sites, termination sequences, polyadenylation sequences, and introns.

[0101] As used herein, "exogenous environmental conditions" refer to settings or circumstances under which the promoter described herein is directly or indirectly induced. The phrase "exogenous environmental conditions" is meant to refer to the environmental conditions external to the bacteria, but endogenous or native to a mammalian subject. Thus, "exogenous" and "endogenous" may be used interchangeably to refer to environmental conditions in which the environmental conditions are endogenous to a mammalian body, but external or exogenous to a bacterial cell. In some embodiments, the exogenous environmental conditions are specific to the gut of a mammal. In some embodiments, the exogenous environmental conditions are specific to the upper gastrointestinal tract of a mammal. In some embodiments, the exogenous environmental conditions are specific to the lower gastrointestinal tract of a mammal. In some embodiments, the exogenous

environmental conditions are specific to the small intestine of a mammal. In some embodiments, the exogenous environmental condition is an environment in which ROS is present. In some embodiments, the exogenous environmental condition is an environment in which RNS is present.

[0102] In some embodiments, the exogenous environmental conditions are low-oxygen or anaerobic conditions such as the environment of the mammalian gut. In some embodiments, exogenous environmental conditions refer to the presence of molecules or metabolites that are specific to the mammalian gut in a healthy or disease state, e.g., propionate. In some embodiments, the gene or gene cassette for producing a therapeutic molecule is operably linked to an oxygen level-dependent promoter. Bacteria have evolved transcription factors that are capable of sensing oxygen levels. Different signaling pathways may be triggered by different oxygen levels and occur with different kinetics. As used herein, an "oxygen level-dependent promoter" or "oxygen level-dependent regulatory region" refers to a nucleic acid sequence to which one or more oxygen level-sensing transcription factors is capable of binding, wherein the binding and/or activation of the corresponding transcription factor activates downstream gene expression.

[0103] In some embodiments, the gene or gene cassette for producing a therapeutic molecule is operably linked to an oxygen level-dependent regulatory region such that the therapeutic molecule is expressed in low-oxygen, microaerobic, or anaerobic conditions. For example, the oxygen level-dependent regulatory region is operably linked to a butyrogenic or other gene cassette or gene sequence(s) (e.g., any of the genes described herein); in low-oxygen conditions, the oxygen level-dependent regulatory region is activated by a corresponding oxygen level-sensing transcription factor, thereby driving expression of the butyrogenic or other gene cassette or gene sequence(s). Examples of oxygen level-dependent transcription factors include, but are not limited to, FNR, ANR, and DNR. Corresponding FNR-responsive promoters, ANR-responsive promoters, and DNR-responsive promoters are known in the art (*see, e.g.,* Castiglione et al., 2009; Eiglmeier et al., 1989; Galimand et al., 1991; Hasegawa et al.,

1998; Hoeren et al., 1993; Salmon et al., 2003), and non-limiting examples are shown in **Table 1**.

Table 1. Examples of transcription factors and responsive genes and regulatory regions

Transcription Factor	**Examples of responsive genes, promoters, and/or regulatory regions:**
FNR	*nirB, ydfZ, pdhR, focA, ndH, hlyE, narK, narX, narG, yfiD, tdcD*
ANR	*arcDABC*
DNR	*norb, norC*

[0104] As used herein, "reactive nitrogen species" and "RNS" are used interchangeably to refer to highly active molecules, ions, and/or radicals derived from molecular nitrogen. RNS can cause deleterious cellular effects such as nitrosative stress. RNS include, but are not limited to, nitric oxide (NO•), peroxynitrite or peroxynitrite anion ($ONOO^-$), nitrogen dioxide ($•NO_2$), dinitrogen trioxide (N_2O_3), peroxynitrous acid (ONOOH), and nitroperoxycarbonate ($ONOOCO_2^-$) (unpaired electrons denoted by •). Bacteria have evolved transcription factors that are capable of sensing RNS levels. Different RNS signaling pathways are triggered by different RNS levels and occur with different kinetics.

[0105] As used herein, "RNS-inducible regulatory region" refers to a nucleic acid sequence to which one or more RNS-sensing transcription factors is capable of binding, wherein the binding and/or activation of the corresponding transcription factor activates downstream gene expression; in the presence of RNS, the transcription factor binds to and/or activates the regulatory region. In some embodiments, the RNS-inducible regulatory region comprises a promoter sequence. In some embodiments, the transcription factor senses RNS and subsequently binds to the RNS-inducible regulatory region, thereby activating downstream gene expression. In alternate embodiments, the transcription factor is bound to the RNS-inducible regulatory region in the absence of RNS; in the presence of RNS, the transcription factor undergoes a conformational change, thereby activating downstream gene expression. The RNS-inducible regulatory region may be operatively linked to a gene or gene cassette, e.g., a butyrogenic or other gene

cassette or gene sequence(s), e.g., any of the genes described herein. For example, in the presence of RNS, a transcription factor senses RNS and activates a corresponding RNS-inducible regulatory region, thereby driving expression of an operatively linked gene sequence or gene cassette. Thus, RNS induces expression of the gene or gene cassette.

[0106] As used herein, "RNS-derepressible regulatory region" refers to a nucleic acid sequence to which one or more RNS-sensing transcription factors is capable of binding, wherein the binding of the corresponding transcription factor represses downstream gene expression; in the presence of RNS, the transcription factor does not bind to and does not repress the regulatory region. In some embodiments, the RNS-derepressible regulatory region comprises a promoter sequence. The RNS-derepressible regulatory region may be operatively linked to a gene or gene cassette, e.g., a butyrogenic or other gene cassette or gene sequence(s). For example, in the presence of RNS, a transcription factor senses RNS and no longer binds to and/or represses the regulatory region, thereby derepressing an operatively linked gene sequence or gene cassette. Thus, RNS derepresses expression of the gene or gene cassette.

[0107] As used herein, "RNS-repressible regulatory region" refers to a nucleic acid sequence to which one or more RNS-sensing transcription factors is capable of binding, wherein the binding of the corresponding transcription factor represses downstream gene expression; in the presence of RNS, the transcription factor binds to and represses the regulatory region. In some embodiments, the RNS-repressible regulatory region comprises a promoter sequence. In some embodiments, the transcription factor that senses RNS is capable of binding to a regulatory region that overlaps with part of the promoter sequence. In alternate embodiments, the transcription factor that senses RNS is capable of binding to a regulatory region that is upstream or downstream of the promoter sequence. The RNS-repressible regulatory region may be operatively linked to a gene sequence or gene cassette. For example, in the presence of RNS, a transcription factor senses RNS and binds to a corresponding RNS-repressible regulatory region, thereby blocking expression of an operatively linked gene sequence or gene cassette. Thus, RNS represses expression of the gene or gene cassette.

[0108] As used herein, a "RNS-responsive regulatory region" refers to a RNS-inducible regulatory region, a RNS-repressible regulatory region, and/or a RNS-derepressible regulatory region. In some embodiments, the RNS-responsive regulatory region comprises a promoter sequence. Each regulatory region is capable of binding at least one corresponding RNS-sensing transcription factor. Examples of transcription factors that sense RNS and their corresponding RNS-responsive genes, promoters, and/or regulatory regions include, but are not limited to, those shown in **Table 2**.

Table 2. Examples of RNS-sensing transcription factors and RNS-responsive genes

RNS-sensing transcription factor:	**Primarily capable of sensing:**	**Examples of responsive genes, promoters, and/or regulatory regions:**
NsrR	NO	*norB, aniA, nsrR, hmpA, ytfE, ygbA, hcp, hcr, nrfA, aox*
NorR	NO	*norVW, norR*
DNR	NO	*norCB, nir, nor, nos*

[0109] As used herein, "reactive oxygen species" and "ROS" are used interchangeably to refer to highly active molecules, ions, and/or radicals derived from molecular oxygen. ROS can be produced as byproducts of aerobic respiration or metal-catalyzed oxidation and may cause deleterious cellular effects such as oxidative damage. ROS include, but are not limited to, hydrogen peroxide (H_2O_2), organic peroxide (ROOH), hydroxyl ion (OH^-), hydroxyl radical (•OH), superoxide or superoxide anion ($•O_2^-$), singlet oxygen ($^1O_2$), ozone (O_3), carbonate radical, peroxide or peroxyl radical ($•O_2^{-2}$), hypochlorous acid (HOCl), hypochlorite ion (OCl^-), sodium hypochlorite (NaOCl), nitric oxide (NO•), and peroxynitrite or peroxynitrite anion ($ONOO^-$) (unpaired electrons denoted by •). Bacteria have evolved transcription factors that are capable of sensing ROS levels. Different ROS signaling pathways are triggered by different ROS levels and occur with different kinetics (Marinho et al., 2014).

[0110] As used herein, "ROS-inducible regulatory region" refers to a nucleic acid sequence to which one or more ROS-sensing transcription factors is capable of binding, wherein the binding and/or activation of the corresponding transcription factor activates downstream gene expression; in the presence of ROS, the transcription factor binds to

and/or activates the regulatory region. In some embodiments, the ROS-inducible regulatory region comprises a promoter sequence. In some embodiments, the transcription factor senses ROS and subsequently binds to the ROS-inducible regulatory region, thereby activating downstream gene expression. In alternate embodiments, the transcription factor is bound to the ROS-inducible regulatory region in the absence of ROS; in the presence of ROS, the transcription factor undergoes a conformational change, thereby activating downstream gene expression. The ROS-inducible regulatory region may be operatively linked to a gene sequence or gene cassette, e.g., a butyrogenic gene cassette. For example, in the presence of ROS, a transcription factor, e.g., OxyR, senses ROS and activates a corresponding ROS-inducible regulatory region, thereby driving expression of an operatively linked gene sequence or gene cassette. Thus, ROS induces expression of the gene or gene cassette.

[0111] As used herein, "ROS-derepressible regulatory region" refers to a nucleic acid sequence to which one or more ROS-sensing transcription factors is capable of binding, wherein the binding of the corresponding transcription factor represses downstream gene expression; in the presence of ROS, the transcription factor does not bind to and does not repress the regulatory region. In some embodiments, the ROS-derepressible regulatory region comprises a promoter sequence. The ROS-derepressible regulatory region may be operatively linked to a gene or gene cassette, e.g., a butyrogenic or other gene cassette or gene sequence(s) described herein. For example, in the presence of ROS, a transcription factor, e.g., OhrR, senses ROS and no longer binds to and/or represses the regulatory region, thereby derepressing an operatively linked gene sequence or gene cassette. Thus, ROS derepresses expression of the gene or gene cassette.

[0112] As used herein, "ROS-repressible regulatory region" refers to a nucleic acid sequence to which one or more ROS-sensing transcription factors is capable of binding, wherein the binding of the corresponding transcription factor represses downstream gene expression; in the presence of ROS, the transcription factor binds to and represses the regulatory region. In some embodiments, the ROS-repressible regulatory region comprises a promoter sequence. In some embodiments, the transcription factor that

senses ROS is capable of binding to a regulatory region that overlaps with part of the promoter sequence. In alternate embodiments, the transcription factor that senses ROS is capable of binding to a regulatory region that is upstream or downstream of the promoter sequence. The ROS-repressible regulatory region may be operatively linked to a gene sequence or gene cassette. For example, in the presence of ROS, a transcription factor, e.g., PerR, senses ROS and binds to a corresponding ROS-repressible regulatory region, thereby blocking expression of an operatively linked gene sequence or gene cassette. Thus, ROS represses expression of the gene or gene cassette.

[0113] As used herein, a "ROS-responsive regulatory region" refers to a ROS-inducible regulatory region, a ROS-repressible regulatory region, and/or a ROS-derepressible regulatory region. In some embodiments, the ROS-responsive regulatory region comprises a promoter sequence. Each regulatory region is capable of binding at least one corresponding ROS-sensing transcription factor. Examples of transcription factors that sense ROS and their corresponding ROS-responsive genes, promoters, and/or regulatory regions include, but are not limited to, those shown in **Table 3**.

Table 3. Examples of ROS-sensing transcription factors and ROS-responsive genes

ROS-sensing transcription factor:	**Primarily capable of sensing:**	**Examples of responsive genes, promoters, and/or regulatory regions:**
OxyR	H_2O_2	*ahpC; ahpF; dps; dsbG; fhuF; flu; fur; gor; grxA; hemH; katG; oxyS; sufA; sufB; sufC; sufD; sufE; sufS; trxC; uxuA; yaaA; yaeH; yaiA; ybjM; ydcH; ydeN; ygaQ; yljA; ytfK*
PerR	H_2O_2	*katA; ahpCF; mrgA; zoaA; fur; hemAXCDBL; srfA*
OhrR	Organic peroxides NaOCl	*ohrA*
SoxR	$\bullet O_2^-$ NO• (also capable of sensing H_2O_2)	*soxS*
RosR	H_2O_2	*rbtT; tnp16a; rluC1; tnp5a; mscL; tnp2d; phoD; tnp15b; pstA; tnp5b; xylC; gabD1; rluC2; cgtS9; azlC; narKGHJI; rosR*

[0114] As used herein, a "tunable regulatory region" refers to a nucleic acid sequence under direct or indirect control of a transcription factor and which is capable of activating, repressing, derepressing, or otherwise controlling gene expression relative to levels of an inducer. In some embodiments, the tunable regulatory region comprises a promoter sequence. The inducer may be RNS, or other inducer described herein, and the tunable regulatory region may be a RNS-responsive regulatory region or other responsive regulatory region described herein. The tunable regulatory region may be operatively linked to a gene sequence(s) or gene cassette, e.g., a butyrogenic or other gene cassette or gene sequence(s). For example, in one specific embodiment, the tunable regulatory region is a RNS-derepressible regulatory region, and when RNS is present, a RNS-sensing transcription factor no longer binds to and/or represses the regulatory region, thereby permitting expression of the operatively linked gene or gene cassette. In this instance, the tunable regulatory region derepresses gene or gene cassette expression relative to RNS levels. Each gene or gene cassette may be operatively linked to a tunable regulatory region that is directly or indirectly controlled by a transcription factor that is capable of sensing at least one RNS.

[0115] As used herein, a "non-native" nucleic acid sequence refers to a nucleic acid sequence not normally present in a bacterium, e.g., an extra copy of an endogenous sequence, or a heterologous sequence such as a sequence from a different species, strain, or substrain of bacteria, or a sequence that is modified and/or mutated as compared to the unmodified sequence from bacteria of the same subtype. In some embodiments, the non-native nucleic acid sequence is a synthetic, non-naturally occurring sequence (*see, e.g.,* Purcell et al., 2013). The non-native nucleic acid sequence may be a regulatory region, a promoter, a gene, and/or one or more genes in gene cassette. In some embodiments, "non-native" refers to two or more nucleic acid sequences that are not found in the same relationship to each other in nature. The non-native nucleic acid sequence may be present on a plasmid or chromosome. In addition, multiple copies of any regulatory region, promoter, gene, and/or gene cassette may be present in the bacterium, wherein one or more copies of the regulatory region, promoter, gene, and/or gene cassette may be mutated or otherwise altered as described

herein. In some embodiments, the genetically engineered bacteria are engineered to comprise multiple copies of the same regulatory region, promoter, gene, and/or gene cassette in order to enhance copy number or to comprise multiple different components of a gene cassette performing multiple different functions or to comprise one or more copies of different regulatory regions, promoters, genes, and/or gene cassette to produce engineered bacteria that express more than one therapeutic molecule and/or perform more than one function.

[0116] In some embodiments, the genetically engineered bacteria of the invention comprise a gene cassette that is operably linked to a directly or indirectly inducible promoter that is not associated with said gene cassette in nature, e.g., a FNR-responsive promoter operably linked to a butyrogenic gene cassette.

[0117] "Constitutive promoter" refers to a promoter that is capable of facilitating continuous transcription of a coding sequence or gene under its control and/or to which it is operably linked. Constitutive promoters and functional variants are well known in the art and include, but are not limited to, BBa_J23100, a constitutive *Escherichia coli* σ^S promoter (e.g., an osmY promoter (International Genetically Engineered Machine (iGEM) Registry of Standard Biological Parts Name BBa_J45992; BBa_J45993)), a constitutive *Escherichia coli* σ^{32} promoter (e.g., htpG heat shock promoter (BBa_J45504)), a constitutive *Escherichia coli* σ^{70} promoter (e.g., lacq promoter (BBa_J54200; BBa_J56015), *E. coli* CreABCD phosphate sensing operon promoter (BBa_J64951), GlnRS promoter (BBa_K088007), lacZ promoter (BBa_K119000; BBa_K119001); M13K07 gene I promoter (BBa_M13101); M13K07 gene II promoter (BBa_M13102), M13K07 gene III promoter (BBa_M13103), M13K07 gene IV promoter (BBa_M13104), M13K07 gene V promoter (BBa_M13105), M13K07 gene VI promoter (BBa_M13106), M13K07 gene VIII promoter (BBa_M13108), M13110 (BBa_M13110)), a constitutive *Bacillus subtilis* σ^A promoter (e.g., promoter veg (BBa_K143013), promoter 43 (BBa_K143013), P_{liaG} (BBa_K823000), P_{lepA} (BBa_K823002), P_{veg} (BBa_K823003)), a constitutive *Bacillus subtilis* σ^B promoter (e.g., promoter ctc (BBa_K143010), promoter gsiB (BBa_K143011)), a *Salmonella* promoter (e.g., Pspv2 from *Salmonella* (BBa_K112706), Pspv from *Salmonella* (BBa_K112707)), a bacteriophage T7 promoter (e.g., T7 promoter (BBa_I712074;

BBa_I719005; BBa_J34814; BBa_J64997; BBa_K113010; BBa_K113011; BBa_K113012; BBa_R0085; BBa_R0180; BBa_R0181; BBa_R0182; BBa_R0183; BBa_Z0251; BBa_Z0252; BBa_Z0253)), and a bacteriophage SP6 promoter (e.g., SP6 promoter (BBa_J64998)).

[0118] "Gut" refers to the organs, glands, tracts, and systems that are responsible for the transfer and digestion of food, absorption of nutrients, and excretion of waste. In humans, the gut comprises the gastrointestinal (GI) tract, which starts at the mouth and ends at the anus, and additionally comprises the esophagus, stomach, small intestine, and large intestine. The gut also comprises accessory organs and glands, such as the spleen, liver, gallbladder, and pancreas. The upper gastrointestinal tract comprises the esophagus, stomach, and duodenum of the small intestine. The lower gastrointestinal tract comprises the remainder of the small intestine, i.e., the jejunum and ileum, and all of the large intestine, i.e., the cecum, colon, rectum, and anal canal. Bacteria can be found throughout the gut, e.g., in the gastrointestinal tract, and particularly in the intestines.

[0119] "Non-pathogenic bacteria" refer to bacteria that are not capable of causing disease or harmful responses in a host. In some embodiments, non-pathogenic bacteria are Gram-negative bacteria. In some embodiments, non-pathogenic bacteria are Gram-positive bacteria. In some embodiments, non-pathogenic bacteria are commensal bacteria, which are present in the indigenous microbiota of the gut. Examples of non-pathogenic bacteria include, but are not limited to *Bacillus, Bacteroides, Bifidobacterium*, *Brevibacteria, Clostridium, Enterococcus, Escherichia, Lactobacillus, Lactococcus, Saccharomyces,* and *Staphylococcus,* e.g., *Bacillus coagulans*, *Bacillus subtilis, Bacteroides fragilis, Bacteroides subtilis, Bacteroides thetaiotaomicron, Bifidobacterium bifidum*, *Bifidobacterium infantis, Bifidobacterium lactis, Bifidobacterium longum, Clostridium butyricum, Enterococcus faecium*, *Escherichia coli, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus johnsonii, Lactobacillus paracasei*, *Lactobacillus plantarum, Lactobacillus reuteri, Lactobacillus rhamnosus, Lactococcus lactis,* and *Saccharomyces boulardii* (Sonnenborn et al., 2009; Dinleyici et al., 2014; U.S. Patent No. 6,835,376; U.S. Patent No. 6,203,797; U.S. Patent No. 5,589,168; U.S. Patent No. 7,731,976). Non-pathogenic bacteria also include

commensal bacteria, which are present in the indigenous microbiota of the gut. Naturally pathogenic bacteria may be genetically engineered to reduce or eliminate pathogenicity.

[0120] "Probiotic" is used to refer to live, non-pathogenic microorganisms, e.g., bacteria, which can confer health benefits to a host organism that contains an appropriate amount of the microorganism. In some embodiments, the host organism is a mammal. In some embodiments, the host organism is a human. Some species, strains, and/or subtypes of non-pathogenic bacteria are currently recognized as probiotic. Examples of probiotic bacteria include, but are not limited to, *Bifidobacteria*, *Escherichia*, *Lactobacillus,* and *Saccharomyces,* e.g., *Bifidobacterium bifidum, Enterococcus faecium, Escherichia coli, Escherichia coli* strain Nissle, *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus paracasei*, *Lactobacillus plantarum*, and *Saccharomyces boulardii* (Dinleyici et al., 2014; U.S. Patent No. 5,589,168; U.S. Patent No. 6,203,797; U.S. Patent 6,835,376). The probiotic may be a variant or a mutant strain of bacterium (Arthur et al., 2012; Cuevas-Ramos et al., 2010; Olier et al., 2012; Nougayrede et al., 2006). Non-pathogenic bacteria may be genetically engineered to enhance or improve desired biological properties, e.g., survivability. Non-pathogenic bacteria may be genetically engineered to provide probiotic properties. Probiotic bacteria may be genetically engineered to enhance or improve probiotic properties.

[0121] As used herein, "stably maintained", "stably expressed" or "stable" bacterium is used to refer to a bacterial host cell carrying non-native genetic material, e.g., a butyrogenic or other gene cassette or gene sequence(s), which is incorporated into the host genome or propagated on a self-replicating extra-chromosomal plasmid, such that the non-native genetic material is retained, expressed, and/or propagated. The stable bacterium is capable of survival and/or growth *in vitro*, e.g., in medium, and/or *in vivo*, e.g., in the gut. For example, the stable bacterium may be a genetically modified bacterium comprising a butyrogenic or other gene cassette or gene sequence(s), in which the plasmid or chromosome carrying the butyrogenic or other gene cassette or gene sequence(s) is stably maintained in the host cell, such that the gene cassette or gene sequence(s) can be expressed in the host cell, and the host cell is capable of survival

and/or growth *in vitro* and/or *in vivo*. In some embodiments, copy number affects the stability of expression of the non-native genetic material. In some embodiments, copy number affects the level of expression of the non-native genetic material.

[0122] As used herein, the term "treat" and its cognates refer to an amelioration of a disease or disorder, or at least one discernible symptom thereof. In another embodiment, "treat" refers to an amelioration of at least one measurable physical parameter, not necessarily discernible by the patient. In another embodiment, "treat" refers to inhibiting the progression of a disease or disorder, either physically (e.g., stabilization of a discernible symptom), physiologically (e.g., stabilization of a physical parameter), or both. In another embodiment, "treat" refers to slowing the progression or reversing the progression of a disease or disorder. As used herein, "prevent" and its cognates refer to delaying the onset or reducing the risk of acquiring a given disease or disorder.

[0123] Those in need of treatment may include individuals already having a particular medical disorder, as well as those at risk of having, or who may ultimately acquire the disorder. The need for treatment is assessed, for example, by the presence of one or more risk factors associated with the development of a disorder, the presence or progression of a disorder, or likely receptiveness to treatment of a subject having the disorder. Treating autoimmune disorders and/or diseases and conditions associated with gut inflammation and/or compromised gut barrier function may encompass reducing or eliminating excess inflammation and/or associated symptoms, and does not necessarily encompass the elimination of the underlying disease or disorder. In some instances, the "initial colonization of the newborn intestine is particularly relevant to the proper development of the host's immune and metabolic functions and to determine disease risk in early and later life" (Sanz et al., 2015). In some embodiments, early intervention (e.g., prenatal, perinatal, neonatal) using the genetically engineered bacteria of the invention may be sufficient to prevent or delay the onset of the disease or disorder.

[0124] As used herein a "pharmaceutical composition" refers to a preparation of genetically engineered bacteria of the invention with other components such as a physiologically suitable carrier and/or excipient.

[0125] The phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which may be used interchangeably refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered bacterial compound. An adjuvant is included under these phrases.

[0126] The term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples include, but are not limited to, calcium bicarbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils, polyethylene glycols, and surfactants, including, for example, polysorbate 20.

[0127] The terms “therapeutically effective dose” and “therapeutically effective amount” are used to refer to an amount of a compound that results in prevention, delay of onset of symptoms, or amelioration of symptoms of a condition, e.g., inflammation, diarrhea. A therapeutically effective amount may, for example, be sufficient to treat, prevent, reduce the severity, delay the onset, and/or reduce the risk of occurrence of one or more symptoms of an autoimmune disorder and/or a disease or condition associated with gut inflammation and/or compromised gut barrier function. A therapeutically effective amount, as well as a therapeutically effective frequency of administration, can be determined by methods known in the art and discussed below.

[0128] The articles “a” and “an,” as used herein, should be understood to mean “at least one,” unless clearly indicated to the contrary.

[0129] The phrase “and/or,” when used between elements in a list, is intended to mean either (1) that only a single listed element is present, or (2) that more than one element of the list is present. For example, “A, B, and/or C” indicates that the selection may be A alone; B alone; C alone; A and B; A and C; B and C; or A, B, and C. The phrase “and/or” may be used interchangeably with “at least one of” or “one or more of” the elements in a list.

Bacteria

[0130] The genetically engineered bacteria of the invention are capable of producing a one or more non-native anti-inflammation and/or gut barrier function

enhancer molecule(s). In some embodiments, the genetically engineered bacteria are naturally non-pathogenic bacteria. In some embodiments, the genetically engineered bacteria are commensal bacteria. In some embodiments, the genetically engineered bacteria are probiotic bacteria. In some embodiments, the genetically engineered bacteria are naturally pathogenic bacteria that are modified or mutated to reduce or eliminate pathogenicity. In some embodiments, non-pathogenic bacteria are Gram-negative bacteria. In some embodiments, non-pathogenic bacteria are Gram-positive bacteria. Exemplary bacteria include, but are not limited to *Bacillus, Bacteroides, Bifidobacterium*, *Brevibacteria, Clostridium, Enterococcus, Escherichia coli, Lactobacillus, Lactococcus, Saccharomyces,* and *Staphylococcus,* e.g., *Bacillus coagulans*, *Bacillus subtilis, Bacteroides fragilis, Bacteroides subtilis, Bacteroides thetaiotaomicron, Bifidobacterium bifidum*, *Bifidobacterium infantis, Bifidobacterium lactis, Bifidobacterium longum, Clostridium butyricum, Enterococcus faecium*, *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus johnsonii, Lactobacillus paracasei*, *Lactobacillus plantarum, Lactobacillus reuteri, Lactobacillus rhamnosus, Lactococcus lactis, Saccharomyces boulardii, Clostridium* clusters *IV* and *XIVa* of *Firmicutes* (including species of Eubacterium)*, Roseburia, Faecalibacterium, Enterobacter, Faecalibacterium prausnitzii, Clostridium difficile, Subdoligranulum, Clostridium sporogenes, Campylobacter jejuni, Clostridium saccharolyticum, Klebsiella, Citrobacter, Pseudobutyrivibrio,* and *Ruminococcus.* In certain embodiments, the genetically engineered bacteria are selected from the group consisting of *Bacteroides fragilis, Bacteroides thetaiotaomicron, Bacteroides subtilis, Bifidobacterium bifidum, Bifidobacterium infantis, Bifidobacterium lactis, Clostridium butyricum, Escherichia coli* Nissle*, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus reuteri,* and *Lactococcus lactis.* In some embodiments, the genetically engineered bacterium is a Gram-positive bacterium, e.g., *Clostridium*, that is naturally capable of producing high levels of butyrate. In some embodiments, the genetically engineered bacterium is selected from the group consisting of *C. butyricum ZJUCB, C. butyricum S21, C. thermobutyricum ATCC 49875, C. beijerinckii, C. populeti ATCC 35295, C. tyrobutyricum JM1, C. tyrobutyricum CIP 1-776, C. tyrobutyricum ATCC 25755, C. tyrobutyricum CNRZ*

596, and *C. tyrobutyricum ZJU 8235*. In some embodiments, the genetically engineered bacterium is *C. butyricum CBM588*, a probiotic bacterium that is highly amenable to protein secretion and has demonstrated efficacy in treating IBD (Kanai et al., 2015). In some embodiments, the genetically engineered bacterium is *Bacillus*, a probiotic bacterium that is highly genetically tractable and has been a popular chassis for industrial protein production; in some embodiments, the bacterium has highly active secretion and/or no toxic byproducts (Cutting, 2011).

[0131] In some embodiments, the genetically engineered bacteria are *Escherichia coli* strain Nissle 1917 (*E. coli* Nissle), a Gram-negative bacterium of the *Enterobacteriaceae* family that has evolved into one of the best characterized probiotics (Ukena et al., 2007). The strain is characterized by its complete harmlessness (Schultz, 2008), and has GRAS (generally recognized as safe) status (Reister et al., 2014, emphasis added). Genomic sequencing confirmed that *E. coli* Nissle lacks prominent virulence factors (e.g., *E. coli* α-hemolysin, P-fimbrial adhesins) (Schultz, 2008). In addition, it has been shown that *E. coli* Nissle does not carry pathogenic adhesion factors, does not produce any enterotoxins or cytotoxins, is not invasive, and is not uropathogenic. (Sonnenborn et al., 2009). As early as in 1917, *E. coli* Nissle was packaged into medicinal capsules, called Mutaflor, for therapeutic use. *E. coli* Nissle has since been used to treat ulcerative colitis in humans *in vivo* (Rembacken et al., 1999), to treat inflammatory bowel disease, Crohn's disease, and pouchitis in humans *in vivo* (Schultz, 2008), and to inhibit enteroinvasive *Salmonella, Legionella, Yersinia,* and *Shigella in vitro* (Altenhoefer et al., 2004). It is commonly accepted that *E. coli* Nissle's therapeutic efficacy and safety have convincingly been proven (Ukena et al., 2007). In some embodiments, the genetically engineered bacteria are *E. coli* Nissle and are naturally capable of promoting tight junctions and gut barrier function. In some embodiments, the genetically engineered bacteria are *E. coli* and are highly amenable to recombinant protein technologies.

[0132] One of ordinary skill in the art would appreciate that the genetic modifications disclosed herein may be adapted for other species, strains, and subtypes of bacteria. It is known, for example, that the clostridial butyrogenic pathway genes are widespread in the genome-sequenced clostridia and related species (Aboulnaga et al.,

2013). Furthermore, genes from one or more different species of bacteria can be introduced into one another, e.g., the butyrogenic genes from *Peptoclostridium difficile* have been expressed in *Escherichia coli* (Aboulnaga et al., 2013).

[0133] Unmodified *E. coli* Nissle and the genetically engineered bacteria of the invention may be destroyed, e.g., by defense factors in the gut or blood serum (Sonnenborn et al., 2009) or by activation of a kill switch, several hours or days after administration. Thus, the genetically engineered bacteria may require continued administration. Residence time *in vivo* may be calculated for the genetically engineered bacteria. In some embodiments, the residence time is calculated for a human subject.

Anti-inflammation and/or gut barrier function enhancer molecules

[0134] The genetically engineered bacteria comprise one or more gene sequence(s) and/or gene cassette(s) for producing a non-native anti-inflammation and/or gut barrier function enhancer molecule. In some embodiments, the genetically engineered bacteria comprise one or more gene sequence(s) for producing a non-native anti-inflammation and/or gut barrier function enhancer molecule. For example, the genetically engineered bacteria may comprise two or more gene sequence(s) for producing a non-native anti-inflammation and/or gut barrier function enhancer molecule. In some embodiments, the two or more gene sequences are multiple copies of the same gene. In some emodiments, the two or more gene sequences are sequences encoding different genes. In some emodiments, the two or more gene sequences are sequences encoding multiple copies of one or more different genes. In some embodiments, the genetically engineered bacteria comprise one or more gene cassette(s) for producing a non-native anti-inflammation and/or gut barrier function enhancer molecule. For example, the genetically engineered bacteria may comprise two or more gene cassette(s) for producing a non-native anti-inflammation and/or gut barrier function enhancer molecule. In some embodiments, the two or more gene cassettes are multiple copies of the same gene cassette. In some emodiments, the two or more gene cassettes are different gene cassettes for producing either the same or different anti-inflammation and/or gut barrier function enhancer molecule(s). In some emodiments, the two or more gene cassettes are gene cassettes for producing multiple copies of one

or more different anti-inflammation and/or gut barrier function enhancer molecule(s). In some embodiments, the anti-inflammation and/or gut barrier function enhancer molecule is selected from the group consisting of a short-chain fatty acid, butyrate, propionate, acetate, IL-2, IL-22, superoxide dismutase (SOD), GLP-2, GLP-1, IL-10, IL-27, TGF-β1, TGF-β2, N-acylphosphatidylethanolamines (NAPEs), elafin (also known as peptidase inhibitor 3 or SKALP), trefoil factor, melatonin, PGD_2, kynurenic acid, and kynurenine. A molecule may be primarily anti-inflammatory, e.g., IL-10, or primarily gut barrier function enhancing, e.g., GLP-2. Alternatively, a molecule may be both anti-inflammatory and gut barrier function enhancing.

[0135] In some embodiments, the genetically engineered bacteria of the invention express one or more anti-inflammation and/or gut barrier function enhancer molecule(s) that is encoded by a single gene, e.g., the molecule is elafin and encoded by the *PI3* gene, or the molecule is interleukin-10 and encoded by the *IL10* gene. In alternate embodiments, the genetically engineered bacteria of the invention encode one or more an anti-inflammation and/or gut barrier function enhancer molecule(s), e.g., butyrate, that is synthesized by a biosynthetic pathway requiring multiple genes.

[0136] The one or more gene sequence(s) and/or gene cassette(s) may be expressed on a high-copy plasmid, a low-copy plasmid, or a chromosome. In some embodiments, expression from the plasmid may be useful for increasing expression of the anti-inflammation and/or gut barrier function enhancer molecule(s). In some embodiments, expression from the chromosome may be useful for increasing stability of expression of the anti-inflammation and/or gut barrier function enhancer molecule(s). In some embodiments, the gene Sequence(s)or gene cassette(s) for producing the anti-inflammation and/or gut barrier function enhancer molecule(s) is integrated into the bacterial chromosome at one or more integration sites in the genetically engineered bacteria. For example, one or more copies of the butyrate biosynthesis gene cassette may be integrated into the bacterial chromosome. In some embodiments, the gene sequence(s) or gene cassette(s) for producing the anti-inflammation and/or gut barrier function enhancer molecule(s) is expressed from a plasmid in the genetically engineered bacteria. In some embodiments, the gene sequence(s) or gene cassette(s) for producing

the anti-inflammation and/or gut barrier function enhancer molecule(s) is inserted into the bacterial genome at one or more of the following insertion sites in *E. coli* Nissle: *malE/K, araC/BAD, lacZ, thyA, malP/T.* Any suitable insertion site may be used (*see, e.g.,* **Fig. 51** for exemplary insertion sites). The insertion site may be anywhere in the genome, e.g., in a gene required for survival and/or growth, such as *thyA* (to create an auxotroph); in an active area of the genome, such as near the site of genome replication; and/or in between divergent promoters in order to reduce the risk of unintended transcription, such as between AraB and AraC of the arabinose operon.

[0137] In some embodiments, the genetically engineered bacteria of the invention comprise one or more butyrogenic gene cassette(s) and are capable of producing butyrate. The genetically engineered bacteria may include any suitable set of butyrogenic genes (*see, e.g.,* **Table 4**). Unmodified bacteria comprising butyrate biosynthesis genes are known and include, but are not limited to, *Peptoclostridium, Clostridium, Fusobacterium, Butyrivibrio, Eubacterium,* and *Treponema*, and these endogenous butyrate biosynthesis pathways may be a source of genes for the genetically engineered bacteria of the invention. In some embodiments, the genetically engineered bacteria of the invention comprise butyrate biosynthesis genes from a different species, strain, or substrain of bacteria. In some embodiments, the genetically engineered bacteria comprise the eight genes of the butyrate biosynthesis pathway from *Peptoclostridium difficile,* e.g., *Peptoclostridium difficile* strain 630: *bcd2, etfB3, etfA3, thiA1, hbd, crt2, pbt,* and *buk* (Aboulnaga et al., 2013), and are capable of producing butyrate under inducing conditions. *Peptoclostridium difficile* strain 630 and strain 1296 are both capable of producing butyrate, but comprise different nucleic acid sequences for *etfA3, thiA1, hbd, crt2, pbt,* and *buk*. In some embodiments, the genetically engineered bacteria comprise a combination of butyrogenic genes from different species, strains, and/or substrains of bacteria, and are capable of producing butyrate under inducing conditions. For example, in some embodiments, the genetically engineered bacteria comprise *bcd2, etfB3, etfA3,* and *thiA1* from *Peptoclostridium difficile* strain 630, and *hbd, crt2, pbt,* and *buk* from *Peptoclostridium difficile* strain 1296.

[0138] The gene products of the *bcd2, etfA3,* and *etfB3* genes in *Clostridium difficile* form a complex that converts crotonyl-CoA to butyryl-CoA, which may function as an oxygen-dependent co-oxidant. In some embodiments, because the genetically engineered bacteria of the invention are designed to produce butyrate in a microaerobic or oxygen-limited environment, e.g., the mammalian gut, oxygen dependence could have a negative effect on butyrate production in the gut. It has been shown that a single gene from *Treponema denticola* (*ter,* encoding *trans*-2-enoynl-CoA reductase) can functionally replace this three-gene complex in an oxygen-independent manner. In some embodiments, the genetically engineered bacteria comprise a *ter* gene, e.g., from *Treponema denticola,* which can functionally replace all three of the *bcd2, etfB3,* and *etfA3* genes, e.g., from *Peptoclostridium difficile*. In this embodiment, the genetically engineered bacteria comprise *thiA1, hbd, crt2, pbt,* and *buk*, e.g., from *Peptoclostridium difficile,* and *ter*, e.g., from *Treponema denticola,* and are capable of producing butyrate in low-oxygen conditions (*see, e.g.*, Table 4). In some embodiments, the genetically engineered bacteria comprise genes for aerobic butyrate biosynthesis and/or genes for anaerobic or microaerobic butyrate biosynthesis. In some embodiments, the genetically engineered bacteria of the invention comprise *thiA1, hbd, crt2, pbt,* and *buk*, e.g., from *Peptoclostridium difficile; ter*, e.g., from *Treponema denticola;* one or more of *bcd2, etfB3,* and *etfA3*, e.g., from *Peptoclostridium difficile;* and produce butyrate under inducing conditions. Alternatively, the *tesB* gene from *Escherichia coli* is capable of functionally replacing *pbt* and *buk* genes from *Peptoclostridium difficile*. Thus, in some embodiments, a butyrogenic gene cassette may comprise *thiA1*, *hbd and crt2* from *Peptoclostridium difficile*, *ter* from *Treponema denticola* and *tesB from E. coli.* In some embodiments, one or more of the butyrate biosynthesis genes may be functionally replaced or modified, e.g., codon optimized. In some embodiments, the butyrogenic gene cassette comprises genes for the aerobic biosynthesis of butyrate and/or genes for the anaerobic or microaerobic biosynthesis of butyrate. In some embodiments, one or more of the butyrate biosynthesis genes is functionally replaced, modified, and/or mutated in order to enhance stability and/or increase butyrate production in low-oxygen conditions. In some embodiments, the local production of butyrate induces the

differentiation of regulatory T cells in the gut and/or promotes the barrier function of colonic epithelial cells. Exemplary butyrate gene cassettes are shown in Figs. 1, 3, 4, 5, 6, 7, and 8.

[0139] In some embodiments, the genetically engineered bacteria are capable of expressing the butyrate biosynthesis cassette and producing butyrate under inducing conditions. The genes may be codon-optimized, and translational and transcriptional elements may be added. **Table 4** depicts the nucleic acid sequences of exemplary genes in the butyrate biosynthesis gene cassette.

[0140] In some embodiments, the genetically engineered bacteria comprise the nucleic acid sequence of any one of SEQ ID NOs: 1-10 or a functional fragment thereof. In some embodiments, the genetically engineered bacteria comprise a nucleic acid sequence that, but for the redundancy of the genetic code, encodes the same polypeptide as any one of SEQ ID NOs: 1-10 or a functional fragment thereof. In some embodiments, the genetically engineered bacteria comprise a nucleic acid sequence that encodes a polypeptide of any one of SEQ ID NOs: 11-20 or a functional fragment thereof. In some embodiments, genetically engineered bacteria comprise a nucleic acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homologous to the DNA sequence of any one of SEQ ID NOs: 1-10 or a functional fragment thereof. In some embodiments, genetically engineered bacteria comprise a nucleic acid that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homologous to the nucleic acid sequence that encodes a polypeptide of any one of SEQ ID NOs: 11-20 or a functional fragment thereof.

Table 4

Gene sequence	0123456789012345678901234567890123456789012345678901234567890123456789
bcd2 (SEQ ID NO: 1)	ATGGATTTAAATTCTAAAAAATATCAGATGCTTAAAGAGCTATATGTAAG CTTCGCTGAAAATGAAGTTAAACCTTTAGCAACAGAACTTGATGAAGAAG AAAGATTTCCTTATGAAACAGTGGAAAAAATGGCAAAAGCAGGAATGATG GGTATACCATATCCAAAAGAATATGGTGGAGAAGGTGGAGACACTGTAGG ATATATAATGGCAGTTGAAGAATTGTCTAGAGTTTGTGGTACTACAGGAG TTATATTATCAGCTCATACATCTCTTGGCTCATGGCCTATATATCAATAT GGTAATGAAGAACAAAAACAAAAATTCTTAAGACCACTAGCAAGTGGAGA AAAATTAGGAGCATTTGGTCTTACTGAGCCTAATGCTGGTACAGATGCGT CTGGCCAACAAACAACTGCTGTTTTAGACGGGGATGAATACATACTTAAT GGCTCAAAAATATTTATAACAAACGCAATAGCTGGTGACATATATGTAGT

Gene sequence	0123456789012345678901234567890123456789012345678
	AATGGCAATGACTGATAAATCTAAGGGGAACAAAGGAATATCAGCATTTA TAGTTGAAAAAGGAACTCCTGGGTTTAGCTTTGGAGTTAAAGAAAAGAAA ATGGGTATAAGAGGTTCAGCTACGAGTGAATTAATATTTGAGGATTGCAG AATACCTAAAGAAAATTTACTTGGAAAAGAAGGTCAAGGATTTAAGATAG CAATGTCTACTCTTGATGGTGGTAGAATTGGTATAGCTGCACAAGCTTTA GGTTTAGCACAAGGTGCTCTTGATGAAACTGTTAAATATGTAAAAGAAAG AGTACAATTTGGTAGACCATTATCAAAATTCCAAAATACACAATTCCAAT TAGCTGATATGGAAGTTAAGGTACAAGCGGCTAGACACCTTGTATATCAA GCAGCTATAAATAAAGACTTAGGAAAACCTTATGGAGTAGAAGCAGCAAT GGCAAAATTATTTGCAGCTGAAACAGCTATGGAAGTTACTACAAAAGCTG TACAACTTCATGGAGGATATGGATACACTCGTGACTATCCAGTAGAAAGA ATGATGAGAGATGCTAAGATAACTGAAATATATGAAGGAACTAGTGAAGT TCAAAGAATGGTTATTTCAGGAAAACTATTAAAATAG
etfB3 (SEQ ID NO: 2)	ATGAATATAGTCGTTTGTATAAAACAAGTTCCAGATACAACAGAAGTTAA ACTAGATCCTAATACAGGTACTTTAATTAGAGATGGAGTACCAAGTATAA TAAACCCTGATGATAAAGCAGGTTTAGAAGAAGCTATAAAATTAAAAGAA GAAATGGGTGCTCATGTAACTGTTATAACAATGGGACCTCCTCAAGCAGA TATGGCTTTAAAAGAAGCTTTAGCAATGGGTGCAGATAGAGGTATATTAT TAACAGATAGAGCATTTGCGGGTGCTGATACTTGGGCAACTTCATCAGCA TTAGCAGGAGCATTAAAAAATATAGATTTTGATATTATAATAGCTGGAAG ACAGGCGATAGATGGAGATACTGCACAAGTTGGACCTCAAATAGCTGAAC ATTTAAATCTTCCATCAATAACATATGCTGAAGAAATAAAAACTGAAGGT GAATATGTATTAGTAAAAAGACAATTTGAAGATTGTTGCCATGACTTAAA AGTTAAAATGCCATGCCTTATAACAACTCTTAAAGATATGAACACACCAA GATACATGAAAGTTGGAAGAATATATGATGCTTTCGAAAATGATGTAGTA GAAACATGGACTGTAAAAGATATAGAAGTTGACCCTTCTAATTTAGGTCT TAAAGGTTCTCCAACTAGTGTATTTAAATCATTTACAAAATCAGTTAAAC CAGCTGGTACAATATACAATGAAGATGCGAAAACATCAGCTGGAATTATC ATAGATAAATTAAAAGAGAAGTATATCATATAA
etfA3 (SEQ ID NO: 3)	ATGGGTAACGTTTTAGTAGTAATAGAACAAAGAGAAAATGTAATTCAAAC TGTTTCTTTAGAATTACTAGGAAAGGCTACAGAAATAGCAAAAGATTATG ATACAAAAGTTTCTGCATTACTTTTAGGTAGTAAGGTAGAAGGTTTAATA GATACATTAGCACACTATGGTGCAGATGAGGTAATAGTAGTAGATGATGA AGCTTTAGCAGTGTATACAACTGAACCATATACAAAAGCAGCTTATGAAG CAATAAAAGCAGCTGACCCTATAGTTGTATTATTTGGTGCAACTTCAATA GGTAGAGATTTAGCGCCTAGAGTTTCTGCTAGAATACATACAGGTCTTAC TGCTGACTGTACAGGTCTTGCAGTAGCTGAAGATACAAAATTATTATTAA TGACAAGACCTGCCTTTGGTGGAAATATAATGGCAACAATAGTTTGTAAA GATTTCAGACCTCAAATGTCTACAGTTAGACCAGGGGTTATGAAGAAAAA TGAACCTGATGAAACTAAAGAAGCTGTAATTAACCGTTTCAAGGTAGAAT TTAATGATGCTGATAAATTAGTTCAAGTTGTACAAGTAATAAAAGAAGCT AAAAAACAAGTTAAAATAGAAGATGCTAAGATATTAGTTTCTGCTGGACG TGGAATGGGTGGAAAAGAAAACTTAGACATACTTTATGAATTAGCTGAAA TTATAGGTGGAGAAGTTTCTGGTTCTCGTGCCACTATAGATGCAGGTTGG TTAGATAAAGCAAGACAAGTTGGTCAAACTGGTAAAACTGTAAGACCAGA CCTTTATATAGCATGTGGTATATCTGGAGCAATACAACATATAGCTGGTA TGGAAGATGCTGAGTTTATAGTTGCTATAAATAAAAATCCAGAAGCTCCA

Gene sequence	0123456789012345678901234567890123456789012345678
	ATATTTAAATATGCTGATGTTGGTATAGTTGGAGATGTTCATAAAGTGCT TCCAGAACTTATCAGTCAGTTAAGTGTTGCAAAAGAAAAAGGTGAAGTTT TAGCTAACTAA
thiA1 (SEQ ID NO: 4)	ATGAGAGAAGTAGTAATTGCCAGTGCAGCTAGAACAGCAGTAGGAAGTTT TGGAGGAGCATTTAAATCAGTTTCAGCGGTAGAGTTAGGGGTAACAGCAG CTAAAGAAGCTATAAAAAGAGCTAACATAACTCCAGATATGATAGATGAA TCTCTTTTAGGGGGAGTACTTACAGCAGGTCTTGGACAAAATATAGCAAG ACAAATAGCATTAGGAGCAGGAATACCAGTAGAAAAACCAGCTATGACTA TAAATATAGTTTGTGGTTCTGGATTAAGATCTGTTTCAATGGCATCTCAA CTTATAGCATTAGGTGATGCTGATATAATGTTAGTTGGTGGAGCTGAAAA CATGAGTATGTCTCCTTATTTAGTACCAAGTGCGAGATATGGTGCAAGAA TGGGTGATGCTGCTTTTGTTGATTCAATGATAAAAGATGGATTATCAGAC ATATTTAATAACTATCACATGGGTATTACTGCTGAAAACATAGCAGAGCA ATGGAATATAACTAGAGAAGAACAAGATGAATTAGCTCTTGCAAGTCAAA ATAAAGCTGAAAAAGCTCAAGCTGAAGGAAAATTTGATGAAGAAATAGTT CCTGTTGTTATAAAAGGAAGAAAAGGTGACACTGTAGTAGATAAAGATGA ATATATTAAGCCTGGCACTACAATGGAGAAACTTGCTAAGTTAAGACCTG CATTTAAAAAAGATGGAACAGTTACTGCTGGTAATGCATCAGGAATAAAT GATGGTGCTGCTATGTTAGTAGTAATGGCTAAAGAAAAAGCTGAAGAACT AGGAATAGAGCCTCTTGCAACTATAGTTTCTTATGGAACAGCTGGTGTTG ACCCTAAAATAATGGGATATGGACCAGTTCCAGCAACTAAAAAAGCTTTA GAAGCTGCTAATATGACTATTGAAGATATAGATTTAGTTGAAGCTAATGA GGCATTTGCTGCCCAATCTGTAGCTGTAATAAGAGACTTAAATATAGATA TGAATAAAGTTAATGTTAATGGTGGAGCAATAGCTATAGGACATCCAATA GGATGCTCAGGAGCAAGAATACTTACTACACTTTTATATGAAATGAAGAG AAGAGATGCTAAAACTGGTCTTGCTACACTTTGTATAGGCGGTGGAATGG GAACTACTTTAATAGTTAAGAGATAG
hbd (SEQ ID NO: 5)	ATGAAATTAGCTGTAATAGGTAGTGGAACTATGGGAAGTGGTATTGTACA AACTTTTGCAAGTTGTGGACATGATGTATGTTTAAAGAGTAGAACTCAAG GTGCTATAGATAAATGTTTAGCTTTATTAGATAAAAATTTAACTAAGTTA GTTACTAAGGGAAAAATGGATGAAGCTACAAAAGCAGAAATATTAAGTCA TGTTAGTTCAACTACTAATTATGAAGATTTAAAAGATATGGATTTAATAA TAGAAGCATCTGTAGAAGACATGAATATAAAGAAAGATGTTTTCAAGTTA CTAGATGAATTATGTAAAGAAGATACTATCTTGGCAACAAATACTTCATC ATTATCTATAACAGAAATAGCTTCTTCTACTAAGCGCCCAGATAAAGTTA TAGGAATGCATTTCTTTAATCCAGTTCCTATGATGAAATTAGTTGAAGTT ATAAGTGGTCAGTTAACATCAAAAGTTACTTTTGATACAGTATTTGAATT ATCTAAGAGTATCAATAAAGTACCAGTAGATGTATCTGAATCTCCTGGAT TTGTAGTAAATAGAATACTTATACCTATGATAAATGAAGCTGTTGGTATA TATGCAGATGGTGTTGCAAGTAAAGAAGAAATAGATGAAGCTATGAAATT AGGAGCAAACCATCCAATGGGACCACTAGCATTAGGTGATTTAATCGGAT TAGATGTTGTTTTAGCTATAATGAACGTTTTATATACTGAATTTGGAGAT

Gene sequence	0123456789012345678901234567890123456789012345678
	ACTAAATATAGACCTCATCCACTTTTAGCTAAAATGGTTAGAGCTAATCA ATTAGGAAGAAAAACTAAGATAGGATTCTATGATTATAATAAATAA
crt2 (SEQ ID NO: 6)	ATGAGTACAAGTGATGTTAAAGTTTATGAGAATGTAGCTGTTGAAGTAGA TGGAAATATATGTACAGTGAAAATGAATAGACCTAAAGCCCTTAATGCAA TAAATTCAAAGACTTTAGAAGAACTTTATGAAGTATTTGTAGATATTAAT AATGATGAAACTATTGATGTTGTAATATTGACAGGGGAAGGAAAGGCATT TGTAGCTGGAGCAGATATTGCATACATGAAAGATTTAGATGCTGTAGCTG CTAAAGATTTTAGTATCTTAGGAGCAAAAGCTTTTGGAGAAATAGAAAAT AGTAAAAAAGTAGTGATAGCTGCTGTAAACGGATTTGCTTTAGGTGGAGG ATGTGAACTTGCAATGGCATGTGATATAAGAATTGCATCTGCTAAAGCTA AATTTGGTCAGCCAGAAGTAACTCTTGGAATAACTCCAGGATATGGAGGA ACTCAAAGGCTTACAAGATTGGTTGGAATGGCAAAAGCAAAAGAATTAAT CTTTACAGGTCAAGTTATAAAAGCTGATGAAGCTGAAAAAATAGGGCTAG TAAATAGAGTCGTTGAGCCAGACATTTTAATAGAAGAAGTTGAGAAATTA GCTAAGATAATAGCTAAAAATGCTCAGCTTGCAGTTAGATACTCTAAAGA AGCAATACAACTTGGTGCTCAAACTGATATAAATACTGGAATAGATATAG AATCTAATTTATTTGGTCTTTGTTTTTCAACTAAAGACCAAAAAGAAGGA ATGTCAGCTTTCGTTGAAAAGAGAGAAGCTAACTTTATAAAAGGGTAA
pbt (SEQ ID NO: 7)	ATGAGAAGTTTTGAAGAAGTAATTAAGTTTGCAAAAGAAAGAGGACCTAA AACTATATCAGTAGCATGTTGCCAAGATAAAGAAGTTTTAATGGCAGTTG AAATGGCTAGAAAAGAAAAAATAGCAAATGCCATTTTAGTAGGAGATATA GAAAAGACTAAAGAAATTGCAAAAAGCATAGACATGGATATCGAAAATTA TGAACTGATAGATATAAAAGATTTAGCAGAAGCATCTCTAAAATCTGTTG AATTAGTTTCACAAGGAAAAGCCGACATGGTAATGAAAGGCTTAGTAGAC ACATCAATAATACTAAAAGCAGTTTTAAATAAAGAAGTAGGTCTTAGAAC TGGAAATGTATTAAGTCACGTAGCAGTATTTGATGTAGAGGGATATGATA GATTATTTTTCGTAACTGACGCAGCTATGAACTTAGCTCCTGATACAAAT ACTAAAAAGCAAATCATAGAAAATGCTTGCACAGTAGCACATTCATTAGA TATAAGTGAACCAAAAGTTGCTGCAATATGCGCAAAAGAAAAAGTAAATC CAAAAATGAAAGATACAGTTGAAGCTAAAGAACTAGAAGAAATGTATGAA AGAGGAGAAATCAAAGGTTGTATGGTTGGTGGGCCTTTTGCAATTGATAA TGCAGTATCTTTAGAAGCAGCTAAACATAAAGGTATAAATCATCCTGTAG CAGGACGAGCTGATATATTATTAGCCCCAGATATTGAAGGTGGTAACATA TTATATAAAGCTTTGGTATTCTTCTCAAAATCAAAAAATGCAGGAGTTAT AGTTGGGGCTAAAGCACCAATAATATTAACTTCTAGAGCAGACAGTGAAG AAACTAAACTAAACTCAATAGCTTTAGGTGTTTTAATGGCAGCAAAGGCA TAA
buk (SEQ ID NO: 8)	ATGAGCAAAATATTTAAAATCTTAACAATAAATCCTGGTTCGACATCAAC TAAAATAGCTGTATTTGATAATGAGGATTTAGTATTTGAAAAAACTTTAA GACATTCTTCAGAAGAAATAGGAAAATATGAGAAGGTGTCTGACCAATTT GAATTTCGTAAACAAGTAATAGAAGAAGCTCTAAAAGAAGGTGGAGTAAA AACATCTGAATTAGATGCTGTAGTAGGTAGAGGAGGACTTCTTAAACCTA TAAAAGGTGGTACTTATTCAGTAAGTGCTGCTATGATTGAAGATTTAAAA GTGGGAGTTTTAGGAGAACACGCTTCAAACCTAGGTGGAATAATAGCAAA

Gene sequence	0123456789012345678901234567890123456789012345678
	ACAAATAGGTGAAGAAGTAAATGTTCCTTCATACATAGTAGACCCTGTTG TTGTAGATGAATTAGAAGATGTTGCTAGAATTTCTGGTATGCCTGAAATA AGTAGAGCAAGTGTAGTACATGCTTTAAATCAAAAGGCAATAGCAAGAAG ATATGCTAGAGAAATAAACAAGAAATATGAAGATATAAATCTTATAGTTG CACACATGGGTGGAGGAGTTTCTGTTGGAGCTCATAAAAATGGTAAAATA GTAGATGTTGCAAACGCATTAGATGGAGAAGGACCTTTCTCTCCAGAAAG AAGTGGTGGACTACCAGTAGGTGCATTAGTAAAAATGTGCTTTAGTGGAA AATATACTCAAGATGAAATTAAAAAGAAAATAAAAGGTAATGGCGGACTA GTTGCATACTTAAACACTAATGATGCTAGAGAAGTTGAAGAAAGAATTGA AGCTGGTGATGAAAAAGCTAAATTAGTATATGAAGCTATGGCATATCAAA TCTCTAAAGAAATAGGAGCTAGTGCTGCAGTTCTTAAGGGAGATGTAAAA GCAATATTATTAACTGGTGGAATCGCATATTCAAAAATGTTTACAGAAAT GATTGCAGATAGAGTTAAATTTATAGCAGATGTAAAAGTTTATCCAGGTG AAGATGAAATGATTGCATTAGCTCAAGGTGGACTTAGAGTTTTAACTGGT GAAGAAGAGGCTCAAGTTTATGATAACTAA
ter (SEQ ID NO: 9)	ATGATCGTAAAACCTATGGTACGCAACAATATCTGCCTGAACGCCCATCC TCAGGGCTGCAAGAAGGGAGTGGAAGATCAGATTGAATATACCAAGAAAC GCATTACCGCAGAAGTCAAAGCTGGCGCAAAAGCTCCAAAAAACGTTCTG GTGCTTGGCTGCTCAAATGGTTACGGCCTGGCGAGCCGCATTACTGCTGC GTTCGGATACGGGGCTGCGACCATCGGCGTGTCCTTTGAAAAAGCGGGTT CAGAAACCAAATATGGTACACCGGGATGGTACAATAATTTGGCATTTGAT GAAGCGGCAAAACGCGAGGGTCTTTATAGCGTGACGATCGACGGCGATGC GTTTTCAGACGAGATCAAGGCCCAGGTAATTGAGGAAGCCAAAAAAAAAG GTATCAAATTTGATCTGATCGTATACAGCTTGGCCAGCCCAGTACGTACT GATCCTGATACAGGTATCATGCACAAAAGCGTTTTGAAACCCTTTGGAAA AACGTTCACAGGCAAAACAGTAGATCCGTTTACTGGCGAGCTGAAGGAAA TCTCCGCGGAACCAGCAAATGACGAGGAAGCAGCCGCCACTGTTAAAGTT ATGGGGGGTGAAGATTGGGAACGTTGGATTAAGCAGCTGTCGAAGGAAGG CCTCTTAGAAGAAGGCTGTATTACCTTGGCCTATAGTTATATTGGCCCTG AAGCTACCCAAGCTTTGTACCGTAAAGGCACAATCGGCAAGGCCAAAGAA CACCTGGAGGCCACAGCACACCGTCTCAACAAAGAGAACCCGTCAATCCG TGCCTTCGTGAGCGTGAATAAAGGCCTGGTAACCCGCGCAAGCGCCGTAA TCCCGGTAATCCCTCTGTATCTCGCCAGCTTGTTCAAAGTAATGAAAGAG AAGGGCAATCATGAAGGTTGTATTGAACAGATCACGCGTCTGTACGCCGA GCGCCTGTACCGTAAAGATGGTACAATTCCAGTTGATGAGGAAAATCGCA TTCGCATTGATGATTGGGAGTTAGAAGAAGACGTCCAGAAAGCGGTATCC GCGTTGATGGAGAAAGTCACGGGTGAAAACGCAGAATCTCTCACTGACTT AGCGGGGTACCGCCATGATTTCTTAGCTAGTAACGGCTTTGATGTAGAAG GTATTAATTATGAAGCGGAAGTTGAACGCTTCGACCGTATCTGA
tesB (SEQ ID NO:10)	ATGAGTCAGGCGCTAAAAAATTTACTGACATTGTTAAATCTGGAAAAAAT TGAGGAAGGACTCTTTCGCGGCCAGAGTGAAGATTTAGGTTTACGCCAGG TGTTTGGCGGCCAGGTCGTGGGTCAGGCCTTGTATGCTGCAAAAGAGACC GTCCCTGAAGAGCGGCTGGTACATTCGTTTCACAGCTACTTTCTTCGCCC TGGCGATAGTAAGAAGCCGATTATTTATGATGTCGAAACGCTGCGTGACG GTAACAGCTTCAGCGCCCGCCGGGTTGCTGCTATTCAAAACGGCAAACCG ATTTTTTATATGACTGCCTCTTTCCAGGCACCAGAAGCGGGTTTCGAACA TCAAAAAACAATGCCGTCCGCGCCAGCGCCTGATGGCCTCCCTTCGGAAA

Gene sequence	01234567890123456789012345678901234567890123456789
	CGCAAATCGCCCAATCGCTGGCGCACCTGCTGCCGCCAGTGCTGAAAGAT AAATTCATCTGCGATCGTCCGCTGGAAGTCCGTCCGGTGGAGTTTCATAA CCCACTGAAAGGTCACGTCGCAGAACCACATCGTCAGGTGTGGATCCGCG CAAATGGTAGCGTGCCGGATGACCTGCGCGTTCATCAGTATCTGCTCGGT TACGCTTCTGATCTTAACTTCCTGCCGGTAGCTCTACAGCCGCACGGCAT CGGTTTTCTCGAACCGGGGATTCAGATTGCCACCATTGACCATTCCATGT GGTTCCATCGCCCGTTTAATTTGAATGAATGGCTGCTGTATAGCGTGGAG AGCACCTCGGCGTCCAGCGCACGTGGCTTTGTGCGCGGTGAGTTTTATAC CCAAGACGGCGTACTGGTTGCCTCGACCGTTCAGGAAGGGGTGATGCGTA ATCACAATTAA

Table 5

Amino acid sequence	01234567890123456789012345678901234567890123456789
bcd2 (SEQ ID NO: 11)	MDLNSKKYQMLKELYVSFAENEVKPLATELDEEERFPYETVEKMAKAGMM GIPYPKEYGGEGGDTVGYIMAVEELSRVCGTTGVILSAHTSLGSWPIYQY GNEEQKQKFLRPLASGEKLGAFGLTEPNAGTDASGQQTTAVLDGDEYILN GSKIFITNAIAGDIYVVMAMTDKSKGNKGISAFIVEKGTPGFSFGVKEKK MGIRGSATSELIFEDCRIPKENLLGKEGQGFKIAMSTLDGGRIGIAAQAL GLAQGALDETVKYVKERVQFGRPLSKFQNTQFQLADMEVKVQAARHLVYQ AAINKDLGKPYGVEAAMAKLFAAETAMEVTTKAVQLHGGYGYTRDYPVER MMRDAKITEIYEGTSEVQRMVISGKLLK
etfB3 (SEQ ID NO: 12)	MNIVVCIKQVPDTTEVKLDPNTGTLIRDGVPSIINPDDKAGLEEAIKLKE EMGAHVTVITMGPPQADMALKEALAMGADRGILLTDRAFAGADTWATSSA LAGALKNIDFDIIIAGRQAIDGDTAQVGPQIAEHLNLPSITYAEEIKTEG EYVLVKRQFEDCCHDLKVKMPCLITTLKDMNTPRYMKVGRIYDAFENDVV ETWTVKDIEVDPSNLGLKGSPTSVFKSFTKSVKPAGTIYNEDAKTSAGII IDKLKEKYII
etfA3 (SEQ ID NO: 13)	MGNVLVVIEQRENVIQTVSLELLGKATEIAKDYDTKVSALLLGSKVEGLI DTLAHYGADEVIVVDDEALAVYTTEPYTKAAYEAIKAADPIVVLFGATSI GRDLAPRVSARIHTGLTADCTGLAVAEDTKLLLMTRPAFGGNIMATIVCK DFRPQMSTVRPGVMKKNEPDETKEAVINRFKVEFNDADKLVQVVQVIKEA KKQVKIEDAKILVSAGRGMGGKENLDILYELAEIIGGEVSGSRATIDAGW LDKARQVGQTGKTVRPDLYIACGISGAIQHIAGMEDAEFIVAINKNPEAP IFKYADVGIVGDVHKVLPELISQLSVAKEKGEVLAN
thiA1 (SEQ ID NO: 14)	MREVVIASAARTAVGSFGGAFKSVSAVELGVTAAKEAIKRANITPDMIDE SLLGGVLTAGLGQNIARQIALGAGIPVEKPAMTINIVCGSGLRSVSMASQ LIALGDADIMLVGGAENMSMSPYLVPSARYGARMGDAAFVDSMIKDGLSD IFNNYHMGITAENIAEQWNITREEQDELALASQNKAEKAQAEGKFDEEIV PVVIKGRKGDTVVDKDEYIKPGTTMEKLAKLRPAFKKDGTVTAGNASGIN DGAAMLVVMAKEKAEELGIEPLATIVSYGTAGVDPKIMGYGPVPATKKAL

Amino acid sequence	0123456789012345678901234567890123456789012345678
	EAANMTIEDIDLVEANEAFAAQSVAVIRDLNIDMNKVNVNGGAIAIGHPI GCSGARILTTLLYEMKRRDAKTGLATLCIGGGMGTTLIVKR
hbd (SEQ ID NO: 15)	MKLAVIGSGTMGSGIVQTFASCGHDVCLKSRTQGAIDKCLALLDKNLTKL VTKGKMDEATKAEILSHVSSTTNYEDLKDMDLIIEASVEDMNIKKDVFKL LDELCKEDTILATNTSSLSITEIASSTKRPDKVIGMHFFNPVPMMKLVEV ISGQLTSKVTFDTVFELSKSINKVPVDVSESPGFVVNRILIPMINEAVGI YADGVASKEEIDEAMKLGANHPMGPLALGDLIGLDVVLAIMNVLYTEFGD TKYRPHPLLAKMVRANQLGRKTKIGFYDYNK
crt2 (SEQ ID NO: 16)	MSTSDVKVYENVAVEVDGNICTVKMNRPKALNAINSKTLEELYEVFVDIN NDETIDVVILTGEGKAFVAGADIAYMKDLDAVAAKDFSILGAKAFGEIEN SKKVVIAAVNGFALGGGCELAMACDIRIASAKAKFGQPEVTLGITPGYGG TQRLTRLVGMAKAKELIFTGQVIKADEAEKIGLVNRVVEPDILIEEVEKL AKIIAKNAQLAVRYSKEAIQLGAQTDINTGIDIESNLFGLCFSTKDQKEG MSAFVEKREANFIKG
pbt (SEQ ID NO: 17)	MRSFEEVIKFAKERGPKTISVACCQDKEVLMAVEMARKEKIANAILVGDI EKTKEIAKSIDMDIENYELIDIKDLAEASLKSVELVSQGKADMVMKGLVD TSIILKAVLNKEVGLRTGNVLSHVAVFDVEGYDRLFFVTDAAMNLAPDTN TKKQIIENACTVAHSLDISEPKVAAICAKEKVNPKMKDTVEAKELEEMYE RGEIKGCMVGGPFAIDNAVSLEAAKHKGINHPVAGRADILLAPDIEGGNI LYKALVFFSKSKNAGVIVGAKAPIILTSRADSEETKLNSIALGVLMAAKA
buk (SEQ ID NO: 18)	MSKIFKILTINPGSTSTKIAVFDNEDLVFEKTLRHSSEEIGKYEKVSDQF EFRKQVIEEALKEGGVKTSELDAVVGRGGLLKPIKGGTYSVSAAMIEDLK VGVLGEHASNLGGIIAKQIGEEVNVPSYIVDPVVVDELEDVARISGMPEI SRASVVHALNQKAIARRYAREINKKYEDINLIVAHMGGGVSVGAHKNGKI VDVANALDGEGPFSPERSGGLPVGALVKMCFSGKYTQDEIKKKIKGNGGL VAYLNTNDAREVEERIEAGDEKAKLVYEAMAYQISKEIGASAAVLKGDVK AILLTGGIAYSKMFTEMIADRVKFIADVKVYPGEDEMIALAQGGLRVLTG EEEAQVYDN
ter (SEQ ID NO: 19)	MIVKPMVRNNICLNAHPQGCKKGVEDQIEYTKKRITAEVKAGAKAPKNVL VLGCSNGYGLASRITAAFGYGAATIGVSFEKAGSETKYGTPGWYNNLAFD EAAKREGLYSVTIDGDAFSDEIKAQVIEEAKKKGIKFDLIVYSLASPVRT DPDTGIMHKSVLKPFGKTFTGKTVDPFTGELKEISAEPANDEEAAATVKV MGGEDWERWIKQLSKEGLLEEGCITLAYSYIGPEATQALYRKGTIGKAKE HLEATAHRLNKENPSIRAFVSVNKGLVTRASAVIPVIPLYLASLFKVMKE KGNHEGCIEQITRLYAERLYRKDGTIPVDEENRIRIDDWELEEDVQKAVS ALMEKVTGENAESLTDLAGYRHDFLASNGFDVEGINYEAEVERFDRI
tesB (SEQ ID NO:20)	MSQALKNLLTLLNLEKIEEGLFRGQSEDLGLRQVFGGQVVGQALYAAKET VPEERLVHSFHSYFLRPGDSKKPIIYDVETLRDGNSFSARRVAAIQNGKP IFYMTASFQAPEAGFEHQKTMPSAPAPDGLPSETQIAQSLAHLLPPVLKD KFICDRPLEVRPVEFHNPLKGHVAEPHRQVWIRANGSVPDDLRVHQYLLG

Amino acid sequence	01234567890123456789012345678901234567890123456789
	YASDLNFLPVALQPHGIGFLEPGIQIATIDHSMWFHRPFNLNEWLLYSVE STSASSARGFVRGEFYTQDGVLVASTVQEGVMRNHN

[0141] In some embodiments, the genetically engineered bacteria of the invention comprise a propionate gene cassette and are capable of producing propionate. The genetically engineered bacteria may express any suitable set of propionate biosynthesis genes (*see, e.g.,* **Table 6**). Unmodified bacteria that are capable of producing propionate via an endogenous propionate biosynthesis pathway include, but are not limited to, *Clostridium propionicum*, *Megasphaera elsdenii*, and *Prevotella ruminicola*, and these endogenous propionate biosynthesis pathways may be a source of genes for the genetically engineered bacteria of the invention. In some embodiments, the genetically engineered bacteria of the invention comprise propionate biosynthesis genes from a different species, strain, or substrain of bacteria. In some embodiments, the genetically engineered bacteria comprise the genes *pct, lcd,* and *acr* from *Clostridium propionicum.* In some embodiments, the genetically engineered bacteria comprise acrylate pathway genes for propionate biosynthesis, e.g., *pct*, *lcdA*, *lcdB*, *lcdC*, *etfA*, *acrB*, and *acrC*. In alternate embodiments, the genetically engineered bacteria comprise pyruvate pathway genes for propionate biosynthesis, e.g., $thrA^{fbr}$, *thrB*, *thrC*, $ilvA^{fbr}$, *aceE*, *aceF*, and *lpd*, and optionally further comprise *tesB.* The genes may be codon-optimized, and translational and transcriptional elements may be added. **Table 6** depicts the nucleic acid sequences of exemplary genes in the propionate biosynthesis gene cassette.

