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(54) Title: BACTERIA ENGINEERED TO TREAT DISEASES THAT BENEFIT FROM REDUCED GUT INFLAMMATION AND/OR TIGHTENED GUT MUCOSAL BARRIER

(57) Abrégé/Abstract:
Genetically engineered bacteria, pharmaceutical compositions thereof, and methods of treating or preventing autoimmune disorders, inhibiting inflammatory mechanisms in the gut, and/or tightening gut mucosal barrier function are disclosed.

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(54) Title: BACTERIA ENGINEERED TO TREAT DISEASES THAT BENEFIT FROM REDUCED GUT INFLAMMATION AND/OR TIGHTENED GUT MUCOSAL BARRIER

(57) Abstract: Genetically engineered bacteria, pharmaceutical compositions thereof, and methods of treating or preventing autoimmune disorders, inhibiting inflammatory mechanisms in the gut, and/or tightening gut mucosal barrier function are disclosed.

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LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

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# JUMBO APPLICATIONS/PATENTS

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

[01] This application is a continuation-in-part of PCT Application No. PCT/US2016/020530, filed March 2, 2016; and claims the benefit of U.S. Provisional Application No. 62/248,814 filed October 30, 2015; U.S. Provisional Application No. 62/248,825 filed October 30, 2015; U.S. Provisional Application No. 62/248,805 filed October 30, 2015; U.S. Provisional Application No. 62/256,042 filed November 16, 2015; U.S. Provisional Application No. 62/256,044 filed November 16, 2015; U.S. Provisional Application No. 62/256,048 filed November 16, 2015; U.S. Provisional Application No. 62/291,461 filed February 4, 2016; U.S. Provisional Application No. 62/291,470 filed February 4, 2016; U.S. Provisional Application No. 62/291,468 filed February 4, 2016; and U.S. Application No. 14/998,376, filed December 22, 2015; which are incorporated herein by reference in their entirety to provide continuity of disclosure.

[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.

## Summary

[08] The genetically engineered bacteria disclosed herein are capable of producing therapeutic anti-inflammation and/or gut barrier enhancer molecules. In some embodiments, 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. 1A, FIG. 1B, FIG 1C, FIG. 1D, FIG. 1E, and FIG. 1F** depict schematics of *E. coli* that are genetically engineered to express a propionate biosynthesis cassette (**FIG. 1A**), a butyrate biosynthesis cassette (**FIG. 1B**), an acetate biosynthesis cassette (**FIG. 1C**), a cassette for the expression of **GLP-2** (**FIG. 1D**), a cassette for the expression of human IL-10 (**FIG. 1E**) under the control of a FNR-responsive promoter. The genetically engineered E coli depicted in FIG. 1D, FIG. 1E, and FIG. 1F may further comprise a secretion system for secretion of the expressed polypeptide out of the cell.

[011] **FIG. 2A, FIG. 2B, FIG. 2C, and FIG.2D** depict schematics of a butyrate production pathway and schematics of different butyrate producing circuits. **FIG. 2A** depicts a metabolic pathway for butyrate production. **FIG. 2B** and **FIG. 2C** depict schematics of two different exemplary butyrate producing circuits, both under the control of a tetracycline inducible promoter. **FIG. 2B** depicts a bdc2 butyrate cassette under control of tet promoter on a plasmid. A "bdc2 cassette" or "bdc2 butyrate cassette" refres to a butyrate producing cassette that comprises at least the following genes: bcd2, etfB3, etfA3, hbd, crt2, pbt, and buk genes. **FIG. 2C** depicts a ter butyrate cassette (ter gene replaces the bcd2, etfB3, and etfA3 genes) under control of tet promoter on a plasmid. A "ter cassette" or "ter butyrate cassette" refers to a butyrate producing cassete that comprises at least the following genes: ter, thiA1, hbd, crt2, pbt, buk**. FIG. 2D** depicts a schematic of a third exemplary butyrate gene cassette under the control of a tetracycline inducible promoter, specifically, a tesB butyrate cassette (ter gene is present and tesB gene replaces the pbt gene and the buk gene) under control of tet promoter on a plasmid. A "tes or tesB cassette or "tes or tesB butyrate cassette" refers to a butyrate producing cassette that comprises at least ter, thiA1, hbd, crt2, and tesB genes. An alternative butyrate cassette of the disclosure comprises at least bcd2, etfB3, etfA3, thiA1, hbd, crt2, and tesB genes. In some embodiments, the tes or tesB cassette is under control of an inducible promoter other than tetracycline. Exemplary inducible promoters which may control the expression of the tesB cassette include oxygen level-dependent promoters (*e.g.*, FNR-inducible promoter), promoters induced by inflammation or an inflammatory response (RNS, ROS promoters), and promoters induced by a metabolite that may or may not be naturally present (e.g., can be exogenously added) in the gut, e.g., arabinose and tetracycline.

[012] **FIG. 3A, FIG. 3B, FIG. 3C, FIG. 3D, FIG. 3E, and FIG. 3F** depict schematics of the gene organization of exemplary bacteria of the disclosure. **FIG. 3A** and **FIG. 3B** depict the gene organization of an exemplary engineered bacterium of the invention and its induction of butyrate production under low-oxygen conditions. **FIG. 3A** depicts relatively low butyrate production under aerobic conditions in which oxygen ($O_2$) prevents (indicated by "X") FNR (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*; white boxes) is expressed.

**FIG. 3B** depicts increased butyrate production under low-oxygen or anaerobic conditions due to FNR dimerizing (two boxed "FNR"s), binding to the FNR-responsive promoter, and inducing expression of the butyrate biosynthesis enzymes, which leads to the production of butyrate. **FIG. 3C** and **FIG. 3D** depict the gene organization of an exemplary recombinant bacterium of the invention and its derepression in the presence of nitric oxide (NO). In **FIG. 3C**, in the absence of NO, the NsrR transcription factor (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) is expressed. In **FIG. 3D**, 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 black arrows and black squiggles) and ultimately to the production of butyrate.

[013] **FIG. 3E and FIG. 3F** depict the gene organization of an exemplary recombinant bacterium of the invention and its induction in the presence of H2O2. In **FIG. 3E**, in the absence of H2O2, the OxyR transcription factor (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) is expressed. In **FIG. 3F**, in the presence of H2O2, the OxyR transcription factor interacts with H2O2 and is then capable of inducing the oxyS promoter. This leads to expression of the butyrate biosynthesis enzymes (indicated by black arrows and black squiggles) and ultimately to the production of butyrate.

[014] **FIG. 4A, FIG. 4B, FIG. 4C, FIG. 4D, FIG. 4E, and FIG. 4F** depict schematics of the gene organization of exemplary bacteria of the disclosure. **FIG. 4A** and **FIG. 4B** depict the gene organization of another exemplary engineered bacterium of the invention and its induction of butyrate production under low-oxygen conditions using a different butyrate circuit from that shown in **FIG. 3**. **FIG. 4A** depicts relatively low butyrate production under aerobic conditions in which oxygen ($O_2$) prevents (indicated by "X") FNR (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*; white boxes) is expressed. **FIG. 4B** depicts increased butyrate production under low-oxygen or anaerobic conditions due to FNR dimerizing (two boxed "FNR"s), binding to the FNR-responsive promoter, and inducing expression of the

butyrate biosynthesis enzymes, which leads to the production of butyrate. **FIG. 4C** and **FIG. 4D** depict the gene organization of another exemplary recombinant bacterium of the invention and its derepression in the presence of NO. In **FIG. 4C**, in the absence of NO, the NsrR transcription factor (circle, "NsrR") binds to and represses a corresponding regulatory region. Therefore, none of the butyrate biosynthesis enzymes (*ter, thiA1, hbd, crt2, pbt, buk*) is expressed. In **FIG. 4D**, 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 black arrows and black squiggles) and ultimately to the production of butyrate. **FIG. 4E** and **FIG. 4F** depict the gene organization of another exemplary recombinant bacterium of the invention and its induction in the presence of $H_2O_2$. In **FIG. 4E**, in the absence of $H_2O_2$, the OxyR transcription factor (circle, "OxyR") binds to, but does not induce, the *oxyS* promoter. Therefore, none of the butyrate biosynthesis enzymes (*ter, thiA1, hbd, crt2, pbt, buk*) is expressed. In **FIG. 4F**, 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 black arrows and black squiggles) and ultimately to the production of butyrate.

[015] **FIG. 5A, FIG. 5B, FIG. 5C, FIG. 5D, FIG. 5E, and FIG. 5F** depict schematics of the gene organization of exemplary bacteria of the disclosure. **FIG. 5A** and **FIG. 5B** depict the gene organization of an exemplary recombinant bacterium of the invention and its induction under low-oxygen conditions. **FIG. 5A** depicts relatively low butyrate production under aerobic conditions in which oxygen ($O_2$) prevents (indicated by "X") FNR (boxed "FNR") from dimerizing and activating the FNR-responsive promoter ("FNR promoter"). Therefore, none of the butyrate biosynthesis enzymes (*ter, thiA1, hbd, crt2,* and *tesB*) is expressed. **FIG. 5B** depicts increased butyrate production under low-oxygen conditions due to FNR dimerizing (two boxed "FNR"s), binding to the FNR-responsive promoter, and inducing expression of the butyrate biosynthesis enzymes, which leads to the production of butyrate. **FIG. 5C** and **FIG. 5D** depict the gene organization of another exemplary recombinant bacterium of the invention and its derepression in the presence of NO. In **FIG. 5C**, in the absence of NO, the NsrR transcription factor ( "NsrR") binds to and represses a corresponding regulatory region. Therefore, none of the butyrate biosynthesis enzymes (*ter, thiA1, hbd, crt2, tesB*) is expressed. In **FIG. 5D**, 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 black arrows and black squiggles) and ultimately to the production of butyrate. **FIG. 5E** and **FIG. 5F** depict the gene organization of another exemplary recombinant bacterium of the invention and its induction in the presence of $H_2O_2$. In **FIG. 5E**, in the absence of $H_2O_2$, the OxyR transcription factor (circle, "OxyR") binds to, but does not induce, the *oxyS* promoter. Therefore, none of the butyrate biosynthesis enzymes (*ter, thiA1, hbd, crt2, tesB*) is expressed. In **Figs. 6F**, 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 black arrows and black squiggles) and ultimately to the production of butyrate.

[016] **FIG. 6A** and **FIG. 6B** depict schematics of the gene organization of exemplary bacteria of the disclosure for inducible propionate production. **FIG. 6A** depicts relatively low propionate production under aerobic conditions in which oxygen ($O_2$) prevents (indicated by "X") FNR (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*) is expressed. **FIG. 6B** depicts increased propionate production under low-oxygen or anaerobic conditions due to FNR dimerizing (two boxed "FNR"s), binding to the FNR-responsive promoter, and inducing expression of the propionate biosynthesis enzymes, which leads to the production of propionate. In other embodiments, propionate production is induced by NO or $H_2O_2$ as depicted and described for the butyrate cassette(s) in the preceding **FIG. 3C-3F, FIG. 4C-4F, FIG. 5C-5F**.

[017] **FIG. 7** depicts an exemplary propionate biosynthesis gene cassette.

[018] **FIG. 8A, FIG. 8B, and FIG. 8C** depict schematics of the gene organization of exemplary bacteria of the disclosure for inducible propionate production. **FIG. 8A** depicts relatively low propionate production under aerobic conditions in which oxygen ($O_2$) prevents (indicated by "X") FNR (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*) is expressed. **FIG. 8B** depicts increased propionate production under low-oxygen or anaerobic

conditions due to FNR dimerizing (two boxed "FNR"s), binding to the FNR-responsive promoter, and inducing expression of the propionate biosynthesis enzymes, which leads to the production of propionate. **FIG. 8C** depicts an exemplary propionate biosynthesis gene cassette. In other embodiments, propionate production is induced by NO or $H_2O_2$ as depicted and described for the butyrate cassette(s) in the preceding **FIG. 3C-3F, FIG. 4C-4F, FIG. 5C-5F**.

[019] **FIG. 9A and FIG. 9B** depict schematics of the gene organization of exemplary bacteria of the disclosure for inducible propionate production. **FIG. 9A** depicts relatively low propionate production under aerobic conditions in which oxygen ($O_2$) prevents (indicated by "X") FNR (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*) is expressed. **FIG. 9B** depicts increased propionate production under low-oxygen or anaerobic conditions due to FNR dimerizing (two boxed "FNR"s), binding to the FNR-responsive promoter, and inducing expression of the propionate biosynthesis enzymes, which leads to the production of propionate. In other embodiments, propionate production is induced by NO or $H_2O_2$ as depicted and described for the butyrate cassette(s) in the preceding **FIG. 3C-3F, FIG. 4C-4F, FIG. 5C-5F**.

[020] **FIG. 10A, FIG. 10B, and FIG. 10C** depict schematics of the sleeping beauty pathway and the gene organization of an exemplary bacterium of the disclosure. **FIG. 10A** depicts a schematic of a genetically engineered sleeping beauty metabolic pathway from E. coli for propionate production. The SBM pathway is cyclical and composed of a series of biochemical conversions forming propionate as a fermentative product while regenerating the starting molecule of succinyl-CoA. **FIG. 10B** and **FIG. 10C** depict schematics of the gene organization of another exemplary engineered bacterium of the invention and its induction of propionate production under low-oxygen conditions. **FIG. 10B** depicts relatively low propionate production under aerobic conditions in which oxygen ($O_2$) prevents (indicated by "X") FNR (boxed "FNR") from dimerizing and activating the FNR-responsive promoter ("FNR promoter"). Therefore, none of the propionate biosynthesis enzymes (*sbm, ygfD, ygfG, ygfH*) is expressed. **FIG. 10C** depicts increased propionate production under low-oxygen or anaerobic conditions due to FNR dimerizing (two boxed "FNR"s), binding to the FNR-responsive promoter,

and inducing expression of the propionate biosynthesis enzymes, which leads to the production of propionate. In other embodiments, propionate production is induced by NO or $H_2O_2$ as depicted and described for the butyrate cassette(s) in the preceding **FIG. 3C-3F, FIG. 4C-4F, FIG. 5C-5F**.

[021] **Fig. 11** depicts a bar graph showing butyrate production of butyrate producing strains of the disclosure. **FIG. 11** 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). 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.

[022] **FIG. 12** depicts a bar graph showing butyrate production of butyrate producing strains of the disclosure. **FIG. 12** 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.

[023] **FIG. 13** depicts a graph of butyrate production using different butyrate-producing circuits comprising a *nuoB* gene deletion. Strains depicted are BW25113 comprising a bcd-butyrate cassette, with or without a nuoB deletion, and BW25113 comprising a ter-butyrate cassette, with or without a nuoB deletion. Strains with deletion are labeled with nuoB. The NuoB gene deletion results in greater levels of butyrate production as compared to a wild-type parent control in butyrate producing strains. 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.

[024] **FIG. 14A, FIG. 14B, FIG.14C, and FIG. 14D**depict schematics and graphs showing butyrate or biomarker production of a butyrate producing circuit under the control of an FNR promoter. **FIG. 14A** depicts a schematic showing a butyrate producing circuit under the control of an FNR promoter. **FIG. 14B** depicts a bar graph of anaerobic induction of butyrate production. FNR-responsive promoters were fused to butyrate cassettes containing either the bcd or ter circuits. Transformed cells were grown in LB to early log and placed in anaerobic chamber for 4 hours to induce expression of butyrate genes. Cells were washed and resuspended in minimal media w/ 0.5% glucose and incubated microaerobically to monitor butyrate production over time. SYN-501 led to significant butyrate production under anaerobic conditions. **FIG. 14C** depicts SYN-501 in the presence and absence of glucose and oxygen *in vitro*. SYN-501 comprises pSC101 PydfZ-ter butyrate plasmid; SYN-500 comprises pSC101 PydfZ-bcd butyrate plasmid; SYN-506 comprises pSC101 nirB-bcd butyrate plasmid. **FIG. 14D** depict levels of mouse lipocalin 2 (left) and calprotectin (right) quantified by ELISA using the fecal samples in an *in vivo* model. SYN-501 reduces inflammation and/or protects gut barrier function as compared to wild type Nissle control.

[025] **FIG. 15** 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.

[026] **FIG. 16** 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.

[027] **FIG. 17** depicts a scatter graph of butyrate concentrations in the feces of mice gavaged with either H2O, 100 mM butyrate in H20, streptomycin resistant Nissle control or SYN501 comprising a PydfZ-ter ->pbt-buk butyrate plasmid. Significantly greater levels of butyrate were detected in the feces of the mice gavaged with SYN501 as compared mice gavaged with the Nissle control or those given water only. Levels are close to 2 mM and higher than the levels seen in the mice fed with H20 (+) 200 mM butyrate.

[028] **FIG. 18** depicts a bar graph comparing butyrate concentrations produced in vitro by the butyrate cassette plasmid strain SYN501 as compared to Clostridia butyricum MIYARISAN (a Japanese probiotic strain), Clostridium tyrobutyricum VPI 5392 (Type

Strain), and Clostridium butyricum NCTC 7423 (Type Strain) under aerobic and anaerobic conditions at the indicated timepoints. The Nissle strain comprising the butyrate cassette produces butyrate levels comparable to *Clostridium spp.* in RCM media.

[029] **FIG. 19** depicts a bar graph showing butyrate concentrations produced in vitro by strains comprising chromsolmally integrated butyrate copies as compared to plasmid copies. Integrated butyrate strains, SYN1001 and SYN1002 (both integrated at the agaI/rsml locus) gave comparable butyrate production to the plasmid strain SYN501.

[030] **FIG. 20A and FIG. 20B** depicts the construction and gene organization of an exemplary plasmids. **FIG. 20A** depicts the construction and gene organization of an exemplary plasmids comprising a gene encoding NsrR, a regulatory sequence from *norB,* and a butyrogenic gene cassette (pLogic031-nsrR-norB-butyrate construct). **FIG. 20B** 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).

[031] **FIG. 21** 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).

[032] **FIG. 22** 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).

[033] **FIG. 23** depicts the construction and gene organization of an exemplary plasmid comprising an *oxyS* promoter and butyrogenic gene cassette (pLogic031-oxyS-butyrogenic gene cassette).

[034] **FIG. 24** depicts the construction and gene organization of another exemplary plasmid comprising an *oxyS* promoter and butyrogenic gene cassette (pLogic046-oxyS- butyrogenic gene cassette).

[035] **FIG. 25** depicts a schematic illustrating a strategy for increasing butyrate and acetate production in engineered bacteria. Aerobic metabolism through the citric acid cycle (TCA cycle) (crossed out) is inactive in the anaerobic environment of the colon. E. coli makes high levels of acetate as an end production of fermentation. To improve

acetate production, while still maintaining highlevels of butyrate production, targeted deletion can be introduced to prevent the production of unnecessary metabolic fermentative byproducts (thereby simultaneously increasing butyrate and acetate production). Non-limiting examples of competing routes (shown in in rounded boxes) are frdA (converts phosphoenolpyruvate to succinate), ldhA (converts pyruvate to lactate) and adhE (converts Acetyl-CoA to Ethanol). Deletions of interest therefore include deletion of adhE, ldh, and frd. Thus, in certain embodiments, the genetically engineered bacteria further comprise mutations and/or deletions in one or more of frdA, ldhA, and adhE.

[036] **FIG. 26A** and **FIG. 26B** depict bar graphs showing Acetate/Butyrate production in 0.5% glucose MOPS (pH6.8) (**FIG. 26A**) and Acetate/Butyrate production in 0.5% glucuronic acid MOPS (pH6.3) (**FIG. 26B**). Deletions in deletions in endogenous adhE (Aldehyde-alcohol dehydrogenase) and ldh (lactate dehydrogenase) were introduced into Nissle strains with either integrated FNRS ter-tesB or FNRS-ter-pbt-buk butyrate cassettes.

[037] **FIG. 27** depicts a schematic of an exemplary propionate biosynthesis gene cassette.

[038] **FIG. 28** depicts a schematic of a construct comprising the sleeping beauty mutase operon from E. coli under the control of a heterologous FnrS promoter.

[039] **FIG. 29** depicts a bar graph of proprionate concentrations produced in vitro by the wild type E coli BW25113 strain and a BW25113 strain which comprises the endogenous SBM operon under the control of the FnrS promoter, as depicted in the schematic in **FIG. 28**.

[040] **FIG. 30A, FIG. 30B**, and **FIG. 30C** depict schematics of the gene organization of exemplary circuits of the disclosure for the expression of therapeutic polypeptides, which are secreted using components of the flagellar type III secretion system. A therapeutic polypeptide of interest, such as, GLP-2, IL-10, and IL-22, is assembled behind a fliC-5'UTR, and is driven by the native fliC and/or fliD promoter (**FIG. 30A** and **FIG. 30B**) or a tet-inducible promoter (**FIG. 30C**). In alternate embodiments, an inducible promoter such as oxygen level-dependent promoters (*e.g.*, FNR-inducible promoter), promoters induced by IBD specific molecules or promoters induced by inflammation or an inflammatory response (RNS, ROS promoters), and promoters induced by a metabolite that may or may not be naturally present (e.g., can be

exogenously added) in the gut, e.g., arabinose can be used. The therapeutic polypeptide of interest is either expressed from a plasmid (e.g., a medium copy plasmid) or integrated into fliC loci (thereby deleting all or a portion of fliC and/or fliD). Optionally, an N terminal part of FliC is included in the construct, as shown in **FIG. 30B** and **FIG. 30D**.