[0142] In some embodiments, the genetically engineered bacteria comprise the nucleic acid sequence of any one of SEQ ID NOs: 21-34 and 10 or a functional fragment thereof. In some embodiments, the genetically engineered bacteria comprise a nucleic acid sequence that encodes a polypeptide of any one of SEQ ID NOs: 35-48 and 20 or a functional fragment thereof. In some embodiments, genetically engineered bacteria comprise a nucleic acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homologous to the DNA sequence of any one of SEQ ID NOs: 21-34 and 10 or a functional fragment thereof. In some

embodiments, genetically engineered bacteria comprise a nucleic acid that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homologous to the nucleic acid sequence that encodes a polypeptide of any one of SEQ ID NOs: 35-48 and 20 or a functional fragment thereof.

Table 6

Gene sequence	0123456789012345678901234567890123456789012345678
pct SEQ ID NO: 21	ATGCGCAAAGTGCCGATTATCACGGCTGACGAGGCCGCAAAACTGATCAA GGACGGCGACACCGTGACAACTAGCGGCTTTGTGGGTAACGCGATCCCTG AGGCCCTTGACCGTGCAGTCGAAAAGCGTTTCCTGGAAACGGGCGAACCG AAGAACATTACTTATGTATATTGCGGCAGTCAGGGCAATCGCGACGGTCG TGGCGCAGAACATTTCGCGCATGAAGGCCTGCTGAAACGTTATATCGCTG GCCATTGGGCGACCGTCCCGGCGTTAGGGAAAATGGCCATGGAGAATAAA ATGGAGGCCTACAATGTCTCTCAGGGCGCCTTGTGTCATCTCTTTCGCGA TATTGCGAGCCATAAACCGGGTGTGTTCACGAAAGTAGGAATCGGCACCT TCATTGATCCACGTAACGGTGGTGGGAAGGTCAACGATATTACCAAGGAA GATATCGTAGAACTGGTGGAAATTAAAGGGCAGGAATACCTGTTTTATCC GGCGTTCCCGATCCATGTCGCGCTGATTCGTGGCACCTATGCGGACGAGA GTGGTAACATCACCTTTGAAAAAGAGGTAGCGCCTTTGGAAGGGACTTCT GTCTGTCAAGCGGTGAAGAACTCGGGTGGCATTGTCGTGGTTCAGGTTGA GCGTGTCGTCAAAGCAGGCACGCTGGATCCGCGCCATGTGAAAGTTCCGG GTATCTATGTAGATTACGTAGTCGTCGCGGATCCGGAGGACCATCAACAG TCCCTTGACTGCGAATATGATCCTGCCCTTAGTGGAGAGCACCGTCGTCC GGAGGTGGTGGGTGAACCACTGCCTTTATCCGCGAAGAAAGTCATCGGCC GCCGTGGCGCGATTGAGCTCGAGAAAGACGTTGCAGTGAACCTTGGGGTA GGTGCACCTGAGTATGTGGCCTCCGTGGCCGATGAAGAAGGCATTGTGGA TTTTATGACTCTCACAGCGGAGTCCGGCGCTATCGGTGGCGTTCCAGCCG GCGGTGTTCGCTTTGGGGCGAGCTACAATGCTGACGCCTTGATCGACCAG GGCTACCAATTTGATTATTACGACGGTGGGGGTCTGGATCTTTGTTACCT GGGTTTAGCTGAATGCGACGAAAAGGGTAATATCAATGTTAGCCGCTTCG GTCCTCGTATCGCTGGGTGCGGCGGATTCATTAACATTACCCAAAACACG CCGAAAGTCTTCTTTTGTGGGACCTTTACAGCCGGGGGGCTGAAAGTGAA AATTGAAGATGGTAAGGTGATTATCGTTCAGGAAGGGAAACAGAAGAAAT TCCTTAAGGCAGTGGAGCAAATCACCTTTAATGGAGACGTGGCCTTAGCG AACAAGCAACAAGTTACCTACATCACGGAGCGTTGCGTCTTCCTCCTCAA AGAAGACGGTTTACACCTTTCGGAAATCGCGCCAGGCATCGATCTGCAGA CCCAGATTTTGGATGTTATGGACTTTGCCCCGATCATTGATCGTGACGCA AACGGGCAGATTAAACTGATGGACGCGGCGTTATTCGCAGAAGGGCTGAT GGGCTTGAAAGAAATGAAGTCTTAA

Gene sequence	0123456789012345678901234567890123456789012345678
lcdA SEQ ID NO: 22	ATGAGCTTAACCCAAGGCATGAAAGCTAAACAACTGTTAGCATACTTTCA GGGTAAAGCCGATCAGGATGCACGTGAAGCGAAAGCCCGCGGTGAGCTGG TCTGCTGGTCGGCGTCAGTCGCGCCGCCGGAATTTTGCGTAACAATGGGC ATTGCCATGATCTACCCGGAGACTCATGCAGCGGGCATCGGTGCCCGCAA AGGTGCGATGGACATGCTGGAAGTTGCGGACCGCAAAGGCTACAACGTGG ATTGTTGTTCCTACGGCCGTGTAAATATGGGTTACATGGAATGTTTAAAA GAAGCCGCCATCACGGGCGTCAAGCCGGAAGTTTTGGTTAATTCCCCTGC TGCTGACGTTCCGCTTCCCGATTTGGTGATTACGTGTAATAATATCTGTA ACACGCTGCTGAAATGGTACGAAAACTTAGCAGCAGAACTCGATATTCCT TGCATCGTGATCGACGTACCGTTTAATCATACCATGCCGATTCCGGAATA TGCCAAGGCCTACATCGCGGACCAGTTCCGCAATGCAATTTCTCAGCTGG AAGTTATTTGTGGCCGTCCGTTCGATTGGAAGAAATTTAAGGAGGTCAAA GATCAGACCCAGCGTAGCGTATACCACTGGAACCGCATTGCCGAGATGGC GAAATACAAGCCTAGCCCGCTGAACGGCTTCGATCTGTTCAATTACATGG CGTTAATCGTGGCGTGCCGCAGCCTGGATTATGCAGAAATTACCTTTAAA GCGTTCGCGGACGAATTAGAAGAGAATTTGAAGGCGGGTATCTACGCCTT TAAAGGTGCGGAAAAAACGCGCTTTCAATGGGAAGGTATCGCGGTGTGGC CACATTTAGGTCACACGTTTAAATCTATGAAGAATCTGAATTCGATTATG ACCGGTACGGCATACCCCGCCCTTTGGGACCTGCACTATGACGCTAACGA CGAATCTATGCACTCTATGGCTGAAGCGTACACCCGTATTTATATTAATA CTTGTCTGCAGAACAAAGTAGAGGTCCTGCTTGGGATCATGGAAAAAGGC CAGGTGGATGGTACCGTATATCATCTGAATCGCAGCTGCAAACTGATGAG TTTCCTGAACGTGGAAACGGCTGAAATTATTAAAGAGAAGAACGGTCTTC CTTACGTCTCCATTGATGGCGATCAGACCGATCCTCGCGTTTTTTCTCCG GCCCAGTTTGATACCCGTGTTCAGGCCCTGGTTGAGATGATGGAGGCCAA TATGGCGGCAGCGGAATAA
lcdB SEQ ID NO: 23	ATGTCACGCGTGGAGGCAATCCTGTCGCAGCTGAAAGATGTCGCCGCGAA TCCGAAAAAAGCCATGGATGACTATAAAGCTGAAACAGGTAAGGGCGCGG TTGGTATCATGCCGATCTACAGCCCCGAAGAAATGGTACACGCCGCTGGC TATTTGCCGATGGGAATCTGGGGCGCCCAGGGCAAAACGATTAGTAAAGC GCGCACCTATCTGCCTGCTTTTGCCTGCAGCGTAATGCAGCAGGTTATGG AATTACAGTGCGAGGGCGCGTATGATGACCTGTCCGCAGTTATTTTTAGC GTACCGTGCGACACTCTCAAATGTCTTAGCCAGAAATGGAAAGGTACGTC CCCAGTGATTGTATTTACGCATCCGCAGAACCGCGGATTAGAAGCGGCGA ACCAATTCTTGGTTACCGAGTATGAACTGGTAAAAGCACAACTGGAATCA GTTCTGGGTGTGAAAATTTCAAACGCCGCCCTGGAAAATTCGATTGCAAT TTATAACGAGAATCGTGCCGTGATGCGTGAGTTCGTGAAAGTGGCAGCGG ACTATCCTCAAGTCATTGACGCAGTGAGCCGCCACGCGGTTTTTAAAGCG CGCCAGTTTATGCTTAAGGAAAAACATACCGCACTTGTGAAAGAACTGAT CGCTGAGATTAAAGCAACGCCAGTCCAGCCGTGGGACGGAAAAAAGGTTG TAGTGACGGGCATTCTGTTGGAACCGAATGAGTTATTAGATATCTTTAAT GAGTTTAAGATCGCGATTGTTGATGATGATTTAGCGCAGGAAAGCCGTCA GATCCGTGTTGACGTTCTGGACGGAGAAGGCGGACCGCTCTACCGTATGG CTAAAGCGTGGCAGCAAATGTATGGCTGCTCGCTGGCAACCGACACCAAG AAGGGTCGCGGCCGTATGTTAATTAACAAAACGATTCAGACCGGTGCGGA CGCTATCGTAGTTGCAATGATGAAGTTTTGCGACCCAGAAGAATGGGATT ATCCGGTAATGTACCGTGAATTTGAAGAAAAAGGGGTCAAATCACTTATG

Gene sequence	01234567890123456789012345678901234567890123456789
	ATTGAGGTGGATCAGGAAGTATCGTCTTTCGAACAGATTAAAACCCGTCT GCAGTCATTCGTCGAAATGCTTTAA
lcdC SEQ ID NO: 24	ATGTATACCTTGGGGATTGATGTCGGTTCTGCCTCTAGTAAAGCGGTGAT TCTGAAAGATGGAAAAGATATTGTCGCTGCCGAGGTTGTCCAAGTCGGTA CCGGCTCCTCGGGTCCCCAACGCGCACTGGACAAAGCCTTTGAAGTCTCT GGCTTAAAAAAGGAAGACATCAGCTACACAGTAGCTACGGGCTATGGGCG CTTCAATTTTAGCGACGCGGATAAACAGATTTCGGAAATTAGCTGTCATG CCAAAGGCATTTATTTCTTAGTACCAACTGCGCGCACTATTATTGACATT GGCGGCCAAGATGCGAAAGCCATCCGCCTGGACGACAAGGGGGGTATTAA GCAATTCTTCATGAATGATAAATGCGCGGCGGGCACGGGGCGTTTCCTGG AAGTCATGGCTCGCGTACTTGAAACCACCCTGGATGAAATGGCTGAACTG GATGAACAGGCGACTGACACCGCTCCCATTTCAAGCACCTGCACGGTTTT CGCCGAAAGCGAAGTAATTAGCCAATTGAGCAATGGTGTCTCACGCAACA ACATCATTAAAGGTGTCCATCTGAGCGTTGCGTCACGTGCGTGTGGTCTG GCGTATCGCGGCGGTTTGGAGAAAGATGTTGTTATGACAGGTGGCGTGGC AAAAAATGCAGGGGTGGTGCGCGCGGTGGCGGGCGTTCTGAAGACCGATG TTATCGTTGCTCCGAATCCTCAGACGACCGGTGCACTGGGGGCAGCGCTG TATGCTTATGAGGCCGCCCAGAAGAAGTA
etfA SEQ ID NO: 25	ATGGCCTTCAATAGCGCAGATATTAATTCTTTCCGCGATATTTGGGTGTT TTGTGAACAGCGTGAGGGCAAACTGATTAACACCGATTTCGAATTAATTA GCGAAGGTCGTAAACTGGCTGACGAACGCGGAAGCAAACTGGTTGGAATT TTGCTGGGGCACGAAGTTGAAGAAATCGCAAAAGAATTAGGCGGCTATGG TGCGGACAAGGTAATTGTGTGCGATCATCCGGAACTTAAATTTTACACTA CGGATGCTTATGCCAAAGTTTTATGTGACGTCGTGATGGAAGAGAAACCG GAGGTAATTTTGATCGGTGCCACCAACATTGGCCGTGATCTCGGACCGCG TTGTGCTGCACGCTTGCACACGGGGCTGACGGCTGATTGCACGCACCTGG ATATTGATATGAATAAATATGTGGACTTTCTTAGCACCAGTAGCACCTTG GATATCTCGTCGATGACTTTCCCTATGGAAGATACAAACCTTAAAATGAC GCGCCCTGCATTTGGCGGACATCTGATGGCAACGATCATTTGTCCACGCT TCCGTCCCTGTATGAGCACAGTGCGCCCCGGAGTGATGAAGAAAGCGGAG TTCTCGCAGGAGATGGCGCAAGCATGTCAAGTAGTGACCCGTCACGTAAA TTTGTCGGATGAAGACCTTAAAACTAAAGTAATTAATATCGTGAAGGAAA CGAAAAAGATTGTGGATCTGATCGGCGCAGAAATTATTGTGTCAGTTGGT CGTGGTATCTCGAAAGATGTCCAAGGTGGAATTGCACTGGCTGAAAAACT TGCGGACGCATTTGGTAACGGTGTCGTGGGCGGCTCGCGCGCAGTGATTG ATTCCGGCTGGTTACCTGCGGATCATCAGGTTGGACAAACCGGTAAGACC GTGCACCCGAAAGTCTACGTGGCGCTGGGTATTAGTGGGGCTATCCAGCA TAAGGCTGGGATGCAAGACTCTGAACTGATCATTGCCGTCAACAAAGACG AAACGGCGCCTATCTTCGACTGCGCCGATTATGGCATCACCGGTGATTTA TTTAAAATCGTACCGATGATGATCGACGCGATCAAAGAGGGTAAAAACGC ATGA

Gene sequence	0123456789012345678901234567890123456789012345678
acrB SEQ ID NO: 26	ATGCGCATCTATGTGTGTGTGAAACAAGTCCCAGATACGAGCGGCAAGGT GGCCGTTAACCCTGATGGGACCCTTAACCGTGCCTCAATGGCAGCGATTA TTAACCCGGACGATATGTCCGCGATCGAACAGGCATTAAAACTGAAAGAT GAAACCGGATGCCAGGTTACGGCGCTTACGATGGGTCCTCCTCCTGCCGA GGGCATGTTGCGCGAAATTATTGCAATGGGGGCCGACGATGGTGTGCTGA TTTCGGCCCGTGAATTTGGGGGGTCCGATACCTTCGCAACCAGTCAAATT ATTAGCGCGGCAATCCATAAATTAGGCTTAAGCAATGAAGACATGATCTT TTGCGGTCGTCAGGCCATTGACGGTGATACGGCCCAAGTCGGCCCTCAAA TTGCCGAAAAACTGAGCATCCCACAGGTAACCTATGGCGCAGGAATCAAA AAATCTGGTGATTTAGTGCTGGTGAAGCGTATGTTGGAGGATGGTTATAT GATGATCGAAGTCGAAACTCCATGTCTGATTACCTGCATTCAGGATAAAG CGGTAAAACCACGTTACATGACTCTCAACGGTATTATGGAATGCTACTCC AAGCCGCTCCTCGTTCTCGATTACGAAGCACTGAAAGATGAACCGCTGAT CGAACTTGATACCATTGGGCTTAAAGGCTCCCCGACGAATATCTTTAAAT CGTTTACGCCGCCTCAGAAAGGCGTTGGTGTCATGCTCCAAGGCACCGAT AAGGAAAAAGTCGAGGATCTGGTGGATAAGCTGATGCAGAAACATGTCAT CTAA
acrC SEQ ID NO: 27	ATGTTCTTACTGAAGATTAAAAAAGAACGTATGAAACGCATGGACTTTAG TTTAACGCGTGAACAGGAGATGTTAAAAAAACTGGCGCGTCAGTTTGCTG AGATCGAGCTGGAACCGGTGGCCGAAGAGATTGATCGTGAGCACGTTTTT CCTGCAGAAAACTTTAAGAAGATGGCGGAAATTGGCTTAACCGGCATTGG TATCCCGAAAGAATTTGGTGGCTCCGGTGGAGGCACCCTGGAGAAGGTCA TTGCCGTGTCAGAATTCGGCAAAAAGTGTATGGCCTCAGCTTCCATTTTA AGCATTCATCTTATCGCGCCGCAGGCAATCTACAAATATGGGACCAAAGA ACAGAAAGAGACGTACCTGCCGCGTCTTACCAAAGGTGGTGAACTGGGCG CCTTTGCGCTGACAGAACCAAACGCCGGAAGCGATGCCGGCGCGGTAAAA ACGACCGCGATTCTGGACAGCCAGACAAACGAGTACGTGCTGAATGGCAC CAAATGCTTTATCAGCGGGGGCGGGCGCGCGGGTGTTCTTGTAATTTTTG CGCTTACTGAACCGAAAAAAGGTCTGAAAGGGATGAGCGCGATTATCGTG GAGAAAGGGACCCCGGGCTTCAGCATCGGCAAGGTGGAGAGCAAGATGGG GATCGCAGGTTCGGAAACCGCGGAACTTATCTTCGAAGATTGTCGCGTTC CGGCTGCCAACCTTTTAGGTAAAGAAGGCAAAGGCTTTAAAATTGCTATG GAAGCCCTGGATGGCGCCCGTATTGGCGTGGGCGCTCAAGCAATCGGAAT TGCCGAGGGGGCGATCGACCTGAGTGTGAAGTACGTTCACGAGCGCATTC AATTTGGTAAACCGATCGCGAATCTGCAGGGAATTCAATGGTATATCGCG GATATGGCGACCAAAACCGCCGCGGCACGCGCACTTGTTGAGTTTGCAGC GTATCTTGAAGACGCGGGTAAACCGTTCACAAAGGAATCTGCTATGTGCA AGCTGAACGCCTCCGAAAACGCGCGTTTTGTGACAAATTTAGCTCTGCAG ATTCACGGGGGTTACGGTTATATGAAAGATTATCCGTTAGAGCGTATGTA TCGCGATGCTAAGATTACGGAAATTTACGAGGGGACATCAGAAATCCATA AGGTGGTGATTGCGCGTGAAGTAATGAAACGCTAA
thrA^{fbr} SEQ ID NO: 28	ATGCGAGTGTTGAAGTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTT TCTGCGTGTTGCCGATATTCTGGAAAGCAATGCCAGGCAGGGGCAGGTGG CCACCGTCCTCTCTGCCCCCGCCAAAATCACCAACCACCTGGTGGCGATG ATTGAAAAAACCATTAGCGGCCAGGATGCTTTACCCAATATCAGCGATGC CGAACGTATTTTTGCCGAACTTTTGACGGGACTCGCCGCCGCCCAGCCGG GGTTCCCGCTGGCGCAATTGAAAACTTTCGTCGATCAGGAATTTGCCCAA

Gene sequence	0123456789012345678901234567890123456789012345678
	ATAAAACATGTCCTGCATGGCATTAGTTTGTTGGGGCAGTGCCCGGATAG CATCAACGCTGCGCTGATTTGCCGTGGCGAGAAAATGTCGATCGCCATTA TGGCCGGCGTATTAGAAGCGCGCGGTCACAACGTTACTGTTATCGATCCG GTCGAAAAACTGCTGGCAGTGGGGCATTACCTCGAATCTACCGTCGATAT TGCTGAGTCCACCCGCCGTATTGCGGCAAGCCGCATTCCGGCTGATCACA TGGTGCTGATGGCAGGTTTCACCGCCGGTAATGAAAAAGGCGAACTGGTG GTGCTTGGACGCAACGGTTCCGACTACTCTGCTGCGGTGCTGGCTGCCTG TTTACGCGCCGATTGTTGCGAGATTTGGACGGACGTTGACGGGGTCTATA CCTGCGACCCGCGTCAGGTGCCCGATGCGAGGTTGTTGAAGTCGATGTCC TACCAGGAAGCGATGGAGCTTTCCTACTTCGGCGCTAAAGTTCTTCACCC CCGCACCATTACCCCCATCGCCCAGTTCCAGATCCCTTGCCTGATTAAAA ATACCGGAAATCCTCAAGCACCAGGTACGCTCATTGGTGCCAGCCGTGAT GAAGACGAATTACCGGTCAAGGGCATTTCCAATCTGAATAACATGGCAAT GTTCAGCGTTTCTGGTCCGGGGATGAAAGGGATGGTCGGCATGGCGGCGC GCGTCTTTGCAGCGATGTCACGCGCCCGTATTTCCGTGGTGCTGATTACG CAATCATCTTCCGAATACAGCATCAGTTTCTGCGTTCCACAAAGCGACTG TGTGCGAGCTGAACGGGCAATGCAGGAAGAGTTCTACCTGGAACTGAAAG AAGGCTTACTGGAGCCGCTGGCAGTGACGGAACGGCTGGCCATTATCTCG GTGGTAGGTGATGGTATGCGCACCTTGCGTGGGATCTCGGCGAAATTCTT TGCCGCACTGGCCCGCGCCAATATCAACATTGTCGCCATTGCTCAGAGAT CTTCTGAACGCTCAATCTCTGTCGTGGTAAATAACGATGATGCGACCACT GGCGTGCGCGTTACTCATCAGATGCTGTTCAATACCGATCAGGTTATCGA AGTGTTTGTGATTGGCGTCGGTGGCGTTGGCGGTGCGCTGCTGGAGCAAC TGAAGCGTCAGCAAAGCTGGCTGAAGAATAAACATATCGACTTACGTGTC TGCGGTGTTGCCAACTCGAAGGCTCTGCTCACCAATGTACATGGCCTTAA TCTGGAAAACTGGCAGGAAGAACTGGCGCAAGCCAAAGAGCCGTTTAATC TCGGGCGCTTAATTCGCCTCGTGAAAGAATATCATCTGCTGAACCCGGTC ATTGTTGACTGCACTTCCAGCCAGGCAGTGGCGGATCAATATGCCGACTT CCTGCGCGAAGGTTTCCACGTTGTCACGCCGAACAAAAAGGCCAACACCT CGTCGATGGATTACTACCATCAGTTGCGTTATGCGGCGGAAAAATCGCGG CGTAAATTCCTCTATGACACCAACGTTGGGGCTGGATTACCGGTTATTGA GAACCTGCAAAATCTGCTCAATGCAGGTGATGAATTGATGAAGTTCTCCG GCATTCTTTCTGGTTCGCTTTCTTATATCTTCGGCAAGTTAGACGAAGGC ATGAGTTTCTCCGAGGCGACCACGCTGGCGCGGGAAATGGGTTATACCGA ACCGGACCCGCGAGATGATCTTTCTGGTATGGATGTGGCGCGTAAACTAT TGATTCTCGCTCGTGAAACGGGACGTGAACTGGAGCTGGCGGATATTGAA ATTGAACCTGTGCTGCCCGCAGAGTTTAACGCCGAGGGTGATGTTGCCGC TTTTATGGCGAATCTGTCACAACTCGACGATCTCTTTGCCGCGCGCGTGG CGAAGGCCCGTGATGAAGGAAAAGTTTTGCGCTATGTTGGCAATATTGAT GAAGATGGCGTCTGCCGCGTGAAGATTGCCGAAGTGGATGGTAATGATCC GCTGTTCAAAGTGAAAAATGGCGAAAACGCCCTGGCCTTCTATAGCCACT ATTATCAGCCGCTGCCGTTGGTACTGCGCGGATATGGTGCGGGCAATGAC GTTACAGCTGCCGGTGTCTTTGCTGATCTGCTACGTACCCTCTCATGGAA GTTAGGAGTCTGA

Gene sequence	0123456789012345678901234567890123456789012345678
thrB SEQ ID NO: 29	ATGGTTAAAGTTTATGCCCCGGCTTCCAGTGCCAATATGAGCGTCGGGTT TGATGTGCTCGGGGCGGCGGTGACACCTGTTGATGGTGCATTGCTCGGAG ATGTAGTCACGGTTGAGGCGGCAGAGACATTCAGTCTCAACAACCTCGGA CGCTTTGCCGATAAGCTGCCGTCAGAACCACGGGAAAATATCGTTTATCA GTGCTGGGAGCGTTTTTGCCAGGAACTGGGTAAGCAAATTCCAGTGGCGA TGACCCTGGAAAAGAATATGCCGATCGGTTCGGGCTTAGGCTCCAGTGCC TGTTCGGTGGTCGCGGCGCTGATGGCGATGAATGAACACTGCGGCAAGCC GCTTAATGACACTCGTTTGCTGGCTTTGATGGGCGAGCTGGAAGGCCGTA TCTCCGGCAGCATTCATTACGACAACGTGGCACCGTGTTTTCTCGGTGGT ATGCAGTTGATGATCGAAGAAAACGACATCATCAGCCAGCAAGTGCCAGG GTTTGATGAGTGGCTGTGGGTGCTGGCGTATCCGGGGATTAAAGTCTCGA CGGCAGAAGCCAGGGCTATTTTACCGGCGCAGTATCGCCGCCAGGATTGC ATTGCGCACGGGCGACATCTGGCAGGCTTCATTCACGCCTGCTATTCCCG TCAGCCTGAGCTTGCCGCGAAGCTGATGAAAGATGTTATCGCTGAACCCT ACCGTGAACGGTTACTGCCAGGCTTCCGGCAGGCGCGGCAGGCGGTCGCG GAAATCGGCGCGGTAGCGAGCGGTATCTCCGGCTCCGGCCCGACCTTGTT CGCTCTGTGTGACAAGCCGGAAACCGCCCAGCGCGTTGCCGACTGGTTGG GTAAGAACTACCTGCAAAATCAGGAAGGTTTTGTTCATATTTGCCGGCTG GATACGGCGGGCGCACGAGTACTGGAAAACTAA
thrC SEQ ID NO: 30	ATGAAACTCTACAATCTGAAAGATCACAACGAGCAGGTCAGCTTTGCGCA AGCCGTAACCCAGGGGTTGGGCAAAAATCAGGGGCTGTTTTTTCCGCACG ACCTGCCGGAATTCAGCCTGACTGAAATTGATGAGATGCTGAAGCTGGAT TTTGTCACCCGCAGTGCGAAGATCCTCTCGGCGTTTATTGGTGATGAAAT CCCACAGGAAATCCTGGAAGAGCGCGTGCGCGCGGCGTTTGCCTTCCCGG CTCCGGTCGCCAATGTTGAAAGCGATGTCGGTTGTCTGGAATTGTTCCAC GGGCCAACGCTGGCATTTAAAGATTTCGGCGGTCGCTTTATGGCACAAAT GCTGACCCATATTGCGGGTGATAAGCCAGTGACCATTCTGACCGCGACCT CCGGTGATACCGGAGCGGCAGTGGCTCATGCTTTCTACGGTTTACCGAAT GTGAAAGTGGTTATCCTCTATCCACGAGGCAAAATCAGTCCACTGCAAGA AAAACTGTTCTGTACATTGGGCGGCAATATCGAAACTGTTGCCATCGACG GCGATTTCGATGCCTGTCAGGCGCTGGTGAAGCAGGCGTTTGATGATGAA GAACTGAAAGTGGCGCTAGGGTTAAACTCGGCTAACTCGATTAACATCAG CCGTTTGCTGGCGCAGATTTGCTACTACTTTGAAGCTGTTGCGCAGCTGC CGCAGGAGACGCGCAACCAGCTGGTTGTCTCGGTGCCAAGCGGAAACTTC GGCGATTTGACGGCGGGTCTGCTGGCGAAGTCACTCGGTCTGCCGGTGAA ACGTTTTATTGCTGCGACCAACGTGAACGATACCGTGCCACGTTTCCTGC ACGACGGTCAGTGGTCACCCAAAGCGACTCAGGCGACGTTATCCAACGCG ATGGACGTGAGTCAGCCGAACAACTGGCCGCGTGTGGAAGAGTTGTTCCG CCGCAAAATCTGGCAACTGAAAGAGCTGGGTTATGCAGCCGTGGATGATG AAACCACGCAACAGACAATGCGTGAGTTAAAAGAACTGGGCTACACTTCG GAGCCGCACGCTGCCGTAGCTTATCGTGCGCTGCGTGATCAGTTGAATCC AGGCGAATATGGCTTGTTCCTCGGCACCGCGCATCCGGCGAAATTTAAAG AGAGCGTGGAAGCGATTCTCGGTGAAACGTTGGATCTGCCAAAAGAGCTG GCAGAACGTGCTGATTTACCCTTGCTTTCACATAATCTGCCCGCCGATTT TGCTGCGTTGCGTAAATTGATGATGAATCATCAGTAA

Gene sequence	0123456789012345678901234567890123456789012345678
ilvA^{fbr} SEQ ID NO: 31	ATGAGTGAAACATACGTGTCTGAGAAAAGTCCAGGAGTGATGGCTAGCGG AGCGGAGCTGATTCGTGCCGCCGACATTCAAACGGCGCAGGCACGAATTT CCTCCGTCATTGCACCAACTCCATTGCAGTATTGCCCTCGTCTTTCTGAG GAAACCGGAGCGGAAATCTACCTTAAGCGTGAGGATCTGCAGGATGTTCG TTCCTACAAGATCCGCGGTGCGCTGAACTCTGGAGCGCAGCTCACCCAAG AGCAGCGCGATGCAGGTATCGTTGCCGCATCTGCAGGTAACCATGCCCAG GGCGTGGCCTATGTGTGCAAGTCCTTGGGCGTTCAGGGACGCATCTATGT TCCTGTGCAGACTCCAAAGCAAAAGCGTGACCGCATCATGGTTCACGGCG GAGAGTTTGTCTCCTTGGTGGTCACTGGCAATAACTTCGACGAAGCATCG GCTGCAGCGCATGAAGATGCAGAGCGCACCGGCGCAACGCTGATCGAGCC TTTCGATGCTCGCAACACCGTCATCGGTCAGGGCACCGTGGCTGCTGAGA TCTTGTCGCAGCTGACTTCCATGGGCAAGAGTGCAGATCACGTGATGGTT CCAGTCGGCGGTGGCGGACTTCTTGCAGGTGTGGTCAGCTACATGGCTGA TATGGCACCTCGCACTGCGATCGTTGGTATCGAACCAGCGGGAGCAGCAT CCATGCAGGCTGCATTGCACAATGGTGGACCAATCACTTTGGAGACTGTT GATCCCTTTGTGGACGGCGCAGCAGTCAAACGTGTCGGAGATCTCAACTA CACCATCGTGGAGAAGAACCAGGGTCGCGTGCACATGATGAGCGCGACCG AGGGCGCTGTGTGTACTGAGATGCTCGATCTTTACCAAAACGAAGGCATC ATCGCGGAGCCTGCTGGCGCGCTGTCTATCGCTGGGTTGAAGGAAATGTC CTTTGCACCTGGTTCTGCAGTGGTGTGCATCATCTCTGGTGGCAACAACG ATGTGCTGCGTTATGCGGAAATCGCTGAGCGCTCCTTGGTGCACCGCGGT TTGAAGCACTACTTCTTGGTGAACTTCCCGCAAAAGCCTGGTCAGTTGCG TCACTTCCTGGAAGATATCCTGGGACCGGATGATGACATCACGCTGTTTG AGTACCTCAAGCGCAACAACCGTGAGACCGGTACTGCGTTGGTGGGTATT CACTTGAGTGAAGCATCAGGATTGGATTCTTTGCTGGAACGTATGGAGGA ATCGGCAATTGATTCCCGTCGCCTCGAGCCGGGCACCCCTGAGTACGAAT ACTTGACCTAA
aceE SEQ ID NO: 32	ATGTCAGAACGTTTCCCAAATGACGTGGATCCGATCGAAACTCGCGACTG GCTCCAGGCGATCGAATCGGTCATCCGTGAAGAAGGTGTTGAGCGTGCTC AGTATCTGATCGACCAACTGCTTGCTGAAGCCCGCAAAGGCGGTGTAAAC GTAGCCGCAGGCACAGGTATCAGCAACTACATCAACACCATCCCCGTTGA AGAACAACCGGAGTATCCGGGTAATCTGGAACTGGAACGCCGTATTCGTT CAGCTATCCGCTGGAACGCCATCATGACGGTGCTGCGTGCGTCGAAAAAA GACCTCGAACTGGGCGGCCATATGGCGTCCTTCCAGTCTTCCGCAACCAT TTATGATGTGTGCTTTAACCACTTCTTCCGTGCACGCAACGAGCAGGATG GCGGCGACCTGGTTTACTTCCAGGGCCACATCTCCCCGGGCGTGTACGCT CGTGCTTTCCTGGAAGGTCGTCTGACTCAGGAGCAGCTGGATAACTTCCG TCAGGAAGTTCACGGCAATGGCCTCTCTTCCTATCCGCACCCGAAACTGA TGCCGGAATTCTGGCAGTTCCCGACCGTATCTATGGGTCTGGGTCCGATT GGTGCTATTTACCAGGCTAAATTCCTGAAATATCTGGAACACCGTGGCCT GAAAGATACCTCTAAACAAACCGTTTACGCGTTCCTCGGTGACGGTGAAA TGGACGAACCGGAATCCAAAGGTGCGATCACCATCGCTACCCGTGAAAAA CTGGATAACCTGGTCTTCGTTATCAACTGTAACCTGCAGCGTCTTGACGG CCCGGTCACCGGTAACGGCAAGATCATCAACGAACTGGAAGGCATCTTCG AAGGTGCTGGCTGGAACGTGATCAAAGTGATGTGGGGTAGCCGTTGGGAT GAACTGCTGCGTAAGGATACCAGCGGTAAACTGATCCAGCTGATGAACGA AACCGTTGACGGCGACTACCAGACCTTCAAATCGAAAGATGGTGCGTACG

Gene sequence	0123456789012345678901234567890123456789012345678
	TTCGTGAACACTTCTTCGGTAAATATCCTGAAACCGCAGCACTGGTTGCA GACTGGACTGACGAGCAGATCTGGGCACTGAACCGTGGTGGTCACGATCC GAAGAAAATCTACGCTGCATTCAAGAAAGCGCAGGAAACCAAAGGCAAAG CGACAGTAATCCTTGCTCATACCATTAAAGGTTACGGCATGGGCGACGCG GCTGAAGGTAAAAACATCGCGCACCAGGTTAAGAAAATGAACATGGACGG TGTGCGTCATATCCGCGACCGTTTCAATGTGCCGGTGTCTGATGCAGATA TCGAAAAACTGCCGTACATCACCTTCCCGGAAGGTTCTGAAGAGCATACC TATCTGCACGCTCAGCGTCAGAAACTGCACGGTTATCTGCCAAGCCGTCA GCCGAACTTCACCGAGAAGCTTGAGCTGCCGAGCCTGCAAGACTTCGGCG CGCTGTTGGAAGAGCAGAGCAAAGAGATCTCTACCACTATCGCTTTCGTT CGTGCTCTGAACGTGATGCTGAAGAACAAGTCGATCAAAGATCGTCTGGT ACCGATCATCGCCGACGAAGCGCGTACTTTCGGTATGGAAGGTCTGTTCC GTCAGATTGGTATTTACAGCCCGAACGGTCAGCAGTACACCCCGCAGGAC CGCGAGCAGGTTGCTTACTATAAAGAAGACGAGAAAGGTCAGATTCTGCA GGAAGGGATCAACGAGCTGGGCGCAGGTTGTTCCTGGCTGGCAGCGGCGA CCTCTTACAGCACCAACAATCTGCCGATGATCCCGTTCTACATCTATTAC TCGATGTTCGGCTTCCAGCGTATTGGCGATCTGTGCTGGGCGGCTGGCGA CCAGCAAGCGCGTGGCTTCCTGATCGGCGGTACTTCCGGTCGTACCACCC TGAACGGCGAAGGTCTGCAGCACGAAGATGGTCACAGCCACATTCAGTCG CTGACTATCCCGAACTGTATCTCTTACGACCCGGCTTACGCTTACGAAGT TGCTGTCATCATGCATGACGGTCTGGAGCGTATGTACGGTGAAAAACAAG AGAACGTTTACTACTACATCACTACGCTGAACGAAAACTACCACATGCCG GCAATGCCGGAAGGTGCTGAGGAAGGTATCCGTAAAGGTATCTACAAACT CGAAACTATTGAAGGTAGCAAAGGTAAAGTTCAGCTGCTCGGCTCCGGTT CTATCCTGCGTCACGTCCGTGAAGCAGCTGAGATCCTGGCGAAAGATTAC GGCGTAGGTTCTGACGTTTATAGCGTGACCTCCTTCACCGAGCTGGCGCG TGATGGTCAGGATTGTGAACGCTGGAACATGCTGCACCCGCTGGAAACTC CGCGCGTTCCGTATATCGCTCAGGTGATGAACGACGCTCCGGCAGTGGCA TCTACCGACTATATGAAACTGTTCGCTGAGCAGGTCCGTACTTACGTACC GGCTGACGACTACCGCGTACTGGGTACTGATGGCTTCGGTCGTTCCGACA GCCGTGAGAACCTGCGTCACCACTTCGAAGTTGATGCTTCTTATGTCGTG GTTGCGGCGCTGGGCGAACTGGCTAAACGTGGCGAAATCGATAAGAAAGT GGTTGCTGACGCAATCGCCAAATTCAACATCGATGCAGATAAAGTTAACC CGCGTCTGGCGTAA
aceF SEQ ID NO: 33	ATGGCTATCGAAATCAAAGTACCGGACATCGGGGCTGATGAAGTTGAAAT CACCGAGATCCTGGTCAAAGTGGGCGACAAAGTTGAAGCCGAACAGTCGC TGATCACCGTAGAAGGCGACAAAGCCTCTATGGAAGTTCCGTCTCCGCAG GCGGGTATCGTTAAAGAGATCAAAGTCTCTGTTGGCGATAAAACCCAGAC CGGCGCACTGATTATGATTTTCGATTCCGCCGACGGTGCAGCAGACGCTG CACCTGCTCAGGCAGAAGAGAAGAAAGAAGCAGCTCCGGCAGCAGCACCA GCGGCTGCGGCGGCAAAAGACGTTAACGTTCCGGATATCGGCAGCGACGA AGTTGAAGTGACCGAAATCCTGGTGAAAGTTGGCGATAAAGTTGAAGCTG AACAGTCGCTGATCACCGTAGAAGGCGACAAGGCTTCTATGGAAGTTCCG GCTCCGTTTGCTGGCACCGTGAAAGAGATCAAAGTGAACGTGGGTGACAA AGTGTCTACCGGCTCGCTGATTATGGTCTTCGAAGTCGCGGGTGAAGCAG GCGCGGCAGCTCCGGCCGCTAAACAGGAAGCAGCTCCGGCAGCGGCCCCT GCACCAGCGGCTGGCGTGAAAGAAGTTAACGTTCCGGATATCGGCGGTGA

Gene sequence	0123456789012345678901234567890123456789012345678
	CGAAGTTGAAGTGACTGAAGTGATGGTGAAAGTGGGCGACAAAGTTGCCG CTGAACAGTCACTGATCACCGTAGAAGGCGACAAAGCTTCTATGGAAGTT CCGGCGCCGTTTGCAGGCGTCGTGAAGGAACTGAAAGTCAACGTTGGCGA TAAAGTGAAAACTGGCTCGCTGATTATGATCTTCGAAGTTGAAGGCGCAG CGCCTGCGGCAGCTCCTGCGAAACAGGAAGCGGCAGCGCCGGCACCGGCA GCAAAAGCTGAAGCCCCGGCAGCAGCACCAGCTGCGAAAGCGGAAGGCAA ATCTGAATTTGCTGAAAACGACGCTTATGTTCACGCGACTCCGCTGATCC GCCGTCTGGCACGCGAGTTTGGTGTTAACCTTGCGAAAGTGAAGGGCACT GGCCGTAAAGGTCGTATCCTGCGCGAAGACGTTCAGGCTTACGTGAAAGA AGCTATCAAACGTGCAGAAGCAGCTCCGGCAGCGACTGGCGGTGGTATCC CTGGCATGCTGCCGTGGCCGAAGGTGGACTTCAGCAAGTTTGGTGAAATC GAAGAAGTGGAACTGGGCCGCATCCAGAAAATCTCTGGTGCGAACCTGAG CCGTAACTGGGTAATGATCCCGCATGTTACTCACTTCGACAAAACCGATA TCACCGAGTTGGAAGCGTTCCGTAAACAGCAGAACGAAGAAGCGGCGAAA CGTAAGCTGGATGTGAAGATCACCCCGGTTGTCTTCATCATGAAAGCCGT TGCTGCAGCTCTTGAGCAGATGCCTCGCTTCAATAGTTCGCTGTCGGAAG ACGGTCAGCGTCTGACCCTGAAGAAATACATCAACATCGGTGTGGCGGTG GATACCCCGAACGGTCTGGTTGTTCCGGTATTCAAAGACGTCAACAAGAA AGGCATCATCGAGCTGTCTCGCGAGCTGATGACTATTTCTAAGAAAGCGC GTGACGGTAAGCTGACTGCGGGCGAAATGCAGGGCGGTTGCTTCACCATC TCCAGCATCGGCGGCCTGGGTACTACCCACTTCGCGCCGATTGTGAACGC GCCGGAAGTGGCTATCCTCGGCGTTTCCAAGTCCGCGATGGAGCCGGTGT GGAATGGTAAAGAGTTCGTGCCGCGTCTGATGCTGCCGATTTCTCTCTCC TTCGACCACCGCGTGATCGACGGTGCTGATGGTGCCCGTTTCATTACCAT CATTAACAACACGCTGTCTGACATTCGCCGTCTGGTGATGTAA
lpd SEQ ID NO: 34	ATGAGTACTGAAATCAAAACTCAGGTCGTGGTACTTGGGGCAGGCCCCGC AGGTTACTCCGCTGCCTTCCGTTGCGCTGATTTAGGTCTGGAAACCGTAA TCGTAGAACGTTACAACACCCTTGGCGGTGTTTGCCTGAACGTCGGCTGT ATCCCTTCTAAAGCACTGCTGCACGTAGCAAAAGTTATCGAAGAAGCCAA AGCGCTGGCTGAACACGGTATCGTCTTCGGCGAACCGAAAACCGATATCG ACAAGATTCGTACCTGGAAAGAGAAAGTGATCAATCAGCTGACCGGTGGT CTGGCTGGTATGGCGAAAGGCCGCAAAGTCAAAGTGGTCAACGGTCTGGG TAAATTCACCGGGGCTAACACCCTGGAAGTTGAAGGTGAGAACGGCAAAA CCGTGATCAACTTCGACAACGCGATCATTGCAGCGGGTTCTCGCCCGATC CAACTGCCGTTTATTCCGCATGAAGATCCGCGTATCTGGGACTCCACTGA CGCGCTGGAACTGAAAGAAGTACCAGAACGCCTGCTGGTAATGGGTGGCG GTATCATCGGTCTGGAAATGGGCACCGTTTACCACGCGCTGGGTTCACAG ATTGACGTGGTTGAAATGTTCGACCAGGTTATCCCGGCAGCTGACAAAGA CATCGTTAAAGTCTTCACCAAGCGTATCAGCAAGAAATTCAACCTGATGC TGGAAACCAAAGTTACCGCCGTTGAAGCGAAAGAAGACGGCATTTATGTG ACGATGGAAGGCAAAAAAGCACCCGCTGAACCGCAGCGTTACGACGCCGT GCTGGTAGCGATTGGTCGTGTGCCGAACGGTAAAAACCTCGACGCAGGCA AAGCAGGCGTGGAAGTTGACGACCGTGGTTTCATCCGCGTTGACAAACAG CTGCGTACCAACGTACCGCACATCTTTGCTATCGGCGATATCGTCGGTCA ACCGATGCTGGCACACAAAGGTGTTCACGAAGGTCACGTTGCCGCTGAAG TTATCGCCGGTAAGAAACACTACTTCGATCCGAAAGTTATCCCGTCCATC GCCTATACCAAACCAGAAGTTGCATGGGTGGGTCTGACTGAGAAAGAAGC

Gene sequence	01234567890123456789012345678901234567890123456789
	GAAAGAGAAAGGCATCAGCTATGAAACCGCCACCTTCCCGTGGGCTGCTT CTGGTCGTGCTATCGCTTCCGACTGCGCAGACGGTATGACCAAGCTGATT TTCGACAAAGAATCTCACCGTGTGATCGGTGGTGCGATTGTCGGTACTAA CGGCGGCGAGCTGCTGGGTGAAATCGGCCTGGCAATCGAAATGGGTTGTG ATGCTGAAGACATCGCACTGACCATCCACGCGCACCCGACTCTGCACGAG TCTGTGGGCCTGGCGGCAGAAGTGTTCGAAGGTAGCATTACCGACCTGCC GAACCCGAAAGCGAAGAAGAAGTAA
tesB SEQ ID NO: 10	ATGAGTCAGGCGCTAAAAAATTTACTGACATTGTTAAATCTGGAAAAAAT TGAGGAAGGACTCTTTCGCGGCCAGAGTGAAGATTTAGGTTTACGCCAGG TGTTTGGCGGCCAGGTCGTGGGTCAGGCCTTGTATGCTGCAAAAGAGACC GTCCCTGAAGAGCGGCTGGTACATTCGTTTCACAGCTACTTTCTTCGCCC TGGCGATAGTAAGAAGCCGATTATTTATGATGTCGAAACGCTGCGTGACG GTAACAGCTTCAGCGCCCGCCGGGTTGCTGCTATTCAAAACGGCAAACCG ATTTTTTATATGACTGCCTCTTTCCAGGCACCAGAAGCGGGTTTCGAACA TCAAAAAACAATGCCGTCCGCGCCAGCGCCTGATGGCCTCCCTTCGGAAA CGCAAATCGCCCAATCGCTGGCGCACCTGCTGCCGCCAGTGCTGAAAGAT AAATTCATCTGCGATCGTCCGCTGGAAGTCCGTCCGGTGGAGTTTCATAA CCCACTGAAAGGTCACGTCGCAGAACCACATCGTCAGGTGTGGATCCGCG CAAATGGTAGCGTGCCGGATGACCTGCGCGTTCATCAGTATCTGCTCGGT TACGCTTCTGATCTTAACTTCCTGCCGGTAGCTCTACAGCCGCACGGCAT CGGTTTTCTCGAACCGGGGATTCAGATTGCCACCATTGACCATTCCATGT GGTTCCATCGCCCGTTTAATTTGAATGAATGGCTGCTGTATAGCGTGGAG AGCACCTCGGCGTCCAGCGCACGTGGCTTTGTGCGCGGTGAGTTTTATAC CCAAGACGGCGTACTGGTTGCCTCGACCGTTCAGGAAGGGGTGATGCGTA ATCACAATTAA

Table 7

Amino acid sequence	01234567890123456789012345678901234567890123456789
pct SEQ ID NO: 35	MRKVPIITADEAAKLIKDGDTVTTSGFVGNAIPEALDRAVEKRFLETGEP KNITYVYCGSQGNRDGRGAEHFAHEGLLKRYIAGHWATVPALGKMAMENK MEAYNVSQGALCHLFRDIASHKPGVFTKVGIGTFIDPRNGGGKVNDITKE DIVELVEIKGQEYLFYPAFPIHVALIRGTYADESGNITFEKEVAPLEGTS VCQAVKNSGGIVVVQVERVVKAGTLDPRHVKVPGIYVDYVVVADPEDHQQ SLDCEYDPALSGEHRRPEVVGEPLPLSAKKVIGRRGAIELEKDVAVNLGV GAPEYVASVADEEGIVDFMTLTAESGAIGGVPAGGVRFGASYNADALIDQ GYQFDYYDGGGLDLCYLGLAECDEKGNINVSRFGPRIAGCGGFINITQNT PKVFFCGTFTAGGLKVKIEDGKVIIVQEGKQKKFLKAVEQITFNGDVALA NKQQVTYITERCVFLLKEDGLHLSEIAPGIDLQTQILDVMDFAPIIDRDA NGQIKLMDAALFAEGLMGLKEMKS

Amino acid sequence	0123456789012345678901234567890123456789012345678
lcdA SEQ ID NO: 36	MSLTQGMKAKQLLAYFQGKADQDAREAKARGELVCWSASVAPPEFCVTMG IAMIYPETHAAGIGARKGAMDMLEVADRKGYNVDCCSYGRVNMGYMECLK EAAITGVKPEVLVNSPAADVPLPDLVITCNNICNTLLKWYENLAAELDIP CIVIDVPFNHTMPIPEYAKAYIADQFRNAISQLEVICGRPFDWKKFKEVK DQTQRSVYHWNRIAEMAKYKPSPLNGFDLFNYMALIVACRSLDYAEITFK AFADELEENLKAGIYAFKGAEKTRFQWEGIAVWPHLGHTFKSMKNLNSIM TGTAYPALWDLHYDANDESMHSMAEAYTRIYINTCLQNKVEVLLGIMEKG QVDGTVYHLNRSCKLMSFLNVETAEIIKEKNGLPYVSIDGDQTDPRVFSP AQFDTRVQALVEMMEANMAAAE
lcdB SEQ ID NO: 37	MSRVEAILSQLKDVAANPKKAMDDYKAETGKGAVGIMPIYSPEEMVHAAG YLPMGIWGAQGKTISKARTYLPAFACSVMQQVMELQCEGAYDDLSAVIFS VPCDTLKCLSQKWKGTSPVIVFTHPQNRGLEAANQFLVTEYELVKAQLES VLGVKISNAALENSIAIYNENRAVMREFVKVAADYPQVIDAVSRHAVFKA RQFMLKEKHTALVKELIAEIKATPVQPWDGKKVVVTGILLEPNELLDIFN EFKIAIVDDDLAQESRQIRVDVLDGEGGPLYRMAKAWQQMYGCSLATDTK KGRGRMLINKTIQTGADAIVVAMMKFCDPEEWDYPVMYREFEEKGVKSLM IEVDQEVSSFEQIKTRLQSFVEML
lcdC SEQ ID NO: 38	MYTLGIDVGSASSKAVILKDGKDIVAAEVVQVGTGSSGPQRALDKAFEVS GLKKEDISYTVATGYGRFNFSDADKQISEISCHAKGIYFLVPTARTIIDI GGQDAKAIRLDDKGGIKQFFMNDKCAAGTGRFLEVMARVLETTLDEMAEL DEQATDTAPISSTCTVFAESEVISQLSNGVSRNNIIKGVHLSVASRACGL AYRGGLEKDVVMTGGVAKNAGVVRAVAGVLKTDVIVAPNPQTTGALGAAL YAYEAAQKK
etfA SEQ ID NO: 39	MAFNSADINSFRDIWVFCEQREGKLINTDFELISEGRKLADERGSKLVGI LLGHEVEEIAKELGGYGADKVIVCDHPELKFYTTDAYAKVLCDVVMEEKP EVILIGATNIGRDLGPRCAARLHTGLTADCTHLDIDMNKYVDFLSTSSTL DISSMTFPMEDTNLKMTRPAFGGHLMATIICPRFRPCMSTVRPGVMKKAE FSQEMAQACQVVTRHVNLSDEDLKTKVINIVKETKKIVDLIGAEIIVSVG RGISKDVQGGIALAEKLADAFGNGVVGGSRAVIDSGWLPADHQVGQTGKT VHPKVYVALGISGAIQHKAGMQDSELIIAVNKDETAPIFDCADYGITGDL FKIVPMMIDAIKEGKNA
acrB SEQ ID NO: 40	MRIYVCVKQVPDTSGKVAVNPDGTLNRASMAAIINPDDMSAIEQALKLKD ETGCQVTALTMGPPPAEGMLREIIAMGADDGVLISAREFGGSDTFATSQI ISAAIHKLGLSNEDMIFCGRQAIDGDTAQVGPQIAEKLSIPQVTYGAGIK KSGDLVLVKRMLEDGYMMIEVETPCLITCIQDKAVKPRYMTLNGIMECYS KPLLVLDYEALKDEPLIELDTIGLKGSPTNIFKSFTPPQKGVGVMLQGTD KEKVEDLVDKLMQKHVI

Amino acid sequence	0123456789012345678901234567890123456789012345678
acrC SEQ ID NO: 41	MFLLKIKKERMKRMDFSLTREQEMLKKLARQFAEIELEPVAEEIDREHVF PAENFKKMAEIGLTGIGIPKEFGGSGGGTLEKVIAVSEFGKKCMASASIL SIHLIAPQAIYKYGTKEQKETYLPRLTKGGELGAFALTEPNAGSDAGAVK TTAILDSQTNEYVLNGTKCFISGGGRAGVLVIFALTEPKKGLKGMSAIIV EKGTPGFSIGKVESKMGIAGSETAELIFEDCRVPAANLLGKEGKGFKIAM EALDGARIGVGAQAIGIAEGAIDLSVKYVHERIQFGKPIANLQGIQWYIA DMATKTAAARALVEFAAYLEDAGKPFTKESAMCKLNASENARFVTNLALQ IHGGYGYMKDYPLERMYRDAKITEIYEGTSEIHKVVIAREVMKR
thrA^{fbr} SEQ ID NO: 42	MRVLKFGGTSVANAERFLRVADILESNARQGQVATVLSAPAKITNHLVAM IEKTISGQDALPNISDAERIFAELLTGLAAAQPGFPLAQLKTFVDQEFAQ IKHVLHGISLLGQCPDSINAALICRGEKMSIAIMAGVLEARGHNVTVIDP VEKLLAVGHYLESTVDIAESTRRIAASRIPADHMVLMAGFTAGNEKGELV VLGRNGSDYSAAVLAACLRADCCEIWTDVDGVYTCDPRQVPDARLLKSMS YQEAMELSYFGAKVLHPRTITPIAQFQIPCLIKNTGNPQAPGTLIGASRD EDELPVKGISNLNNMAMFSVSGPGMKGMVGMAARVFAAMSRARISVVLIT QSSSEYSISFCVPQSDCVRAERAMQEEFYLELKEGLLEPLAVTERLAIIS VVGDGMRTLRGISAKFFAALARANINIVAIAQRSSERSISVVVNNDDATT GVRVTHQMLFNTDQVIEVFVIGVGGVGGALLEQLKRQQSWLKNKHIDLRV CGVANSKALLTNVHGLNLENWQEELAQAKEPFNLGRLIRLVKEYHLLNPV IVDCTSSQAVADQYADFLREGFHVVTPNKKANTSSMDYYHQLRYAAEKSR RKFLYDTNVGAGLPVIENLQNLLNAGDELMKFSGILSGSLSYIFGKLDEG MSFSEATTLAREMGYTEPDPRDDLSGMDVARKLLILARETGRELELADIE IEPVLPAEFNAEGDVAAFMANLSQLDDLFAARVAKARDEGKVLRYVGNID EDGVCRVKIAEVDGNDPLFKVKNGENALAFYSHYYQPLPLVLRGYGAGND VTAAGVFADLLRTLSWKLGV
thrB SEQ ID NO: 43	MVKVYAPASSANMSVGFDVLGAAVTPVDGALLGDVVTVEAAETFSLNNLG RFADKLPSEPRENIVYQCWERFCQELGKQIPVAMTLEKNMPIGSGLGSSA CSVVAALMAMNEHCGKPLNDTRLLALMGELEGRISGSIHYDNVAPCFLGG MQLMIEENDIISQQVPGFDEWLWVLAYPGIKVSTAEARAILPAQYRRQDC IAHGRHLAGFIHACYSRQPELAAKLMKDVIAEPYRERLLPGFRQARQAVA EIGAVASGISGSGPTLFALCDKPETAQRVADWLGKNYLQNQEGFVHICRL DTAGARVLEN
thrC SEQ ID NO: 44	MKLYNLKDHNEQVSFAQAVTQGLGKNQGLFFPHDLPEFSLTEIDEMLKLD FVTRSAKILSAFIGDEIPQEILEERVRAAFAFPAPVANVESDVGCLELFH GPTLAFKDFGGRFMAQMLTHIAGDKPVTILTATSGDTGAAVAHAFYGLPN VKVVILYPRGKISPLQEKLFCTLGGNIETVAIDGDFDACQALVKQAFDDE ELKVALGLNSANSINISRLLAQICYYFEAVAQLPQETRNQLVVSVPSGNF GDLTAGLLAKSLGLPVKRFIAATNVNDTVPRFLHDGQWSPKATQATLSNA MDVSQPNNWPRVEELFRRKIWQLKELGYAAVDDETTQQTMRELKELGYTS EPHAAVAYRALRDQLNPGEYGLFLGTAHPAKFKESVEAILGETLDLPKEL AERADLPLLSHNLPADFAALRKLMMNHQ

Amino acid sequence	0123456789012345678901234567890123456789012345678
ilvA^{fbr} SEQ ID NO: 45	MSETYVSEKSPGVMASGAELIRAADIQTAQARISSVIAPTPLQYCPRLSE ETGAEIYLKREDLQDVRSYKIRGALNSGAQLTQEQRDAGIVAASAGNHAQ GVAYVCKSLGVQGRIYVPVQTPKQKRDRIMVHGGEFVSLVVTGNNFDEAS AAAHEDAERTGATLIEPFDARNTVIGQGTVAAEILSQLTSMGKSADHVMV PVGGGGLLAGVVSYMADMAPRTAIVGIEPAGAASMQAALHNGGPITLETV DPFVDGAAVKRVGDLNYTIVEKNQGRVHMMSATEGAVCTEMLDLYQNEGI IAEPAGALSIAGLKEMSFAPGSAVVCIISGGNNDVLRYAEIAERSLVHRG LKHYFLVNFPQKPGQLRHFLEDILGPDDDITLFEYLKRNNRETGTALVGI HLSEASGLDSLLERMEESAIDSRRLEPGTPEYEYLT
aceE SEQ ID NO: 46	MSERFPNDVDPIETRDWLQAIESVIREEGVERAQYLIDQLLAEARKGGVN VAAGTGISNYINTIPVEEQPEYPGNLELERRIRSAIRWNAIMTVLRASKK DLELGGHMASFQSSATIYDVCFNHFFRARNEQDGGDLVYFQGHISPGVYA RAFLEGRLTQEQLDNFRQEVHGNGLSSYPHPKLMPEFWQFPTVSMGLGPI GAIYQAKFLKYLEHRGLKDTSKQTVYAFLGDGEMDEPESKGAITIATREK LDNLVFVINCNLQRLDGPVTGNGKIINELEGIFEGAGWNVIKVMWGSRWD ELLRKDTSGKLIQLMNETVDGDYQTFKSKDGAYVREHFFGKYPETAALVA DWTDEQIWALNRGGHDPKKIYAAFKKAQETKGKATVILAHTIKGYGMGDA AEGKNIAHQVKKMNMDGVRHIRDRFNVPVSDADIEKLPYITFPEGSEEHT YLHAQRQKLHGYLPSRQPNFTEKLELPSLQDFGALLEEQSKEISTTIAFV RALNVMLKNKSIKDRLVPIIADEARTFGMEGLFRQIGIYSPNGQQYTPQD REQVAYYKEDEKGQILQEGINELGAGCSWLAAATSYSTNNLPMIPFYIYY SMFGFQRIGDLCWAAGDQQARGFLIGGTSGRTTLNGEGLQHEDGHSHIQS LTIPNCISYDPAYAYEVAVIMHDGLERMYGEKQENVYYYITTLNENYHMP AMPEGAEEGIRKGIYKLETIEGSKGKVQLLGSGSILRHVREAAEILAKDY GVGSDVYSVTSFTELARDGQDCERWNMLHPLETPRVPYIAQVMNDAPAVA STDYMKLFAEQVRTYVPADDYRVLGTDGFGRSDSRENLRHHFEVDASYVV VAALGELAKRGEIDKKVVADAIAKFNIDADKVNPRLA
aceF SEQ ID NO: 47	MAIEIKVPDIGADEVEITEILVKVGDKVEAEQSLITVEGDKASMEVPSPQ AGIVKEIKVSVGDKTQTGALIMIFDSADGAADAAPAQAEEKKEAAPAAAP AAAAAKDVNVPDIGSDEVEVTEILVKVGDKVEAEQSLITVEGDKASMEVP APFAGTVKEIKVNVGDKVSTGSLIMVFEVAGEAGAAAPAAKQEAAPAAAP APAAGVKEVNVPDIGGDEVEVTEVMVKVGDKVAAEQSLITVEGDKASMEV PAPFAGVVKELKVNVGDKVKTGSLIMIFEVEGAAPAAAPAKQEAAAPAPA AKAEAPAAAPAAKAEGKSEFAENDAYVHATPLIRRLAREFGVNLAKVKGT GRKGRILREDVQAYVKEAIKRAEAAPAATGGGIPGMLPWPKVDFSKFGEI EEVELGRIQKISGANLSRNWVMIPHVTHFDKTDITELEAFRKQQNEEAAK RKLDVKITPVVFIMKAVAAALEQMPRFNSSLSEDGQRLTLKKYINIGVAV DTPNGLVVPVFKDVNKKGIIELSRELMTISKKARDGKLTAGEMQGGCFTI SSIGGLGTTHFAPIVNAPEVAILGVSKSAMEPVWNGKEFVPRLMLPISLS FDHRVIDGADGARFITIINNTLSDIRRLVM

Amino acid sequence	0123456789012345678901234567890123456789012345678
lpd SEQ ID NO: 48	MSTEIKTQVVVLGAGPAGYSAAFRCADLGLETVIVERYNTLGGVCLNVGC IPSKALLHVAKVIEEAKALAEHGIVFGEPKTDIDKIRTWKEKVINQLTGG LAGMAKGRKVKVVNGLGKFTGANTLEVEGENGKTVINFDNAIIAAGSRPI QLPFIPHEDPRIWDSTDALELKEVPERLLVMGGGIIGLEMGTVYHALGSQ IDVVEMFDQVIPAADKDIVKVFTKRISKKFNLMLETKVTAVEAKEDGIYV TMEGKKAPAEPQRYDAVLVAIGRVPNGKNLDAGKAGVEVDDRGFIRVDKQ LRTNVPHIFAIGDIVGQPMLAHKGVHEGHVAAEVIAGKKHYFDPKVIPSI AYTKPEVAWVGLTEKEAKEKGISYETATFPWAASGRAIASDCADGMTKLI FDKESHRVIGGAIVGTNGGELLGEIGLAIEMGCDAEDIALTIHAHPTLHE SVGLAAEVFEGSITDLPNPKAKKK
tesB SEQ ID NO: 20	MSQALKNLLTLLNLEKIEEGLFRGQSEDLGLRQVFGGQVVGQALYAAKET VPEERLVHSFHSYFLRPGDSKKPIIYDVETLRDGNSFSARRVAAIQNGKP IFYMTASFQAPEAGFEHQKTMPSAPAPDGLPSETQIAQSLAHLLPPVLKD KFICDRPLEVRPVEFHNPLKGHVAEPHRQVWIRANGSVPDDLRVHQYLLG YASDLNFLPVALQPHGIGFLEPGIQIATIDHSMWFHRPFNLNEWLLYSVE STSASSARGFVRGEFYTQDGVLVASTVQEGVMRNHN

[0143] In some embodiments, one or more of the propionate biosynthesis genes is a synthetic propionate biosynthesis gene. In some embodiments, one or more of the propionate biosynthesis genes is an *E. coli* propionate biosynthesis gene. In some embodiments, one or more of the propionate biosynthesis genes is a *C. glutamicum* propionate biosynthesis gene. In some embodiments, one or more of the propionate biosynthesis genes is a *C. propionicum* propionate biosynthesis gene. The propionate gene cassette may comprise genes for the aerobic biosynthesis of propionate and/or genes for the anaerobic or microaerobic biosynthesis of propionate. One or more of the propionate biosynthesis genes may be functionally replaced or modified, e.g., codon optimized. In some embodiments, the genetically engineered bacteria comprise a combination of propionate biosynthesis genes from different species, strains, and/or substrains of bacteria, and are capable of producing propionate under inducing conditions. In some embodiments, one or more of the propionate biosynthesis genes is functionally replaced, modified, and/or mutated in order to enhance stability and/or increase propionate production under inducing conditions. In some embodiments, the genetically engineered bacteria are capable of expressing the propionate biosynthesis cassette and producing propionate under inducing conditions.

[0144] In some embodiments, the genetically engineered bacteria of the invention comprise an acetate gene cassette and are capable of producing acetate. The genetically engineered bacteria may include any suitable set of acetate biosynthesis genes. Unmodified bacteria comprising acetate biosynthesis genes are known in the art and are capable of consuming various substrates to produce acetate under aerobic and/or anaerobic conditions (*see, e.g.,* Ragsdale, 2008), and these endogenous acetate biosynthesis pathways may be a source of genes for the genetically engineered bacteria of the invention. In some embodiments, the genetically engineered bacteria of the invention comprise acetate biosynthesis genes from a different species, strain, or substrain of bacteria. In some embodiments, the native acetate biosynthesis genes in the genetically engineered bacteria are enhanced. In some embodiments, the genetically engineered bacteria comprise aerobic acetate biosynthesis genes, e.g., from *Escherichia coli*. In some embodiments, the genetically engineered bacteria comprise anaerobic acetate biosynthesis genes, e.g., from *Acetitomaculum, Acetoanaerobium, Acetohalobium, Acetonema, Balutia, Butyribacterium, Clostridium, Moorella, Oxobacter, Sporomusa,* and/or *Thermoacetogenium*. The genetically engineered bacteria may comprise genes for aerobic acetate biosynthesis or genes for anaerobic or microaerobic acetate biosynthesis. In some embodiments, the genetically engineered bacteria comprise both aerobic and anaerobic or microaerobic acetate biosynthesis genes. In some embodiments, the genetically engineered bacteria comprise a combination of acetate biosynthesis genes from different species, strains, and/or substrains of bacteria, and are capable of producing acetate. In some embodiments, one or more of the acetate biosynthesis genes is functionally replaced, modified, and/or mutated in order to enhance stability and/or acetate production. In some embodiments, the genetically engineered bacteria are capable of expressing the acetate biosynthesis cassette and producing acetate under inducing conditions. In some embodiments, the genetically engineered bacteria are capable of producing an alternate short-chain fatty acid.

[0145] In some embodiments, the genetically engineered bacteria of the invention are capable of producing IL-10. Interleukin-10 (IL-10) is a class 2 cytokine, a category which includes cytokines, interferons, and interferon-like molecules, such as IL-

19, IL-20, IL-22, IL-24, IL-26, IL-28A, IL-28B, IL-29, IFN-α, IFN-β, IFN-δ, IFN-ε, IFN-κ, IFN-τ, IFN-ω, and limitin. IL-10 is an anti-inflammatory cytokine that signals through two receptors, IL-10R1 and IL-10R2. Deficiencies in IL-10 and/or its receptors are associated with IBD and intestinal sensitivity (Nielsen, 2014). Bacteria expressing IL-10 or protease inhibitors may ameliorate conditions such as Crohn's disease and ulcerative colitis (Simpson et al., 2014). The genetically engineered bacteria may comprise any suitable gene encoding IL-10, e.g., human IL-10. In some embodiments, the gene encoding IL-10 is modified and/or mutated, e.g., to enhance stability, increase IL-10 production, and/or increase anti-inflammatory potency under inducing conditions. In some embodiments, the genetically engineered bacteria are capable of producing IL-10 under inducing conditions, e.g., under a condition(s) associated with inflammation. In some embodiments, the genetically engineered bacteria are capable of producing IL-10 in low-oxygen conditions. In some embodiments, the genetically engineered bacteria comprise a nucleic acid sequence that encodes IL-10. In some embodiments, the genetically engineered bacteria comprise a nucleic acid sequence comprising SEQ ID NO: 49 or a functional fragment thereof. In some embodiments, genetically engineered bacteria comprise a nucleic acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homologous to a nucleic acid sequence comprising SEQ ID NO: 49 or a functional fragment thereof.

IL-10 (SEQ ID NO: 49):

ATG AGC CCC GGA CAG GGA ACT CAA AGC GAG AAC AGC TGC ACA CAT TTT CC
A GGTAAT CTT CCA AAT ATG CTT CGT GAC TTG CGT GAC GCT TTC TCT CGCGT
G AAA ACC TTT TTT CAG ATG AAG GAT CAG TTA GAT AAT CTG CTG CTG AAA
GAA TCG CTT CTTGAG GAC TTC AAG GGA TAT CTG GGA TGT CAG GCG TTATCT
GAG ATG ATT CAG TTT TAT TTG GAA GAA GTT ATG CCC CAG GCT GAG AAT CA
A GAC CCT GAC ATC AAA GCGCAT GTG AAT AGC CTG GGC GAG AAT CTGAAG AC
A CTG CGC CTG CGT CTT CGC CGC TGT CAC CGT TTT CTG CCT TGC GAA AAT
AAG AGT AAG GCC GTT GAG CAA GTG AAAAAT GCT TTC AAC AAG TTACAA GAA
AAA GGG ATT TAC AAA GCT ATG TCT GAG TTT GAC ATT TTC ATT AAT TAC AT
T GAG GCC TAC ATG ACT ATG AAG ATT CGC AAT

[0146] In some embodiments, the genetically engineered bacteria are capable of producing IL-2. Interleukin 2 (IL-2) mediates autoimmunity by preserving health of

regulatory T cells (Treg). Treg cells, including those expressing Foxp3, typically suppress effector T cells that are active against self-antigens, and in doing so, can dampen autoimmune activity. IL-2 functions as a cytokine to enhance Treg cell differentiation and activity while diminished IL-2 activity can promote autoimmunity events. IL-2 is generated by activated CD4+ T cells, and by other immune mediators including activated CD8+ T cells, activated dendritic cells, natural killer cells, and NK T cells. IL-2 binds to IL-2R, which is composed of three chains including CD25, CD122, and CD132. IL-2 promotes growth of Treg cells in the thymus, while preserving their function and activity in systemic circulation. Treg cell activity plays an intricate role in the IBD setting, with murine studies suggesting a protective role in disease pathogenesis. In some embodiments, the genetically engineered bacteria comprise a nucleic acid sequence encoding SEQ ID NO: 50 or a functional fragment thereof. In some embodiments, genetically engineered bacteria comprise a nucleic acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homologous to a nucleic acid sequence encoding SEQ ID NO: 50 or a functional fragment thereof. In some embodiments, the genetically engineered bacteria are capable of producing IL-2 under inducing conditions, e.g., under a condition(s) associated with inflammation. In some embodiments, the genetically engineered bacteria are capable of producing IL-2 in low-oxygen conditions.

SEQ ID NO: 50
MAPTSSSTKK TQLQLEHLLL DLQMILNGIN NYKNPKLTRM LTFKFYMPKK ATELKHLQCL EEELKPLEEV LNLAQSKNFH LRPRDLISNI NVIVLELKGS ETTFMCEYAD ETATIVEFLN RWITFCQSII STLT

[0147] In some embodiments, the genetically engineered bacteria are capable of producing IL-22. Interleukin 22 (IL-22) cytokine can be produced by dendritic cells, lymphoid tissue inducer-like cells, natural killer cells and expressed on adaptive lymphocytes. Through initiation of Jak-STAT signaling pathways, IL-22 expression can trigger expression of antimicrobial compounds as well as a range of cell growth related pathways, both of which enhance tissue repair mechanisms. IL-22 is critical in promoting

intestinal barrier fidelity and healing, while modulating inflammatory states. Murine models have demonstrated improved intestinal inflammation states following administration of Il-22. Additionally, IL-22 activates STAT3 signaling to promote enhanced mucus production to preserve barrier function. IL-22's association with IBD susceptibility genes may modulate phenotypic expression of disease as well. In some embodiments, the genetically engineered bacteria comprise a nucleic acid sequence encoding SEQ ID NO: 51 or a functional fragment thereof. In some embodiments, genetically engineered bacteria comprise a nucleic acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homologous to a nucleic acid sequence encoding SEQ ID NO: 51 or a functional fragment thereof. In some embodiments, the genetically engineered bacteria are capable of producing IL-22 under inducing conditions, e.g., under a condition(s) associated with inflammation. In some embodiments, the genetically engineered bacteria are capable of producing IL-22 in low-oxygen conditions.

SEQ ID NO: 51
MAALQKSVSS FLMGTLATSC LLLLALLVQG GAAAPISSHC RLDKSNFQQP YITNRTFMLA KEASLADNNT DVRLIGEKLF HGVSMSERCY LMKQVLNFTL EEVLFPQSDR FQPYMQEVVP FLARLSNRLS TCHIEGDDLH IQRNVQKLKD TVKKLGESGE IKAIGELDLL FMSLRNACI

[0148] In some embodiments, the genetically engineered bacteria are capable of producing IL-27. Interleukin 27 (IL-27) cytokine is predominately expressed by activated antigen presenting cells, while IL-27 receptor is found on a range of cells including T cells, NK cells, among others. In particular, IL-27 suppresses development of pro-inflammatory T helper 17 (Th17) cells, which play a critical role in IBD pathogenesis. Further, IL-27 can promote differentiation of IL-10 producing Tr1 cells and enhance IL-10 output, both of which have anti-inflammatory effects. IL-27 has protective effects on epithelial barrier function via activation of MAPK and STAT signaling within intestinal epithelial cells. Additionally, IL-27 enhances production of antibacterial proteins that curb bacterial growth. Improvement in barrier function and reduction in bacterial growth suggest a favorable role for IL-27 in IBD pathogenesis. In some embodiments, the genetically

engineered bacteria comprise a nucleic acid sequence encoding SEQ ID NO: 52 or a functional fragment thereof. In some embodiments, genetically engineered bacteria comprise a nucleic acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homologous to a nucleic acid sequence encoding SEQ ID NO: 52 or a functional fragment thereof. In some embodiments, the genetically engineered bacteria are capable of producing IL-27 under inducing conditions, e.g., under a condition(s) associated with inflammation. In some embodiments, the genetically engineered bacteria are capable of producing IL-27 in low-oxygen conditions.

SEQ ID NO: 52
MGQTAGDLGW RLSLLLLPLL LVQAGVWGFP RPPGRPQLSL QELRREFTVS
LHLARKLLSE VRGQAHRFAE SHLPGVNLYL LPLGEQLPDV SLTFQAWRRL
SDPERLCFIS TTLQPFHALL GGLGTQGRWT NMERMQLWAM RLDLRDLQRH
LRFQVLAAGF NLPEEEEEEE EEEEEERKGL LPGALGSALQ GPAQVSWPQL
LSTYRLLHSL ELVLSRAVRE LLLLSKAGHS VWPLGFPTLS PQP

[0149] In some embodiments, the genetically engineered bacteria of the invention are capable of producing SOD. Increased ROS levels contribute to pathophysiology of inflammatory bowel disease. Increased ROS levels may lead to enhanced expression of vascular cell adhesion molecule 1 (VCAM-1), which can facilitate translocation of inflammatory mediators to disease affected tissue, and result in a greater degree of inflammatory burden. Antioxidant systems including superoxide dismutase (SOD) can function to mitigate overall ROS burden. However, studies indicate that the expression of SOD in the setting of IBD may be compromised, e.g., produced at lower levels in IBD, thus allowing disease pathology to proceed. Further studies have shown that supplementation with SOD to rats within a colitis model is associated with reduced colonic lipid peroxidation and endothelial VCAM-1 expression as well as overall improvement in inflammatory environment. Thus, in some embodiments, the genetically engineered bacteria comprise a nucleic acid sequence encoding SEQ ID NO: 52 or a functional fragment thereof. In some embodiments, genetically engineered bacteria comprise a nucleic acid sequence that is at least about 80%, at least about 85%, at least

about 90%, at least about 95%, or at least about 99% homologous to a nucleic acid sequence encoding SEQ ID NO: 53 or a functional fragment thereof. In some embodiments, the genetically engineered bacteria are capable of producing SOD under inducing conditions, e.g., under a condition(s) associated with inflammation. In some embodiments, the genetically engineered bacteria are capable of producing SOD in low-oxygen conditions.

SEQ ID NO: 53
MATKAVCVLK GDGPVQGIIN FEQKESNGPV KVWGSIKGLT EGLHGFHVHE FGDNTAGCTS AGPHFNPLSR KHGGPKDEER HVGDLGNVTA DKDGVADVSI EDSVISLSGD HCIIGRTLVV HEKADDLGKG GNEESTKTGN AGSRLACGVI GIAQ

[0150] In some embodiments, the genetically engineered bacteria are capable of producing GLP-2 or proglucagon. Glucagon-like peptide 2 (GLP-2) is produced by intestinal endocrine cells and stimulates intestinal growth and enhances gut barrier function. GLP-2 administration has therapeutic potential in treating IBD, short bowel syndrome, and small bowel enteritis (Yazbeck et al., 2009). The genetically engineered bacteria may comprise any suitable gene encoding GLP-2 or proglucagon, e.g., human GLP-2 or proglucagon. In some embodiments, a protease inhibitor, e.g., an inhibitor of dipeptidyl peptidase, is also administered to decrease GLP-2 degradation. In some embodiments, the genetically engineered bacteria express a degradation resistant GLP-2 analog, e.g., Teduglutide (Yazbeck et al., 2009). In some embodiments, the gene encoding GLP-2 or proglucagon is modified and/or mutated, e.g., to enhance stability, increase GLP-2 production, and/or increase gut barrier enhancing potency under inducing conditions. In some embodiments, the genetically engineered bacteria of the invention are capable of producing GLP-2 or proglucagon under inducing conditions. GLP-2 administration in a murine model of IBD is associated with reduced mucosal damage and inflammation, as well as a reduction in inflammatory mediators, such as TNF-α and IFN-γ. Further, GLP-2 supplementation may also lead to reduced mucosal myeloperoxidase in colitis/ileitis models. In some embodiments, the genetically engineered bacteria comprise a nucleic acid sequence encoding SEQ ID NO: 54 or a

functional fragment thereof. In some embodiments, genetically engineered bacteria comprise a nucleic acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homologous to a nucleic acid sequence encoding SEQ ID NO: 54 or a functional fragment thereof. In some embodiments, the genetically engineered bacteria are capable of producing GLP-2 under inducing conditions, e.g., under a condition(s) associated with inflammation. In some embodiments, the genetically engineered bacteria are capable of producing GLP-2 in low-oxygen conditions.

SEQ ID NO: 54
HADGSFSDEMNTILDNLAARDFINWLIQTKITD

[0151] In some embodiments, the genetically engineered bacteria are capable of producing kynurenine. Kynurenine is a metabolite produced in the first, rate-limiting step of tryptophan catabolism. This step involves the conversion of tryptophan to kynurenine, and may be catalyzed by the ubiquitously-expressed enzyme indoleamine 2,3-dioxygenase (IDO-1), or by tryptophan dioxygenase (TDO), an enzyme which is primarily localized to the liver (Alvarado et al., 2015). Biopsies from human patients with IBD show elevated levels of IDO-1 expression compared to biopsies from healthy individuals, particularly near sites of ulceration (Ferdinande et al., 2008; Wolf et al., 2004). IDO-1 enzyme expression is similarly upregulated in trinitrobenzene sulfonic acid- and dextran sodium sulfate-induced mouse models of IBD; inhibition of IDO-1 significantly augments the inflammatory response caused by each inducer (Ciorba et al., 2010; Gurtner et al., 2003; Matteoli et al., 2010). Kynurenine has also been shown to directly induce apoptosis in neutrophils (El-Zaatari et al., 2014). Together, these observations suggest that IDO-1 and kynurenine play a role in limiting inflammation. The genetically engineered bacteria may comprise any suitable gene for producing kynurenine. In some embodiments, the genetically engineered bacteria may comprise a gene or gene cassette for producing a tryptophan transporter, a gene or gene cassette for producing IDO-1, and a gene or gene cassette for producing TDO. In some embodiments, the gene for producing kynurenine is modified and/or mutated, e.g., to enhance stability, increase kynurenine production, and/or increase anti-inflammatory

potency under inducing conditions. In some embodiments, the engineered bacteria have enhanced uptake or import of tryptophan, e.g., comprise a transporter or other mechanism for increasing the uptake of tryptophan into the bacterial cell. In some embodiments, the genetically engineered bacteria are capable of producing kynurenine under inducing conditions, e.g., under a condition(s) associated with inflammation. In some embodiments, the genetically engineered bacteria are capable of producing kynurenine in low-oxygen conditions.

[0152] In some embodiments, the genetically engineered bacteria are capable of producing kynurenic acid. Kynurenic acid is produced from the irreversible transamination of kynurenine in a reaction catalyzed by the enzyme kynurenine-oxoglutarate transaminase. Kynurenic acid acts as an antagonist of ionotropic glutamate receptors (Turski et al., 2013). While glutamate is known to be a major excitatory neurotransmitter in the central nervous system, there is now evidence to suggest an additional role for glutamate in the peripheral nervous system. For example, the activation of NMDA glutamate receptors in the major nerve supply to the GI tract (i.e., the myenteric plexus) leads to an increase in gut motility (Forrest et al., 2003), but rats treated with kynurenic acid exhibit decreased gut motility and inflammation in the early phase of acute colitis (Varga et al., 2010). Thus, the elevated levels of kynurenic acid reported in IBD patients may represent a compensatory response to the increased activation of enteric neurons (Forrest et al., 2003). The genetically engineered bacteria may comprise any suitable gene for producing kynurenic acid. In some embodiments, the gene for producing kynurenic acid is modified and/or mutated, e.g., to enhance stability, increase kynurenic acid production, and/or increase anti-inflammatory potency under inducing conditions. In some embodiments, the genetically engineered bacteria are capable of producing kynurenic acid under inducing conditions, e.g., under a condition(s) associated with inflammation. In some embodiments, the genetically engineered bacteria are capable of producing kynurenic acid in low-oxygen conditions.

[0153] In some embodiments, the genetically engineered bacteria are capable of producing IL-19, IL-20, and/or IL-24. In some embodiments, the genetically engineered bacteria are capable of producing IL-19, IL-20, and/or IL-24 under inducing conditions,

e.g., under a condition(s) associated with inflammation. In some embodiments, the genetically engineered bacteria are capable of producing IL-19, IL-20 and/or IL-24 in low-oxygen conditions.

[0154] In some embodiments, the genetically engineered bacteria of the invention are capable of producing a molecule that is capable of inhibiting a pro-inflammatory molecule. The genetically engineered bacteria may express any suitable inhibitory molecule, e.g., a single-chain variable fragment (scFv), antisense RNA, siRNA, or shRNA, that is capable of neutralizing one or more pro-inflammatory molecules, e.g., TNF, IFN-γ, IL-1β, IL-6, IL-8, IL-17, IL-18, IL-21, IL-23, IL-26, IL-32, Arachidonic acid, prostaglandins (e.g., PGE_2), PGI_2, serotonin, thromboxanes (e.g., TXA_2), leukotrienes (e.g., LTB_4), hepoxillin A_3, or chemokines (Keates et al., 2008; Ahmad et al., 2012). The genetically engineered bacteria may inhibit one or more pro-inflammatory molecules, e.g., TNF, IL-17. In some embodiments, the genetically engineered bacteria are capable of modulating one or more molecule(s) shown in Table 8. In some embodiments, the genetically engineered bacteria are capable of inhibiting, removing, degrading, and/or metabolizing one or more inflammatory molecules.

Table 8

Metabolites	**Related bacteria**	**Potential biological functions**
Bile acids: cholate, hyocholate, deoxycholate, chenodeoxycholate, a-muricholate, b-muricholate, w-muricholate, taurocholate, glycocholate, taurochenoxycholate, glycochenodeoxycholate, taurocholate, tauro-a-muricholate, tauro-b-muricholate, lithocholate, ursodeoxycholate, hyodeoxycholate, glycodeoxylcholate	Lactobacillus, Bifidobacteria, Enterobacter, Bacteroides, Clostridium	Absorb dietary fats and lipid-soluble vitamins, facilitate lipid absorption, maintain intestinal barrier function, signal systemic endocrine functions to regulate triglycerides, cholesterol, glucose and energy homeostasis.
Choline metabolites: methylamine, dimethylamine, trimethylamine, trimethylamine-N-oxide, dimethylglycine, betaine	Faecalibacterium prausnitzii, Bifidobacterium	Modulate lipid metabolism and glucose homeostasis. Involved in nonalcoholic fatty liver disease, dietary induced obesity, diabetes, and cardiovascular disease.
Phenolic, benzoyl, and phenyl derivatives: benzoic acid, hippuric acid, 2-hydroxyhippuric acid, 2-hydroxybenzoic acid, 3-	Clostridium difficile, F. prausnitzii, Bifidobacterium, Subdoligranulum,	Detoxification of xenobiotics; indicate gut microbial composition and activity; utilize polyphenols. Urinary hippuric acid may be a biomarker of hypertension and

hydroxyhippuric acid, 3-hydroxybenzoic acid, 4 hydroxybenzoic acid, 3hydroxyphenylpropionate, 4-hydroxyphenylpropionate, 3-hydroxycinnamate, 4-methylphenol, tyrosine, phenylalanine, 4-cresol, 4-cresyl sulfate, 4-cresyl glucuronide, 4-hydroxyphenylacetate	Lactobacillus	obesity in humans. Urinary 4-hydroxyphenylacetate, 4-cresol, and phenylacetate are elevated in colorectal cancer. Urinary 4-cresyl sulfate is elevated in children with severe autism.
Indole derivatives: N-acetyltryptophan, indoleacetate, indoleacetylglycine (IAG), indole, indoxyl sulfate, indole-3-propionate, melatonin, melatonin 6-sulfate, serotonin, 5-hydroxyindole	Clostridium sporogenes, E. coli	Protect against stress-induced lesions in the GI tract; modulate expression of proinflammatory genes, increase expression of anti-inflammatory genes, strengthen epithelial cell barrier properties. Implicated in GI pathologies, brain-gut axis, and a few neurological conditions.
Vitamins: vitamin K, vitamin B12, biotin, folate, thiamine, riboflavin, pyridoxine	Bifidobacterium	Provide complementary endogenous sources of vitamins, strengthen immune function, exert epigenetic effects to regulate cell proliferation.
Polyamines: putrescine, cadaverine, spermidine, spermine	Campylobacter jejuni, Clostridium saccharolyticum	Exert genotoxic effects on the host, anti-inflammatory and antitumoral effects. Potential tumor markers.
Lipids: conjugated fatty acids, LPS, peptidoglycan, acylglycerols, sphingomyelin, cholesterol, phosphatidylcholines, phosphoethanolamines, triglycerides	Bifidobacterium, Roseburia, Lactobacillus, Klebsiella, Enterobacter, Citrobacter, Clostridium	Impact intestinal permeability, activate intestinebrain- liver neural axis to regulate glucose homeostasis; LPS induces chronic systemic inflammation; conjugated fatty acids improve hyperinsulinemia, enhance the immune system and alter lipoprotein profiles.
Others: D-lactate, formate, methanol, ethanol, succinate, lysine, glucose, urea, a-ketoisovalerate, creatine, creatinine, endocannabinoids, 2-arachidonoylglycerol (2-AG), N-arachidonoylethanolamide, LPS	Bacteroides, Pseudobutyrivibrio, Ruminococcus, Faecalibacterium	Direct or indirect synthesis or utilization of compounds or modulation of linked pathways including endocannabinoid system.

[0155] In some embodiments, the genetically engineered bacteria are capable of producing an anti-inflammation and/or gut barrier enhancer molecule and further producing a molecule that is capable of inhibiting an inflammatory molecule. In some embodiments, the genetically engineered bacteria of the invention are capable of producing an anti-inflammation and/or gut barrier enhancer molecule and further

producing an enzyme that is capable of degrading an inflammatory molecule. For example, the genetically engineered bacteria of the invention are capable of expressing a gene cassette for producing butyrate, as well as a molecule or biosynthetic pathway for inhibiting, removing, degrading, and/or metabolizing an inflammatory molecule, e.g., PGE_2.

[0156] RNA interference (RNAi) is a post-transcriptional gene silencing mechanism in plants and animals. RNAi is activated when microRNA (miRNA), double-stranded RNA (dsRNA), or short hairpin RNA (shRNA) is processed into short interfering RNA (siRNA) duplexes (Keates et al., 2008). RNAi can be "activated *in vitro* and *in vivo* by non-pathogenic bacteria engineered to manufacture and deliver shRNA to target cells" such as mammalian cells (Keates et al., 2008). In some embodiments, the genetically engineered bacteria of the invention induce RNAi-mediated gene silencing of one or more pro-inflammatory molecules in low-oxygen conditions. In some embodiments, the genetically engineered bacteria produce siRNA targeting TNF in low-oxygen conditions.

[0157] Single-chain variable fragments (scFv) are "widely used antibody fragments… produced in prokaryotes" (Frenzel et al., 2013). scFv lacks the constant domain of a traditional antibody and expresses the antigen-binding domain as a single peptide. Bacteria such as *Escherichia coli* are capable of producing scFv that target pro-inflammatory cytokines, e.g., TNF (Hristodorov et al., 2014). In some embodiments, the genetically engineered bacteria of the invention express a binding protein for neutralizing one or more pro-inflammatory molecules in low-oxygen conditions. In some embodiments, the genetically engineered bacteria produce scFv targeting TNF in low-oxygen conditions. In some embodiments, the genetically engineered bacteria produce both scFv and siRNA targeting one or more pro-inflammatory molecules in low-oxygen conditions (*see, e.g.,* Xiao et al., 2014).

[0158] One of skill in the art would appreciate that additional genes and gene cassettes capable of producing anti-inflammation and/or gut barrier function enhancer molecules are known in the art and may be expressed by the genetically engineered bacteria of the invention. In some embodiments, the gene or gene cassette for producing a therapeutic molecule also comprises additional transcription and translation

elements, e.g., a ribosome binding site, to enhance expression of the therapeutic molecule.

[0159] In some embodiments, the genetically engineered bacteria produce two or more anti-inflammation and/or gut barrier function enhancer molecules. In certain embodiments, the two or more molecules behave synergistically to reduce gut inflammation and/or enhance gut barrier function. In some embodiments, the genetically engineered bacteria express at least one anti-inflammation molecule and at least one gut barrier function enhancer molecule. In certain embodiments, the genetically engineered bacteria express IL-10 and GLP-2. In alternate embodiments, the genetically engineered bacteria express IL-10 and butyrate.

[0160] In some embodiments, the genetically engineered bacteria are capable of producing IL-2, IL-10, IL-22, IL-27, propionate, and butyrate. In some embodiments, the genetically engineered bacteria are capable of producing IL-10, IL-27, GLP-2, and butyrate. In some embodiments, the genetically engineered bacteria are capable of producing GLP-2, IL-10, IL-22, SOD, butyrate, and propionate. In some embodiments, the genetically engineered bacteria are capable of GLP-2, IL-2, IL-10, IL-22, IL-27, SOD, butyrate, and propionate. Any suitable combination of therapeutic molecules may be produced by the genetically engineered bacteria.

Inducible regulatory regions

Oxygen level-dependent regulation

[0161] The genetically engineered bacteria of the invention comprise a promoter that is directly or indirectly induced by exogenous environmental conditions. In some embodiments, a gene or gene cassette for producing an anti-inflammation and/or gut barrier function enhancer molecule is operably linked to an oxygen level-dependent promoter or regulatory region comprising said promoter. In some embodiments, the gene or gene cassette is operably linked to an oxygen level-dependent promoter such that the therapeutic molecule is expressed in low-oxygen, microaerobic, or anaerobic conditions. For example, in low-oxygen conditions, the oxygen level-dependent promoter is activated by a corresponding oxygen level-sensing transcription factor, thereby driving production of the therapeutic molecule.

[0162] In certain embodiments, the genetically engineered bacteria comprise a gene or a gene cassette for producing an anti-inflammation and/or gut barrier function enhancer molecule expressed under the control of a fumarate and nitrate reductase regulator (FNR)-responsive promoter, an anaerobic regulation of arginine deiminiase and nitrate reduction (ANR)-responsive promoter, or a dissimilatory nitrate respiration regulator (DNR)-responsive promoter, which are capable of being regulated by the transcription factors FNR, ANR, or DNR, respectively.

[0163] In certain embodiments, the genetically engineered bacteria comprise a FNR-responsive promoter. In *E. coli,* FNR is a major transcriptional activator that controls the switch from aerobic to anaerobic metabolism (Unden et al., 1997). In the anaerobic state, FNR dimerizes into an active DNA binding protein that activates hundreds of genes responsible for adapting to anaerobic growth. In the aerobic state, FNR is prevented from dimerizing by oxygen and is inactive. In some embodiments, multiple distinct FNR nucleic acid sequences are inserted in the genetically engineered bacteria.

[0164] In alternate embodiments, the promoter is an alternate oxygen level-dependent promoter, e.g., DNR (Trunk et al., 2010) or ANR (Ray et al., 1997). In *P. aeruginosa,* the anaerobic regulation of ANR is "required for the expression of physiological functions which are inducible under oxygen-limiting or anaerobic conditions" (Sawers, 1991; Winteler et al., 1996). *P. aeruginosa* ANR is homologous with *E. coli* FNR, and "the consensus FNR site (TTGAT----ATCAA) was recognized efficiently by ANR and FNR" (Winteler et al., 1996). Like FNR, in the anaerobic state, ANR activates numerous genes responsible for adapting to anaerobic growth. In the aerobic state, ANR is inactive. *Pseudomonas fluorescens, Pseudomonas putida, Pseudomonas syringae*, and *Pseudomonas mendocina* all have functional analogs of ANR (Zimmermann et al., 1991). Promoters that are regulated by ANR are known in the art, e.g., the promoter of the *arcDABC* operon (*see, e.g.,* Hasegawa et al., 1998).

[0165] The FNR family also includes the dissimilatory nitrate respiration regulator (DNR) (Arai et al., 1995), a transcription factor which is required in conjunction with ANR for "anaerobic nitrate respiration of *Pseudomonas aeruginosa*" (Hasegawa et al., 1998). For certain genes, the FNR-binding motifs "are probably recognized only by DNR"

(Hasegawa et al., 1998). In some embodiments, gene expression is further optimized by methods known in the art, e.g., by optimizing ribosomal binding sites and/or increasing mRNA stability.

[0166] FNR promoter sequences are known in the art, and any suitable FNR promoter sequence(s) may be used in the genetically engineered bacteria of the invention. Any suitable FNR promoter(s) may be combined with any suitable gene or gene cassette for producing an anti-inflammation and/or gut barrier function enhancer molecule. Non-limiting FNR promoter sequences are provided in **Table 9**. In some embodiments, the genetically engineered bacteria of the invention comprise one or more of: SEQ ID NO: 55, SEQ ID NO: 56, *nirB1* promoter (SEQ ID NO: 57), *nirB2* promoter (SEQ ID NO: 58), *nirB3* promoter (SEQ ID NO: 59), *ydfZ* promoter (SEQ ID NO: 60), *nirB* promoter fused to a strong ribosome binding site (SEQ ID NO: 61), *ydfZ* promoter fused to a strong ribosome binding site (SEQ ID NO: 62), *fnrS*, an anaerobically induced small RNA gene (*fnrS1* promoter SEQ ID NO: 63 or *fnrS2* promoter SEQ ID NO: 64), *nirB* promoter fused to a *crp* binding site (SEQ ID NO: 65), and *fnrS* fused to a *crp* binding site (SEQ ID NO: 66). In some embodiments, genetically engineered bacteria comprise a nucleic acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homologous to the DNA sequence of SEQ ID NO: 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, or 66, or a functional fragment thereof.

Table 9

FNR-responsive regulatory region	12345678901234567890123456789012345678901234567890
SEQ ID NO: 55	ATCCCCATCACTCTTGATGGAGATCAATTCCCCAAGCTGCTAGAGCGTTACCTTGCCCTTAAACATTAGCAATGTCGATTTATCAGAGGGCCGACAGGCTCCCACAGGAGAAAACCG
SEQ ID NO: 56	CTCTTGATCGTTATCAATTCCCACGCTGTTTCAGAGCGTTACCTTGCCCTTAAACATTAGCAATGTCGATTTATCAGAGGGCCGACAGGCTCCCACAGGAGAAAACCG

nirB1 SEQ ID NO: 57	GTCAGCATAACACCCTGACCTCTCATTAATTGTTCATGCCGGGCGGCACT ATCGTCGTCCGGCCTTTTCCTCTCTTACTCTGCTACGTACATCTATTTCT ATAAATCCGTTCAATTTGTCTGTTTTTTGCACAAACATGAAATATCAGAC AATTCCGTGACTTAAGAAAATTTATACAAATCAGCAATATACCCCTTAAG GAGTATATAAAGGTGAATTTGATTTACATCAATAAGCGGGGTTGCTGAAT CGTTAAGGTAGGCGGTAATAGAAAAGAAATCGAGGCAAAA
nirB2 SEQ ID NO: 58	CGGCCCGATCGTTGAACATAGCGGTCCGCAGGCGGCACTGCTTACAGCAA ACGGTCTGTACGCTGTCGTCTTTGTGATGTGCTTCCTGTTAGGTTTCGTC AGCCGTCACCGTCAGCATAACACCCTGACCTCTCATTAATTGCTCATGCC GGACGGCACTATCGTCGTCCGGCCTTTTCCTCTCTTCCCCCGCTACGTGC ATCTATTTCTATAAACCCGCTCATTTTGTCTATTTTTTGCACAAACATGA AATATCAGACAATTCCGTGACTTAAGAAAATTTATACAAATCAGCAATAT ACCCATTAAGGAGTATATAAAGGTGAATTTGATTTACATCAATAAGCGGG GTTGCTGAATCGTTAAGGTAGGCGGTAATAGAAAAGAAATCGAGGCAAAA atgtttgtttaactttaagaaggagatatacat
nirB3 SEQ ID NO: 59	GTCAGCATAACACCCTGACCTCTCATTAATTGCTCATGCCGGACGGCACT ATCGTCGTCCGGCCTTTTCCTCTCTTCCCCCGCTACGTGCATCTATTTCT ATAAACCCGCTCATTTTGTCTATTTTTTGCACAAACATGAAATATCAGAC AATTCCGTGACTTAAGAAAATTTATACAAATCAGCAATATACCCATTAAG GAGTATATAAAGGTGAATTTGATTTACATCAATAAGCGGGGTTGCTGAAT CGTTAAGGTAGGCGGTAATAGAAAAGAAATCGAGGCAAAA
ydfZ SEQ ID NO: 60	ATTTCCTCTCATCCCATCCGGGGTGAGAGTCTTTTCCCCCGACTTATGGC TCATGCATGCATCAAAAAAGATGTGAGCTTGATCAAAAACAAAAAATATT TCACTCGACAGGAGTATTTATATTGCGCCCGTTACGTGGGCTTCGACTGT AAATCAGAAAGGAGAAAACACCT
nirB+RBS SEQ ID NO: 61	GTCAGCATAACACCCTGACCTCTCATTAATTGTTCATGCCGGGCGGCACT ATCGTCGTCCGGCCTTTTCCTCTCTTACTCTGCTACGTACATCTATTTCT ATAAATCCGTTCAATTTGTCTGTTTTTTGCACAAACATGAAATATCAGAC AATTCCGTGACTTAAGAAAATTTATACAAATCAGCAATATACCCCTTAAG GAGTATATAAAGGTGAATTTGATTTACATCAATAAGCGGGGTTGCTGAAT CGTTAA**GGATCC**CTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATA TACAT
ydfZ+RBS SEQ ID NO: 62	CATTTCCTCTCATCCCATCCGGGGTGAGAGTCTTTTCCCCCGACTTATGG CTCATGCATGCATCAAAAAAGATGTGAGCTTGATCAAAAACAAAAAATAT TTCACTCGACAGGAGTATTTATATTGCGCCC**GGATCC**CTCTAGAAATAAT TTTGTTTAACTTTAAGAAGGAGATATACAT

fnrS1 SEQ ID NO: 63	AGTTGTTCTTATTGGTGGTGTTGCTTTATGGTTGCATCGTAGTAAATGGT TGTAACAAAAGCAATTTTTCCGGCTGTCTGTATACAAAAACGCCGTAAAG TTTGAGCGAAGTCAATAAACTCTCTACCCATTCAGGGCAATATCTCTCTT **GGATCC**CTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACAT
fnrS2 SEQ ID NO: 64	AGTTGTTCTTATTGGTGGTGTTGCTTTATGGTTGCATCGTAGTAAATGGT TGTAACAAAAGCAATTTTTCCGGCTGTCTGTATACAAAAACGCCGCAAAG TTTGAGCGAAGTCAATAAACTCTCTACCCATTCAGGGCAATATCTCTCTT GGATCCAAAGTGAACTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGA TATACAT
nirB+crp SEQ ID NO: 65	TCGTCTTTGTGATGTGCTTCCTGTTAGGTTTCGTCAGCCGTCACCGTCAG CATAACACCCTGACCTCTCATTAATTGCTCATGCCGGACGGCACTATCGT CGTCCGGCCTTTTCCTCTCTTCCCCCGCTACGTGCATCTATTTCTATAAA CCCGCTCATTTTGTCTATTTTTTGCACAAACATGAAATATCAGACAATTC CGTGACTTAAGAAAATTTATACAAATCAGCAATATACCCATTAAGGAGTA TATAAAGGTGAATTTGATTTACATCAATAAGCGGGGTTGCTGAATCGTTA AGGTAGaaatgtgatctagttcacatttGCGGTAATAGAAAAGAAATCGA GGCAAAA*atgtttgtttaactttaagaaggagatatacat*
fnrS+crp SEQ ID NO: 66	AGTTGTTCTTATTGGTGGTGTTGCTTTATGGTTGCATCGTAGTAAATGGT TGTAACAAAAGCAATTTTTCCGGCTGTCTGTATACAAAAACGCCGCAAAG TTTGAGCGAAGTCAATAAACTCTCTACCCATTCAGGGCAATATCTCTCaa atgtgatctagttcacatttttgtttaactttaagaaggagatatacat

[0167] In other embodiments, the gene or gene cassette for producing an anti-inflammation and/or gut barrier function enhancer molecule is expressed under the control of an oxygen level-dependent promoter fused to a binding site for a transcriptional activator, e.g., CRP. CRP (cyclic AMP receptor protein or catabolite activator protein or CAP) plays a major regulatory role in bacteria by repressing genes responsible for the uptake, metabolism, and assimilation of less favorable carbon sources when rapidly metabolizable carbohydrates, such as glucose, are present (Wu et al., 2015). This preference for glucose has been termed glucose repression, as well as carbon catabolite repression (Deutscher, 2008; Görke and Stülke, 2008). In some embodiments, the gene or gene cassette for producing an anti-inflammation and/or gut barrier function enhancer molecule is controlled by an oxygen level-dependent promoter fused to a CRP binding site. In some embodiments, the gene or gene cassette for producing an anti-

inflammation and/or gut barrier function enhancer molecule is controlled by a FNR promoter fused to a CRP binding site. In these embodiments, cyclic AMP binds to CRP when no glucose is present in the environment. This binding causes a conformational change in CRP, and allows CRP to bind tightly to its binding site. CRP binding then activates transcription of the gene or gene cassette by recruiting RNA polymerase to the FNR promoter via direct protein-protein interactions. In the presence of glucose, cyclic AMP does not bind to CRP and transcription of the gene or gene cassette for producing an anti-inflammation and/or gut barrier function enhancer molecule is repressed. In some embodiments, an oxygen level-dependent promoter (e.g., an FNR promoter) fused to a binding site for a transcriptional activator is used to ensure that the gene or gene cassette for producing an anti-inflammation and/or gut barrier function enhancer molecule is not expressed under anaerobic conditions when sufficient amounts of glucose are present, e.g., by adding glucose to growth media *in vitro*.

[0168] In some embodiments, the genetically engineered bacteria comprise an oxygen level-dependent promoter from a different species, strain, or substrain of bacteria. In some embodiments, the genetically engineered bacteria comprise an oxygen level-sensing transcription factor, e.g., FNR, ANR or DNR, from a different species, strain, or substrain of bacteria. In some embodiments, the genetically engineered bacteria comprise an oxygen level-sensing transcription factor and corresponding promoter from a different species, strain, or substrain of bacteria. The heterologous oxygen level-dependent transcription factor and/or promoter may increase the production of the anti-inflammation and/or gut barrier enhancer molecule in low-oxygen conditions, as compared to the native transcription factor and promoter in the bacteria under the same conditions. In certain embodiments, the non-native oxygen level-dependent transcription factor is a FNR protein from *N. gonorrhoeae* (*see, e.g.,* Isabella et al., 2011). In some embodiments, the corresponding wild-type transcription factor is deleted or mutated to reduce or eliminate wild-type activity. In alternate embodiments, the corresponding wild-type transcription factor is left intact and retains wild-type activity. In some embodiments, the heterologous transcription factor minimizes or eliminates off-

target effects on endogenous regulatory regions and genes in the genetically engineered bacteria.

[0169] In some embodiments, the genetically engineered bacteria comprise a wild-type gene encoding an oxygen level-dependent transcription factor, such as FNR, ANR or DNR, and a corresponding promoter that is mutated relative to the wild-type promoter from bacteria of the same subtype. The mutated promoter increases the production of an anti-inflammation and/or gut barrier enhancer molecule in low-oxygen conditions, as compared to the wild-type promoter under the same conditions. In some embodiments, the genetically engineered bacteria comprise a wild-type oxygen level-dependent promoter, e.g., a FNR-, ANR- or DNR-responsive promoter, and a corresponding transcription factor that is mutated relative to the wild-type transcription factor from bacteria of the same subtype. The mutant transcription factor increases the expression of the anti-inflammation and/or gut barrier enhancer molecule in low-oxygen conditions, as compared to the wild-type transcription factor under the same conditions. In certain embodiments, the mutant oxygen level-dependent transcription factor is a FNR protein comprising amino acid substitutions that enhance dimerization and FNR activity (*see, e.g.,* Moore et al., 2006). In some embodiments, both the oxygen level-sensing transcription factor and corresponding promoter are mutated relative to the wild-type sequences from bacteria of the same subtype in order to increase expression of the anti-inflammation and/or gut barrier enhancer molecule in low-oxygen conditions.

[0170] In some embodiments, the genetically engineered bacteria of the invention comprise a gene encoding an oxygen level-sensing transcription factor, e.g., FNR, ANR or DNR, that is controlled by its native promoter, an inducible promoter, a promoter that is stronger than the native promoter, e.g., a GlnRS promoter, a P(Bla) promoter, or a constitutive promoter. In some instances, it may be advantageous to express the oxygen level-dependent transcription factor under the control of an inducible promoter in order to enhance expression stability. In some embodiments, expression of the oxygen level-dependent transcription factor is controlled by a different promoter than the promoter that controls expression of the therapeutic molecule. In some embodiments, expression of the oxygen level-dependent transcription factor is

controlled by the same promoter that controls expression of the therapeutic molecule. In some embodiments, the oxygen level-dependent transcription factor and therapeutic molecule are divergently transcribed from a promoter region.

[0171] In some embodiments, the genetically engineered bacteria of the invention comprise multiple copies of the endogenous gene encoding the oxygen level-sensing transcription factor, e.g., the *fnr* gene. In some embodiments, the gene encoding the oxygen level-sensing transcription factor is present on a plasmid. In some embodiments, the gene encoding the oxygen level-sensing transcription factor and the gene or gene cassette for producing the therapeutic molecule are present on different plasmids. In some embodiments, the gene encoding the oxygen level-sensing transcription factor and the gene or gene cassette for producing the therapeutic molecule are present on the same plasmid. In some embodiments, the gene encoding the oxygen level-sensing transcription factor is present on a chromosome. In some embodiments, the gene encoding the oxygen level-sensing transcription factor and the gene or gene cassette for producing the therapeutic molecule are present on different chromosomes. In some embodiments, the gene encoding the oxygen level-sensing transcription factor and the gene or gene cassette for producing the therapeutic molecule are present on the same chromosome.

[0172] In some embodiments, the gene or gene cassette for producing the anti-inflammation and/or gut barrier function enhancer molecule is present on a plasmid and operably linked to a promoter that is induced by low-oxygen conditions. In some embodiments, the gene or gene cassette for producing the anti-inflammation and/or gut barrier function enhancer molecule is present in the chromosome and operably linked to a promoter that is induced by low-oxygen conditions. In some embodiments, the gene or gene cassette for producing the anti-inflammation and/or gut barrier function enhancer molecule is present on a chromosome and operably linked to a promoter that is induced by exposure to tetracycline. In some embodiments, the gene or gene cassette for producing the anti-inflammation and/or gut barrier function enhancer molecule is present on a plasmid and operably linked to a promoter that is induced by exposure to tetracycline. In some embodiments, expression is further optimized by methods known

in the art, e.g., by optimizing ribosomal binding sites, manipulating transcriptional regulators, and/or increasing mRNA stability.

[0173] In some embodiments, the genetically engineered bacteria comprise a stably maintained plasmid or chromosome carrying the gene(s) or gene cassette(s) capable of producing an anti-inflammation and/or gut barrier function enhancer molecule, such that the gene(s) or gene cassette(s) can be expressed in the host cell, and the host cell is capable of survival and/or growth *in vitro*, e.g., in medium, and/or *in vivo*, e.g., in the gut. In some embodiments, a bacterium may comprise multiple copies of the gene or gene cassette for producing the anti-inflammation and/or gut barrier function enhance molecule. In some embodiments, the gene or gene cassette is expressed on a low-copy plasmid. In some embodiments, the low-copy plasmid may be useful for increasing stability of expression. In some embodiments, the low-copy plasmid may be useful for decreasing leaky expression under non-inducing conditions. In some embodiments, the gene or gene cassette is expressed on a high-copy plasmid. In some embodiments, the high-copy plasmid may be useful for increasing gene or gene cassette expression. In some embodiments, gene or gene cassette is expressed on a chromosome.

[0174] In some embodiments, the genetically engineered bacteria may comprise multiple copies of the gene(s) or gene cassette(s) capable of producing an anti-inflammation and/or gut barrier function enhancer molecule. In some embodiments, the gene(s) or gene cassette(s) capable of producing an anti-inflammation and/or gut barrier function enhancer molecule is present on a plasmid and operably linked to an oxygen level-dependent promoter. In some embodiments, the gene(s) or gene cassette(s) capable of producing an anti-inflammation and/or gut barrier function enhancer molecule is present in a chromosome and operably linked to an oxygen level-dependent promoter.

[0175] In some embodiments, the genetically engineered bacteria of the invention produce at least one anti-inflammation and/or gut barrier enhancer molecule in low-oxygen conditions to reduce local gut inflammation by at least about 1.5-fold, at least about 2-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at

least about 30-fold, at least about 50-fold, at least about 100-fold, at least about 200-fold, at least about 300-fold, at least about 400-fold, at least about 500-fold, at least about 600-fold, at least about 700-fold, at least about 800-fold, at least about 900-fold, at least about 1,000-fold, or at least about 1,500-fold as compared to unmodified bacteria of the same subtype under the same conditions. Inflammation may be measured by methods known in the art, e.g., counting disease lesions using endoscopy; detecting T regulatory cell differentiation in peripheral blood, e.g., by fluorescence activated sorting; measuring T regulatory cell levels; measuring cytokine levels; measuring areas of mucosal damage; assaying inflammatory biomarkers, e.g., by qPCR; PCR arrays; transcription factor phosphorylation assays; immunoassays; and/or cytokine assay kits (Mesoscale, Cayman Chemical, Qiagen).

[0176] In some embodiments, the genetically engineered bacteria produce at least about 1.5-fold, at least about 2-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 30-fold, at least about 50-fold, at least about 100-fold, at least about 200-fold, at least about 300-fold, at least about 400-fold, at least about 500-fold, at least about 600-fold, at least about 700-fold, at least about 800-fold, at least about 900-fold, at least about 1,000-fold, or at least about 1,500-fold more of an anti-inflammation and/or gut barrier enhancer molecule in low-oxygen conditions than unmodified bacteria of the same subtype under the same conditions. Certain unmodified bacteria will not have detectable levels of the anti-inflammation and/or gut barrier enhancer molecule. In embodiments using genetically modified forms of these bacteria, the anti-inflammation and/or gut barrier enhancer molecule will be detectable in low-oxygen conditions.

[0177] In certain embodiments, the anti-inflammation and/or gut barrier enhancer molecule is butyrate. Methods of measuring butyrate levels, e.g., by mass spectrometry, gas chromatography, high-performance liquid chromatography (HPLC), are known in the art (*see, e.g.,* Aboulnaga et al., 2013). In some embodiments, butyrate is measured as butyrate level/bacteria optical density (OD). In some embodiments, measuring the activity and/or expression of one or more gene products in the butyrogenic gene cassette serves as a proxy measurement for butyrate production. In

some embodiments, the bacterial cells of the invention are harvested and lysed to measure butyrate production. In alternate embodiments, butyrate production is measured in the bacterial cell medium. In some embodiments, the genetically engineered bacteria produce at least about 1 nM/OD, at least about 10 nM/OD, at least about 100 nM/OD, at least about 500 nM/OD, at least about 1 μM/OD, at least about 10 μM/OD, at least about 100 μM/OD, at least about 500 μM/OD, at least about 1 mM/OD, at least about 2 mM/OD, at least about 3 mM/OD, at least about 5 mM/OD, at least about 10 mM/OD, at least about 20 mM/OD, at least about 30 mM/OD, or at least about 50 mM/OD of butyrate in low-oxygen conditions.

[0178] In certain embodiments, the anti-inflammation and/or gut barrier enhancer molecule is propionate. Methods of measuring propionate levels, e.g., by mass spectrometry, gas chromatography, high-performance liquid chromatography (HPLC), are known in the art (*see, e.g.,* Hillman, 1978; Lukovac et al., 2014). In some embodiments, measuring the activity and/or expression of one or more gene products in the propionate gene cassette serves as a proxy measurement for propionate production. In some embodiments, the bacterial cells of the invention are harvested and lysed to measure propionate production. In alternate embodiments, propionate production is measured in the bacterial cell medium. In some embodiments, the genetically engineered bacteria produce at least about 1 μM, at least about 10 μM, at least about 100 μM, at least about 500 μM, at least about 1 mM, at least about 2 mM, at least about 3 mM, at least about 5 mM, at least about 10 mM, at least about 15 mM, at least about 20 mM, at least about 30 mM, at least about 40 mM, or at least about 50 mM of propionate in low-oxygen conditions.

RNS-dependent regulation

[0179] In some embodiments, the genetically engineered bacteria of the invention comprise a tunable regulatory region that is directly or indirectly controlled by a transcription factor that is capable of sensing at least one reactive nitrogen species. The tunable regulatory region is operatively linked to a gene or gene cassette capable of directly or indirectly driving the expression of an anti-inflammation and/or gut barrier function enhancer molecule, thus controlling expression of the molecule relative to RNS

levels. For example, the tunable regulatory region is a RNS-inducible regulatory region, and the molecule is butyrate; when RNS is present, e.g., in an inflamed tissue, a RNS-sensing transcription factor binds to and/or activates the regulatory region and drives expression of the butyrogenic gene cassette, thereby producing butyrate, which exerts anti-inflammation and/or gut barrier enhancing effects. Subsequently, when inflammation is ameliorated, RNS levels are reduced, and butyrate production is decreased or eliminated.

[0180] In some embodiments, the tunable regulatory region is a RNS-inducible regulatory region; in the presence of RNS, a transcription factor senses RNS and activates the RNS-inducible regulatory region, thereby driving expression of an operatively linked gene or gene cassette. In some embodiments, the transcription factor senses RNS and subsequently binds to the RNS-inducible regulatory region, thereby activating downstream gene expression. In alternate embodiments, the transcription factor is bound to the RNS-inducible regulatory region in the absence of RNS; when the transcription factor senses RNS, it undergoes a conformational change, thereby inducing downstream gene expression.

[0181] In some embodiments, the tunable regulatory region is a RNS-inducible regulatory region, and the transcription factor that senses RNS is NorR. NorR "is an NO-responsive transcriptional activator that regulates expression of the *norVW* genes encoding flavorubredoxin and an associated flavoprotein, which reduce NO to nitrous oxide" (Spiro 2006). The genetically engineered bacteria of the invention may comprise any suitable RNS-responsive regulatory region from a gene that is activated by NorR. Genes that are capable of being activated by NorR are known in the art (*see, e.g.,* Spiro, 2006; Vine et al., 2011; Karlinsey et al., 2012; Table 1). In certain embodiments, the genetically engineered bacteria of the invention comprise a RNS-inducible regulatory region from *norVW* that is operatively linked to a gene or gene cassette, e.g., a butyrogenic gene cassette. In the presence of RNS, a NorR transcription factor senses RNS and activates to the *norVW* regulatory region, thereby driving expression of the operatively linked butyrogenic gene cassette and producing butyrate.

[0182] In some embodiments, the tunable regulatory region is a RNS-inducible regulatory region, and the transcription factor that senses RNS is DNR. DNR (dissimilatory nitrate respiration regulator) "promotes the expression of the *nir,* the *nor* and the *nos* genes" in the presence of nitric oxide (Castiglione et al., 2009). The genetically engineered bacteria of the invention may comprise any suitable RNS-responsive regulatory region from a gene that is activated by DNR. Genes that are capable of being activated by DNR are known in the art (*see, e.g.,* Castiglione et al., 2009; Giardina et al., 2008). In certain embodiments, the genetically engineered bacteria of the invention comprise a RNS-inducible regulatory region from *norCB* that is operatively linked to a gene or gene cassette, e.g., a butyrogenic gene cassette. In the presence of RNS, a DNR transcription factor senses RNS and activates to the *norCB* regulatory region, thereby driving expression of the operatively linked butyrogenic gene cassette and producing butyrate. In some embodiments, the DNR is *Pseudomonas aeruginosa* DNR.

[0183] In some embodiments, the tunable regulatory region is a RNS-derepressible regulatory region, and binding of a corresponding transcription factor represses downstream gene expression; in the presence of RNS, the transcription factor no longer binds to the regulatory region, thereby derepressing the operatively linked gene or gene cassette.

[0184] In some embodiments, the tunable regulatory region is a RNS-derepressible regulatory region, and the transcription factor that senses RNS is NsrR. NsrR is "an Rrf2-type transcriptional repressor [that] can sense NO and control the expression of genes responsible for NO metabolism" (Isabella et al., 2009). The genetically engineered bacteria of the invention may comprise any suitable RNS-responsive regulatory region from a gene that is repressed by NsrR. In some embodiments, the NsrR is *Neisseria gonorrhoeae* NsrR. Genes that are capable of being repressed by NsrR are known in the art (*see, e.g.,* Isabella et al., 2009; Dunn et al., 2010; Table 1). In certain embodiments, the genetically engineered bacteria of the invention comprise a RNS-derepressible regulatory region from *norB* that is operatively linked to a gene or gene cassette, e.g., a butyrogenic gene cassette. In the presence of RNS, an NsrR transcription factor senses RNS and no longer binds to the *norB* regulatory region,

thereby derepressing the operatively linked butyrogenic gene cassette and producing butyrate.

[0185] In some embodiments, it is advantageous for the genetically engineered bacteria to express a RNS-sensing transcription factor that does not regulate the expression of a significant number of native genes in the bacteria. In some embodiments, the genetically engineered bacterium of the invention expresses a RNS-sensing transcription factor from a different species, strain, or substrain of bacteria, wherein the transcription factor does not bind to regulatory sequences in the genetically engineered bacterium of the invention. In some embodiments, the genetically engineered bacterium of the invention is *Escherichia coli,* and the RNS-sensing transcription factor is NsrR, e.g., from is *Neisseria gonorrhoeae*, wherein the *Escherichia coli* does not comprise binding sites for said NsrR. In some embodiments, the heterologous transcription factor minimizes or eliminates off-target effects on endogenous regulatory regions and genes in the genetically engineered bacteria.

[0186] In some embodiments, the tunable regulatory region is a RNS-repressible regulatory region, and binding of a corresponding transcription factor represses downstream gene expression; in the presence of RNS, the transcription factor senses RNS and binds to the RNS-repressible regulatory region, thereby repressing expression of the operatively linked gene or gene cassette. In some embodiments, the RNS-sensing transcription factor is capable of binding to a regulatory region that overlaps with part of the promoter sequence. In alternate embodiments, the RNS-sensing transcription factor is capable of binding to a regulatory region that is upstream or downstream of the promoter sequence.

[0187] In these embodiments, the genetically engineered bacteria may comprise a two repressor activation regulatory circuit, which is used to express an anti-inflammation and/or gut barrier function enhancer molecule. The two repressor activation regulatory circuit comprises a first RNS-sensing repressor and a second repressor, which is operatively linked to a gene or gene cassette, e.g., a butyrogenic gene cassette. In one aspect of these embodiments, the RNS-sensing repressor inhibits transcription of the second repressor, which inhibits the transcription of the gene or gene

cassette. Examples of second repressors useful in these embodiments include, but are not limited to, TetR, C1, and LexA. In the absence of binding by the first repressor (which occurs in the absence of RNS), the second repressor is transcribed, which represses expression of the gene or gene cassette, e.g., a butyrogenic gene cassette. In the presence of binding by the first repressor (which occurs in the presence of RNS), expression of the second repressor is repressed, and the gene or gene cassette, e.g., a butyrogenic gene cassette, is expressed.

[0188] A RNS-responsive transcription factor may induce, derepress, or repress gene expression depending upon the regulatory region sequence used in the genetically engineered bacteria. One or more types of RNS-sensing transcription factors and corresponding regulatory region sequences may be present in genetically engineered bacteria. In some embodiments, the genetically engineered bacteria comprise one type of RNS-sensing transcription factor, e.g., NsrR, and one corresponding regulatory region sequence, e.g., from *norB*. In some embodiments, the genetically engineered bacteria comprise one type of RNS-sensing transcription factor, e.g., NsrR, and two or more different corresponding regulatory region sequences, e.g., from *norB* and *aniA*. In some embodiments, the genetically engineered bacteria comprise two or more types of RNS-sensing transcription factors, e.g., NsrR and NorR, and two or more corresponding regulatory region sequences, e.g., from *norB* and *norR*, respectively. One RNS-responsive regulatory region may be capable of binding more than one transcription factor. In some embodiments, the genetically engineered bacteria comprise two or more types of RNS-sensing transcription factors and one corresponding regulatory region sequence. Nucleic acid sequences of several RNS-regulated regulatory regions are known in the art (*see, e.g.,* Spiro, 2006; Isabella et al., 2009; Dunn et al., 2010; Vine et al., 2011; Karlinsey et al., 2012).

[0189] In some embodiments, the genetically engineered bacteria of the invention comprise a gene encoding a RNS-sensing transcription factor, e.g., the *nsrR* gene, that is controlled by its native promoter, an inducible promoter, a promoter that is stronger than the native promoter, e.g., the GlnRS promoter or the P(Bla) promoter, or a constitutive promoter. In some instances, it may be advantageous to express the RNS-

sensing transcription factor under the control of an inducible promoter in order to enhance expression stability. In some embodiments, expression of the RNS-sensing transcription factor is controlled by a different promoter than the promoter that controls expression of the therapeutic molecule. In some embodiments, expression of the RNS-sensing transcription factor is controlled by the same promoter that controls expression of the therapeutic molecule. In some embodiments, the RNS-sensing transcription factor and therapeutic molecule are divergently transcribed from a promoter region.

[0190] In some embodiments, the genetically engineered bacteria of the invention comprise a gene for a RNS-sensing transcription factor from a different species, strain, or substrain of bacteria. In some embodiments, the genetically engineered bacteria comprise a RNS-responsive regulatory region from a different species, strain, or substrain of bacteria. In some embodiments, the genetically engineered bacteria comprise a RNS-sensing transcription factor and corresponding RNS-responsive regulatory region from a different species, strain, or substrain of bacteria. The heterologous RNS-sensing transcription factor and regulatory region may increase the transcription of genes operatively linked to said regulatory region in the presence of RNS, as compared to the native transcription factor and regulatory region from bacteria of the same subtype under the same conditions.

[0191] In some embodiments, the genetically engineered bacteria comprise a RNS-sensing transcription factor, NsrR, and corresponding regulatory region, *nsrR*, from *Neisseria gonorrhoeae.* In some embodiments, the native RNS-sensing transcription factor, e.g., NsrR, is left intact and retains wild-type activity. In alternate embodiments, the native RNS-sensing transcription factor, e.g., NsrR, is deleted or mutated to reduce or eliminate wild-type activity.

[0192] In some embodiments, the genetically engineered bacteria of the invention comprise multiple copies of the endogenous gene encoding the RNS-sensing transcription factor, e.g., the *nsrR* gene. In some embodiments, the gene encoding the RNS-sensing transcription factor is present on a plasmid. In some embodiments, the gene encoding the RNS-sensing transcription factor and the gene or gene cassette for producing the therapeutic molecule are present on different plasmids. In some

embodiments, the gene encoding the RNS-sensing transcription factor and the gene or gene cassette for producing the therapeutic molecule are present on the same plasmid. In some embodiments, the gene encoding the RNS-sensing transcription factor is present on a chromosome. In some embodiments, the gene encoding the RNS-sensing transcription factor and the gene or gene cassette for producing the therapeutic molecule are present on different chromosomes. In some embodiments, the gene encoding the RNS-sensing transcription factor and the gene or gene cassette for producing the therapeutic molecule are present on the same chromosome.

[0193] In some embodiments, the genetically engineered bacteria comprise a wild-type gene encoding a RNS-sensing transcription factor, e.g., the *NsrR* gene, and a corresponding regulatory region, e.g., a *norB* regulatory region, that is mutated relative to the wild-type regulatory region from bacteria of the same subtype. The mutated regulatory region increases the expression of the anti-inflammation and/or gut barrier enhancer molecule in the presence of RNS, as compared to the wild-type regulatory region under the same conditions. In some embodiments, the genetically engineered bacteria comprise a wild-type RNS-responsive regulatory region, e.g., the *norB* regulatory region, and a corresponding transcription factor, e.g., NsrR, that is mutated relative to the wild-type transcription factor from bacteria of the same subtype. The mutant transcription factor increases the expression of the anti-inflammation and/or gut barrier enhancer molecule in the presence of RNS, as compared to the wild-type transcription factor under the same conditions. In some embodiments, both the RNS-sensing transcription factor and corresponding regulatory region are mutated relative to the wild-type sequences from bacteria of the same subtype in order to increase expression of the anti-inflammation and/or gut barrier enhancer molecule in the presence of RNS.

[0194] Nucleic acid sequences of exemplary RNS-regulated constructs comprising a gene encoding NsrR and a *norB* promoter are shown in **Table 10** and **Table 11**. **Table 10** depicts the nucleic acid sequence of an exemplary RNS-regulated construct comprising a gene encoding *nsrR*, a regulatory region of *norB*, and a butyrogenic gene cassette (pLogic031-nsrR-norB-butyrate construct; SEQ ID NO: 67). The sequence encoding NsrR is **underlined and bolded**, and the NsrR binding site, i.e., a regulatory

region of *norB* is boxed. **Table 11** depicts the nucleic acid sequence of an exemplary RNS-regulated construct comprising a gene encoding *nsrR*, a regulatory region of *norB*, and a butyrogenic gene cassette (pLogic046-nsrR-norB-butyrate construct; SEQ ID NO: 68). The sequence encoding NsrR is **underlined and bolded**, and the NsrR binding site, i.e., a regulatory region of *norB* is boxed. Nucleic acid sequences of tetracycline-regulated constructs comprising a *tet* promoter are shown in **Table 12** and **Table 13**. **Table 12** depicts the nucleic acid sequence of an exemplary tetracycline-regulated construct comprising a *tet* promoter and a butyrogenic gene cassette (pLogic031-tet-butyrate construct; SEQ ID NO: 69). The sequence encoding TetR is underlined, and the overlapping tetR/tetA promoters are boxed. **Table 13** depicts the nucleic acid sequence of an exemplary tetracycline-regulated construct comprising a *tet* promoter and a butyrogenic gene cassette (pLogic046-tet-butyrate construct; SEQ ID NO: 70). The sequence encoding TetR is underlined, and the overlapping tetR/tetA promoters are boxed. In some embodiments, genetically engineered bacteria comprise a nucleic acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homologous to the DNA sequence of SEQ ID NO: 67, 68, 69, or 70, or a functional fragment thereof.