[041] **FIG. 31A and FIG. 31B** depict schematics of the gene organization of exemplary circuits of the disclosure for the expression of therapeutic polypeptides, which are secreted via a diffusible outer membrane (DOM) system. The therapeutic polypeptide of interest is fused to a prototypical N-terminal Sec-dependent secretion signal or Tat-dependent secretion signal, which is is cleaved upon secretion into the periplasmic space. Exemplary secretion tags include sec-dependent PhoA, OmpF, OmpA, cvaC, and Tat-dependent tags (TorA, FdnG, DmsA). In certain embodiments, the genetically engineered bacteria comprise deletions in one or more of lpp, pal, tolA, and/or nlpI. Optionally, periplasmic proteases are also deleted, including, but not limited to, degP and ompT, e.g., to increase stability of the polypeptide in the periplasm. A FRT-KanR-FRT cassette is used for downstream integration. Expression is driven by a tet promoter (**FIG. 31A**) or an inducible promoter, such as oxygen level-dependent promoters (*e.g.*, FNR-inducible promoter, **FIG. 31B**), promoters induced by IBD specific molecules or promoters induced by inflammation or an inflammatory response (RNS, ROS promoters), and promoters induced by a metabolite that may or may not be naturally present (e.g., can be exogenously added) in the gut, e.g., arabinose.

[042] **FIG. 32A, FIG. 32B, FIG. 32C, FIG. 32D, and FIG. 32E** depict schematics of non-limiting examples of constructs for the expression of GLP2 for bacterial secretion. **FIG. 32A** depicts a schematic of a human GLP2 construct inserted into the FliC locus, under the control of the native FliC promoter. **FIG. 32B** depicts a schematic of a human GLP2 construct, including the N terminal 20 amino acids of FliC, inserted into the FliC locus under the control of the native FliC promoter. **FIG. 32C** depicts a schematic of a human GLP2 construct, including the N-terminal 20 amino acids of FliC, inserted into the FliC locus under the control of a tet inducible promoter. **FIG. 32D** depicts a schematic of a human GLP2 construct with a N terminal OmpF secretion tag (sec-dependent secretion system) under the control of a tet inducible promoter. **FIG. 32E** depicts a schematic of a human GLP2 construct with a N terminal TorA secretion tag (tat secretion system) under the control of a tet inducible promoter.

[043] **FIG. 33A** and **FIG. 33B** depict line graphs of ELISA results. **FIG. 33A** depicts a line graph, showing an phopho-STAT3 (Tyr705) ELISA conducted on extracts from serum-starved Colo205 cells treated with supernatants from engineered bacteria comprising a PAL deletion and an integrated construct encoding hIL-22 with a phoA secretion tag. The data demonstrate that hIL-22 secreted from the engineered bacteria is functionally active. **FIG. 33B** depicts a line graph, showing an phopho-STAT3 (Tyr705) ELISA showing a antibody completion assay. Extracts from Colo205 cells were treated with the bacterial supernatants from the IL-22 overexpressing strain preincubated with increasing concentrations of neutralizing anti-IL-22 antibody. The data demonstrated that phospho-Stat3 signal induced by the secreted hIL-22 is competed away by the hIL-22 antibody MAB7821.

[044] **FIG. 34** depicts a schematic of tryptophan metabolism along the kynurenine and the serotonin arms in humans. The abbreviations for the enzymes are as follows: 3-HAO: 3-hydroxyl-anthranilate 3,4-dioxidase; AAAD: aromatic –amino acid decarboxylase; ACMSD, alpha-amino-beta-carboxymuconate-epsilon-semialdehyde decarboxylase; HIOMT, hydroxyl-O-methyltransferase; IDO, indoleamine 2,3-dioxygenase; KAT, kynurenine amino transferases I-III; KMO: kynurenine 3-monooxygenase; KYNU, kynureninase; NAT, N-acetyltransferase; TDO, tryptophan 2,3-dioxygenase; TPH, tryptophan hydroxylase; QPRT, quinolinic acid phosphoribosyl transferase.

[045] **FIG. 35** depicts a schematic of bacterial tryptophan catabolism machinery, which is genetically and functionally homologous to IDO1 enzymatic activity, as described in Vujkovic-Cvijin et al., Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism; Sci Transl Med. 2013 July 10; 5(193): 193ra91, the contents of which is herein incorporated by reference in its entirety. In certain embodiments of the disclosure, the genetically engineered bacteria comprise gene cassettes comprising one or more of the bacterial tryptophan metabolism enzymes depicted in **FIG. 35**. In certain embodiments, the genetically engineered bacteria comprise one or more gene cassettes which produce one or more of the metabolites depicted in **FIG. 35,** including but not limited to, kynurenine, indole-3-aldehyde, indole-3-acetic acid, and/or indole-3 acetaldehyde.

[046] **FIG. 36A and FIG. 36B** depict schematics of indole metabolite mode of action (**FIG.36A)** and indole biosynthesis (**FIG. 36B)**. **FIG.36A** depicts a schematic of molecular mechanisms of action of indole and its metabolites on host physiology and disease. Tryptophan catabolized by bacteria to yield indole and other indole metabolites, e.g., Indole-3-propionate (IPA) and Indole-3-aldehyde (I3A), in the gut lumen. IPA acts on intestinal cells via pregnane X receptors (PXR) to maintain mucosal homeostasis and barrier function. I3A acts on the aryl hydrocarbon receptor (AhR) found on intestinal immune cells and promotes IL-22 production. Activation of AhR plays a crucial role in gut immunity, such as in maintaining the epithelial barrier function and promoting immune tolerance to promote microbial commensalism while protecting against pathogenic infections. Indole has a number of roles, such as a signaling molecule to intestinal L cells to produce glucagon-like protein 1 (GLP-1) or as a ligand for AhR (Zhang et al. Genome Med. 2016; 8: 46). **FIG. 36B** depicts a schematic of the trypophan catabolic pathway/indole biosynthesis pathways. Host and microbiota metabolites with AhR agonistic activity are in in diamond and circled, respectively (see, e.g., Lamas et al., CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands; Nature Medicine 22, 598–605 (2016). In certain embodiments of the disclosure, the genetically engineered bacteria comprise gene cassettes comprising one or more of the bacterial tryptophan metabolism enzymes which catalyze the reactions shown in **FIGs. 36A** and **36B**. In certain embodiments, the genetically engineered bacteria comprise one or more gene cassettes which produce one or more of the metabolites depicted in **FIGs. 36A** and **36B**, including but not limited to, kynurenine, indole-3-aldehyde, indole-3-acetic acid, and/or indole-3 acetaldehyde.

[047] **FIG. 37A** and **FIG. 37B** depict diagrams of bacterial tryptophan metabolism pathways. **FIG. 37A** depicts a schematic of the bacterial tryptophan metabolism, as described, e.g., in Enzymes are numbered as follows 1) Trp 2,3 dioxygenase (EC 1.13.11.11); 2) kynurenine formidase (EC 3.5.1.49); 3) kynureninase (EC 3.7.1.3); 4) tryptophanase (EC 4.1.99.1); 5) Trp aminotransferase (EC 2.6.1.27); 6) indole lactate dehydrogenase (EC1.1.1.110); 7) Trp decarboxylase (EC 4.1.1.28); 8) tryptamine oxidase (EC 1.4.3.4); 9) Trp side chain oxidase (EC 4.1.1.43); 10) indole acetaldehyde dehydrogenase (EC 1.2.1.3); 11) indole acetic acid oxidase; 13) Trp 2-monooxygenase (EC 1.13.12.3); and 14) indole acetamide hydrolase (EC 3.5.1.0). The

dotted lines (———) indicate a spontaneous reaction. **FIG. 37B** Depicts a schematic of tryptophan derived pathways. Known AHR agonists are with asterisk. Abbreviations are as follows. Trp: Tryptophan; TrA: Tryptamine; IAAld: Indole-3-acetaldehyde; IAA: Indole-3-acetic acid; FICZ: 6-formylindolo(3,2-b)carbazole; IPyA: Indole-3-pyruvic acid; IAM: Indole-3-acetamine; IAOx: Indole-3-acetaldoxime; IAN: Indole-3-acetonitrile; N-formyl Kyn: N-formylkynurenine;; Kyn:Kynurenine; KynA: Kynurenic acid; I3C: Indole-3-carbinol; IAld: Indole-3-aldehyde; DIM: 3,3'-Diindolylmethane; ICZ: Indolo(3,2-b)carbazole. Enzymes are numbered as follows: 1. EC 1.13.11.11 (Tdo2, Bna2), EC 1.13.11.11 (Ido1); 2. EC 4.1.1.28 (Tdc); 3. EC 1.4.3.22, EC 1.4.3.4 (TynA); 4. EC 1.2.1.3 (lad1), EC 1.2.3.7 (Aao1); 5. EC 3.5.1.9 (Afmid Bna3); 6. EC 2.6.1.7 (Cclb1, Cclb2, Aadat, Got2); 7. EC 1.4.99.1 (TnaA); 8. EC 1.14.13.125 (CYP79B2, CYP79B3); 9. EC 1.4.3.2 (StaO), EC 2.6.1.27 (Aro9, aspC), EC 2.6.1.99 (Taa1), EC 1.4.1.19 (TrpDH); 10. EC 1.13.12.3 (laaM); 11. EC 4.1.1.74 (IpdC); 12. EC 1.14.13.168 (Yuc2); 13. EC 3.5.1.4 (IaaH); 14. EC 3.5.5.1. (Nit1); 15. EC 4.2.1.84 (Nit1); 16. EC 4.99.1.6 (CYP71A13); 17. EC 3.2.1.147 (Pen2). In certain embodiments of the disclosure, the genetically engineered bacteria comprise gene cassettes comprising one or more of the bacterial tryptophan metabolism enzymes depicted in **FIGs. 37A** and **37B**. In certain embodiments, the genetically engineered bacteria comprise one or more gene cassettes which produce one or more of the metabolites depicted in **FIGs. 37A** and **37B**. In certain embodiments, the one or more cassettes are on a plasmid; in other embodiments, the cassettes are integrated into the genome. In certain embodiments the one or more cassettes are under the control of inducible promoters which are induced under low-oxygen conditions, in the presence of certain molecules or metabolites, in the presence of molecules or metabolites associated with inflammation or an inflammatory response, or in the presence of some other metabolite that may or may not be present in the gut, such as arabinose.

[048] **FIG. 38** depicts a schematic of the E. coli tryptophan synthesis pathway. In Escherichia coli, tryptophan is biosynthesized from chorismate, the principal common precursor of the aromatic amino acids tryptophan, tyrosine and phenylalanine, as well as the essential compounds tetrahydrofolate, ubiquinone-8, menaquinone-8 and enterobactin (enterochelin), as shown in the superpathway of chorismate metabolism. Five genes encode five enzymes that catalyze tryptophan biosynthesis from chorismate. The five genes trpE trpD trpC trpB trpA form a single transcription unit, the trp operon. A weak

internal promoter also exists within the trpD structural gene that provides low, constitutive levels of mRNA.

[049] **FIG. 39** shows a schematic depicting an exemplary Tryptophan circuit. Tryptophan is produced from the Chorismate precursor through expression of the trpE, trpG-D (also referred to as trpD), trpC-F (also referred to as trpC), trpB and trpA genes. Optional knockout of the tryptophan Repressor trpR is also depicted. Optional production of the Chorismate precursor through expression of aroG/F/H and aroB, aroD, aroE, aroK and aroC genes is also shown. All of these genes are optionally expressed from an inducible promoter, *e.g.*, a FNR-inducible promoter. The bacteria may also include an auxotrophy, *e.g.*, deletion of thyA (Δ thyA; thymidine dependence). The bacteria may also include gene sequence(s) for yddG to express YddG to assist in the exportation of tryptophan. Non limiting example of a bacterial strain is listed.

[050] **FIG. 40** depicts one embodiment of the disclosure in which the E. coli TRP synthesis enzymes are expressed from a construct under the control of a tetracycline inducible system.

[051] **FIG. 41A, FIG. 41B, FIG. 41C, FIG. 41D, FIG. 41E, FIG. 41F, FIG. 41G, and FIG. 41H** depict schematics of non-limiting examples of embodiments of the disclosure. In all embodiments, optionally gene(s) which encode exporters may also be included. **FIG. 41A** depicts one embodiment of the disclosure, in which the genetically engineered bacteria produce tryptamine from tryptophan. The optional circuits for tryptophan production are as depicted and described in **FIG. 39**. The strain optionally comprises additional circuits as depicted and/or described in **FIG. 45A** and/or **FIG. 45B.** Alternatively, optionally, tryptophan can be imported through a transporter. In addition, the genetically engineered bacteria comprise a circuit for Tryptophan decarboxylase, e.g., from *Catharanthus roseus*, which converts tryptophan to tryptamine, e.g., under the control of an inducible promoter e.g., an FNR promoter. **FIG. 41B** depicts one embodiment of the disclosure, in which the genetically engineered bacteria produce indole-3-acetaldehyde and FICZ from tryptophan. The optional circuits for tryptophan production are as depicted and described in **FIG. 39**. The strain optionally comprises additional circuits as depicted and/or described in **FIG. 45A** and/or **FIG. 45B.** Alternatively, optionally, tryptophan can be imported through a transporter. In addition, the genetically engineered bacteria comprise a circuit for aro9 ( L-tryptophan

aminotransferase, e.g., from S. cerevisae) or aspC (aspartate aminotransferase, e.g., from E. coli, or taa1 (L-tryptophan-pyruvate aminotransferase, e.g., from Arabidopsis thaliana) or staO (L-tryptophan oxidase, e.g., from streptomyces sp. TP-A0274) or trpDH (Tryptophan dehydrogenase, e.g., from Nostoc punctiforme NIES-2108) and ipdC (Indole-3-pyruvate decarboxylase, e.g., from Enterobacter cloacae) which together produce indole-3-acetaldehyde and FICZ from tryptophan, e.g., under the control of an inducible promoter e.g., an FNR promoter. **FIG. 41C** depicts one embodiment of the disclosure, in which the genetically engineered bacteria produce indole-3-acetaldehyde and FICZ from tryptophan. The optional circuits for tryptophan production are as depicted and described in **FIG. 39**. The strain optionally comprises additional circuits as depicted and/or described in **FIG. 45A** and/or **FIG. 45B.** Alternatively, optionally, tryptophan can be imported through a transporter. In addition, the genetically engineered bacteria comprise a circuit comprising tdc (Tryptophan decarboxylase, e.g., from Catharanthus roseus), and tynA (Monoamine oxidase, e.g., from E. coli) , which converts tryptophan to indole-3-acetaldehyde and FICZ, e.g., under the control of an inducible promoter e.g., an FNR promoter. **FIG. 41D** depicts one embodiment of the disclosure, in which the genetically engineered bacteria produce indole-3-acetonitrile from tryptophan. The optional circuits for tryptophan production are as depicted and described in **FIG. 39**. The strain optionally comprises additional circuits as depicted and/or described in **FIG. 45A** and/or **FIG. 45B.** Alternatively, optionally, tryptophan can be imported through a transporter. In addition, the genetically engineered bacteria comprise a circuit for cyp79B2, (tryptophan N-monooxygenase, e.g., from Arabidopsis thaliana) or cyp79B3 (tryptophan N-monooxygenase, e.g., from Arabidopsis thaliana), which together convert tryptophan to indole-3-acetonitrile, e.g., under the control of an inducible promoter e.g., an FNR promoter. **FIG. 41E** depicts one embodiment of the disclosure, in which the genetically engineered bacteria produce kynurenine from tryptophan. The optional circuits for tryptophan production are as depicted and described in **FIG. 39**. The strain optionally comprises additional circuits as depicted and/or described in **FIG. 45A** and/or **FIG. 45B.** Alternatively, optionally, tryptophan can be imported through a transporter. In addition, the genetically engineered bacteria comprise a circuit comprising IDO1(indoleamine 2,3-dioxygenase, e.g., from homo sapiens or TDO2 (tryptophan 2,3-dioxygenase, e.g., from homo sapiens) or BNA2 (indoleamine 2,3-dioxygenase, e.g., from S. cerevisiae) and

Afmid: Kynurenine formamidase, e.g., from mouse) or BNA3 (kynurenine--oxoglutarate transaminase, e.g., from S. cerevisae) which together convert tryptophan to kynurenine, e.g., under the control of an inducible promoter e.g., an FNR promoter. **FIG. 41F** depicts one embodiment of the disclosure, in which the genetically engineered bacteria produce kynureninic acid from tryptophan. The optional circuits for tryptophan production are as depicted and described in **FIG. 39**. The strain optionally comprises additional circuits as depicted and/or described in **FIG. 45A** and/or **FIG. 45B.** Alternatively, optionally, tryptophan can be imported through a transporter. In addition, the genetically engineered bacteria comprise a circuit comprising IDO1(indoleamine 2,3-dioxygenase, e.g., from homo sapiens or TDO2 (tryptophan 2,3-dioxygenase, e.g., from homo sapiens) or BNA2 (indoleamine 2,3-dioxygenase, e.g., from S. cerevisiae) and Afmid: Kynurenine formamidase, e.g., from mouse) or BNA3 (kynurenine--oxoglutarate transaminase, e.g., from S. cerevisae) and GOT2 (Aspartate aminotransferase, mitochondrial, e.g.,from homo sapiens or AADAT (Kynurenine/alpha-aminoadipate aminotransferase, mitochondrial, e.g., from homo sapiens), or CCLB1 (Kynurenine--oxoglutarate transaminase 1, e.g., from homo sapiens) or CCLB2 (kynurenine--oxoglutarate transaminase 3, e.g., from homo sapiens, which together produce kynureninic acid from tryptophan, under the control of an inducible promoter, e.g., an FNR promoter. **FIG. 41G** depicts one embodiment of the disclosure, in which the genetically engineered bacteria produce indole from tryptophan. The optional circuits for tryptophan production are as depicted and described in **FIG. 39**. The strain optionally comprises additional circuits as depicted and/or described in **FIG. 45A** and/or **FIG. 45B.** Alternatively, optionally, tryptophan can be imported through a transporter. In addition, the genetically engineered bacteria comprise a circuit for tnaA (tryptophanase, e.g., from E. coli), which converts tryptophan to indole, e.g., under the control of an inducible promoter e.g., an FNR promoter. **FIG. 41H** depicts one embodiment of the disclosure, in which the genetically engineered bacteria produce indole-3-carbinol, indole-3-aldehyde, 3,3' diindolylmethane (DIM), indolo(3,2-b) carbazole (ICZ) from indole glucosinolate taken up through the diet. The genetically engineered bacteria comprise a circuit comprising pne2 (myrosinase, e.g., from Arabidopsis thaliana) under the control of an inducible promoter, e.g. an FNR promoter. The engineered bacterium shown in any of **FIG. 41A, FIG. 41B, FIG. 41C, FIG. 41D, FIG. 41E, FIG. 41F, FIG. 41G and FIG. 41H** may also have an auxotrophy, e.g., in one

example, the *thyA* gene can be been mutated in the *E. coli Nissle* genome, so thymidine must be supplied in the culture medium to support growth.

[052] **FIG. 42A, FIG. 42B, FIG. 42C, FIG. 42D, and FIG. 42E** depict schematics of exemplary embodiments of the disclosure, in which the genetically engineered bacteria convert tryptophan into indole-3-acetic acid. In **FIG. 42A**, the optional circuits for tryptophan production are as depicted and described in **FIG. 39**. The strain optionally comprises additional circuits as depicted and/or described in **FIG. 45A** and/or **FIG. 45B.** Alternatively, optionally, tryptophan can be imported through a transporter. In addition, the genetically engineered bacteria comprise a circuit comprising aro9 ( L-tryptophan aminotransferase, e.g., from S. cerevisae) or aspC (aspartate aminotransferase, e.g., from E. coli, or taa1 (L-tryptophan-pyruvate aminotransferase, e.g., from Arabidopsis thaliana) or staO (L-tryptophan oxidase, e.g., from streptomyces sp. TP-A0274) or trpDH (Tryptophan dehydrogenase, e.g., from Nostoc punctiforme NIES-2108) and ipdC (Indole-3-pyruvate decarboxylase, e.g., from Enterobacter cloacae) and iad1 ( Indole-3-acetaldehyde dehydrogenase, e.g., from Ustilago maydis) or AAO1 (Indole-3-acetaldehyde oxidase, e.g., from Arabidopsis thaliana) which together produce indole-3-acetic acid from tryptophan, e.g., under the control of an inducible promoter e.g., an FNR promoter. In **FIG. 42B** the optional circuits for tryptophan production are as depicted and described in **FIG. 39**. The strain optionally comprises additional circuits as depicted and/or described in **FIG. 45A** and/or **FIG. 45B.** Alternatively, optionally, tryptophan can be imported through a transporter. In addition, the genetically engineered bacteria comprise a circuit comprising tdc (Tryptophan decarboxylase, e.g.,from Catharanthus roseus) ot tynA (Monoamine oxidase, e.g., from E. coli) and or iad1 (Indole-3-acetaldehyde dehydrogenase, e.g., from Ustilago maydis) or AAO1 (Indole-3-acetaldehyde oxidase, e.g., from Arabidopsis thaliana), e.g., under the control of an inducible promoter e.g., an FNR promoter. In **FIG. 42C** the optional circuits for tryptophan production are as depicted and described in **FIG. 39**. The strain optionally comprises additional circuits as depicted and/or described in **FIG. 45A** and/or **FIG. 45B.** Alternatively, optionally, tryptophan can be imported through a transporter. In addition, the genetically engineered bacteria comprise a circuit comprising aro9 ( L-tryptophan aminotransferase, e.g., from S. cerevisae) or aspC (aspartate aminotransferase, e.g., from E. coli, or taa1 (L-tryptophan-pyruvate aminotransferase, e.g., from Arabidopsis thaliana)

or staO (L-tryptophan oxidase, e.g., from streptomyces sp. TP-A0274) or trpDH (Tryptophan dehydrogenase, e.g., from Nostoc punctiforme NIES-2108) and yuc2 ( indole-3-pyruvate monoxygenase, e.g., from Arabidopsis thaliana) e.g., under the control of an inducible promoter e.g., an FNR promoter. In **FIG. 42D** the optional circuits for tryptophan production are as depicted and described in **FIG. 39**. The strain optionally comprises additional circuits as depicted and/or described in **FIG. 45A** and/or **FIG. 45B.** Alternatively, optionally, tryptophan can be imported through a transporter. In addition, the genetically engineered bacteria comprise a circuit comprising IaaM (Tryptophan 2-monooxygenase e.g., from Pseudomonas savastanoi) and iaaH (Indoleacetamide hydrolase, e.g., from Pseudomonas savastanoi), e.g., under the control of an inducible promoter e.g., an FNR promoter. In **FIG. 42E** the optional circuits for tryptophan production are as depicted and described in **FIG. 39**. The strain optionally comprises additional circuits as depicted and/or described in **FIG. 45A** and/or **FIG. 45B.** Alternatively, optionally, tryptophan can be imported through a transporter. In addition, the genetically engineered bacteria comprise a circuit comprising cyp79B2 (tryptophan N-monooxygenase, e.g., from Arabidopsis thaliana) or cyp79B3 (tryptophan N-monooxygenase, e.g., from Arabidopsis thaliana and cyp71a13 (indoleacetaldoxime dehydratase, e.g., from Arabidopis thaliana) and nit1 (Nitrilase, e.g., from Arabidopsis thaliana) and iaaH (Indoleacetamide hydrolase, e.g., from Pseudomonas savastanoi), e.g., under the control of an inducible promoter e.g., an FNR promoter. the engineered bacterium shown in any of **FIG. 42A, FIG. 42B, FIG. 42C, FIG. 42D, and FIG. 42E** may also have an auxotrophy, e.g., in one example, the *thyA* gene can be been mutated in the *E. coli Nissle* genome, so thymidine must be supplied in the culture medium to support growth.