Table 10

Nucleotide sequences of pLogic031-nsrR-norB-butyrate construct (SEQ ID NO: 67)
ttatta**tcgcaccgcaatcgggattttcgattcataaagcaggtcgtaggtcggcttgtt gagcaggtcttgcagcgtgaaaccgtccagatacgtgaaaaacgacttcattgcaccgcc gagtatgcccgtcagccggcaggacggcgtaatcaggcattcgttgttcgggcccataca ctcgaccagctgcatcggttcgaggtggcggacgaccgcgccgatattgatgcgttcggg cggcgcggccagcctcagcccgccgcctttcccgcgtacgctgtgcaagaacccgccttt gaccagcgcggtaaccactttcatcaaatggcttttggaaatgccgtaggtcgaggcgat ggtggcgatattgaccagcgcgtcgtcgttgacggcggtgtagatgaggacgcgcagccc gtagtcggtatgttgggtcagatacat**acaacctccttagtacatgcaaaattatttcta gagcaacatacgagccggaagcataaagtgtaaagcctggggtgcctaatgagttgagtt gaggaattataacaggaagaaatattcctcatacgcttgtaattcctctatggttgttga caattaatcatcggctcgtataatgtataacattcatattttgtgaattttaaactctag aaataattttgtttaactttaagaaggagatatacatatggatttaaattctaaaaaata tcagatgcttaaagagctatatgtaagcttcgctgaaaatgaagttaaacctttagcaac agaacttgatgaagaagaaagatttccttatgaaacagtggaaaaaatggcaaaagcagg aatgatgggtataccatatccaaaagaatatggtggagaaggtggagacactgtaggata tataatggcagttgaagaattgtctagagtttgtggtactacaggagttatattatcagc tcatacatctcttggctcatggcctatatatcaatatggtaatgaagaacaaaaacaaaa attcttaagaccactagcaagtggagaaaaattaggagcatttggtcttactgagcctaa

Nucleotide sequences of pLogic031-nsrR-norB-butyrate construct (SEQ ID NO: 67)
tgctggtacagatgcgtctggccaacaaacaactgctgttttagacggggatgaatacat acttaatggctcaaaaatatttataacaaacgcaatagctggtgacatatatgtagtaat ggcaatgactgataaatctaaggggaacaaaggaatatcagcatttatagttgaaaaagg aactcctgggtttagctttggagttaaagaaaagaaaatgggtataagaggttcagctac gagtgaattaatatttgaggattgcagaatacctaaagaaaatttacttggaaaagaagg tcaaggatttaagatagcaatgtctactcttgatggtggtagaattggtatagctgcaca agctttaggtttagcacaaggtgctcttgatgaaactgttaaatatgtaaaagaaagagt acaatttggtagaccattatcaaaattccaaaatacacaattccaattagctgatatgga agttaaggtacaagcggctagacaccttgtatatcaagcagctataaataaagacttagg aaaaccttatggagtagaagcagcaatggcaaaattatttgcagctgaaacagctatgga agttactacaaaagctgtacaacttcatggaggatatggatacactcgtgactatccagt agaaagaatgatgagagatgctaagataactgaaatatatgaaggaactagtgaagttca aagaatggttatttcaggaaaactattaaaatagtaagaaggagatatacatatggagga aggatttatgaatatagtcgtttgtataaaacaagttccagatacaacagaagttaaact agatcctaatacaggtactttaattagagatggagtaccaagtataataaaccctgatga taaagcaggtttagaagaagctataaaattaaaagaagaaatgggtgctcatgtaactgt tataacaatgggacctcctcaagcagatatggctttaaaagaagctttagcaatgggtgc agatagaggtatattattaacagatagagcatttgcgggtgctgatacttgggcaacttc atcagcattagcaggagcattaaaaaatatagattttgatattataatagctggaagaca ggcgatagatggagatactgcacaagttggacctcaaatagctgaacatttaaatcttcc atcaataacatatgctgaagaaataaaaactgaaggtgaatatgtattagtaaaaagaca atttgaagattgttgccatgacttaaaagttaaaatgccatgccttataacaactcttaa agatatgaacacaccaagatacatgaaagttggaagaatatatgatgctttcgaaaatga tgtagtagaaacatggactgtaaaagatatagaagttgacccttctaatttaggtcttaa aggttctccaactagtgtatttaaatcatttacaaaatcagttaaaccagctggtacaat atacaatgaagatgcgaaaacatcagctggaattatcatagataaattaaaagagaagta tatcatataataagaaggagatatacatatgggtaacgttttagtagtaatagaacaaag agaaaatgtaattcaaactgtttctttagaattactaggaaaggctacagaaatagcaaa agattatgatacaaaagtttctgcattacttttaggtagtaaggtagaaggtttaataga tacattagcacactatggtgcagatgaggtaatagtagtagatgatgaagctttagcagt gtatacaactgaaccatatacaaaagcagcttatgaagcaataaaagcagctgaccctat agttgtattatttggtgcaacttcaataggtagagatttagcgcctagagtttctgctag aatacatacaggtcttactgctgactgtacaggtcttgcagtagctgaagatacaaaatt attattaatgacaagacctgcctttggtggaaatataatggcaacaatagtttgtaaaga tttcagacctcaaatgtctacagttagaccaggggttatgaagaaaaatgaacctgatga aactaaagaagctgtaattaaccgtttcaaggtagaatttaatgatgctgataaattagt tcaagttgtacaagtaataaaagaagctaaaaaacaagttaaaatagaagatgctaagat attagtttctgctggacgtggaatgggtggaaaagaaaacttagacatactttatgaatt agctgaaattataggtggagaagtttctggttctcgtgccactatagatgcaggttggtt agataaagcaagacaagttggtcaaactggtaaaactgtaagaccagacctttatatagc atgtggtatatctggagcaatacaacatatagctggtatggaagatgctgagtttatagt tgctataaataaaaatccagaagctccaatatttaaatatgctgatgttggtatagttgg agatgttcataaagtgcttccagaacttatcagtcagttaagtgttgcaaaagaaaaagg tgaagttttagctaactaataagaaggagatatacatatgagagaagtagtaattgccag tgcagctagaacagcagtaggaagttttggaggagcatttaaatcagtttcagcggtaga gttaggggtaacagcagctaaagaagctataaaaagagctaacataactccagatatgat agatgaatctcttttaggggagtacttacagcaggtcttggacaaaatatagcaagaca

Nucleotide sequences of pLogic031-nsrR-norB-butyrate construct (SEQ ID NO: 67)
aatagcattaggagcaggaataccagtagaaaaaccagctatgactataaatatagtttg tggttctggattaagatctgtttcaatggcatctcaacttatagcattaggtgatgctga tataatgttagttggtggagctgaaaacatgagtatgtctccttatttagtaccaagtgc gagatatggtgcaagaatgggtgatgctgcttttgttgattcaatgataaaagatggatt atcagacatatttaataactatcacatgggtattactgctgaaaacatagcagagcaatg gaatataactagagaagaacaagatgaattagctcttgcaagtcaaaataaagctgaaaa agctcaagctgaaggaaaatttgatgaagaaatagttcctgttgttataaaaggaagaaa aggtgacactgtagtagataaagatgaatatattaagcctggcactacaatggagaaact tgctaagttaagacctgcatttaaaaaagatggaacagttactgctggtaatgcatcagg aataaatgatggtgctgctatgttagtagtaatggctaaagaaaaagctgaagaactagg aatagagcctcttgcaactatagtttcttatggaacagctggtgttgaccctaaaataat gggatatggaccagttccagcaactaaaaaagctttagaagctgctaatatgactattga agatatagatttagttgaagctaatgaggcatttgctgcccaatctgtagctgtaataag agacttaaatatagatatgaataaagttaatgttaatggtggagcaatagctataggaca tccaataggatgctcaggagcaagaatacttactacacttttatatgaaatgaagagaag agatgctaaaactggtcttgctacactttgtataggcggtggaatgggaactactttaat agttaagagatagtaagaaggagatatacatatgaaattagctgtaataggtagtggaac tatgggaagtggtattgtacaaacttttgcaagttgtggacatgatgtatgtttaaagag tagaactcaaggtgctatagataaatgtttagctttattagataaaaatttaactaagtt agttactaagggaaaaatggatgaagctacaaaagcagaaatattaagtcatgttagttc aactactaattatgaagatttaaaagatatggatttaataatagaagcatctgtagaaga catgaatataaagaaagatgttttcaagttactagatgaattatgtaaagaagatactat cttggcaacaaatacttcatcattatctataacagaaatagcttcttctactaagcgccc agataaagttataggaatgcatttctttaatccagttcctatgatgaaattagttgaagt tataagtggtcagttaacatcaaaagttacttttgatacagtatttgaattatctaagag tatcaataaagtaccagtagatgtatctgaatctcctggatttgtagtaaatagaatact tatacctatgataaatgaagctgttggtatatatgcagatggtgttgcaagtaaagaaga aatagatgaagctatgaaattaggagcaaaccatccaatgggaccactagcattaggtga tttaatcggattagatgttgttttagctataatgaacgttttatatactgaatttggaga tactaaatatagacctcatccacttttagctaaaatggttagagctaatcaattaggaag aaaaactaagataggattctatgattataataaataataagaaggagatatacatatgag tacaagtgatgttaaagtttatgagaatgtagctgttgaagtagatggaaatatatgtac agtgaaaatgaatagacctaaagcccttaatgcaataaattcaaagactttagaagaact ttatgaagtatttgtagatattaataatgatgaaactattgatgttgtaatattgacagg ggaaggaaaggcatttgtagctggagcagatattgcatacatgaaagatttagatgctgt agctgctaaagattttagtatcttaggagcaaaagcttttggagaaatagaaaatagtaa aaaagtagtgatagctgctgtaaacggatttgctttaggtggaggatgtgaacttgcaat ggcatgtgatataagaattgcatctgctaaagctaaatttggtcagccagaagtaactct tggaataactccaggatatggaggaactcaaaggcttacaagattggttggaatggcaaa agcaaaagaattaatctttacaggtcaagttataaaagctgatgaagctgaaaaaatagg gctagtaaatagagtcgttgagccagacattttaatagaagaagttgagaaattagctaa gataatagctaaaaatgctcagcttgcagttagatactctaaagaagcaatacaacttgg tgctcaaactgatataaatactggaatagatatagaatctaatttatttggtctttgttt ttcaactaaagaccaaaaagaaggaatgtcagctttcgttgaaaagagagaagctaactt tataaaagggtaataagaaggagatatacatatgagaagttttgaagaagtaattaagtt tgcaaaagaaagaggacctaaaactatatcagtagcatgttgccaagataaagaagtttt aatggcagttgaaatggctagaaaagaaaaaatagcaaatgccattttagtaggagatat

Nucleotide sequences of pLogic031-nsrR-norB-butyrate construct (SEQ ID NO: 67)
agaaaagactaaagaaattgcaaaaagcatagacatggatatcgaaaattatgaactgat agatataaaagatttagcagaagcatctctaaaatctgttgaattagtttcacaaggaaa agccgacatggtaatgaaaggcttagtagacacatcaataatactaaaagcagttttaaa taaagaagtaggtcttagaactggaaatgtattaagtcacgtagcagtatttgatgtaga gggatatgatagattatttttcgtaactgacgcagctatgaacttagctcctgatacaaa tactaaaaagcaaatcatagaaaatgcttgcacagtagcacattcattagatataagtga accaaaagttgctgcaatatgcgcaaaagaaaaagtaaatccaaaaatgaaagatacagt tgaagctaaagaactagaagaaatgtatgaaagaggagaaatcaaaggttgtatggttgg tgggccttttgcaattgataatgcagtatctttagaagcagctaaacataaaggtataaa tcatcctgtagcaggacgagctgatatattattagccccagatattgaaggtggtaacat attatataaagctttggtattcttctcaaaatcaaaaaatgcaggagttatagttggggc taaagcaccaataatattaacttctagagcagacagtgaagaaactaaactaaactcaat agctttaggtgttttaatggcagcaaaggcataataagaaggagatatacatatgagcaa aatatttaaaatcttaacaataaatcctggttcgacatcaactaaaatagctgtatttga taatgaggatttagtatttgaaaaaactttaagacattcttcagaagaaataggaaaata tgagaaggtgtctgaccaatttgaatttcgtaaacaagtaatagaagaagctctaaaaga aggtggagtaaaaacatctgaattagatgctgtagtaggtagaggaggacttcttaaacc tataaaaggtggtacttattcagtaagtgctgctatgattgaagatttaaaagtgggagt tttaggagaacacgcttcaaacctaggtggaataatagcaaaacaaataggtgaagaagt aaatgttccttcatacatagtagaccctgttgttgtagatgaattagaagatgttgctag aatttctggtatgcctgaaataagtagagcaagtgtagtacatgctttaaatcaaaaggc aatagcaagaagatatgctagagaaataaacaagaaatatgaagatataaatcttatagt tgcacacatgggtggaggagtttctgttggagctcataaaaatggtaaaatagtagatgt tgcaaacgcattagatggagaaggacctttctctccagaaagaagtggtggactaccagt aggtgcattagtaaaaatgtgctttagtggaaaatatactcaagatgaaattaaaaagaa aataaaaggtaatggcggactagttgcatacttaaacactaatgatgctagagaagttga agaaagaattgaagctggtgatgaaaaagctaaattagtatatgaagctatggcatatca aatctctaaagaaataggagctagtgctgcagttcttaagggagatgtaaaagcaatatt attaactggtggaatcgcatattcaaaaatgtttacagaaatgattgcagatagagttaa atttatagcagatgtaaaagtttatccaggtgaagatgaaatgattgcattagctcaagg tggacttagagttttaactggtgaagaagaggctcaagtttatgataactaataa

Table 11

Nucleotide sequences of pLogic046-nsrR-norB-butyrate construct (SEQ ID NO: 68)
ttatta**tcgcaccgcaatcgggattttcgattcataaagcaggtcgtaggtcggcttgtt gagcaggtcttgcagcgtgaaaccgtccagatacgtgaaaaacgacttcattgcaccgcc gagtatgcccgtcagccggcaggacggcgtaatcaggcattcgttgttcgggcccataca ctcgaccagctgcatcggttcgaggtggcggacgaccgcgccgatattgatgcgttcggg cggcgcggccagcctcagcccgccgcctttcccgcgtacgctgtgcaagaacccgccttt gaccagcgcggtaaccactttcatcaaatggcttttggaaatgccgtaggtcgaggcgat ggtggcgatattgaccagcgcgtcgtcgttgacggcggtgtagatgaggacgcgcagccc gtagtcggtatgttgggtcagatacat**acaacctccttagtacatgcaaaattatttcta gagcaacatacgagccggaagcataaagtgtaaagcctggggtgcctaatgagttgagtt gaggaattataacaggaagaaatattcctcatacgcttgtaattcctctatggttgttga

Nucleotide sequences of pLogic046-nsrR-norB-butyrate construct (SEQ ID NO: 68)

caattaatcatcggctcgtataatgtataacattcatattttgtgaattttaaactctag
aaataattttgtttaactttaagaaggagatatacatatgatcgtaaaacctatggtacg
caacaatatctgcctgaacgcccatcctcagggctgcaagaagggagtggaagatcagat
tgaatataccaagaaacgcattaccgcagaagtcaaagctggcgcaaaagctccaaaaaa
cgttctggtgcttggctgctcaaatggttacggcctggcgagccgcattactgctgcgtt
cggatacggggctgcgaccatcggcgtgtcctttgaaaaagcgggttcagaaaccaaata
tggtacaccgggatggtacaataatttggcatttgatgaagcggcaaaacgcgagggtct
ttatagcgtgacgatcgacggcgatgcgttttcagacgagatcaaggcccaggtaattga
ggaagccaaaaaaaaaggtatcaaatttgatctgatcgtatacagcttggccagcccagt
acgtactgatcctgatacaggtatcatgcacaaaagcgttttgaaacccttggaaaaac
gttcacaggcaaaacagtagatccgtttactggcgagctgaaggaaatctccgcggaacc
agcaaatgacgaggaagcagccgccactgttaaagttatgggggtgaagattgggaacg
ttggattaagcagctgtcgaaggaaggcctcttagaagaaggctgtattaccttggccta
tagttatattggccctgaagctacccaagctttgtaccgtaaaggcacaatcggcaaggc
caaagaacacctggaggccacagcacaccgtctcaacaaagagaacccgtcaatccgtgc
cttcgtgagcgtgaataaaggcctggtaacccgcgcaagcgccgtaatcccggtaatccc
tctgtatctcgccagcttgttcaaagtaatgaaagagaagggcaatcatgaaggttgtat
tgaacagatcacgcgtctgtacgccgagcgcctgtaccgtaaagatggtacaattccagt
tgatgaggaaaatcgcattcgcattgatgattgggagttagaagaagacgtccagaaagc
ggtatccgcgttgatggagaaagtcacgggtgaaaacgcagaatctctcactgacttagc
ggggtaccgccatgatttcttagctagtaacggctttgatgtagaaggtattaattatga
agcggaagttgaacgcttcgaccgtatctgataagaaggagatatacatatgagagaagt
agtaattgccagtgcagctagaacagcagtaggaagttttggaggagcatttaaatcagt
ttcagcggtagagttaggggtaacagcagctaaagaagctataaaaagagctaacataac
tccagatatgatagatgaatctcttttaggggagtacttacagcaggtcttggacaaaa
tatagcaagacaaatagcattaggagcaggaataccagtagaaaaaccagctatgactat
aaatatagtttgtggttctggattaagatctgtttcaatggcatctcaacttatagcatt
aggtgatgctgatataatgttagttggtggagctgaaaacatgagtatgtctccttattt
agtaccaagtgcgagatatggtgcaagaatgggtgatgctgcttttgttgattcaatgat
aaaagatggattatcagacatatttaataactatcacatgggtattactgctgaaaacat
agcagagcaatggaatataactagagaagaacaagatgaattagctcttgcaagtcaaaa
taaagctgaaaaagctcaagctgaaggaaaatttgatgaagaaatagttcctgttgttat
aaaaggaagaaaaggtgacactgtagtagataaagatgaatatattaagcctggcactac
aatggagaaacttgctaagttaagacctgcatttaaaaaagatggaacagttactgctgg
taatgcatcaggaataaatgatggtgctgctatgttagtagtaatggctaaagaaaaagc
tgaagaactaggaatagagcctcttgcaactatagtttcttatggaacagctggtgttga
ccctaaaataatgggatatggaccagttccagcaactaaaaaagctttagaagctgctaa
tatgactattgaagatatagatttagttgaagctaatgaggcatttgctgcccaatctgt
agctgtaataagagacttaaatatagatatgaataaagttaatgttaatggtggagcaat
agctataggacatccaataggatgctcaggagcaagaatacttactacacttttatatga
aatgaagagaagagatgctaaaactggtcttgctacactttgtataggcggtggaatggg
aactactttaatagttaagagatagtaagaaggagatatacatatgaaattagctgtaat
aggtagtggaactatgggaagtggtattgtacaaacttttgcaagttgtggacatgatgt
atgtttaaagagtagaactcaaggtgctatagataaatgtttagctttattagataaaaa
tttaactaagttagttactaagggaaaaatggatgaagctacaaaagcagaaatattaag
tcatgttagttcaactactaattatgaagatttaaaagatatggatttaataatagaagc
atctgtagaagacatgaatataaagaaagatgttttcaagttactagatgaattatgtaa

Nucleotide sequences of pLogic046-nsrR-norB-butyrate construct (SEQ ID NO: 68)
agaagatactatcttggcaacaaatacttcatcattatctataacagaaatagcttcttc
tactaagcgcccagataaagttataggaatgcatttctttaatccagttcctatgatgaa
attagttgaagttataagtggtcagttaacatcaaaagttacttttgatacagtatttga
attatctaagagtatcaataaagtaccagtagatgtatctgaatctcctggatttgtagt
aaatagaatacttatacctatgataaatgaagctgttggtatatatgcagatggtgttgc
aagtaaagaagaaatagatgaagctatgaaattaggagcaaaccatccaatgggaccact
agcattaggtgatttaatcggattagatgttgttttagctataatgaacgttttatatac
tgaatttggagatactaaatatagacctcatccacttttagctaaaatggttagagctaa
tcaattaggaagaaaaactaagataggattctatgattataataaataataagaaggaga
tatacatatgagtacaagtgatgttaaagtttatgagaatgtagctgttgaagtagatgg
aaatatatgtacagtgaaaatgaatagacctaaagcccttaatgcaataaattcaaagac
tttagaagaactttatgaagtatttgtagatattaataatgatgaaactattgatgttgt
aatattgacaggggaaggaaaggcatttgtagctggagcagatattgcatacatgaaaga
tttagatgctgtagctgctaaagattttagtatcttaggagcaaaagcttttggagaaat
agaaaatagtaaaaaagtagtgatagctgctgtaaacggatttgctttaggtggaggatg
tgaacttgcaatggcatgtgatataagaattgcatctgctaaagctaaatttggtcagcc
agaagtaactcttggaataactccaggatatggaggaactcaaaggcttacaagattggt
tggaatggcaaaagcaaaagaattaatctttacaggtcaagttataaaagctgatgaagc
tgaaaaaatagggctagtaaatagagtcgttgagccagacattttaatagaagaagttga
gaaattagctaagataatagctaaaaatgctcagcttgcagttagatactctaaagaagc
aatacaacttggtgctcaaactgatataaatactggaatagatatagaatctaatttatt
tggtctttgtttttcaactaaagaccaaaaagaaggaatgtcagctttcgttgaaaagag
agaagctaactttataaaagggtaataagaaggagatatacatatgagaagttttgaaga
agtaattaagtttgcaaaagaaagaggacctaaaactatatcagtagcatgttgccaaga
taaagaagttttaatggcagttgaaatggctagaaaagaaaaaatagcaaatgccatttt
agtaggagatatagaaaagactaaagaaattgcaaaaagcatagacatggatatcgaaaa
ttatgaactgatagatataaaagatttagcagaagcatctctaaaatctgttgaattagt
ttcacaaggaaaagccgacatggtaatgaaaggcttagtagacacatcaataatactaaa
agcagttttaaataaagaagtaggtcttagaactggaaatgtattaagtcacgtagcagt
atttgatgtagagggatatgatagattatttttcgtaactgacgcagctatgaacttagc
tcctgatacaaatactaaaaagcaaatcatagaaaatgcttgcacagtagcacattcatt
agatataagtgaaccaaaagttgctgcaatatgcgcaaaagaaaaagtaaatccaaaaat
gaaagatacagttgaagctaaagaactagaagaaatgtatgaaagaggagaaatcaaagg
ttgtatggttggtgggccttttgcaattgataatgcagtatctttagaagcagctaaaca
taaaggtataaatcatcctgtagcaggacgagctgatatattattagccccagatattga
aggtggtaacatattatataaagctttggtattcttctcaaaatcaaaaaatgcaggagt
tatagttggggctaaagcaccaataatattaacttctagagcagacagtgaagaaactaa
actaaactcaatagctttaggtgttttaatggcagcaaaggcataataagaaggagatat
acatatgagcaaaatatttaaaatcttaacaataaatcctggttcgacatcaactaaaat
agctgtatttgataatgaggatttagtatttgaaaaaactttaagacattcttcagaaga
aataggaaaatatgagaaggtgtctgaccaatttgaatttcgtaaacaagtaatagaaga
agctctaaaagaaggtggagtaaaaacatctgaattagatgctgtagtaggtagaggagg
acttcttaaacctataaaaggtggtacttattcagtaagtgctgctatgattgaagattt
aaaagtgggagttttaggagaacacgcttcaaacctaggtggaataatagcaaaacaaat
aggtgaagaagtaaatgttccttcatacatagtagaccctgttgttgtagatgaattaga
agatgttgctagaatttctggtatgcctgaaataagtagagcaagtgtagtacatgcttt
aaatcaaaaggcaatagcaagaagatatgctagagaaataaacaagaaatatgaagatat

Nucleotide sequences of pLogic046-nsrR-norB-butyrate construct (SEQ ID NO: 68)
aaatcttatagttgcacacatgggtggaggagtttctgttggagctcataaaaatggtaa aatagtagatgttgcaaacgcattagatggagaaggacctttctctccagaaagaagtgg tggactaccagtaggtgcattagtaaaaatgtgctttagtggaaaatatactcaagatga aattaaaaagaaaataaaaggtaatggcggactagttgcatacttaaacactaatgatgc tagagaagttgaagaaagaattgaagctggtgatgaaaaagctaaattagtatatgaagc tatggcatatcaaatctctaaagaaataggagctagtgctgcagttcttaagggagatgt aaaagcaatattattaactggtggaatcgcatattcaaaaatgtttacagaaatgattgc agatagagttaaatttatagcagatgtaaaagtttatccaggtgaagatgaaatgattgc attagctcaaggtggacttagagttttaactggtgaagaagaggctcaagtttatgataa ctaataa

Table 12

Nucleotide sequences of pLogic031-tet-butyrate construct (SEQ ID NO: 69)
1 gtaaaacgac ggccagtgaa ttcgttaaga cccactttca catttaagtt gtttttctaa 61 tccgcatatg atcaattcaa ggccgaataa gaaggctggc tctgcacctt ggtgatcaaa 121 taattcgata gcttgtcgta ataatggcgg catactatca gtagtaggtg tttccctttc 181 ttctttagcg acttgatgct cttgatcttc caatacgcaa cctaaagtaa aatgccccac 241 agcgctgagt gcatataatg cattctctag tgaaaaacct tgttggcata aaaaggctaa 301 ttgattttcg agagtttcat actgtttttc tgtaggccgt gtacctaaat gtacttttgc 361 tccatcgcga tgacttagta aagcacatct aaaactttta gcgttattac gtaaaaaatc 421 ttgccagctt tccccttcta aagggcaaaa gtgagtatgg tgcctatcta acatctcaat 481 ggctaaggcg tcgagcaaag cccgcttatt ttttacatgc caatacaatg taggctgctc 541 tacacctagc ttctgggcga gtttacgggt tgttaaacct tcgattccga cctcattaag 601 cagctctaat gcgctgttaa tcactttact tttatctaat ctagacatca ttaattccta 661 attttgttg acactctatc attgatagag ttattttacc actccctatc agtgatagag 721 aaaagtgaac tctagaaata attttgttta actttaagaa ggagatatac atatggattt 781 aaattctaaa aaatatcaga tgcttaaaga gctatatgta agcttcgctg aaaatgaagt 841 taaaccttta gcaacagaac ttgatgaaga agaaagattt ccttatgaaa cagtggaaaa 901 aatggcaaaa gcaggaatga tgggtatacc atatccaaaa gaatatggtg gagaaggtgg 961 agacactgta ggatatataa tggcagttga agaattgtct agagtttgtg gtactacagg

Nucleotide sequences of pLogic031-tet-butyrate construct (SEQ ID NO: 69)

```
     1021 agttatatta tcagctcata catctcttgg ctcatggcct atatatcaat
atggtaatga
     1081 agaacaaaaa caaaaattct taagaccact agcaagtgga gaaaaattag
gagcatttgg
     1141 tcttactgag cctaatgctg gtacagatgc gtctggccaa caaacaactg
ctgttttaga
     1201 cggggatgaa tacatactta atggctcaaa aatatttata acaaacgcaa
tagctggtga
     1261 catatatgta gtaatggcaa tgactgataa atctaagggg aacaaaggaa
tatcagcatt
     1321 tatagttgaa aaaggaactc ctgggtttag ctttggagtt aaagaaaaga
aaatgggtat
     1381 aagaggttca gctacgagtg aattaatatt tgaggattgc agaataccta
aagaaaattt
     1441 acttggaaaa gaaggtcaag gatttaagat agcaatgtct actcttgatg
gtggtagaat
     1501 tggtatagct gcacaagctt taggtttagc acaaggtgct cttgatgaaa
ctgttaaata
     1561 tgtaaaagaa agagtacaat ttggtagacc attatcaaaa ttccaaaata
cacaattcca
     1621 attagctgat atggaagtta aggtacaagc ggctagacac cttgtatatc
aagcagctat
     1681 aaataaagac ttaggaaaac cttatggagt agaagcagca atggcaaaat
tatttgcagc
     1741 tgaaacagct atggaagtta ctacaaaagc tgtacaactt catggaggat
atggatacac
     1801 tcgtgactat ccagtagaaa gaatgatgag agatgctaag ataactgaaa
tatatgaagg
     1861 aactagtgaa gttcaaagaa tggttatttc aggaaaacta ttaaaatagt
aagaaggaga
     1921 tatacatatg gaggaaggat ttatgaatat agtcgtttgt ataaaacaag
ttccagatac
     1981 aacagaagtt aaactagatc ctaatacagg tactttaatt agagatggag
taccaagtat
     2041 aataaaccct gatgataaag caggtttaga agaagctata aaattaaaag
aagaaatggg
     2101 tgctcatgta actgttataa caatgggacc tcctcaagca gatatggctt
taaaagaagc
     2161 tttagcaatg ggtgcagata gaggtatatt attaacagat agagcatttg
cgggtgctga
     2221 tacttgggca acttcatcag cattagcagg agcattaaaa aatatagatt
ttgatattat
     2281 aatagctgga agacaggcga tagatggaga tactgcacaa gttggacctc
aaatagctga
     2341 acatttaaat cttccatcaa taacatatgc tgaagaaata aaaactgaag
gtgaatatgt
     2401 attagtaaaa agacaatttg aagattgttg ccatgactta aaagttaaaa
tgccatgcct
     2461 tataacaact cttaaagata tgaacacacc aagatacatg aaagttggaa
gaatatatga
     2521 tgctttcgaa aatgatgtag tagaaacatg gactgtaaaa gatatagaag
ttgacccttc
```

Nucleotide sequences of pLogic031-tet-butyrate construct (SEQ ID NO: 69)

```
     2581 taatttaggt cttaaaggtt ctccaactag tgtatttaaa tcatttacaa
aatcagttaa
     2641 accagctggt acaatataca atgaagatgc gaaaacatca gctggaatta
tcatagataa
     2701 attaaaagag aagtatatca tataataaga aggagatata catatgggta
acgttttagt
     2761 agtaatagaa caaagagaaa atgtaattca aactgtttct ttagaattac
taggaaaggc
     2821 tacagaaata gcaaaagatt atgatacaaa agtttctgca ttacttttag
gtagtaaggt
     2881 agaaggttta atagatacat tagcacacta tggtgcagat gaggtaatag
tagtagatga
     2941 tgaagcttta gcagtgtata caactgaacc atatacaaaa gcagcttatg
aagcaataaa
     3001 agcagctgac cctatagttg tattatttgg tgcaacttca ataggtagag
atttagcgcc
     3061 tagagtttct gctagaatac atacaggtct tactgctgac tgtacaggtc
ttgcagtagc
     3121 tgaagataca aaattattat taatgacaag acctgccttt ggtggaaata
taatggcaac
     3181 aatagtttgt aaagatttca gacctcaaat gtctacagtt agaccagggg
ttatgaagaa
     3241 aaatgaacct gatgaaacta aagaagctgt aattaaccgt ttcaaggtag
aatttaatga
     3301 tgctgataaa ttagttcaag ttgtacaagt aataaaagaa gctaaaaaac
aagttaaaat
     3361 agaagatgct aagatattag tttctgctgg acgtggaatg ggtggaaaag
aaaacttaga
     3421 catactttat gaattagctg aaattatagg tggagaagtt tctggttctc
gtgccactat
     3481 agatgcaggt tggttagata aagcaagaca agttggtcaa actggtaaaa
ctgtaagacc
     3541 agacctttat atagcatgtg gtatatctgg agcaatacaa catatagctg
gtatggaaga
     3601 tgctgagttt atagttgcta taaataaaaa tccagaagct ccaatattta
aatatgctga
     3661 tgttggtata gttggagatg ttcataaagt gcttccagaa cttatcagtc
agttaagtgt
     3721 tgcaaaagaa aaaggtgaag ttttagctaa ctaataagaa ggagatatac
atatgagaga
     3781 agtagtaatt gccagtgcag ctagaacagc agtaggaagt tttggaggag
catttaaatc
     3841 agtttcagcg gtagagttag gggtaacagc agctaaagaa gctataaaaa
gagctaacat
     3901 aactccagat atgatagatg aatctctttt agggggagta cttacagcag
gtcttggaca
     3961 aaatatagca agacaaatag cattaggagc aggaatacca gtagaaaaac
cagctatgac
     4021 tataaatata gtttgtggtt ctggattaag atctgtttca atggcatctc
aacttatagc
     4081 attaggtgat gctgatataa tgttagttgg tggagctgaa aacatgagta
tgtctcctta
```

Nucleotide sequences of pLogic031-tet-butyrate construct (SEQ ID NO: 69)

```
     4141 tttagtacca agtgcgagat atggtgcaag aatgggtgat gctgcttttg
ttgattcaat
     4201 gataaaagat ggattatcag acatatttaa taactatcac atgggtatta
ctgctgaaaa
     4261 catagcagag caatggaata taactagaga agaacaagat gaattagctc
ttgcaagtca
     4321 aaataaagct gaaaaagctc aagctgaagg aaaatttgat gaagaaatag
ttcctgttgt
     4381 tataaaagga agaaaaggtg acactgtagt agataaagat gaatatatta
agcctggcac
     4441 tacaatggag aaacttgcta agttaagacc tgcatttaaa aaagatggaa
cagttactgc
     4501 tggtaatgca tcaggaataa atgatggtgc tgctatgtta gtagtaatgg
ctaaagaaaa
     4561 agctgaagaa ctaggaatag agcctcttgc aactatagtt tcttatggaa
cagctggtgt
     4621 tgaccctaaa ataatgggat atggaccagt tccagcaact aaaaaagctt
tagaagctgc
     4681 taatatgact attgaagata tagatttagt tgaagctaat gaggcatttg
ctgcccaatc
     4741 tgtagctgta ataagagact taaatataga tatgaataaa gttaatgtta
atggtggagc
     4801 aatagctata ggacatccaa taggatgctc aggagcaaga atacttacta
cacttttata
     4861 tgaaatgaag agaagagatg ctaaaactgg tcttgctaca ctttgtatag
gcggtggaat
     4921 gggaactact ttaatagtta agagatagta agaaggagat atacatatga
aattagctgt
     4981 aataggtagt ggaactatgg gaagtggtat tgtacaaact tttgcaagtt
gtggacatga
     5041 tgtatgttta aagagtagaa ctcaaggtgc tatagataaa tgtttagctt
tattagataa
     5101 aaatttaact aagttagtta ctaagggaaa aatggatgaa gctacaaaag
cagaaatatt
     5161 aagtcatgtt agttcaacta ctaattatga agatttaaaa gatatggatt
taataataga
     5221 agcatctgta gaagacatga atataaagaa agatgttttc aagttactag
atgaattatg
     5281 taaagaagat actatcttgg caacaaatac ttcatcatta tctataacag
aaatagcttc
     5341 ttctactaag cgcccagata aagttatagg aatgcatttc tttaatccag
ttcctatgat
     5401 gaaattagtt gaagttataa gtggtcagtt aacatcaaaa gttacttttg
atacagtatt
     5461 tgaattatct aagagtatca ataaagtacc agtagatgta tctgaatctc
ctggatttgt
     5521 agtaaataga atacttatac ctatgataaa tgaagctgtt ggtatatatg
cagatggtgt
     5581 tgcaagtaaa gaagaaatag atgaagctat gaaattagga gcaaaccatc
caatgggacc
     5641 actagcatta ggtgatttaa tcggattaga tgttgtttta gctataatga
acgttttata
```

Nucleotide sequences of pLogic031-tet-butyrate construct (SEQ ID NO: 69)

```
     5701 tactgaattt ggagatacta aatatagacc tcatccactt ttagctaaaa
tggttagagc
     5761 taatcaatta ggaagaaaaa ctaagatagg attctatgat tataataaat
aataagaagg
     5821 agatatacat atgagtacaa gtgatgttaa agtttatgag aatgtagctg
ttgaagtaga
     5881 tggaaatata tgtacagtga aaatgaatag acctaaagcc cttaatgcaa
taaattcaaa
     5941 gactttagaa gaactttatg aagtatttgt agatattaat aatgatgaaa
ctattgatgt
     6001 tgtaatattg acaggggaag gaaaggcatt tgtagctgga gcagatattg
catacatgaa
     6061 agatttagat gctgtagctg ctaaagattt tagtatctta ggagcaaaag
cttttggaga
     6121 aatagaaaat agtaaaaaag tagtgatagc tgctgtaaac ggatttgctt
taggtggagg
     6181 atgtgaactt gcaatggcat gtgatataag aattgcatct gctaaagcta
aatttggtca
     6241 gccagaagta actcttggaa taactccagg atatggagga actcaaaggc
ttacaagatt
     6301 ggttggaatg gcaaaagcaa aagaattaat ctttacaggt caagttataa
aagctgatga
     6361 agctgaaaaa atagggctag taaatagagt cgttgagcca gacattttaa
tagaagaagt
     6421 tgagaaatta gctaagataa tagctaaaaa tgctcagctt gcagttagat
actctaaaga
     6481 agcaatacaa cttggtgctc aaactgatat aaatactgga atagatatag
aatctaattt
     6541 atttggtctt tgtttttcaa ctaaagacca aaaagaagga atgtcagctt
tcgttgaaaa
     6601 gagagaagct aactttataa aagggtaata agaaggagat atacatatga
gaagttttga
     6661 agaagtaatt aagtttgcaa aagaaagagg acctaaaact atatcagtag
catgttgcca
     6721 agataaagaa gttttaatgg cagttgaaat ggctagaaaa gaaaaaatag
caaatgccat
     6781 tttagtagga gatatagaaa agactaaaga aattgcaaaa agcatagaca
tggatatcga
     6841 aaattatgaa ctgatagata taaaagattt agcagaagca tctctaaaat
ctgttgaatt
     6901 agtttcacaa ggaaaagccg acatggtaat gaaaggctta gtagacacat
caataatact
     6961 aaaagcagtt ttaaataaag aagtaggtct tagaactgga aatgtattaa
gtcacgtagc
     7021 agtatttgat gtagagggat atgatagatt atttttcgta actgacgcag
ctatgaactt
     7081 agctcctgat acaaatacta aaaagcaaat catagaaaat gcttgcacag
tagcacattc
     7141 attagatata agtgaaccaa aagttgctgc aatatgcgca aaagaaaaag
taaatccaaa
     7201 aatgaaagat acagttgaag ctaaagaact agaagaaatg tatgaaagag
gagaaatcaa
```

Nucleotide sequences of pLogic031-tet-butyrate construct (SEQ ID NO: 69)
7261 aggttgtatg gttggtgggc cttttgcaat tgataatgca gtatctttag aagcagctaa 7321 acataaaggt ataaatcatc ctgtagcagg acgagctgat atattattag ccccagatat 7381 tgaaggtggt aacatattat ataaagcttt ggtattcttc tcaaaatcaa aaaatgcagg 7441 agttatagtt ggggctaaag caccaataat attaacttct agagcagaca gtgaagaaac 7501 taaactaaac tcaatagctt taggtgtttt aatggcagca aaggcataat aagaaggaga 7561 tatacatatg agcaaaatat ttaaaatctt aacaataaat cctggttcga catcaactaa 7621 aatagctgta tttgataatg aggatttagt atttgaaaaa actttaagac attcttcaga 7681 agaaatagga aaatatgaga aggtgtctga ccaatttgaa tttcgtaaac aagtaataga 7741 agaagctcta aaagaaggtg gagtaaaaac atctgaatta gatgctgtag taggtagagg 7801 aggacttctt aaacctataa aaggtggtac ttattcagta agtgctgcta tgattgaaga 7861 tttaaaagtg ggagttttag gagaacacgc ttcaaaccta ggtggaataa tagcaaaaca 7921 aataggtgaa gaagtaaatg ttccttcata catagtagac cctgttgttg tagatgaatt 7981 agaagatgtt gctagaattt ctggtatgcc tgaaataagt agagcaagtg tagtacatgc 8041 tttaaatcaa aaggcaatag caagaagata tgctagagaa ataaacaaga aatatgaaga 8101 tataaatctt atagttgcac acatgggtgg aggagtttct gttggagctc ataaaaatgg 8161 taaaatagta gatgttgcaa acgcattaga tggagaagga cctttctctc cagaaagaag 8221 tggtggacta ccagtaggtg cattagtaaa aatgtgcttt agtggaaaat atactcaaga 8281 tgaaattaaa aagaaaataa aaggtaatgg cggactagtt gcatacttaa acactaatga 8341 tgctagagaa gttgaagaaa gaattgaagc tggtgatgaa aaagctaaat tagtatatga 8401 agctatggca tatcaaatct ctaaagaaat aggagctagt gctgcagttc ttaagggaga 8461 tgtaaaagca atattattaa ctggtggaat cgcatattca aaaatgttta cagaaatgat 8521 tgcagataga gttaaattta tagcagatgt aaaagtttat ccaggtgaag atgaaatgat 8581 tgcattagct caaggtggac ttagagtttt aactggtgaa gaagaggctc aagtttatga 8641 taactaataa

Table 13

Nucleotide sequences of pLogic046-tet-butyrate construct (SEQ ID NO: 70)

```
        1 gtaaaacgac ggccagtgaa ttcgttaaga cccactttca catttaagtt
gtttttctaa
       61 tccgcatatg atcaattcaa ggccgaataa gaaggctggc tctgcacctt
ggtgatcaaa
      121 taattcgata gcttgtcgta ataatggcgg catactatca gtagtaggtg
tttccctttc
      181 ttctttagcg acttgatgct cttgatcttc caatacgcaa cctaaagtaa
aatgccccac
      241 agcgctgagt gcatataatg cattctctag tgaaaaacct tgttggcata
aaaaggctaa
      301 ttgattttcg agagtttcat actgtttttc tgtaggccgt gtacctaaat
gtacttttgc
      361 tccatcgcga tgacttagta aagcacatct aaaactttta gcgttattac
gtaaaaaatc
      421 ttgccagctt tccccttcta aagggcaaaa gtgagtatgg tgcctatcta
acatctcaat
      481 ggctaaggcg tcgagcaaag cccgcttatt ttttacatgc caatacaatg
taggctgctc
      541 tacacctagc ttctgggcga gtttacgggt tgttaaacct tcgattccga
cctcattaag
      601 cagctctaat gcgctgttaa tcactttact tttatctaat ctagacatca
ttaattccta

      661 attttgttg acactctatc attgatagag ttattttacc actccctatc
agtgatagag
      721 aaaagtgaac tctagaaata attttgttta actttaagaa ggagatatac
atatgatcgt
      781 aaaacctatg gtacgcaaca atatctgcct gaacgcccat cctcagggct
gcaagaaggg
      841 agtggaagat cagattgaat ataccaagaa acgcattacc gcagaagtca
aagctggcgc
      901 aaaagctcca aaaaacgttc tggtgcttgg ctgctcaaat ggttacggcc
tggcgagccg
      961 cattactgct gcgttcggat acggggctgc gaccatcggc gtgtcctttg
aaaaagcggg
     1021 ttcagaaacc aaatatggta caccgggatg gtacaataat ttggcatttg
atgaagcggc
     1081 aaaacgcgag ggtctttata gcgtgacgat cgacggcgat gcgttttcag
acgagatcaa
     1141 ggcccaggta attgaggaag ccaaaaaaaa aggtatcaaa tttgatctga
tcgtatacag
     1201 cttggccagc ccagtacgta ctgatcctga tacaggtatc atgcacaaaa
gcgttttgaa
     1261 acccttttgga aaaacgttca caggcaaaac agtagatccg tttactggcg
agctgaagga
     1321 aatctccgcg gaaccagcaa atgacgagga agcagccgcc actgttaaag
ttatgggggg
     1381 tgaagattgg gaacgttgga ttaagcagct gtcgaaggaa ggcctcttag
aagaaggctg
     1441 tattaccttg gcctatagtt atattggccc tgaagctacc caagctttgt
accgtaaagg
     1501 cacaatcggc aaggccaaag aacacctgga ggccacagca caccgtctca
acaaagagaa
     1561 cccgtcaatc cgtgccttcg tgagcgtgaa taaaggcctg gtaacccgcg
caagcgccgt
     1621 aatcccggta atccctctgt atctcgccag cttgttcaaa gtaatgaaag
```

Nucleotide sequences of pLogic046-tet-butyrate construct (SEQ ID NO: 70)

```
agaagggcaa
     1681 tcatgaaggt tgtattgaac agatcacgcg tctgtacgcc gagcgcctgt
accgtaaaga
     1741 tggtacaatt ccagttgatg aggaaaatcg cattcgcatt gatgattggg
agttagaaga
     1801 agacgtccag aaagcggtat ccgcgttgat ggagaaagtc acgggtgaaa
acgcagaatc
     1861 tctcactgac ttagcggggt accgccatga tttcttagct agtaacggct
ttgatgtaga
     1921 aggtattaat tatgaagcgg aagttgaacg cttcgaccgt atctgataag
aaggagatat
     1981 acatatgaga gaagtagtaa ttgccagtgc agctagaaca gcagtaggaa
gttttggagg
     2041 agcatttaaa tcagtttcag cggtagagtt aggggtaaca gcagctaaag
aagctataaa
     2101 aagagctaac ataactccag atatgataga tgaatctctt ttaggggggag
tacttacagc
     2161 aggtcttgga caaaatatag caagacaaat agcattagga gcaggaatac
cagtagaaaa
     2221 accagctatg actataaata tagtttgtgg ttctggatta agatctgttt
caatggcatc
     2281 tcaacttata gcattaggtg atgctgatat aatgttagtt ggtggagctg
aaaacatgag
     2341 tatgtctcct tatttagtac caagtgcgag atatggtgca agaatgggtg
atgctgcttt
     2401 tgttgattca atgataaaag atggattatc agacatattt aataactatc
acatgggtat
     2461 tactgctgaa aacatagcag agcaatggaa tataactaga gaagaacaag
atgaattagc
     2521 tcttgcaagt caaaataaag ctgaaaaagc tcaagctgaa ggaaaatttg
atgaagaaat
     2581 agttcctgtt gttataaaag gaagaaaagg tgacactgta gtagataaag
atgaatatat
     2641 taagcctggc actacaatgg agaaacttgc taagttaaga cctgcattta
aaaaagatgg
     2701 aacagttact gctggtaatg catcaggaat aaatgatggt gctgctatgt
tagtagtaat
     2761 ggctaaagaa aaagctgaag aactaggaat agagcctctt gcaactatag
tttcttatgg
     2821 aacagctggt gttgacccta aaataatggg atatggacca gttccagcaa
ctaaaaaagc
     2881 tttagaagct gctaatatga ctattgaaga tatagattta gttgaagcta
atgaggcatt
     2941 tgctgcccaa tctgtagctg taataagaga cttaaatata gatatgaata
aagttaatgt
     3001 taatggtgga gcaatagcta taggacatcc aataggatgc tcaggagcaa
gaatacttac
     3061 tacactttta tatgaaatga agagaagaga tgctaaaact ggtcttgcta
cactttgtat
     3121 aggcggtgga atgggaacta ctttaatagt taagagatag taagaaggag
atatacatat
     3181 gaaattagct gtaataggta gtggaactat gggaagtggt attgtacaaa
cttttgcaag
     3241 ttgtggacat gatgtatgtt taaagagtag aactcaaggt gctatagata
aatgtttagc
     3301 tttattagat aaaaatttaa ctaagttagt tactaaggga aaaatggatg
aagctacaaa
```

Nucleotide sequences of pLogic046-tet-butyrate construct (SEQ ID NO: 70)

```
     3361 agcagaaata ttaagtcatg ttagttcaac tactaattat gaagatttaa
aagatatgga
     3421 tttaataata gaagcatctg tagaagacat gaatataaag aaagatgttt
tcaagttact
     3481 agatgaatta tgtaaagaag atactatctt ggcaacaaat acttcatcat
tatctataac
     3541 agaaatagct tcttctacta agcgcccaga taaagttata ggaatgcatt
tctttaatcc
     3601 agttcctatg atgaaattag ttgaagttat aagtggtcag ttaacatcaa
aagttacttt
     3661 tgatacagta tttgaattat ctaagagtat caataaagta ccagtagatg
tatctgaatc
     3721 tcctggattt gtagtaaata gaatacttat acctatgata aatgaagctg
ttggtatata
     3781 tgcagatggt gttgcaagta aagaagaaat agatgaagct atgaaattag
gagcaaacca
     3841 tccaatggga ccactagcat taggtgattt aatcggatta gatgttgttt
tagctataat
     3901 gaacgtttta tatactgaat ttggagatac taaatataga cctcatccac
ttttagctaa
     3961 aatggttaga gctaatcaat taggaagaaa aactaagata ggattctatg
attataataa
     4021 ataataagaa ggagatatac atatgagtac aagtgatgtt aaagtttatg
agaatgtagc
     4081 tgttgaagta gatggaaata tatgtacagt gaaaatgaat agacctaaag
cccttaatgc
     4141 aataaattca aagactttag aagaacttta tgaagtattt gtagatatta
ataatgatga
     4201 aactattgat gttgtaatat tgacagggga aggaaaggca tttgtagctg
gagcagatat
     4261 tgcatacatg aaagatttag atgctgtagc tgctaaagat tttagtatct
taggagcaaa
     4321 agcttttgga gaaatagaaa atagtaaaaa agtagtgata gctgctgtaa
acggatttgc
     4381 tttaggtgga ggatgtgaac ttgcaatggc atgtgatata agaattgcat
ctgctaaagc
     4441 taaatttggt cagccagaag taactcttgg aataactcca ggatatggag
gaactcaaag
     4501 gcttacaaga ttggttggaa tggcaaaagc aaaagaatta atctttacag
gtcaagttat
     4561 aaaagctgat gaagctgaaa aaatagggct agtaaataga gtcgttgagc
cagacatttt
     4621 aatagaagaa gttgagaaat tagctaagat aatagctaaa aatgctcagc
ttgcagttag
     4681 atactctaaa gaagcaatac aacttggtgc tcaaactgat ataaatactg
gaatagatat
     4741 agaatctaat ttatttggtc tttgtttttc aactaaagac caaaaagaag
gaatgtcagc
     4801 tttcgttgaa aagagagaag ctaactttat aaaagggtaa taagaaggag
atatacatat
     4861 gagaagtttt gaagaagtaa ttaagtttgc aaaagaaaga ggacctaaaa
ctatatcagt
     4921 agcatgttgc caagataaag aagttttaat ggcagttgaa atggctagaa
aagaaaaaat
     4981 agcaaatgcc attttagtag gagatataga aaagactaaa gaaattgcaa
aaagcataga
     5041 catggatatc gaaaattatg aactgataga tataaaagat ttagcagaag
```

Nucleotide sequences of pLogic046-tet-butyrate construct (SEQ ID NO: 70)

```
catctctaaa
     5101 atctgttgaa ttagtttcac aaggaaaagc cgacatggta atgaaaggct
tagtagacac
     5161 atcaataata ctaaaagcag ttttaaataa agaagtaggt cttagaactg
gaaatgtatt
     5221 aagtcacgta gcagtatttg atgtagaggg atatgataga ttatttttcg
taactgacgc
     5281 agctatgaac ttagctcctg atacaaatac taaaaagcaa atcatagaaa
atgcttgcac
     5341 agtagcacat tcattagata taagtgaacc aaaagttgct gcaatatgcg
caaaagaaaa
     5401 agtaaatcca aaaatgaaag atacagttga agctaaagaa ctagaagaaa
tgtatgaaag
     5461 aggagaaatc aaaggttgta tggttggtgg gccttttgca attgataatg
cagtatcttt
     5521 agaagcagct aaacataaag gtataaatca tcctgtagca ggacgagctg
atatattatt
     5581 agccccagat attgaaggtg gtaacatatt atataaagct ttggtattct
tctcaaaatc
     5641 aaaaaatgca ggagttatag ttggggctaa agcaccaata atattaactt
ctagagcaga
     5701 cagtgaagaa actaaactaa actcaatagc tttaggtgtt ttaatggcag
caaaggcata
     5761 ataagaagga gatatacata tgagcaaaat atttaaaatc ttaacaataa
atcctggttc
     5821 gacatcaact aaaatagctg tatttgataa tgaggattta gtatttgaaa
aaactttaag
     5881 acattcttca gaagaaatag gaaaatatga gaaggtgtct gaccaatttg
aatttcgtaa
     5941 acaagtaata gaagaagctc taaaagaagg tggagtaaaa acatctgaat
tagatgctgt
     6001 agtaggtaga ggaggacttc ttaaacctat aaaaggtggt acttattcag
taagtgctgc
     6061 tatgattgaa gatttaaaag tgggagtttt aggagaacac gcttcaaacc
taggtggaat
     6121 aatagcaaaa caaataggtg aagaagtaaa tgttccttca tacatagtag
accctgttgt
     6181 tgtagatgaa ttagaagatg ttgctagaat ttctggtatg cctgaaataa
gtagagcaag
     6241 tgtagtacat gctttaaatc aaaaggcaat agcaagaaga tatgctagag
aaataaacaa
     6301 gaaatatgaa gatataaatc ttatagttgc acacatgggt ggaggagttt
ctgttggagc
     6361 tcataaaaat ggtaaaatag tagatgttgc aaacgcatta gatggagaag
gacctttctc
     6421 tccagaaaga agtggtggac taccagtagg tgcattagta aaaatgtgct
ttagtggaaa
     6481 atatactcaa gatgaaatta aaaagaaaat aaaaggtaat ggcggactag
ttgcatactt
     6541 aaacactaat gatgctagag aagttgaaga aagaattgaa gctggtgatg
aaaaagctaa
     6601 attagtatat gaagctatgg catatcaaat ctctaaagaa ataggagcta
gtgctgcagt
     6661 tcttaaggga gatgtaaaag caatattatt aactggtgga atcgcatatt
caaaaatgtt
     6721 tacagaaatg attgcagata gagttaaatt tatagcagat gtaaaagttt
atccaggtga
```

Nucleotide sequences of pLogic046-tet-butyrate construct (SEQ ID NO: 70)
6781 agatgaaatg attgcattag ctcaaggtgg acttagagtt ttaactggtg aagaagaggc 6841 tcaagtttat gataactaat aa

[0195] In some embodiments, the gene or gene cassette for producing the anti-inflammation and/or gut barrier function enhancer molecule is present on a plasmid and operably linked to a promoter that is induced by RNS. In some embodiments, the gene or gene cassette for producing the anti-inflammation and/or gut barrier function enhancer molecule is present in the chromosome and operably linked to a promoter that is induced by RNS. In some embodiments, the gene or gene cassette for producing the anti-inflammation and/or gut barrier function enhancer molecule is present on a chromosome and operably linked to a promoter that is induced by exposure to tetracycline. In some embodiments, the gene or gene cassette for producing the anti-inflammation and/or gut barrier function enhancer molecule is present on a plasmid and operably linked to a promoter that is induced by exposure to tetracycline. In some embodiments, expression is further optimized by methods known in the art, e.g., by optimizing ribosomal binding sites, manipulating transcriptional regulators, and/or increasing mRNA stability.

[0196] In some embodiments, the genetically engineered bacteria comprise a stably maintained plasmid or chromosome carrying the gene(s) or gene cassette(s) capable of producing an anti-inflammation and/or gut barrier function enhancer molecule, such that the gene(s) or gene cassette(s) can be expressed in the host cell, and the host cell is capable of survival and/or growth *in vitro*, e.g., in medium, and/or *in vivo*, e.g., in the gut. In some embodiments, a bacterium may comprise multiple copies of the gene or gene cassette for producing the anti-inflammation and/or gut barrier function enhance molecule. In some embodiments, gene or gene cassette is expressed on a low-copy plasmid. In some embodiments, the low-copy plasmid may be useful for increasing stability of expression. In some embodiments, the low-copy plasmid may be useful for decreasing leaky expression under non-inducing conditions. In some embodiments, gene or gene cassette is expressed on a high-copy plasmid. In some embodiments, the high-

copy plasmid may be useful for increasing gene or gene cassette expression. In some embodiments, gene or gene cassette is expressed on a chromosome.

[0197] In some embodiments, the genetically engineered bacteria may comprise multiple copies of the gene(s) or gene cassette(s) capable of producing an anti-inflammation and/or gut barrier function enhancer molecule. In some embodiments, the gene(s) or gene cassette(s) capable of producing an anti-inflammation and/or gut barrier function enhancer molecule is present on a plasmid and operatively linked to a RNS-responsive regulatory region. In some embodiments, the gene(s) or gene cassette(s) capable of producing an anti-inflammation and/or gut barrier function enhancer molecule is present in a chromosome and operatively linked to a RNS-responsive regulatory region.

[0198] In some embodiments, any of the gene(s) or gene cassette(s) of the present disclosure may be integrated into the bacterial chromosome at one or more integration sites. For example, one or more copies of the butyrogenic gene cassette may be integrated into the bacterial chromosome. Having multiple copies of the butyrogenic gene cassette integrated into the chromosome allows for greater production of the butyrate and also permits fine-tuning of the level of expression. Alternatively, different circuits described herein, such as any of the kill-switch circuits, in addition to the therapeutic gene(s) or gene cassette(s) could be integrated into the bacterial chromosome at one or more different integration sites to perform multiple different functions.

[0199] In some embodiments, the genetically engineered bacteria of the invention produce at least one anti-inflammation and/or gut barrier enhancer molecule in the presence of RNS to reduce local gut inflammation by at least about 1.5-fold, at least about 2-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 30-fold, at least about 50-fold, at least about 100-fold, at least about 200-fold, at least about 300-fold, at least about 400-fold, at least about 500-fold, at least about 600-fold, at least about 700-fold, at least about 800-fold, at least about 900-fold, at least about 1,000-fold, or at least about 1,500-fold as compared to unmodified bacteria of the same subtype under the same conditions. Inflammation may be

measured by methods known in the art, e.g., counting disease lesions using endoscopy; detecting T regulatory cell differentiation in peripheral blood, e.g., by fluorescence activated sorting; measuring T regulatory cell levels; measuring cytokine levels; measuring areas of mucosal damage; assaying inflammatory biomarkers, e.g., by qPCR; PCR arrays; transcription factor phosphorylation assays; immunoassays; and/or cytokine assay kits (Mesoscale, Cayman Chemical, Qiagen).

[0200] In some embodiments, the genetically engineered bacteria produce at least about 1.5-fold, at least about 2-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 30-fold, at least about 50-fold, at least about 100-fold, at least about 200-fold, at least about 300-fold, at least about 400-fold, at least about 500-fold, at least about 600-fold, at least about 700-fold, at least about 800-fold, at least about 900-fold, at least about 1,000-fold, or at least about 1,500-fold more of an anti-inflammation and/or gut barrier enhancer molecule in the presence of RNS than unmodified bacteria of the same subtype under the same conditions. Certain unmodified bacteria will not have detectable levels of the anti-inflammation and/or gut barrier enhancer molecule. In embodiments using genetically modified forms of these bacteria, the anti-inflammation and/or gut barrier enhancer molecule will be detectable in the presence of RNS.

[0201] In certain embodiments, the anti-inflammation and/or gut barrier enhancer molecule is butyrate. Methods of measuring butyrate levels, e.g., by mass spectrometry, gas chromatography, high-performance liquid chromatography (HPLC), are known in the art (*see, e.g.,* Aboulnaga et al., 2013). In some embodiments, butyrate is measured as butyrate level/bacteria optical density (OD). In some embodiments, measuring the activity and/or expression of one or more gene products in the butyrogenic gene cassette serves as a proxy measurement for butyrate production. In some embodiments, the bacterial cells of the invention are harvested and lysed to measure butyrate production. In alternate embodiments, butyrate production is measured in the bacterial cell medium. In some embodiments, the genetically engineered bacteria produce at least about 1 nM/OD, at least about 10 nM/OD, at least about 100 nM/OD, at least about 500 nM/OD, at least about 1 μM/OD, at least about 10

μM/OD, at least about 100 μM/OD, at least about 500 μM/OD, at least about 1 mM/OD, at least about 2 mM/OD, at least about 3 mM/OD, at least about 5 mM/OD, at least about 10 mM/OD, at least about 20 mM/OD, at least about 30 mM/OD, or at least about 50 mM/OD of butyrate in the presence of RNS.

ROS-dependent regulation

[0202] In some embodiments, the genetically engineered bacteria of the invention comprise a tunable regulatory region that is directly or indirectly controlled by a transcription factor that is capable of sensing at least one reactive oxygen species. The tunable regulatory region is operatively linked to a gene or gene cassette capable of directly or indirectly driving the expression of an anti-inflammation and/or gut barrier function enhancer molecule, thus controlling expression of the molecule relative to ROS levels. For example, the tunable regulatory region is a ROS-inducible regulatory region, and the molecule is butyrate; when ROS is present, e.g., in an inflamed tissue, a ROS-sensing transcription factor binds to and/or activates the regulatory region and drives expression of the butyrogenic gene cassette, thereby producing butyrate, which exerts anti-inflammation and/or gut barrier enhancing effects. Subsequently, when inflammation is ameliorated, ROS levels are reduced, and butyrate production is decreased or eliminated.

[0203] In some embodiments, the tunable regulatory region is a ROS-inducible regulatory region; in the presence of ROS, a transcription factor senses ROS and activates the ROS-inducible regulatory region, thereby driving expression of an operatively linked gene or gene cassette. In some embodiments, the transcription factor senses ROS and subsequently binds to the ROS-inducible regulatory region, thereby activating downstream gene expression. In alternate embodiments, the transcription factor is bound to the ROS-inducible regulatory region in the absence of ROS; when the transcription factor senses ROS, it undergoes a conformational change, thereby inducing downstream gene expression.

[0204] In some embodiments, the tunable regulatory region is a ROS-inducible regulatory region, and the transcription factor that senses ROS is OxyR. OxyR "functions primarily as a global regulator of the peroxide stress response" and is capable of

regulating dozens of genes, e.g., "genes involved in H_2O_2 detoxification (*katE, ahpCF*), heme biosynthesis (*hemH*), reductant supply (*grxA, gor, trxC*), thiol-disulfide isomerization (*dsbG*), Fe-S center repair (*sufA-E, sufS*), iron binding (*yaaA*), repression of iron import systems (*fur*)" and "OxyS, a small regulatory RNA" (Dubbs et al., 2012). The genetically engineered bacteria of the invention may comprise any suitable ROS-responsive regulatory region from a gene that is activated by OxyR. Genes that are capable of being activated by OxyR are known in the art (*see, e.g.,* Zheng et al., 2001; Dubbs et al., 2012; Table 1). In certain embodiments, the genetically engineered bacteria of the invention comprise a ROS-inducible regulatory region from *oxyS* that is operatively linked to a gene or gene cassette, e.g., a butyrogenic gene cassette. In the presence of ROS, e.g., H_2O_2, an OxyR transcription factor senses ROS and activates to the *oxyS* regulatory region, thereby driving expression of the operatively linked butyrogenic gene cassette and producing butyrate. In some embodiments, OxyR is encoded by an *E. coli oxyR* gene. In some embodiments, the *oxyS* regulatory region is an *E. coli oxyS* regulatory region. In some embodiments, the ROS-inducible regulatory region is selected from the regulatory region of *katG*, *dps*, and *ahpC*.

[0205] In alternate embodiments, the tunable regulatory region is a ROS-inducible regulatory region, and the corresponding transcription factor that senses ROS is SoxR. When SoxR is "activated by oxidation of its [2Fe–2S] cluster, it increases the synthesis of SoxS, which then activates its target gene expression" (Koo et al., 2003). "SoxR is known to respond primarily to superoxide and nitric oxide" (Koo et al., 2003), and is also capable of responding to H_2O_2. The genetically engineered bacteria of the invention may comprise any suitable ROS-responsive regulatory region from a gene that is activated by SoxR. Genes that are capable of being activated by SoxR are known in the art (*see, e.g.,* Koo et al., 2003; Table 1). In certain embodiments, the genetically engineered bacteria of the invention comprise a ROS-inducible regulatory region from *soxS* that is operatively linked to a gene or gene cassette, e.g., a butyrogenic gene cassette. In the presence of ROS, the SoxR transcription factor senses ROS and activates the *soxS* regulatory region, thereby driving expression of the operatively linked butyrogenic gene cassette and producing butyrate.

[0206] In some embodiments, the tunable regulatory region is a ROS-derepressible regulatory region, and binding of a corresponding transcription factor represses downstream gene expression; in the presence of ROS, the transcription factor no longer binds to the regulatory region, thereby derepressing the operatively linked gene or gene cassette.

[0207] In some embodiments, the tunable regulatory region is a ROS-derepressible regulatory region, and the transcription factor that senses ROS is OhrR. OhrR "binds to a pair of inverted repeat DNA sequences overlapping the *ohrA* promoter site and thereby represses the transcription event," but oxidized OhrR is "unable to bind its DNA target" (Duarte et al., 2010). OhrR is a "transcriptional repressor [that]... senses both organic peroxides and NaOCl" (Dubbs et al., 2012) and is "weakly activated by H_2O_2 but it shows much higher reactivity for organic hydroperoxides" (Duarte et al., 2010). The genetically engineered bacteria of the invention may comprise any suitable ROS-responsive regulatory region from a gene that is repressed by OhrR. Genes that are capable of being repressed by OhrR are known in the art (*see, e.g.,* Dubbs et al., 2012; Table 1). In certain embodiments, the genetically engineered bacteria of the invention comprise a ROS-derepressible regulatory region from *ohrA* that is operatively linked to a gene or gene cassette, e.g., a butyrogenic gene cassette. In the presence of ROS, e.g., NaOCl, an OhrR transcription factor senses ROS and no longer binds to the *ohrA* regulatory region, thereby derepressing the operatively linked butyrogenic gene cassette and producing butyrate.

[0208] OhrR is a member of the MarR family of ROS-responsive regulators. "Most members of the MarR family are transcriptional repressors and often bind to the -10 or -35 region in the promoter causing a steric inhibition of RNA polymerase binding" (Bussmann et al., 2010). Other members of this family are known in the art and include, but are not limited to, OspR, MgrA, RosR, and SarZ. In some embodiments, the transcription factor that senses ROS is OspR, MgRA, RosR, and/or SarZ, and the genetically engineered bacteria of the invention comprises one or more corresponding regulatory region sequences from a gene that is repressed by OspR, MgRA, RosR, and/or

SarZ. Genes that are capable of being repressed by OspR, MgRA, RosR, and/or SarZ are known in the art (*see, e.g.,* Dubbs et al., 2012).

[0209] In some embodiments, the tunable regulatory region is a ROS-derepressible regulatory region, and the corresponding transcription factor that senses ROS is RosR. RosR is "a MarR-type transcriptional regulator" that binds to an "18-bp inverted repeat with the consensus sequence TTGTTGAYRYRTCAACWA" and is "reversibly inhibited by the oxidant H_2O_2" (Bussmann et al., 2010). RosR is capable of repressing numerous genes and putative genes, including but not limited to "a putative polyisoprenoid-binding protein (cg1322, gene upstream of and divergent from *rosR*), a sensory histidine kinase (*cgtS9*), a putative transcriptional regulator of the Crp/FNR family (cg3291), a protein of the glutathione S-transferase family (cg1426), two putative FMN reductases (cg1150 and cg1850), and four putative monooxygenases (cg0823, cg1848, cg2329, and cg3084)" (Bussmann et al., 2010). The genetically engineered bacteria of the invention may comprise any suitable ROS-responsive regulatory region from a gene that is repressed by RosR. Genes that are capable of being repressed by RosR are known in the art (*see, e.g.,* Bussmann et al., 2010; Table 1). In certain embodiments, the genetically engineered bacteria of the invention comprise a ROS-derepressible regulatory region from *cgtS9* that is operatively linked to a gene or gene cassette, e.g., a butyrogenic gene cassette. In the presence of ROS, e.g., H_2O_2, a RosR transcription factor senses ROS and no longer binds to the *cgtS9* regulatory region, thereby derepressing the operatively linked butyrogenic gene cassette and producing butyrate.

[0210] In some embodiments, it is advantageous for the genetically engineered bacteria to express a ROS-sensing transcription factor that does not regulate the expression of a significant number of native genes in the bacteria. In some embodiments, the genetically engineered bacterium of the invention expresses a ROS-sensing transcription factor from a different species, strain, or substrain of bacteria, wherein the transcription factor does not bind to regulatory sequences in the genetically engineered bacterium of the invention. In some embodiments, the genetically engineered bacterium of the invention is *Escherichia coli,* and the ROS-sensing

transcription factor is RosR, e.g., from *Corynebacterium glutamicum*, wherein the *Escherichia coli* does not comprise binding sites for said RosR. In some embodiments, the heterologous transcription factor minimizes or eliminates off-target effects on endogenous regulatory regions and genes in the genetically engineered bacteria.

[0211] In some embodiments, the tunable regulatory region is a ROS-repressible regulatory region, and binding of a corresponding transcription factor represses downstream gene expression; in the presence of ROS, the transcription factor senses ROS and binds to the ROS-repressible regulatory region, thereby repressing expression of the operatively linked gene or gene cassette. In some embodiments, the ROS-sensing transcription factor is capable of binding to a regulatory region that overlaps with part of the promoter sequence. In alternate embodiments, the ROS-sensing transcription factor is capable of binding to a regulatory region that is upstream or downstream of the promoter sequence.

[0212] In some embodiments, the tunable regulatory region is a ROS-repressible regulatory region, and the transcription factor that senses ROS is PerR. In *Bacillus subtilis*, PerR "when bound to DNA, represses the genes coding for proteins involved in the oxidative stress response (*katA, ahpC,* and *mrgA*), metal homeostasis (*hemAXCDBL, fur,* and *zoaA*) and its own synthesis (*perR*)" (Marinho et al., 2014). PerR is a "global regulator that responds primarily to H_2O_2" (Dubbs et al., 2012) and "interacts with DNA at the *per* box, a specific palindromic consensus sequence (TTATAATNATTATAA) residing within and near the promoter sequences of PerR-controlled genes" (Marinho et al., 2014). PerR is capable of binding a regulatory region that "overlaps part of the promoter or is immediately downstream from it" (Dubbs et al., 2012). The genetically engineered bacteria of the invention may comprise any suitable ROS-responsive regulatory region from a gene that is repressed by PerR. Genes that are capable of being repressed by PerR are known in the art (*see, e.g.,* Dubbs et al., 2012; Table 1).

[0213] In these embodiments, the genetically engineered bacteria may comprise a two repressor activation regulatory circuit, which is used to express an anti-inflammation and/or gut barrier function enhancer molecule. The two repressor activation regulatory circuit comprises a first ROS-sensing repressor, e.g., PerR, and a

second repressor, e.g., TetR, which is operatively linked to a gene or gene cassette, e.g., a butyrogenic gene cassette. In one aspect of these embodiments, the ROS-sensing repressor inhibits transcription of the second repressor, which inhibits the transcription of the gene or gene cassette. Examples of second repressors useful in these embodiments include, but are not limited to, TetR, C1, and LexA. In some embodiments, the ROS-sensing repressor is PerR. In some embodiments, the second repressor is TetR. In this embodiment, a PerR-repressible regulatory region drives expression of TetR, and a TetR-repressible regulatory region drives expression of the gene or gene cassette, e.g., a butyrogenic gene cassette. In the absence of PerR binding (which occurs in the absence of ROS), *tetR* is transcribed, and TetR represses expression of the gene or gene cassette, e.g., a butyrogenic gene cassette. In the presence of PerR binding (which occurs in the presence of ROS), *tetR* expression is repressed, and the gene or gene cassette, e.g., a butyrogenic gene cassette, is expressed.

[0214] A ROS-responsive transcription factor may induce, derepress, or repress gene expression depending upon the regulatory region sequence used in the genetically engineered bacteria. For example, although "OxyR is primarily thought of as a transcriptional activator under oxidizing conditions… OxyR can function as either a repressor or activator under both oxidizing and reducing conditions" (Dubbs et al., 2012), and OxyR "has been shown to be a repressor of its own expression as well as that of *fhuF* (encoding a ferric ion reductase) and *flu* (encoding the antigen 43 outer membrane protein)" (Zheng et al., 2001). The genetically engineered bacteria of the invention may comprise any suitable ROS-responsive regulatory region from a gene that is repressed by OxyR. In some embodiments, OxyR is used in a two repressor activation regulatory circuit, as described above. Genes that are capable of being repressed by OxyR are known in the art (*see, e.g.,* Zheng et al., 2001; Table 1). Or, for example, although RosR is capable of repressing a number of genes, it is also capable of activating certain genes, e.g., the *narKGHJI* operon. In some embodiments, the genetically engineered bacteria comprise any suitable ROS-responsive regulatory region from a gene that is activated by RosR. In addition, "PerR-mediated positive regulation has also been observed…and appears to involve PerR binding to distant upstream sites" (Dubbs et al., 2012). In some

embodiments, the genetically engineered bacteria comprise any suitable ROS-responsive regulatory region from a gene that is activated by PerR.

[0215] One or more types of ROS-sensing transcription factors and corresponding regulatory region sequences may be present in genetically engineered bacteria. For example, "OhrR is found in both Gram-positive and Gram-negative bacteria and can coreside with either OxyR or PerR or both" (Dubbs et al., 2012). In some embodiments, the genetically engineered bacteria comprise one type of ROS-sensing transcription factor, e.g., OxyR, and one corresponding regulatory region sequence, e.g., from *oxyS*. In some embodiments, the genetically engineered bacteria comprise one type of ROS-sensing transcription factor, e.g., OxyR, and two or more different corresponding regulatory region sequences, e.g., from *oxyS* and *katG*. In some embodiments, the genetically engineered bacteria comprise two or more types of ROS-sensing transcription factors, e.g., OxyR and PerR, and two or more corresponding regulatory region sequences, e.g., from *oxyS* and *katA*, respectively. One ROS-responsive regulatory region may be capable of binding more than one transcription factor. In some embodiments, the genetically engineered bacteria comprise two or more types of ROS-sensing transcription factors and one corresponding regulatory region sequence.

[0216] Nucleic acid sequences of several exemplary OxyR-regulated regulatory regions are shown in **Table 14**. OxyR binding sites are **underlined and bolded**. In some embodiments, genetically engineered bacteria comprise a nucleic acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homologous to the DNA sequence of SEQ ID NO: 71, 72, 73, or 74, or a functional fragment thereof.

Table 14: Nucleotide sequences of exemplary OxyR-regulated regulatory regions

Regulatory sequence	0123456789012345678901234567890123456789012345678 9

Regulatory sequence	0123456789012345678901234567890123456789012345678
katG (SEQ ID NO: 71)	TGTGGCTTTTATGAAAATCACACAGTGATCACAAATTTTAAACAGAGCAC AAAATGCTGCCTCGAAATGAGGGCGGGAAAATAAGGTTATCAGCCTTGTT TTCTCCCTCATTACTTGAAGGATATGAAGCTAAAACCCTTTTTTATAAAG CATTTGTCCGAATTCGGACATAATCAAAAAAGCTTAATTAAGATCAATTT GATCTACATCTCTTTAACCAACAATATGTAAGATCTCAACTATCGCATCCG TGGATTAATTCAATTATAACTTCTCTCTAACGCTGTGTATCGTAACGGT AACACTGTAGAGGGGAGCACATTGATGCGAATTCATTAAAGAGGAGAAAG GTACC
dps (SEQ ID NO: 72)	TTCCGAAAATTCCTGGCGAGCAGATAAATAAGAATTGTTCTTATCAATAT ATCTAACTCATTGAATCTTTATTAGTTTTGTTTTTCACGCTTGTTACCAC TATTAGTGTGATAGGAACAGCCAGAATAGCGGAACACATAGCCGGTGCTA TACTTAATCTCGTTAATTACTGGGACATAACATCAAGAGGATATGAAATT CGAATTCATTAAAGAGGAGAAAGGTACC
ahpC (SEQ ID NO: 73)	GCTTAGATCAGGTGATTGCCCTTTGTTTATGAGGGTGTTGTAATCCATGT CGTTGTTGCATTTGTAAGGGCAACACCTCAGCCTGCAGGCAGGCACTGAA GATACCAAAGGGTAGTTCAGATTACACGGTCACCTGGAAAGGGGGCCATT TTACTTTTTATCGCCGCTGGCGGTGCAAAGTTCACAAAGTTGTCTTACGA AGGTTGTAAGGTAAAACTTATCGATTTGATAATGGAAACGCATTAGCCGA ATCGGCAAAAATTGGTTACCTTACATCTCATCGAAAACACGGAGGAAGTA TAGATGCGAATTCATTAAAGAGGAGAAAGGTACC
oxyS (SEQ ID NO: 74)	CTCGAGTTCATTATCCATCCTCCATCGCCACGATAGTTCATGGCGATAGG TAGAATAGCAATGAACGATTATCCCTATCAAGCATTCTGACTGATAATTG CTCACACGAATTCATTAAAGAGGAGAAAGGTACC

[0217] In some embodiments, the genetically engineered bacteria of the invention comprise a gene encoding a ROS-sensing transcription factor, e.g., the *oxyR* gene, that is controlled by its native promoter, an inducible promoter, a promoter that is stronger than the native promoter, e.g., the GlnRS promoter or the P(Bla) promoter, or a constitutive promoter. In some instances, it may be advantageous to express the ROS-sensing transcription factor under the control of an inducible promoter in order to enhance expression stability. In some embodiments, expression of the ROS-sensing transcription factor is controlled by a different promoter than the promoter that controls expression of the therapeutic molecule. In some embodiments, expression of the ROS-sensing transcription factor is controlled by the same promoter that controls expression

of the therapeutic molecule. In some embodiments, the ROS-sensing transcription factor and therapeutic molecule are divergently transcribed from a promoter region.