[053] **FIG. 43A and FIG 43B** depict schematics of cicuits for the production of indole metabolites. **FIG. 43A** depicts a schematic of an indole-3-propionic acid (IPA) synthesis circuit. IPA produced by the gut micro bioata has a significant positive effect on barrier integrity. IPA does not signal through AhR, but rather through a different receptor (PXR) (Venkatesh et al., Symbiotic Bacterial Metabolites Regulate Gastrointestinal Barrier Function via the Xenobiotic Sensor PXR and Toll-like Receptor 4; Immunity 41, 296–310, August 21, 2014). In some embodiments, IPA can be produced in a synthetic circuit by expressing two enzymes, a tryptophan ammonia lyase and an indole-3-acrylate

reductase (e.g., Tryptophan ammonia lyase (WAL) (e.g., from *Rubrivivax benzoatilyticus*) and indole-3-acrylate reductase (e.g., from *Clostridum botulinum*). Tryptophan ammonia lyase converts tryptophan to indole-3-acrylic acid, and indole-3-acrylate reductase converts indole-3-acrylic acid into IPA. Without wishing to be bound by theory, no oxygen is needed for this reaction, allowing it to proceed under low or no oxygen conditions, e.g., as those found in the mammalian gut. The strains further comprise optional circuits for tryptophan production are as depicted and described in **FIG. 39** and/or **FIG. 45A** and/or **FIG. 45B.**

**FIG. 43B** depicts a schematic of another indole-3-propionic acid (IPA) synthesis circuit. Enzymes are as follows: 1. TrpDH: tryptophan dehydrogenase, e.g., from from Nostoc punctiforme NIES-2108; FldH1/FldH2: indole-3-lactate dehydrogenase, e.g., from Clostridium sporogenes; FldA: indole-3-propionyl-CoA:indole-3-lactate CoA transferase, e.g., from Clostridium sporogenes; FldBC: indole-3-lactate dehydratase, e.g., from Clostridium sporogenes; FldD: indole-3-acrylyl-CoA reductase, e.g., from Clostridium sporogenes; AcuI: acrylyl-CoA reductase, e.g., from Rhodobacter sphaeroides. Tryptophan dehydrogenase (EC 1.4.1.19) is an enzyme that catalyzes the reversible chemical reaction converting L-tryptophan, NAD(P) and water to (indol-3-yl)pyruvate, $NH_3$, NAD(P)H and $H^+$. Indole-3-lactate dehydrogenase ((EC 1.1.1.110, e.g., Clostridium sporogenes or Lactobacillus casei) converts (indol-3yl)pyruvate and NADH and H+ to indole-3-lactate and NAD+. Indole-3-propionyl-CoA:indole-3-lactate CoA transferase (FldA) converts indole-3-lactate and indol-3-propionyl-CoA to indole-3-propionic acid and indole-3-lactate-CoA. Indole-3-acrylyl-CoA reductase (FldD) and acrylyl-CoA reductase (AcuI) convert indole-3-acrylyl-CoA to indole-3-propionyl-CoA. Indole-3-lactate dehydratase (FldBC) converts indole-3-lactate-CoA to indole-3-acrylyl-CoA. The strains further comprise optional circuits for tryptophan production are as depicted and described in **FIG. 39** and/or **FIG. 45A** and/or **FIG. 45B.**

**FIG.44A and FIG. 44B and FIG. 44C** depict bar graphs showing tryptophan production by various engineered bacterial strains. **FIG.44A** depicts a bar graph showing tryptophan production by various tryptophan producing strains. The data show expressing a feedback resistant form of AroG ($AroG^{fbr}$) is necessary to get tryptophan production. Additionally, using a feedback resistant trpE ($trpE^{fbr}$) has a positive effect on tryptophan production. **FIG. 44B** shows tryptophan production from a strain comprising a tet-

trpE$^{fbr}$DCBA, tet-aroG$^{fbr}$ construct, comparing glucose and glucuronate as carbon sources in the presence and absence of oxygen. It takes E. coli two molecules of phosphoenolpyruvate (PEP) to produce one molecule of tryptophan. When glucose is used as the carbon source, 50% of all available PEP is used to import glucose into the cell through the PTS system (Phosphotransferase system). Tryptophan production is improved by using a non-PTS sugar (glucuronate) aerobically. The data also show the positive effect of deleting tnaA (only at early time point aerobically). **FIG. 44C** depicts a bar graph showing improved tryptophan production by engineered strain comprising *ΔtrpRΔtnaA, tet-trpE$^{fbr}$DCBA, tet-aroG$^{fbr}$* through the addition of serine.

[054] **FIG. 45A, FIG. 45B, FIG. 45C, FIG. 45D, and FIG. 45E** depict schematics of exemplary embodiments of the disclosure, in which the genetically engineered bacteria comprise circuits for the production of tryptophan, tryptamine, indole acetic acid, and indole propionic acid. Any of the gene(s), gene sequence(s) and/or gene circuit(s) or cassette(s) are optionally expressed from an inducible promoter. Exemplary inducible promoters which may control the expression of the gene(s), gene sequence(s) and/or gene circuit(s) or cassette(s) include oxygen level-dependent promoters (*e.g.*, FNR-inducible promoter), promoters induced by inflammation or an inflammatory response (RNS, ROS promoters), and promoters induced by a metabolite that may or may not be naturally present (e.g., can be exogenously added) in the gut, e.g., arabinose and tetracycline. The bacteria may also include an auxotrophy, *e.g.*, deletion of thyA (Δ thyA; thymidine dependence). **FIG. 45A** depicts a tryptophan producing strain, in which tryptophan is produced from the chorismate precursor through expression of the trpE, trpG-D, trpC-F, trpB and trpA genes. AroG and TrpE are replaced with feedback resistant versions to improve tryptophan production. Optionally, bacteria may comprise any of the transporters and/or additional tryptophan circuits depicted in **FIG. 39** and/or described in the description of **FIG. 39** and/or **FIG. 45B**. Optionally, Trp Repressor and/or the tnaA gene (encoding a tryptophanase converting Trp into indole) are deleted to further increase levels of tryptophan produced. The bacteria may also include gene sequence(s) for yddG to express YddG to assist in the exportation of tryptophan. **FIG. 45B** depicts a tryptophan producing strain, in which tryptophan is produced from the chorismate precursor through expression of the trpE, trpG-D, trpC-F, trpB and trpA genes. AroG and TrpE are replaced with feedback resistant versions to improve tryptophan production. The strain further

comprises either a wild type or a feedback resistant SerA gene. Escherichia coli serA-encoded 3-phosphoglycerate (3PG) dehydrogenase catalyzes the first step of the major phosphorylated pathway of L-serine (Ser) biosynthesis. This step is an oxidation of 3PG to 3-phosphohydroxypyruvate (3PHP) with the concomitant reduction of NAD1 to NADH. E. coli uses one serine for each tryptophan produced. As a result, by expressing serA, tryptophan production is improved. Optionally, bacteria may comprise any of the transporters and/or additional tryptophan circuits depicted in **FIG. 39** and/or described in the description of **FIG. 39**. Optionally, Trp Repressor and/or the tnaA gene (encoding a tryptophanase converting Trp into indole) are deleted to further increase levels of tryptophan produced. The bacteria may also include gene sequence(s) for yddG to express YddG to assist in the exportation of tryptophan. **FIG. 45C** depicts non-limiting example of a tryptamine producing strain. Tryptophan is optionally produced from chorismate precursor, and the strain optionally comprises additional circuits as depicted and/or described in **FIG. 45A** and/or **FIG. 45B** and/or **FIG. 39**. Additionally, the strain comprises tdc (tryptophan decarboxylase, e.g., from *Catharanthus roseus*), which converts tryptophan into tryptamine. **FIG. 45D** depicts a non-limiting example of an indole-3-acetate producing strain. Tryptophan optionally is produced from chorismate precursor, and the strain optionally comprises additional circuits as depicted and/or described in **FIG. 45A** and/or **FIG. 45B** and/or **FIG. 39**. Additionally, the strain comprises trpDH (Tryptophan dehydrogenase, e.g., from *Nostoc punctiforme* NIES-2108) and ipdC (Indole-3-pyruvate decarboxylase, e.g., from Enterobacter cloacae) which together produce indole-3-acetaldehyde and FICZ though an (indol-3yl)pyruvate intermediate, and iad1 (Indole-3-acetaldehyde dehydrogenase, e.g., from Ustilago maydis), which converts indole-3-acetaldehyde into indole-3-acetate. **FIG. 45E** depicts a non-limiting example of an indole-3-propionate-producing strain. Tryptophan is optionally produced from chorismate precursor, and the strain optionally comprises additional circuits as depicted and/or described in **FIG. 45A** and/or **FIG. 45B** and/or **FIG. 39**. Additionally, the strain comprises a circuit as described in **FIG. 44**, comprising trpDH (Tryptophan dehydrogenase, e.g., from Nostoc punctiforme NIES-2108, which produces (indol-3yl)pyruvate from tryptophan), fldA (indole-3-propionyl-CoA:indole-3-lactate CoA transferase, e.g., from Clostridium sporogenes, which converts converts indole-3-lactate and indol-3-propionyl-CoA to indole-3-propionic acid and indole-3-lactate-CoA), fldB

and fldC (indole-3-lactate dehydratase e.g., from Clostridium sporogenes, which converts indole-3-lactate-CoA to indole-3-acrylyl-CoA) fldD and/or AcuI: (indole-3-acrylyl-CoA reductase, e.g., from Clostridium sporogenes and/or acrylyl-CoA reductase, e.g., from Rhodobacter sphaeroides, which convert indole-3-acrylyl-CoA to indole-3-propionyl-CoA). The circuits further comprise *fldH1 and/or fldH2 (*indole-3-lactate dehydrogenase 1 and/or 2, e.g., from Clostridium sporogenes), which converts (indol-3-yl)pyruvate into indole-3-lactate).

[055] **FIG. 46A, FIG. 46B, FIG. 46C, FIG. 46D, FIG. 46E** depict schematics of non-limiting examples of genetically engineered bacteria of the disclosure which comprises one or more gene sequence(s) and/or gene cassette(s) as described herein.

[056] **FIG. 47** depicts a map of integration sites within the *E. coli* 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.

[057] **FIG. 48** depicts an exemplary schematic of the E. coli 1917 Nissle chromosome comprising multiple mechanisms of action (MoAs).

[058] **FIG. 49A and FIG. 49B** depict schematics of bacterial chromosomes, for example the *E. coli* Nissle 1917 Chromosome. For example, **FIG. 49A** depicts a schematic of an engineered bacterium comprising, a circuit for butyrate production, a circuit for propionate production, and a circuit for production of one or more interleukins relevant to IBD. **Fig. 49B** depicts a schematic of an engineered bacterium comprising three circuits, a circuit for butyrate production, a circuit for GLP-2 expression and and a circuit for production of one or more interleukins relevant to IBD.

[059] **FIG. 50** 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.

[060] **FIG. 51** 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.

[061] **FIG. 52** 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.

[062] **FIG. 53** depicts a schematic of the outer and inner membranes of a gram-negative bacterium, and several deletion targets for generating a leaky or destabilized outer membrane, thereby facilitating the translocation of a therapeutic polypeptides to the extracellular space, e.g., therapeutic polypeptides of eukaryotic origin containing disulphide bonds. Deactivating mutations of one or more genes encoding a protein that tethers the outer membrane to the peptidoglycan skeleton, e.g., lpp, ompC, ompA, ompF, tolA, tolB, pal, and/or one or more genes encoding a periplasmic protease, e.g., degS, degP, nlpl, generates a leaky phenotype. Combinations of mutations may synergistically enhance the leaky phenotype.

[063] **FIG. 54** 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-inducible 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-

inducible promoter, drives expression of a regulatory factor, e.g. T7 polymerase, that then activates the expression of the tagged therapeutic peptide (hexagons).

[064] **FIGs. 55A- 55C** depict other non-limiting embodiments 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 ParaBAD promoter ($P_{araBAD}$), which induces expression of the Tet repressor (TetR) 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. 55A** 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. **FIG. 55B** 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. **FIG. 55C** 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 the Tet repressor (TetR) 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 either under the control of a constitutive promoter or an inducible promoter (e.g., AraC promoter) in this circuit.

[065] **FIG. 56** depicts one non-limiting embodiment of the disclosure, where an exogenous environmental condition 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.

[066] **FIG. 57** depicts another non-limiting embodiment of the disclosure, where an exogenous environmental condition 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.

[067] **FIG. 58** depicts another non-limiting embodiment of the disclosure, where an exogenous environmental condition 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.

[068] **FIG. 59** depicts one non-limiting embodiment of the disclosure, where an exogenous environmental condition or one or more environmental signals activates expression of a heterologous gene and a first recombinase from an inducible promoter or inducible promoters. The recombinase then flips a second recombinase from an inverted orientation to an active conformation. The activated second recombinase flips the 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.

[069] **FIG. 60** 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., "GeneGuard: A Modular Plasmid System Designed for Biosafety," ACS Synthetic Biology (2015) 4: 307-316.

[070] **FIG. 61** depicts β-galactosidase levels in samples comprising bacteria harboring a low-copy plasmid expressing *lacZ* from an FNR-responsive promoter selected from the exemplary FNR promoters shown in **Table 25** (Pfnr1-5). Different FNR-responsive promoters were used to create a library of anaerobic-inducible reporters with a variety of expression levels and dynamic ranges. These promoters included strong ribosome binding sites. Bacterial cultures were grown in either aerobic ($+O_2$) or anaerobic conditions ($-O_2$). Samples were removed at 4 hrs and the promoter activity based on β-galactosidase levels was analyzed by performing standard β-galactosidase colorimetric assays.

[071] **FIGs. 62A-62C** depict a schematic representation of the *lacZ* gene under the control of an exemplary FNR promoter ($P_{fnrS}$) and corresponding graphical data. **FIGs. 62A** depicts a schematic representation of the *lacZ* gene under the control of an exemplary FNR promoter ($P_{fnrS}$). *LacZ* encodes the β-galactosidase enzyme and is a common reporter gene in bacteria. **FIG. 62B** depicts FNR promoter activity as a function of β-galactosidase activity in SYN340. SYN340, an engineered bacterial strain harboring a low-copy *fnrS-lacZ* fusion gene, was grown in the presence or absence of oxygen. Values for standard β-galactosidase colorimetric assays are expressed in Miller units

(Miller, 1972). These data suggest that the *fnrS* promoter begins to drive high-level gene expression within 1 hr under anaerobic conditions. **FIG. 62C** depicts the growth of bacterial cell cultures expressing *lacZ* over time, both in the presence and absence of oxygen.

[072] **FIGs. 63A-63D** depict bar graphs, schematic, and dot blot, respectively, showing the structure or activity of reporter constructs. **FIG. 63A** and **FIG. 63B** depict bar graphs of reporter constructs activity. **FIG. 69A** depicts a graph of an ATC-inducible reporter construct expression and **FIG. 63B** depicts a graph of a nitric oxide-inducible reporter construct expression. 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. **FIG. 63C** depicts a schematic of the constructs. **FIG. 63D** 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. DSS-treated mice serve as exemplary models for HE. As in HE subjects, the guts of mice are damaged by supplementing drinking water with 2-3% dextran sodium sulfate (DSS). Chemiluminescent is shown for NsrR-regulated promoters induced in DSS-treated mice.

[073] **FIG. 64** depicts a graph of Nissle residence *in vivo*. Streptomycin-resistant Nissle was administered to mice via oral gavage without antibiotic pre-treatment. Fecal pellets from 6 total mice were monitored post-administration to determine the amount of administered Nissle still residing within the mouse gastrointestinal tract. The bars represent the number of bacteria administered to the mice. The line represents the number of Nissle recovered from the fecal samples each day for 10 consecutive days.

[074] **FIG. 65** depicts a bar graph of residence over time for streptomycin resistant Nissle in various compartments of the intestinal tract at 1, 4, 8, 12, 24, and 30 hours post gavage. Mice were treated with approximately 109 CFU, and at each timepoint, animals (n=4) were euthanized, and intestine, cecum, and colon were removed. The small intestine was cut into three sections, and the large intestine and colon each into two sections. Intestinal effluents gathered and CFUs in each compartment were determined by serial dilution plating.

[075] **FIG. 66A and FIG. 66B** depict a schematic diagrams of a wild-type clbA construct (**FIG. 66A**) and a schematic diagram of a clbA knockout construct (**FIG. 66B**).

[076] **FIG. 67** depicts a schematic of a design-build-test cycle. Steps are as follows: 1: Define the disease pathway; 2. Identify target metabolites; 3. Design genetic circuits; 4. Build synthetic biotic; 5. Activate circuit in vivo; 6. Characterize circuit activation kinetics; 7. Optimize in vitro productivity to disease threshold; 8. Test optimize circuit in animla disease model; 9. Assimilate into the microbiome; 10. Develop understanding of in vivo PK and dosing regimen.

[077] **FIG. 68** depicts a schematic of non-limiting manufacturing processes for upstream and downstream production of the genetically engineered bacteria of the present disclosure. Step 1 depicts the parameters for starter culture 1 (SC1): loop full – glycerol stock, duration overnight, temperature 37° C, shaking at 250 rpm. Step 2 depicts the parameters for starter culture 2 (SC2): 1/100 dilution from SC1, duration 1.5 hours, temperature 37° C, shaking at 250 rpm. Step 3 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. Step 4 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. Step 5 depicts the parameters for vial fill/storage: 1-2 mL aliquots, -80° C.

## Description of Embodiments

[078] 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). In some embodiments, 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.

[079] 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.

[080] 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.

[081] 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.

[082] 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.

[083] 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), GLP-2 and analogs, GLP-1, IL-10, IL-27, TGF-β1, TGF-β2, N-acylphosphatidylethanolamines (NAPEs), elafin (also called peptidase inhibitor 3 and SKALP), trefoil factor, melatonin, tryptophan, $PGD_2$, and kynurenic acid, indole metabolites, and other tryptophan metabolites, 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. Such molecules also include AHR agonists (e.g., which result in IL-22 production, e.g., indole acetic acid, indole-3-aldehyde, and indole) and and PXR agonists (e.g., IPA), as described herein. Such molecules also include HDAC inhibitors (e.g., butyrate), activators of GPR41 and/or GPR43 (e.g., butyrate and/or propionate and/or acetate), activtators of GPR109A (e.g., butyrate), inhibitors of NF-kappaB signaling (e.g., butyrate), and modulators of PPARgamma (e.g., butyrate), activators of AMPK signaling (e.g., acetate), and modulators of GLP-1 secretion. Such molecules also include hydroxyl radical scavengers and antioxidants (e.g., IPA). 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. In some instances, the "anti-inflammation molecules" and/or "gut barrier function enhancer molecules" are referred to herein as "effector molecules" or "therapeutic molecules" or "therapeutic polypeptides".

[084] As used herein, the term "recombinant microorganism" refers to a microorganism, e.g., bacterial, yeast, or viral cell, or bacteria, yeast, or virus, that has been genetically modified from its native state. Thus, a "recombinant bacterial cell" or "recombinant bacteria" refers to a bacterial cell or bacteria that have been genetically modified from their native state. For instance, a recombinant bacterial cell may have nucleotide insertions, nucleotide deletions, nucleotide rearrangements, and nucleotide modifications introduced into their DNA. These genetic modifications may be present in the chromosome of the bacteria or bacterial cell, or on a plasmid in the bacteria or bacterial cell. Recombinant bacterial cells disclosed herein may comprise exogenous nucleotide sequences on plasmids. Alternatively, recombinant bacterial cells may comprise exogenous nucleotide sequences stably incorporated into their chromosome.

[085] A "programmed or engineered microorganism" refers to a microorganism, e.g., bacterial or viral cell, or bacteria or virus, that has been genetically modified from its native state to perform a specific function. Thus, a "programmed or engineered bacterial cell" or "programmed or engineered bacteria" refers to a bacterial cell or bacteria that has been genetically modified from its native state to perform a specific function. In certain embodiments, the programmed or engineered bacterial cell has been modified to express one or more proteins, for example, one or more proteins that have a therapeutic activity or serve a therapeutic purpose. The programmed or engineered bacterial cell may additionally have the ability to stop growing or to destroy itself once the protein(s) of interest have been expressed.

[086] As used herein, the term "gene" refers to a nucleic acid fragment that encodes a protein or fragment thereof, optionally including regulatory sequences

preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. In one embodiment, a "gene" does not include regulatory sequences preceding and following the coding sequence. A "native gene" refers to a gene as found in nature, optionally with its own regulatory sequences preceding and following the coding sequence. A "chimeric gene" refers to any gene that is not a native gene, optionally comprising regulatory sequences preceding and following the coding sequence, wherein the coding sequences and/or the regulatory sequences, in whole or in part, are not found together in nature. Thus, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory and coding sequences that are derived from the same source, but arranged differently than is found in nature.

[087] As used herein, the term "gene sequence" is meant to refer to a genetic sequence, e.g., a nucleic acid sequence. The gene sequence or genetic sequence is meant to include a complete gene sequence or a partial gene sequence. The gene sequence or genetic sequence is meant to include sequence that encodes a protein or polypeptide and is also menat to include genetic sequence that does not encode a protein or polypeptide, e.g., a regulatory sequence, leader sequence, signal sequence, or other non-protein coding sequence.

[088] In some embodiments, the term "gene" or "gene sequence" 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.

[089] As used herein, a "heterologous" gene or "heterologous sequence" refers to a nucleotide sequence that is not normally found in a given cell in nature. As used herein, a heterologous sequence encompasses a nucleic acid sequence that is exogenously introduced into a given cell and can be a native sequence (naturally found or expressed in

the cell) or non-native sequence (not naturally found or expressed in the cell) and can be a natural or wild-type sequence or a variant, non-natural, or synthetic sequence. "Heterologous gene" includes a native gene, or fragment thereof, that has been introduced into the host cell in a form that is different from the corresponding native gene. For example, a heterologous gene may include a native coding sequence that is a portion of a chimeric gene to include non-native regulatory regions that is reintroduced into the host cell. A heterologous gene may also include a native gene, or fragment thereof, introduced into a non-native host cell. Thus, a heterologous gene may be foreign or native to the recipient cell; a nucleic acid sequence that is naturally found in a given cell but expresses an unnatural amount of the nucleic acid and/or the polypeptide which it encodes; and/or two or more nucleic acid sequences that are not found in the same relationship to each other in nature. As used herein, the term "endogenous gene" refers to a native gene in its natural location in the genome of an organism. As used herein, the term "transgene" refers to a gene that has been introduced into the host organism, *e.g.,* host bacterial cell, genome.

[090] As used herein, a "non-native" nucleic acid sequence refers to a nucleic acid sequence not normally present in a microorganism, 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 virus, or a sequence that is modified and/or mutated as compared to the unmodified sequence from bacteria or virus 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 some embodiments, the genetically engineered microorganism of the disclosure comprises a gene that is operably linked to a promoter that is not associated with said gene in nature. For example, in some embodiments, the genetically engineered bacteria disclosed herein comprise a gene that is operably linked to a directly or indirectly inducible promoter that is not associated with said gene in nature, *e.g.,* an FNR responsive promoter (or other promoter disclosed herein) operably linked to an anti-inflammatory or gut barrier enhancer molecule. In some embodiments, the genetically engineered virus of the disclosure

comprises a gene that is operably linked to a directly or indirectly inducible promoter that is not associated with said gene in nature, e.g., a promoter operably linked to a gene encoding an anti-inflammatory or gut barrier enhancer molecule.

[091] As used herein, the term "coding region" refers to a nucleotide sequence that codes for a specific amino acid sequence. The term "regulatory sequence" refers to a nucleotide sequence located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influences the transcription, RNA processing, RNA stability, or translation of the associated coding sequence. Examples of regulatory sequences include, but are not limited to, promoters, translation leader sequences, effector binding sites, signal sequences, and stem-loop structures. In one embodiment, the regulatory sequence comprises a promoter, *e.g.*, an FNR responsive promoter or other promoter disclosed herein.

[092] 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-inflammatory or gut barrier enhancer molecule. 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.

[093] A "butyrogenic gene cassette," "butyrate biosynthesis gene cassette," and "butyrate operon" 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. 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. The butyrogenic gene cassette may comprise genes for the aerobic biosynthesis of butyrate and/or genes for the anaerobic or microaerobic biosynthesis of butyrate. In another example of a butyrate gene cassette, the *pbt* and *buk* genes are replaced with *tesB* (e.g., from E coli). Thus a butyrogenic gene cassette may comprise ter, thiA1, hbd, crt2, and tesB.

[094] Likewise, a "propionate gene cassette" or "propionate operon" refers 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. 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, and Kandasamy 2012 Engineering Escherichia coli with acrylate pathway genes for propionic acid synthesis and its impact on mixed-acid fermentation). This operon catalyses the reduction of lactate to propionate. Dehydration of (*R*)-lactoyl-CoA leads to the production of the intermediate acryloyl-CoA by lactoyl-CoA dehydratase (LcdABC). Acrolyl-CoA is converted to propionyl-CoA by acrolyl-CoA reductase (EtfA, AcrBC). In some embodiments, the rate limiting step

catalyzed by the enzymes encoded by *etfA*, *acrB* and *acrC*, are replaced by the *acuI* gene from *R. sphaeroides*. This gene product catalyzes the NADPH-dependent acrylyl-CoA reduction to produce propionyl-CoA (Acrylyl-Coenzyme A Reductase, an Enzyme Involved in the Assimilation of 3-Hydroxypropionate by Rhodobacter sphaeroides; Asao 2013). Thus the propionate cassette comprises *pct*, *lcdA*, *lcdB*, *lcdC*, and *acuI*. In another embodiment, the homolog of AcuI in *E coli*, YhdH is used (see.e.g., Structure of Escherichia coli YhdH, a putative quinone oxidoreductase. Sulzenbacher 2004). This the propionate cassette comprises *pct*, *lcdA*, *lcdB*, lcdC, and yhdH. In alternate embodiments, the propionate gene cassette comprises pyruvate pathway propionate biosynthesis genes (see, e.g., Tseng et al., 2012), e.g., thrAfbr, thrB, thrC, ilvAfbr, aceE, aceF, and lpd, which encode homoserine dehydrogenase 1, homoserine kinase, L-threonine synthase, L-threonine dehydratase, pyruvate dehydrogenase, dihydrolipoamide acetyltrasferase, and dihydrolipoyl dehydrogenase, respectively. In some embodiments, the propionate gene cassette further comprises tesB, which encodes acyl-CoA thioesterase.

[095] In another example of a propionate gene cassette comprises the genes of the Sleeping Beauty Mutase operon, e.g., from *E. coli* (sbm, ygfD, ygfG, ygfH). Recently, this pathway has been considered and utilized for the high yield industrial production of propionate from glycerol (Akawi et al., Engineering Escherichia coli for high‐level production of propionate; J Ind Microbiol Biotechnol (2015) 42:1057–1072, the contents of which is herein incorporated by reference in its entirety). In addition, as described herein, it has been found that this pathway is also suitable for production of proprionate from glucose, e.g. by the genetically engineered bacteria of the disclosure. The SBM pathway is cyclical and composed of a series of biochemical conversions forming propionate as a fermentative product while regenerating the starting molecule of succinyl-CoA. Sbm (methylmalonyl-CoA mutase) converts succinyl CoA to L-methylmalonylCoA, YgfD is a Sbm-interacting protein kinase with GTPase activity, ygfG (methylmalonylCoA decarboxylase) converts L-methylmalonylCoA into PropionylCoA, and ygfH (propionyl-CoA/succinylCoA transferase) converts propionylCoA into propionate and succinate into succinylCoA (Sleeping beauty mutase (sbm) is expressed and interacts with ygfd in Escherichia coli; Froese 2009). This pathway is very similar to the oxidative propionate pathway of Propionibacteria, which also converts succinate to propionate. Succinyl-CoA is converted to R-methylmalonyl-CoA by methymalonyl-CoA mutase (mutAB). This is in

turn converted to S-methylmalonyl-CoA via methymalonyl-CoA epimerase (GI:18042134). There are three genes which encode methylmalonyl-CoA carboxytransferase (mmdA, PFREUD_18870, bccp) which converts methylmalonyl-CoA to propionyl-CoA.

[096] 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.

[097] An "acetate gene cassette" or "acetate operon" 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 et al., 2008). 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.

[098] Each gene or gene cassette may be present on a plasmid or bacterial chromosome. 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 or to comprise multiple different components of a gene cassette performing multiple different functions.

[099] Each gene or gene cassette may be operably linked to a promoter that is induced under low-oxygen conditions. "Operably linked" refers a nucleic acid sequence, e.g., a gene or gene cassette for producing an anti-inflammatory 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 "Operably linked" refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is affected by the other. A regulatory element is operably linked with a coding sequence when it is capable of affecting the expression of the gene coding sequence, regardless of the distance between the regulatory element and the coding sequence. More specifically, operably linked refers to a nucleic acid sequence, *e.g.*, a gene encoding an anti-inflammatory or gut barrier enhancer molecule, that is joined to a regulatory sequence in a manner which allows expression of the nucleic acid sequence, *e.g.*, the gene encoding the anti-inflammatory or gut barrier enhancer molecule. In other words, the regulatory sequence acts in cis. In one embodiment, a gene may be "directly linked" to a regulatory sequence in a manner which allows expression of the gene. In another embodiment, a gene may be "indirectly linked" to a regulatory sequence in a manner which allows expression of the gene. In one embodiment, two or more genes may be directly or indirectly linked to a regulatory sequence in a manner which allows expression of the two or more genes. A regulatory region or sequence 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.

[0100] A "promoter" as used herein, refers to a nucleotide sequence that is capable of controlling the expression of a coding sequence or gene. Promoters are generally located 5' of the sequence that they regulate. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from promoters found in nature, and/or comprise synthetic nucleotide segments. Those skilled in the art will readily ascertain that different promoters may regulate expression of a coding sequence or gene in response to a particular stimulus, e.g., in a cell- or tissue-specific manner, in

response to different environmental or physiological conditions, or in response to specific compounds. Prokaryotic promoters are typically classified into two classes: inducible and constitutive. A "constitutive promoter" refers to a promoter that allows for continual transcription of the coding sequence or gene under its control.

[0101] "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 variants are well known in the art and include, but are not limited to, Ptac promoter, BBa_J23100, a constitutive Escherichia coli σS 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 σ32 promoter (e.g., htpG heat shock promoter (BBa_J45504)), a constitutive Escherichia coli σ70 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 σA promoter (e.g., promoter veg (BBa_K143013), promoter 43 (BBa_K143013), PliaG (BBa_K823000), PlepA (BBa_K823002), Pveg (BBa_K823003)), a constitutive Bacillus subtilis σB 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)).

[0102] 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. An "inducible promoter" refers to a promoter that initiates increased levels of transcription of the coding sequence or gene under its control in response to a stimulus or an exogenous environmental condition. A "directly inducible

promoter" refers to a regulatory region, wherein the regulatory region is operably linked to a gene encoding a protein or polypeptide, where, in the presence of an inducer of said regulatory region, the protein or polypeptide 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 first gene encoding a first protein, polypeptide, or factor, e.g., a transcriptional regulator, which is capable of regulating a second regulatory region that is operably linked to a second gene, the second regulatory region may be activated or repressed, thereby activating or repressing expression of the second gene. Both a directly inducible promoter and an indirectly inducible promoter are encompassed by "inducible promoter." Exemplary inducible promoters described herein include oxygen level-dependent promoters (e.g., FNR-inducible promoter), promoters induced by inflammation or an inflammatory response (RNS, ROS promoters), and promoters induced by a metabolite that may or may not be naturally present (e.g., can be exogenously added) in the gut, e.g., arabinose and tetracycline. Examples of inducible promoters include, but are not limited to, an FNR responsive promoter, a ParaC promoter, a ParaBAD promoter, and a PTetR promoter, each of which are described in more detail herein. Examples of other inducible promoters are provided herein below.

[0103] As used herein, "stably maintained" or "stable" bacterium is used to refer to a bacterial host cell carrying non-native genetic material, e.g., a gene encoding one or more anti-inflammation and/or gut barrier enhancer molecule(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 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 engineered bacterium comprising a gene encoding a encoding a payload, e.g., one or more anti-inflammation and/or gut barrier enhancer molecule(s), in which the plasmid or chromosome carrying the gene is stably maintained in the bacterium, such that the payload can be expressed in the bacterium, and the bacterium 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.

[0104] As used herein, the term "expression" refers to the transcription and stable accumulation of sense (mRNA) or anti-sense RNA derived from a nucleic acid, and/or to translation of an mRNA into a polypeptide.

[0105] As used herein, the term "plasmid" or "vector" refers to an extrachromosomal nucleic acid, e.g., DNA, construct that is not integrated into a bacterial cell's genome. Plasmids are usually circular and capable of autonomous replication. Plasmids may be low-copy, medium-copy, or high-copy, as is well known in the art. Plasmids may optionally comprise a selectable marker, such as an antibiotic resistance gene, which helps select for bacterial cells containing the plasmid and which ensures that the plasmid is retained in the bacterial cell. A plasmid disclosed herein may comprise a nucleic acid sequence encoding a heterologous gene, e.g., a gene encoding an anti-inflammatory or gut barrier enhancer molecule.

[0106] As used herein, the term "transform" or "transformation" refers to the transfer of a nucleic acid fragment into a host bacterial cell, resulting in genetically-stable inheritance. Host bacterial cells comprising the transformed nucleic acid fragment are referred to as "recombinant" or "transgenic" or "transformed" organisms.

[0107] The term "genetic modification," as used herein, refers to any genetic change. Exemplary genetic modifications include those that increase, decrease, or abolish the expression of a gene, including, for example, modifications of native chromosomal or extrachromosomal genetic material. Exemplary genetic modifications also include the introduction of at least one plasmid, modification, mutation, base deletion, base addition, base substitution, and/or codon modification of chromosomal or extrachromosomal genetic sequence(s), gene over-expression, gene amplification, gene suppression, promoter modification or substitution, gene addition (either single or multi-copy), antisense expression or suppression, or any other change to the genetic elements of a host cell, whether the change produces a change in phenotype or not. Genetic modification can include the introduction of a plasmid, e.g., a plasmid comprising an anti-inflammatory or gut barrier enhancer molecule operably linked to a promoter, into a bacterial cell. Genetic modification can also involve a targeted replacement in the chromosome, e.g., to replace a native gene promoter with an inducible promoter, regulated promoter, strong promoter, or constitutive promoter. Genetic modification can also involve gene amplification, e.g.,

introduction of at least one additional copy of a native gene into the chromosome of the cell. Alternatively, chromosomal genetic modification can involve a genetic mutation.

[0108] As used herein, the term "genetic mutation" refers to a change or changes in a nucleotide sequence of a gene or related regulatory region that alters the nucleotide sequence as compared to its native or wild-type sequence. Mutations include, for example, substitutions, additions, and deletions, in whole or in part, within the wild-type sequence. Such substitutions, additions, or deletions can be single nucleotide changes (e.g., one or more point mutations), or can be two or more nucleotide changes, which may result in substantial changes to the sequence. Mutations can occur within the coding region of the gene as well as within the non-coding and regulatory sequence of the gene. The term "genetic mutation" is intended to include silent and conservative mutations within a coding region as well as changes which alter the amino acid sequence of the polypeptide encoded by the gene. A genetic mutation in a gene coding sequence may, for example, increase, decrease, or otherwise alter the activity (e.g., enzymatic activity) of the gene's polypeptide product. A genetic mutation in a regulatory sequence may increase, decrease, or otherwise alter the expression of sequences operably linked to the altered regulatory sequence.

[0109] As used herein, the term "transporter" is meant to refer to a mechanism, e.g., protein, proteins, or protein complex, for importing a molecule, e.g., amino acid, peptide (di-peptide, tri-peptide, polypeptide, etc), toxin, metabolite, substrate, as well as other biomolecules into the microorganism from the extracellular milieu.

[0110] As used herein, the phrase "exogenous environmental condition" or "exogenous environment signal" refers to settings, circumstances, stimuli, or biological molecules under which a promoter described herein is directly or indirectly induced. The phrase "exogenous environmental conditions" is meant to refer to the environmental conditions external to the engineered micororganism, but endogenous or native to the host subject environment. 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 an intact microorganism 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 conditions are low-oxygen, microaerobic, or anaerobic conditions, such as the environment of the mammalian gut. In some embodiments, exogenous environmental conditions are molecules or metabolites that are specific to the mammalian gut, e.g., propionate. In some embodiments, the exogenous environmental condition is a tissue-specific or disease-specific metabolite or molecule(s). In some embodiments, the exogenous environmental condition is specific to an inflammatory disease. In some embodiments, the exogenous environmental condition is a low-pH environment. In some embodiments, the genetically engineered microorganism of the disclosure comprises a pH-dependent promoter. In some embodiments, the genetically engineered microorganism of the diclosure comprise an oxygen level-dependent promoter. In some aspects, 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. 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.

[0111] Examples of oxygen level-dependent transcription factors include, but are not limited to, FNR (fumarate and nitrate reductase), ANR, and DNR. Corresponding FNR-responsive promoters, ANR (anaerobic nitrate respiration)-responsive promoters, and DNR (dissimilatory nitrate respiration regulator)-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**.

[0112] In a non-limiting example, a promoter (PfnrS) was derived from the E. coli Nissle fumarate and nitrate reductase gene S (fnrS) that is known to be highly expressed under conditions of low or no environmental oxygen (Durand and Storz, 2010; Boysen et al, 2010). The PfnrS promoter is activated under anaerobic conditions by the global transcriptional regulator FNR that is naturally found in Nissle. Under anaerobic conditions, FNR forms a dimer and binds to specific sequences in the promoters of

specific genes under its control, thereby activating their expression. However, under aerobic conditions, oxygen reacts with iron-sulfur clusters in FNR dimers and converts them to an inactive form. In this way, the PfnrS inducible promoter is adopted to modulate the expression of proteins or RNA. PfnrS is used interchangeably in this application as FNRS, fnrs, FNR, P-FNRS promoter and other such related designations to indicate the promoter PfnrS.

**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* |

[0113] 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 for the production of one or more payloads, 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.

[0114] In some embodiments, the exogenous environmental conditions are the presence or absence of reactive oxygen species (ROS). In other embodiments, the

exogenous environmental conditions are the presence or absence of reactive nitrogen species (RNS). In some embodiments, exogenous environmental conditions are biological molecules that are involved in the inflammatory response, for example, molecules present in an inflammatory disorder of the gut. In some embodiments, the exogenous environmental conditions or signals exist naturally or are naturally absent in the environment in which the recombinant bacterial cell resides. In some embodiments, the exogenous environmental conditions or signals are artificially created, for example, by the creation or removal of biological conditions and/or the administration or removal of biological molecules.

[0115] In some embodiments, the exogenous environmental condition(s) and/or signal(s) stimulates the activity of an inducible promoter. In some embodiments, the exogenous environmental condition(s) and/or signal(s) that serves to activate the inducible promoter is not naturally present within the gut of a mammal. In some embodiments, the inducible promoter is stimulated by a molecule or metabolite that is administered in combination with the pharmaceutical composition of the disclosure, for example, tetracycline, arabinose, or any biological molecule that serves to activate an inducible promoter. In some embodiments, the exogenous environmental condition(s) and/or signal(s) is added to culture media comprising a recombinant bacterial cell of the disclosure. In some embodiments, the exogenous environmental condition that serves to activate the inducible promoter is naturally present within the gut of a mammal (for example, low oxygen or anaerobic conditions, or biological molecules involved in an inflammatory response). In some embodiments, the loss of exposure to an exogenous environmental condition (for example, *in vivo*) inhibits the activity of an inducible promoter, as the exogenous environmental condition is not present to induce the promoter (for example, an aerobic environment outside the gut). "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.

[0116] "Microorganism" refers to an organism or microbe of microscopic, submicroscopic, or ultramicroscopic size that typically consists of a single cell. Examples of microrganisms include bacteria, viruses, parasites, fungi, certain algae, yeast, e.g., Saccharomyces, and protozoa. In some aspects, the microorganism is engineered ("engineered microorganism") to produce one or more therpauetic molecules, e.g., an antinflammatory or barrier enhancer molecule. In certain embodiments, the engineered microorganism is an engineered bacterium. In certain embodiments, the engineered microorganism is an engineered virus.

[0117] "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 do not contain lipopolysaccharides (LPS). In some embodiments, non-pathogenic bacteria are commensal bacteria. Examples of non-pathogenic bacteria include, but are not limited to certain strains belonging to the genus *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*, *Escherichia coli, Escherichia coli Nissle, 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. In one embodiment, the disclosure further includes non-pathogenic *Saccharomyces*, such as *Saccharomyces boulardii*. Naturally pathogenic bacteria may be genetically engineered to reduce or eliminate pathogenicity.

[0118] "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. In some embodiments, the probiotic bacteria are Gram-negative bacteria. In some embodiments, the probiotic bacteria are Gram-positive bacteria. Some species, strains, and/or subtypes of non-pathogenic bacteria are currently recognized as probiotic bacteria. Examples of probiotic bacteria include, but are not limited to, certain strains belonging to the genus *Bifidobacteria*, *Escherichia Coli*, *Lactobacillus,* and S*accharomyces e.g., Bifidobacterium bifidum, Enterococcus faecium, Escherichia coli* strain Nissle, *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus paracasei*, and *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.

[0119] As used herein, the term "modulate" and its cognates means to alter, regulate, or adjust positively or negatively a molecular or physiological readout, outcome, or process, to effect a change in said readout, outcome, or process as compared to a normal, average, wild-type, or baseline measurement. Thus, for example, "modulate" or "modulation" includes up-regulation and down-regulation. A non-limiting example of modulating a readout, outcome, or process is effecting a change or alteration in the normal or baseline functioning, activity, expression, or secretion of a biomolecule (e.g. a protein, enzyme, cytokine, growth factor, hormone, metabolite, short chain fatty acid, or other compound). Another non-limiting example of modulating a readout, outcome, or process is effecting a change in the amount or level of a biomolecule of interest, e.g. in the serum and/or the gut lumen. In another non-limiting example, modulating a readout, outcome, or process relates to a phenotypic change or alteration in one or more disease symptoms. Thus, "modulate" is used to refer to an increase, decrease, masking, altering, overriding or restoring the normal functioning, activity, or levels of a readout, outcome or process (e.g,

biomolecule of interest, and/or molecular or physiological process, and/or a phenotypic change in one or more disease symptoms).