[0218] In some embodiments, the genetically engineered bacteria of the invention comprise a gene for a ROS-sensing transcription factor from a different species, strain, or substrain of bacteria. In some embodiments, the genetically engineered bacteria comprise a ROS-responsive regulatory region from a different species, strain, or substrain of bacteria. In some embodiments, the genetically engineered bacteria comprise a ROS-sensing transcription factor and corresponding ROS-responsive regulatory region from a different species, strain, or substrain of bacteria. The heterologous ROS-sensing transcription factor and regulatory region may increase the transcription of genes operatively linked to said regulatory region in the presence of ROS, as compared to the native transcription factor and regulatory region from bacteria of the same subtype under the same conditions.

[0219] In some embodiments, the genetically engineered bacteria comprise a ROS-sensing transcription factor, OxyR, and corresponding regulatory region, *oxyS*, from *Escherichia coli*. In some embodiments, the native ROS-sensing transcription factor, e.g., OxyR, is left intact and retains wild-type activity. In alternate embodiments, the native ROS-sensing transcription factor, e.g., OxyR, is deleted or mutated to reduce or eliminate wild-type activity.

[0220] In some embodiments, the genetically engineered bacteria of the invention comprise multiple copies of the endogenous gene encoding the ROS-sensing transcription factor, e.g., the *oxyR* gene. In some embodiments, the gene encoding the ROS-sensing transcription factor is present on a plasmid. In some embodiments, the gene encoding the ROS-sensing transcription factor and the gene or gene cassette for producing the therapeutic molecule are present on different plasmids. In some embodiments, the gene encoding the ROS-sensing transcription factor and the gene or gene cassette for producing the therapeutic molecule are present on the same. In some embodiments, the gene encoding the ROS-sensing transcription factor is present on a chromosome. In some embodiments, the gene encoding the ROS-sensing transcription factor and the gene or gene cassette for producing the therapeutic molecule are present

on different chromosomes. In some embodiments, the gene encoding the ROS-sensing transcription factor and the gene or gene cassette for producing the therapeutic molecule are present on the same chromosome.

[0221] In some embodiments, the genetically engineered bacteria comprise a wild-type gene encoding a ROS-sensing transcription factor, e.g., the *soxR* gene, and a corresponding regulatory region, e.g., a *soxS* regulatory region, that is mutated relative to the wild-type regulatory region from bacteria of the same subtype. The mutated regulatory region increases the expression of the anti-inflammation and/or gut barrier enhancer molecule in the presence of ROS, as compared to the wild-type regulatory region under the same conditions. In some embodiments, the genetically engineered bacteria comprise a wild-type ROS-responsive regulatory region, e.g., the *oxyS* regulatory region, and a corresponding transcription factor, e.g., OxyR, that is mutated relative to the wild-type transcription factor from bacteria of the same subtype. The mutant transcription factor increases the expression of the anti-inflammation and/or gut barrier enhancer molecule in the presence of ROS, as compared to the wild-type transcription factor under the same conditions. In some embodiments, both the ROS-sensing transcription factor and corresponding regulatory region are mutated relative to the wild-type sequences from bacteria of the same subtype in order to increase expression of the anti-inflammation and/or gut barrier enhancer molecule in the presence of ROS.

[0222] Nucleic acid sequences of exemplary ROS-regulated constructs comprising an *oxyS* promoter are shown in **Table 15** and **Table 16**. The nucleic acid sequence of an exemplary construct encoding OxyR is shown in **Table 17**. Nucleic acid sequences of tetracycline-regulated constructs comprising a *tet* promoter are shown in **Table 18** and **Table 19**. **Table 15** depicts the nucleic acid sequence of an exemplary ROS-regulated construct comprising an *oxyS* promoter and a butyrogenic gene cassette (pLogic031-oxyS-butyrate construct; SEQ ID NO: 75). **Table 16** depicts the nucleic acid sequence of an exemplary ROS-regulated construct comprising an *oxyS* promoter and a butyrogenic gene cassette (pLogic046-oxyS-butyrate construct; SEQ ID NO: 76). **Table 17** depicts the nucleic acid sequence of an exemplary construct encoding OxyR (pZA22-oxyR construct; SEQ ID NO: 77). **Table 18** depicts the nucleic acid sequence of an exemplary tetracycline-

regulated construct comprising a *tet* promoter and a butyrogenic gene cassette (pLogic031-tet-butyrate construct; SEQ ID NO: 78). The sequence encoding TetR is underlined, and the overlapping tetR/tetA promoters are boxed. **Table 19** depicts the nucleic acid sequence of an exemplary tetracycline-regulated construct comprising a *tet* promoter and a butyrogenic gene cassette (pLogic046-tet-butyrate construct; SEQ ID NO: 79). The sequence encoding TetR is underlined, and the overlapping tetR/tetA promoters are boxed.

Table 15

Nucleotide sequences of pLogic031-oxyS-butyrate construct (SEQ ID NO: 75)

```
        1 ctcgagttca ttatccatcc tccatcgcca cgatagttca tggcgatagg
tagaatagca
       61 atgaacgatt atccctatca agcattctga ctgataattg ctcacacgaa
ttcattaaag
      121 aggagaaagg taccatggat ttaaattcta aaaaatatca gatgcttaaa
gagctatatg
      181 taagcttcgc tgaaaatgaa gttaaacctt tagcaacaga acttgatgaa
gaagaaagat
      241 ttccttatga aacagtggaa aaaatggcaa aagcaggaat gatgggtata
ccatatccaa
      301 aagaatatgg tggagaaggt ggagacactg taggatatat aatggcagtt
gaagaattgt
      361 ctagagtttg tggtactaca ggagttatat tatcagctca tacatctctt
ggctcatggc
      421 ctatatatca atatggtaat gaagaacaaa aacaaaaatt cttaagacca
ctagcaagtg
      481 gagaaaaatt aggagcattt ggtcttactg agcctaatgc tggtacagat
gcgtctggcc
      541 aacaaacaac tgctgtttta gacggggatg aatacatact taatggctca
aaaatattta
      601 taacaaacgc aatagctggt gacatatatg tagtaatggc aatgactgat
aaatctaagg
      661 ggaacaaagg aatatcagca tttatagttg aaaaaggaac tcctgggttt
agctttggag
      721 ttaaagaaaa gaaaatgggt ataagaggtt cagctacgag tgaattaata
tttgaggatt
      781 gcagaatacc taaagaaaat ttacttggaa aagaaggtca aggatttaag
atagcaatgt
      841 ctactcttga tggtggtaga attggtatag ctgcacaagc tttaggttta
gcacaaggtg
      901 ctcttgatga aactgttaaa tatgtaaaag aaagagtaca atttggtaga
ccattatcaa
      961 aattccaaaa tacacaattc caattagctg atatggaagt taaggtacaa
gcggctagac
     1021 accttgtata tcaagcagct ataaataaag acttaggaaa accttatgga
gtagaagcag
     1081 caatggcaaa attatttgca gctgaaacag ctatggaagt tactacaaaa
gctgtacaac
     1141 ttcatggagg atatggatac actcgtgact atccagtaga aagaatgatg
agagatgcta
     1201 agataactga aatatatgaa ggaactagtg aagttcaaag aatggttatt
tcaggaaaac
```

Nucleotide sequences of pLogic031-oxyS-butyrate construct (SEQ ID NO: 75)

```
     1261 tattaaaata gtaagaagga gatatacata tggaggaagg atttatgaat
atagtcgttt
     1321 gtataaaaca agttccagat acaacagaag ttaaactaga tcctaataca
ggtactttaa
     1381 ttagagatgg agtaccaagt ataataaacc ctgatgataa agcaggttta
gaagaagcta
     1441 taaaattaaa agaagaaatg ggtgctcatg taactgttat aacaatggga
cctcctcaag
     1501 cagatatggc tttaaaagaa gctttagcaa tgggtgcaga tagaggtata
ttattaacag
     1561 atagagcatt tgcgggtgct gatacttggg caacttcatc agcattagca
ggagcattaa
     1621 aaaatataga ttttgatatt ataatagctg gaagacaggc gatagatgga
gatactgcac
     1681 aagttggacc tcaaatagct gaacatttaa atcttccatc aataacatat
gctgaagaaa
     1741 taaaaactga aggtgaatat gtattagtaa aaagacaatt tgaagattgt
tgccatgact
     1801 taaaagttaa aatgccatgc cttataacaa ctcttaaaga tatgaacaca
ccaagataca
     1861 tgaaagttgg aagaatatat gatgctttcg aaaatgatgt agtagaaaca
tggactgtaa
     1921 aagatataga agttgaccct tctaatttag gtcttaaagg ttctccaact
agtgtattta
     1981 aatcatttac aaaatcagtt aaaccagctg gtacaatata caatgaagat
gcgaaaacat
     2041 cagctggaat tatcatagat aaattaaaag agaagtatat catataataa
gaaggagata
     2101 tacatatggg taacgtttta gtagtaatag aacaaagaga aaatgtaatt
caaactgttt
     2161 ctttagaatt actaggaaag gctacagaaa tagcaaaaga ttatgataca
aaagtttctg
     2221 cattactttt aggtagtaag gtagaaggtt taatagatac attagcacac
tatggtgcag
     2281 atgaggtaat agtagtagat gatgaagctt tagcagtgta tacaactgaa
ccatatacaa
     2341 aagcagctta tgaagcaata aaagcagctg accctatagt tgtattattt
ggtgcaactt
     2401 caataggtag agatttagcg cctagagttt ctgctagaat acatacaggt
cttactgctg
     2461 actgtacagg tcttgcagta gctgaagata caaaattatt attaatgaca
agacctgcct
     2521 ttggtggaaa tataatggca acaatagttt gtaaagattt cagacctcaa
atgtctacag
     2581 ttagaccagg ggttatgaag aaaaatgaac ctgatgaaac taaagaagct
gtaattaacc
     2641 gtttcaaggt agaatttaat gatgctgata aattagttca agttgtacaa
gtaataaaag
     2701 aagctaaaaa acaagttaaa atagaagatg ctaagatatt agtttctgct
ggacgtggaa
     2761 tgggtggaaa agaaaactta gacatacttt atgaattagc tgaaattata
ggtggagaag
     2821 tttctggttc tcgtgccact atagatgcag gttggttaga taaagcaaga
caagttggtc
     2881 aaactggtaa aactgtaaga ccagaccttt atatagcatg tggtatatct
ggagcaatac
     2941 aacatatagc tggtatggaa gatgctgagt ttatagttgc tataaataaa
```

Nucleotide sequences of pLogic031-oxyS-butyrate construct (SEQ ID NO: 75)

```
aatccagaag
     3001 ctccaatatt taaatatgct gatgttggta tagttggaga tgttcataaa
gtgcttccag
     3061 aacttatcag tcagttaagt gttgcaaaag aaaaaggtga agttttagct
aactaataag
     3121 aaggagatat acatatgaga gaagtagtaa ttgccagtgc agctagaaca
gcagtaggaa
     3181 gttttggagg agcatttaaa tcagtttcag cggtagagtt aggggtaaca
gcagctaaag
     3241 aagctataaa aagagctaac ataactccag atatgataga tgaatctctt
ttagggggag
     3301 tacttacagc aggtcttgga caaaatatag caagacaaat agcattagga
gcaggaatac
     3361 cagtagaaaa accagctatg actataaata tagtttgtgg ttctggatta
agatctgttt
     3421 caatggcatc tcaacttata gcattaggtg atgctgatat aatgttagtt
ggtggagctg
     3481 aaaacatgag tatgtctcct tatttagtac caagtgcgag atatggtgca
agaatgggtg
     3541 atgctgcttt tgttgattca atgataaaag atggattatc agacatattt
aataactatc
     3601 acatgggtat tactgctgaa aacatagcag agcaatggaa tataactaga
gaagaacaag
     3661 atgaattagc tcttgcaagt caaaataaag ctgaaaaagc tcaagctgaa
ggaaaatttg
     3721 atgaagaaat agttcctgtt gttataaaag gaagaaaagg tgacactgta
gtagataaag
     3781 atgaatatat taagcctggc actacaatgg agaaacttgc taagttaaga
cctgcattta
     3841 aaaaagatgg aacagttact gctggtaatg catcaggaat aaatgatggt
gctgctatgt
     3901 tagtagtaat ggctaaagaa aaagctgaag aactaggaat agagcctctt
gcaactatag
     3961 tttcttatgg aacagctggt gttgacccta aaataatggg atatggacca
gttccagcaa
     4021 ctaaaaaagc tttagaagct gctaatatga ctattgaaga tatagattta
gttgaagcta
     4081 atgaggcatt tgctgcccaa tctgtagctg taataagaga cttaaatata
gatatgaata
     4141 aagttaatgt taatggtgga gcaatagcta taggacatcc aataggatgc
tcaggagcaa
     4201 gaatacttac tacactttta tatgaaatga agagaagaga tgctaaaact
ggtcttgcta
     4261 cactttgtat aggcggtgga atgggaacta ctttaatagt taagagatag
taagaaggag
     4321 atatacatat gaaattagct gtaataggta gtggaactat gggaagtggt
attgtacaaa
     4381 cttttgcaag ttgtggacat gatgtatgtt taaagagtag aactcaaggt
gctatagata
     4441 aatgtttagc tttattagat aaaaatttaa ctaagttagt tactaaggga
aaaatggatg
     4501 aagctacaaa agcagaaata ttaagtcatg ttagttcaac tactaattat
gaagatttaa
     4561 aagatatgga tttaataata gaagcatctg tagaagacat gaatataaag
aaagatgttt
     4621 tcaagttact agatgaatta tgtaaagaag atactatctt ggcaacaaat
acttcatcat
```

Nucleotide sequences of pLogic031-oxyS-butyrate construct (SEQ ID NO: 75)

```
     4681 tatctataac agaaatagct tcttctacta agcgcccaga taaagttata
ggaatgcatt
     4741 tctttaatcc agttcctatg atgaaattag ttgaagttat aagtggtcag
ttaacatcaa
     4801 aagttacttt tgatacagta tttgaattat ctaagagtat caataaagta
ccagtagatg
     4861 tatctgaatc tcctggattt gtagtaaata gaatacttat acctatgata
aatgaagctg
     4921 ttggtatata tgcagatggt gttgcaagta aagaagaaat agatgaagct
atgaaattag
     4981 gagcaaacca tccaatggga ccactagcat taggtgattt aatcggatta
gatgttgttt
     5041 tagctataat gaacgtttta tatactgaat ttggagatac taaatataga
cctcatccac
     5101 ttttagctaa aatggttaga gctaatcaat taggaagaaa aactaagata
ggattctatg
     5161 attataataa ataataagaa ggagatatac atatgagtac aagtgatgtt
aaagtttatg
     5221 agaatgtagc tgttgaagta gatggaaata tatgtacagt gaaaatgaat
agacctaaag
     5281 cccttaatgc aataaattca aagactttag aagaacttta tgaagtattt
gtagatatta
     5341 ataatgatga aactattgat gttgtaatat tgacagggga aggaaaggca
tttgtagctg
     5401 gagcagatat tgcatacatg aaagatttag atgctgtagc tgctaaagat
tttagtatct
     5461 taggagcaaa agcttttgga gaaatagaaa atagtaaaaa agtagtgata
gctgctgtaa
     5521 acggatttgc tttaggtgga ggatgtgaac ttgcaatggc atgtgatata
agaattgcat
     5581 ctgctaaagc taaatttggt cagccagaag taactcttgg aataactcca
ggatatggag
     5641 gaactcaaag gcttacaaga ttggttggaa tggcaaaagc aaaagaatta
atctttacag
     5701 gtcaagttat aaaagctgat gaagctgaaa aaatagggct agtaaataga
gtcgttgagc
     5761 cagacatttt aatagaagaa gttgagaaat tagctaagat aatagctaaa
aatgctcagc
     5821 ttgcagttag atactctaaa gaagcaatac aacttggtgc tcaaactgat
ataaatactg
     5881 gaatagatat agaatctaat ttatttggtc tttgtttttc aactaaagac
caaaaagaag
     5941 gaatgtcagc tttcgttgaa aagagagaag ctaactttat aaaagggtaa
taagaaggag
     6001 atatacatat gagaagtttt gaagaagtaa ttaagtttgc aaaagaaaga
ggacctaaaa
     6061 ctatatcagt agcatgttgc caagataaag aagttttaat ggcagttgaa
atggctagaa
     6121 aagaaaaaat agcaaatgcc attttagtag gagatataga aaagactaaa
gaaattgcaa
     6181 aaagcataga catggatatc gaaaattatg aactgataga tataaaagat
ttagcagaag
     6241 catctctaaa atctgttgaa ttagtttcac aaggaaaagc cgacatggta
atgaaaggct
     6301 tagtagacac atcaataata ctaaaagcag ttttaaataa agaagtaggt
cttagaactg
     6361 gaaatgtatt aagtcacgta gcagtatttg atgtagaggg atatgataga
```

Nucleotide sequences of pLogic031-oxyS-butyrate construct (SEQ ID NO: 75)

```
ttatttttcg
     6421 taactgacgc agctatgaac ttagctcctg atacaaatac taaaaagcaa
atcatagaaa
     6481 atgcttgcac agtagcacat tcattagata taagtgaacc aaaagttgct
gcaatatgcg
     6541 caaaagaaaa agtaaatcca aaaatgaaag atacagttga agctaaagaa
ctagaagaaa
     6601 tgtatgaaag aggagaaatc aaaggttgta tggttggtgg gccttttgca
attgataatg
     6661 cagtatcttt agaagcagct aaacataaag gtataaatca tcctgtagca
ggacgagctg
     6721 atatattatt agccccagat attgaaggtg gtaacatatt atataaagct
ttggtattct
     6781 tctcaaaatc aaaaaatgca ggagttatag ttggggctaa agcaccaata
atattaactt
     6841 ctagagcaga cagtgaagaa actaaactaa actcaatagc tttaggtgtt
ttaatggcag
     6901 caaaggcata ataagaagga gatatacata tgagcaaaat atttaaaatc
ttaacaataa
     6961 atcctggttc gacatcaact aaaatagctg tatttgataa tgaggattta
gtatttgaaa
     7021 aaactttaag acattcttca gaagaaatag gaaaatatga gaaggtgtct
gaccaatttg
     7081 aatttcgtaa acaagtaata gaagaagctc taaaagaagg tggagtaaaa
acatctgaat
     7141 tagatgctgt agtaggtaga ggaggacttc ttaaacctat aaaaggtggt
acttattcag
     7201 taagtgctgc tatgattgaa gatttaaaag tgggagtttt aggagaacac
gcttcaaacc
     7261 taggtggaat aatagcaaaa caaataggtg aagaagtaaa tgttccttca
tacatagtag
     7321 accctgttgt tgtagatgaa ttagaagatg ttgctagaat ttctggtatg
cctgaaataa
     7381 gtagagcaag tgtagtacat gctttaaatc aaaaggcaat agcaagaaga
tatgctagag
     7441 aaataaacaa gaaatatgaa gatataaatc ttatagttgc acacatgggt
ggaggagttt
     7501 ctgttggagc tcataaaaat ggtaaaatag tagatgttgc aaacgcatta
gatggagaag
     7561 gacctttctc tccagaaaga agtggtggac taccagtagg tgcattagta
aaaatgtgct
     7621 ttagtggaaa atatactcaa gatgaaatta aaaagaaaat aaaaggtaat
ggcggactag
     7681 ttgcatactt aaacactaat gatgctagag aagttgaaga aagaattgaa
gctggtgatg
     7741 aaaaagctaa attagtatat gaagctatgg catatcaaat ctctaaagaa
ataggagcta
     7801 gtgctgcagt tcttaaggga gatgtaaaag caatattatt aactggtgga
atcgcatatt
     7861 caaaaatgtt tacagaaatg attgcagata gagttaaatt tatagcagat
gtaaaagttt
     7921 atccaggtga agatgaaatg attgcattag ctcaaggtgg acttagagtt
ttaactggtg
     7981 aagaagaggc tcaagtttat gataactaat aa
```

Table 16

Nucleotide sequences of pLogic046-oxyS-butyrate construct (SEQ ID NO: 76)

```
        1 ctcgagttca ttatccatcc tccatcgcca cgatagttca tggcgatagg
tagaatagca
       61 atgaacgatt atccctatca agcattctga ctgataattg ctcacacgaa
ttcattaaag
      121 aggagaaagg taccatgatc gtaaaaccta tggtacgcaa caatatctgc
ctgaacgccc
      181 atcctcaggg ctgcaagaag ggagtggaag atcagattga atataccaag
aaacgcatta
      241 ccgcagaagt caaagctggc gcaaaagctc caaaaaacgt tctggtgctt
ggctgctcaa
      301 atggttacgg cctggcgagc cgcattactg ctgcgttcgg atacggggct
gcgaccatcg
      361 gcgtgtcctt tgaaaaagcg ggttcagaaa ccaaatatgg tacaccggga
tggtacaata
      421 atttggcatt tgatgaagcg gcaaaacgcg agggtcttta tagcgtgacg
atcgacggcg
      481 atgcgttttc agacgagatc aaggcccagg taattgagga agccaaaaaa
aaaggtatca
      541 aatttgatct gatcgtatac agcttggcca gcccagtacg tactgatcct
gatacaggta
      601 tcatgcacaa aagcgttttg aaaccctttg gaaaaacgtt cacaggcaaa
acagtagatc
      661 cgtttactgg cgagctgaag gaaatctccg cggaaccagc aaatgacgag
gaagcagccg
      721 ccactgttaa agttatgggg ggtgaagatt gggaacgttg gattaagcag
ctgtcgaagg
      781 aaggcctctt agaagaaggc tgtattacct tggcctatag ttatattggc
cctgaagcta
      841 cccaagcttt gtaccgtaaa ggcacaatcg gcaaggccaa agaacacctg
gaggccacag
      901 cacaccgtct caacaaagag aacccgtcaa tccgtgcctt cgtgagcgtg
aataaaggcc
      961 tggtaacccg cgcaagcgcc gtaatcccgg taatccctct gtatctcgcc
agcttgttca
     1021 aagtaatgaa agagaagggc aatcatgaag gttgtattga acagatcacg
cgtctgtacg
     1081 ccgagcgcct gtaccgtaaa gatggtacaa ttccagttga tgaggaaaat
cgcattcgca
     1141 ttgatgattg ggagttagaa gaagacgtcc agaaagcggt atccgcgttg
atggagaaag
     1201 tcacgggtga aaacgcagaa tctctcactg acttagcggg gtaccgccat
gatttcttag
     1261 ctagtaacgg ctttgatgta gaaggtatta attatgaagc ggaagttgaa
cgcttcgacc
     1321 gtatctgata agaaggagat atacatatga gagaagtagt aattgccagt
gcagctagaa
     1381 cagcagtagg aagttttgga ggagcattta aatcagtttc agcggtagag
ttaggggtaa
     1441 cagcagctaa agaagctata aaaagagcta acataactcc agatatgata
gatgaatctc
     1501 ttttaggggg agtacttaca gcaggtcttg gacaaaatat agcaagacaa
atagcattag
     1561 gagcaggaat accagtagaa aaaccagcta tgactataaa tatagtttgt
ggttctggat
     1621 taagatctgt ttcaatggca tctcaactta tagcattagg tgatgctgat
ataatgttag
     1681 ttggtggagc tgaaaacatg agtatgtctc cttatttagt accaagtgcg
```

Nucleotide sequences of pLogic046-oxyS-butyrate construct (SEQ ID NO: 76)

```
agatatggtg
     1741 caagaatggg tgatgctgct tttgttgatt caatgataaa agatggatta
tcagacatat
     1801 ttaataacta tcacatgggt attactgctg aaaacatagc agagcaatgg
aatataacta
     1861 gagaagaaca agatgaatta gctcttgcaa gtcaaaataa agctgaaaaa
gctcaagctg
     1921 aaggaaaatt tgatgaagaa atagttcctg ttgttataaa aggaagaaaa
ggtgacactg
     1981 tagtagataa agatgaatat attaagcctg gcactacaat ggagaaactt
gctaagttaa
     2041 gacctgcatt taaaaaagat ggaacagtta ctgctggtaa tgcatcagga
ataaatgatg
     2101 gtgctgctat gttagtagta atggctaaag aaaaagctga agaactagga
atagagcctc
     2161 ttgcaactat agtttcttat ggaacagctg gtgttgaccc taaaataatg
ggatatggac
     2221 cagttccagc aactaaaaaa gctttagaag ctgctaatat gactattgaa
gatatagatt
     2281 tagttgaagc taatgaggca tttgctgccc aatctgtagc tgtaataaga
gacttaaata
     2341 tagatatgaa taaagttaat gttaatggtg gagcaatagc tataggacat
ccaataggat
     2401 gctcaggagc aagaatactt actacacttt tatatgaaat gaagagaaga
gatgctaaaa
     2461 ctggtcttgc tacactttgt ataggcggtg gaatgggaac tactttaata
gttaagagat
     2521 agtaagaagg agatatacat atgaaattag ctgtaatagg tagtggaact
atgggaagtg
     2581 gtattgtaca aacttttgca agttgtggac atgatgtatg tttaaagagt
agaactcaag
     2641 gtgctataga taaatgttta gctttattag ataaaaattt aactaagtta
gttactaagg
     2701 gaaaaatgga tgaagctaca aaagcagaaa tattaagtca tgttagttca
actactaatt
     2761 atgaagattt aaaagatatg gatttaataa tagaagcatc tgtagaagac
atgaatataa
     2821 agaaagatgt tttcaagtta ctagatgaat tatgtaaaga agatactatc
ttggcaacaa
     2881 atacttcatc attatctata acagaaatag cttcttctac taagcgccca
gataaagtta
     2941 taggaatgca tttctttaat ccagttccta tgatgaaatt agttgaagtt
ataagtggtc
     3001 agttaacatc aaaagttact tttgatacag tatttgaatt atctaagagt
atcaataaag
     3061 taccagtaga tgtatctgaa tctcctggat ttgtagtaaa tagaatactt
atacctatga
     3121 taaatgaagc tgttggtata tatgcagatg gtgttgcaag taaagaagaa
atagatgaag
     3181 ctatgaaatt aggagcaaac catccaatgg gaccactagc attaggtgat
ttaatcggat
     3241 tagatgttgt tttagctata atgaacgttt tatatactga atttggagat
actaaatata
     3301 gacctcatcc acttttagct aaaatggtta gagctaatca attaggaaga
aaaactaaga
     3361 taggattcta tgattataat aaataataag aaggagatat acatatgagt
acaagtgatg
```

Nucleotide sequences of pLogic046-oxyS-butyrate construct (SEQ ID NO: 76)

```
     3421 ttaaagttta tgagaatgta gctgttgaag tagatggaaa tatatgtaca
gtgaaaatga
     3481 atagacctaa agcccttaat gcaataaatt caaagacttt agaagaactt
tatgaagtat
     3541 ttgtagatat taataatgat gaaactattg atgttgtaat attgacaggg
gaaggaaagg
     3601 catttgtagc tggagcagat attgcataca tgaaagattt agatgctgta
gctgctaaag
     3661 attttagtat cttaggagca aaagcttttg gagaaataga aaatagtaaa
aaagtagtga
     3721 tagctgctgt aaacggattt gctttaggtg gaggatgtga acttgcaatg
gcatgtgata
     3781 taagaattgc atctgctaaa gctaaatttg gtcagccaga agtaactctt
ggaataactc
     3841 caggatatgg aggaactcaa aggcttacaa gattggttgg aatggcaaaa
gcaaaagaat
     3901 taatctttac aggtcaagtt ataaaagctg atgaagctga aaaaatagggg
ctagtaaata
     3961 gagtcgttga gccagacatt ttaatagaag aagttgagaa attagctaag
ataatagcta
     4021 aaaatgctca gcttgcagtt agatactcta aagaagcaat acaacttggt
gctcaaactg
     4081 atataaatac tggaatagat atagaatcta atttatttgg tctttgtttt
tcaactaaag
     4141 accaaaaaga aggaatgtca gctttcgttg aaaagagaga agctaacttt
ataaaagggt
     4201 aataagaagg agatatacat atgagaagtt ttgaagaagt aattaagttt
gcaaaagaaa
     4261 gaggacctaa aactatatca gtagcatgtt gccaagataa agaagtttta
atggcagttg
     4321 aaatggctag aaaagaaaaa atagcaaatg ccattttagt aggagatata
gaaaagacta
     4381 aagaaattgc aaaaagcata gacatggata tcgaaaatta tgaactgata
gatataaaag
     4441 atttagcaga agcatctcta aaatctgttg aattagtttc acaaggaaaa
gccgacatgg
     4501 taatgaaagg cttagtagac acatcaataa tactaaaagc agttttaaat
aaagaagtag
     4561 gtcttagaac tggaaatgta ttaagtcacg tagcagtatt tgatgtagag
ggatatgata
     4621 gattattttt cgtaactgac gcagctatga acttagctcc tgatacaaat
actaaaaagc
     4681 aaatcataga aaatgcttgc acagtagcac attcattaga tataagtgaa
ccaaaagttg
     4741 ctgcaatatg cgcaaaagaa aaagtaaatc caaaaatgaa agatacagtt
gaagctaaag
     4801 aactagaaga aatgtatgaa agaggagaaa tcaaaggttg tatggttggt
gggccttttg
     4861 caattgataa tgcagtatct ttagaagcag ctaaacataa aggtataaat
catcctgtag
     4921 caggacgagc tgatatatta ttagccccag atattgaagg tggtaacata
ttatataaag
     4981 ctttggtatt cttctcaaaa tcaaaaaatg caggagttat agttggggct
aaagcaccaa
     5041 taatattaac ttctagagca gacagtgaag aaactaaact aaactcaata
gctttaggtg
     5101 ttttaatggc agcaaaggca taataagaag gagatataca tatgagcaaa
```

Nucleotide sequences of pLogic046-oxyS-butyrate construct (SEQ ID NO: 76)

```
atatttaaaa
     5161 tcttaacaat aaatcctggt tcgacatcaa ctaaaatagc tgtatttgat
aatgaggatt
     5221 tagtatttga aaaaacttta agacattctt cagaagaaat aggaaaatat
gagaaggtgt
     5281 ctgaccaatt tgaatttcgt aaacaagtaa tagaagaagc tctaaaagaa
ggtggagtaa
     5341 aaacatctga attagatgct gtagtaggta gaggaggact tcttaaacct
ataaaaggtg
     5401 gtacttattc agtaagtgct gctatgattg aagatttaaa agtgggagtt
ttaggagaac
     5461 acgcttcaaa cctaggtgga ataatagcaa aacaaatagg tgaagaagta
aatgttcctt
     5521 catacatagt agaccctgtt gttgtagatg aattagaaga tgttgctaga
atttctggta
     5581 tgcctgaaat aagtagagca agtgtagtac atgctttaaa tcaaaaggca
atagcaagaa
     5641 gatatgctag agaaataaac aagaaatatg aagatataaa tcttatagtt
gcacacatgg
     5701 gtggaggagt ttctgttgga gctcataaaa atggtaaaat agtagatgtt
gcaaacgcat
     5761 tagatggaga aggacctttc tctccagaaa gaagtggtgg actaccagta
ggtgcattag
     5821 taaaaatgtg ctttagtgga aaatatactc aagatgaaat taaaaagaaa
ataaaaggta
     5881 atggcggact agttgcatac ttaaacacta atgatgctag agaagttgaa
gaaagaattg
     5941 aagctggtga tgaaaaagct aaattagtat atgaagctat ggcatatcaa
atctctaaag
     6001 aaataggagc tagtgctgca gttcttaagg gagatgtaaa agcaatatta
ttaactggtg
     6061 gaatcgcata ttcaaaaatg tttacagaaa tgattgcaga tagagttaaa
tttatagcag
     6121 atgtaaaagt ttatccaggt gaagatgaaa tgattgcatt agctcaaggt
ggacttagag
     6181 ttttaactgg tgaagaagag gctcaagttt atgataacta ataa
```

Table 17

Nucleotide sequences of pZA22-oxyR constrcut (SEQ ID NO: 77)

```
        1 ctcgagatgc tagcaattgt gagcggataa caattgacat tgtgagcgga
taacaagata
       61 ctgagcacat cagcaggacg cactgacctt aattaaaaga attcattaaa
gaggagaaag
      121 gtaccatgaa tattcgtgat cttgagtacc tggtggcatt ggctgaacac
cgccattttc
      181 ggcgtgcggc agattcctgc cacgttagcc agccgacgct tagcgggcaa
attcgtaagc
      241 tggaagatga gctgggcgtg atgttgctgg agcggaccag ccgtaaagtg
ttgttcaccc
      301 aggcgggaat gctgctggtg gatcaggcgc gtaccgtgct gcgtgaggtg
aaagtcctta
      361 aagagatggc aagccagcag ggcgagacga tgtccggacc gctgcacatt
ggtttgattc
      421 ccacagttgg accgtacctg ctaccgcata ttatccctat gctgcaccag
```

Nucleotide sequences of pZA22-oxyR constrcut (SEQ ID NO: 77)

```
acctttccaa
      481 agctggaaat gtatctgcat gaagcacaga cccaccagtt actggcgcaa
ctggacagcg
      541 gcaaactcga ttgcgtgatc ctcgcgctgg tgaaagagag cgaagcattc
attgaagtgc
      601 cgttgtttga tgagccaatg ttgctggcta tctatgaaga tcacccgtgg
gcgaaccgcg
      661 aatgcgtacc gatggccgat ctggcagggg aaaaactgct gatgctggaa
gatggtcact
      721 gtttgcgcga tcaggcaatg ggtttctgtt ttgaagccgg ggcggatgaa
gatacacact
      781 tccgcgcgac cagcctggaa actctgcgca acatggtggc ggcaggtagc
gggatcactt
      841 tactgccagc gctggctgtg ccgccggagc gcaaacgcga tggggttgtt
tatctgccgt
      901 gcattaagcc ggaaccacgc cgcactattg gcctggttta tcgtcctggc
tcaccgctgc
      961 gcagccgcta tgagcagctg gcagaggcca tccgcgcaag aatggatggc
catttcgata
     1021 aagttttaaa acaggcggtt taaggatccc atggtacgcg tgctagaggc
atcaaataaa
     1081 acgaaaggct cagtcgaaag actgggcctt tcgttttatc tgttgtttgt
cggtgaacgc
     1141 tctcctgagt aggacaaatc cgccgcccta gacctagggg atatattccg
cttcctcgct
     1201 cactgactcg ctacgctcgg tcgttcgact gcggcgagcg gaaatggctt
acgaacgggg
     1261 cggagatttc ctggaagatg ccaggaagat acttaacagg gaagtgagag
ggccgcggca
     1321 aagccgtttt tccataggct ccgcccccct gacaagcatc acgaaatctg
acgctcaaat
     1381 cagtggtggc gaaacccgac aggactataa agataccagg cgtttccccc
tggcggctcc
     1441 ctcgtgcgct ctcctgttcc tgcctttcgg tttaccggtg tcattccgct
gttatggccg
     1501 cgtttgtctc attccacgcc tgacactcag ttccgggtag gcagttcgct
ccaagctgga
     1561 ctgtatgcac gaacccccgg ttcagtccga ccgctgcgcc ttatccggta
actatcgtct
     1621 tgagtccaac ccggaaagac atgcaaaagc accactggca gcagccactg
gtaattgatt
     1681 tagaggagtt agtcttgaag tcatgcgccg gttaaggcta aactgaaagg
acaagttttg
     1741 gtgactgcgc tcctccaagc cagttacctc ggttcaaaga gttggtagct
cagagaacct
     1801 tcgaaaaacc gccctgcaag gcggtttttt cgttttcaga gcaagagatt
acgcgcagac
     1861 caaaacgatc tcaagaagat catcttatta atcagataaa atatttctag
atttcagtgc
     1921 aatttatctc ttcaaatgta gcacctgaag tcagccccat acgatataag
ttgttactag
     1981 tgcttggatt ctcaccaata aaaaacgccc ggcggcaacc gagcgttctg
aacaaatcca
     2041 gatggagttc tgaggtcatt actggatcta tcaacaggag tccaagcgag
ctctcgaacc
     2101 ccagagtccc gctcagaaga actcgtcaag aaggcgatag aaggcgatgc
gctgcgaatc
```

Nucleotide sequences of pZA22-oxyR constrcut (SEQ ID NO: 77)
2161 gggagcggcg ataccgtaaa gcacgaggaa gcggtcagcc cattcgccgc caagctcttc 2221 agcaatatca cgggtagcca acgctatgtc ctgatagcgg tccgccacac ccagccggcc 2281 acagtcgatg aatccagaaa agcggccatt ttccaccatg atattcggca agcaggcatc 2341 gccatgggtc acgacgagat cctcgccgtc gggcatgcgc gccttgagcc tggcgaacag 2401 ttcggctggc gcgagcccct gatgctcttc gtccagatca tcctgatcga caagaccggc 2461 ttccatccga gtacgtgctc gctcgatgcg atgtttcgct tggtggtcga atgggcaggt 2521 agccggatca agcgtatgca gccgccgcat tgcatcagcc atgatggata ctttctcggc 2581 aggagcaagg tgagatgaca ggagatcctg ccccggcact tcgcccaata gcagccagtc 2641 ccttcccgct tcagtgacaa cgtcgagcac agctgcgcaa ggaacgcccg tcgtggccag 2701 ccacgatagc cgcgctgcct cgtcctgcag ttcattcagg gcaccggaca ggtcggtctt 2761 gacaaaaaga accgggcgcc cctgcgctga cagccggaac acggcggcat cagagcagcc 2821 gattgtctgt tgtgcccagt catagccgaa tagcctctcc acccaagcgg ccggagaacc 2881 tgcgtgcaat ccatcttgtt caatcatgcg aaacgatcct catcctgtct cttgatcaga 2941 tcttgatccc ctgcgccatc agatccttgg cggcaagaaa gccatccagt ttactttgca 3001 gggcttccca accttaccag agggcgcccc agctggcaat tccgacgtct aagaaaccat 3061 tattatcatg acattaacct ataaaaatag gcgtatcacg aggccctttc gtcttcac

Table 18

Nucleotide sequences of pLogic031-tet-butyrate construct (SEQ ID NO: 78)
1 gtaaaacgac ggccagtgaa ttcgttaaga cccactttca catttaagtt gtttttctaa 61 tccgcatatg atcaattcaa ggccgaataa gaaggctggc tctgcacctt ggtgatcaaa 121 taattcgata gcttgtcgta ataatggcgg catactatca gtagtaggtg tttccctttc 181 ttctttagcg acttgatgct cttgatcttc caatacgcaa cctaaagtaa aatgccccac 241 agcgctgagt gcatataatg cattctctag tgaaaaacct tgttggcata aaaaggctaa 301 ttgattttcg agagtttcat actgtttttc tgtaggccgt gtacctaaat gtacttttgc 361 tccatcgcga tgacttagta aagcacatct aaaactttta gcgttattac gtaaaaaatc 421 ttgccagctt tccccttcta aagggcaaaa gtgagtatgg tgcctatcta acatctcaat 481 ggctaaggcg tcgagcaaag cccgcttatt ttttacatgc caatacaatg taggctgctc 541 tacacctagc ttctgggcga gtttacgggt tgttaaacct tcgattccga

Nucleotide sequences of pLogic031-tet-butyrate construct (SEQ ID NO: 78)

cctcattaag
601 cagctctaat gcgctgttaa tcactttact tttatctaat ctagacatca
ttaattccta

661 attttt gttg acactctatc attgatagag ttattttacc actccctatc
agtgatagag
721 aaaagtgaac tctagaaata attttgttta actttaagaa ggagatatac
atatggattt
781 aaattctaaa aaatatcaga tgcttaaaga gctatatgta agcttcgctg
aaaatgaagt
841 taaaccttta gcaacagaac ttgatgaaga agaaagattt ccttatgaaa
cagtggaaaa
901 aatggcaaaa gcaggaatga tgggtatacc atatccaaaa gaatatggtg
gagaaggtgg
961 agacactgta ggatatataa tggcagttga agaattgtct agagtttgtg
gtactacagg
1021 agttatatta tcagctcata catctcttgg ctcatggcct atatatcaat
atggtaatga
1081 agaacaaaaa caaaaattct taagaccact agcaagtgga gaaaaattag
gagcatttgg
1141 tcttactgag cctaatgctg gtacagatgc gtctggccaa caaacaactg
ctgttttaga
1201 cggggatgaa tacatactta atggctcaaa aatatttata acaaacgcaa
tagctggtga
1261 catatatgta gtaatggcaa tgactgataa atctaagggg aacaaaggaa
tatcagcatt
1321 tatagttgaa aaaggaactc ctgggtttag ctttggagtt aaagaaaaga
aaatgggtat
1381 aagaggttca gctacgagtg aattaatatt tgaggattgc agaataccta
aagaaaattt
1441 acttggaaaa gaaggtcaag gatttaagat agcaatgtct actcttgatg
gtggtagaat
1501 tggtatagct gcacaagctt taggtttagc acaaggtgct cttgatgaaa
ctgttaaata
1561 tgtaaaagaa agagtacaat ttggtagacc attatcaaaa ttccaaaata
cacaattcca
1621 attagctgat atggaagtta aggtacaagc ggctagacac cttgtatatc
aagcagctat
1681 aaataaagac ttaggaaaac cttatggagt agaagcagca atggcaaaat
tatttgcagc
1741 tgaaacagct atggaagtta ctacaaaagc tgtacaactt catggaggat
atggatacac
1801 tcgtgactat ccagtagaaa gaatgatgag agatgctaag ataactgaaa
tatatgaagg
1861 aactagtgaa gttcaaagaa tggttatttc aggaaaacta ttaaaatagt
aagaaggaga
1921 tatacatatg gaggaaggat ttatgaatat agtcgtttgt ataaaacaag
ttccagatac
1981 aacagaagtt aaactagatc ctaatacagg tactttaatt agagatggag
taccaagtat
2041 aataaaccct gatgataaag caggtttaga agaagctata aaattaaaag
aagaaatggg
2101 tgctcatgta actgttataa caatgggacc tcctcaagca gatatggctt
taaaagaagc
2161 tttagcaatg ggtgcagata gaggtatatt attaacagat agagcatttg
cgggtgctga

Nucleotide sequences of pLogic031-tet-butyrate construct (SEQ ID NO: 78)

```
     2221 tacttgggca acttcatcag cattagcagg agcattaaaa aatatagatt
ttgatattat
     2281 aatagctgga agacaggcga tagatggaga tactgcacaa gttggacctc
aaatagctga
     2341 acatttaaat cttccatcaa taacatatgc tgaagaaata aaaactgaag
gtgaatatgt
     2401 attagtaaaa agacaatttg aagattgttg ccatgactta aaagttaaaa
tgccatgcct
     2461 tataacaact cttaaagata tgaacacacc aagatacatg aaagttggaa
gaatatatga
     2521 tgctttcgaa aatgatgtag tagaaacatg gactgtaaaa gatatagaag
ttgacccttc
     2581 taatttaggt cttaaaggtt ctccaactag tgtatttaaa tcatttacaa
aatcagttaa
     2641 accagctggt acaatataca atgaagatgc gaaaacatca gctggaatta
tcatagataa
     2701 attaaaagag aagtatatca tataataaga aggagatata catatgggta
acgttttagt
     2761 agtaatagaa caaagagaaa atgtaattca aactgtttct ttagaattac
taggaaaggc
     2821 tacagaaata gcaaaagatt atgatacaaa agtttctgca ttacttttag
gtagtaaggt
     2881 agaaggttta atagatacat tagcacacta tggtgcagat gaggtaatag
tagtagatga
     2941 tgaagcttta gcagtgtata caactgaacc atatacaaaa gcagcttatg
aagcaataaa
     3001 agcagctgac cctatagttg tattatttgg tgcaacttca ataggtagag
atttagcgcc
     3061 tagagtttct gctagaatac atacaggtct tactgctgac tgtacaggtc
ttgcagtagc
     3121 tgaagataca aaattattat taatgacaag acctgccttt ggtggaaata
taatggcaac
     3181 aatagtttgt aaagatttca gacctcaaat gtctacagtt agaccagggg
ttatgaagaa
     3241 aaatgaacct gatgaaacta aagaagctgt aattaaccgt ttcaaggtag
aatttaatga
     3301 tgctgataaa ttagttcaag ttgtacaagt aataaaagaa gctaaaaaac
aagttaaaat
     3361 agaagatgct aagatattag tttctgctgg acgtggaatg ggtggaaaag
aaaacttaga
     3421 catactttat gaattagctg aaattatagg tggagaagtt tctggttctc
gtgccactat
     3481 agatgcaggt tggttagata aagcaagaca agttggtcaa actggtaaaa
ctgtaagacc
     3541 agacctttat atagcatgtg gtatatctgg agcaatacaa catatagctg
gtatggaaga
     3601 tgctgagttt atagttgcta taaataaaaa tccagaagct ccaatattta
aatatgctga
     3661 tgttggtata gttggagatg ttcataaagt gcttccagaa cttatcagtc
agttaagtgt
     3721 tgcaaaagaa aaaggtgaag ttttagctaa ctaataagaa ggagatatac
atatgagaga
     3781 agtagtaatt gccagtgcag ctagaacagc agtaggaagt tttggaggag
catttaaatc
     3841 agtttcagcg gtagagttag gggtaacagc agctaaagaa gctataaaaa
gagctaacat
     3901 aactccagat atgatagatg aatctctttt agggggagta cttacagcag
```

Nucleotide sequences of pLogic031-tet-butyrate construct (SEQ ID NO: 78)

```
gtcttggaca
     3961 aaatatagca agacaaatag cattaggagc aggaatacca gtagaaaaac
cagctatgac
     4021 tataaatata gtttgtggtt ctggattaag atctgtttca atggcatctc
aacttatagc
     4081 attaggtgat gctgatataa tgttagttgg tggagctgaa aacatgagta
tgtctcctta
     4141 tttagtacca agtgcgagat atggtgcaag aatgggtgat gctgcttttg
ttgattcaat
     4201 gataaaagat ggattatcag acatatttaa taactatcac atgggtatta
ctgctgaaaa
     4261 catagcagag caatggaata taactagaga agaacaagat gaattagctc
ttgcaagtca
     4321 aaataaagct gaaaaagctc aagctgaagg aaaatttgat gaagaaatag
ttcctgttgt
     4381 tataaaagga agaaaaggtg acactgtagt agataaagat gaatatatta
agcctggcac
     4441 tacaatggag aaacttgcta agttaagacc tgcatttaaa aaagatggaa
cagttactgc
     4501 tggtaatgca tcaggaataa atgatggtgc tgctatgtta gtagtaatgg
ctaaagaaaa
     4561 agctgaagaa ctaggaatag agcctcttgc aactatagtt tcttatggaa
cagctggtgt
     4621 tgaccctaaa ataatgggat atggaccagt tccagcaact aaaaaagctt
tagaagctgc
     4681 taatatgact attgaagata tagatttagt tgaagctaat gaggcatttg
ctgcccaatc
     4741 tgtagctgta ataagagact taaatataga tatgaataaa gttaatgtta
atggtggagc
     4801 aatagctata ggacatccaa taggatgctc aggagcaaga atacttacta
cacttttata
     4861 tgaaatgaag agaagagatg ctaaaactgg tcttgctaca ctttgtatag
gcggtggaat
     4921 gggaactact ttaatagtta agagatagta agaaggagat atacatatga
aattagctgt
     4981 aataggtagt ggaactatgg gaagtggtat tgtacaaact tttgcaagtt
gtggacatga
     5041 tgtatgttta aagagtagaa ctcaaggtgc tatagataaa tgtttagctt
tattagataa
     5101 aaatttaact aagttagtta ctaagggaaa aatggatgaa gctacaaaag
cagaaatatt
     5161 aagtcatgtt agttcaacta ctaattatga agatttaaaa gatatggatt
taataataga
     5221 agcatctgta gaagacatga atataaagaa agatgttttc aagttactag
atgaattatg
     5281 taaagaagat actatcttgg caacaaatac ttcatcatta tctataacag
aaatagcttc
     5341 ttctactaag cgcccagata aagttatagg aatgcatttc tttaatccag
ttcctatgat
     5401 gaaattagtt gaagttataa gtggtcagtt aacatcaaaa gttacttttg
atacagtatt
     5461 tgaattatct aagagtatca ataaagtacc agtagatgta tctgaatctc
ctggatttgt
     5521 agtaaataga atacttatac ctatgataaa tgaagctgtt ggtatatatg
cagatggtgt
     5581 tgcaagtaaa gaagaaatag atgaagctat gaaattagga gcaaaccatc
caatgggacc
```

Nucleotide sequences of pLogic031-tet-butyrate construct (SEQ ID NO: 78)

```
     5641 actagcatta ggtgatttaa tcggattaga tgttgtttta gctataatga
acgttttata
     5701 tactgaattt ggagatacta aatatagacc tcatccactt ttagctaaaa
tggttagagc
     5761 taatcaatta ggaagaaaaa ctaagatagg attctatgat tataataaat
aataagaagg
     5821 agatatacat atgagtacaa gtgatgttaa agtttatgag aatgtagctg
ttgaagtaga
     5881 tggaaatata tgtacagtga aaatgaatag acctaaagcc cttaatgcaa
taaattcaaa
     5941 gactttagaa gaactttatg aagtatttgt agatattaat aatgatgaaa
ctattgatgt
     6001 tgtaatattg acaggggaag gaaaggcatt tgtagctgga gcagatattg
catacatgaa
     6061 agatttagat gctgtagctg ctaaagattt tagtatctta ggagcaaaag
cttttggaga
     6121 aatagaaaat agtaaaaaag tagtgatagc tgctgtaaac ggatttgctt
taggtggagg
     6181 atgtgaactt gcaatggcat gtgatataag aattgcatct gctaaagcta
aatttggtca
     6241 gccagaagta actcttggaa taactccagg atatggagga actcaaaggc
ttacaagatt
     6301 ggttggaatg gcaaaagcaa aagaattaat ctttacaggt caagttataa
aagctgatga
     6361 agctgaaaaa atagggctag taaatagagt cgttgagcca gacattttaa
tagaagaagt
     6421 tgagaaatta gctaagataa tagctaaaaa tgctcagctt gcagttagat
actctaaaga
     6481 agcaatacaa cttggtgctc aaactgatat aaatactgga atagatatag
aatctaattt
     6541 atttggtctt tgtttttcaa ctaaagacca aaaagaagga atgtcagctt
tcgttgaaaa
     6601 gagagaagct aactttataa aagggtaata agaaggagat atacatatga
gaagttttga
     6661 agaagtaatt aagtttgcaa aagaaagagg acctaaaact atatcagtag
catgttgcca
     6721 agataaagaa gttttaatgg cagttgaaat ggctagaaaa gaaaaaatag
caaatgccat
     6781 tttagtagga gatatagaaa agactaaaga aattgcaaaa agcatagaca
tggatatcga
     6841 aaattatgaa ctgatagata taaaagattt agcagaagca tctctaaaat
ctgttgaatt
     6901 agtttcacaa ggaaaagccg acatggtaat gaaaggctta gtagacacat
caataatact
     6961 aaaagcagtt ttaaataaag aagtaggtct tagaactgga aatgtattaa
gtcacgtagc
     7021 agtatttgat gtagagggat atgatagatt atttttcgta actgacgcag
ctatgaactt
     7081 agctcctgat acaaatacta aaaagcaaat catagaaaat gcttgcacag
tagcacattc
     7141 attagatata agtgaaccaa aagttgctgc aatatgcgca aaagaaaaag
taaatccaaa
     7201 aatgaaagat acagttgaag ctaaagaact agaagaaatg tatgaaagag
gagaaatcaa
     7261 aggttgtatg gttggtgggc cttttgcaat tgataatgca gtatctttag
aagcagctaa
     7321 acataaaggt ataaatcatc ctgtagcagg acgagctgat atattattag
```

Nucleotide sequences of pLogic031-tet-butyrate construct (SEQ ID NO: 78)
ccccagatat 7381 tgaaggtggt aacatattat ataaagcttt ggtattcttc tcaaaatcaa aaaatgcagg 7441 agttatagtt ggggctaaag caccaataat attaacttct agagcagaca gtgaagaaac 7501 taaactaaac tcaatagctt taggtgtttt aatggcagca aaggcataat aagaaggaga 7561 tatacatatg agcaaaatat ttaaaatctt aacaataaat cctggttcga catcaactaa 7621 aatagctgta tttgataatg aggatttagt atttgaaaaa actttaagac attcttcaga 7681 agaaatagga aaatatgaga aggtgtctga ccaatttgaa tttcgtaaac aagtaataga 7741 agaagctcta aaagaaggtg gagtaaaaac atctgaatta gatgctgtag taggtagagg 7801 aggacttctt aaacctataa aaggtggtac ttattcagta agtgctgcta tgattgaaga 7861 tttaaaagtg ggagttttag gagaacacgc ttcaaaccta ggtggaataa tagcaaaaca 7921 aataggtgaa gaagtaaatg ttccttcata catagtagac cctgttgttg tagatgaatt 7981 agaagatgtt gctagaattt ctggtatgcc tgaaataagt agagcaagtg tagtacatgc 8041 tttaaatcaa aaggcaatag caagaagata tgctagagaa ataaacaaga aatatgaaga 8101 tataaatctt atagttgcac acatgggtgg aggagtttct gttggagctc ataaaaatgg 8161 taaaatagta gatgttgcaa acgcattaga tggagaagga cctttctctc cagaaagaag 8221 tggtggacta ccagtaggtg cattagtaaa aatgtgcttt agtggaaaat atactcaaga 8281 tgaaattaaa aagaaaataa aaggtaatgg cggactagtt gcatacttaa acactaatga 8341 tgctagagaa gttgaagaaa gaattgaagc tggtgatgaa aaagctaaat tagtatatga 8401 agctatggca tatcaaatct ctaaagaaat aggagctagt gctgcagttc ttaagggaga 8461 tgtaaaagca atattattaa ctggtggaat cgcatattca aaaatgttta cagaaatgat 8521 tgcagataga gttaaattta tagcagatgt aaaagtttat ccaggtgaag atgaaatgat 8581 tgcattagct caaggtggac ttagagtttt aactggtgaa gaagaggctc aagtttatga 8641 taactaataa

Table 19

Nucleotide sequences of pLogic046-tet-butyrate construct (SEQ ID NO: 79)
1 gtaaaacgac ggccagtgaa ttcgttaaga cccactttca catttaagtt gtttttctaa 61 tccgcatatg atcaattcaa ggccgaataa gaaggctggc tctgcacctt ggtgatcaaa 121 taattcgata gcttgtcgta ataatggcgg catactatca gtagtaggtg tttccctttc

Nucleotide sequences of pLogic046-tet-butyrate construct (SEQ ID NO: 79)

```
       181 ttctttagcg acttgatgct cttgatcttc caatacgcaa cctaaagtaa
aatgccccac
       241 agcgctgagt gcatataatg cattctctag tgaaaaacct tgttggcata
aaaaggctaa
       301 ttgattttcg agagtttcat actgtttttc tgtaggccgt gtacctaaat
gtacttttgc
       361 tccatcgcga tgacttagta aagcacatct aaaactttta gcgttattac
gtaaaaaatc
       421 ttgccagctt tccccttcta aagggcaaaa gtgagtatgg tgcctatcta
acatctcaat
       481 ggctaaggcg tcgagcaaag cccgcttatt ttttacatgc caatacaatg
taggctgctc
       541 tacacctagc ttctgggcga gtttacgggt tgttaaacct tcgattccga
cctcattaag
       601 cagctctaat gcgctgttaa tcactttact tttatctaat ctagacatca
ttaattccta

       661 attttgttg acactctatc attgatagag ttattttacc actccctatc
agtgatagag
       721 aaaagtgaac tctagaaata attttgttta actttaagaa ggagatatac
atatgatcgt
       781 aaaacctatg gtacgcaaca atatctgcct gaacgcccat cctcagggct
gcaagaaggg
       841 agtggaagat cagattgaat ataccaagaa acgcattacc gcagaagtca
aagctggcgc
       901 aaaagctcca aaaaacgttc tggtgcttgg ctgctcaaat ggttacggcc
tggcgagccg
       961 cattactgct gcgttcggat acggggctgc gaccatcggc gtgtcctttg
aaaaagcggg
      1021 ttcagaaacc aaatatggta caccgggatg gtacaataat ttggcatttg
atgaagcggc
      1081 aaaacgcgag ggtctttata gcgtgacgat cgacggcgat gcgttttcag
acgagatcaa
      1141 ggcccaggta attgaggaag ccaaaaaaaa aggtatcaaa tttgatctga
tcgtatacag
      1201 cttggccagc ccagtacgta ctgatcctga tacaggtatc atgcacaaaa
gcgttttgaa
      1261 acccttttgga aaaacgttca caggcaaaac agtagatccg tttactggcg
agctgaagga
      1321 aatctccgcg gaaccagcaa atgacgagga agcagccgcc actgttaaag
ttatgggggg
      1381 tgaagattgg gaacgttgga ttaagcagct gtcgaaggaa ggcctcttag
aagaaggctg
      1441 tattaccttg gcctatagtt atattggccc tgaagctacc caagctttgt
accgtaaagg
      1501 cacaatcggc aaggccaaag aacacctgga ggccacagca caccgtctca
acaaagagaa
      1561 cccgtcaatc cgtgccttcg tgagcgtgaa taaaggcctg gtaacccgcg
caagcgccgt
      1621 aatcccggta atccctctgt atctcgccag cttgttcaaa gtaatgaaag
agaagggcaa
      1681 tcatgaaggt tgtattgaac agatcacgcg tctgtacgcc gagcgcctgt
accgtaaaga
      1741 tggtacaatt ccagttgatg aggaaaatcg cattcgcatt gatgattggg
agttagaaga
      1801 agacgtccag aaagcggtat ccgcgttgat ggagaaagtc acgggtgaaa
```

Nucleotide sequences of pLogic046-tet-butyrate construct (SEQ ID NO: 79)

```
acgcagaatc
     1861 tctcactgac ttagcggggt accgccatga tttcttagct agtaacggct
ttgatgtaga
     1921 aggtattaat tatgaagcgg aagttgaacg cttcgaccgt atctgataag
aaggagatat
     1981 acatatgaga gaagtagtaa ttgccagtgc agctagaaca gcagtaggaa
gttttggagg
     2041 agcatttaaa tcagtttcag cggtagagtt aggggtaaca gcagctaaag
aagctataaa
     2101 aagagctaac ataactccag atatgataga tgaatctctt ttagggggag
tacttacagc
     2161 aggtcttgga caaaatatag caagacaaat agcattagga gcaggaatac
cagtagaaaa
     2221 accagctatg actataaata tagtttgtgg ttctggatta agatctgttt
caatggcatc
     2281 tcaacttata gcattaggtg atgctgatat aatgttagtt ggtggagctg
aaaacatgag
     2341 tatgtctcct tatttagtac caagtgcgag atatggtgca agaatgggtg
atgctgcttt
     2401 tgttgattca atgataaaag atggattatc agacatattt aataactatc
acatgggtat
     2461 tactgctgaa aacatagcag agcaatggaa tataactaga gaagaacaag
atgaattagc
     2521 tcttgcaagt caaaataaag ctgaaaaagc tcaagctgaa ggaaaatttg
atgaagaaat
     2581 agttcctgtt gttataaaag gaagaaaagg tgacactgta gtagataaag
atgaatatat
     2641 taagcctggc actacaatgg agaaacttgc taagttaaga cctgcattta
aaaaagatgg
     2701 aacagttact gctggtaatg catcaggaat aaatgatggt gctgctatgt
tagtagtaat
     2761 ggctaaagaa aaagctgaag aactaggaat agagcctctt gcaactatag
tttcttatgg
     2821 aacagctggt gttgacccta aaataatggg atatggacca gttccagcaa
ctaaaaaagc
     2881 tttagaagct gctaatatga ctattgaaga tatagattta gttgaagcta
atgaggcatt
     2941 tgctgcccaa tctgtagctg taataagaga cttaaatata gatatgaata
aagttaatgt
     3001 taatggtgga gcaatagcta taggacatcc aataggatgc tcaggagcaa
gaatacttac
     3061 tacactttta tatgaaatga agagaagaga tgctaaaact ggtcttgcta
cactttgtat
     3121 aggcggtgga atgggaacta ctttaatagt taagagatag taagaaggag
atatacatat
     3181 gaaattagct gtaataggta gtggaactat gggaagtggt attgtacaaa
cttttgcaag
     3241 ttgtggacat gatgtatgtt taaagagtag aactcaaggt gctatagata
aatgtttagc
     3301 tttattagat aaaaatttaa ctaagttagt tactaaggga aaaatggatg
aagctacaaa
     3361 agcagaaata ttaagtcatg ttagttcaac tactaattat gaagatttaa
aagatatgga
     3421 tttaataata gaagcatctg tagaagacat gaatataaag aaagatgttt
tcaagttact
     3481 agatgaatta tgtaaagaag atactatctt ggcaacaaat acttcatcat
tatctataac
```

Nucleotide sequences of pLogic046-tet-butyrate construct (SEQ ID NO: 79)

```
     3541 agaaatagct tcttctacta agcgcccaga taaagttata ggaatgcatt
tctttaatcc
     3601 agttcctatg atgaaattag ttgaagttat aagtggtcag ttaacatcaa
aagttacttt
     3661 tgatacagta tttgaattat ctaagagtat caataaagta ccagtagatg
tatctgaatc
     3721 tcctggattt gtagtaaata gaatacttat acctatgata aatgaagctg
ttggtatata
     3781 tgcagatggt gttgcaagta aagaagaaat agatgaagct atgaaattag
gagcaaacca
     3841 tccaatggga ccactagcat taggtgattt aatcggatta gatgttgttt
tagctataat
     3901 gaacgtttta tatactgaat ttggagatac taaatataga cctcatccac
ttttagctaa
     3961 aatggttaga gctaatcaat taggaagaaa aactaagata ggattctatg
attataataa
     4021 ataataagaa ggagatatac atatgagtac aagtgatgtt aaagtttatg
agaatgtagc
     4081 tgttgaagta gatggaaata tatgtacagt gaaaatgaat agacctaaag
cccttaatgc
     4141 aataaattca aagactttag aagaacttta tgaagtattt gtagatatta
ataatgatga
     4201 aactattgat gttgtaatat tgacagggga aggaaaggca tttgtagctg
gagcagatat
     4261 tgcatacatg aaagatttag atgctgtagc tgctaaagat tttagtatct
taggagcaaa
     4321 agcttttgga gaaatagaaa atagtaaaaa agtagtgata gctgctgtaa
acggatttgc
     4381 tttaggtgga ggatgtgaac ttgcaatggc atgtgatata agaattgcat
ctgctaaagc
     4441 taaatttggt cagccagaag taactcttgg aataactcca ggatatggag
gaactcaaag
     4501 gcttacaaga ttggttggaa tggcaaaagc aaaagaatta atctttacag
gtcaagttat
     4561 aaaagctgat gaagctgaaa aaatagggct agtaaataga gtcgttgagc
cagacatttt
     4621 aatagaagaa gttgagaaat tagctaagat aatagctaaa aatgctcagc
ttgcagttag
     4681 atactctaaa gaagcaatac aacttggtgc tcaaactgat ataaatactg
gaatagatat
     4741 agaatctaat ttatttggtc tttgtttttc aactaaagac caaaaagaag
gaatgtcagc
     4801 tttcgttgaa aagagagaag ctaactttat aaaagggtaa taagaaggag
atatacatat
     4861 gagaagtttt gaagaagtaa ttaagtttgc aaaagaaaga ggacctaaaa
ctatatcagt
     4921 agcatgttgc caagataaag aagttttaat ggcagttgaa atggctagaa
aagaaaaaat
     4981 agcaaatgcc attttagtag gagatataga aaagactaaa gaaattgcaa
aaagcataga
     5041 catggatatc gaaaattatg aactgataga tataaaagat ttagcagaag
catctctaaa
     5101 atctgttgaa ttagtttcac aaggaaaagc cgacatggta atgaaaggct
tagtagacac
     5161 atcaataata ctaaaagcag ttttaaataa agaagtaggt cttagaactg
gaaatgtatt
     5221 aagtcacgta gcagtatttg atgtagaggg atatgataga ttatttttcg
```

Nucleotide sequences of pLogic046-tet-butyrate construct (SEQ ID NO: 79)

```
taactgacgc
     5281 agctatgaac ttagctcctg atacaaatac taaaaagcaa atcatagaaa
atgcttgcac
     5341 agtagcacat tcattagata taagtgaacc aaaagttgct gcaatatgcg
caaaagaaaa
     5401 agtaaatcca aaaatgaaag atacagttga agctaaagaa ctagaagaaa
tgtatgaaag
     5461 aggagaaatc aaaggttgta tggttggtgg gccttttgca attgataatg
cagtatcttt
     5521 agaagcagct aaacataaag gtataaatca tcctgtagca ggacgagctg
atatattatt
     5581 agccccagat attgaaggtg gtaacatatt atataaagct ttggtattct
tctcaaaatc
     5641 aaaaaatgca ggagttatag ttggggctaa agcaccaata atattaactt
ctagagcaga
     5701 cagtgaagaa actaaactaa actcaatagc tttaggtgtt ttaatggcag
caaaggcata
     5761 ataagaagga gatatacata tgagcaaaat atttaaaatc ttaacaataa
atcctggttc
     5821 gacatcaact aaaatagctg tatttgataa tgaggattta gtatttgaaa
aaactttaag
     5881 acattcttca gaagaaatag gaaaatatga gaaggtgtct gaccaatttg
aatttcgtaa
     5941 acaagtaata gaagaagctc taaaagaagg tggagtaaaa acatctgaat
tagatgctgt
     6001 agtaggtaga ggaggacttc ttaaacctat aaaaggtggt acttattcag
taagtgctgc
     6061 tatgattgaa gatttaaaag tgggagtttt aggagaacac gcttcaaacc
taggtggaat
     6121 aatagcaaaa caaataggtg aagaagtaaa tgttccttca tacatagtag
accctgttgt
     6181 tgtagatgaa ttagaagatg ttgctagaat ttctggtatg cctgaaataa
gtagagcaag
     6241 tgtagtacat gctttaaatc aaaaggcaat agcaagaaga tatgctagag
aaataaacaa
     6301 gaaatatgaa gatataaatc ttatagttgc acacatgggt ggaggagttt
ctgttggagc
     6361 tcataaaaat ggtaaaatag tagatgttgc aaacgcatta gatggagaag
gacctttctc
     6421 tccagaaaga agtggtggac taccagtagg tgcattagta aaaatgtgct
ttagtggaaa
     6481 atatactcaa gatgaaatta aaaagaaaat aaaaggtaat ggcggactag
ttgcatactt
     6541 aaacactaat gatgctagag aagttgaaga aagaattgaa gctggtgatg
aaaaagctaa
     6601 attagtatat gaagctatgg catatcaaat ctctaaagaa ataggagcta
gtgctgcagt
     6661 tcttaaggga gatgtaaaag caatattatt aactggtgga atcgcatatt
caaaaatgtt
     6721 tacagaaatg attgcagata gagttaaatt tatagcagat gtaaaagttt
atccaggtga
     6781 agatgaaatg attgcattag ctcaaggtgg acttagagtt ttaactggtg
aagaagaggc
     6841 tcaagtttat gataactaat aa
```

[0119] In some embodiments, the gene or gene cassette for producing the anti-inflammation and/or gut barrier function enhancer molecule is present on a plasmid and operably linked to a promoter that is induced by ROS. In some embodiments, the gene or gene cassette for producing the anti-inflammation and/or gut barrier function enhancer molecule is present in the chromosome and operably linked to a promoter that is induced by ROS. In some embodiments, the gene or gene cassette for producing the anti-inflammation and/or gut barrier function enhancer molecule is present on a chromosome and operably linked to a promoter that is induced by exposure to tetracycline. In some embodiments, the gene or gene cassette for producing the anti-inflammation and/or gut barrier function enhancer molecule is present on a plasmid and operably linked to a promoter that is induced by exposure to tetracycline. In some embodiments, expression is further optimized by methods known in the art, e.g., by optimizing ribosomal binding sites, manipulating transcriptional regulators, and/or increasing mRNA stability.

[0120] In some embodiments, the genetically engineered bacteria comprise a stably maintained plasmid or chromosome carrying the gene(s) or gene cassette(s) capable of producing an anti-inflammation and/or gut barrier function enhancer molecule, such that the gene(s) or gene cassette(s) can be expressed in the host cell, and the host cell is capable of survival and/or growth *in vitro*, e.g., in medium, and/or *in vivo*, e.g., in the gut. In some embodiments, a bacterium may comprise multiple copies of the gene or gene cassette for producing the anti-inflammation and/or gut barrier function enhance molecule. In some embodiments, gene or gene cassette is expressed on a low-copy plasmid. In some embodiments, the low-copy plasmid may be useful for increasing stability of expression. In some embodiments, the low-copy plasmid may be useful for decreasing leaky expression under non-inducing conditions. In some embodiments, gene or gene cassette is expressed on a high-copy plasmid. In some embodiments, the high-copy plasmid may be useful for increasing gene or gene cassette expression. In some embodiments, gene or gene cassette is expressed on a chromosome.

[0121] In some embodiments, the genetically engineered bacteria may comprise multiple copies of the gene(s) or gene cassette(s) capable of producing an anti-

inflammation and/or gut barrier function enhancer molecule. In some embodiments, the gene(s) or gene cassette(s) capable of producing an anti-inflammation and/or gut barrier function enhancer molecule is present on a plasmid and operatively linked to a ROS-responsive regulatory region. In some embodiments, the gene(s) or gene cassette(s) capable of producing an anti-inflammation and/or gut barrier function enhancer molecule is present in a chromosome and operatively linked to a ROS-responsive regulatory region.