[0120] As used herein, the term "auxotroph" or "auxotrophic" refers to an organism that requires a specific factor, *e.g.,* an amino acid, a sugar, or other nutrient) to support its growth. An "auxotrophic modification" is a genetic modification that causes the organism to die in the absence of an exogenously added nutrient essential for survival or growth because it is unable to produce said nutrient. As used herein, the term "essential gene" refers to a gene which is necessary to for cell growth and/or survival. Essential genes are described in more detail *infra* and include, but are not limited to, DNA synthesis genes (such as *thyA*), cell wall synthesis genes (such as *dapA*), and amino acid genes (such as *serA* and *metA*).

[0121] As used herein, the terms "modulate" and "treat" a disease and their cognates refer to an amelioration of a disease, disorder, and/or condition, or at least one discernible symptom thereof. In another embodiment, "modulate" and "treat" refer to an amelioration of at least one measurable physical parameter, not necessarily discernible by the patient. In another embodiment, "modulate" and "treat" refer to inhibiting the progression of a disease, disorder, and/or condition, either physically (e.g., stabilization of a discernible symptom), physiologically (e.g., stabilization of a physical parameter), or both. In another embodiment, "modulate" and "treat" refer to slowing the progression or reversing the progression of a disease, disorder, and/or condition. As used herein, "prevent" and its cognates refer to delaying the onset or reducing the risk of acquiring a given disease, disorder and/or condition or a symptom associated with such disease, disorder, and/or condition.

[0122] 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.

[0123] Treating the diseases described herein may encompass increasing levels of butyrate, increasing levels of acetate, increasing levels of butyrate and increasing GLP-2, IL-22, and/o rIL-10, and/or modulating levels of tryptophan and/or its metabolites (e.g., kynurenine), and/or providing any other anti-inflammation and/or gut barrier enhancer molecule and does not necessarily encompass the elimination of the underlying disease.

[0124] As used herein a "pharmaceutical composition" refers to a preparation of genetically engineered microorganism of the disclosure, e.g., genetically engineered bacteria or virus, 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 or viral 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, sodium 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.an autoimmune disorder. 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 a 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] As used herein, the term "bacteriostatic” or "cytostatic” refers to a molecule or protein which is capable of arresting, retarding, or inhibiting the growth, division, multiplication or replication of recombinant bacterial cell of the disclosure.

[0129] As used herein, the term “bactericidal” refers to a molecule or protein which is capable of killing the recombinant bacterial cell of the disclosure.

[0130] As used herein, the term “toxin” refers to a protein, enzyme, or polypeptide fragment thereof, or other molecule which is capable of arresting, retarding, or inhibiting the growth, division, multiplication or replication of the recombinant bacterial cell of the disclosure, or which is capable of killing the recombinant bacterial cell of the disclosure. The term “toxin” is intended to include bacteriostatic proteins and bactericidal proteins. The term “toxin” is intended to include, but not limited to, lytic proteins, bacteriocins (*e.g.*, microcins and colicins), gyrase inhibitors, polymerase inhibitors, transcription inhibitors, translation inhibitors, DNases, and RNases. The term “anti-toxin” or “antitoxin,” as used herein, refers to a protein or enzyme which is capable of inhibiting the activity of a toxin. The term anti-toxin is intended to include, but not limited to, immunity modulators, and inhibitors of toxin expression. Examples of toxins and antitoxins are known in the art and described in more detail *infra*.

[0131] As used herein, “payload” refers to one or more molecules of interest to be produced by a genetically engineered microorganism, such as a bacteria or a virus. In some embodiments, the payload is a therapeutic payload, e.g. and antiinflammatory or gut barrier enhancer molecule, e.g. butyrate, acetate, propionate, GLP-2, IL-10, IL-22, IL-2, other interleukins, and/or tryptophan and/or one or more of its metabolites. In some embodiments, the payload is a regulatory molecule, e.g., a transcriptional regulator such as FNR. In some embodiments, the payload comprises a regulatory element, such as a promoter or a repressor. In some embodiments, the payload comprises an inducible promoter, such as from FNRS. In some embodiments the payload comprises a repressor element, such as a kill switch. In some embodiments the payload comprises an antibiotic resistance gene or genes. In some embodiments, the payload is encoded by a gene, multiple genes, gene cassette, or an operon. In alternate embodiments, the payload is produced by a biosynthetic or biochemical pathway, wherein the biosynthetic or biochemical pathway may optionally be endogenous to the microorganism. In alternate embodiments, the payload is produced by a biosynthetic or biochemical pathway, wherein the biosynthetic or biochemical pathway is not endogenous to the microorganism. In some embodiments, the genetically engineered microorganism comprises two or more payloads.

[0132] As used herein, the term “conventional treatment” or “conventional therapy” refers to treatment or therapy that is currently accepted, considered current standard of care, and/or used by most healthcare professionals for treating a disease or disorder associated with BCAA. It is different from alternative or complementary therapies, which are not as widely used.

[0133] As used herein, the term “polypeptide” includes “polypeptide” as well as “polypeptides,” and refers to a molecule composed of amino acid monomers linearly linked by amide bonds (i.e., peptide bonds). The term “polypeptide” refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, “peptides,” “dipeptides,” “tripeptides, “oligopeptides,” “protein,” “amino acid chain,” or any other term used to refer to a chain or chains of two or more amino acids, are included within the definition of “polypeptide,” and the term “polypeptide” may be used instead of, or interchangeably with any of these terms. The term “polypeptide” is also intended to refer to the products of post-expression modifications of the polypeptide, including but not limited to glycosylation, acetylation, phosphorylation, amidation, derivatization, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide may be derived from a natural biological source or produced by recombinant technology. In other embodiments, the polypeptide is produced by the genetically engineered bacteria or virus of the current invention. A polypeptide of the invention may be of a size of about 3 or more, 5 or more, 10 or more, 20 or more, 25 or more, 50 or more, 75 or more, 100 or more, 200 or more, 500 or more, 1,000 or more, or 2,000 or more amino acids. Polypeptides may have a defined three-dimensional structure, although they do not necessarily have such structure. Polypeptides with a defined three-dimensional structure are referred to as folded, and polypeptides, which do not possess a defined three-dimensional structure, but rather can adopt a large number of different conformations, are referred to as unfolded. The term “peptide” or “polypeptide” may refer to an amino acid sequence that corresponds to a protein or a portion of a protein or may refer to an amino acid sequence that corresponds with non-protein sequence, e.g., a sequence selected from a regulatory peptide sequence, leader peptide sequence, signal peptide sequence, linker peptide sequence, and other peptide sequence.

[0134] An “isolated” polypeptide or a fragment, variant, or derivative thereof refers to a polypeptide that is not in its natural milieu. No particular level of purification is

required. Recombinantly produced polypeptides and proteins expressed in host cells, including but not limited to bacterial or mammalian cells, are considered isolated for purposed of the invention, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique. Recombinant peptides, polypeptides or proteins refer to peptides, polypeptides or proteins produced by recombinant DNA techniques, i.e. produced from cells, microbial or mammalian, transformed by an exogenous recombinant DNA expression construct encoding the polypeptide. Proteins or peptides expressed in most bacterial cultures will typically be free of glycan. Fragments, derivatives, analogs or variants of the foregoing polypeptides, and any combination thereof are also included as polypeptides. The terms "fragment," "variant," "derivative" and "analog" include polypeptides having an amino acid sequence sufficiently similar to the amino acid sequence of the original peptide and include any polypeptides, which retain at least one or more properties of the corresponding original polypeptide. Fragments of polypeptides of the present invention include proteolytic fragments, as well as deletion fragments. Fragments also include specific antibody or bioactive fragments or immunologically active fragments derived from any polypeptides described herein. Variants may occur naturally or be non-naturally occurring. Non-naturally occurring variants may be produced using mutagenesis methods known in the art. Variant polypeptides may comprise conservative or non-conservative amino acid substitutions, deletions or additions.

[0135] Polypeptides also include fusion proteins. As used herein, the term "variant" includes a fusion protein, which comprises a sequence of the original peptide or sufficiently similar to the original peptide. As used herein, the term "fusion protein" refers to a chimeric protein comprising amino acid sequences of two or more different proteins. Typically, fusion proteins result from well known in vitro recombination techniques. Fusion proteins may have a similar structural function (but not necessarily to the same extent), and/or similar regulatory function (but not necessarily to the same extent), and/or similar biochemical function (but not necessarily to the same extent) and/or immunological activity (but not necessarily to the same extent) as the individual original proteins which are the components of the fusion proteins."Derivatives" include but are not limited to peptides, which contain one or more naturally occurring amino acid derivatives of the twenty standard amino acids. "Similarity" between two peptides is determined by

comparing the amino acid sequence of one peptide to the sequence of a second peptide. An amino acid of one peptide is similar to the corresponding amino acid of a second peptide if it is identical or a conservative amino acid substitution. Conservative substitutions include those described in Dayhoff, M. O., ed., The Atlas of Protein Sequence and Structure 5, National Biomedical Research Foundation, Washington, D.C. (1978), and in Argos, EMBO J. 8 (1989), 779-785. For example, amino acids belonging to one of the following groups represent conservative changes or substitutions: -Ala, Pro, Gly, Gln, Asn, Ser, Thr; -Cys, Ser, Tyr, Thr; -Val, Ile, Leu, Met, Ala, Phe; -Lys, Arg, His; -Phe, Tyr, Trp, His; and -Asp, Glu.

[0136] An antibody generally refers to a polypeptide of the immunoglobulin family or a polypeptide comprising fragments of an immunoglobulin that is capable of noncovalently, reversibly, and in a specific manner binding a corresponding antigen. An exemplary antibody structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD), connected through a disulfide bond. The recognized immunoglobulin genes include the κ, λ, α, γ, δ, ε, and μ constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either κ or λ. Heavy chains are classified as γ, μ, α, δ, or ε, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD, and IgE, respectively. The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (VL) and variable heavy chain (VH) refer to these regions of light and heavy chains respectively.

[0137] As used herein, the term "antibody" or “antibodies”is meant to encompasses all variations of antibody and fragments thereof that possess one or more particular binding specificities. Thus, the term “antibody” or “antibodies” is meant to include full length antibodies, chimeric antibodies, humanized antibodies, single chain antibodies (ScFv, camelids), Fab, Fab', multimeric versions of these fragments (e.g., F(ab')2), single domain antibodies (sdAB, VHH framents), heavy chain antibodies (HCAb), nanobodies, diabodies, and minibodies. Antibodies can have more than one binding specificity, e.g., be bispecific. The term “antibody” is also meant to include so-called antibody mimetics. Antibody mimetics refers to small molecules, e.g., 3-30 kDa, which can be single amino acid chain molecules, which can specifically bind antigens but

do not have an antibody-related structure. Antibody mimetics, include, but are not limited to, Affibody molecules (Z domain of Protein A), Affilins (Gamma-B crystalline), Ubiquitin, Affimers (Cystatin), Affitins (Sac7d (from Sulfolobus acidocaldarius), Alphabodies (Triple helix coiled coil), Anticalins (Lipocalins), Avimers (domains of various membrane receptors), DARPins (Ankyrin repeat motif), Fynomers (SH3 domain of Fyn), Kunitz domain peptides Kunitz domains of various protease inhibitors), Ecallantide (Kalbitor), and Monobodies. In certain aspects, the term "antibody" or "antibodies" is meant to refer to a single chain antibody(ies), single domain antibody(ies), and camelid antibody(ies). Utility of antibodies in the treatment of cancer and additional anti cancer antibodies can for example be found in Scott et al., Antibody Therapy for Cancer, Nature Reviews Cancer April 2012 Volume 12, incorporated by reference in its entirety.

[0138] A "single-chain antibody" or "single-chain antibodies" typically refers to a peptide comprising a heavy chain of an immunoglobulin, a light chain of an immunoglobulin, and optionally a linker or bond, such as a disulfide bond. The single-chain antibody lacks the constant Fc region found in traditional antibodies. In some embodiments, the single-chain antibody is a naturally occurring single-chain antibody, e.g., a camelid antibody. In some embodiments, the single-chain antibody is a synthetic, engineered, or modified single-chain antibody. In some embodiments, the single-chain antibody is capable of retaining substantially the same antigen specificity as compared to the original immunoglobulin despite the addition of a linker and the removal of the constant regions. In some aspects, the single chain antibody can be a "scFv antibody", which refers to a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of immunoglobulins (without any constant regions), optionally connected with a short linker peptide of ten to about 25 amino acids, as described, for example, in U.S. Patent No. 4,946,778, the contents of which is herein incorporated by reference in its entirety. The Fv fragment is the smallest fragment that holds a binding site of an antibody, which binding site may, in some aspects, maintain the specificity of the original antibody. Techniques for the production of single chain antibodies are described in U.S. Patent No. 4,946,778. The Vh and VL sequences of the scFv can be connected via the N-terminus of the VH connecting to the C-terminus of the VL or via the C-terminus of the VH connecting to the N-terminus of the VL. ScFv fragments are independent folding entities

that can be fused indistinctively on either end to other epitope tags or protein domains. Linkers of varying length can be used to link the Vh and VL sequences, which the linkers can be glycine rich (provides flexibility) and serine or threonine rich (increases solubility). Short linkers may prevent association of the two domains and can result in multimers (diabodies, tribodies, etc.). Long linkers may result in proteolysis or weak domain association (described in Voelkel et al el., 2011). Linkers of length between 15 and 20 amino acids or 18 and 20 amino acids are most often used. Additional non-limiting examples of linkers, including other flexible linkers are described in Chen et al., 2013 (Adv Drug Deliv Rev. 2013 Oct 15; 65(10): 1357–1369. Fusion Protein Linkers: Property, Design and Functionality), the contents of which is herein incorporated by reference in its entirety. Flexible linkers are also rich in small or polar amino acids such as Glycine and Serine, but can contain additional amino acids such as Threonine and Alanine to maintain flexibility, as well as polar amino acids such as Lysine and Glutamate to improve solubility. Exemplary linkers include, but are not limited to, (Gly-Gly-Gly-Gly-Ser)n, KESGSVSSEQLAQFRSLD and EGKSSGSGSESKST, (Gly)8, and Gly and Ser rich flexible linker, GSAGSAAGSGEF. "Single chain antibodies" as used herein also include single-domain antibodies, which include camelid antibodies and other heavy chain antibodies, light chain antibodies, including nanobodies and single domains VH or VL domains derived from human, mouse or other species. Single domain antibodies may be derived from any species including, but not limited to mouse, human, camel, llama, fish, shark, goat, rabbit, and bovine. Single domain antibodies include domain antigen-binding units which have a camelid scaffold, derived from camels, llamas, or alpacas. Camelids produce functional antibodies devoid of light chains. The heavy chain variable (VH) domain folds autonomously and functions independently as an antigen-binding unit. Its binding surface involves only three CDRs as compared to the six CDRs in classical antigen-binding molecules (Fabs) or single chain variable fragments (scFvs). Camelid antibodies are capable of attaining binding affinities comparable to those of conventional antibodies. Camelid scaffold-based antibodies can be produced using methods well known in the art. Cartilaginous fishes also have heavy-chain antibodies (IgNAR, 'immunoglobulin new antigen receptor'), from which single-domain antibodies called VNAR fragments can be obtained. Alternatively, the dimeric variable domains from IgG from humans or mice

can be split into monomers. Nanobodies are single chain antibodies derived from light chains. The term "single chain antibody" also refers to antibody mimetics.

[0139] In some embodiments, the antibodies expressed by the engineered microorganisms are bispecfic. In certain embodiments, a bispecific antibody molecule comprises a scFv, or fragment thereof, have binding specificity for a first epitope and a scFv, or fragment thereof, have binding specificity for a second epitope. Antigen-binding fragments or antibody portions include bivalent scFv (diabody), bispecific scFv antibodies where the antibody molecule recognizes two different epitopes, single binding domains (dAbs), and minibodies. Monomeric single-chain diabodies (scDb) are readily assembled in bacterial and mammalian cells and show improved stability under physiological conditions (Voelkel et al., 2001 and references therein; Protein Eng. (2001) 14 (10): 815-823 (describes optimized linker sequences for the expression of monomeric and dimeric bispecific single-chain diabodies).

[0140] As used herein, the term "sufficiently similar" means a first amino acid sequence that contains a sufficient or minimum number of identical or equivalent amino acid residues relative to a second amino acid sequence such that the first and second amino acid sequences have a common structural domain and/or common functional activity. For example, amino acid sequences that comprise a common structural domain that is at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100%, identical are defined herein as sufficiently similar. Preferably, variants will be sufficiently similar to the amino acid sequence of the peptides of the invention. Such variants generally retain the functional activity of the peptides of the present invention. Variants include peptides that differ in amino acid sequence from the native and wt peptide, respectively, by way of one or more amino acid deletion(s), addition(s), and/or substitution(s). These may be naturally occurring variants as well as artificially designed ones.

[0141] As used herein the term "linker", "linker peptide" or "peptide linkers" or "linker" refers to synthetic or non-native or non-naturally-occurring amino acid sequences that connect or link two polypeptide sequences, e.g., that link two polypeptide domains.

As used herein the term "synthetic" refers to amino acid sequences that are not naturally occurring. Exemplary linkers are described herein. Additional exemplary linkers are provided in US 20140079701, the contents of which are herein incorporated by reference in its entirety.

[0142] As used herein the term "codon-optimized" refers to the modification of codons in the gene or coding regions of a nucleic acid molecule to reflect the typical codon usage of the host organism without altering the polypeptide encoded by the nucleic acid molecule. Such optimization includes replacing at least one, or more than one, or a significant number, of codons with one or more codons that are more frequently used in the genes of the host organism. A "codon-optimized sequence" refers to a sequence, which was modified from an existing coding sequence, or designed, for example, to improve translation in an expression host cell or organism of a transcript RNA molecule transcribed from the coding sequence, or to improve transcription of a coding sequence. Codon optimization includes, but is not limited to, processes including selecting codons for the coding sequence to suit the codon preference of the expression host organism. Many organisms display a bias or preference for use of particular codons to code for insertion of a particular amino acid in a growing polypeptide chain. Codon preference or codon bias, differences in codon usage between organisms, is allowed by the degeneracy of the genetic code, and is well documented among many organisms. Codon bias often correlates with the efficiency of translation of messenger RNA (mRNA), which is in turn believed to be dependent on, inter alia, the properties of the codons being translated and the availability of particular transfer RNA (tRNA) molecules. The predominance of selected tRNAs in a cell is generally a reflection of the codons used most frequently in peptide synthesis. Accordingly, genes can be tailored for optimal gene expression in a given organism based on codon optimization.

[0143] As used herein, the terms "secretion system" or "secretion protein" refers to a native or non-native secretion mechanism capable of secreting or exporting a biomolecule, e.g., polypeptide from the microbial, e.g., bacterial cytoplasm. The secretion system may comprise a single protein or may comprise two or more proteins assembled in a complex e.g.,HlyBD. Non-limiting examples of secretion systems for gram negative bacteria include the modified type III flagellar, type I (e.g., hemolysin secretion system), type II, type IV, type V, type VI, and type VII secretion systems, resistance-nodulation-

division (RND) multi-drug efflux pumps, various single membrane secretion systems. Non-liming examples of secretion systems for gram positive bacteria include Sec and TAT secretion systems. In some embodiments, the polypeptide to be secreted include a "secretion tag" of either RNA or peptide origin to direct the polypeptide to specific secretion systems. In some embodiments, the secretion system is able to remove this tag before secreting the polyppetide from the engineered bacteria. For example, in Type V auto-secretion-mediated secretion the N-terminal peptide secretion tag is removed upon translocation of the "passenger" peptide from the cytoplasm into the periplasmic compartment by the native Sec system. Further, once the auto-secretor is translocated across the outer membrane the C-terminal secretion tag can be removed by either an autocatalytic or protease-catalyzed e.g., OmpT cleavage thereby releasing the antinflammatory or barrier enhancer molecule(s) into the extracellular milieu. In some embodiments, the secretion system involves the generation of a "leaky" or de-stabilized outer membrane, which may be accomplished by deleting or mutagenizing genes responsible for tethering the outer membrane to the rigid peptidoglycan skeleton, including for example, lpp, ompC, ompA, ompF, tolA, tolB, pal, degS, degP, and nlpl. Lpp functions as the primary 'staple' of the bacterial cell wall to the peptidoglycan. TolA-PAL and OmpA complexes function similarly to Lpp and are other deletion targets to generate a leaky phenotype. Additionally, leaky phenotypes have been observed when periplasmic proteases, such as degS, degP or nlpI, are deactivated. Thus, in some embodiments, the engineered bacteria have one or more deleted or mutated membrane genes, e.g., selected from lpp, ompA, ompA, ompF, tolA, tolB, and pal genes. In some embodiments, the engineered bacteria have one or more deleted or mutated periplasmic protease genes, e.g., selected from degS, degP, and nlpl. In some embodiments, the engineered bacteria have one or more deleted or mutated gene(s), selected from lpp, ompA, ompA, ompF, tolA, tolB, pal, degS, degP, and nlpl genes.

[0144] The articles "a" and "an," as used herein, should be understood to mean "at least one," unless clearly indicated to the contrary.

[0145] 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.

[0146] Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50.

Bacteria

[0147] The genetically engineered microorganisms, or programmed microorganisms, such as genetically engineered bacteria of the disclosure are capable of producing one or more non-native anti-inflammation and/or gut barrier function enhancer molecules. In certain embodiments, the genetically engineered bacteria are obligate anaerobic bacteria. In certain embodiments, the genetically engineered bacteria are facultative anaerobic bacteria. In certain embodiments, the genetically engineered bacteria are aerobic bacteria. In some embodiments, the genetically engineered bacteria are Gram-positive bacteria. In some embodiments, the genetically engineered bacteria are Gram-positive bacteria and lack LPS. In some embodiments, the genetically engineered bacteria are Gram-negative bacteria. In some embodiments, the genetically engineered bacteria are Gram-positive and obligate anaerobic bacteria. In some embodiments, the genetically engineered bacteria are Gram-positive and facultative anaerobic bacteria. In some embodiments, the genetically engineered bacteria are 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. Exemplary bacteria include, but are not limited to, Bacillus, Bacteroides, Bifidobacterium, Brevibacteria, Caulobacter, Clostridium, Enterococcus, Escherichia coli, Lactobacillus, Lactococcus, Listeria, Mycobacterium, Saccharomyces, Salmonella, Staphylococcus, Streptococcus, Vibrio, Bacillus coagulans, Bacillus subtilis, Bacteroides fragilis, Bacteroides subtilis, Bacteroides thetaiotaomicron, Bifidobacterium adolescentis, Bifidobacterium bifidum, Bifidobacterium breve UCC2003, Bifidobacterium infantis, Bifidobacterium lactis, Bifidobacterium longum, Clostridium acetobutylicum, Clostridium butyricum,

Clostridium butyricum M-55, Clostridium cochlearum, Clostridium felsineum, Clostridium histolyticum, Clostridium multifermentans, Clostridium novyi-NT, Clostridium paraputrificum, Clostridium pasteureanum, Clostridium pectinovorum, Clostridium perfringens, Clostridium roseum, Clostridium sporogenes, Clostridium tertium, Clostridium tetani, Clostridium tyrobutyricum, Corynebacterium parvum, Escherichia coli MG1655, Escherichia coli Nissle 1917, Listeria monocytogenes, Mycobacterium bovis, Salmonella choleraesuis, Salmonella typhimurium, and Vibrio cholera. In certain embodiments, the genetically engineered bacteria are selected from the group consisting of Enterococcus faecium, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus johnsonii, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus reuteri, Lactobacillus rhamnosus, Lactococcus lactis, and 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 the genetically engineered bacteria are selected from Bacteroides fragilis, Bacteroides thetaiotaomicron, Bacteroides subtilis, Bifidobacterium bifidum, Bifidobacterium infantis, Bifidobacterium lactis, Clostridium butyricum, Escherichia coli, Escherichia coli Nissle, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus reuteri, and Lactococcus lactis

[0148] 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).

[0149] In one embodiment, the bacterial cell is a Bacteroides fragilis bacterial cell. In one embodiment, the bacterial cell is a Bacteroides thetaiotaomicron bacterial cell. In one embodiment, the bacterial cell is a Bacteroides subtilis bacterial cell. In one embodiment, the bacterial cell is a Bifidobacterium bifidum bacterial cell. In one embodiment, the bacterial cell is a Bifidobacterium infantis bacterial cell. In one embodiment, the bacterial cell is a Bifidobacterium lactis bacterial cell. In one embodiment, the bacterial cell is a Clostridium butyricum bacterial cell. In one embodiment, the bacterial cell is an Escherichia coli bacterial cell. In one embodiment, the bacterial cell is a Lactobacillus acidophilus bacterial cell. In one embodiment, the bacterial cell is a Lactobacillus plantarum bacterial cell. In one embodiment, the bacterial cell is a Lactobacillus reuteri bacterial cell. In one embodiment, the bacterial cell is a Lactococcus lactis bacterial cell.

[0150] 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 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.

[0151] 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 fromPeptoclostridium difficile have been expressed in Escherichia coli (Aboulnaga et al., 2013).

[0152] . In one embodiment, the recombinant bacterial cell does not colonize the subject having the disorder. 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. In some embodiments, residence time in vivo is calculated for the genetically engineered bacteria of the invention, e.g. as described herein.

[0153] In some embodiments, the bacterial cell is a genetically engineered bacterial cell. In another embodiment, the bacterial cell is a recombinant bacterial cell. In some embodiments, the disclosure comprises a colony of bacterial cells disclosed herein.

[0154] In another aspect, the disclosure provides a recombinant bacterial culture which comprises bacterial cells disclosed herein.

[0155] In some embodiments, the genetically engineered bacteria comprising an anti-inflammatory or gut barrier enhancer molecule further comprise 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 antitoxin. 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 antitoxin. 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 ParaBAD. In some embodiments, the genetically engineered bacteria further comprise one or more genes encoding an antitoxin.

[0156] In some embodiments, the genetically engineered bacteria is an auxotroph comprising gene sequence encoding an anti-inflammatory or gut barrier enhancer molecule and further comprises a kill-switch circuit, such as any of the kill-switch circuits described herein.

[0157] In some embodiments of the above described genetically engineered bacteria, the gene encoding an anti-inflammatory or gut barrier enhancer molecule is present on a plasmid in the bacterium. In some embodiments, the gene sequence(s) encoding an anti-inflammatory or gut barrier enhancer molecule is present in the bacterial chromosome. In some embodiments, a gene sequence encoding a secretion protein or protein complex, such as any of the secretion systems disclosed herein, for secreting a biomolecule (e.g. an anti-inflammatory or gut barrier enhancer molecule), is present on a plasmid in the bacterium. In some embodiments, the gene sequence encoding a secretion protein or protein complex for secreting a biomolecule, such as any of the secretion systems disclosed herein, is present in the bacterial chromosome. In some embodiments, the gene sequence(s) encoding an antibiotic resistance gene is present on a plasmid in the bacterium. In some embodiments, the gene sequence(s) encoding an antibiotic resistance gene is present in the bacterial chromosome.

Anti-inflammation and/or gut barrier function enhancer molecules

[0158] 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 (human or viral), IL-27, TGF-β1, TGF-β2, N-acylphosphatidylethanolamines (NAPEs), elafin (also known as peptidase inhibitor 3 or SKALP), trefoil factor, melatonin, PGD2, kynurenic acid, kynurenine, typtophan metabolite, indole, indole metabolite, 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, AHR agonist (e.g., indole acetic acid, indole-3-aldehyde, and indole), PXR agonist (e.g., IPA), HDAC inhibitor (e.g., butyrate), GPR41 and/or GPR43 activator (e.g., butyrate and/or propionate and/or acetate), GPR109A activator (e.g., butyrate), inhibitor of NF-kappaB signaling (e.g., butyrate), modulator of PPARgamma (e.g., butyrate), activator of AMPK signaling (e.g., acetate), modulator of GLP-1 secretion, and hydroxyl radical scavengers and antioxidants (e.g., IPA). 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.

[0159] 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.

[0160] 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.

Short chain Fatty Acids and Tryptophan Metabolites

[0161] One strategy in the treatment, prevention, and/or management of inflammatory bowel disorders may include approaches to help maintain and/or reestablish gut barrier function, e.g. through the prevention, treatment and/or management of

inflammatory events at the root of increased permeability, e.g. through the administration of anti-inflammatory effectors.

[0162] For example, leading metabolites that play gut-protective roles are short chain fatty acids, e.g. acetate, butyrate and propionate, and those derived from tryptophan metabolism. These metabolites have been shown to play a major role in the prevention of inflammatory disease. As such one approach in the treatment, prevention, and/or management of gut barrier health may be to provide a treatment which contains one or more of such metabolites.

[0163] For example, butyrate and other SCFA, e.g., derived from the microbiota, are known to promote maintaining intestinal integrity (e.g., as reviewed in Thorburn et al., Diet, Metabolites, and "Western-Lifestyle" Inflammatory Diseases; Immunity Volume 40, Issue 6, 19 June 2014, Pages 833–842). (A) SCFA-induced promotion of mucus by gut epithelial cells, possibly through signaling through metabolite sensing GPCRs; (B) SCFA-induced secretion of IgA by B cells; (C) SCFA-induced promotion of tissue repair and wound healing; (D) SCFA-induced promotion of Treg cell development in the gut in a process that presumably facilitates immunological tolerance; (E) SCFA- mediated enhancement of epithelial integrity in a process dependent on inflammasome activation (e.g., via NALP3) and IL-18 production; and (F) anti-inflammatory effects, inhibition of inflammatory cytokine production (e.g., TNF, Il-6, and IFN-gamma), and inhibition of NF-κB. Many of these actions of SCFAs in gut homeostatis can be ascribed to GPR43 and GPR109A, which are expressed by the colonic epithelium, by inflammatory leukocytes (e.g. neutrophils and marcophages) and by Treg cells. These receptors signal through G proteins, coupled to MAPK, PI3K and mTOR, as well as a separate arrestin- pathway, leading to NFkappa B inhibition. Other effects can be ascribed to SCFA-mediated HDAC inhibition, e.g. butyrate, which may regulate macrophage function and promote TReg cells.

[0164] In addition, a number of trptophan metabolites, including kynurenine and kynurenic acid, as well as several indoles, such as indole-3 aldehhyde, indole-3 propionic acid, and several other indole metabolites (which can be derived from microbiota or the diet) described infra, have been shown to be essential for gut homeostais and promote gut-barrier health. These metabolites bind to aryl hydrocarbon receptor (Ahr). After agonist binding, AhR translocates to the nucleus, where it forms a heterodimer with AhR nuclear

translocator (ARNT). AhR-dependent gene expression includes genes involved in the production of mediators important for gut homeostasis; these mediators include IL-22, antimicrobicidal factors, increased Th17 cell activity, and the maintenance of intraepithelial lymphocytes and RORγt+ innate lymphoid cells.

[0165] Tryptophan can also be transported across the epithelium by transport machinery comprising angiotensin I converting enzyme 2 (Ace2). Tryptophan is degraded to kynurenine, another AhR agonist, by the immune-regulatory enzyme indoleamine 2,3-dioxygenase (IDO), which is linked to suppression of T cell responses, promotion of Treg cells, and immune tolerance. Moreover, a number of tryptophan metabolites, including kynurenic acid and niacin, agonize metabolite-sensing GPCRs, such as GPR35 and GPR109A and thus multiple elements of tryptophan catabolism facilitate gut homeostasis.

[0166] In addition, some indole metabolites, e.g., indole 3-propionic acid (IPA), may exert their effect an acitvating ligand of Pregnane X receptor (PXR), which is thought to play a key role as an essential regulator of intestinal barrier function, through downregulation of TLR4 signaling (Venkatesh et al., 2014 Symbiotic Bacterial Metabolites Regulate Gastrointestinal Barrier Function via the Xenobiotic Sensor PXR and Toll-like Receptor 4; Immunity 41, 296–310, August 21, 2014). As a result, indole levels may through the activation of PXR regulate and balance the levels of TLR4 expression to promote homeostasis and gut barrier health.

[0167] Thus, in some embodiments, the genetically engineered bacteria of the disclosure produce one or more short chain fatty acids and/or one or more tryprophan metabolites

*Butyrate*

[0168] In some embodiments, the genetically engineered bacteria of the invention comprise a butyrogenic gene cassette and are capable of producing butyrate under particular exogenous environmental conditions. The genetically engineered bacteria may include any suitable set of butyrogenic genes (*see, e.g.,* **Table 2 and Table 3**). Unmodified bacteria comprising butyrate biosynthesis genes are known and include, but are not limited to, *Peptoclostridium, Clostridium, Fusobacterium, Butyrivibrio, Eubacterium,* and *Treponema*. 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. *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. 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. 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. In another example of a butyrate gene cassette, the pbt and buk genes are replaced with tesB (e.g., from E coli). Thus a butyrogenic gene cassette may comprise ter, thiA1, hbd, crt2, and tesB.n some embodiments, the genetically engineered bacteria are capable of expressing the butyrate biosynthesis cassette and producing butyrate in low-oxygen conditions, in the presence of certain molecules or metabolites, in the presence of molecules or metabolites associated with inflammation or an inflammatory response, or in the presence of some other metabolite that may or may not be present in the gut, such as arabinose. One or more of the butyrate biosynthesis genes may be functionally replaced or modified, e.g., codon optimized.

[0169] In some embodiments, additional genes may be mutated or knocked out, to further increase the levels of butyrate production. Production under anaerobic conditions depends on endogenous NADH pools. Therefore, the flux through the butyrate pathway may be enhanced by eliminating competing routes for NADH utilization. Non-limiting examples of such competing routes are frdA (converts phosphoenolpyruvate to succinate), ldhA (converts pyruvate to lactate) and adhE (converts Acetyl-CoA to Ethanol). Thus, in certain embodiments, the genetically engineered bacteria further comprise mutations and/or deletions in one or more of frdA, ldhA, and adhE.

[0170] **Table 2** depicts the nucleic acid sequences of exemplary genes in exemplary butyrate biosynthesis gene cassettes.

**Table 2. Exemplary Butyrate Cassette Sequences**

| Description | Sequence |
|---|---|
| *bcd2* **SEQ ID NO: 1** | ATGGATTTAAATTCTAAAAAATATCAGATGCTTAAAGAGCTATATGTAAG<br>CTTCGCTGAAAATGAAGTTAAACCTTTAGCAACAGAACTTGATGAAGAAG<br>AAAGATTTCCTTATGAAACAGTGGAAAAAATGGCAAAAGCAGGAATGATG<br>GGTATACCATATCCAAAAGAATATGGTGGAGAAGGTGGAGACACTGTAGG<br>ATATATAATGGCAGTTGAAGAATTGTCTAGAGTTTGTGGTACTACAGGAG<br>TTATATTATCAGCTCATACATCTCTTGGCTCATGGCCTATATATCAATAT<br>GGTAATGAAGAACAAAAACAAAAATTCTTAAGACCACTAGCAAGTGGAGA<br>AAAATTAGGAGCATTTGGTCTTACTGAGCCTAATGCTGGTACAGATGCGT<br>CTGGCCAACAAACAACTGCTGTTTTAGACGGGGATGAATACATACTTAAT<br>GGCTCAAAAATATTTATAACAAACGCAATAGCTGGTGACATATATGTAGT<br>AATGGCAATGACTGATAAATCTAAGGGGAACAAAGGAATATCAGCATTTA<br>TAGTTGAAAAAGGAACTCCTGGGTTTAGCTTTGGAGTTAAAGAAAAGAAA<br>ATGGGTATAAGAGGTTCAGCTACGAGTGAATTAATATTTGAGGATTGCAG<br>AATACCTAAAGAAAATTTACTTGGAAAAGAAGGTCAAGGATTTAAGATAG<br>CAATGTCTACTCTTGATGGTGGTAGAATTGGTATAGCTGCACAAGCTTTA<br>GGTTTAGCACAAGGTGCTCTTGATGAAACTGTTAAATATGTAAAAGAAAG<br>AGTACAATTTGGTAGACCATTATCAAAATTCCAAAATACACAATTCCAAT<br>TAGCTGATATGGAAGTTAAGGTACAAGCGGCTAGACACCTTGTATATCAA<br>GCAGCTATAAATAAAGACTTAGGAAAACCTTATGGAGTAGAAGCAGCAAT<br>GGCAAAATTATTTGCAGCTGAAACAGCTATGGAAGTTACTACAAAAGCTG<br>TACAACTTCATGGAGGATATGGATACACTCGTGACTATCCAGTAGAAAGA<br>ATGATGAGAGATGCTAAGATAACTGAAATATATGAAGGAACTAGTGAAGT<br>TCAAAGAATGGTTATTTCAGGAAAACTATTAAAATAG |
| *etfB3* **SEQ ID NO: 2** | ATGAATATAGTCGTTTGTATAAAACAAGTTCCAGATACAACAGAAGTTAA<br>ACTAGATCCTAATACAGGTACTTTAATTAGAGATGGAGTACCAAGTATAA<br>TAAACCCTGATGATAAAGCAGGTTTAGAAGAAGCTATAAAATTAAAAGAA<br>GAAATGGGTGCTCATGTAACTGTTATAACAATGGGACCTCCTCAAGCAGA<br>TATGGCTTTAAAAGAAGCTTTAGCAATGGGTGCAGATAGAGGTATATTAT<br>TAACAGATAGAGCATTTGCGGGTGCTGATACTTGGGCAACTTCATCAGCA<br>TTAGCAGGAGCATTAAAAAATATAGATTTTGATATTATAATAGCTGGAAG<br>ACAGGCGATAGATGGAGATACTGCACAAGTTGGACCTCAAATAGCTGAAC<br>ATTTAAATCTTCCATCAATAACATATGCTGAAGAAATAAAAACTGAAGGT<br>GAATATGTATTAGTAAAAAGACAATTTGAAGATTGTTGCCATGACTTAAA<br>AGTTAAAATGCCATGCCTTATAACAACTCTTAAAGATATGAACACACCAA<br>GATACATGAAAGTTGGAAGAATATATGATGCTTTCGAAAATGATGTAGTA<br>GAAACATGGACTGTAAAAGATATAGAAGTTGACCCTTCTAATTTAGGTCT<br>TAAAGGTTCTCCAACTAGTGTATTTAAATCATTTACAAAATCAGTTAAAC<br>CAGCTGGTACAATATACAATGAAGATGCGAAAACATCAGCTGGAATTATC<br>ATAGATAAATTAAAAGAGAAGTATATCATATAA |
| *etfA3* **SEQ ID NO: 3** | ATGGGTAACGTTTTAGTAGTAATAGAACAAAGAGAAAATGTAATTCAAAC<br>TGTTTCTTTAGAATTACTAGGAAAGGCTACAGAAATAGCAAAAGATTATG<br>ATACAAAAGTTTCTGCATTACTTTTAGGTAGTAAGGTAGAAGGTTTAATA |

| Description | Sequence |
|---|---|
| | GATACATTAGCACACTATGGTGCAGATGAGGTAATAGTAGTAGATGATGA<br>AGCTTTAGCAGTGTATACAACTGAACCATATACAAAAGCAGCTTATGAAG<br>CAATAAAAGCAGCTGACCCTATAGTTGTATTATTTGGTGCAACTTCAATA<br>GGTAGAGATTTAGCGCCTAGAGTTTCTGCTAGAATACATACAGGTCTTAC<br>TGCTGACTGTACAGGTCTTGCAGTAGCTGAAGATACAAAATTATTATTAA<br>TGACAAGACCTGCCTTTGGTGGAAATATAATGGCAACAATAGTTTGTAAA<br>GATTTCAGACCTCAAATGTCTACAGTTAGACCAGGGGTTATGAAGAAAAA<br>TGAACCTGATGAAACTAAAGAAGCTGTAATTAACCGTTTCAAGGTAGAAT<br>TTAATGATGCTGATAAATTAGTTCAAGTTGTACAAGTAATAAAAGAAGCT<br>AAAAAACAAGTTAAAATAGAAGATGCTAAGATATTAGTTTCTGCTGGACG<br>TGGAATGGGTGGAAAAGAAAACTTAGACATACTTTATGAATTAGCTGAAA<br>TTATAGGTGGAGAAGTTTCTGGTTCTCGTGCCACTATAGATGCAGGTTGG<br>TTAGATAAAGCAAGACAAGTTGGTCAAACTGGTAAAACTGTAAGACCAGA<br>CCTTTATATAGCATGTGGTATATCTGGAGCAATACAACATATAGCTGGTA<br>TGGAAGATGCTGAGTTTATAGTTGCTATAAATAAAAATCCAGAAGCTCCA<br>ATATTTAAATATGCTGATGTTGGTATAGTTGGAGATGTTCATAAAGTGCT<br>TCCAGAACTTATCAGTCAGTTAAGTGTTGCAAAAGAAAAAGGTGAAGTTT<br>TAGCTAACTAA |
| *thiA1*<br>**SEQ ID NO: 4** | ATGAGAGAAGTAGTAATTGCCAGTGCAGCTAGAACAGCAGTAGGAAGTTT<br>TGGAGGAGCATTTAAATCAGTTTCAGCGGTAGAGTTAGGGGTAACAGCAG<br>CTAAAGAAGCTATAAAAAGAGCTAACATAACTCCAGATATGATAGATGAA<br>TCTCTTTTAGGGGGAGTACTTACAGCAGGTCTTGGACAAAATATAGCAAG<br>ACAAATAGCATTAGGAGCAGGAATACCAGTAGAAAAACCAGCTATGACTA<br>TAAATATAGTTTGTGGTTCTGGATTAAGATCTGTTTCAATGGCATCTCAA<br>CTTATAGCATTAGGTGATGCTGATATAATGTTAGTTGGTGGAGCTGAAAA<br>CATGAGTATGTCTCCTTATTTAGTACCAAGTGCGAGATATGGTGCAAGAA<br>TGGGTGATGCTGCTTTTGTTGATTCAATGATAAAAGATGGATTATCAGAC<br>ATATTTAATAACTATCACATGGGTATTACTGCTGAAAACATAGCAGAGCA<br>ATGGAATATAACTAGAGAAGAACAAGATGAATTAGCTCTTGCAAGTCAAA<br>ATAAAGCTGAAAAAGCTCAAGCTGAAGGAAAATTTGATGAAGAAATAGTT<br>CCTGTTGTTATAAAAGGAAGAAAAGGTGACACTGTAGTAGATAAAGATGA<br>ATATATTAAGCCTGGCACTACAATGGAGAAACTTGCTAAGTTAAGACCTG<br>CATTTAAAAAAGATGGAACAGTTACTGCTGGTAATGCATCAGGAATAAAT<br>GATGGTGCTGCTATGTTAGTAGTAATGGCTAAAGAAAAAGCTGAAGAACT<br>AGGAATAGAGCCTCTTGCAACTATAGTTTCTTATGGAACAGCTGGTGTTG<br>ACCCTAAAATAATGGGATATGGACCAGTTCCAGCAACTAAAAAAGCTTTA<br>GAAGCTGCTAATATGACTATTGAAGATATAGATTTAGTTGAAGCTAATGA<br>GGCATTTGCTGCCCAATCTGTAGCTGTAATAAGAGACTTAAATATAGATA<br>TGAATAAAGTTAATGTTAATGGTGGAGCAATAGCTATAGGACATCCAATA<br>GGATGCTCAGGAGCAAGAATACTTACTACACTTTTATATGAAATGAAGAG<br>AAGAGATGCTAAAACTGGTCTTGCTACACTTTGTATAGGCGGTGGAATGG<br>GAACTACTTTAATAGTTAAGAGATAG |
| *hbd*<br>**SEQ ID NO: 5** | ATGAAATTAGCTGTAATAGGTAGTGGAACTATGGGAAGTGGTATTGTACA<br>AACTTTTGCAAGTTGTGGACATGATGTATGTTTAAAGAGTAGAACTCAAG<br>GTGCTATAGATAAATGTTTAGCTTTATTAGATAAAAATTTAACTAAGTTA<br>GTTACTAAGGGAAAAATGGATGAAGCTACAAAAGCAGAAATATTAAGTCA<br>TGTTAGTTCAACTACTAATTATGAAGATTTAAAAGATATGGATTTAATAA |