[0122] In some embodiments, the genetically engineered bacteria of the invention produce at least one anti-inflammation and/or gut barrier enhancer molecule in the presence of ROS to reduce local gut inflammation by at least about 1.5-fold, at least about 2-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 30-fold, at least about 50-fold, at least about 100-fold, at least about 200-fold, at least about 300-fold, at least about 400-fold, at least about 500-fold, at least about 600-fold, at least about 700-fold, at least about 800-fold, at least about 900-fold, at least about 1,000-fold, or at least about 1,500-fold as compared to unmodified bacteria of the same subtype under the same conditions. Inflammation may be measured by methods known in the art, e.g., counting disease lesions using endoscopy; detecting T regulatory cell differentiation in peripheral blood, e.g., by fluorescence activated sorting; measuring T regulatory cell levels; measuring cytokine levels; measuring areas of mucosal damage; assaying inflammatory biomarkers, e.g., by qPCR; PCR arrays; transcription factor phosphorylation assays; immunoassays; and/or cytokine assay kits (Mesoscale, Cayman Chemical, Qiagen).

[0123] In some embodiments, the genetically engineered bacteria produce at least about 1.5-fold, at least about 2-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 30-fold, at least about 50-fold, at least about 100-fold, at least about 200-fold, at least about 300-fold, at least about 400-fold, at least about 500-fold, at least about 600-fold, at least about 700-fold, at least about 800-fold, at least about 900-fold, at least about 1,000-fold, or at least about 1,500-fold more of an anti-inflammation and/or gut barrier enhancer molecule in the presence of ROS than unmodified bacteria of the same subtype under the same conditions. Certain unmodified

bacteria will not have detectable levels of the anti-inflammation and/or gut barrier enhancer molecule. In embodiments using genetically modified forms of these bacteria, the anti-inflammation and/or gut barrier enhancer molecule will be detectable in the presence of ROS.

[0124] In certain embodiments, the anti-inflammation and/or gut barrier enhancer molecule is butyrate. Methods of measuring butyrate levels, e.g., by mass spectrometry, gas chromatography, high-performance liquid chromatography (HPLC), are known in the art (*see, e.g.,* Aboulnaga et al., 2013). In some embodiments, butyrate is measured as butyrate level/bacteria optical density (OD). In some embodiments, measuring the activity and/or expression of one or more gene products in the butyrogenic gene cassette serves as a proxy measurement for butyrate production. In some embodiments, the bacterial cells of the invention are harvested and lysed to measure butyrate production. In alternate embodiments, butyrate production is measured in the bacterial cell medium. In some embodiments, the genetically engineered bacteria produce at least about 1 nM/OD, at least about 10 nM/OD, at least about 100 nM/OD, at least about 500 nM/OD, at least about 1 μM/OD, at least about 10 μM/OD, at least about 100 μM/OD, at least about 500 μM/OD, at least about 1 mM/OD, at least about 2 mM/OD, at least about 3 mM/OD, at least about 5 mM/OD, at least about 10 mM/OD, at least about 20 mM/OD, at least about 30 mM/OD, or at least about 50 mM/OD of butyrate in the presence of ROS.

Multiple mechanisms of action

[0223] In some embodiments, the bacteria are genetically engineered to include multiple mechanisms of action (MOAs), e.g., circuits producing multiple copies of the same product (e.g., to enhance copy number) or circuits performing multiple different functions. In some embodiments, the genetically engineered bacteria are capable of producing IL-2, IL-10, IL-22, IL-27, propionate, and butyrate. In some embodiments, the genetically engineered bacteria are capable of producing IL-10, IL-27, GLP-2, and butyrate. In some embodiments, the genetically engineered bacteria are capable of producing GLP-2, IL-10, IL-22, SOD, butyrate, and propionate. In some embodiments, the genetically engineered bacteria are capable of GLP-2, IL-2, IL-10, IL-22, IL-27, SOD,

butyrate, and propionate. Any suitable combination of therapeutic molecules may be produced by the genetically engineered bacteria. Examples of insertion sites include, but are not limited to, *malE/K, insB/I, araC/BAD, lacZ, dapA, cea,* and other shown in **Fig. 51**. For example, the genetically engineered bacteria may include four copies of GLP-2 inserted at four different insertion sites, e.g., *malE/K, insB/I, araC/BAD,* and *lacZ.* Alternatively, the genetically engineered bacteria may include three copies of GLP-2 inserted at three different insertion sites, e.g., *malE/K, insB/I,* and *lacZ*, and three copies of a butyrate gene cassette inserted at three different insertion sites, e.g., *dapA, cea,* and *araC/BAD.*

Secretion

[0224] In some embodiments, the genetically engineered bacteria further comprise a native secretion mechanism (e.g., Gram-positive bacteria) or non-native secretion mechanism (e.g., Gram-negative bacteria) that is capable of secreting the the anti-inflammation and/or gut barrier enhancer molecule from the bacterial cytoplasm. Many bacteria have evolved sophisticated secretion systems to transport substrates across the bacterial cell envelope. Substrates, such as small molecules, proteins, and DNA, may be released into the extracellular space or periplasm (such as the gut lumen or other space), injected into a target cell, or associated with the bacterial membrane.

[0225] In Gram-negative bacteria, secretion machineries may span one or both of the inner and outer membranes. In some embodiments, the genetically engineered bacteria further comprise a non-native double membrane-spanning secretion system. Double membrane-spanning secretion systems include, but are not limited to, the type I secretion system (T1SS), the type II secretion system (T2SS), the type III secretion system (T3SS), the type IV secretion system (T4SS), the type VI secretion system (T6SS), and the resistance-nodulation-division (RND) family of multi-drug efflux pumps (Pugsley, 1993; Gerlach et al., 2007; Collinson et al., 2015; Costa et al., 2015; Reeves et al., 2015; WO2014138324A1, incorporated herein by reference). Examples of such secretion systems are shown in **Figs. 68-71**. Mycobacteria, which have a Gram-negative-like cell envelope, may also encode a type VII secretion system (T7SS) (Stanley et al., 2003). With the exception of the T2SS, double membrane-spanning secretions generally transport

substrates from the bacterial cytoplasm directly into the extracellular space or into the target cell. In contrast, the T2SS and secretion systems that span only the outer membrane may use a two-step mechanism, wherein substrates are first translocated to the periplasm by inner membrane-spanning transporters, and then transferred to the outer membrane or secreted into the extracellular space. Outer membrane-spanning secretion systems include, but are not limited to, the type V secretion or autotransporter system (T5SS), the curli secretion system, and the chaperone-usher pathway for pili assembly (Saier, 2006; Costa et al., 2015).

[0226] In some embodiments, the genetically engineered bacteria of the invention further comprise a type III or a type III-like secretion system (T3SS) from *Shigella, Salmonella, E. coli, Bivrio, Burkholderia, Yersinia, Chlamydia,* or *Pseudomonas.* The T3SS is capable of transporting a protein from the bacterial cytoplasm to the host cytoplasm through a needle complex. The T3SS may be modified to secrete the molecule from the bacterial cytoplasm, but not inject the molecule into the host cytoplasm. Thus, the molecule is secreted into the gut lumen or other extracellular space. In some embodiments, the genetically engineered bacteria comprise said modified T3SS and are capable of secreting the anti-inflammation and/or gut barrier enhancer molecule from the bacterial cytoplasm. In some embodiments, the secreted molecule comprises a type III secretion sequence that allows the molecule to be secreted from the bacteria.

[0227] In some embodiments, a flagellar type III secretion pathway is used to secrete the anti-inflammation and/or gut barrier enhancer molecule. In some embodiments, an incomplete flagellum is used to secrete a therapeutic molecule by recombinantly fusing the molecule to an N-terminal flagellar secretion signal of a native flagellar component. In this manner, the intracellularly expressed chimeric molecule can be mobilized across the inner and outer membranes into the surrounding host environment.

[0228] In some embodiments, a type V autotransporter secretion system is used to secrete the anti-inflammation and/or gut barrier enhancer molecule. Due to the simplicity of the machinery and capacity to handle relatively large protein fluxes, the type V secretion system is attractive for the extracellular production of recombinant proteins.

As shown in **Fig. 69**, a therapeutic peptide (star) can be fused to an N-terminal secretion signal, a linker, and the beta-domain of an autotransporter. The N-terminal signal sequence directs the protein to the SecA-YEG machinery which moves the protein across the inner membrane into the periplasm, followed by subsequent cleavage of the signal sequence. The beta-domain is recruited to the Bam complex ('Beta-barrel assembly machinery') where the beta-domain is folded and inserted into the outer membrane as a beta-barrel structure. The therapeutic peptide is thread through the hollow pore of the beta-barrel structure ahead of the linker sequence. Once exposed to the extracellular environment, the therapeutic peptide can be freed from the linker system by an autocatalytic cleavage (left side of Bam complex) or by targeting of a membrane-associated peptidase (black scissors; right side of Bam complex) to a complimentary protease cut site in the linker. Thus, in some embodiments, the secreted molecule, such as a heterologous protein or peptide comprises an N-terminal secretion signal, a linker, and beta-domain of an autotransporter so as to allow the molecule to be secreted from the bacteria.

[0229] In some embodiments, a hemolysin-based secretion system is used to secrete the anti-inflammation and/or gut barrier enhancer molecule. Type I secretion systems offer the advantage of translocating their passenger peptide directly from the cytoplasm to the extracellular space, obviating the two-step process of other secretion types. **Fig. 71** shows the alpha-hemolysin (HlyA) of uropathogenic *Escherichia coli*. This pathway uses HlyB, an ATP-binding cassette transporter; HlyD, a membrane fusion protein; and TolC, an outer membrane protein. The assembly of these three proteins forms a channel through both the inner and outer membranes. Natively, this channel is used to secrete HlyA, however, to secrete the therapeutic molecule of the present disclosure, the secretion signal-containing C-terminal portion of HlyA is fused to the C-terminal portion of a therapeutic molecule (star) to mediate secretion of this molecule.

[0230] In alternate embodiments, the genetically engineered bacteria further comprise a non-native single membrane-spanning secretion system. Single membrane-spanning transporters may act as a component of a secretion system, or may export substrates independently. Such transporters include, but are not limited to, ATP-binding

cassette translocases, flagellum/virulence-related translocases, conjugation-related translocases, the general secretory system (e.g., the SecYEG complex in *E. coli*), the accessory secretory system in mycobacteria and several types of Gram-positive bacteria (e.g., *Bacillus anthracis*, *Lactobacillus johnsonii*, *Corynebacterium glutamicum*, *Streptococcus gordonii*, *Staphylococcus aureus*), and the twin-arginine translocation (TAT) system (Saier, 2006; Rigel and Braunstein, 2008; Albiniak et al., 2013). It is known that the general secretory and TAT systems can both export substrates with cleavable N-terminal signal peptides into the periplasm, and have been explored in the context of biopharmaceutical production. The TAT system may offer particular advantages, however, in that it is able to transport folded substrates, thus eliminating the potential for premature or incorrect folding. In certain embodiments, the genetically engineered bacteria comprise a TAT or a TAT-like system and are capable of secreting the anti-inflammation and/or gut barrier enhancer molecule from the bacterial cytoplasm. One of ordinary skill in the art would appreciate that the secretion systems disclosed herein may be modified to act in different species, strains, and subtypes of bacteria, and/or adapted to deliver different effector molecules.

Essential genes and auxotrophs

[0231] As used herein, the term "essential gene" refers to a gene which is necessary to for cell growth and/or survival. Bacterial essential genes are well known to one of ordinary skill in the art, and can be identified by directed deletion of genes and/or random mutagenesis and screening (*see, e.g.,* Zhang and Lin, 2009, DEG 5.0, a database of essential genes in both prokaryotes and eukaryotes, Nucl. Acids Res., 37:D455-D458 and Gerdes et al., Essential genes on metabolic maps, Curr. Opin. Biotechnol., 17(5):448-456, the entire contents of each of which are expressly incorporated herein by reference).

[0232] An "essential gene" may be dependent on the circumstances and environment in which an organism lives. For example, a mutation of, modification of, or excision of an essential gene may result in the genetically engineered bacteria of the disclosure becoming an auxotroph. An auxotrophic modification is intended to cause

bacteria to die in the absence of an exogenously added nutrient essential for survival or growth because they lack the gene(s) necessary to produce that essential nutrient.

[0233] An auxotrophic modification is intended to cause bacteria to die in the absence of an exogenously added nutrient essential for survival or growth because they lack the gene(s) necessary to produce that essential nutrient. In some embodiments, any of the genetically engineered bacteria described herein also comprise a deletion or mutation in a gene required for cell survival and/or growth. In one embodiment, the essential gene is a DNA synthesis gene, for example, *thyA*. In another embodiment, the essential gene is a cell wall synthesis gene, for example, *dapA*. In yet another embodiment, the essential gene is an amino acid gene, for example, *serA* or *MetA*. Any gene required for cell survival and/or growth may be targeted, including but not limited to, *cysE, glnA, ilvD, leuB, lysA, serA, metA, glyA, hisB, ilvA, pheA, proA, thrC, trpC, tyrA, thyA, uraA, dapA, dapB, dapD, dapE, dapF, flhD, metB, metC, proAB,* and *thi1*, as long as the corresponding wild-type gene product is not produced in the bacteria. For example, thymine is a nucleic acid that is required for bacterial cell growth; in its absence, bacteria undergo cell death. The *thyA* gene encodes thimidylate synthetase, an enzyme that catalyzes the first step in thymine synthesis by converting dUMP to dTMP (Sat et al., 2003). In some embodiments, the bacterial cell of the disclosure is a thyA auxotroph in which the *thyA* gene is deleted and/or replaced with an unrelated gene. A thyA auxotroph can grow only when sufficient amounts of thymine are present, e.g., by adding thymine to growth media *in vitro*, or in the presence of high thymine levels found naturally in the human gut *in vivo*. In some embodiments, the bacterial cell of the disclosure is auxotrophic in a gene that is complemented when the bacterium is present in the mammalian gut. Without sufficient amounts of thymine, the thyA auxotroph dies. In some embodiments, the auxotrophic modification is used to ensure that the bacterial cell does not survive in the absence of the auxotrophic gene product (e.g., outside of the gut).

[0234] Diaminopimelic acid (DAP) is an amino acid synthetized within the lysine biosynthetic pathway and is required for bacterial cell wall growth (Meadow et al., 1959; Clarkson et al., 1971). In some embodiments, any of the genetically engineered bacteria

described herein is a dapD auxotroph in which the *dapD* gene is deleted and/or replaced with an unrelated gene. A dapD auxotroph can grow only when sufficient amounts of DAP are present, e.g., by adding DAP to growth media *in vitro*. Without sufficient amounts of DAP, the dapD auxotroph dies. In some embodiments, the auxotrophic modification is used to ensure that the bacterial cell does not survive in the absence of the auxotrophic gene product (e.g., outside of the gut).

[0235] In other embodiments, the genetically engineered bacterium of the present disclosure is a uraA auxotroph in which the *uraA* gene is deleted and/or replaced with an unrelated gene. The *uraA* gene codes for UraA, a membrane-bound transporter that facilitates the uptake and subsequent metabolism of the pyrimidine uracil (Andersen et al., 1995). An uraA auxotroph can grow only when sufficient amounts of uracil are present, e.g., by adding uracil to growth media *in vitro*. Without sufficient amounts of uracil, the uraA auxotroph dies. In some embodiments, auxotrophic modifications are used to ensure that the bacteria do not survive in the absence of the auxotrophic gene product (e.g., outside of the gut).

[0236] In complex communities, it is possible for bacteria to share DNA. In very rare circumstances, an auxotrophic bacterial strain may receive DNA from a non-auxotrophic strain, which repairs the genomic deletion and permanently rescues the auxotroph. Therefore, engineering a bacterial strain with more than one auxotroph may greatly decrease the probability that DNA transfer will occur enough times to rescue the auxotrophy. In some embodiments, the genetically engineered bacteria of the invention comprise a deletion or mutation in two or more genes required for cell survival and/or growth.

[0237] Other examples of essential genes include, but are not limited to *yhbV, yagG, hemB, secD, secF, ribD, ribE, thiL, dxs, ispA, dnaX, adk, hemH, lpxH, cysS, fold, rplT, infC, thrS, nadE, gapA, yeaZ, aspS, argS, pgsA, yefM, metG, folE, yejM, gyrA, nrdA, nrdB, folC, accD, fabB, gltX, ligA, zipA, dapE, dapA, der, hisS, ispG, suhB, tadA, acpS, era, rnc, ftsB, eno, pyrG, chpR, lgt, fbaA, pgk, yqgD, metK, yqgF, plsC, ygiT, pare, ribB, cca, ygjD, tdcF, yraL, yihA, ftsN, murI, murB, birA, secE, nusG, rplJ, rplL, rpoB, rpoC, ubiA, plsB, lexA, dnaB, ssb, alsK, groS, psd, orn, yjeE, rpsR, chpS, ppa, valS, yjgP, yjgQ, dnaC, ribF, lspA,*

ispH, dapB, folA, imp, yabQ, ftsL, ftsI, murE, murF, mraY, murD, ftsW, murG, murC, ftsQ, ftsA, ftsZ, lpxC, secM, secA, can, folK, hemL, yadR, dapD, map, rpsB, infB ,nusA, ftsH, obgE, rpmA, rplU, ispB, murA, yrbB, yrbK, yhbN, rpsI, rplM, degS, mreD, mreC, mreB, accB, accC, yrdC, def, fmt, rplQ, rpoA, rpsD, rpsK, rpsM, entD, mrdB, mrdA, nadD, hlepB, rpoE, pssA, yfiO, rplS, trmD, rpsP, ffh, grpE, yfjB, csrA, ispF, ispD, rplW, rplD, rplC, rpsJ, fusA, rpsG, rpsL, trpS, yrfF, asd, rpoH, ftsX, ftsE, ftsY, frr, dxr, ispU, rfaK, kdtA, coaD, rpmB, dfp, dut, gmk, spot, gyrB, dnaN, dnaA, rpmH, rnpA, yidC, tnaB, glmS, glmU, wzyE, hemD, hemC, yigP, ubiB, ubiD, hemG, secY, rplO, rpmD, rpsE, rplR, rplF, rpsH, rpsN, rplE, rplX, rplN, rpsQ, rpmC, rplP, rpsC, rplV, rpsS, rplB, cdsA, yaeL, yaeT, lpxD, fabZ, lpxA, lpxB, dnaE, accA, tilS, proS, yafF, tsf, pyrH, olA, rlpB, leuS, lnt, glnS, fldA, cydA, infA, cydC, ftsK, lolA, serS, rpsA, msbA, lpxK, kdsB, mukF, mukE, mukB, asnS, fabA, mviN, rne, yceQ, fabD, fabG, acpP, tmk, holB, lolC, lolD, lolE, purB, ymfK, minE, mind, pth, rsA, ispE, lolB, hemA, prfA, prmC, kdsA, topA, ribA, fabI, racR, dicA, ydfB, tyrS, ribC, ydiL, pheT, pheS, yhhQ, bcsB, glyQ, yibJ, and *gpsA*. Other essential genes are known to those of ordinary skill in the art.

[0238] In some embodiments, the genetically engineered bacterium of the present disclosure is a synthetic ligand-dependent essential gene (SLiDE) bacterial cell. SLiDE bacterial cells are synthetic auxotrophs with a mutation in one or more essential genes that only grow in the presence of a particular ligand (*see* Lopez and Anderson "Synthetic Auxotrophs with Ligand-Dependent Essential Genes for a BL21 (DE3) Biosafety Strain," ACS Synthetic Biology (2015) DOI: 10.1021/acssynbio.5b00085, the entire contents of which are expressly incorporated herein by reference).

[0239] In some embodiments, the SLiDE bacterial cell comprises a mutation in an essential gene. In some embodiments, the essential gene is selected from the group consisting of *pheS, dnaN, tyrS, metG,* and *adk*. In some embodiments, the essential gene is *dnaN* comprising one or more of the following mutations: H191N, R240C, I317S, F319V, L340T, V347I, and S345C. In some embodiments, the essential gene is *dnaN* comprising the mutations H191N, R240C, I317S, F319V, L340T, V347I, and S345C. In some embodiments, the essential gene is *pheS* comprising one or more of the following mutations: F125G, P183T, P184A, R186A, and I188L. In some embodiments, the essential gene is *pheS* comprising the mutations F125G, P183T, P184A, R186A, and I188L. In some

embodiments, the essential gene is *tyrS* comprising one or more of the following mutations: L36V, C38A, and F40G. In some embodiments, the essential gene is *tyrS* comprising the mutations L36V, C38A, and F40G. In some embodiments, the essential gene is *metG* comprising one or more of the following mutations: E45Q, N47R, I49G, and A51C. In some embodiments, the essential gene is *metG* comprising the mutations E45Q, N47R, I49G, and A51C. In some embodiments, the essential gene is *adk* comprising one or more of the following mutations: I4L, L5I, and L6G. In some embodiments, the essential gene is *adk* comprising the mutations I4L, L5I, and L6G.

[0240] In some embodiments, the genetically engineered bacterium is complemented by a ligand. In some embodiments, the ligand is selected from the group consisting of benzothiazole, indole, 2-aminobenzothiazole, indole-3-butyric acid, indole-3-acetic acid, and L-histidine methyl ester. For example, bacterial cells comprising mutations in *metG* (E45Q, N47R, I49G, and A51C) are complemented by benzothiazole, indole, 2-aminobenzothiazole, indole-3-butyric acid, indole-3-acetic acid, or L-histidine methyl ester. Bacterial cells comprising mutations in *dnaN* (H191N, R240C, I317S, F319V, L340T, V347I, and S345C) are complemented by benzothiazole, indole or 2-aminobenzothiazole. Bacterial cells comprising mutations in *pheS* (F125G, P183T, P184A, R186A, and I188L) are complemented by benzothiazole or 2-aminobenzothiazole. Bacterial cells comprising mutations in *tyrS* (L36V, C38A, and F40G) are complemented by benzothiazole or 2-aminobenzothiazole. Bacterial cells comprising mutations in *adk* (I4L, L5I, and L6G) are complemented by benzothiazole or indole.

[0241] In some embodiments, the genetically engineered bacterium comprises more than one mutant essential gene that renders it auxotrophic to a ligand. In some embodiments, the bacterial cell comprises mutations in two essential genes. For example, in some embodiments, the bacterial cell comprises mutations in *tyrS* (L36V, C38A, and F40G) and *metG* (E45Q, N47R, I49G, and A51C). In other embodiments, the bacterial cell comprises mutations in three essential genes. For example, in some embodiments, the bacterial cell comprises mutations in *tyrS* (L36V, C38A, and F40G), *metG* (E45Q, N47R, I49G, and A51C), and *pheS* (F125G, P183T, P184A, R186A, and I188L).

[0242] In some embodiments, the genetically engineered bacterium is a conditional auxotroph whose essential gene(s) is replaced using the arabinose system shown in **Figs. 57-61.**

[0243] In some embodiments, the genetically engineered bacterium of the disclosure is an auxotroph and also comprises kill-switch circuitry, such as any of the kill-switch components and systems described herein. For example, the genetically engineered bacteria may comprise a deletion or mutation in an essential gene required for cell survival and/or growth, for example, in a DNA synthesis gene, for example, *thyA*, a cell wall synthesis gene, for example, *dapA* and/or an amino acid gene, for example, *serA* or *MetA* and may also comprise a toxin gene that is regulated by one or more transcriptional activators that are expressed in response to an environmental condition(s) and/or signal(s) (such as the described arabinose system) or regulated by one or more recombinases that are expressed upon sensing an exogenous environmental condition(s) and/or signal(s) (such as the recombinase systems described herein). Other embodiments are described in Wright et al., "GeneGuard: A Modular Plasmid System Designed for Biosafety," ACS Synthetic Biology (2015) 4: 307-316, the entire contents of which are expressly incorporated herein by reference). In some embodiments, the genetically engineered bacterium of the disclosure is an auxotroph and also comprises kill-switch circuitry, such as any of the kill-switch components and systems described herein, as well as another biosecurity system, such a conditional origin of replication (Wright et al., 2015). In other embodiments, auxotrophic modifications may also be used to screen for mutant bacteria that produce the anti-inflammation and/or gut barrier enhancer molecule. In some embodiments, the genetically engineered bacteria further comprise an antibiotic resistance gene.

Genetic regulatory circuits

[0244] In some embodiments, the genetically engineered bacteria comprise multi-layered genetic regulatory circuits for expressing the constructs described herein (*see, e.g.,* U.S. Provisional Application No. 62/184,811, incorporated herein by reference in its entirety). The genetic regulatory circuits are useful to screen for mutant bacteria that produce an anti-inflammation and/or gut barrier enhancer molecule or rescue an

auxotroph. In certain embodiments, the invention provides methods for selecting genetically engineered bacteria that produce one or more genes of interest.

[0245] In some embodiments, the invention provides genetically engineered bacteria comprising a gene or gene cassette for producing a therapeutic molecule (e.g., butyrate) and a T7 polymerase-regulated genetic regulatory circuit. For example, the genetically engineered bacteria comprise a first gene encoding a T7 polymerase, wherein the first gene is operably linked to a FNR-responsive promoter; a second gene or gene cassette for producing a therapeutic molecule (e.g., butyrate), wherein the second gene or gene cassette is operably linked to a T7 promoter that is induced by the T7 polymerase; and a third gene encoding an inhibitory factor, lysY, that is capable of inhibiting the T7 polymerase. In the presence of oxygen, FNR does not bind the FNR-responsive promoter, and the therapeutic molecule (e.g., butyrate) is not expressed. *LysY* is expressed constitutively (P-lac constitutive) and further inhibits T7 polymerase. In the absence of oxygen, FNR dimerizes and binds to the FNR-responsive promoter, T7 polymerase is expressed at a level sufficient to overcome lysY inhibition, and the therapeutic molecule (e.g., butyrate) is expressed. In some embodiments, the *lysY* gene is operably linked to an additional FNR binding site. In the absence of oxygen, FNR dimerizes to activate T7 polymerase expression as described above, and also inhibits *lysY* expression.

[0246] In some embodiments, the invention provides genetically engineered bacteria comprising a gene or gene cassette for producing a therapeutic molecule (e.g., butyrate) and a protease-regulated genetic regulatory circuit. For example, the genetically engineered bacteria comprise a first gene encoding an mf-lon protease, wherein the first gene is operably linked to a FNR-responsive promoter; a second gene or gene cassette for producing a therapeutic molecule operably linked to a Tet regulatory region (TetO); and a third gene encoding an mf-lon degradation signal linked to a Tet repressor (TetR), wherein the TetR is capable of binding to the Tet regulatory region and repressing expression of the second gene or gene cassette. The mf-lon protease is capable of recognizing the mf-lon degradation signal and degrading the TetR. In the presence of oxygen, FNR does not bind the FNR-responsive promoter, the repressor is

not degraded, and the therapeutic molecule is not expressed. In the absence of oxygen, FNR dimerizes and binds the FNR-responsive promoter, thereby inducing expression of the mf-lon protease. The mf-lon protease recognizes the mf-lon degradation signal and degrades the TetR, and the therapeutic molecule is expressed.

[0247] In some embodiments, the invention provides genetically engineered bacteria comprising a gene or gene cassette for producing a therapeutic molecule and a repressor-regulated genetic regulatory circuit. For example, the genetically engineered bacteria comprise a first gene encoding a first repressor, wherein the first gene is operably linked to a FNR-responsive promoter; a second gene or gene cassette for producing a therapeutic molecule operably linked to a first regulatory region comprising a constitutive promoter; and a third gene encoding a second repressor, wherein the second repressor is capable of binding to the first regulatory region and repressing expression of the second gene or gene cassette. The third gene is operably linked to a second regulatory region comprising a constitutive promoter, wherein the first repressor is capable of binding to the second regulatory region and inhibiting expression of the second repressor. In the presence of oxygen, FNR does not bind the FNR-responsive promoter, the first repressor is not expressed, the second repressor is expressed, and the therapeutic molecule is not expressed. In the absence of oxygen, FNR dimerizes and binds the FNR-responsive promoter, the first repressor is expressed, the second repressor is not expressed, and the therapeutic molecule is expressed.

[0248] Examples of repressors useful in these embodiments include, but are not limited to, ArgR, TetR, ArsR, AscG, LacI, CscR, DeoR, DgoR, FruR, GalR, GatR, CI, LexA, RafR, QacR, and PtxS (US20030166191).

[0249] In some embodiments, the invention provides genetically engineered bacteria comprising a gene or gene cassette for producing a therapeutic molecule and a regulatory RNA-regulated genetic regulatory circuit. For example, the genetically engineered bacteria comprise a first gene encoding a regulatory RNA, wherein the first gene is operably linked to a FNR-responsive promoter, and a second gene or gene cassette for producing a therapeutic molecule. The second gene or gene cassette is operably linked to a constitutive promoter and further linked to a nucleotide sequence

capable of producing an mRNA hairpin that inhibits translation of the therapeutic molecule. The regulatory RNA is capable of eliminating the mRNA hairpin and inducing translation via the ribosomal binding site. In the presence of oxygen, FNR does not bind the FNR-responsive promoter, the regulatory RNA is not expressed, and the mRNA hairpin prevents the therapeutic molecule from being translated. In the absence of oxygen, FNR dimerizes and binds the FNR-responsive promoter, the regulatory RNA is expressed, the mRNA hairpin is eliminated, and the therapeutic molecule is expressed.

[0250] In some embodiments, the invention provides genetically engineered bacteria comprising a gene or gene cassette for producing a therapeutic molecule and a CRISPR-regulated genetic regulatory circuit. For example, the genetically engineered bacteria comprise a Cas9 protein; a first gene encoding a CRISPR guide RNA, wherein the first gene is operably linked to a FNR-responsive promoter; a second gene or gene cassette for producing a therapeutic molecule, wherein the second gene or gene cassette is operably linked to a regulatory region comprising a constitutive promoter; and a third gene encoding a repressor operably linked to a constitutive promoter, wherein the repressor is capable of binding to the regulatory region and repressing expression of the second gene or gene cassette. The third gene is further linked to a CRISPR target sequence that is capable of binding to the CRISPR guide RNA, wherein said binding to the CRISPR guide RNA induces cleavage by the Cas9 protein and inhibits expression of the repressor. In the presence of oxygen, FNR does not bind the FNR-responsive promoter, the guide RNA is not expressed, the repressor is expressed, and the therapeutic molecule is not expressed. In the absence of oxygen, FNR dimerizes and binds the FNR-responsive promoter, the guide RNA is expressed, the repressor is not expressed, and the therapeutic molecule is expressed.

[0251] In some embodiments, the invention provides genetically engineered bacteria comprising a gene or gene cassette for producing a therapeutic molecule and a recombinase-regulated genetic regulatory circuit. For example, the genetically engineered bacteria comprise a first gene encoding a recombinase, wherein the first gene is operably linked to a FNR-responsive promoter, and a second gene or gene cassette for producing a therapeutic molecule operably linked to a constitutive promoter.

The second gene or gene cassette is inverted in orientation (3' to 5') and flanked by recombinase binding sites, and the recombinase is capable of binding to the recombinase binding sites to induce expression of the second gene or gene cassette by reverting its orientation (5' to 3'). In the presence of oxygen, FNR does not bind the FNR-responsive promoter, the recombinase is not expressed, the gene or gene cassette remains in the 3' to 5' orientation, and no functional therapeutic molecule is produced. In the absence of oxygen, FNR dimerizes and binds the FNR-responsive promoter, the recombinase is expressed, the gene or gene cassette is reverted to the 5' to 3' orientation, and a functional therapeutic molecule is produced.

[0252] In some embodiments, the invention provides genetically engineered bacteria comprising a gene or gene cassette for producing a therapeutic molecule and a polymerase- and recombinase-regulated genetic regulatory circuit. For example, the genetically engineered bacteria comprise a first gene encoding a recombinase, wherein the first gene is operably linked to a FNR-responsive promoter; a second gene or gene cassette for producing a therapeutic molecule operably linked to a T7 promoter; a third gene encoding a T7 polymerase, wherein the T7 polymerase is capable of binding to the T7 promoter and inducing expression of the therapeutic molecule. The third gene encoding the T7 polymerase is inverted in orientation (3' to 5') and flanked by recombinase binding sites, and the recombinase is capable of binding to the recombinase binding sites to induce expression of the T7 polymerase gene by reverting its orientation (5' to 3'). In the presence of oxygen, FNR does not bind the FNR-responsive promoter, the recombinase is not expressed, the T7 polymerase gene remains in the 3' to 5' orientation, and the therapeutic molecule is not expressed. In the absence of oxygen, FNR dimerizes and binds the FNR-responsive promoter, the recombinase is expressed, the T7 polymerase gene is reverted to the 5' to 3' orientation, and the therapeutic molecule is expressed.

[0253] Synthetic gene circuits expressed on plasmids may function well in the short term but lose ability and/or function in the long term (Danino et al., 2015). In some embodiments, the genetically engineered bacteria comprise stable circuits for expressing genes of interest over prolonged periods. In some embodiments, the genetically

engineered bacteria are capable of producing a therapeutic molecule and further comprise a toxin-anti-toxin system that simultaneously produces a toxin (hok) and a short-lived anti-toxin (sok), wherein loss of the plasmid causes the cell to be killed by the long-lived toxin (Danino et al., 2015). In some embodiments, the genetically engineered bacteria further comprise *alp7* from *B. subtilis* plasmid pL20 and produces filaments that are capable of pushing plasmids to the poles of the cells in order to ensure equal segregation during cell division (Danino et al., 2015).

Host-plasmid mutual dependency

[0254] In some embodiments, the genetically engineered bacteria of the invention also comprise a plasmid that has been modified to create a host-plasmid mutual dependency. In certain embodiments, the mutually dependent host-plasmid platform is GeneGuard (Wright et al., 2015). In some embodiments, the GeneGuard plasmid comprises (i) a conditional origin of replication, in which the requisite replication initiator protein is provided *in trans*; (ii) an auxotrophic modification that is rescued by the host via genomic translocation and is also compatible for use in rich media; and/or (iii) a nucleic acid sequence which encodes a broad-spectrum toxin. The toxin gene may be used to select against plasmid spread by making the plasmid DNA itself disadvantageous for strains not expressing the anti-toxin (e.g., a wild-type bacterium). In some embodiments, the GeneGuard plasmid is stable for at least 100 generations without antibiotic selection. In some embodiments, the GeneGuard plasmid does not disrupt growth of the host. The GeneGuard plasmid is used to greatly reduce unintentional plasmid propagation in the genetically engineered bacteria of the invention.

[0255] The mutually dependent host-plasmid platform may be used alone or in combination with other biosafety mechanisms, such as those described herein (e.g., kill switches, auxotrophies). In some embodiments, the genetically engineered bacteria comprise a GeneGuard plasmid. In other embodiments, the genetically engineered bacteria comprise a GeneGuard plasmid and/or one or more kill switches. In other embodiments, the genetically engineered bacteria comprise a GeneGuard plasmid and/or one or more auxotrophies. In still other embodiments, the genetically engineered

bacteria comprise a GeneGuard plasmid, one or more kill switches, and/or one or more auxotrophies.

[0256] Synthetic gene circuits express on plasmids may function well in the short term but lose ability and/or function in the long term (Danino et al., 2015). In some embodiments, the genetically engineered bacteria comprise stable circuits for expressing genes of interest over prolonged periods. In some embodiments, the genetically engineered bacteria are capable of producing an anti-inflammation and/or gut enhancer molecule and further comprise a toxin-anti-toxin system that simultaneously produces a toxin (hok) and a short-lived anti-toxin (sok), wherein loss of the plasmid causes the cell to be killed by the long-lived toxin (Danino et al., 2015; **Fig. 66**). In some embodiments, the genetically engineered bacteria further comprise *alp7* from *B. subtilis* plasmid pL20 and produces filaments that are capable of pushing plasmids to the poles of the cells in order to ensure equal segregation during cell division (Danino et al., 2015).

Kill switch

[0257] In some embodiments, the genetically engineered bacteria of the invention also comprise a kill switch (*see, e.g.,* U.S. Provisional Application Nos. 62/183,935, 62/263,329, and 62/277,654, each of which is incorporated herein by reference in their entireties). The kill switch is intended to actively kill genetically engineered bacteria in response to external stimuli. As opposed to an auxotrophic mutation where bacteria die because they lack an essential nutrient for survival, the kill switch is triggered by a particular factor in the environment that induces the production of toxic molecules within the microbe that cause cell death.

[0258] Bacteria comprising kill switches have been engineered for *in vitro* research purposes, e.g., to limit the spread of a biofuel-producing microorganism outside of a laboratory environment. Bacteria engineered for *in vivo* administration to treat a disease may also be programmed to die at a specific time after the expression and delivery of a heterologous gene or genes, for example, an anti-inflammation and/or gut barrier enhancer molecule, or after the subject has experienced the therapeutic effect. For example, in some embodiments, the kill switch is activated to kill the bacteria after a period of time following expression of the anti-inflammation and/or gut barrier enhancer

molecule, e.g., GLP-1. In some embodiments, the kill switch is activated in a delayed fashion following expression of the anti-inflammation and/or gut barrier enhancer molecule. Alternatively, the bacteria may be engineered to die after the bacterium has spread outside of a disease site. Specifically, it may be useful to prevent long-term colonization of subjects by the microorganism, spread of the microorganism outside the area of interest (for example, outside the gut) within the subject, or spread of the microorganism outside of the subject into the environment (for example, spread to the environment through the stool of the subject). Examples of such toxins that can be used in kill-switches include, but are not limited to, bacteriocins, lysins, and other molecules that cause cell death by lysing cell membranes, degrading cellular DNA, or other mechanisms. Such toxins can be used individually or in combination. The switches that control their production can be based on, for example, transcriptional activation (toggle switches; *see, e.g.,* Gardner et al., 2000), translation (riboregulators), or DNA recombination (recombinase-based switches), and can sense environmental stimuli such as anaerobiosis or reactive oxygen species. These switches can be activated by a single environmental factor or may require several activators in AND, OR, NAND and NOR logic configurations to induce cell death. For example, an AND riboregulator switch is activated by tetracycline, isopropyl β-D-1-thiogalactopyranoside (IPTG), and arabinose to induce the expression of lysins, which permeabilize the cell membrane and kill the cell. IPTG induces the expression of the endolysin and holin mRNAs, which are then derepressed by the addition of arabinose and tetracycline. All three inducers must be present to cause cell death. Examples of kill switches are known in the art (Callura et al., 2010).

[0259] Kill-switches can be designed such that a toxin is produced in response to an environmental condition or external signal (e.g., the bacteria is killed in response to an external cue) or, alternatively designed such that a toxin is produced once an environmental condition no longer exists or an external signal is ceased.

[0260] Thus, in some embodiments, the genetically engineered bacteria of the disclosure are further programmed to die after sensing an exogenous environmental signal, for example, in low-oxygen conditions, in the presence of ROS, or in the presence

of RNS. In some embodiments, the genetically engineered bacteria of the present disclosure comprise one or more genes encoding one or more recombinase(s), whose expression is induced in response to an environmental condition or signal and causes one or more recombination events that ultimately leads to the expression of a toxin which kills the cell. In some embodiments, the at least one recombination event is the flipping of an inverted heterologous gene encoding a bacterial toxin which is then constitutively expressed after it is flipped by the first recombinase. In one embodiment, constitutive expression of the bacterial toxin kills the genetically engineered bacterium. In these types of kill-switch systems once the engineered bacterial cell senses the exogenous environmental condition and expresses the heterologous gene of interest, the recombinant bacterial cell is no longer viable.

[0261] In another embodiment in which the genetically engineered bacteria of the present disclosure express one or more recombinase(s) in response to an environmental condition or signal causing at least one recombination event, the genetically engineered bacterium further expresses a heterologous gene encoding an anti-toxin in response to an exogenous environmental condition or signal. In one embodiment, the at least one recombination event is flipping of an inverted heterologous gene encoding a bacterial toxin by a first recombinase. In one embodiment, the inverted heterologous gene encoding the bacterial toxin is located between a first forward recombinase recognition sequence and a first reverse recombinase recognition sequence. In one embodiment, the heterologous gene encoding the bacterial toxin is constitutively expressed after it is flipped by the first recombinase. In one embodiment, the anti-toxin inhibits the activity of the toxin, thereby delaying death of the genetically engineered bacterium. In one embodiment, the genetically engineered bacterium is killed by the bacterial toxin when the heterologous gene encoding the anti-toxin is no longer expressed when the exogenous environmental condition is no longer present.

[0262] In another embodiment, the at least one recombination event is flipping of an inverted heterologous gene encoding a second recombinase by a first recombinase, followed by the flipping of an inverted heterologous gene encoding a bacterial toxin by

the second recombinase. In one embodiment, the inverted heterologous gene encoding the second recombinase is located between a first forward recombinase recognition sequence and a first reverse recombinase recognition sequence. In one embodiment, the inverted heterologous gene encoding the bacterial toxin is located between a second forward recombinase recognition sequence and a second reverse recombinase recognition sequence. In one embodiment, the heterologous gene encoding the second recombinase is constitutively expressed after it is flipped by the first recombinase. In one embodiment, the heterologous gene encoding the bacterial toxin is constitutively expressed after it is flipped by the second recombinase. In one embodiment, the genetically engineered bacterium is killed by the bacterial toxin. In one embodiment, the genetically engineered bacterium further expresses a heterologous gene encoding an anti-toxin in response to the exogenous environmental condition. In one embodiment, the anti-toxin inhibits the activity of the toxin when the exogenous environmental condition is present, thereby delaying death of the genetically engineered bacterium. In one embodiment, the genetically engineered bacterium is killed by the bacterial toxin when the heterologous gene encoding the anti-toxin is no longer expressed when the exogenous environmental condition is no longer present.

[0263] In one embodiment, the at least one recombination event is flipping of an inverted heterologous gene encoding a second recombinase by a first recombinase, followed by flipping of an inverted heterologous gene encoding a third recombinase by the second recombinase, followed by flipping of an inverted heterologous gene encoding a bacterial toxin by the third recombinase.

[0264] In one embodiment, the at least one recombination event is flipping of an inverted heterologous gene encoding a first excision enzyme by a first recombinase. In one embodiment, the inverted heterologous gene encoding the first excision enzyme is located between a first forward recombinase recognition sequence and a first reverse recombinase recognition sequence. In one embodiment, the heterologous gene encoding the first excision enzyme is constitutively expressed after it is flipped by the first recombinase. In one embodiment, the first excision enzyme excises a first essential

gene. In one embodiment, the programmed recombinant bacterial cell is not viable after the first essential gene is excised.

[0265] In one embodiment, the first recombinase further flips an inverted heterologous gene encoding a second excision enzyme. In one embodiment, the inverted heterologous gene encoding the second excision enzyme is located between a second forward recombinase recognition sequence and a second reverse recombinase recognition sequence. In one embodiment, the heterologous gene encoding the second excision enzyme is constitutively expressed after it is flipped by the first recombinase. In one embodiment, the genetically engineered bacterium dies or is no longer viable when the first essential gene and the second essential gene are both excised. In one embodiment, the genetically engineered bacterium dies or is no longer viable when either the first essential gene is excised or the second essential gene is excised by the first recombinase.

[0266] In one embodiment, the genetically engineered bacterium dies after the at least one recombination event occurs. In another embodiment, the genetically engineered bacterium is no longer viable after the at least one recombination event occurs.

[0267] In any of these embodiment, the recombinase can be a recombinase selected from the group consisting of: BxbI, PhiC31, TP901, BxbI, PhiC31, TP901, HK022, HP1, R4, Int1, Int2, Int3, Int4, Int5, Int6, Int7, Int8, Int9, Int10, Int11, Int12, Int13, Int14, Int15, Int16, Int17, Int18, Int19, Int20, Int21, Int22, Int23, Int24, Int25, Int26, Int27, Int28, Int29, Int30, Int31, Int32, Int33, and Int34, or a biologically active fragment thereof.

[0268] In the above-described kill-switch circuits, a toxin is produced in the presence of an environmental factor or signal. In another aspect of kill-switch circuitry, a toxin may be repressed in the presence of an environmental factor (not produced) and then produced once the environmental condition or external signal is no longer present. Such kill switches are called repression-based kill switches and represent systems in which the bacterial cells are viable only in the presence of an external factor or signal, such as arabinose or other sugar. Exemplary kill switch designs in which the toxin is repressed in the presence of an external factor or signal (and activated once the external

signal is removed) is shown in **Figs. 57, 60, 65**. The disclosure provides recombinant bacterial cells which express one or more heterologous gene(s) upon sensing arabinose or other sugar in the exogenous environment. In this aspect, the recombinant bacterial cells contain the *araC* gene, which encodes the AraC transcription factor, as well as one or more genes under the control of the araBAD promoter. In the absence of arabinose, the AraC transcription factor adopts a conformation that represses transcription of genes under the control of the araBAD promoter. In the presence of arabinose, the AraC transcription factor undergoes a conformational change that allows it to bind to and activate the araBAD promoter, which induces expression of the desired gene, for example *tetR*, which represses expression of a toxin gene. In this embodiment, the toxin gene is repressed in the presence of arabinose or other sugar. In an environment where arabinose is not present, the *tetR* gene is not activated and the toxin is expressed, thereby killing the bacteria. The arabinose system can also be used to express an essential gene, in which the essential gene is only expressed in the presence of arabinose or other sugar and is not expressed when arabinose or other sugar is absent from the environment.

[0269] Thus, in some embodiments in which one or more heterologous gene(s) are expressed upon sensing arabinose in the exogenous environment, the one or more heterologous genes are directly or indirectly under the control of the araBAD promoter (P_{araBAD}). In some embodiments, the expressed heterologous gene is selected from one or more of the following: a heterologous therapeutic gene, a heterologous gene encoding an anti-toxin, a heterologous gene encoding a repressor protein or polypeptide, for example, a TetR repressor, a heterologous gene encoding an essential protein not found in the bacterial cell, and/or a heterologous encoding a regulatory protein or polypeptide.

[0270] Arabinose inducible promoters are known in the art, including P_{ara}, P_{araB}, P_{araC}, and P_{araBAD}. In one embodiment, the arabinose inducible promoter is from *E. coli*. In some embodiments, the P_{araC} promoter and the P_{araBAD} promoter operate as a bidirectional promoter, with the P_{araBAD} promoter controlling expression of a heterologous gene(s) in one direction, and the P_{araC} (in close proximity to, and on the

opposite strand from the P_{araBAD} promoter), controlling expression of a heterologous gene(s) in the other direction. In the presence of arabinose, transcription of both heterologous genes from both promoters is induced. However, in the absence of arabinose, transcription of both heterologous genes from both promoters is not induced.

[0271] In one exemplary embodiment of the disclosure, the genetically engineered bacteria of the present disclosure contains a kill-switch having at least the following sequences: a P_{araBAD} promoter operably linked to a heterologous gene encoding a Tetracycline Repressor Protein (TetR), a P_{araC} promoter operably linked to a heterologous gene encoding AraC transcription factor, and a heterologous gene encoding a bacterial toxin operably linked to a promoter which is repressed by the Tetracycline Repressor Protein (P_{TetR}). In the presence of arabinose, the AraC transcription factor activates the P_{araBAD} promoter, which activates transcription of the TetR protein which, in turn, represses transcription of the toxin. In the absence of arabinose, however, AraC suppresses transcription from the the P_{araBAD} promoter and no TetR protein is expressed. In this case, expression of the heterologous toxin gene is activated, and the toxin is expressed. The toxin builds up in the recombinant bacterial cell, and the recombinant bacterial cell is killed. In one embodiment, the *araC* gene encoding the AraC transcription factor is under the control of a constitutive promoter and is therefore constitutively expressed.

[0272] In one embodiment of the disclosure, the genetically engineered bacterium further comprises an anti-toxin under the control of a constitutive promoter. In this situation, in the presence of arabinose, the toxin is not expressed due to repression by TetR protein, and the anti-toxin protein builds-up in the cell. However, in the absence of arabinose, TetR protein is not expressed, and expression of the toxin is induced. The toxin begins to build-up within the recombinant bacterial cell. The recombinant bacterial cell is no longer viable once the toxin protein is present at either equal or greater amounts than that of the anti-toxin protein in the cell, and the recombinant bacterial cell will be killed by the toxin.

[0273] In another embodiment of the disclosure, the genetically engineered bacterium further comprises an anti-toxin under the control of the P_{araBAD} promoter. In

this situation, in the presence of arabinose, TetR and the anti-toxin are expressed, the anti-toxin builds up in the cell, and the toxin is not expressed due to repression by TetR protein. However, in the absence of arabinose, both the TetR protein and the anti-toxin are not expressed, and expression of the toxin is induced. The toxin begins to build-up within the recombinant bacterial cell. The recombinant bacterial cell is no longer viable once the toxin protein is expressed, and the recombinant bacterial cell will be killed by the toxin.

[0274] In another exemplary embodiment of the disclosure, the genetically engineered bacteria of the present disclosure contains a kill-switch having at least the following sequences: a P_{araBAD} promoter operably linked to a heterologous gene encoding an essential polypeptide not found in the recombinant bacterial cell (and required for survival), and a P_{araC} promoter operably linked to a heterologous gene encoding AraC transcription factor. In the presence of arabinose, the AraC transcription factor activates the P_{araBAD} promoter, which activates transcription of the heterologous gene encoding the essential polypeptide, allowing the recombinant bacterial cell to survive. In the absence of arabinose, however, AraC suppresses transcription from the the P_{araBAD} promoter and the essential protein required for survival is not expressed. In this case, the recombinant bacterial cell dies in the absence of arabinose. In some embodiments, the sequence of P_{araBAD} promoter operably linked to a heterologous gene encoding an essential polypeptide not found in the recombinant bacterial cell can be present in the bacterial cell in conjunction with the TetR/toxin kill-switch system described directly above. In some embodiments, the sequence of P_{araBAD} promoter operably linked to a heterologous gene encoding an essential polypeptide not found in the recombinant bacterial cell can be present in the bacterial cell in conjunction with the TetR/toxin/anti-toxin kill-switch system described directly above.

[0275] In yet other embodiments, the bacteria may comprise a plasmid stability system with a plasmid that produces both a short-lived anti-toxin and a long-lived toxin. In this system, the bacterial cell produces equal amounts of toxin and anti-toxin to neutralize the toxin. However, if/when the cell loses the plasmid, the short-lived anti-

toxin begins to decay. When the anti-toxin decays completely the cell dies as a result of the longer-lived toxin killing it.

[0276] In some embodiments, the engineered bacteria of the present disclosure further comprise the gene(s) encoding the components of any of the above-described kill-switch circuits.

[0277] In any of the above-described embodiments, the bacterial toxin may be selected from the group consisting of a lysin, Hok, Fst, TisB, LdrD, Kid, SymE, MazF, FlmA, Ibs, XCV2162, dinJ, CcdB, MazF, ParE, YafO, Zeta, hicB, relB, yhaV, yoeB, chpBK, hipA, microcin B, microcin B17, microcin C, microcin C7-C51, microcin J25, microcin ColV, microcin 24, microcin L, microcin D93, microcin L, microcin E492, microcin H47, microcin I47, microcin M, colicin A, colicin E1, colicin K, colicin N, colicin U, colicin B, colicin Ia, colicin Ib, colicin 5, colicin10, colicin S4, colicin Y, colicin E2, colicin E7, colicin E8, colicin E9, colicin E3, colicin E4, colicin E6, colicin E5, colicin D, colicin M, and cloacin DF13, or a biologically active fragment thereof.

[0278] In any of the above-described embodiments, the anti-toxin may be selected from the group consisting of an anti-lysin, Sok, RNAII, IstR, RdlD, Kis, SymR, MazE, FlmB, Sib, ptaRNA1, yafQ, CcdA, MazE, ParD, yafN, Epsilon, HicA, relE, prlF, yefM, chpBI, hipB, MccE, $MccE^{CTD}$, MccF, Cai, ImmE1, Cki, Cni, Cui, Cbi, Iia, Imm, Cfi, Im10, Csi, Cyi, Im2, Im7, Im8, Im9, Im3, Im4, ImmE6, cloacin immunity protein (Cim), ImmE5, ImmD, and Cmi, or a biologically active fragment thereof.

[0279] In one embodiment, the bacterial toxin is bactericidal to the genetically engineered bacterium. In one embodiment, the bacterial toxin is bacteriostatic to the genetically engineered bacterium.

[0280] In some embodiments, the genetically engineered bacterium provided herein is an auxotroph. In one embodiment, the genetically engineered bacterium is an auxotroph selected from a *cysE, glnA, ilvD, leuB, lysA, serA, metA, glyA, hisB, ilvA, pheA, proA, thrC, trpC, tyrA, thyA, uraA, dapA, dapB, dapD, dapE, dapF, flhD, metB, metC, proAB,* and *thi1* auxotroph. In some embodiments, the engineered bacteria have more than one auxotrophy, for example, they may be a Δ*thyA* and Δ*dapA* auxotroph.

[0281] In some embodiments, the genetically engineered bacterium provided herein further comprises a kill-switch circuit, such as any of the kill-switch circuits provided herein. For example, in some embodiments, the genetically engineered bacteria further comprise one or more genes encoding one or more recombinase(s) under the control of an inducible promoter and an inverted toxin sequence. In some embodiments, the genetically engineered bacteria further comprise one or more genes encoding an anti-toxin. In some embodiments, the engineered bacteria further comprise one or more genes encoding one or more recombinase(s) under the control of an inducible promoter and one or more inverted excision genes, wherein the excision gene(s) encode an enzyme that deletes an essential gene. In some embodiments, the genetically engineered bacteria further comprise one or more genes encoding an anti-toxin. In some embodiments, the engineered bacteria further comprise one or more genes encoding a toxin under the control of a promoter having a TetR repressor binding site and a gene encoding the TetR under the control of an inducible promoter that is induced by arabinose, such as P_{araBAD}. In some embodiments, the genetically engineered bacteria further comprise one or more genes encoding an anti-toxin.

[0282] In some embodiments, the genetically engineered bacterium is an auxotroph comprising a therapeutic payload and further comprises a kill-switch circuit, such as any of the kill-switch circuits described herein.

[0283] In some embodiments of the above described genetically engineered bacteria, the gene or gene cassette for producing the anti-inflammation and/or gut barrier enhancer molecule is present on a plasmid in the bacterium and operatively linked on the plasmid to the inducible promoter. In other embodiments, the gene or gene cassette for producing the anti-inflammation and/or gut barrier enhancermolecule is present in the bacterial chromosome and is operatively linked in the chromosome to the inducible promoter.

Mutagenesis

[0284] In some embodiments, the inducible promoter is operably linked to a detectable product, e.g., GFP, and can be used to screen for mutants. In some embodiments, the inducible promoter is mutagenized, and mutants are selected based

upon the level of detectable product, e.g., by flow cytometry, fluorescence-activated cell sorting (FACS) when the detectable product fluoresces. In some embodiments, one or more transcription factor binding sites is mutagenized to increase or decrease binding. In alternate embodiments, the wild-type binding sites are left intact and the remainder of the regulatory region is subjected to mutagenesis. In some embodiments, the mutant promoter is inserted into the genetically engineered bacteria of the invention to increase expression of the anti-inflammation and/or gut barrier enhancer molecule under inducing conditions, as compared to unmutated bacteria of the same subtype under the same conditions. In some embodiments, the inducible promoter and/or corresponding transcription factor is a synthetic, non-naturally occurring sequence.

[0285] In some embodiments, the gene encoding an anti-inflammation and/or gut barrier enhancer molecule is mutated to increase expression and/or stability of said molecule under inducing conditions, as compared to unmutated bacteria of the same subtype under the same conditions. In some embodiments, one or more of the genes in a gene cassette for producing an anti-inflammation and/or gut barrier enhancer molecule is mutated to increase expression of said molecule under inducing conditions, as compared to unmutated bacteria of the same subtype under the same conditions.

Pharmaceutical compositions and formulations

[0286] Pharmaceutical compositions comprising the genetically engineered bacteria described herein may be used to inhibit inflammatory mechanisms in the gut, restore and tighten gut mucosal barrier function, and/or treat or prevent autoimmune disorders. Pharmaceutical compositions comprising one or more genetically engineered bacteria, alone or in combination with prophylactic agents, therapeutic agents, and/or pharmaceutically acceptable carriers are provided. In certain embodiments, the pharmaceutical composition comprises one species, strain, or subtype of bacteria that are engineered to comprise the genetic modifications described herein, e.g., to produce an anti-inflammation and/or gut barrier enhancer molecule. In alternate embodiments, the pharmaceutical composition comprises two or more species, strains, and/or subtypes of bacteria that are each engineered to comprise the genetic modifications described herein, e.g., to produce an anti-inflammation and/or gut barrier enhancer molecule.

[0287] The pharmaceutical compositions described herein may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into compositions for pharmaceutical use. Methods of formulating pharmaceutical compositions are known in the art (*see, e.g.,* "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA). In some embodiments, the pharmaceutical compositions are subjected to tabletting, lyophilizing, direct compression, conventional mixing, dissolving, granulating, levigating, emulsifying, encapsulating, entrapping, or spray drying to form tablets, granulates, nanoparticles, nanocapsules, microcapsules, microtablets, pellets, or powders, which may be enterically coated or uncoated. Appropriate formulation depends on the route of administration.

[0288] The genetically engineered bacteria described herein may be formulated into pharmaceutical compositions in any suitable dosage form (e.g., liquids, capsules, sachet, hard capsules, soft capsules, tablets, enteric coated tablets, suspension powders, granules, or matrix sustained release formations for oral administration) and for any suitable type of administration (e.g., oral, topical, injectable, immediate-release, pulsatile-release, delayed-release, or sustained release). Suitable dosage amounts for the genetically engineered bacteria may range from about 10^5 to 10^{12} bacteria, e.g., approximately 10^5 bacteria, approximately 10^6 bacteria, approximately 10^7 bacteria, approximately 10^8 bacteria, approximately 10^9 bacteria, approximately 10^{10} bacteria, approximately 10^{11} bacteria, or approximately 10^{11} bacteria. The composition may be administered once or more daily, weekly, or monthly. The composition may be administered before, during, or following a meal. In one embodiment, the pharmaceutical composition is administered before the subject eats a meal. In one embodiment, the pharmaceutical composition is administered currently with a meal. In one embodiment, the pharmaceutical composition is administered after the subject eats a meal.

[0289] The genetically engineered bacteria may be formulated into pharmaceutical compositions comprising one or more pharmaceutically acceptable carriers, thickeners, diluents, buffers, buffering agents, surface active agents, neutral or

cationic lipids, lipid complexes, liposomes, penetration enhancers, carrier compounds, and other pharmaceutically acceptable carriers or agents. For example, the pharmaceutical composition may include, but is not limited to, the addition of calcium bicarbonate, sodium bicarbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils, polyethylene glycols, and surfactants, including, for example, polysorbate 20. In some embodiments, the genetically engineered bacteria of the invention may be formulated in a solution of sodium bicarbonate, e.g., 1 molar solution of sodium bicarbonate (to buffer an acidic cellular environment, such as the stomach, for example). The genetically engineered bacteria may be administered and formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0290] The genetically engineered bacteria disclosed herein may be administered topically and formulated in the form of an ointment, cream, transdermal patch, lotion, gel, shampoo, spray, aerosol, solution, emulsion, or other form well known to one of skill in the art. *See, e.g.,* "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA. In an embodiment, for non-sprayable topical dosage forms, viscous to semi-solid or solid forms comprising a carrier or one or more excipients compatible with topical application and having a dynamic viscosity greater than water are employed. Suitable formulations include, but are not limited to, solutions, suspensions, emulsions, creams, ointments, powders, liniments, salves, etc., which may be sterilized or mixed with auxiliary agents (e.g., preservatives, stabilizers, wetting agents, buffers, or salts) for influencing various properties, e.g., osmotic pressure. Other suitable topical dosage forms include sprayable aerosol preparations wherein the active ingredient in combination with a solid or liquid inert carrier, is packaged in a mixture with a pressurized volatile (e.g., a gaseous propellant, such as freon) or in a squeeze bottle. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms. Examples of such additional ingredients are well known in the art. In one

embodiment, the pharmaceutical composition comprising the recombinant bacteria of the invention may be formulated as a hygiene product. For example, the hygiene product may be an antibacterial formulation, or a fermentation product such as a fermentation broth. Hygiene products may be, for example, shampoos, conditioners, creams, pastes, lotions, and lip balms.

[0291] The genetically engineered bacteria disclosed herein may be administered orally and formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, etc. Pharmacological compositions for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients include, but are not limited to, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose compositions such as maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP) or polyethylene glycol (PEG). Disintegrating agents may also be added, such as cross-linked polyvinylpyrrolidone, agar, alginic acid or a salt thereof such as sodium alginate.

[0292] Tablets or capsules can be prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone, hydroxypropyl methylcellulose, carboxymethylcellulose, polyethylene glycol, sucrose, glucose, sorbitol, starch, gum, kaolin, and tragacanth); fillers (e.g., lactose, microcrystalline cellulose, or calcium hydrogen phosphate); lubricants (e.g., calcium, aluminum, zinc, stearic acid, polyethylene glycol, sodium lauryl sulfate, starch, sodium benzoate, L-leucine, magnesium stearate, talc, or silica); disintegrants (e.g., starch, potato starch, sodium starch glycolate, sugars, cellulose derivatives, silica powders); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well known in the art. A coating shell may be present, and common membranes include, but are not limited to, polylactide, polyglycolic acid, polyanhydride, other biodegradable polymers, alginate-polylysine-alginate (APA), alginate-polymethylene-co-guanidine-alginate (A-PMCG-A), hydroymethylacrylate-methyl

methacrylate (HEMA-MMA), multilayered HEMA-MMA-MAA, polyacrylonitrilevinylchloride (PAN-PVC), acrylonitrile/sodium methallylsulfonate (AN-69), polyethylene glycol/poly pentamethylcyclopentasiloxane/polydimethylsiloxane (PEG/PD5/PDMS), poly N,N- dimethyl acrylamide (PDMAAm), siliceous encapsulates, cellulose sulphate/sodium alginate/polymethylene-co-guanidine (CS/A/PMCG), cellulose acetate phthalate, calcium alginate, k-carrageenan-locust bean gum gel beads, gellan-xanthan beads, poly(lactide-co-glycolides), carrageenan, starch poly-anhydrides, starch polymethacrylates, polyamino acids, and enteric coating polymers.

[0293] In some embodiments, the genetically engineered bacteria are enterically coated for release into the gut or a particular region of the gut, for example, the large intestine. The typical pH profile from the stomach to the colon is about 1-4 (stomach), 5.5-6.0 (duodenum), 7.3-8.0 (ileum), and 5.5-6.5 (colon). In some diseases, the pH profile may be modified. In some embodiments, the coating is degraded in specific pH environments in order to specify the site of release. In some embodiments, at least two coatings are used. In some embodiments, the outside coating and the inside coating are degraded at different pH levels.

[0294] Liquid preparations for oral administration may take the form of solutions, syrups, suspensions, or a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable agents such as suspending agents (e.g., sorbitol syrup, cellulose derivatives, or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol, or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring, and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated for slow release, controlled release, or sustained release of the genetically engineered bacteria described herein.

[0295] In one embodiment, the genetically engineered bacteria of the disclosure may be formulated in a composition suitable for administration to pediatric subjects. As is well known in the art, children differ from adults in many aspects, including different

rates of gastric emptying, pH, gastrointestinal permeability, etc. (Ivanovska et al., *Pediatrics*, 134(2):361-372, 2014). Moreover, pediatric formulation acceptability and preferences, such as route of administration and taste attributes, are critical for achieving acceptable pediatric compliance. Thus, in one embodiment, the composition suitable for administration to pediatric subjects may include easy-to-swallow or dissolvable dosage forms, or more palatable compositions, such as compositions with added flavors, sweeteners, or taste blockers. In one embodiment, a composition suitable for administration to pediatric subjects may also be suitable for administration to adults.

[0296] In one embodiment, the composition suitable for administration to pediatric subjects may include a solution, syrup, suspension, elixir, powder for reconstitution as suspension or solution, dispersible/effervescent tablet, chewable tablet, gummy candy, lollipop, freezer pop, troche, chewing gum, oral thin strip, orally disintegrating tablet, sachet, soft gelatin capsule, sprinkle oral powder, or granules. In one embodiment, the composition is a gummy candy, which is made from a gelatin base, giving the candy elasticity, desired chewy consistency, and longer shelf-life. In some embodiments, the gummy candy may also comprise sweeteners or flavors.

[0297] In one embodiment, the composition suitable for administration to pediatric subjects may include a flavor. As used herein, "flavor" is a substance (liquid or solid) that provides a distinct taste and aroma to the formulation. Flavors also help to improve the palatability of the formulation. Flavors include, but are not limited to, strawberry, vanilla, lemon, grape, bubble gum, and cherry.

[0298] In certain embodiments, the genetically engineered bacteria may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

[0299] In another embodiment, the pharmaceutical composition comprising the recombinant bacteria of the invention may be a comestible product, for example, a food product. In one embodiment, the food product is milk, concentrated milk, fermented milk (yogurt, sour milk, frozen yogurt, lactic acid bacteria-fermented beverages), milk powder, ice cream, cream cheeses, dry cheeses, soybean milk, fermented soybean milk, vegetable-fruit juices, fruit juices, sports drinks, confectionery, candies, infant foods (such as infant cakes), nutritional food products, animal feeds, or dietary supplements. In one embodiment, the food product is a fermented food, such as a fermented dairy product. In one embodiment, the fermented dairy product is yogurt. In another embodiment, the fermented dairy product is cheese, milk, cream, ice cream, milk shake, or kefir. In another embodiment, the recombinant bacteria of the invention are combined in a preparation containing other live bacterial cells intended to serve as probiotics. In another embodiment, the food product is a beverage. In one embodiment, the beverage is a fruit juice-based beverage or a beverage containing plant or herbal extracts. In another embodiment, the food product is a jelly or a pudding. Other food products suitable for administration of the recombinant bacteria of the invention are well known in the art. For example, see U.S. 2015/0359894 and US 2015/0238545, the entire contents of each of which are expressly incorporated herein by reference. In yet another embodiment, the pharmaceutical composition of the invention is injected into, sprayed onto, or sprinkled onto a food product, such as bread, yogurt, or cheese.

[0300] In some embodiments, the composition is formulated for intraintestinal administration, intrajejunal administration, intraduodenal administration, intraileal administration, gastric shunt administration, or intracolic administration, via nanoparticles, nanocapsules, microcapsules, or microtablets, which are enterically coated or uncoated. The pharmaceutical compositions may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides. The compositions may be suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain suspending, stabilizing and/or dispersing agents.

[0301] The genetically engineered bacteria described herein may be administered intranasally, formulated in an aerosol form, spray, mist, or in the form of drops, and conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant (e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas). Pressurized aerosol dosage units may be determined by providing a valve to deliver a metered amount. Capsules and cartridges (e.g., of gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0302] The genetically engineered bacteria may be administered and formulated as depot preparations. Such long acting formulations may be administered by implantation or by injection, including intravenous injection, subcutaneous injection, local injection, direct injection, or infusion. For example, the compositions may be formulated with suitable polymeric or hydrophobic materials (e.g., as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives (e.g., as a sparingly soluble salt).

[0303] In some embodiments, disclosed herein are pharmaceutically acceptable compositions in single dosage forms. Single dosage forms may be in a liquid or a solid form. Single dosage forms may be administered directly to a patient without modification or may be diluted or reconstituted prior to administration. In certain embodiments, a single dosage form may be administered in bolus form, e.g., single injection, single oral dose, including an oral dose that comprises multiple tablets, capsule, pills, etc. In alternate embodiments, a single dosage form may be administered over a period of time, e.g., by infusion.

[0304] Single dosage forms of the pharmaceutical composition may be prepared by portioning the pharmaceutical composition into smaller aliquots, single dose containers, single dose liquid forms, or single dose solid forms, such as tablets, granulates, nanoparticles, nanocapsules, microcapsules, microtablets, pellets, or powders, which may be enterically coated or uncoated. A single dose in a solid form may

be reconstituted by adding liquid, typically sterile water or saline solution, prior to administration to a patient.

[0305] In other embodiments, the composition can be delivered in a controlled release or sustained release system. In one embodiment, a pump may be used to achieve controlled or sustained release. In another embodiment, polymeric materials can be used to achieve controlled or sustained release of the therapies of the present disclosure (*see*, *e.g.,* U.S. Patent No. 5,989,463). Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N- vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. The polymer used in a sustained release formulation may be inert, free of leachable impurities, stable on storage, sterile, and biodegradable. In some embodiments, a controlled or sustained release system can be placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose. Any suitable technique known to one of skill in the art may be used.

[0306] Dosage regimens may be adjusted to provide a therapeutic response. Dosing can depend on several factors, including severity and responsiveness of the disease, route of administration, time course of treatment (days to months to years), and time to amelioration of the disease. For example, a single bolus may be administered at one time, several divided doses may be administered over a predetermined period of time, or the dose may be reduced or increased as indicated by the therapeutic situation. The specification for the dosage is dictated by the unique characteristics of the active compound and the particular therapeutic effect to be achieved. Dosage values may vary with the type and severity of the condition to be alleviated. For any particular subject, specific dosage regimens may be adjusted over time according to the individual need and the professional judgment of the treating clinician. Toxicity and therapeutic efficacy of compounds provided herein can be determined by standard pharmaceutical procedures in cell culture or animal models. For example, LD_{50}, ED_{50}, EC_{50}, and IC_{50} may be

determined, and the dose ratio between toxic and therapeutic effects (LD_{50}/ED_{50}) may be calculated as the therapeutic index. Compositions that exhibit toxic side effects may be used, with careful modifications to minimize potential damage to reduce side effects. Dosing may be estimated initially from cell culture assays and animal models. The data obtained from *in vitro* and *in vivo* assays and animal studies can be used in formulating a range of dosage for use in humans.