| Description | Sequence |
|---|---|
| | TAGAAGCATCTGTAGAAGACATGAATATAAAGAAAGATGTTTTCAAGTTA<br>CTAGATGAATTATGTAAAGAAGATACTATCTTGGCAACAAATACTTCATC<br>ATTATCTATAACAGAAATAGCTTCTTCTACTAAGCGCCCAGATAAAGTTA<br>TAGGAATGCATTTCTTTAATCCAGTTCCTATGATGAAATTAGTTGAAGTT<br>ATAAGTGGTCAGTTAACATCAAAAGTTACTTTTGATACAGTATTTGAATT<br>ATCTAAGAGTATCAATAAAGTACCAGTAGATGTATCTGAATCTCCTGGAT<br>TTGTAGTAAATAGAATACTTATACCTATGATAAATGAAGCTGTTGGTATA<br>TATGCAGATGGTGTTGCAAGTAAAGAAGAAATAGATGAAGCTATGAAATT<br>AGGAGCAAACCATCCAATGGGACCACTAGCATTAGGTGATTTAATCGGAT<br>TAGATGTTGTTTTAGCTATAATGAACGTTTTATATACTGAATTTGGAGAT<br>ACTAAATATAGACCTCATCCACTTTTAGCTAAAATGGTTAGAGCTAATCA<br>ATTAGGAAGAAAAACTAAGATAGGATTCTATGATTATAATAAATAA |
| *crt2*<br>**SEQ ID NO: 6** | ATGAGTACAAGTGATGTTAAAGTTTATGAGAATGTAGCTGTTGAAGTAGA<br>TGGAAATATATGTACAGTGAAAATGAATAGACCTAAAGCCCTTAATGCAA<br>TAAATTCAAAGACTTTAGAAGAACTTTATGAAGTATTTGTAGATATTAAT<br>AATGATGAAACTATTGATGTTGTAATATTGACAGGGGAAGGAAAGGCATT<br>TGTAGCTGGAGCAGATATTGCATACATGAAAGATTTAGATGCTGTAGCTG<br>CTAAAGATTTTAGTATCTTAGGAGCAAAAGCTTTTGGAGAAATAGAAAAT<br>AGTAAAAAAGTAGTGATAGCTGCTGTAAACGGATTTGCTTTAGGTGGAGG<br>ATGTGAACTTGCAATGGCATGTGATATAAGAATTGCATCTGCTAAAGCTA<br>AATTTGGTCAGCCAGAAGTAACTCTTGGAATAACTCCAGGATATGGAGGA<br>ACTCAAAGGCTTACAAGATTGGTTGGAATGGCAAAAGCAAAAGAATTAAT<br>CTTTACAGGTCAAGTTATAAAAGCTGATGAAGCTGAAAAAATAGGGCTAG<br>TAAATAGAGTCGTTGAGCCAGACATTTTAATAGAAGAAGTTGAGAAATTA<br>GCTAAGATAATAGCTAAAAATGCTCAGCTTGCAGTTAGATACTCTAAAGA<br>AGCAATACAACTTGGTGCTCAAACTGATATAAATACTGGAATAGATATAG<br>AATCTAATTTATTTGGTCTTTGTTTTTCAACTAAAGACCAAAAAGAAGGA<br>ATGTCAGCTTTCGTTGAAAAGAGAGAAGCTAACTTTATAAAAGGGTAA |
| *pbt*<br>**SEQ ID NO: 7** | ATGAGAAGTTTTGAAGAAGTAATTAAGTTTGCAAAAGAAAGAGGACCTAA<br>AACTATATCAGTAGCATGTTGCCAAGATAAAGAAGTTTTAATGGCAGTTG<br>AAATGGCTAGAAAAGAAAAAATAGCAAATGCCATTTTAGTAGGAGATATA<br>GAAAAGACTAAAGAAATTGCAAAAAGCATAGACATGGATATCGAAAATTA<br>TGAACTGATAGATATAAAAGATTTAGCAGAAGCATCTCTAAAATCTGTTG<br>AATTAGTTTCACAAGGAAAAGCCGACATGGTAATGAAAGGCTTAGTAGAC<br>ACATCAATAATACTAAAAGCAGTTTTAAATAAAGAAGTAGGTCTTAGAAC<br>TGGAAATGTATTAAGTCACGTAGCAGTATTTGATGTAGAGGGATATGATA<br>GATTATTTTTCGTAACTGACGCAGCTATGAACTTAGCTCCTGATACAAAT<br>ACTAAAAAGCAAATCATAGAAAATGCTTGCACAGTAGCACATTCATTAGA<br>TATAAGTGAACCAAAAGTTGCTGCAATATGCGCAAAAGAAAAAGTAAATC<br>CAAAAATGAAAGATACAGTTGAAGCTAAAGAACTAGAAGAAATGTATGAA<br>AGAGGAGAAATCAAAGGTTGTATGGTTGGTGGGCCTTTTGCAATTGATAA<br>TGCAGTATCTTTAGAAGCAGCTAAACATAAAGGTATAAATCATCCTGTAG<br>CAGGACGAGCTGATATATTATTAGCCCCAGATATTGAAGGTGGTAACATA<br>TTATATAAAGCTTTGGTATTCTTCTCAAAATCAAAAAATGCAGGAGTTAT<br>AGTTGGGGCTAAAGCACCAATAATATTAACTTCTAGAGCAGACAGTGAAG<br>AAACTAAACTAAACTCAATAGCTTTAGGTGTTTTAATGGCAGCAAAGGCA<br>TAA |

| Description | Sequence |
|---|---|
| *buk* **SEQ ID NO: 8** | ATGAGCAAAATATTTAAAATCTTAACAATAAATCCTGGTTCGACATCAAC<br>TAAAATAGCTGTATTTGATAATGAGGATTTAGTATTTGAAAAAACTTTAA<br>GACATTCTTCAGAAGAAATAGGAAAATATGAGAAGGTGTCTGACCAATTT<br>GAATTTCGTAAACAAGTAATAGAAGAAGCTCTAAAAGAAGGTGGAGTAAA<br>AACATCTGAATTAGATGCTGTAGTAGGTAGAGGAGGACTTCTTAAACCTA<br>TAAAAGGTGGTACTTATTCAGTAAGTGCTGCTATGATTGAAGATTTAAAA<br>GTGGGAGTTTTAGGAGAACACGCTTCAAACCTAGGTGGAATAATAGCAAA<br>ACAAATAGGTGAAGAAGTAAATGTTCCTTCATACATAGTAGACCCTGTTG<br>TTGTAGATGAATTAGAAGATGTTGCTAGAATTTCTGGTATGCCTGAAATA<br>AGTAGAGCAAGTGTAGTACATGCTTTAAATCAAAAGGCAATAGCAAGAAG<br>ATATGCTAGAGAAATAAACAAGAAATATGAAGATATAAATCTTATAGTTG<br>CACACATGGGTGGAGGAGTTTCTGTTGGAGCTCATAAAAATGGTAAAATA<br>GTAGATGTTGCAAACGCATTAGATGGAGAAGGACCTTTCTCTCCAGAAAG<br>AAGTGGTGGACTACCAGTAGGTGCATTAGTAAAAATGTGCTTTAGTGGAA<br>AATATACTCAAGATGAAATTAAAAAGAAAATAAAAGGTAATGGCGGACTA<br>GTTGCATACTTAAACACTAATGATGCTAGAGAAGTTGAAGAAAGAATTGA<br>AGCTGGTGATGAAAAAGCTAAATTAGTATATGAAGCTATGGCATATCAAA<br>TCTCTAAAGAAATAGGAGCTAGTGCTGCAGTTCTTAAGGGAGATGTAAAA<br>GCAATATTATTAACTGGTGGAATCGCATATTCAAAAATGTTTACAGAAAT<br>GATTGCAGATAGAGTTAAATTTATAGCAGATGTAAAAGTTTATCCAGGTG<br>AAGATGAAATGATTGCATTAGCTCAAGGTGGACTTAGAGTTTTAACTGGT<br>GAAGAAGAGGCTCAAGTTTATGATAACTAA |
| *ter* **SEQ ID NO: 9** | ATGATCGTAAAACCTATGGTACGCAACAATATCTGCCTGAACGCCCATCC<br>TCAGGGCTGCAAGAAGGGAGTGGAAGATCAGATTGAATATACCAAGAAAC<br>GCATTACCGCAGAAGTCAAAGCTGGCGCAAAAGCTCCAAAAAACGTTCTG<br>GTGCTTGGCTGCTCAAATGGTTACGGCCTGGCGAGCCGCATTACTGCTGC<br>GTTCGGATACGGGGCTGCGACCATCGGCGTGTCCTTTGAAAAAGCGGGTT<br>CAGAAACCAAATATGGTACACCGGGATGGTACAATAATTTGGCATTTGAT<br>GAAGCGGCAAAACGCGAGGGTCTTTATAGCGTGACGATCGACGGCGATGC<br>GTTTTCAGACGAGATCAAGGCCCAGGTAATTGAGGAAGCCAAAAAAAAAG<br>GTATCAAATTTGATCTGATCGTATACAGCTTGGCCAGCCCAGTACGTACT<br>GATCCTGATACAGGTATCATGCACAAAAGCGTTTTGAAACCCTTTGGAAA<br>AACGTTCACAGGCAAAACAGTAGATCCGTTTACTGGCGAGCTGAAGGAAA<br>TCTCCGCGGAACCAGCAAATGACGAGGAAGCAGCCGCCACTGTTAAAGTT<br>ATGGGGGGTGAAGATTGGGAACGTTGGATTAAGCAGCTGTCGAAGGAAGG<br>CCTCTTAGAAGAAGGCTGTATTACCTTGGCCTATAGTTATATTGGCCCTG<br>AAGCTACCCAAGCTTTGTACCGTAAAGGCACAATCGGCAAGGCCAAAGAA<br>CACCTGGAGGCCACAGCACACCGTCTCAACAAAGAGAACCCGTCAATCCG<br>TGCCTTCGTGAGCGTGAATAAAGGCCTGGTAACCCGCGCAAGCGCCGTAA<br>TCCCGGTAATCCCTCTGTATCTCGCCAGCTTGTTCAAAGTAATGAAAGAG<br>AAGGGCAATCATGAAGGTTGTATTGAACAGATCACGCGTCTGTACGCCGA<br>GCGCCTGTACCGTAAAGATGGTACAATTCCAGTTGATGAGGAAAATCGCA<br>TTCGCATTGATGATTGGGAGTTAGAAGAAGACGTCCAGAAAGCGGTATCC<br>GCGTTGATGGAGAAAGTCACGGGTGAAAACGCAGAATCTCTCACTGACTT<br>AGCGGGGTACCGCCATGATTTCTTAGCTAGTAACGGCTTTGATGTAGAAG<br>GTATTAATTATGAAGCGGAAGTTGAACGCTTCGACCGTATCTGA |

| Description | Sequence |
|---|---|
| *tesB*<br>**SEQ ID NO: 10** | ATGAGTCAGGCGCTAAAAAATTTACTGACATTGTTAAATCTGGAAAAAAT<br>TGAGGAAGGACTCTTTCGCGGCCAGAGTGAAGATTTAGGTTTACGCCAGG<br>TGTTTGGCGGCCAGGTCGTGGGTCAGGCCTTGTATGCTGCAAAAGAGACC<br>GTCCCTGAAGAGCGGCTGGTACATTCGTTTCACAGCTACTTTCTTCGCCC<br>TGGCGATAGTAAGAAGCCGATTATTTATGATGTCGAAACGCTGCGTGACG<br>GTAACAGCTTCAGCGCCCGCCGGGTTGCTGCTATTCAAAACGGCAAACCG<br>ATTTTTTATATGACTGCCTCTTTCCAGGCACCAGAAGCGGGTTTCGAACA<br>TCAAAAAACAATGCCGTCCGCGCCAGCGCCTGATGGCCTCCCTTCGGAAA<br>CGCAAATCGCCCAATCGCTGGCGCACCTGCTGCCGCCAGTGCTGAAAGAT<br>AAATTCATCTGCGATCGTCCGCTGGAAGTCCGTCCGGTGGAGTTTCATAA<br>CCCACTGAAAGGTCACGTCGCAGAACCACATCGTCAGGTGTGGATCCGCG<br>CAAATGGTAGCGTGCCGGATGACCTGCGCGTTCATCAGTATCTGCTCGGT<br>TACGCTTCTGATCTTAACTTCCTGCCGGTAGCTCTACAGCCGCACGGCAT<br>CGGTTTTCTCGAACCGGGGATTCAGATTGCCACCATTGACCATTCCATGT<br>GGTTCCATCGCCCGTTTAATTTGAATGAATGGCTGCTGTATAGCGTGGAG<br>AGCACCTCGGCGTCCAGCGCACGTGGCTTTGTGCGCGGTGAGTTTTATAC<br>CCAAGACGGCGTACTGGTTGCCTCGACCGTTCAGGAAGGGGTGATGCGTA<br>ATCACAATTAA |

[0171] Exemplary polypeptide sequences for the production of butyrate by the genetically engineered bacteria are provided in **Table 3**.

**Table 3. Exemplary Polypeptide Sequences for Butyrate Production**

| Description | Sequence |
|---|---|
| Bcd2<br>**SEQ ID NO: 11** | MDLNSKKYQMLKELYVSFAENEVKPLATELDEEER<br>FPYETVEKMAKAGMMGIPYPKEYGGEGGDTVGYIM<br>AVEELSRVCGTTGVILSAHTSLGSWPIYQYGNEEQK<br>QKFLRPLASGEKLGAFGLTEPNAGTDASGQQTTAVL<br>DGDEYILNGSKIFITNAIAGDIYVVMAMTDKSKGNK<br>GISAFIVEKGTPGFSFGVKEKKMGIRGSATSELIFEDC<br>RIPKENLLGKEGQGFKIAMSTLDGGRIGIAAQALGLA<br>QGALDETVKYVKERVQFGRPLSKFQNTQFQLADME<br>VKVQAARHLVYQAAINKDLGKPYGVEAAMAKLFA<br>AETAMEVTTKAVQLHGGYGYTRDYPVERMMRDAK<br>ITEIYEGTSEVQRMVISGKLLK |
| etfB3<br>**SEQ ID NO: 12** | MNIVVCIKQVPDTTEVKLDPNTGTLIRDGVPSIINPDD<br>KAGLEEAIKLKEEMGAHVTVITMGPPQADMALKEA<br>LAMGADRGILLTDRAFAGADTWATSSALAGALKNI<br>DFDIIIAGRQAIDGDTAQVGPQIAEHLNLPSITYAEEIK<br>TEGEYVLVKRQFEDCCHDLKVKMPCLITTLKDMNT<br>PRYMKVGRIYDAFENDVVETWTVKDIEVDPSNLGL<br>KGSPTSVFKSFTKSVKPAGTIYNEDAKTSAGIIIDKLK<br>EKYII |
| etfA3 | MGNVLVVIEQRENVIQTVSLELLGKATEIAKDYDTK |

| | |
|---|---|
| **SEQ ID NO: 13** | VSALLLGSKVEGLIDTLAHYGADEVIVVDDEALAVY TTEPYTKAAYEAIKAADPIVVLFGATSIGRDLAPRVS ARIHTGLTADCTGLAVAEDTKLLLMTRPAFGGNIMA TIVCKDFRPQMSTVRPGVMKKNEPDETKEAVINRFK VEFNDADKLVQVVQVIKEAKKQVKIEDAKILVSAGR GMGGKENLDILYELAEIIGGEVSGSRATIDAGWLDK ARQVGQTGKTVRPDLYIACGISGAIQHIAGMEDAEFI VAINKNPEAPIFKYADVGIVGDVHKVLPELISQLSVA KEKGEVLAN |
| Ter<br>**SEQ ID NO: 14** | MIVKPMVRNNICLNAHPQGCKKGVEDQIEYTKKRIT AEVKAGAKAPKNVLVLGCSNGYGLASRITAAFGYG AATIGVSFEKAGSETKYGTPGWYNNLAFDEAAKRE GLYSVTIDGDAFSDEIKAQVIEEAKKKGIKFDLIVYSL ASPVRTDPDTGIMHKSVLKPFGKTFTGKTVDPFTGEL KEISAEPANDEEAAATVKVMGGEDWERWIKQLSKE GLLEEGCITLAYSYIGPEATQALYRKGTIGKAKEHLE ATAHRLNKENPSIRAFVSVNKGLVTRASAVIPVIPLY LASLFKVMKEKGNHEGCIEQITRLYAERLYRKDGTIP VDEENRIRIDDWELEEDVQKAVSALMEKVTGENAES LTDLAGYRHDFLASNGFDVEGINYEAEVERFDRI |
| ThiA<br>**SEQ ID NO: 15** | MREVVIASAARTAVGSFGGAFKSVSAVELGVTAAK EAIKRANITPDMIDESLLGGVLTAGLGQNIARQIALG AGIPVEKPAMTINIVCGSGLRSVSMASQLIALGDADI MLVGGAENMSMSPYLVPSARYGARMGDAAFVDSM IKDGLSDIFNNYHMGITAENIAEQWNITREEQDELAL ASQNKAEKAQAEGKFDEEIVPVVIKGRKGDTVVDK DEYIKPGTTMEKLAKLRPAFKKDGTVTAGNASGIND GAAMLVVMAKEKAEELGIEPLATIVSYGTAGVDPKI MGYGPVPATKKALEAANMTIEDIDLVEANEAFAAQ SVAVIRDLNIDMNKVNVNGGAIAIGHPIGCSGARILT TLLYEMKRRDAKTGLATLCIGGGMGTTLIVKR |
| Hbd<br>**SEQ ID NO: 16** | MKLAVIGSGTMGSGIVQTFASCGHDVCLKSRTQGAI DKCLALLDKNLTKLVTKGKMDEATKAEILSHVSSTT NYEDLKDMDLIIEASVEDMNIKKDVFKLLDELCKED TILATNTSSLSITEIASSTKRPDKVIGMHFFNPVPMMK LVEVISGQLTSKVTFDTVFELSKSINKVPVDVSESPGF VVNRILIPMINEAVGIYADGVASKEEIDEAMKLGAN HPMGPLALGDLIGLDVVLAIMNVLYTEFGDTKYRPH PLLAKMVRANQLGRKTKIGFYDYNK |
| Crt2<br>**SEQ ID NO: 17** | MSTSDVKVYENVAVEVDGNICTVKMNRPKALNAIN SKTLEELYEVFVDINNDETIDVVILTGEGKAFVAGAD IAYMKDLDAVAAKDFSILGAKAFGEIENSKKVVIAA VNGFALGGGCELAMACDIRIASAKAKFGQPEVTLGI TPGYGGTQRLTRLVGMAKAKELIFTGQVIKADEAEK IGLVNRVVEPDILIEEVEKLAKIIAKNAQLAVRYSKE AIQLGAQTDINTGIDIESNLFGLCFSTKDQKEGMSAF VEKREANFIKG |

| | |
|---|---|
| Pbt<br>**SEQ ID NO: 18** | MRSFEEVIKFAKERGPKTISVACCQDKEVLMAVEMA<br>RKEKIANAILVGDIEKTKEIAKSIDMDIENYELIDIKD<br>LAEASLKSVELVSQGKADMVMKGLVDTSIILKAVLN<br>KEVGLRTGNVLSHVAVFDVEGYDRLFFVTDAAMNL<br>APDTNTKKQIIENACTVAHSLDISEPKVAAICAKEKV<br>NPKMKDTVEAKELEEMYERGEIKGCMVGGPFAIDN<br>AVSLEAAKHKGINHPVAGRADILLAPDIEGGNILYKA<br>LVFFSKSKNAGVIVGAKAPIILTSRADSEETKLNSIAL<br>GVLMAAKA |
| Buk<br>**SEQ ID NO: 19** | MSKIFKILTINPGSTSTKIAVFDNEDLVFEKTLRHSSE<br>EIGKYEKVSDQFEFRKQVIEEALKEGGVKTSELDAV<br>VGRGGLLKPIKGGTYSVSAAMIEDLKVGVLGEHASN<br>LGGIIAKQIGEEVNVPSYIVDPVVVDELEDVARISGM<br>PEISRASVVHALNQKAIARRYAREINKKYEDINLIVA<br>HMGGGVSVGAHKNGKIVDVANALDGEGPFSPERSG<br>GLPVGALVKMCFSGKYTQDEIKKKIKGNGGLVAYL<br>NTNDAREVEERIEAGDEKAKLVYEAMAYQISKEIGA<br>SAAVLKGDVKAILLTGGIAYSKMFTEMIADRVKFIA<br>DVKVYPGEDEMIALAQGGLRVLTGEEEAQVYDN |
| TesB<br>**SEQ ID NO: 20** | MSQALKNLLTLLNLEKIEEGLFRGQSEDLGLRQVFG<br>GQVVGQALYAAKETVPEERLVHSFHSYFLRPGDSKK<br>PIIYDVETLRDGNSFSARRVAAIQNGKPIFYMTASFQ<br>APEAGFEHQKTMPSAPAPDGLPSETQIAQSLAHLLPP<br>VLKDKFICDRPLEVRPVEFHNPLKGHVAEPHRQVWI<br>RANGSVPDDLRVHQYLLGYASDLNFLPVALQPHGIG<br>FLEPGIQIATIDHSMWFHRPFNLNEWLLYSVESTSAS<br>SARGFVRGEFYTQDGVLVASTVQEGVMRNHN* |

[0172] 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 produce butyrate in low-oxygen conditions, in the presence of certain molecules or metabolites , in the presence of molecules or metabolites associated with inflammation or an inflammatory response, or in the presence of some other metabolite that may or may not be present in the gut, such as arabinose..