[0307] The ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water-free concentrate in a hermetically sealed container such as an ampoule or sachet indicating the quantity of active agent. If the mode of administration is by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0308] The pharmaceutical compositions may be packaged in a hermetically sealed container such as an ampoule or sachet indicating the quantity of the agent. In one embodiment, one or more of the pharmaceutical compositions is supplied as a dry sterilized lyophilized powder or water-free concentrate in a hermetically sealed container and can be reconstituted (e.g., with water or saline) to the appropriate concentration for administration to a subject. In an embodiment, one or more of the prophylactic or therapeutic agents or pharmaceutical compositions is supplied as a dry sterile lyophilized powder in a hermetically sealed container stored between 2 °C and 8 °C and administered within 1 hour, within 3 hours, within 5 hours, within 6 hours, within 12 hours, within 24 hours, within 48 hours, within 72 hours, or within one week after being reconstituted. Cryoprotectants can be included for a lyophilized dosage form, principally 0-10% sucrose (optimally 0.5-1.0%). Other suitable cryoprotectants include trehalose and lactose. Other suitable bulking agents include glycine and arginine, either of which can be included at a concentration of 0-0.05%, and polysorbate-80 (optimally included at a concentration of 0.005-0.01%). Additional surfactants include but are not limited to polysorbate 20 and BRIJ surfactants. The pharmaceutical composition may be prepared as an injectable solution and can further comprise an agent useful as an adjuvant, such as those used to increase absorption or dispersion, e.g., hyaluronidase.

Methods of treatment

[0309] Another aspect of the invention provides methods of treating autoimmune disorders, diarrheal diseases, IBD, related diseases, and other diseases that benefit from reduced gut inflammation and/or enhanced gut barrier function. In some embodiments, the invention provides for the use of at least one genetically engineered species, strain, or subtype of bacteria described herein for the manufacture of a medicament. In some embodiments, the invention provides for the use of at least one genetically engineered species, strain, or subtype of bacteria described herein for the manufacture of a medicament for treating autoimmune disorders, diarrheal diseases, IBD, related diseases, and other diseases that benefit from reduced gut inflammation and/or enhanced gut barrier function. In some embodiments, the invention provides at least one genetically engineered species, strain, or subtype of bacteria described herein for use in treating autoimmune disorders, diarrheal diseases, IBD, related diseases, and other diseases that benefit from reduced gut inflammation and/or enhanced gut barrier function.

[0310] In some embodiments, the diarrheal disease is selected from the group consisting of acute watery diarrhea, e.g., cholera, acute bloody diarrhea, e.g., dysentery, and persistent diarrhea. In some embodiments, the IBD or related disease is selected from the group consisting of Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, diversion colitis, Behcet's disease, intermediate colitis, short bowel syndrome, ulcerative proctitis, proctosigmoiditis, left-sided colitis, pancolitis, and fulminant colitis. In some embodiments, the disease or condition is an autoimmune disorder selected from the group consisting of acute disseminated encephalomyelitis (ADEM), acute necrotizing hemorrhagic leukoencephalitis, Addison's disease, agammaglobulinemia, alopecia areata, amyloidosis, ankylosing spondylitis, anti-GBM/anti-TBM nephritis, antiphospholipid syndrome (APS), autoimmune angioedema, autoimmune aplastic anemia, autoimmune dysautonomia, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune hyperlipidemia, autoimmune immunodeficiency, autoimmune inner ear disease (AIED), autoimmune myocarditis, autoimmune oophoritis, autoimmune pancreatitis, autoimmune retinopathy,

autoimmune thrombocytopenic purpura (ATP), autoimmune thyroid disease, autoimmune urticarial, axonal & neuronal neuropathies, Balo disease, Behcet's disease, bullous pemphigoid, cardiomyopathy, Castleman disease, celiac disease, Chagas disease, chronic inflammatory demyelinating polyneuropathy (CIDP), chronic recurrent multifocal ostomyelitis (CRMO), Churg-Strauss syndrome, cicatricial pemphigoid/benign mucosal pemphigoid, Crohn's disease, Cogan's syndrome, cold agglutinin disease, congenital heart block, Coxsackie myocarditis, CREST disease, essential mixed cryoglobulinemia, demyelinating neuropathies, dermatitis herpetiformis, dermatomyositis, Devic's disease (neuromyelitis optica), discoid lupus, Dressler's syndrome, endometriosis, eosinophilic esophagitis, eosinophilic fasciitis, erythema nodosum, experimental allergic encephalomyelitis, Evans syndrome, fibrosing alveolitis, giant cell arteritis (temporal arteritis), giant cell myocarditis, glomerulonephritis, Goodpasture's syndrome, granulomatosis with polyangiitis (GPA), Graves' disease, Guillain-Barre syndrome, Hashimoto's encephalitis, Hashimoto's thyroiditis, hemolytic anemia, Henoch-Schonlein purpura, herpes gestationis, hypogammaglobulinemia, idiopathic thrombocytopenic purpura (ITP), IgA nephropathy, IgG4-related sclerosing disease, immunoregulatory lipoproteins, inclusion body myositis, interstitial cystitis, juvenile arthritis, juvenile idiopathic arthritis, juvenile myositis, Kawasaki syndrome, Lambert-Eaton syndrome, leukocytoclastic vasculitis, lichen planus, lichen sclerosus, ligneous conjunctivitis, linear IgA disease (LAD), lupus (systemic lupus erythematosus), chronic Lyme disease, Meniere's disease, microscopic polyangiitis, mixed connective tissue disease (MCTD), Mooren's ulcer, Mucha-Habermann disease, multiple sclerosis, myasthenia gravis, myositis, narcolepsy, neuromyelitis optica (Devic's), neutropenia, ocular cicatricial pemphigoid, optic neuritis, palindromic rheumatism, PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcus), paraneoplastic cerebellar degeneration, paroxysmal nocturnal hemoglobinuria (PNH), Parry Romberg syndrome, Parsonnage-Turner syndrome, pars planitis (peripheral uveitis), pemphigus, peripheral neuropathy, perivenous encephalomyelitis, pernicious anemia, POEMS syndrome, polyarteritis nodosa, type I, II, & III autoimmune polyglandular syndromes, polymyalgia rheumatic, polymyositis, postmyocardial infarction syndrome, postpericardiotomy

syndrome, progesterone dermatitis, primary biliary cirrhosis, primary sclerosing cholangitis, psoriasis, psoriatic arthritis, idiopathic pulmonary fibrosis, pyoderma gangrenosum, pure red cell aplasia, Raynaud's phenomenon, reactive arthritis, reflex sympathetic dystrophy, Reiter's syndrome, relapsing polychondritis, restless legs syndrome, retroperitoneal fibrosis, rheumatic fever, rheumatoid arthritis, sarcoidosis, Schmidt syndrome, scleritis, scleroderma, Sjogren's syndrome, sperm & testicular autoimmunity, stiff person syndrome, subacute bacterial endocarditis (SBE), Susac's syndrome, sympathetic ophthalmia, Takayasu's arteritis, temporal arteritis/giant cell arteritis, thrombocytopenic purpura (TTP), Tolosa-Hunt syndrome, transverse myelitis, type 1 diabetes, asthma, ulcerative colitis, undifferentiated connective tissue disease (UCTD), uveitis, vasculitis, vesiculobullous dermatosis, vitiligo, and Wegener's granulomatosis. In some embodiments, the invention provides methods for reducing, ameliorating, or eliminating one or more symptom(s) associated with these diseases, including but not limited to diarrhea, bloody stool, mouth sores, perianal disease, abdominal pain, abdominal cramping, fever, fatigue, weight loss, iron deficiency, anemia, appetite loss, weight loss, anorexia, delayed growth, delayed pubertal development, and inflammation of the skin, eyes, joints, liver, and bile ducts. In some embodiments, the invention provides methods for reducing gut inflammation and/or enhancing gut barrier function, thereby ameliorating or preventing a systemic autoimmune disorder, e.g., asthma (Arrieta et al., 2015).

[0311] The method may comprise preparing a pharmaceutical composition with at least one genetically engineered species, strain, or subtype of bacteria described herein, and administering the pharmaceutical composition to a subject in a therapeutically effective amount. In some embodiments, the genetically engineered bacteria of the invention are administered orally in a liquid suspension. In some embodiments, the genetically engineered bacteria of the invention are lyophilized in a gel cap and administered orally. In some embodiments, the genetically engineered bacteria of the invention are administered via a feeding tube. In some embodiments, the genetically engineered bacteria of the invention are administered rectally, e.g., by enema. In some embodiments, the genetically engineered bacteria of the invention are

administered topically, intraintestinally, intrajejunally, intraduodenally, intraileally, and/or intracolically.

[0312] In certain embodiments, the pharmaceutical composition described herein is administered to reduce gut inflammation, enhance gut barrier function, and/or treat or prevent an autoimmune disorder in a subject. In some embodiments, the methods of the present disclosure may reduce gut inflammation in a subject by at least about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or more as compared to levels in an untreated or control subject. In some embodiments, the methods of the present disclosure may enhance gut barrier function in a subject by at least about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or more as compared to levels in an untreated or control subject. In some embodiments, changes in inflammation and/or gut barrier function are measured by comparing a subject before and after administration of the pharmaceutical composition. In some embodiments, the method of treating or ameliorating the autoimmune disorder and/or the disease or condition associated with gut inflammation and/or compromised gut barrier function allows one or more symptoms of the disease or condition to improve by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more.

[0313] Before, during, and after the administration of the pharmaceutical composition, gut inflammation and/or barrier function in the subject may be measured in a biological sample, such as blood, serum, plasma, urine, fecal matter, peritoneal fluid, intestinal mucosal scrapings, a sample collected from a tissue, and/or a sample collected from the contents of one or more of the following: the stomach, duodenum, jejunum, ileum, cecum, colon, rectum, and anal canal. In some embodiments, the methods may include administration of the compositions of the invention to enhance gut barrier function and/or to reduce gut inflammation to baseline levels, e.g., levels comparable to those of a healthy control, in a subject. In some embodiments, the methods may include administration of the compositions of the invention to reduce gut inflammation to undetectable levels in a subject, or to less than about 1%, 2%, 5%, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, or 80% of the subject's levels prior to treatment. In some embodiments, the methods may include administration of the compositions of the

invention to enhance gut barrier function in a subject by about 1%, 2%, 5%, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, 100% or more of the subject's levels prior to treatment.

[0314] In certain embodiments, the genetically engineered bacteria are *E. coli* Nissle. The genetically engineered bacteria may be destroyed, e.g., by defense factors in the gut or blood serum (Sonnenborn et al., 2009) or by activation of a kill switch, several hours or days after administration. Thus, the pharmaceutical composition comprising the genetically engineered bacteria may be re-administered at a therapeutically effective dose and frequency. In alternate embodiments, the genetically engineered bacteria are not destroyed within hours or days after administration and may propagate and colonize the gut.

[0315] The pharmaceutical composition may be administered alone or in combination with one or more additional therapeutic agents, e.g., corticosteroids, aminosalicylates, anti-inflammatory agents. In some embodiments, the pharmaceutical composition is administered in conjunction with an anti-inflammatory drug (e.g., mesalazine, prednisolone, methylprednisolone, butesonide), an immunosuppressive drug (e.g., azathioprine, 6-mercaptopurine, methotrexate, cyclosporine, tacrolimus), an antibiotic (e.g., metronidazole, ornidazole, clarithromycin, rifaximin, ciprofloxacin, anti-TB), other probiotics, and/or biological agents (e.g., infliximab, adalimumab, certolizumab pegol) (Triantafillidis et al., 2011). An important consideration in the selection of the one or more additional therapeutic agents is that the agent(s) should be compatible with the genetically engineered bacteria of the invention, e.g., the agent(s) must not kill the bacteria. The dosage of the pharmaceutical composition and the frequency of administration may be selected based on the severity of the symptoms and the progression of the disorder. The appropriate therapeutically effective dose and/or frequency of administration can be selected by a treating clinician.

Treatment *in vivo*

[0316] The genetically engineered bacteria of the invention may be evaluated *in vivo,* e.g., in an animal model. Any suitable animal model of a disease or condition associated with gut inflammation, compromised gut barrier function, and/or an

autoimmune disorder may be used (*see, e.g.,* Mizoguchi, 2012). The animal model may be a mouse model of IBD, e.g., a $CD45RB^{Hi}$ T cell transfer model or a dextran sodium sulfate (DSS) model. The animal model may be a mouse model of type 1 diabetes (T1D), and T1D may be induced by treatment with streptozotocin.

[0317] Colitis is characterized by inflammation of the inner lining of the colon, and is one form of IBD. In mice, modeling colitis often involves the aberrant expression of T cells and/or cytokines. One exemplary mouse model of IBD can be generated by sorting CD4+ T cells according to their levels of CD45RB expression, and adoptively transferring CD4+ T cells with high CD45RB expression from normal donor mice into immunodeficient mice. Non-limiting examples of immunodeficient mice that may be used for transfer include severe combined immunodeficient (SCID) mice (Morrissey et al., 1993; Powrie et al., 1993), and recombination activating gene 2 (RAG2)-deficient mice (Corazza et al., 1999). The transfer of $CD45RB^{Hi}$ T cells into immunodeficient mice, e.g., via intravenous or intraperitoneal injection, results in epithelial cell hyperplasia, tissue damage, and severe mononuclear cell infiltration within the colon (Byrne et al., 2005; Dohi et al., 2004; Wei et al., 2005). In some embodiments, the genetically engineered bacteria of the invention may be evaluated in a $CD45RB^{Hi}$ T cell transfer mouse model of IBD.

[0318] Another exemplary animal model of IBD can be generated by supplementing the drinking water of mice with dextran sodium sulfate (DSS) (Martinez et al., 2006; Okayasu et al., 1990; Whittem et al., 2010). Treatment with DSS results in epithelial damage and robust inflammation in the colon lasting several days. Single treatments may be used to model acute injury, or acute injury followed by repair. Mice treated acutely show signs of acute colitis, including bloody stool, rectal bleeding, diarrhea, and weight loss (Okayasu et al., 1990). In contrast, repeat administration cycles of DSS may be used to model chronic inflammatory disease. Mice that develop chronic colitis exhibit signs of colonic mucosal regeneration, such as dysplasia, lymphoid follicle formation, and shortening of the large intestine (Okayasu et al., 1990). In some embodiments, the genetically engineered bacteria of the invention may be evaluated in a DSS mouse model of IBD.

[0319] In some embodiments, the genetically engineered bacteria of the invention is administered to the animal, e.g., by oral gavage, and treatment efficacy is determined, e.g., by endoscopy, colon translucency, fibrin attachment, mucosal and vascular pathology, and/or stool characteristics. In some embodiments, the animal is sacrificed, and tissue samples are collected and analyzed, e.g., colonic sections are fixed and scored for inflammation and ulceration, and/or homogenized and analyzed for myeloperoxidase activity and cytokine levels (e.g., IL-1β, TNF-α, IL-6, IFN-γ and IL-10).

References

Aboulnaga et al. Effect of an oxygen-tolerant bifurcating butyryl coenzyme A dehydrogenase/ electron-transferring flavoprotein complex from Clostridium difficile on butyrate production in Escherichia coli. J Bact. 2013;195(16):3704-13. PMID: 23772070.

Ahmad et al. scFv antibody: principles and clinical application. Clin Dev Immunol. 2012;2012:980250. PMID: 22474489.

Alavi et al. Treatment of inflammatory bowel disease in a rodent model with the intestinal growth factor glucagon-like peptide-2. J Pediatr Surg. 2000 Jun;35(6):847-51. PMID: 10873024.

Albiniak et al. High-level secretion of a recombinant protein to the culture medium with a Bacillus subtilis twin-arginine translocation system in Escherichia coli. FEBS J. 2013;280(16):3810-21. PMID: 23745597.

Altenhoefer et al. The probiotic Escherichia coli strain Nissle 1917 interferes with invasion of human intestinal epithelial cells by different enteroinvasive bacterial pathogens. FEMS Immunol Med Microbiol. 2004 Apr 9;40(3):223-9. PMID: 15039098.

Alvarado et al. Targeting the Broadly Pathogenic Kynurenine Pathway. 2015. Sandeep, ed. Springer International Publishing: Switzerland.

Appleyard CB, Wallace JL. Reactivation of hapten-induced colitis and its prevention by anti-inflammatory drugs. Am J Physiol. 1995 Jul;269(1 Pt 1):G119-25. PMID: 7631788.

Arai et al. Expression of the nir and nor genes for denitrification of Pseudomonas aeruginosa requires a novel CRP/FNR-related transcriptional regulator, DNR, in addition to ANR. FEBS Lett. 1995 Aug 28;371(1):73-6. PMID: 7664887.

Arrieta et al. Early infancy microbial and metabolic alterations affect risk of childhood asthma. Sci Transl Med. 2015 Sep 30;7(307):307ra152. PMID: 26424567.

Arthur et al. Intestinal inflammation targets cancer-inducing activity of the microbiota. Science. 2012 Oct 5;338(6103):120-3. PMID: 22903521.

Atarashi et al. Induction of colonic regulatory T cells by indigenous Clostridium species. Science. 2011 Jan 21;331(6015):337-41. PMID: 21205640.

Boirivant et al. Oxazolone colitis: a murine model of T helper cell type 2 colitis treatable with antibodies to interleukin 4. J Exp Med. 1998 Nov 16;188(10):1929-39. PMID: 9815270.

Bramhall et al. Quality of methods reporting in animal models of colitis. Inflamm Bowel Dis. 2015 Jun;21(6):1248-59. PMID: 25989337.
Byrne et al. CD4+CD45RBHi T cell transfer induced colitis in mice is accompanied by osteopenia which is treatable with recombinant human osteoprotegerin. Gut. 2005 Jan;54(1):78-86. PMID: 15591508.
Callura et al. Tracking, Tuning and terminating microbial physiology using synthetic riboregulators. Proc Natl Acad Sci. 2010;27(36):15898-903. PMID: 20713708.
Castiglione et al. The transcription factor DNR from Pseudomonas aeruginosa specifically requires nitric oxide and haem for the activation of a target promoter in Escherichia coli. Microbiology. 2009 Sep;155(Pt 9):2838-44. PMID: 19477902.
Chassaing et al. Dextran sulfate sodium (DSS)-induced colitis in mice. Curr Protoc Immunol. 2014 Feb 4;104:Unit 15.25. PMID: 24510619.
Chassaing et al. Fecal lipocalin 2, a sensitive and broadly dynamic non-invasive biomarker for intestinal inflammation. PLoS One. 2012;7(9):e44328. PMID: 22957064.
Ciorba et al. Induction of IDO-1 by immunostimulatory DNA limits severity of experimental colitis. J Immunol. 2010 Apr 1;184(7):3907-16. PMID: 20181893.
Clarkson et al. Diaminopimelic acid and lysine auxotrophs of Pseudomonas aeruginosa 8602. J Gen Microbiol. 1971 May;66(2):161-9. PMID: 4999073.
Cohen et al. Biologic therapies in inflammatory bowel disease. Transl Res. 2014 Jun;163(6):533-56. PMID: 24467968.
Collinson et al. Channel crossing: how are proteins shipped across the bacterial plasma membrane? Philos Trans R Soc Lond B Biol Sci. 2015;370: 20150025. PMID: 26370937.
Corazza et al. Nonlymphocyte-derived tumor necrosis factor is required for induction of colitis in recombination activating gene (RAG)2(-/-) mice upon transfer of CD4(+)CD45RB(hi) T cells. J Exp Med. 1999 Nov 15;190(10):1479-92. PMID: 10562322.
Costa et al. Secretion systems in Gram-negative bacteria: structural and mechanistic insights. Nat Rev Microbiol. 2015;13(6):343-59. PMID: 25978706.
Cuevas-Ramos et al. Escherichia coli induces DNA damage in vivo and triggers genomic instability in mammalian cells. Proc Natl Acad Sci U S A. 2010 Jun 22;107(25):11537-42. PMID: 20534522.
Cutting. Bacillus probiotics. Food Microbiol. 2011 Apr;28(2):214-20. PMID: 21315976.
Danino et al. Programmable probiotics for detection of cancer in urine. Sci Transl Med. 2015 May 27;7(289):289ra84. PMID: 26019220.
Davis-Richardson et al. A model for the role of gut bacteria in the development of autoimmunity for type 1 diabetes. Diabetologia. 2015 Jul;58(7):1386-93. PMID: 25957231.
Dinleyici et al. Saccharomyces boulardii CNCM I-745 in different clinical conditions. Expert Opin Biol Ther. 2014 Nov;14(11):1593-609. PMID: 24995675.
Dohi et al. CD4+CD45RBHi interleukin-4 defective T cells elicit antral gastritis and duodenitis. Am J Pathol. 2004 Oct;165(4):1257-68. PMID: 15466391.
Eiglmeier et al. Molecular genetic analysis of FNR-dependent promoters. Mol Microbiol. 1989 Jul;3(7):869-78. PMID: 2677602.

Elson et al. The C3H/HeJBir mouse model: a high susceptibility phenotype for colitis. Int Rev Immunol. 2000;19(1):63-75. PMID: 10723678.
El-Zaatari et al. Tryptophan catabolism restricts IFN-γ-expressing neutrophils and Clostridium difficile immunopathology. J Immunol. 2014 Jul 15;193(2):807-16. PMID: 24935925.
Erben et al. A guide to histomorphological evaluation of intestinal inflammation in mouse models. Int J Clin Exp Pathol. 2014 Jul 15;7(8):4557-76. PMID: 25197329.
Fasano A, Shea-Donohue T. Mechanisms of disease: the role of intestinal barrier function in the pathogenesis of gastrointestinal autoimmune diseases. Nat Clin Pract Gastroenterol Hepatol. 2005 Sep;2(9):416-22. PMID: 16265432.
Fasano. Leaky gut and autoimmune diseases. Clin Rev Allergy Immunol. 2012 Feb;42(1):71-8. PMID: 22109896.
Ferdinande et al. Inflamed intestinal mucosa features a specific epithelial expression pattern of indoleamine 2,3-dioxygenase. Int J Immunopathol Pharmacol. 2008 Apr-Jun;21(2):289-95. PMID: 18547472.
Forrest et al. Levels of purine, kynurenine and lipid peroxidation products in patients with inflammatory bowel disease. In: Developments in Tryptophan and Serotonin Metabolism. 2003;527:395-400. Allegri et al., ed. Springer Science + Business Media: New York.
Frenzel et al. Expression of recombinant antibodies. Front Immunol. 2013;4:217. PMID: 23908655.
Furusawa et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013;504:446-50. PMID: 24226770.
Galimand et al. Positive FNR-like control of anaerobic arginine degradation and nitrate respiration in Pseudomonas aeruginosa. J Bacteriol. 1991 Mar;173(5):1598-606. PMID: 1900277.
Gardner et al. Construction of a genetic toggle switch in *Escherichia coli*. Nature. 2000;403:339-42. PMID: 10659857.
Garrett et al. Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. Cell. 2007 Oct 5;131(1):33-45. PMID: 17923086.
Gerlach et al. Protein secretion systems and adhesins: the molecular armory of Gram-negative pathogens. Int J Med Microbiol. 2007;297:401–15. PMID: 17482513.
Ghishan et al. Epithelial transport in inflammatory bowel diseases. Inflamm Bowel Dis. 2014 Jun;20(6):1099-109. PMID: 24691115.
Gurtner et al. Inhibition of indoleamine 2,3-dioxygenase augments trinitrobenzene sulfonic acid colitis in mice. Gastroenterology. 2003 Dec;125(6):1762-73. PMID: 14724829.
Hamer et al. Review article: the role of butyrate on colonic function. Aliment Pharmacol Ther. 2008 Jan 15;27(2):104-19. PMID: 17973645.
Hammer et al. Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human beta 2m: an animal model of HLA-B27-associated human disorders. Cell. 1990 Nov 30;63(5):1099-112. PMID: 2257626.
Hasegawa et al. Activation of a consensus FNR-dependent promoter by DNR of Pseudomonas aeruginosa in response to nitrite. FEMS Microbiol Lett. 1998 Sep 15;166(2):213-7. PMID: 9770276.

Hermiston ML, Gordon JI. Inflammatory bowel disease and adenomas in mice expressing a dominant negative N-cadherin. Science. 1995 Nov 17;270(5239):1203-7. PMID: 7502046.

Hetzel et al. Acryloyl-CoA reductase from Clostridium propionicum. An enzyme complex of propionyl-CoA dehydrogenase and electron-transferring flavoprotein. Eur J Biochem. 2003 Mar;270(5):902-10. PMID: 12603323.

Hillman. Simple, rapid method for determination of propionic acid and other short-chain fatty acids in serum. Clin Chem. 1978 May;24(5):800-3. PMID: 647915.

Hoeren et al. Sequence and expression of the gene encoding the respiratory nitrous-oxide reductase from Paracoccus denitrificans. New and conserved structural and regulatory motifs. Eur J Biochem. 1993 Nov 15;218(1):49-57. PMID: 8243476.

Hristodorov et al. Recombinant H22(scFv) blocks CD64 and prevents the capture of anti-TNF monoclonal antibody. A potential strategy to enhance anti-TNF therapy. MAbs. 2014;6(5):1283-9. PMID: 25517313.

Hsu et al. Differential mechanisms in the pathogenesis of autoimmune cholangitis versus inflammatory bowel disease in interleukin-2Ralpha(-/-) mice. Hepatology. 2009 Jan;49(1):133-40. PMID: 19065673.

Ianiro et al. Fecal microbiota transplantation in inflammatory bowel disease: beyond the excitement. Medicine (Baltimore). 2014 Oct;93(19):e97. PMID: 25340496.

Isabella et al. Deep sequencing-based analysis of the anaerobic stimulon in Neisseria gonorrhoeae. BMC Genomics. 2011 Jan 20;12:51. PMID: 21251255.

Iyer SS, Cheng G. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. Crit Rev Immunol. 2012;32(1):23-63. PMID: 22428854.

Johansson et al. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Proc Natl Acad Sci USA. 2008 Sep 30;105(39):15064-9. PMID: 18806221.

Kanai et al. A breakthrough in probiotics: Clostridium butyricum regulates gut homeostasis and anti-inflammatory response in inflammatory bowel disease. J Gastroenterol. 2015 Sep;50(9):928-39. PMID: 25940150.

Keates et al. TransKingdom RNA interference: a bacterial approach to challenges in RNAi therapy and delivery. Biotechnol Genet Eng Rev. 2008;25:113-27. PMID: 21412352.

Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. Nature. 2011 Jun 15;474(7351):307-17. PMID: 21677747.

Kleman et al. Acetate metabolism by Escherichia coli in high-cell-density fermentation. Appl Environ Microbiol. 1994 Nov;60(11):3952-8. PMID: 7993084.

Lerner et al. (a) Changes in intestinal tight junction permeability associated with industrial food additives explain the rising incidence of autoimmune disease. Autoimmun Rev. 2015 Jun;14(6):479-89. PMID: 25676324.

Lerner et al. (b) Rheumatoid arthritis-celiac disease relationship: Joints get that gut feeling. Autoimmun Rev. 2015 Nov;14(11):1038-47. PMID: 26190704.

Low et al. Animal models of ulcerative colitis and their application in drug research. Drug Des Devel Ther. 2013 Nov 12;7:1341-57. PMID: 24250223.

Lukovac et al. Differential modulation by Akkermansia muciniphila and Faecalibacterium prausnitzii of host peripheral lipid metabolism and histone acetylation in mouse gut organoids. MBio. 2014 Aug 12;5(4). pii: e01438-14. PMID: 25118238.

MacPherson BR, Pfeiffer CJ. Experimental production of diffuse colitis in rats. Digestion. 1978;17(2):135-50. PMID: 627326.
Martinez et al. Deletion of Mtgr1 sensitizes the colonic epithelium to dextran sodium sulfate-induced colitis. Gastroenterology. 2006 Aug;131(2):579-88. PMID: 16890610.
Matteoli et al. Gut CD103+ dendritic cells express indoleamine 2,3-dioxygenase which influences T regulatory/T effector cell balance and oral tolerance induction. Gut. 2010 May;59(5):595-604. PMID: 20427394.
Meadow et al. Biosynthesis of diaminopimelic acid and lysine in Escherichia coli. Biochem J. 1959 Jul;72(3):396-400. PMID: 16748796.
Mizoguchi. Animal models of inflammatory bowel disease. Prog Mol Biol Transl Sci. 2012;105:263-320. PMID: 22137435.
Mombaerts et al. Spontaneous development of inflammatory bowel disease in T cell receptor mutant mice. Cell. 1993 Oct 22;75(2):274-82. PMID: 8104709.
Moolenbeek C, Ruitenberg EJ. The "Swiss roll": a simple technique for histological studies of the rodent intestine. Lab Anim. 1981 Jan;15(1):57-9. PMID: 7022018.
Moore et al. Regulation of FNR dimerization by subunit charge repulsion. J Biol Chem. 2006 Nov 3;281(44):33268-75. PMID: 16959764.
Morrissey et al. CD4+ T cells that express high levels of CD45RB induce wasting disease when transferred into congenic severe combined immunodeficient mice. Disease development is prevented by cotransfer of purified CD4+ T cells. J Exp Med. 1993 Jul 1;178(1):237-44. PMID: 8100269.
Mourelle et al. Polyunsaturated phosphatidylcholine prevents stricture formation in a rat model of colitis. Gastroenterology. 1996 Apr;110(4):1093-7. PMID: 8612998.
Nguyen et al. Lymphocyte-dependent and Th2 cytokine-associated colitis in mice deficient in Wiskott-Aldrich syndrome protein. Gastroenterology. 2007 Oct;133(4):1188-97. PMID: 17764675.
Nielsen. New strategies for treatment of inflammatory bowel disease. Front Med (Lausanne). 2014;1:3. PMID: 25685754.
Nougayrede et al. Escherichia coli induces DNA double-strand breaks in eukaryotic cells. Science. 2006 Aug 11;313(5788):848-51. PMID: 16902142.
Ohman et al. Regression of Peyer's patches in G alpha i2 deficient mice prior to colitis is associated with reduced expression of Bcl-2 and increased apoptosis. Gut. 2002 Sep;51(3):392-7. PMID: 12171962.
Okayasu et al. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenterology. 1990 Mar;98(3):694-702. PMID: 1688816.
Olier et al. Genotoxicity of Escherichia coli Nissle 1917 strain cannot be dissociated from its probiotic activity. Gut Microbes. 2012 Nov-Dec;3(6):501-9. PMID: 22895085.
Ostanin et al. T cell transfer model of chronic colitis: concepts, considerations, and tricks of the trade. Am J Physiol Gastrointest Liver Physiol. 2009 Feb;296(2):G135-46. PMID: 19033538.
Paun et al. Immuno-ecology: how the microbiome regulates tolerance and autoimmunity. Curr Opin Immunol. 2015 Oct 10;37:34-9. PMID: 26460968.

Pizarro et al. SAMP1/YitFc mouse strain: a spontaneous model of Crohn's disease-like ileitis. Inflamm Bowel Dis. 2011 Dec;17(12):2566-84. PMID: 21557393.
Powrie et al. Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. Int Immunol. 1993 Nov;5(11):1461-71. PMID: 7903159.
Pugsley. The complete general secretory pathway in gram-negative bacteria. Microbiol Rev. 1993 Mar;57(1):50-108. PMID: 8096622.
Purcell et al. Towards a whole-cell modeling approach for synthetic biology. Chaos. 2013 Jun;23(2):025112. PMID: 23822510.
Ragsdale. Enzymology of the wood-Ljungdahl pathway of acetogenesis. Ann N Y Acad Sci. 2008 Mar;1125:129-36. PMID: 18378591.
Ray et al. The effects of mutation of the anr gene on the aerobic respiratory chain of Pseudomonas aeruginosa. FEMS Microbiol Lett. 1997 Nov 15;156(2):227-32. PMID: 9513270.
Reeves et al. Engineering Escherichia coli into a protein delivery system for mammalian cells. ACS Synth Biol. 2015;4(5):644-54. PMID: 25853840.
Reister et al. Complete genome sequence of the Gram-negative probiotic Escherichia coli strain Nissle 1917. J Biotechnol. 2014 Oct 10;187:106-7. PMID: 25093936.
Rembacken et al. Non-pathogenic Escherichia coli versus mesalazine for the treatment of ulcerative colitis: a randomised trial. Lancet. 1999 Aug 21;354(9179):635-9. PMID: 10466665.
Remington's Pharmaceutical Sciences, 22nd ed. Mack Publishing Co.
Rigel et al. A new twist on an old pathway – accessory Sec systems. Mol Microbiol. 2008 Jul;69(2):291-302. PMID: 18544071.
Sabiu et al. Indomethacin-induced gastric ulceration in rats: Ameliorative roles of Spondias mombin and Ficus exasperata. Pharm Biol. 2016 Jan;54(1):180-6. PMID: 25815713.
Saier. Protein secretion and membrane insertion systems in Gram-negative bacteria. J Membr Biol. 2006;214(2):75-90. PMID: 17546510.
Salmon et al. Global gene expression profiling in Escherichia coli K12. The effects of oxygen availability and FNR. J Biol Chem. 2003 Aug 8;278(32):29837-55. PMID: 12754220.
Sanz et al. Microbiota, inflammation and obesity. Adv Exp Med Biol. 2014;817:291-317. PMID: 24997040.
Sanz et al. Understanding the role of gut microbiome in metabolic disease risk. Pediatr Res. 2015 Jan;77(1-2):236-44. PMID: 25314581.
Sat et al. The Escherichia coli mazEF suicide module mediates thymineless death. J Bacteriol. 2003 Mar;185(6):1803-7. PMID: 12618443.
Satoh et al. New ulcerative colitis model induced by sulfhydryl blockers in rats and the effects of antiinflammatory drugs on the colitis. Jpn J Pharmacol. 1997 Apr;73(4):299-309. PMID: 9165366.
Sawers. Identification and molecular characterization of a transcriptional regulator from Pseudomonas aeruginosa PAO1 exhibiting structural and functional similarity to the FNR protein of Escherichia coli. Mol Microbiol. 1991 Jun;5(6):1469-81. PMID: 1787797.

Schiel-Bengelsdorf et al. Pathway engineering and synthetic biology using acetogens. FEBS Lett. 2012 Jul 16;586(15):2191-8. PMID: 22710156.
Schultz. Clinical use of E. coli Nissle 1917 in inflammatory bowel disease. Inflamm Bowel Dis. 2008 Jul;14(7):1012-8. Review. PMID: 18240278.
Seguí et al. Superoxide dismutase ameliorates TNBS-induced colitis by reducing oxidative stress, adhesion molecule expression, and leukocyte recruitment into the inflamed intestine. J Leukoc Biol. 2004 Sep;76(3):537-44. PMID: 15197232.
Selmer et al. Propionate CoA-transferase from Clostridium propionicum. Cloning of gene and identification of glutamate 324 at the active site. Eur J Biochem. 2002 Jan;269(1):372-80. PMID: 11784332.
Simpson et al. IBD: microbiota manipulation through diet and modified bacteria. Dig Dis. 2014;32 Suppl 1:18-25. PMID: 25531349.
Smith et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science. 2013 Aug 2;341(6145):569-73. PMID: 23828891.
Sonnenborn et al. The non-pathogenic Escherichia coli strain Nissle 1917 – features of a versatile probiotic. Microbial Ecology in Health and Disease. 2009;21:122-58.
Stanley et al. Acute infection and macrophage subversion by Mycobacterium tuberculosis require a specialized secretion system. Proc Natl Acad Sci USA. 2003 Oct;100(22):13001-6. PMID: 14557536.
Sugimoto et al. IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. J Clin Invest. 2008 Feb;118(2):534-44. PMID: 18172556.
Triantafillidis et al. Current and emerging drugs for the treatment of inflammatory bowel disease. Drug Des Devel Ther 5.5 (2011): 185-210.
Trunk et al. Anaerobic adaptation in Pseudomonas aeruginosa: definition of the Anr and Dnr regulons. Environ Microbiol. 2010 Jun;12(6):1719-33. PMID: 20553552.
Tseng et al. Controlled biosynthesis of odd-chain fuels and chemicals via engineered modular metabolic pathways. Proc Natl Acad Sci U S A. 2012 Oct 30;109(44):17925-30. PMID: 23071297.
Turski et al. Kynurenic Acid in the digestive system-new facts, new challenges. Int J Tryptophan Res. 2013 Sep 4;6:47-55. PMID: 24049450.
Ukena et al. Probiotic Escherichia coli Nissle 1917 inhibits leaky gut by enhancing mucosal integrity. PLoS One. 2007 Dec 12;2(12):e1308. PMID: 18074031.
Unden et al. Alternative respiratory pathways of Escherichia coli: energetics and transcriptional regulation in response to electron acceptors. Biochim Biophys Acta. 1997 Jul 4;1320(3):217-34. PMID: 9230919.
Varga et al. N-Methyl-D-aspartate receptor antagonism decreases motility and inflammatory activation in the early phase of acute experimental colitis in the rat. Neurogastroenterol Motil. 2010 Feb;22(2):217-25. PMID: 19735360.
Wagner et al. Semisynthetic diet ameliorates Crohn's disease-like ileitis in TNFΔARE/WT mice through antigen-independent mechanisms of gluten. Inflamm Bowel Dis. 2013 May;19(6):1285-94. PMID: 23567784.
Watanabe et al. Interleukin 7 transgenic mice develop chronic colitis with decreased interleukin 7 protein accumulation in the colonic mucosa. J Exp Med. 1998 Feb 2;187(3):389-402. PMID: 9449719.

Wei et al. Mesenteric B cells centrally inhibit CD4+ T cell colitis through interaction with regulatory T cell subsets. Proc Natl Acad Sci USA. 2005 Feb 8;102(6):2010-15. PMID: 15684084.

Wen et al. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature. 2008 Oct 23;455(7216):1109-13. PMID: 18806780.

Whittem et al. Murine colitis modeling using dextran sulfate sodium (DSS). J Vis Exp. 2010 Jan 19;(35). PMID: 20087313.

Wilk et al. The mdr1a-/- mouse model of spontaneous colitis: a relevant and appropriate animal model to study inflammatory bowel disease. Immunol Res. 2005;31(2):151-9. PMID: 15778512.

Williams GT, Williams WJ. Granulomatous inflammation--a review. J Clin Pathol. 1983 Jul;36(7):723-733. PMID: 6345591.

Winteler et al. The homologous regulators ANR of Pseudomonas aeruginosa and FNR of Escherichia coli have overlapping but distinct specificities for anaerobically inducible promoters. Microbiology. 1996 Mar;142 (Pt 3):685-93. PMID: 8868444.

Wolf et al. Overexpression of indoleamine 2,3-dioxygenase in human inflammatory bowel disease. Clin Immunol. 2004 Oct;113(1):47-55. PMID: 15380529.

Wright et al. GeneGuard: A Modular Plasmid System Designed for Biosafety. ACS Synth Biol. 2015 Mar 20;4(3):307-16. PMID: 24847673.

Xiao et al. Nanoparticles with surface antibody against CD98 and carrying CD98 small interfering RNA reduce colitis in mice. Gastroenterology. 2014 May;146(5):1289-300. PMID: 24503126.

Yazbeck et al. Growth factor based therapies and intestinal disease: is glucagon-like peptide-2 the new way forward? Cytokine Growth Factor Rev. 2009 Apr;20(2):175-84. PMID: 19324585.

Zhang et al. Deletion of interleukin-6 in mice with the dominant negative form of transforming growth factor beta receptor II improves colitis but exacerbates autoimmune cholangitis. Hepatology. 2010 Jul;52(1):215-22. PMID: 20578264.

Zimmermann et al. Anaerobic growth and cyanide synthesis of Pseudomonas aeruginosa depend on anr, a regulatory gene homologous with fnr of Escherichia coli. Mol Microbiol. 1991 Jun;5(6):1483-90. PMID: 1787798.

Examples

[0320] The following examples provide illustrative embodiments of the disclosure. One of ordinary skill in the art will recognize the numerous modifications and variations that may be performed without altering the spirit or scope of the disclosure. Such modifications and variations are encompassed within the scope of the disclosure. The Examples do not in any way limit the disclosure.

Examples

[0321] The following examples provide illustrative embodiments of the disclosure. One of ordinary skill in the art will recognize the numerous modifications and variations that may be performed without altering the spirit or scope of the disclosure. Such modifications and variations are encompassed within the scope of the disclosure. The Examples do not in any way limit the disclosure.

Example 1. Construction of Vectors for Producing Therapeutic Molecules

Butyrate

[0322] To facilitate inducible production of butyrate in *Escherichia coli* Nissle, the eight genes of the butyrate production pathway from *Peptoclostridium difficile* 630 (*bcd2, etfB3, etfA3, thiA1, hbd, crt2, pbt,* and *buk*; NCBI; Table 4), as well as transcriptional and translational elements, are synthesized (Gen9, Cambridge, MA) and cloned into vector pBR322. In some embodiments, the butyrate gene cassette is placed under the control of a FNR regulatory region selected from SEQ ID NOs: 55-66 (Table 9). In certain constructs, the FNR-responsive promoter is further fused to a strong ribosome binding site sequence. For efficient translation of butyrate genes, each synthetic gene in the operon was separated by a 15 base pair ribosome binding site derived from the T7 promoter/translational start site. In certain constructs, the butyrate gene cassette is placed under the control of an RNS-responsive regulatory region, e.g., *norB*, and the bacteria further comprises a gene encoding a corresponding RNS-responsive transcription factor, e.g., *nsrR* (see, e.g., Tables 10 and 11). In certain constructs, the butyrate gene cassette is placed under the control of an ROS-responsive regulatory region, e.g., *oxyS*, and the bacteria further comprises a gene encoding a corresponding ROS-responsive transcription factor, e.g., *oxyR* (see, e.g., Tables 14-17). In certain

constructs, the butyrate gene cassette is placed under the control of a tetracycline-inducible or constitutive promoter.

[0323] The gene products of the *bcd2-etfA3-etfB3* genes form a complex that converts crotonyl-CoA to butyryl-CoA and may exhibit dependence on oxygen as a co-oxidant. Because the recombinant bacteria of the invention are designed to produce butyrate in an oxygen-limited environment (e.g. the mammalian gut), that dependence on oxygen could have a negative effect of butyrate production in the gut. It has been shown that a single gene from *Treponema denticola*, trans-2-enoynl-CoA reductase (*ter,* Table 4), can functionally replace this three gene complex in an oxygen-independent manner. Therefore, a second butyrate gene cassette in which the *ter* gene replaces the *bcd2-etfA3-etfB3* genes of the first butyrate cassette is synthesized (Genewiz, Cambridge, MA). The *ter* gene is codon-optimized for *E. coli* codon usage using Integrated DNA Technologies online codon optimization tool (https://www.idtdna.com/CodonOpt). The second butyrate gene cassette, as well as transcriptional and translational elements, is synthesized (Gen9, Cambridge, MA) and cloned into vector pBR322. In certain constructs, the second butyrate gene cassette is placed under control of a FNR regulatory region as described above (Table 4). In certain constructs, the butyrate gene cassette is placed under the control of an RNS-responsive regulatory region, e.g., *norB*, and the bacteria further comprises a gene encoding a corresponding RNS-responsive transcription factor, e.g., *nsrR* (see, e.g., Tables 10 and 11). In certain constructs, the butyrate gene cassette is placed under the control of an ROS-responsive regulatory region, e.g., *oxyS*, and the bacteria further comprises a gene encoding a corresponding ROS-responsive transcription factor, e.g., *oxyR* (see, e.g., Tables 14-17). In certain constructs, the butyrate gene cassette is placed under the control of a tetracycline-inducible or constitutive promoter.

In a third butyrate gene cassette, the *pbt* and *buk* genes are replaced with *tesB* (SEQ ID NO: 10). TesB is a thioesterase found in E. Coli that cleaves off the butyrate from butyryl-coA, thus obviating the need for pbt-buk (see Fig. 2).

[0324] In one embodiment, *tesB* is placed under the control of a FNR regulatory region selected from SEQ ID NOs: 55-66 (Table 9) In an alternate embodiment, *tesB* is

placed under the control of an RNS-responsive regulatory region, e.g., *norB*, and the bacteria further comprises a gene encoding a corresponding RNS-responsive transcription factor, e.g., *nsrR* (see, e.g., Tables 10 and 11). In yet another embodiment, *tesB* is placed under the control of an ROS-responsive regulatory region, e.g., *oxyS*, and the bacteria further comprises a gene encoding a corresponding ROS-responsive transcription factor, e.g., *oxyR* (see, e.g., Tables 14-17). In certain constructs, the different described butyrate gene cassettes are each placed under the control of a tetracycline-inducible or constitutive promoter. For example, genetically engineered Nissle are generated comprising a butyrate gene cassette in which the *pbt* and *buk* genes are replaced with *tesB* (SEQ ID NO: 10) expressed under the control of a nitric oxide-responsive regulatory (SEQ ID NO: 80). SEQ ID NO: 80 comprises a reverse complement of the *nsrR* repressor gene from *Neisseria gonorrhoeae* (underlined), intergenic region containing divergent promoters controlling *nsrR* and the butyrogenic gene cassette and their respective RBS (bold), and the butyrate genes (*ter-thiA1-hbd-crt2-tesB)* separated by RBS.

SEQ ID NO: 80

ttattatcgcaccgcaatcgggattttcgattcataaagcaggtcgtaggtcggcttgtt
gagcaggtcttgcagcgtgaaaccgtccagatacgtgaaaaacgacttcattgcaccgcc
gagtatgcccgtcagccggcaggacggcgtaatcaggcattcgttgttcgggcccataca
ctcgaccagctgcatcggttcgaggtggcggacgaccgcgccgatattgatgcgttcggg
cggcgcggccagcctcagcccgccgcctttcccgcgtacgctgtgcaagaacccgccttt
gaccagcgcggtaaccactttcatcaaatggcttttggaaatgccgtaggtcgaggcgat
ggtggcgatattgaccagcgcgtcgtcgttgacggcggtgtagatgaggacgcgcagccc
gtagtcggtatgttgggtcagatacat**acaacctccttagtacatgcaaaattatttcta**
gagcaacatacgagccggaagcataaagtgtaaagcctggggtgcctaatgagttgagtt
gaggaattataacaggaagaaatattcctcatacgcttgtaattcctctatggttgttga
caattaatcatcggctcgtataatgtataacattcatattttgtgaattttaaactctag
aaataattttgtttaactttaagaaggagatatacatatgatcgtaaaacctatggtacg
caacaatatctgcctgaacgcccatcctcagggctgcaagaagggagtggaagatcagat
tgaatataccaagaaacgcattaccgcagaagtcaaagctggcgcaaaagctccaaaaaa
cgttctggtgcttggctgctcaaatggttacggcctggcgagccgcattactgctgcgtt
cggatacggggctgcgaccatcggcgtgtcctttgaaaaagcgggttcagaaaccaaata
tggtacaccgggatggtacaataatttggcatttgatgaagcggcaaaacgcgagggtct
ttatagcgtgacgatcgacggcgatgcgttttcagacgagatcaaggcccaggtaattga
ggaagccaaaaaaaaaggtatcaaatttgatctgatcgtatacagcttggccagcccagt
acgtactgatcctgatacaggtatcatgcacaaaagcgttttgaaacccttggaaaaac
gttcacaggcaaaacagtagatccgtttactggcgagctgaaggaaatctccgcggaacc
agcaaatgacgaggaagcagccgccactgttaaagttatgggggtgaagattgggaacg
ttggattaagcagctgtcgaaggaaggcctcttagaagaaggctgtattaccttggccta

tagttatattggccctgaagctacccaagctttgtaccgtaaaggcacaatcggcaaggc
caaagaacacctggaggccacagcacaccgtctcaacaaagagaacccgtcaatccgtgc
cttcgtgagcgtgaataaaggcctggtaacccgcgcaagcgccgtaatcccggtaatccc
tctgtatctcgccagcttgttcaaagtaatgaaagagaagggcaatcatgaaggttgtat
tgaacagatcacgcgtctgtacgccgagcgcctgtaccgtaaagatggtacaattccagt
tgatgaggaaaatcgcattcgcattgatgattgggagttagaagaagacgtccagaaagc
ggtatccgcgttgatggagaaagtcacgggtgaaaacgcagaatctctcactgacttagc
ggggtaccgccatgatttcttagctagtaacggctttgatgtagaaggtattaattatga
agcggaagttgaacgcttcgaccgtatctgataagaaggagatatacatatgagagaagt
agtaattgccagtgcagctagaacagcagtaggaagttttggaggagcatttaaatcagt
ttcagcggtagagttaggggtaacagcagctaaagaagctataaaaagagctaacataac
tccagatatgatagatgaatctcttttaggggagtacttacagcaggtcttggacaaaa
tatagcaagacaaatagcattaggagcaggaataccagtagaaaaaccagctatgactat
aaatatagtttgtggttctggattaagatctgtttcaatggcatctcaacttatagcatt
aggtgatgctgatataatgttagttggtggagctgaaaacatgagtatgtctccttattt
agtaccaagtgcgagatatggtgcaagaatgggtgatgctgcttttgttgattcaatgat
aaaagatggattatcagacatatttaataactatcacatgggtattactgctgaaaacat
agcagagcaatggaatataactagagaagaacaagatgaattagctcttgcaagtcaaaa
taaagctgaaaaagctcaagctgaaggaaaatttgatgaagaaatagttcctgttgttat
aaaaggaagaaaaggtgacactgtagtagataaagatgaatatattaagcctggcactac
aatggagaaacttgctaagttaagacctgcatttaaaaaagatggaacagttactgctgg
taatgcatcaggaataaatgatggtgctgctatgttagtagtaatggctaaagaaaaagc
tgaagaactaggaatagagcctcttgcaactatagtttcttatggaacagctggtgttga
ccctaaaataatgggatatggaccagttccagcaactaaaaaagctttagaagctgctaa
tatgactattgaagatatagatttagttgaagctaatgaggcatttgctgcccaatctgt
agctgtaataagagacttaaatatagatatgaataaagttaatgttaatggtggagcaat
agctataggacatccaataggatgctcaggagcaagaatacttactacacttttatatga
aatgaagagaagagatgctaaaactggtcttgctacactttgtataggcggtggaatggg
aactactttaatagttaagagatagtaagaaggagatatacatatgaaattagctgtaat
aggtagtggaactatgggaagtggtattgtacaaacttttgcaagttgtggacatgatgt
atgtttaaagagtagaactcaaggtgctatagataaatgtttagctttattagataaaaa
tttaactaagttagttactaagggaaaaatggatgaagctacaaaagcagaaatattaag
tcatgttagttcaactactaattatgaagatttaaaagatatggatttaataatagaagc
atctgtagaagacatgaatataaagaaagatgttttcaagttactagatgaattatgtaa
agaagatactatcttggcaacaaatacttcatcattatctataacagaaatagcttcttc
tactaagcgcccagataaagttataggaatgcatttctttaatccagttcctatgatgaa
attagttgaagttataagtggtcagttaacatcaaaagttacttttgatacagtatttga
attatctaagagtatcaataaagtaccagtagatgtatctgaatctcctggatttgtagt
aaatagaatacttatacctatgataaatgaagctgttggtatatatgcagatggtgttgc
aagtaaagaagaaatagatgaagctatgaaattaggagcaaaccatccaatgggaccact
agcattaggtgatttaatcggattagatgttgttttagctataatgaacgttttatatac
tgaatttggagatactaaatatagacctcatccacttttagctaaaatggttagagctaa
tcaattaggaagaaaaactaagataggattctatgattataataaataataagaaggaga
tatacatatgagtacaagtgatgttaaagtttatgagaatgtagctgttgaagtagatgg
aaatatatgtacagtgaaaatgaatagacctaaagcccttaatgcaataaattcaaagac
tttagaagaactttatgaagtatttgtagatattaataatgatgaaactattgatgttgt
aatattgacaggggaaggaaaggcatttgtagctggagcagatattgcatacatgaaaga
tttagatgctgtagctgctaaagattttagtatcttaggagcaaaagcttttggagaaat
agaaaatagtaaaaaagtagtgatagctgctgtaaacggatttgctttaggtggaggatg

tgaacttgcaatggcatgtgatataagaattgcatctgctaaagctaaatttggtcagcc
agaagtaactcttggaataactccaggatatggaggaactcaaaggcttacaagattggt
tggaatggcaaaagcaaaagaattaatctttacaggtcaagttataaaagctgatgaagc
tgaaaaaatagggctagtaaatagagtcgttgagccagacattttaatagaagaagttga
gaaattagctaagataatagctaaaaatgctcagcttgcagttagatactctaaagaagc
aatacaacttggtgctcaaactgatataaatactggaatagatatagaatctaatttatt
tggtctttgtttttcaactaaagaccaaaaagaaggaatgtcagctttcgttgaaaagag
agaagctaactttataaaagggtaataagaaggagatatacatatgAGTCAGGCGCTAAA
AAATTTACTGACATTGTTAAATCTGGAAAAAATTGAGGAAGGACTCTTTCGCGGCCAGAG
TGAAGATTTAGGTTTACGCCAGGTGTTTGGCGGCCAGGTCGTGGGTCAGGCCTTGTATGC
TGCAAAAGAGACCGTCCCTGAAGAGCGGCTGGTACATTCGTTTCACAGCTACTTTCTTCG
CCCTGGCGATAGTAAGAAGCCGATTATTTATGATGTCGAAACGCTGCGTGACGGTAACAG
CTTCAGCGCCCGCCGGGTTGCTGCTATTCAAAACGGCAAACCGATTTTTTATATGACTGC
CTCTTTCCAGGCACCAGAAGCGGGTTTCGAACATCAAAAAACAATGCCGTCCGCGCCAGC
GCCTGATGGCCTCCCTTCGGAAACGCAAATCGCCCAATCGCTGGCGCACCTGCTGCCGCC
AGTGCTGAAAGATAAATTCATCTGCGATCGTCCGCTGGAAGTCCGTCCGGTGGAGTTTCA
TAACCCACTGAAAGGTCACGTCGCAGAACCACATCGTCAGGTGTGGATCCGCGCAAATGG
TAGCGTGCCGGATGACCTGCGCGTTCATCAGTATCTGCTCGGTTACGCTTCTGATCTTAA
CTTCCTGCCGGTAGCTCTACAGCCGCACGGCATCGGTTTTCTCGAACCGGGGATTCAGAT
TGCCACCATTGACCATTCCATGTGGTTCCATCGCCCGTTTAATTTGAATGAATGGCTGCT
GTATAGCGTGGAGAGCACCTCGGCGTCCAGCGCACGTGGCTTTGTGCGCGGTGAGTTTTA
TACCCAAGACGGCGTACTGGTTGCCTCGACCGTTCAGGAAGGGGTGATGCGTAATCACAA
Ttaa

Butyrate, IL-10, IL-22, GLP-2

[0325] In certain constructs, in addition to the butyrate production pathways described above, the *Escherichia coli* Nissle are further engineered to produce one or more molecules selected from IL-10, IL-2, IL-22, IL-27, SOD, kyurenine, kyurenic acid, and GLP-2 using the methods described above. In some embodiments, the bacteria comprise a gene cassette for producing butyrate as described above, and a gene encoding IL-10 (see, e.g., SEQ ID NO: 49). In some embodiments, the bacteria comprise a gene cassette for producing butyrate as described above, and a gene encoding IL-2 (see, e.g., 50). In some embodiments, the bacteria comprise a gene cassette for producing butyrate as described above, and a gene encoding IL-22 (see, e.g., 51). In some embodiments, the bacteria comprise a gene cassette for producing butyrate as described above, and a gene encoding IL-27 (see, e.g., SEQ ID NO: 52). In some embodiments, the bacteria comprise a gene cassette for producing butyrate as described above, and a gene encoding SOD (see, e.g., 53). In some embodiments, the bacteria comprise a gene cassette for producing butyrate as described above, and a gene encoding GLP-2 (see, e.g., SEQ ID NO: 54). In some embodiments, the bacteria comprise a gene cassette for producing butyrate as

described above, and a gene or gene cassette for producing kyurenine or kyurenic acid. In some embodiments, the bacteria comprise a gene cassette for producing butyrate as described above, and a gene encoding IL-10, IL-22, and GLP-2. In one embodiment, each of the genes or gene cassettes is placed under the control of a FNR regulatory region selected from SEQ ID NOs: 55-66 (Table 9). In an alternate embodiment, each of the genes or gene cassettes is placed under the control of an RNS-responsive regulatory region, e.g., *norB*, and the bacteria further comprises a gene encoding a corresponding RNS-responsive transcription factor, e.g., *nsrR* (see, e.g., Tables 10 and 11). In yet another embodiment, each of the genes or gene cassettes is placed under the control of an ROS-responsive regulatory region, e.g., *oxyS*, and the bacteria further comprises a gene encoding a corresponding ROS-responsive transcription factor, e.g., *oxyR* (see, e.g., Tables 14-17). In certain constructs, one or more of the genes is placed under the control of a tetracycline-inducible or constitutive promoter.

Butyrate, Propionate, IL-10, IL-22, IL-2, IL-27

[0326] In certain constructs, in addition to the butyrate production pathways described above, the *Escherichia coli* Nissle are further engineered to produce propionate, and one or more molecules selected from IL-10, IL-2, IL-22, IL-27, SOD, kyurenine, kyurenic acid, and GLP-2 using the methods described above. In certain constructs, in addition to the butyrate production pathways described above, the *Escherichia coli* Nissle are further engineered to produce propionate, and one or more molecules selected from IL-10, IL-2, and IL-22. In certain constructs, in addition to the butyrate production pathways described above, the *Escherichia coli* Nissle are further engineered to produce propionate, and one or more molecules selected from IL-10, IL-2, and IL-27. In some embodiments, the genetically engineered bacteria further comprise acrylate pathway genes for propionate biosynthesis, *pct*, *lcdA*, *lcdB*, *lcdC*, *etfA*, *acrB*, and *acrC*. In an alternate embodiment, the genetically engineered bacteria comprise pyruvate pathway genes for propionate biosynthesis, $thrA^{fbr}$, *thrB*, *thrC*, $ilvA^{fbr}$, *aceE*, *aceF*, and *lpd.* In another alternate embodiment, the genetically engineered bacteria comprise $thrA^{fbr}$, *thrB*, *thrC*, $ilvA^{fbr}$, *aceE*, *aceF*, *lpd*, and *tesB.*

[0327] The bacteria comprise a gene cassette for producing butyrate as described above, a gene cassette for producing propionate as described above, a gene encoding IL-10 (see, e.g., 49), a gene encoding IL-27 (see, e.g., SEQ ID NO: 52), a gene encoding IL-22 (see, e.g., SEQ ID NO: 51), and a gene encoding IL-2 (see, e.g., SEQ ID NO: 50). In one embodiment, each of the genes or gene cassettes is placed under the control of a FNR regulatory region selected from SEQ ID NOs: 55-66 (Table 9). In an alternate embodiment, each of the genes or gene cassettes is placed under the control of an RNS-responsive regulatory region, e.g., *norB*, and the bacteria further comprises a gene encoding a corresponding RNS-responsive transcription factor, e.g., *nsrR* (see, e.g., Tables 10 and 11). In yet another embodiment, each of the genes or gene cassettes is placed under the control of an ROS-responsive regulatory region, e.g., *oxyS*, and the bacteria further comprises a gene encoding a corresponding ROS-responsive transcription factor, e.g., *oxyR* (see, e.g., Tables 14-17). In certain constructs, one or more of the genes is placed under the control of a tetracycline-inducible or constitutive promoter.

Butyrate, Propionate, IL-10, L-22, SOD, GLP-2, kynurenine

[0328] In certain constructs, in addition to the butyrate production pathways described above, the Escherichia *coli* Nissle are further engineered to produce one or more molecules selected from IL-10, IL-22, SOD, GLP-2, and kynurenine using the methods described above. In certain constructs, in addition to the butyrate production pathways described above, the *Escherichia coli* Nissle are further engineered to produce propionate, and one or more molecules selected from IL-10, IL-22, SOD, GLP-2, and kynurenine using the methods described above. In certain constructs, in addition to the butyrate production pathways described above, the *Escherichia coli* Nissle are further engineered to produce IL-10, IL-27, IL-22, SOD, GLP-2, and kynurenine using the methods described above. In certain constructs, in addition to the butyrate production pathways described above, the *Escherichia coli* Nissle are further engineered to produce propionate, IL-10, IL-27, IL-22, SOD, GLP-2, and kynurenine using the methods described above. In some embodiments, the genetically engineered bacteria further comprise acrylate pathway genes for propionate biosynthesis, *pct*, *lcdA*, *lcdB*, *lcdC*, *etfA*, *acrB*, and *acrC*. In an alternate embodiment, the genetically engineered bacteria comprise

pyruvate pathway genes for propionate biosynthesis, *thrA*^{fbr}, *thrB*, *thrC*, *ilvA*^{fbr}, *aceE*, *aceF*, and *lpd.* In another alternate embodiment, the genetically engineered bacteria comprise *thrA*^{fbr}, *thrB*, *thrC*, *ilvA*^{fbr}, *aceE*, *aceF*, *lpd*, and *tesB.*

[0329] The bacteria comprise a gene cassette for producing butyrate as described above, a gene cassette for producing propionate as described above, a gene encoding IL-10 (see, e.g., 49), a gene encoding IL-22 (see, e.g., SEQ ID NO: 51), a gene encoding SOD (see, e.g., SEQ ID NO: 53), a gene encoding GLP-2 (see, e.g., SEQ ID NO: 54), and a gene or gene cassette for producing kynurenine . In one embodiment, each of the genes or gene cassettes is placed under the control of a FNR regulatory region selected from SEQ ID NOs: 55-66 (Table 9). In an alternate embodiment, each of the genes or gene cassettes is placed under the control of an RNS-responsive regulatory region, e.g., *norB*, and the bacteria further comprises a gene encoding a corresponding RNS-responsive transcription factor, e.g., *nsrR* (see, e.g., Tables 10 and 11). In yet another embodiment, each of the genes or gene cassettes is placed under the control of an ROS-responsive regulatory region, e.g., *oxyS*, and the bacteria further comprises a gene encoding a corresponding ROS-responsive transcription factor, e.g., *oxyR* (see, e.g., Tables 14-17). In certain constructs, one or more of the genes is placed under the control of a tetracycline-inducible or constitutive promoter.

Butyrate, Propionate, IL-10, IL-27, IL-22, IL-2, SOD, GLP-2, kynurenine

[0330] In certain constructs, in addition to the butyrate production pathways described above, the *Escherichia coli* Nissle are further engineered to produce one or more molecules selected from IL-10, IL-27, IL-22, IL-2, SOD, GLP-2, and kynurenine using the methods described above. In certain constructs, in addition to the butyrate production pathways described above, the *Escherichia coli* Nissle are further engineered to produce propionate and one or more molecules selected from IL-10, IL-27, IL-22, IL-2, SOD, GLP-2, and kynurenine using the methods described above. In certain constructs, in addition to the butyrate production pathways described above, the *Escherichia coli* Nissle are further engineered to produce IL-10, IL-27, IL-22, SOD, GLP-2, and kynurenine using the methods described above. In some embodiments, the genetically engineered bacteria further comprise acrylate pathway genes for propionate biosynthesis, *pct*, *lcdA*,

lcdB, *lcdC*, *etfA*, *acrB*, and *acrC*. In an alternate embodiment, the genetically engineered bacteria comprise pyruvate pathway genes for propionate biosynthesis, $thrA^{fbr}$, *thrB*, *thrC*, $ilvA^{fbr}$, *aceE*, *aceF*, and *lpd*. In another alternate embodiment, the genetically engineered bacteria comprise $thrA^{fbr}$, *thrB*, *thrC*, $ilvA^{fbr}$, *aceE*, *aceF*, *lpd*, and *tesB*.

[0331] The bacteria comprise a gene cassette for producing butyrate as described above, a gene cassette for producing propionate as described above, a gene encoding IL-10 (see, e.g., 49), a gene encoding IL-27 (see, e.g., SEQ ID NO: 52), a gene encoding IL-22 (see, e.g., SEQ ID NO: 51), a gene encoding IL-2 (see, e.g., SEQ ID NO: 50), a gene encoding SOD (see, e.g., SEQ ID NO: 53), a gene encoding GLP-2 (see, e.g., SEQ ID NO: 54), and a gene or gene cassette for producing kynurenine . In one embodiment, each of the genes or gene cassettes is placed under the control of a FNR regulatory region selected from SEQ ID NOs: 55-66 (Table 9). In an alternate embodiment, each of the genes or gene cassettes is placed under the control of an RNS-responsive regulatory region, e.g., *norB*, and the bacteria further comprises a gene encoding a corresponding RNS-responsive transcription factor, e.g., *nsrR* (see, e.g., Tables 9 and 10). In yet another embodiment, each of the genes or gene cassettes is placed under the control of an ROS-responsive regulatory region, e.g., *oxyS*, and the bacteria further comprises a gene encoding a corresponding ROS-responsive transcription factor, e.g., *oxyR* (see, e.g., Tables 14-17). In certain constructs, one or more of the genes is placed under the control of a tetracycline-inducible or constitutive promoter.

[0332] In some embodiments, bacterial genes may be disrupted or deleted to produce an auxotrophic strain. These include, but are not limited to, genes required for oligonucleotide synthesis, amino acid synthesis, and cell wall synthesis, as shown below.

Amino Acid	Oligonucleotide	Cell wall
cysE	thyA	dapA
glnA	uraA	dapB
ilvD		dapD
leuB		dapE
lysA		dapF
serA		
metA		
glyA		
hisB		
ilvA		
pheA		
proA		
thrC		
trpC		
tyrA		

Example 2. Transforming *E. coli*

[0333] Each plasmid is transformed into *E. coli* Nissle or *E. coli* DH5a. All tubes, solutions, and cuvettes are pre-chilled to 4° C. An overnight culture of *E. coli* Nissle or *E. coli* DH5a is diluted 1:100 in 5 mL of lysogeny broth (LB) and grown until it reached an OD_{600} of 0.4-0.6. The cell culture medium contains a selection marker, e.g., ampicillin, that is suitable for the plasmid. The *E. coli* cells are then centrifuged at 2,000 rpm for 5 min. at 4° C, the supernatant is removed, and the cells are resuspended in 1 mL of 4° C water. The *E. coli* are again centrifuged at 2,000 rpm for 5 min. at 4° C, the supernatant is removed, and the cells are resuspended in 0.5 mL of 4° C water. The *E. coli* are again centrifuged at 2,000 rpm for 5 min. at 4° C, the supernatant is removed, and the cells are finally resuspended in 0.1 mL of 4° C water. The electroporator is set to 2.5 kV. 0.5 µg of one of the above plasmids is added to the cells, mixed by pipetting, and pipetted into a sterile, chilled cuvette. The dry cuvette is placed into the sample chamber, and the electric pulse is applied. One mL of room-temperature SOC media is immediately added, and the mixture is transferred to a culture tube and incubated at 37° C for 1 hr. The cells are spread out on an LB plate containing ampicillin and incubated overnight.

[0334] In alternate embodiments, the butyrate cassette can be inserted into the Nissle genome through homologous recombination (Genewiz, Cambridge, MA).

Organization of the constructs and nucleotide sequences are provided herein. To create a vector capable of integrating the synthesized butyrate cassette construct into the chromosome, Gibson assembly was first used to add 1000bp sequences of DNA homologous to the Nissle lacZ locus into the R6K origin plasmid pKD3. This targets DNA cloned between these homology arms to be integrated into the lacZ locus in the Nissle genome. Gibson assembly was used to clone the fragment between these arms. PCR was used to amplify the region from this plasmid containing the entire sequence of the homology arms, as well as the butyrate cassette between them. This PCR fragment was used to transform electrocompetent Nissle-pKD46, a strain that contains a temperature-sensitive plasmid encoding the lambda red recombinase genes. After transformation, cells were grown out for 2 hours before plating on chloramphenicol at 20ug/mL at 37 degrees C. Growth at 37 degrees C also cures the pKD46 plasmid. Transformants containing cassette were chloramphenicol resistant and lac-minus (lac-).

Example 3. Production of Butyrate in Recombinant E. coli using tet-inducible promoter

[0335] Figures 15-17, 20 and 21 show butyrate cassettes described above under the control of a tet-inducible promoter. Production of butyrate is assessed using the methods described below in Example 4. The tet-inducible cassettes tested include (1) tet-butyrate cassette comprising all eight genes (pLOGIC031); (2) tet-butyrate cassette in which the ter is substituted (pLOGIC046) and (3) tet-butyarte cassette in which tesB is substituted in place of pbt and buk genes. Figure 18 shows butyrate production in strains pLOGIC031 and pLOGIC046 in the presence and absence of oxygen, in which there is no significant difference in butyrate production. Enhanced butyrate production was shown in Nissle in low copy plasmid expressing pLOGIC046 which contain a deletion of the final two genes (ptb-buk) and their replacement with the endogenous E. Coli tesB gene (a thioesterase that cleaves off the butyrate portion from butyryl CoA).

[0336] Overnight cultures of cells were diluted 1:100 in Lb and grown for 1.5 hours until early log phase was reached at which point anhydrous tet was added at a final concentration of 100ng/ml to induce plasmid expression. After 2 hours induction, cells were washed and resuspended in M9 minimal media containing 0.5% glucose at

OD600=0.5. Samples were removed at indicated times and cells spun down. The supernatant was tested for butyrate production using LC-MS. Figure 22 shows butyrate production in strains comprising a tet–butyrate cassette having ter substitution (pLOGIC046) or the tesB substitution (ptb-buk deletion), demonstrating that the tesB substituted strain has greater butyrate production.

[0337] Figure 19 shows the BW25113 strain of E. Coli, which is a common cloning strain and the background of the KEIO collection of E. Coli mutants. NuoB mutants having NuoB deletion were obtained. NuoB is a protein complex involved in the oxidation of NADH during respiratory growth (form of growth requiring electron transport). Preventing the coupling of NADH oxidation to electron transport allows an increase in the amount of NADH being used to support butyrate production. Figure 19 shows that compared with wild-type Nissle, deletion of NuoB results in grater production of butyrate.

pLOGIC046-tesB-butyrate:

gtaaaacgacggccagtgaattcgttaagacccactttcacatttaagttgtttttctaa
tccgcatatgatcaattcaaggccgaataagaaggctggctctgcaccttggtgatcaaa
taattcgatagcttgtcgtaataatggcggcatactatcagtagtaggtgtttccctttc
ttctttagcgacttgatgctcttgatcttccaatacgcaacctaaagtaaaatgccccac
agcgctgagtgcatataatgcattctctagtgaaaaaccttgttggcataaaaaggctaa
ttgattttcgagagtttcatactgtttttctgtaggccgtgtacctaaatgtacttttgc
tccatcgcgatgacttagtaaagcacatctaaaacttttagcgttattacgtaaaaaatc
ttgccagctttccccttctaaagggcaaaagtgagtatggtgcctatctaacatctcaat
ggctaaggcgtcgagcaaagcccgcttatttttacatgccaatacaatgtaggctgctc
tacacctagcttctgggcgagtttacgggttgttaaaccttcgattccgacctcattaag
cagctctaatgcgctgttaatcactttacttttatctaatctagacatcattaattccta
atttttgttgacactctatcattgatagagttatttaccactccctatcagtgatagag
aaaagtgaactctagaaataattttgtttaactttaagaaggagatatacatatgatcgt
aaaacctatggtacgcaacaatatctgcctgaacgcccatcctcagggctgcaagaaggg
agtggaagatcagattgaatataccaagaaacgcattaccgcagaagtcaaagctggcgc
aaaagctccaaaaaacgttctggtgcttggctgctcaaatggttacggcctggcgagccg
cattactgctgcgttcggatacggggctgcgaccatcggcgtgtcctttgaaaaagcggg
ttcagaaaccaaatatggtacaccgggatggtacaataatttggcatttgatgaagcggc
aaaacgcgagggtctttatagcgtgacgatcgacggcgatgcgttttcagacgagatcaa
ggcccaggtaattgaggaagccaaaaaaaaaggtatcaaatttgatctgatcgtatacag
cttggccagcccagtacgtactgatcctgatacaggtatcatgcacaaaagcgttttgaa
acccttggaaaaacgttcacaggcaaaacagtagatccgtttactggcgagctgaagga
aatctccgcggaaccagcaaatgacgaggaagcagccgccactgttaaagttatgggggg
tgaagattgggaacgttggattaagcagctgtcgaaggaaggcctcttagaagaaggctg
tattaccttggcctatagttatattggccctgaagctacccaagctttgtaccgtaaagg
cacaatcggcaaggccaaagaacacctggaggccacagcacaccgtctcaacaaagagaa

cccgtcaatccgtgccttcgtgagcgtgaataaaggcctggtaacccgcgcaagcgccgt
aatcccggtaatccctctgtatctcgccagcttgttcaaagtaatgaaagagaagggcaa
tcatgaaggttgtattgaacagatcacgcgtctgtacgccgagcgcctgtaccgtaaaga
tggtacaattccagttgatgaggaaaatcgcattcgcattgatgattgggagttagaaga
agacgtccagaaagcggtatccgcgttgatggagaaagtcacgggtgaaaacgcagaatc
tctcactgacttagcggggtaccgccatgatttcttagctagtaacggctttgatgtaga
aggtattaattatgaagcggaagttgaacgcttcgaccgtatctgataagaaggagatat
acatatgagagaagtagtaattgccagtgcagctagaacagcagtaggaagttttggagg
agcatttaaatcagtttcagcggtagagttaggggtaacagcagctaaagaagctataaa
aagagctaacataactccagatatgatagatgaatctcttttaggggagtacttacagc
aggtcttggacaaaatatagcaagacaaatagcattaggagcaggaataccagtagaaaa
accagctatgactataaatatagtttgtggttctggattaagatctgtttcaatggcatc
tcaacttatagcattaggtgatgctgatataatgttagttggtggagctgaaaacatgag
tatgtctccttatttagtaccaagtgcgagatatggtgcaagaatgggtgatgctgcttt
tgttgattcaatgataaaagatggattatcagacatatttaataactatcacatgggtat
tactgctgaaaacatagcagagcaatggaatataactagagaagaacaagatgaattagc
tcttgcaagtcaaaataaagctgaaaaagctcaagctgaaggaaaatttgatgaagaaat
agttcctgttgttataaaaggaagaaaaggtgacactgtagtagataaagatgaatatat
taagcctggcactacaatggagaaacttgctaagttaagacctgcatttaaaaaagatgg
aacagttactgctggtaatgcatcaggaataaatgatggtgctgctatgttagtagtaat
ggctaaagaaaaagctgaagaactaggaatagagcctcttgcaactatagtttcttatgg
aacagctggtgttgaccctaaaataatgggatatggaccagttccagcaactaaaaaagc
tttagaagctgctaatatgactattgaagatatagatttagttgaagctaatgaggcatt
tgctgcccaatctgtagctgtaataagagacttaaatatagatatgaataaagttaatgt
taatggtggagcaatagctataggacatccaataggatgctcaggagcaagaatacttac
tacacttttatatgaaatgaagagaagagatgctaaaactggtcttgctacactttgtat
aggcggtggaatgggaactactttaatagttaagagatagtaagaaggagatatacatat
gaaattagctgtaataggtagtggaactatgggaagtggtattgtacaaacttttgcaag
ttgtggacatgatgtatgtttaaagagtagaactcaaggtgctatagataaatgtttagc
tttattagataaaaatttaactaagttagttactaagggaaaaatggatgaagctacaaa
agcagaaatattaagtcatgttagttcaactactaattatgaagatttaaaagatatgga
tttaataatagaagcatctgtagaagacatgaatataaagaaagatgttttcaagttact
agatgaattatgtaaagaagatactatcttggcaacaaatacttcatcattatctataac
agaaatagcttcttctactaagcgcccagataaagttataggaatgcatttctttaatcc
agttcctatgatgaaattagttgaagttataagtggtcagttaacatcaaaagttacttt
tgatacagtatttgaattatctaagagtatcaataaagtaccagtagatgtatctgaatc
tcctggatttgtagtaaatagaatacttatacctatgataaatgaagctgttggtatata
tgcagatggtgttgcaagtaaagaagaaatagatgaagctatgaaattaggagcaaacca
tccaatgggaccactagcattaggtgatttaatcggattagatgttgttttagctataat
gaacgttttatatactgaatttggagatactaaatatagacctcatccacttttagctaa
aatggttagagctaatcaattaggaagaaaaactaagataggattctatgattataataa
ataataagaaggagatatacatatgagtacaagtgatgttaaagtttatgagaatgtagc
tgttgaagtagatggaaatatatgtacagtgaaaatgaatagacctaaagcccttaatgc
aataaattcaaagactttagaagaactttatgaagtatttgtagatattaataatgatga
aactattgatgttgtaatattgacaggggaaggaaaggcatttgtagctggagcagatat
tgcatacatgaaagatttagatgctgtagctgctaaagattttagtatcttaggagcaaa
agcttttggagaaatagaaaatagtaaaaaagtagtgatagctgctgtaaacggatttgc
tttaggtggaggatgtgaacttgcaatggcatgtgatataagaattgcatctgctaaagc
taaatttggtcagccagaagtaactcttggaataactccaggatatggaggaactcaaag

gcttacaagattggttggaatggcaaaagcaaaagaattaatctttacaggtcaagttat
aaaagctgatgaagctgaaaaaatagggctagtaaatagagtcgttgagccagacatttt
aatagaagaagttgagaaattagctaagataatagctaaaaatgctcagcttgcagttag
atactctaaagaagcaatacaacttggtgctcaaactgatataaatactggaatagatat
agaatctaatttatttggtctttgtttttcaactaaagaccaaaaagaaggaatgtcagc
tttcgttgaaaagagagaagctaactttataaaagggtaataagaaggagatatacatat
gAGTCAGGCGCTAAAAAATTTACTGACATTGTTAAATCTGGAAAAAATTGAGGAAGGACT
CTTTCGCGGCCAGAGTGAAGATTTAGGTTTACGCCAGGTGTTTGGCGGCCAGGTCGTGGG
TCAGGCCTTGTATGCTGCAAAAGAGACCGTCCCTGAAGAGCGGCTGGTACATTCGTTTCA
CAGCTACTTTCTTCGCCCTGGCGATAGTAAGAAGCCGATTATTTATGATGTCGAAACGCT
GCGTGACGGTAACAGCTTCAGCGCCCGCCGGGTTGCTGCTATTCAAAACGGCAAACCGAT
TTTTTATATGACTGCCTCTTTCCAGGCACCAGAAGCGGGTTTCGAACATCAAAAAACAAT
GCCGTCCGCGCCAGCGCCTGATGGCCTCCCTTCGGAAACGCAAATCGCCCAATCGCTGGC
GCACCTGCTGCCGCCAGTGCTGAAAGATAAATTCATCTGCGATCGTCCGCTGGAAGTCCG
TCCGGTGGAGTTTCATAACCCACTGAAAGGTCACGTCGCAGAACCACATCGTCAGGTGTG
GATCCGCGCAAATGGTAGCGTGCCGGATGACCTGCGCGTTCATCAGTATCTGCTCGGTTA
CGCTTCTGATCTTAACTTCCTGCCGGTAGCTCTACAGCCGCACGGCATCGGTTTTCTCGA
ACCGGGGATTCAGATTGCCACCATTGACCATTCCATGTGGTTCCATCGCCCGTTTAATTT
GAATGAATGGCTGCTGTATAGCGTGGAGAGCACCTCGGCGTCCAGCGCACGTGGCTTTGT
GCGCGGTGAGTTTTATACCCAAGACGGCGTACTGGTTGCCTCGACCGTTCAGGAAGGGGT
GATGCGTAATCACAATtaa

Example 4. Production of Butyrate in Recombinant E. coli

[0338] Production of butyrate is assessed in *E. coli* Nissle strains containing the butyrate cassettes described above in order to determine the effect of oxygen on butyrate production. All incubations are performed at 37° C. Cultures of *E. coli* strains DH5a and Nissle transformed with the butyrate cassettes are grown overnight in LB and then diluted 1:200 into 4 mL of M9 minimal medium containing 0.5% glucose. The cells are grown with shaking (250 rpm) for 4-6 h and incubated aerobically or anaerobically in a Coy anaerobic chamber (supplying 90% N_2, 5% CO_2, 5%H_2). One mL culture aliquots are prepared in 1.5 mL capped tubes and incubated in a stationary incubator to limit culture aeration. One tube is removed at each time point (0, 1, 2, 4, and 20 hours) and analyzed for butyrate concentration by LC-MS to confirm that butyrate production in these recombinant strains can be achieved in a low-oxygen environment.

[0339] In an alternate embodiment, overnight bacterial cultures were diluted 1:100 into fresh LB and grown for 1.5 hrs to allow entry into early log phase. At this point, long half-life nitric oxide donor (DETA-NO; diethylenetriamine-nitric oxide adduct) was added to cultures at a final concentration of 0.3mM to induce expression from plasmid. After 2 hours of induction, cells were spun down, supernatant was discarded,

and the cells were resuspended in M9 minimal media containing 0.5% glucose. Culture supernatant was then analyzed at indicated time points to assess levels of butyrate production. Genetically engineered Nissle comprising pLogic031-nsrR-norB-butyrate operon construct; SYN133) or (pLogic046-nsrR-norB-butyrate operon construct; SYN145) produce significantly more butyrate as compared to wild-type Nissle (SYN001).

[0340] Genetically engineered Nissle were generated comprising a butyrate gene cassette in which the *pbt* and *buk* genes are replaced with *tesB* (SEQ ID NO: 24) expressed under the control of a tetracycline promoter (pLOGIC046-tesB-butyrate; SEQ ID NO: 81). SEQ ID NO: 81 comprises a reverse complement of the tetR repressor (underlined), an intergenic region containing divergent promoters controlling tetR and the butyrate operon and their respective RBS (bold), and the butyrate genes (*ter-thiA1-hbd-crt2-tesB)* separated by RBS.

SEQ ID NO: 81

gtaaaacgacggccagtgaattcgttaagacccactttcacatttaagttgttttctaa
tccgcatatgatcaattcaaggccgaataagaaggctggctctgcaccttggtgatcaaa
taattcgatagcttgtcgtaataatggcggcatactatcagtagtaggtgtttccctttc
ttctttagcgacttgatgctcttgatcttccaatacgcaacctaaagtaaaatgccccac
agcgctgagtgcatataatgcattctctagtgaaaaaccttgttggcataaaaaggctaa
ttgattttcgagagtttcatactgtttttctgtaggccgtgtacctaaatgtactttgc
tccatcgcgatgacttagtaaagcacatctaaaacttttagcgttattacgtaaaaaatc
ttgccagctttccccttctaaagggcaaaagtgagtatggtgcctatctaacatctcaat
ggctaaggcgtcgagcaaagcccgcttatttttacatgccaatacaatgtaggctgctc
tacacctagcttctgggcgagtttacgggttgttaaaccttcgattccgacctcattaag
cagctctaatgcgctgttaatcactttacttttatctaatctagacat**cattaattccta**
attttgttgacactctatcattgatagagttattttaccactccctatcagtgatagag
aaaagtgaactctagaaataattttgtttaactttaagaaggagatatacatatgatcgt
aaaacctatggtacgcaacaatatctgcctgaacgcccatcctcagggctgcaagaaggg
agtggaagatcagattgaatataccaagaaacgcattaccgcagaagtcaaagctggcgc
aaaagctccaaaaaacgttctggtgcttggctgctcaaatggttacggcctggcgagccg
cattactgctgcgttcggatacggggctgcgaccatcggcgtgtcctttgaaaaagcggg
ttcagaaaccaaatatggtacaccgggatggtacaataatttggcatttgatgaagcggc
aaaacgcgagggtctttatagcgtgacgatcgacggcgatgcgttttcagacgagatcaa
ggcccaggtaattgaggaagccaaaaaaaaaggtatcaaatttgatctgatcgtatacag
cttggccagcccagtacgtactgatcctgatacaggtatcatgcacaaaagcgttttgaa
acccctttggaaaaacgttcacaggcaaaacagtagatccgtttactggcgagctgaagga
aatctccgcggaaccagcaaatgacgaggaagcagccgccactgttaaagttatgggggg
tgaagattgggaacgttggattaagcagctgtcgaaggaaggcctcttagaagaaggctg
tattaccttggcctatagttatattggccctgaagctacccaagctttgtaccgtaaagg
cacaatcggcaaggccaaagaacacctggaggccacagcacaccgtctcaacaaagagaa
cccgtcaatccgtgccttcgtgagcgtgaataaaggcctggtaacccgcgcaagcgccgt
aatcccggtaatccctctgtatctcgccagcttgttcaaagtaatgaaagagaagggcaa

tcatgaaggttgtattgaacagatcacgcgtctgtacgccgagcgcctgtaccgtaaaga
tggtacaattccagttgatgaggaaaatcgcattcgcattgatgattgggagttagaaga
agacgtccagaaagcggtatccgcgttgatggagaaagtcacgggtgaaaacgcagaatc
tctcactgacttagcggggtaccgccatgatttcttagctagtaacggctttgatgtaga
aggtattaattatgaagcggaagttgaacgcttcgaccgtatctgataagaaggagatat
acatatgagagaagtagtaattgccagtgcagctagaacagcagtaggaagttttggagg
agcatttaaatcagtttcagcggtagagttaggggtaacagcagctaaagaagctataaa
aagagctaacataactccagatatgatagatgaatctcttttaggggagtacttacagc
aggtcttggacaaaatatagcaagacaaatagcattaggagcaggaataccagtagaaaa
accagctatgactataaatatagtttgtggttctggattaagatctgtttcaatggcatc
tcaacttatagcattaggtgatgctgatataatgttagttggtggagctgaaaacatgag
tatgtctccttatttagtaccaagtgcgagatatggtgcaagaatgggtgatgctgcttt
tgttgattcaatgataaaagatggattatcagacatatttaataactatcacatgggtat
tactgctgaaaacatagcagagcaatggaatataactagagaagaacaagatgaattagc
tcttgcaagtcaaaataaagctgaaaaagctcaagctgaaggaaaatttgatgaagaaat
agttcctgttgttataaaaggaagaaaaggtgacactgtagtagataaagatgaatatat
taagcctggcactacaatggagaaacttgctaagttaagacctgcatttaaaaaagatgg
aacagttactgctggtaatgcatcaggaataaatgatggtgctgctatgttagtagtaat
ggctaaagaaaaagctgaagaactaggaatagagcctcttgcaactatagtttcttatgg
aacagctggtgttgaccctaaaataatgggatatggaccagttccagcaactaaaaaagc
tttagaagctgctaatatgactattgaagatatagatttagttgaagctaatgaggcatt
tgctgcccaatctgtagctgtaataagagacttaaatatagatatgaataaagttaatgt
taatggtggagcaatagctataggacatccaataggatgctcaggagcaagaatacttac
tacacttttatatgaaatgaagagaagagatgctaaaactggtcttgctacactttgtat
aggcggtggaatgggaactactttaatagttaagagatagtaagaaggagatatacatat
gaaattagctgtaataggtagtggaactatgggaagtggtattgtacaaacttttgcaag
ttgtggacatgatgtatgtttaaagagtagaactcaaggtgctatagataaatgtttagc
tttattagataaaaatttaactaagttagttactaagggaaaaatggatgaagctacaaa
agcagaaatattaagtcatgttagttcaactactaattatgaagatttaaaagatatgga
tttaataatagaagcatctgtagaagacatgaatataaagaaagatgttttcaagttact
agatgaattatgtaaagaagatactatcttggcaacaaatacttcatcattatctataac
agaaatagcttcttctactaagcgcccagataaagttataggaatgcatttctttaatcc
agttcctatgatgaaattagttgaagttataagtggtcagttaacatcaaaagttacttt
tgatacagtatttgaattatctaagagtatcaataaagtaccagtagatgtatctgaatc
tcctggatttgtagtaaatagaatacttatacctatgataaatgaagctgttggtatata
tgcagatggtgttgcaagtaaagaagaaatagatgaagctatgaaattaggagcaaacca
tccaatgggaccactagcattaggtgatttaatcggattagatgttgttttagctataat
gaacgttttatatactgaatttggagatactaaatatagacctcatccacttttagctaa
aatggttagagctaatcaattaggaagaaaaactaagataggattctatgattataataa
ataataagaaggagatatacatatgagtacaagtgatgttaaagtttatgagaatgtagc
tgttgaagtagatggaaatatatgtacagtgaaaatgaatagacctaaagcccttaatgc
aataaattcaaagactttagaagaactttatgaagtatttgtagatattaataatgatga
aactattgatgttgtaatattgacaggggaaggaaaggcatttgtagctggagcagatat
tgcatacatgaaagatttagatgctgtagctgctaaagattttagtatcttaggagcaaa
agcttttggagaaatagaaaatagtaaaaaagtagtgatagctgctgtaaacggatttgc
tttaggtggaggatgtgaacttgcaatggcatgtgatataagaattgcatctgctaaagc
taaatttggtcagccagaagtaactcttggaataactccaggatatggaggaactcaaag
gcttacaagattggttggaatggcaaaagcaaaagaattaatctttacaggtcaagttat
aaaagctgatgaagctgaaaaaatagggctagtaaatagagtcgttgagccagacatttt

aatagaagaagttgagaaattagctaagataatagctaaaaatgctcagcttgcagttag
atactctaaagaagcaatacaacttggtgctcaaactgatataaatactggaatagatat
agaatctaatttatttggtctttgtttttcaactaaagaccaaaaagaaggaatgtcagc
tttcgttgaaaagagagaagctaactttataaaagggtaataagaaggagatatacatat
gAGTCAGGCGCTAAAAAATTTACTGACATTGTTAAATCTGGAAAAAATTGAGGAAGGACT
CTTTCGCGGCCAGAGTGAAGATTTAGGTTTACGCCAGGTGTTTGGCGGCCAGGTCGTGGG
TCAGGCCTTGTATGCTGCAAAAGAGACCGTCCCTGAAGAGCGGCTGGTACATTCGTTTCA
CAGCTACTTTCTTCGCCCTGGCGATAGTAAGAAGCCGATTATTTATGATGTCGAAACGCT
GCGTGACGGTAACAGCTTCAGCGCCCGCCGGGTTGCTGCTATTCAAAACGGCAAACCGAT
TTTTTATATGACTGCCTCTTTCCAGGCACCAGAAGCGGGTTTCGAACATCAAAAAACAAT
GCCGTCCGCGCCAGCGCCTGATGGCCTCCCTTCGGAAACGCAAATCGCCCAATCGCTGGC
GCACCTGCTGCCGCCAGTGCTGAAAGATAAATTCATCTGCGATCGTCCGCTGGAAGTCCG
TCCGGTGGAGTTTCATAACCCACTGAAAGGTCACGTCGCAGAACCACATCGTCAGGTGTG
GATCCGCGCAAATGGTAGCGTGCCGGATGACCTGCGCGTTCATCAGTATCTGCTCGGTTA
CGCTTCTGATCTTAACTTCCTGCCGGTAGCTCTACAGCCGCACGGCATCGGTTTTCTCGA
ACCGGGGATTCAGATTGCCACCATTGACCATTCCATGTGGTTCCATCGCCCGTTTAATTT
GAATGAATGGCTGCTGTATAGCGTGGAGAGCACCTCGGCGTCCAGCGCACGTGGCTTTGT
GCGCGGTGAGTTTTATACCCAAGACGGCGTACTGGTTGCCTCGACCGTTCAGGAAGGGGT
GATGCGTAATCACAATtaa

[0341] Overnight bacterial cultures were diluted 1:100 into fresh LB and grown for 1.5 hrs to allow entry into early log phase. At this point, anhydrous tetracycline (ATC) was added to cultures at a final concentration of 100 ng/mL to induce expression of butyrate genes from plasmid. After 2 hours of induction, cells were spun down, supernatant was discarded, and the cells were resuspended in M9 minimal media containing 0.5% glucose. Culture supernatant was then analyzed at indicated time points to assess levels of butyrate production. Replacement of pbt and buk with tesB leads to greater levels of butyrate production.

[0342] Figure 24 shows butyrate production in strains comprising an FNR-butyrate cassette syn 363 (having the ter substitution) in the presence/absence of glucose and oxygen. Figure 24 Shows that bacteria need both glucose and anaerobic conditions for butyrate production from the FNR promoter. Cells were grown aerobically or anaerobically in media containg no glucose (LB) or in media containing glucose at 0.5% (RMC). Culture samples were taken at indicaed time pints and supernatant fractions were assessed for butyrate concentration using LC-MS. These data show that SYN 363 requires glucose for butyrate production and that in the presence of glucose butyrate production can be enhanced under anaerobic conditions when under the control of the anaerobic FNR-regulated ydfZ promoter.

Example 5. Efficacy of Butyrate-Expressing Bacteria in a Mouse Model of IBD

[0343] Bacteria harboring the butyrate cassettes described above are grown overnight in LB. Bacteria are then diluted 1:100 into LB containing a suitable selection marker, e.g., ampicillin, and grown to an optical density of 0.4-0.5 and then pelleted by centrifugation. Bacteria are resuspended in phosphate buffered saline and 100 microliters is administered by oral gavage to mice. IBD is induced in mice by supplementing drinking water with 3% dextran sodium sulfate for 7 days prior to bacterial gavage. Mice are treated daily for 1 week and bacteria in stool samples are detected by plating stool homogenate on agar plates supplemented with a suitable selection marker, e.g., ampicillin. After 5 days of bacterial treatment, colitis is scored in live mice using endoscopy. Endoscopic damage score is determined by assessing colon translucency, fibrin attachment, mucosal and vascular pathology, and/or stool characteristics. Mice are sacrificed and colonic tissues are isolated. Distal colonic sections are fixed and scored for inflammation and ulceration. Colonic tissue is homogenized and measurements are made for myeloperoxidase activity using an enzymatic assay kit and for cytokine levels (IL-1β, TNF-α, IL-6, IFN-γ and IL-10).

Example 6. Generating a DSS-Induced Mouse Model of IBD

[0344] The genetically engineered bacteria described in Example 1 can be tested in the dextran sodium sulfate (DSS)-induced mouse model of colitis. The administration of DSS to animals results in chemical injury to the intestinal epithelium, allowing proinflammatory intestinal contents (e.g., luminal antigens, enteric bacteria, bacterial products) to disseminate and trigger inflammation (Low et al., 2013). To prepare mice for DSS treatment, mice are labeled using ear punch, or any other suitable labeling method. Labeling individual mice allows the investigator to track disease progression in each mouse, since mice show differential susceptibilities and responsiveness to DSS induction. Mice are then weighed, and if required, the average group weight is equilibrated to eliminate any significant weight differences between groups. Stool is also collected prior to DSS administration, as a control for subsequent assays. Exemplary assays for fecal markers of inflammation (e.g., cytokine levels or myeloperoxidase activity) are described below.

[0345] For DSS administration, a 3% solution of DSS (MP Biomedicals, Santa Ana, CA; Cat. No. 160110) in autoclaved water is prepared. Cage water bottles are then filled with 100 mL of DSS water, and control mice are given the same amount of water without DSS supplementation. This amount is generally sufficient for 5 mice for 2-3 days. Although DSS is stable at room temperature, both types of water are changed every 2 days, or when turbidity in the bottles is observed.

[0346] Acute, chronic, and resolving models of intestinal inflammation are achieved by modifying the dosage of DSS (usually 1-5%) and the duration of DSS administration (Chassaing et al., 2014). For example, acute and resolving colitis may be achieved after a single continuous exposure to DSS over one week or less, whereas chronic colitis is typically induced by cyclical administration of DSS punctuated with recovery periods (e.g., four cycles of DSS treatment for 7 days, followed by 7-10 days of water).

[0347] Figure 27 shows that butyrate produced in vivo in DSS mouse models under the control of an FNR promoter can be gut protective. LCN2 and calprotectin are both a measure of gut barrier disruption (measure by ELISA in this assay). Figure 27 shows that Syn 363 (ter substitution) reduces inflammation and/or protects gut barrier as conmpared to Syn 94 (wildtype Nissle).

Example 7. Monitoring Disease Progression In Vivo

[0348] Following initial administration of DSS, stool is collected from each animal daily, by placing a single mouse in an empty cage (without bedding material) for 15-30 min. However, as DSS administration progresses and inflammation becomes more robust, the time period required for collection increases. Stool samples are collected using sterile forceps, and placed in a microfuge tube. A single pellet is used to monitor occult blood according to the following scoring system: 0, normal stool consistency with negative hemoccult; 1, soft stools with positive hemoccult; 2, very soft stools with traces of blood; and 3, watery stools with visible rectal bleeding. This scale is used for comparative analysis of intestinal bleeding. All remaining stool is reserved for the measurement of inflammatory markers, and frozen at -20 ◦C.

[0349] The body weight of each animal is also measured daily. Body weights may increase slightly during the first three days following initial DSS administration, and then begin to decrease gradually upon initiation of bleeding. For mouse models of acute colitis, DSS is typically administered for 7 days. However, this length of time may be modified at the discretion of the investigator.

Example 8. In Vivo Efficacy of Genetically Engineered Bacteria Following DSS Induction

[0350] The genetically engineered bacteria described in Example 1 can be tested in DSS-induced animal models of IBD. Bacteria are grown overnight in LB supplemented with the appropriate antibiotic. Bacteria are then diluted 1:100 in fresh LB containing selective antibiotic, grown to an optical density of 0.4-0.5, and pelleted by centrifugation. Bacteria are then resuspended in phosphate buffered saline (PBS). IBD is induced in mice by supplementing drinking water with 3% DSS for 7 days prior to bacterial gavage. On day 7 of DSS treatment, 100 µL of bacteria (or vehicle) is administered to mice by oral gavage. Bacterial treatment is repeated once daily for 1 week, and bacteria in stool samples are detected by plating stool homogenate on selective agar plates.

[0351] After 5 days of bacterial treatment, colitis is scored in live mice using the Coloview system (Karl Storz Veterinary Endoscopy, Goleta, CA). In mice under 1.5-2.0% isoflurane anesthesia, colons are inflated with air and approximately 3 cm of the proximal colon can be visualized (Chassaing et al., 2014). Endoscopic damage is scored by assessing colon translucency (score 0-3), fibrin attachment to the bowel wall (score 0-3), mucosal granularity (score 0-3), vascular pathology (score 0-3), stool characteristics (normal to diarrhea; score 0-3), and the presence of blood in the lumen (score 0-3), to generate a maximum score of 18. Mice are sacrificed and colonic tissues are isolated using protocols described in Examples 8 and 9. Distal colonic sections are fixed and scored for inflammation and ulceration. Remaining colonic tissue is homogenized and cytokine levels (e.g., IL-1β, TNF-α, IL-6, IFN-γ, and IL-10), as well as myeloperoxidase activity, are measured using methods described below.

Example 9. Euthanasia Procedures for Rodent Models of IBD

[0352] Four and 24 hours prior to sacrifice, 5-bromo-2'-deooxyuridine (BrdU) (Invitrogen, Waltham, MA; Cat. No. B23151) may be intraperitoneally administered to mice, as recommended by the supplier. BrdU is used to monitor intestinal epithelial cell proliferation and/or migration via immunohistochemistry with standard anti-BrdU antibodies (Abcam, Cambridge, MA).

[0353] On the day of sacrifice, mice are deprived of food for 4 hours, and then gavaged with FITC-dextran tracer (4 kDa, 0.6 mg/g body weight). Fecal pellets are collected, and mice are euthanized 3 hours following FITC-dextran administration. Animals are then cardiac bled to collect hemolysis-free serum. Intestinal permeability correlates with fluorescence intensity of appropriately diluted serum (excitation, 488 nm; emission, 520 nm), and is measured using spectrophotometry. Serial dilutions of a known amount of FITC-dextran in mouse serum are used to prepare a standard curve.

[0354] Alternatively, intestinal inflammation is quantified according to levels of serum keratinocyte-derived chemokine (KC), lipocalin 2, calprotectin, and/or CRP-1. These proteins are reliable biomarkers of inflammatory disease activity, and are measured using DuoSet ELISA kits (R&D Systems, Minneapolis, MN) according to manufacturer's instructions. For these assays, control serum samples are diluted 1:2 or 1:4 for KC, and 1:200 for lipocalin 2. Samples from DSS-treated mice require a significantly higher dilution.

Example 10. Isolation and Preservation of Colonic Tissues

[0355] To isolate intestinal tissues from mice, each mouse is opened by ventral midline incision. The spleen is then removed and weighed. Increased spleen weights generally correlate with the degree of inflammation and/or anemia in the animal. Spleen lysates (100 mg/mL in PBS) plated on non-selective agar plates are also indicative of disseminated intestinal bacteria. The extent of bacterial dissemination should be consistent with any FITC-dextran permeability data.

[0356] Mesenteric lymph nodes are then isolated. These may be used to characterize immune cell populations and/or assay the translocation of gut bacteria. Lymph node enlargement is also a reliable indicator of DSS-induced pathology. Finally,

the colon is removed by lifting the organ with forceps and carefully pulling until the cecum is visible. Colon dissection from severely inflamed DSS-treated mice is particularly difficult, since the inflammatory process causes colonic tissue to thin, shorten, and attach to extraintestinal tissues.

[0357] The colon and cecum are separated from the small intestine at the ileocecal junction, and from the anus at the distal end of the rectum. At this point, the mouse intestine (from cecum to rectum) may be imaged for gross analysis, and colonic length may be measured by straightening (but not stretching) the colon. The colon is then separated from the cecum at the ileocecal junction, and briefly flushed with cold PBS using a 5- or 10-mL syringe (with a feeding needle). Flushing removes any feces and/or blood. However, if histological staining for mucin layers or bacterial adhesion/translocation is ultimately anticipated, flushing the colon with PBS should be avoided. Instead, the colon is immersed in Carnoy's solution (60% ethanol, 30% chloroform, 10% glacial acetic acid; Johansson et al., 2008) to preserve mucosal architecture. The cecum can be discarded, as DSS-induced inflammation is generally not observed in this region.

[0358] After flushing, colon weights are measured. Inflamed colons exhibit reduced weights relative to normal colons due to tissue wasting, and reductions in colon weight correlate with the severity of acute inflammation. In contrast, in chronic models of colitis, inflammation is often associated with increased colon weight. Increased weight may be attributed to focal collections of macrophages, epithelioid cells, and multinucleated giant cells, and/or the accumulation of other cells, such as lymphocytes, fibroblasts, and plasma cells (Williams and Williams, 1983).

[0359] To obtain colon samples for later assays, colons are cut into the appropriate number of pieces. It is important to compare the same region of the colon from different groups of mice when performing any assay. For example, the proximal colon is frozen at -80 ◦C and saved for MPO analysis, the middle colon is stored in RNA later and saved for RNA isolation, and the rectal region is fixed in 10% formalin for histology. Alternatively, washed colons may be cultured ex vivo. Exemplary protocols for each of these assays are described below.

Example 11. Myeloperoxidase Activity Assay

[0360] Granulocyte infiltration in the rodent intestine correlates with inflammation, and is measured by the activity levels of myeloperoxidase, an enzyme abundantly expressed in neutrophil granulocytes. Myeloperoxidase (MPO) activity may be quantified using either o-dianisidine dihydrochloride (Sigma, St. Louis, MO; Cat. No. D3252) or 3,3',5,5'-tetramethylbenzidine (Sigma; Cat. No. T2885) as a substrate.

[0361] Briefly, clean, flushed samples of colonic tissue (50-100 mg) are removed from storage at -80 °C and immediately placed on ice. Samples are then homogenized in 0.5% hexadecyltrimethylammonium bromide (Sigma; Cat. No. H6269) in 50 mM phosphate buffer, pH 6.0. Homogenates are then disrupted for 30 sec by sonication, snap-frozen in dry ice, and thawed for a total of three freeze-thaw cycles before a final sonication for 30 sec.

[0362] For assays with o-dianisidine dihydrochloride, samples are centrifuged for 6 min at high speed (13,400 g) at 4 °C. MPO in the supernatant is then assayed in a 96-well plate by adding 1 mg/mL of o-dianisidine dihydrochloride and 0.5x10-4 % H2O2, and measuring optical density at 450 nm. A brownish yellow color develops slowly over a period of 10-20 min; however, if color development is too rapid, the assay is repeated after further diluting the samples. Human neutrophil MPO (Sigma; Cat. No. M6908) is used as a standard, with a range of 0.5-0.015 units/mL. One enzyme unit is defined as the amount of enzyme needed to degrade 1.0 µmol of peroxide per minute at 25 °C. This assay is used to analyze MPO activity in rodent colonic samples, particularly in DSS-induced tissues.

[0363] For assays with 3,3',5,5'-tetramethylbenzidine (TMB), samples are incubated at 60 °C for 2 hours and then spun down at 4,000 g for 12 min. Enzymatic activity in the supernatant is quantified photometrically at 630 nm. The assay mixture consists of 20 mL supernatant, 10 mL TMB (final concentration, 1.6 mM) dissolved in dimethylsulfoxide, and 70 mL H2O2 (final concentration, 3.0 mM) diluted in 80 mM phosphate buffer, pH 5.4. One enzyme unit is defined as the amount of enzyme that produces an increase of one absorbance unit per minute. This assay is used to analyze

MPO activity in rodent colonic samples, particularly in tissues induced by trinitrobenzene (TNBS) as described herein.

Example 12. RNA Isolation and Gene Expression Analysis

[0364] To gain further mechanistic insights into how the genetically engineered bacteria may reduce gut inflammation in vivo, gene expression is evaluated by semi-quantitative and/or real-time reverse transcription PCR.

[0365] For semi-quantitative analysis, total RNA is extracted from intestinal mucosal samples using the RNeasy isolation kit (Qiagen, Germantown, MD; Cat. No. 74106). RNA concentration and purity are determined based on absorbency measurements at 260 and 280 nm. Subsequently, 1 μg of total RNA is reverse-transcribed, and cDNA is amplified for the following genes: tumor necrosis factor alpha (TNF-α), interferon-gamma (IFN-γ), interleukin-2 (IL-2), or any other gene associated with inflammation. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is used as the internal standard. Polymerase chain reaction (PCR) reactions are performed with a 2-min melting step at 95 °C, then 25 cycles of 30 sec at 94 °C, 30 sec at 63 °C, and 1 min at 75 °C, followed by a final extension step of 5 min at 65 °C. Reverse transcription (RT)-PCR products are separated by size on a 4% agarose gel and stained with ethidium bromide. Relative band intensities are analyzed using standard image analysis software.

[0366] For real-time, quantitative analysis, intestinal samples (50 mg) are stored in RNAlater solution (Sigma; Cat. No. R0901) until RNA extraction. Samples should be kept frozen at -20 °C for long-term storage. On the day of RNA extraction, samples are thawed, or removed from RNAlater, and total RNA is extracted using Trizol (Fisher Scientific, Waltham, MA; Cat. No. 15596026). Any suitable RNA extraction method may be used. When working with DSS-induced samples, it is necessary to remove all polysaccharides (including DSS) using the lithium chloride method (Chassaing et al., 2012). Traces of DSS in colonic tissues are known to interfere with PCR amplification in subsequent steps.

[0367] Primers are designed for various genes and cytokines associated with the immune response using Primer Express® software (Applied Biosystems, Foster City, CA). Following isolation of total RNA, reverse transcription is performed using random

primers, dNTPs, and Superscript® II enzyme (Invitrogen; 18064014). cDNA is then used for real-time PCR with SYBR Green PCR Master Mix (Applied Biosystems; 4309155) and the ABI PRISM 7000 Sequence Detection System (Applied Biosystems), although any suitable detection method may be used. PCR products are validated by melt analysis.

Example 13. Histology

[0368] Standard histological stains are used to evaluate intestinal inflammation at the microscopic level. Hematoxylin-eosin (H&E) stain allows visualization of the quality and dimension of cell infiltrates, epithelial changes, and mucosal architecture (Erben et al., 2014). Periodic Acid-Schiff (PAS) stain is used to stain for carbohydrate macromolecules (e.g., glycogen, glycoproteins, mucins). Goblet cells, for example, are PAS-positive due to the presence of mucin.

[0369] Swiss rolls are recommended for most histological stains, so that the entire length of the rodent intestine may be examined. This is a simple technique in which the intestine is divided into portions, opened longitudinally, and then rolled with the mucosa outwards (Moolenbeek and Ruitenberg, 1981). Briefly, individual pieces of colon are cut longitudinally, wrapped around a toothpick wetted with PBS, and placed in a cassette. Following fixation in 10% formalin for 24 hours, cassettes are stored in 70% ethanol until the day of staining. Formalin-fixed colonic tissue may be stained for BrdU using anti-BrdU antibodies (Abcam). Alternatively, Ki67 may be used to visualize epithelial cell proliferation. For stains using antibodies to more specific targets (e.g., immunohistochemistry, immunofluorescence), frozen sections are fixed in a cryoprotective embedding medium, such as Tissue-Tek® OCT (VWR, Radnor, PA; Cat. No. 25608-930).

[0370] For H&E staining, stained colonic tissues are analyzed by assigning each section four scores of 0-3 based on the extent of epithelial damage, as well as inflammatory infiltration into the mucosa, submucosa, and muscularis/serosa. Each of these scores is multiplied by: 1, if the change is focal; 2, if the change is patchy; and 3, if the change is diffuse. The four individual scores are then summed for each colon, resulting in a total scoring range of 0-36 per animal. Average scores for the control and affected groups are tabulated. Alternative scoring systems are detailed herein.

Example 14. Ex Vivo Culturing of Rodent Colons

[0371] Culturing colons ex vivo may provide information regarding the severity of intestinal inflammation. Longitudinally-cut colons (approximately 1.0 cm) are serially washed three times in Hanks' Balanced Salt Solution with 1.0% penicillin/streptomycin (Fisher; Cat. No. BP295950). Washed colons are then placed in the wells of a 24-well plate, each containing 1.0 mL of serum-free RPMI1640 medium (Fisher; Cat. No. 11875093) with 1.0% penicillin/streptomycin, and incubated at 37 °C with 5.0% CO2 for 24 hours. Following incubation, supernatants are collected and centrifuged for 10 min at 4 °C. Supernatants are stored at -80 °C prior to analysis for proinflammatory cytokines.

Example 15. In Vivo Efficacy of Genetically Engineered Bacteria Following TNBS Induction

[0372] Apart from DSS, the genetically engineered bacteria described in 1 can also be tested in other chemically induced animal models of IBD. Non-limiting examples include those induced by oxazolone (Boirivant et al., 1998), acetic acid (MacPherson and Pfeiffer, 1978), indomethacin (Sabiu et al., 2016), sulfhydryl inhibitors (Satoh et al., 1997), and trinitrobenzene sulfonic acid (TNBS) (Gurtner et al., 2003; Seguí et al., 2004). To determine the efficacy of the genetically engineered bacteria in a TNBS-induced mouse model of colitis, bacteria are grown overnight in LB supplemented with the appropriate antibiotic. Bacteria are then diluted 1:100 in fresh LB containing selective antibiotic, grown to an optical density of 0.4-0.5, and pelleted by centrifugation. Bacteria are resuspended in PBS. IBD is induced in mice by intracolonic administration of 30 mg TNBS in 0.25 mL 50% (vol/vol) ethanol (Seguí et al., 2004). Control mice are administered 0.25 mL saline. Four hours post-induction, 100 µL of bacteria (or vehicle) is administered to mice by oral gavage. Bacterial treatment is repeated once daily for 1 week. Animals are weighed daily.

[0373] After 7 days of bacterial treatment, mice are sacrificed via intraperitoneal administration of thiobutabarbital (100 mg/kg). Colonic tissues are isolated by blunt dissection, rinsed with saline, and weighed. Blood samples are collected by open cardiac puncture under aseptic conditions using a 1-mL syringe, placed in Eppendorf vials, and spun at 1,500 g for 10 min at 4 °C. The supernatant serum is then pipetted into

autoclaved Eppendorf vials and frozen at -80 °C for later assay of IL-6 levels using a quantitative, colorimetric commercial kit (R&D Systems).

[0374] Macroscopic damage is examined under a dissecting microscope by a blinded observer. An established scoring system is used to account for the presence/severity of intestinal adhesions (score 0-2), strictures (score 0-3), ulcers (score 0-3), and wall thickness (score 0-2) (Mourelle et al., 1996). Two colon samples (50 mg) are then excised, snap-frozen in liquid nitrogen, and stored at -80 °C for subsequent myeloperoxidase activity assay. If desired, additional samples are preserved in 10% formalin for histologic grading. Formalin-fixed colonic samples are then embedded in paraffin, and 5 μm sections are stained with H&E. Microscopic inflammation of the colon is assessed on a scale of 0 to 11, according to previously defined criteria (Appleyard and Wallace, 1995).

Example 16. Generating a Cell Transfer Mouse Model of IBD

[0375] The genetically engineered bacteria described in Example 1 can be tested in cell transfer animal models of IBD. One exemplary cell transfer model is the CD45RBHi T cell transfer model of colitis (Bramhall et al., 2015; Ostanin et al., 2009; Sugimoto et al., 2008). This model is generated by sorting CD4+ T cells according to their levels of CD45RB expression, and adoptively transferring CD4+ T cells with high CD45RB expression (referred to as CD45RBHi T cells) from normal donor mice into immunodeficient mice (e.g., SCID or RAG-/- mice). Specific protocols are described below.

Enrichment for CD4 T Cells

[0376] Following euthanization of C57BL/6 wild-type mice of either sex (Jackson Laboratories, Bar Harbor, ME), mouse spleens are removed and placed on ice in a 100 mm Petri dish containing 10-15 mL of FACS buffer (1X PBS without Ca2+/Mg2+, supplemented with 4% fetal calf serum). Spleens are teased apart using two glass slides coated in FACS buffer, until no large pieces of tissue remain. The cell suspension is then withdrawn from the dish using a 10-mL syringe (no needle), and expelled out of the syringe (using a 26-gauge needle) into a 50-mL conical tube placed on ice. The Petri dish is washed with an additional 10 mL of FACS buffer, using the same needle technique,

until the 50-mL conical tube is full. Cells are pelleted by centrifugation at 400 g for 10 min at 4 °C. After the cell pellet is gently disrupted with a stream of FACS buffer, cells are counted. Cells used for counting are kept on ice and saved for single-color staining described in the next section. All other cells (i.e., those remaining in the 50-mL conical tube) are transferred to new 50-mL conical tubes. Each tube should contain a maximum of $25x10^7$ cells.

[0377] To enrich for CD4+ T cells, the Dynal® Mouse CD4 Negative Isolation kit (Invitrogen; Cat. No. 114-15D) is used as per manufacturer's instructions. Any comparable CD4+ T cell enrichment method may be used. Following negative selection, CD4+ cells remain in the supernatant. Supernatant is carefully pipetted into a new 50-mL conical tube on ice, and cells are pelleted by centrifugation at 400 g for 10 min at 4 °C. Cell pellets from all 50-mL tubes are then resuspended, pooled into a single 15-mL tube, and pelleted once more by centrifugation. Finally, cells are resuspended in 1 mL of fresh FACS buffer, and stained with anti-CD4-APC and anti-CD45RB-FITC antibodies.

Fluorescent Labeling of CD4+ T Cells

[0378] To label CD4+ T cells, an antibody cocktail containing appropriate dilutions of pre-titrated anti-CD4-APC and anti-CD45RB-FITC antibodies in FACS buffer (approximately 1 mL cocktail/5x107 cells) is added to a 1.5-mL Eppendorf tube, and the volume is adjusted to 1 mL with FACS buffer. Antibody cocktail is then combined with cells in a 15-mL tube. The tube is capped, gently inverted to ensure proper mixing, and incubated on a rocking platform for 15 min at 4 °C.

[0379] During the incubation period, a 96-well round-bottom staining plate is prepared by transferring equal aliquots of counted cells (saved from the previous section) into each well of the plate that corresponds to single-color control staining. These wells are then filled to 200 µL with FACs buffer, and the cells are pelleted at 300 g for 3 min at 4 °C using a pre-cooled plate centrifuge. Following centrifugation, the supernatant is discarded using a 21-gauge needle attached to a vacuum line, and 100 µL of anti-CD16/32 antibody (Fc receptor-blocking) solution is added to each well to prevent non-specific binding. The plate is incubated on a rocking platform at 4 °C for 15 min. Cells are then washed with 200 µL FACS buffer and pelleted by centrifugation.

Supernatant is aspirated, discarded, and 100 µL of the appropriate antibody (i.e., pre-titrated anti-CD4-APC or anti-CD45RB-FITC) is added to wells corresponding to each single-color control. Cells in unstained control wells are resuspended in 100 µL FACS buffer. The plate is incubated on a rocking platform at 4 °C for 15 min. After two washes, cells are resuspended in 200 µL of FACS buffer, transferred into twelve 75-mm flow tubes containing 150-200 µL of FACS buffer, and the tubes are placed on ice.

[0380] Following incubation, cells in the 15-mL tube containing antibody cocktail are pelleted by centrifugation at 400 *g* for 10 min at 4 °C, and resuspended in FACS buffer to obtain a concentration of 25-50x10^6 cells/mL.

Purification of CD4+ CD45RBHi T Cells

[0381] Cell sorting of CD45RBHi and CD45RBLow populations is performed using flow cytometry. Briefly, a sample of unstained cells is used to establish baseline autofluorescence, and for forward scatter vs. side scatter gating of lymphoid cells. Single-color controls are used to set the appropriate levels of compensation to apply to each fluorochrome. However, with FITC and APC fluorochromes, compensation is generally not required. A single-parameter histogram (gated on singlet lymphoid cells) is then used to gate CD4+ (APC+) singlet cells, and a second singlet-parameter (gated on CD4+ singlet cells) is collected to establish sort gates. The CD45RBHi population is defined as the 40% of cells which exhibit the brightest CD45RB staining, whereas the CD45RBLow population is defined as the 15% of cells with the dimmest CD45RB expression. Each of these populations is sorted individually, and the CD45RBHi cells are used for adoptive transfer.

Adoptive Transfer

[0382] Purified populations of CD4+ CD45RBHi cells are adoptively transferred into 6- to 8-week-old RAG-/- male mice. The collection tubes containing sorted cells are filled with FACS buffer, and the cells are pelleted by centrifugation. The supernatant is then discarded, and cells are resuspended in 500 µL PBS. Resuspended cells are transferred into an injection tube, with a maximum of 5x106 cells per tube, and diluted with cold PBS to a final concentration of 1x106 cells/mL. Injection tubes are kept on ice.

[0383] Prior to injection, recipient mice are weighed and injection tubes are gently inverted several times to mix the cells. Mixed cells (0.5 mL, ~0.5x106 cells) are carefully drawn into a 1-mL syringe with a 26G3/8 needle attached. Cells are then intraperitoneally injected into recipient mice.

Example 17. Efficacy of Genetically Engineered Bacteria in a CD45RBHi T Cell Transfer Model

[0384] To determine whether the genetically engineered bacteria of the disclosure are efficacious in CD45RBHi T cell transfer mice, disease progression following adoptive transfer is monitored by weighing each mouse on a weekly basis. Typically, modest weight increases are observed over the first 3 weeks post-transfer, followed by slow but progressive weight loss over the next 4-5 weeks. Weight loss is generally accompanied by the appearance of loose stools and diarrhea.

[0385] At weeks 4 or 5 post-transfer, as recipient mice begin to develop signs of disease, the genetically engineered bacteria described in Example 1 are grown overnight in LB supplemented with the appropriate antibiotic. Bacteria are then diluted 1:100 in fresh LB containing selective antibiotic, grown to an optical density of 0.4-0.5, and pelleted by centrifugation. Bacteria are resuspended in PBS and 100 µL of bacteria (or vehicle) is administered by oral gavage to CD45RBHi T cell transfer mice. Bacterial treatment is repeated once daily for 1-2 weeks before mice are euthanized. Murine colonic tissues are isolated and analyzed using the procedures described above.

Example 18. Efficacy of Genetically Engineered Bacteria in a Genetic Mouse Model of IBD

[0386] The genetically engineered bacteria described in Example 1 can be tested in genetic (including congenic and genetically modified) animal models of IBD. For example, IL-10 is an anti-inflammatory cytokine and the gene encoding IL-10 is a susceptibility gene for both Crohn's disease and ulcerative colitis (Khor et al., 2011). Functional impairment of IL-10, or its receptor, has been used to create several mouse models for the study of inflammation (Bramhall et al., 2015). IL-10 knockout (IL-10-/-) mice housed under normal conditions develop chronic inflammation in the gut (Iyer and Cheng, 2012).

[0387] To determine whether the genetically engineered bacteria of the disclosure are efficacious in IL-10-/- mice, bacteria are grown overnight in LB supplemented with the appropriate antibiotic. Bacteria are then diluted 1:100 in fresh LB containing selective antibiotic, grown to an optical density of 0.4-0.5, and pelleted by centrifugation. Bacteria are resuspended in PBS and 100 µL of bacteria (or vehicle) is administered by oral gavage to IL-10-/- mice. Bacterial treatment is repeated once daily for 1-2 weeks before mice are euthanized. Murine colonic tissues are isolated and analyzed using the procedures described above.

[0388] Protocols for testing the genetically engineered bacteria are similar for other genetic animal models of IBD. Such models include, but are not limited to, transgenic mouse models, e.g., SAMP1/YitFc (Pizarro et al., 2011), dominant negative N-cadherin mutant (NCAD delta; Hermiston and Gordon, 1995), TNFΔARE (Wagner et al., 2013), IL-7 (Watanabe et al., 1998), C3H/HeJBir (Elson et al., 2000), and dominant negative TGF-β receptor II mutant (Zhang et al., 2010); and knockout mouse models, e.g., TCRα-/- (Mombaerts et al., 1993; Sugimoto et al., 2008), WASP-/- (Nguyen et al., 2007), Mdr1a-/- (Wilk et al., 2005), IL-2 Rα-/- (Hsu et al., 2009), Gαi2-/- (Ohman et al., 2002), and TRUC (Tbet-/-Rag2-/-; Garrett et al., 2007).

Example 19. Efficacy of Genetically Engineered Bacteria in a Transgenic Rat Model of IBD

[0389] The genetically engineered bacteria described in Example 1 can be tested in non-murine animal models of IBD. The introduction of human leukocyte antigen B27 (HLA-B27) and the human β2-microglobulin gene into Fisher (F344) rats induces spontaneous, chronic inflammation in the GI tract (Alavi et al., 2000; Hammer et al., 1990). To investigate whether the genetically engineered bacteria of the invention are capable of ameliorating gut inflammation in this model, bacteria are grown overnight in LB supplemented with the appropriate antibiotic. Bacteria are then diluted 1:100 in fresh LB containing selective antibiotic, grown to an optical density of 0.4-0.5, and pelleted by centrifugation. Bacteria are resuspended in PBS and 100 µL of bacteria (or vehicle) is administered by oral gavage to transgenic F344-HLA-B27 rats. Bacterial treatment is repeated once daily for 2 weeks.

[0390] To determine whether bacterial treatment reduces the gross and histological intestinal lesions normally present in F344-HLA-B27 rats at 25 weeks of age, all animals are sacrificed at day 14 following the initial treatment. The GI tract is then resected from the ligament of Treitz to the rectum, opened along the antimesenteric border, and imaged using a flatbed scanner. Total mucosal damage, reported as a percent of the total surface area damaged, is quantified using standard image analysis software.

[0391] For microscopic analysis, samples (0.5-1.0 cm) are excised from both normal and diseased areas of the small and large intestine. Samples are fixed in formalin and embedded in paraffin before sections (5 μm) are processed for H&E staining. The stained sections are analyzed and scored as follows: 0, no inflammation; 1, mild inflammation extending into the submucosa; 2, moderate inflammation extending into the muscularis propria; and 3, severe inflammation. The scores are combined and reported as mean ± standard error.

Example 19. Butyrate-Producing Bacterial Strain Reduces Gut Inflammation in a Low-Dose DSS-Induced Mouse Model of IBD

[0392] At Day 0, 40 C57BL6 mice (8 weeks of age) were weighed and randomized into the following five treatment groups (n=8 per group): H_2O control (group 1); 0.5% DSS control (group 2); 0.5% DSS + 100 mM butyrate (group 3); 0.5% DSS + SYN94 (group 4); and 0.5% DSS + SYN363 (group 5). After randomization, the cage water for group 3 was changed to water supplemented with butyrate (100 mM), and groups 4 and 5 were administered 100 μL of SYN94 and SYN363 by oral gavage, respectively. At Day 1, groups 4 and 5 were gavaged with bacteria in the morning, weighed, and gavaged again in the evening. Groups 4 and 5 were also gavaged once per day for Day 2 and Day 3.

[0393] At Day 4, groups 4 and 5 were gavaged with bacteria, and then all mice were weighed. Cage water was changed to either H_2O + 0.5% DSS (groups 2, 4, and 5), or H_2O + 0.5% DSS supplemented with 100 mM butyrate (group 3). Mice from groups 4 and 5 were gavaged again in the evening. On Days 5-7, groups 4 and 5 were gavaged with bacteria in the morning, weighed, and gavaged again in the evening.

[0394] At Day 8, all mice were fasted for 4 hours, and groups 4 and 5 were gavaged with bacteria immediately following the removal of food. All mice were then weighed, and gavaged with a single dose of FITC-dextran tracer (4 kDa, 0.6 mg/g body weight). Fecal pellets were collected; however, if colitis was severe enough to prevent feces collection, feces were harvested after euthanization. All mice were euthanized at exactly 3 hours following FITC-dextran administration. Animals were then cardiac bled and blood samples were processed to obtain serum. Levels of mouse lipocalin 2, calprotectin, and CRP-1 were quantified by ELISA, and serum levels of FITC-dextran were analyzed by spectrophotometry (see also Example 8).

[0395] **FIG. 27** shows lipocalin 2 (LCN2) levels in all treatment groups, as demonstrated by ELISA, on Day 8 of the study. Since LCN2 is a biomarker of inflammatory disease activity, these data suggest that SYN363 produces enough butyrate to significantly reduce LCN2 concentrations, as well as gut inflammation, in a low-dose DSS-induced mouse model of IBD.

Example 20. Nitric oxide-inducible reporter constructs

[0396] ATC and nitric oxide-inducible reporter constructs were synthesized (Genewiz, Cambridge, MA). When induced by their cognate inducers, these constructs express GFP, which is detected by monitoring fluorescence in a plate reader at an excitation/emission of 395/509 nm, respectively. Nissle cells harboring plasmids with either the control, ATC-inducible Ptet-GFP reporter construct, or the nitric oxide inducible PnsrR-GFP reporter construct were first grown to early log phase (OD600 of about 0.4-0.6), at which point they were transferred to 96-well microtiter plates containing LB and two-fold decreased inducer (ATC or the long half-life NO donor, DETA-NO (Sigma)). Both ATC and NO were able to induce the expression of GFP in their respective constructs across a range of concentrations (Fig. 28); promoter activity is expressed as relative florescence units. An exemplary sequence of a nitric oxide-inducible reporter construct is shown. The bsrR sequence is **bolded**. The gfp sequence is underlined. The PnsrR (NO regulated promoter and RBS) is *italicized*. The constitutive promoter and RBS are boxed.

[0397] These constructs, when induced by their cognate inducer, lead to high level expression of GFP, which is detected by monitoring fluorescence in a plate reader at an excitation/emission of 395/509 nm, respectively. Nissle cells harboring plasmids with either the ATC-inducible Ptet-GFP reporter construct or the nitric oxide inducible PnsrR-GFP reporter construct were first grown to early log phase (OD600= ~0.4-0.6), at which point they were transferred to 96-well microtiter plates containing LB and 2-fold decreases in inducer (ATC or the long half-life NO donor, DETA-NO (Sigma)). It was observed that both the ATC and NO were able to induce the expression of GFP in their respective construct across a wide range of concentrations. Promoter activity is expressed as relative florescence units.

[0398] Figure 29 shows NO-GFP constructs (the dot blot) E. coli Nissle harboring the nitric oxide inducible NsrR-GFP reporter fusion were grown overnight in LB supplemented with kanamycin. Bacteria were then diluted 1:100 into LB containing kanamycin and grown to an optical density of 0.4-0.5 and then pelleted by centrifugation. Bacteria were resuspended in phosphate buffered saline and 100 microliters were administered by oral gavage to mice. IBD is induced in mice by supplementing drinking water with 2-3% dextran sodium sulfate for 7 days prior to bacterial gavage. At 4 hours post-gavage, mice were sacrificed and bacteria were recovered from colonic samples. Colonic contents were boiled in SDS, and the soluble fractions were used to perform a dot blot for GFP detection (induction of NsrR-regulated promoters). Detection of GFP was performed by binding of anti-GFP antibody conjugated to HRP (horse radish peroxidase). Detection was visualized using Pierce chemiluminescent detection kit. It is shown in the figure that NsrR-regulated promoters are induced in DSS-treated mice, but are not shown to be induced in untreated mice. This is consistent with the role of NsrR in response to NO, and thus inflammation.

[0399] Bacteria harboring a plasmid expressing NsrR under control of a constitutive promoter and the reporter gene *gfp* (green fluorescent protein) under control of an NsrR-inducible promoter were grown overnight in LB supplemented with kanamycin. Bacteria are then diluted 1:100 into LB containing kanamycin and grown to an optical density of about 0.4-0.5 and then pelleted by centrifugation. Bacteria are

resuspended in phosphate buffered saline and 100 microliters were administered by oral gavage to mice. IBD is induced in mice by supplementing drinking water with 2-3% dextran sodium sulfate for 7 days prior to bacterial gavage. At 4 hours post-gavage, mice were sacrificed and bacteria were recovered from colonic samples. Colonic contents were boiled in SDS, and the soluble fractions were used to perform a dot blot for GFP detection (induction of NsrR-regulated promoters) Detection of GFP was performed by binding of anti-GFP antibody conjugated to to HRP (horse radish peroxidase). Detection was visualized using Pierce chemiluminescent detection kit. Fig. 15 shows NsrR-regulated promoters are induced in DSS-treated mice, but not in untreated mice.

CLAIMS

1. A genetically engineered bacterium comprising:

(a) an FNR-responsive promoter, an ANR-responsive promoter or a DNR-responsive promoter that is operably linked to one or more of:

(i) a first gene encoding a non-native anti-inflammation molecule;

(ii) a first gene encoding a non-native gut barrier function enhancer molecule; and

(iii) a gene cassette encoding a biosynthetic pathway, in which the final product of the biosynthetic pathway is selected from the group consisting of an anti-inflammation molecule and a gut barrier function enhancer molecule.

2. A bacterium according to Claim 1, in which the first promoter is an FNR-responsive promoter.

3. A bacterium according to Claim 1 or 2, in which the first gene and/or gene cassette is located on a chromosome in the bacterium.

4. A bacterium according to Claim 1, 2 or 3, in which the first gene and/or gene cassette is located on a plasmid in the bacterium.

5. A bacterium according to any preceding claim, in which the anti-inflammation and/or gut barrier enhancer molecule is selected from a short-chain fatty acid, propionate, butyrate, acetate, IL-10, IL-27, TGF-ß2, TGF-ß1, GLP-2, NAPEs, elafin, and trefoil factor.

6. A bacterium according to any of Claims 1 to 4, in which the anti-inflammation and/or gut barrier enhancer molecule is selected from a scFv, antisense RNA, siRNA, and shRNA directed against a pro-inflammatory molecule selected from TNF-α, IFN-γ, IL-1β, IL-6, IL-8, IL-17, CXCL-8, and CCL2.

7. A bacterium according to any preceding claim, in which the bacterium is a probiotic bacterium.

8. A bacterium according to Claim 7, in which the bacterium is selected from the group consisting of *Bacteroides, Bifidobacterium, Clostridium, Escherichia, Lactobacillus*, and *Lactococcus*.

9. A bacterium according to Claim 8, in which the bacterium is *Escherichia coli* strain Nissle.

10. A bacterium according to any preceding claim, in which the bacterium is an auxotroph in a gene that is complemented when the bacterium is present in a mammalian gut.

11. A bacterium according to Claim 10, in which the mammalian gut is a human gut.

12. A bacterium according to Claim 10 or 11, in which the bacterium is an auxotroph in diaminopimelic acid or an enzyme in the thymine biosynthetic pathway.

13. A bacterium according to any preceding claim, in which the bacterium is further engineered to harbour a second gene coding for a substance toxic to the bacterium, in which the second gene is under the control of a second promoter that is directly or indirectly induced by an environmental factor not naturally present in a mammalian gut.

14. A bacterium according to any preceding claim, in which the bacterium is further engineered to harbour a third gene coding for a substance toxic to the bacterium, in which the third gene is under the control of the first promoter, and in which the expression of the toxic substance is delayed in time as compared to the expression of the anti-inflammation molecule, the gut barrier enhancer molecule, or the gene cassette encoding the biosynthetic pathway.

15. A pharmaceutically acceptable composition comprising a bacterium according to any preceding claim and a pharmaceutically acceptable carrier.

16. A composition according to Claim 15 formulated for oral or rectal administration.

17. Use of a composition according to Claim 15 or 16 in the manufacture of a medicament for treating or preventing an autoimmune disorder.

18. Use of a composition according to Claim 15 or 16 in the manufacture of a medicament for treating an inflammatory bowel disease or a diarrhoeal disease.

19. Use according to Claim 18, in which the autoimmune disorder is selected from the group consisting of acute disseminated encephalomyelitis (ADEM), acute necrotizing hemorrhagic leukoencephalitis, Addison's disease, agammaglobulinemia, alopecia areata, amyloidosis, ankylosing spondylitis, anti-GBM/anti-TBM nephritis, antiphospholipid syndrome (APS), autoimmune angioedema, autoimmune aplastic anemia, autoimmune

dysautonomia, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune hyperlipidemia, autoimmune immunodeficiency, autoimmune inner ear disease (AIED), autoimmune myocarditis, autoimmune oophoritis, autoimmune pancreatitis, autoimmune retinopathy, autoimmune thrombocytopenic purpura (ATP), autoimmune thyroid disease, autoimmune urticarial, Axonal & neuronal neuropathies, Balo disease, Behcet's disease, Bullous pemphigoid, Cardiomyopathy, Castleman disease, Celiac disease, Chagas disease, Chronic inflammatory demyelinating polyneuropathy (CIDP), Chronic recurrent multifocal ostomyelitis (CRMO), Churg-Strauss syndrome, Cicatricial pemphigoid/benign mucosal pemphigoid, Crohn's disease, Cogan syndrome, Cold agglutinin disease, Congenital heart block, Coxsackie myocarditis, CREST disease, Essential mixed cryoglobulinemia, Demyelinating neuropathies, Dermatitis herpetiformis, Dermatomyositis, Devic's disease (neuromyelitis optica), Discoid lupus, Dressler's syndrome, Endometriosis, Eosinophilic esophagitis, Eosinophilic fasciitis, Erythema nodosum, Experimental allergic encephalomyelitis, Evans syndrome, Fibrosing alveolitis, Giant cell arteritis (temporal arteritis), Giant cell myocarditis, Glomerulonephritis, Goodpasture's syndrome, Granulomatosis with Polyangiitis (GPA), Graves' disease, Guillain-Barre syndrome, Hashimoto's encephalitis, Hashimoto's thyroiditis, Hemolytic anemia, Henoch-Schonlein purpura, Herpes gestationis, Hypogammaglobulinemia, Idiopathic thrombocytopenic purpura (ITP), IgA nephropathy, IgG4-related sclerosing disease, Immunoregulatory lipoproteins, Inclusion body myositis, Interstitial cystitis, Juvenile arthritis, Juvenile idiopathic arthritis, Juvenile myositis, Kawasaki syndrome, Lambert-Eaton syndrome, Leukocytoclastic vasculitis, Lichen planus, Lichen sclerosus, Ligneous conjunctivitis, Linear IgA disease (LAD), Lupus (Systemic Lupus Erythematosus), chronic Lyme disease, Meniere's disease, Microscopic polyangiitis, Mixed connective tissue disease (MCTD), Mooren's ulcer, Mucha-Habermann disease, Multiple sclerosis, Myasthenia gravis, Myositis, Narcolepsy, Neuromyelitis optica (Devic's), Neutropenia, Ocular cicatricial pemphigoid, Optic neuritis, Palindromic rheumatism, PANDAS (Pediatric autoimmune Neuropsychiatric Disorders Associated with Streptococcus), Paraneoplastic cerebellar degeneration, Paroxysmal nocturnal hemoglobinuria (PNH), Parry Romberg syndrome, Parsonnage-Turner syndrome, Pars planitis (peripheral uveitis), Pemphigus, Peripheral neuropathy, Perivenous encephalomyelitis, Pernicious anemia, POEMS syndrome, Polyarteritis nodosa, Type I, II, & III autoimmune polyglandular syndromes, Polymyalgia rheumatic, Polymyositis, Postmyocardial infarction syndrome, Postpericardiotomy syndrome, Progesterone dermatitis, Primary biliary cirrhosis,

Primary sclerosing cholangitis, Psoriasis, Psoriatic arthritis, Idiopathic pulmonary fibrosis, Pyoderma gangrenosum, Pure red cell aplasia, Raynauds phenomenon, reactive arthritis, reflex sympathetic dystrophy, Reiter's syndrome, relapsing polychondritis, restless legs syndrome, retroperitoneal fibrosis, rheumatic fever, rheumatoid arthritis, sarcoidosis, Schmidt syndrome, scleritis, scleroderma, Sjogren's syndrome, sperm & testicular autoimmunity, stiff person syndrome, subacute bacterial endocarditis (SBE), Susac's syndrome, sympathetic ophthalmia, Takayasu's arteritis, temporal arteritis/giant cell arteritis, thrombocytopenic purpura (TTP), Tolosa-Hunt syndrome, transverse myelitis, type 1 diabetes, asthma, ulcerative colitis, undifferentiated connective tissue disease (UCTD), uveitis, vasculitis, vesiculobullous dermatosis, vitiligo, and Wegener's granulomatosis.

20. Use according to Claim 19, in which the autoimmune disorder is selected from the group consisting of type 1 diabetes, lupus, rheumatoid arthritis, ulcerative colitis, juvenile arthritis, psoriasis, psoriatic arthritis, celiac disease, and ankylosing spondylitis.

21. Use according to Claim 18, in which the disease or condition is selected from Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, diversion colitis, Behcet's disease, indeterminate colitis, acute watery diarrhoea, cholera, acute bloody diarrhoea, dysentery, persistent diarrhoea, short bowel syndrome, ulcerative proctitis, proctosigmoiditis, left-sided colitis, pancolitis, and fulminant colitis.

22. Use according to Claim 18, in which the inflammatory bowel disease is selected from Crohn's disease and ulcerative colitis.