[0173] 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 in low-oxygen conditions, in the presence of certain molecules or metabolites , in the presence of molecules or metabolites associated with inflammation or an inflammatory response, or in the presence of some other metabolite that may or may not be present in the gut, such as arabinose. 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 the presence of certain molecules or metabolites, in the presence of molecules or metabolites associated with inflammation or an inflammatory response, or in the presence of some other metabolite that may or may not be present in the gut, such as arabinose.

[0174] The gene products of *pbt* and *buk* convert butyrylCoA to Butyrate. In some embodiments, the *pbt* and *buk* genes can be replaced by a *tesB* gene. tesB can be used to cleave off the CoA from butyryl-coA. In one embodiment, the genetically engineered bacteria comprise *bcd2, etfB3, etfA3, thiA1, hbd, and crt2, e.g.*, from *Peptoclostridium difficile,* and *tesB* from *E. Coli* and produce butyrate in low-oxygen conditions, in the presence of molecules or metabolites, in the presence of molecules or metabolites associated with inflammation or an inflammatory response, or in the presence of some other metabolite that may or may not be present in the gut, such as arabinose. In one embodiment, the genetically engineered bacteria comprise *ter* gene (encoding *trans*-2-enoynl-CoA reductase) e.g., from *Treponema denticola, thiA1, hbd, crt2, pbt,* and *buk*, *e.g.*, from *Peptoclostridium difficile,* and *tesB* from *E. Coli* , and produce butyrate in low-oxygen conditions,in the presence of specific molecules or metabolites, in the presence of molecules or metabolites associated with inflammation or an inflammatory response, or in

the presence of some other metabolite that may or may not be present in the gut, such as arabinose. 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 or in the presence of specific molecules or metabolites, or molecules or metabolites associated with condition(s) such as inflammation or an inflammatory response, or in the presence of some other metabolite that may or may not be present in the gut, such as arabinose.

[0175] 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. 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, local butyrate production reduces gut inflammation, a symptom of IBD and other gut related disorders.

[0176] In one embodiment, the *bcd2* gene has at least about 80% identity with **SEQ ID NO: 1**. In another embodiment, the *bcd2* gene has at least about 85% identity with **SEQ ID NO: 1**. In one embodiment, the *bcd2* gene has at least about 90% identity with **SEQ ID NO: 1**. In one embodiment, the *bcd2* gene has at least about 95% identity with **SEQ ID NO: 1**. In another embodiment, the *bcd2* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 1**. Accordingly, in one embodiment, the *bcd2* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 1**. In another embodiment, the *bcd2* gene comprises the sequence of **SEQ ID NO: 1**. In yet another embodiment the *bcd2* gene consists of the sequence of **SEQ ID NO: 1**.

[0177] In one embodiment, the *etfB3* gene has at least about 80% identity with **SEQ ID NO: 2**. In another embodiment, the *etfB3* gene has at least about 85% identity with **SEQ ID NO: 2**. In one embodiment, the *etfB3* gene has at least about 90% identity with **SEQ ID NO: 2**. In one embodiment, the *etfB3* gene has at least about 95% identity with **SEQ ID NO: 2**. In another embodiment, the *etfB3* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 2**. Accordingly, in one embodiment, the *etfB3* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 2**. In

another embodiment, the *etfB3* gene comprises the sequence of **SEQ ID NO: 2**. In yet another embodiment the *etfB3* gene consists of the sequence of **SEQ ID NO: 2**.

[0178] In one embodiment, the *etfA3* gene has at least about 80% identity with **SEQ ID NO: 3**. In another embodiment, the *etfA3* gene has at least about 85% identity with **SEQ ID NO: 3**. In one embodiment, the *etfA3* gene has at least about 90% identity with **SEQ ID NO: 3**. In one embodiment, the *etfA3* gene has at least about 95% identity with **SEQ ID NO: 3**. In another embodiment, the *etfA3* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 3**. Accordingly, in one embodiment, the *etfA3* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 3**. In another embodiment, the *etfA3* gene comprises the sequence of **SEQ ID NO: 3**. In yet another embodiment the *etfA3* gene consists of the sequence of **SEQ ID NO: 3**.

[0179] In one embodiment, the *thiA1* gene has at least about 80% identity with **SEQ ID NO: 4**. In another embodiment, the *thiA1* gene has at least about 85% identity with **SEQ ID NO: 4**. In one embodiment, the *thiA1* gene has at least about 90% identity with **SEQ ID NO: 4**. In one embodiment, the *thiA1* gene has at least about 95% identity with **SEQ ID NO: 4**. In another embodiment, the *thiA1* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 4**. Accordingly, in one embodiment, the *thiA1* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 4**. In another embodiment, the *thiA1* gene comprises the sequence of **SEQ ID NO: 4**. In yet another embodiment the *thiA1* gene consists of the sequence of **SEQ ID NO: 4**.

[0180] In one embodiment, the *hbd* gene has at least about 80% identity with **SEQ ID NO: 5**. In another embodiment, the *hbd* gene has at least about 85% identity with **SEQ ID NO: 5**. In one embodiment, the *hbd* gene has at least about 90% identity with **SEQ ID NO: 5**. In one embodiment, the *hbd* gene has at least about 95% identity with **SEQ ID NO: 5**. In another embodiment, the *hbd* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 5**. Accordingly, in one embodiment, the *hbd* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 5**. In another embodiment, the *hbd* gene comprises the sequence of **SEQ ID NO: 5**. In yet another embodiment the *hbd* gene consists of the sequence of **SEQ ID NO: 5**.

[0181] In one embodiment, the *crt2* gene has at least about 80% identity with **SEQ ID NO: 6**. In another embodiment, the *crt2* gene has at least about 85% identity with **SEQ ID NO: 6**. In one embodiment, the *crt2* gene has at least about 90% identity with **SEQ ID NO: 6**. In one embodiment, the *crt2* gene has at least about 95% identity with **SEQ ID NO: 6**. In another embodiment, the *crt2* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 6**. Accordingly, in one embodiment, the *crt2* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 6**. In another embodiment, the *crt2* gene comprises the sequence of **SEQ ID NO: 6**. In yet another embodiment the *crt2* gene consists of the sequence of **SEQ ID NO: 6**.

[0182] In one embodiment, the *pbt* gene has at least about 80% identity with **SEQ ID NO:** 7. In another embodiment, the *pbt* gene has at least about 85% identity with **SEQ ID NO:** 7. In one embodiment, the *pbt* gene has at least about 90% identity with **SEQ ID NO:** 7. In one embodiment, the *pbt* gene has at least about 95% identity with **SEQ ID NO:** 7. In another embodiment, the *pbt* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO:** 7. Accordingly, in one embodiment, the *pbt* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO:** 7. In another embodiment, the *pbt* gene comprises the sequence of **SEQ ID NO:** 7. In yet another embodiment the *pbt* gene consists of the sequence of **SEQ ID NO:** 7.

[0183] In one embodiment, the *buk* gene has at least about 80% identity with **SEQ ID NO: 8**. In another embodiment, the *buk* gene has at least about 85% identity with **SEQ ID NO: 8**. In one embodiment, the *buk* gene has at least about 90% identity with **SEQ ID NO: 8**. In one embodiment, the *buk* gene has at least about 95% identity with **SEQ ID NO: 8**. In another embodiment, the *buk* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 8**. Accordingly, in one embodiment, the *buk* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 8**. In another embodiment, the *buk* gene comprises the sequence of **SEQ ID NO: 8**. In yet another embodiment the *buk* gene consists of the sequence of **SEQ ID NO: 8**.

[0184] In one embodiment, the *ter* gene has at least about 80% identity with **SEQ ID NO: 9**. In another embodiment, the *ter* gene has at least about 85% identity with **SEQ**

**ID NO: 9**. In one embodiment, the *ter* gene has at least about 90% identity with **SEQ ID NO: 9**. In one embodiment, the *ter* gene has at least about 95% identity with **SEQ ID NO: 9**. In another embodiment, the *ter* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 9**. Accordingly, in one embodiment, the *ter* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 9**. In another embodiment, the *ter* gene comprises the sequence of **SEQ ID NO: 9**. In yet another embodiment the *ter* gene consists of the sequence of **SEQ ID NO: 9**.

[0185] In one embodiment, the *tesB* gene has at least about 80% identity with **SEQ ID NO: 10**. In another embodiment, the *tesB* gene has at least about 85% identity with **SEQ ID NO: 10**. In one embodiment, the *tesB* gene has at least about 90% identity with **SEQ ID NO: 10**. In one embodiment, the *tesB* gene has at least about 95% identity with **SEQ ID NO: 10**. In another embodiment, the *tesB* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 10**. Accordingly, in one embodiment, the *tesB* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 10**. In another embodiment, the *tesB* gene comprises the sequence of **SEQ ID NO: 10**. In yet another embodiment the *tesB* gene consists of the sequence of **SEQ ID NO: 10**.

[0186] In one embodiment, one or more polypeptides encoded by the butyrate circuits and expressed by the genetically engineered bacteria have at least about 80% identity with one or more of SEQ ID NO: 11 through SEQ ID NO: 20. In another embodiment, one or more polypeptides encoded by the butyrate circuits and expressed by the genetically engineered bacteria have at least about 85% identity with one or more of SEQ ID NO: 11 through SEQ ID NO: 20. In one embodiment, one or more polypeptides encoded by the butyrate circuits and expressed by the genetically engineered bacteria have at least about 90% identity with one or more of SEQ ID NO: 11 through SEQ ID NO: 20. In one embodiment, one or more polypeptides encoded by the butyrate circuits and expressed by the genetically engineered bacteria have at least about 95% identity with one or more of SEQ ID NO: 11 through SEQ ID NO: 20. In another embodiment, one or more polypeptides encoded by the butyrate circuits and expressed by the genetically engineered bacteria have at least about 96%, 97%, 98%, or 99% identity with one or more of SEQ ID NO: 11 through SEQ ID NO: 20. Accordingly, in one embodiment, one or more

polypeptides encoded by the butyrate circuits and expressed by the genetically engineered bacteria have at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with one or more of SEQ ID NO: 11 through SEQ ID NO: 20. In another embodiment, one or more polypeptides encoded by the butyrate circuits and expressed by the genetically engineered bacteria one or more polypeptides encoded by the butyrate circuits and expressed by the genetically engineered bacteria comprise the sequence of with one or more of SEQ ID NO: 11 through SEQ ID NO: 20. In yet another embodiment one or more polypeptides encoded by the butyrate circuits and expressed by the genetically engineered bacteria consist of the sequence of with one or more of SEQ ID NO: 11 through SEQ ID NO: 20.

[0187] In some embodiments, one or more of the butyrate biosynthesis genes is a synthetic butyrate biosynthesis gene. In some embodiments, one or more of the butyrate biosynthesis genes is a *Treponema denticola* butyrate biosynthesis gene. In some embodiments, one or more of the butyrate biosynthesis genes is a *C. glutamicum* butyrate biosynthesis gene. In some embodiments, one or more of the butyrate biosynthesis genes is a *Peptoclostridicum difficile* butyrate biosynthesis gene. The butyrate gene cassette may comprise genes for the aerobic biosynthesis of butyrate and/or genes for the anaerobic or microaerobic biosynthesis of butyrate.

[0188] In some embodiments, the genetically engineered bacteria comprise a combination of butyrate biosynthesis genes from different species, strains, and/or substrains of bacteria, and are capable of producing 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 some embodiments, the local production of butyrate reduces food intake and ameliorates improves gut barrier function and reduces inflammation. In some embodiments, the genetically engineered bacteria are capable of expressing the butyrate biosynthesis cassette and producing butyrate in low-oxygen conditions, in the presence of certain molecules or metabolites, in the presence of molecules or metabolites associated with inflammation or an inflammatory response, or in the presence of some other metabolite that may or may not be present in the gut, such as arabinose.

[0189] In one embodiment, the butyrate gene cassette is directly operably linked to a first promoter. In another embodiment, the butyrate gene cassette is indirectly operably

linked to a first promoter. In one embodiment, the promoter is not operably linked with the butyrate gene cassette in nature.

[0190] In some embodiments, the butyrate gene cassette is expressed under the control of a constitutive promoter. In another embodiment, the butyrate gene cassette is expressed under the control of an inducible promoter. In some embodiments, the butyrate gene cassette is expressed under the control of a promoter that is directly or indirectly induced by exogenous environmental conditions. In one embodiment, the butyrate gene cassette is expressed under the control of a promoter that is directly or indirectly induced by low-oxygen or anaerobic conditions, wherein expression of the butyrate gene cassette is activated under low-oxygen or anaerobic environments, such as the environment of the mammalian gut. Inducible promoters are described in more detail *infra*.

[0191] The butyrate gene cassette may be present on a plasmid or chromosome in the bacterial cell. In one embodiment, the butyrate gene cassette is located on a plasmid in the bacterial cell. In another embodiment, the butyrate gene cassette is located in the chromosome of the bacterial cell. In yet another embodiment, a native copy of the butyrate gene cassette is located in the chromosome of the bacterial cell, and a butyrate gene cassette from a different species of bacteria is located on a plasmid in the bacterial cell. In yet another embodiment, a native copy of the butyrate gene cassette is located on a plasmid in the bacterial cell, and a butyrate gene cassette from a different species of bacteria is located on a plasmid in the bacterial cell. In yet another embodiment, a native copy of the butyrate gene cassette is located in the chromosome of the bacterial cell, and a butyrate gene cassette from a different species of bacteria is located in the chromosome of the bacterial cell.

[0192] In some embodiments, the butyrate gene cassette is expressed on a low-copy plasmid. In some embodiments, the butyrate gene cassette is expressed on a high-copy plasmid. In some embodiments, the high-copy plasmid may be useful for increasing expression of butyrate.

*Propionate*

[0193] In alternate embodiments, the genetically engineered bacteria of the invention are capable of producing an anti-inflammatory or gut barrier enhancer molecule,

*e.g.*, propionate, that is synthesized by a biosynthetic pathway requiring multiple genes and/or enzymes.

[0194] In some embodiments, the genetically engineered bacteria of the invention comprise a propionate gene cassette and are capable of producing propionate under particular exogenous environmental conditions. The genetically engineered bacteria may express any suitable set of propionate biosynthesis genes (*see, e.g.,* **Table 4, Table 5, Table 6, Table** 7). 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*. 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 some embodiments, the rate limiting step catalyzed by the Acr enzyme, is replaced by the AcuI from R. sphaeroides, which catalyzes the NADPH-dependent acrylyl-CoA reduction to produce propionyl-CoA. Thus the propionate cassette comprises pct, lcdA, lcdB, lcdC, and acuI. In another embodiment, the homolog of AcuI in E coli, yhdH is used. This the propionate cassette comprises pct, lcdA, lcdB, lcdC, and yhdH. 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*. In another embodiment, the propionate gene cassette comprises the genes of the Sleepting Beauty Mutase operon, e.g., from E. coli (sbm, ygfD, ygfG, ygfH). The SBM pathway is cyclical and composed of a series of biochemical conversions forming propionate as a fermentative product while regenerating the starting molecule of succinyl-CoA. Sbm converts succinyl CoA to L-methylmalonylCoA, ygfG converts L-methylmalonylCoA into PropionylCoA, and ygfH converts propionylCoA into propionate and succinate into succinylCoA.

[0195] This pathway is very similar to the oxidative propionate pathway of Propionibacteria, which also converts succinate to propionate. Succinyl-CoA is converted to R-methylmalonyl-CoA by methymalonyl-CoA mutase (mutAB). This is in turn converted to S-methylmalonyl-CoA via methymalonyl-CoA epimerase (GI:18042134).

There are three genes which encode methylmalonyl-CoA carboxytransferase (mmdA, PFREUD_18870, bccp) which converts methylmalonyl-CoA to propionyl-CoA.

[0196] The genes may be codon-optimized, and translational and transcriptional elements may be added. **Table 4-6** lists the nucleic acid sequences of exemplary genes in the propionate biosynthesis gene cassette. **Table 7** lists the polypeptide sequences expressed by exemplary propionate biosynthesis genes.

**Table 4. Propionate Cassette Sequences (Acrylate Pathway)**

| Gene sequence | Description |
|---|---|
| *pct* **SEQ ID NO: 21** | ATGCGCAAAGTGCCGATTATCACGGCTGACGAGGCCGCAAAAC<br>TGATCAAGGACGGCGACACCGTGACAACTAGCGGCTTTGTGGGT<br>AACGCGATCCCTGAGGCCCTTGACCGTGCAGTCGAAAAGCGTTT<br>CCTGGAAACGGGCGAACCGAAGAACATTACTTATGTATATTGCG<br>GCAGTCAGGGCAATCGCGACGGTCGTGGCGCAGAACATTTCGC<br>GCATGAAGGCCTGCTGAAACGTTATATCGCTGGCCATTGGGCGA<br>CCGTCCCGGCGTTAGGGAAAATGGCCATGGAGAATAAAATGGA<br>GGCCTACAATGTCTCTCAGGGCGCCTTGTGTCATCTCTTTCGCGA<br>TATTGCGAGCCATAAACCGGGTGTGTTCACGAAAGTAGGAATCG<br>GCACCTTCATTGATCCACGTAACGGTGGTGGGAAGGTCAACGAT<br>ATTACCAAGGAAGATATCGTAGAACTGGTGGAAATTAAAGGGC<br>AGGAATACCTGTTTTATCCGGCGTTCCCGATCCATGTCGCGCTG<br>ATTCGTGGCACCTATGCGGACGAGAGTGGTAACATCACCTTTGA<br>AAAAGAGGTAGCGCCTTTGGAAGGGACTTCTGTCTGTCAAGCGG<br>TGAAGAACTCGGGTGGCATTGTCGTGGTTCAGGTTGAGCGTGTC<br>GTCAAAGCAGGCACGCTGGATCCGCGCCATGTGAAAGTTCCGG<br>GTATCTATGTAGATTACGTAGTCGTCGCGGATCCGGAGGACCAT<br>CAACAGTCCCTTGACTGCGAATATGATCCTGCCCTTAGTGGAGA<br>GCACCGTCGTCCGGAGGTGGTGGGTGAACCACTGCCTTTATCCG<br>CGAAGAAAGTCATCGGCCGCCGTGGCGCGATTGAGCTCGAGAA<br>AGACGTTGCAGTGAACCTTGGGGTAGGTGCACCTGAGTATGTGG<br>CCTCCGTGGCCGATGAAGAAGGCATTGTGGATTTTATGACTCTC<br>ACAGCGGAGTCCGGCGCTATCGGTGGCGTTCCAGCCGGCGGTGT<br>TCGCTTTGGGGCGAGCTACAATGCTGACGCCTTGATCGACCAGG<br>GCTACCAATTTGATTATTACGACGGTGGGGGTCTGGATCTTTGTT<br>ACCTGGGTTTAGCTGAATGCGACGAAAAGGGTAATATCAATGTT<br>AGCCGCTTCGGTCCTCGTATCGCTGGGTGCGGCGGATTCATTAA<br>CATTACCCAAAACACGCCGAAAGTCTTCTTTTGTGGGACCTTTA<br>CAGCCGGGGGGCTGAAAGTGAAAATTGAAGATGGTAAGGTGAT<br>TATCGTTCAGGAAGGGAAACAGAAGAAATTCCTTAAGGCAGTG<br>GAGCAAATCACCTTTAATGGAGACGTGGCCTTAGCGAACAAGC<br>AACAAGTTACCTACATCACGGAGCGTTGCGTCTTCCTCCTCAAA<br>GAAGACGGTTTACACCTTTCGGAAATCGCGCCAGGCATCGATCT<br>GCAGACCCAGATTTTGGATGTTATGGACTTTGCCCCGATCATTG<br>ATCGTGACGCAAACGGGCAGATTAAACTGATGGACGCGGCGTT<br>ATTCGCAGAAGGGCTGATGGGCTTGAAAGAAATGAAGTCTTAA |

| Gene sequence | Description |
|---|---|
| *lcdA*<br>**SEQ ID NO: 22** | ATGAGCTTAACCCAAGGCATGAAAGCTAAACAACTGTTAGCAT<br>ACTTTCAGGGTAAAGCCGATCAGGATGCACGTGAAGCGAAAGC<br>CCGCGGTGAGCTGGTCTGCTGGTCGGCGTCAGTCGCGCCGCCGG<br>AATTTTGCGTAACAATGGGCATTGCCATGATCTACCCGGAGACT<br>CATGCAGCGGGCATCGGTGCCCGCAAAGGTGCGATGGACATGC<br>TGGAAGTTGCGGACCGCAAAGGCTACAACGTGGATTGTTGTTCC<br>TACGGCCGTGTAAATATGGGTTACATGGAATGTTTAAAAGAAGC<br>CGCCATCACGGGCGTCAAGCCGGAAGTTTTGGTTAATTCCCCTG<br>CTGCTGACGTTCCGCTTCCCGATTTGGTGATTACGTGTAATAATA<br>TCTGTAACACGCTGCTGAAATGGTACGAAAACTTAGCAGCAGA<br>ACTCGATATTCCTTGCATCGTGATCGACGTACCGTTTAATCATAC<br>CATGCCGATTCCGGAATATGCCAAGGCCTACATCGCGGACCAGT<br>TCCGCAATGCAATTTCTCAGCTGGAAGTTATTTGTGGCCGTCCGT<br>TCGATTGGAAGAAATTTAAGGAGGTCAAAGATCAGACCCAGCG<br>TAGCGTATACCACTGGAACCGCATTGCCGAGATGGCGAAATAC<br>AAGCCTAGCCCGCTGAACGGCTTCGATCTGTTCAATTACATGGC<br>GTTAATCGTGGCGTGCCGCAGCCTGGATTATGCAGAAATTACCT<br>TTAAAGCGTTCGCGGACGAATTAGAAGAGAATTTGAAGGCGGG<br>TATCTACGCCTTTAAAGGTGCGGAAAAAACGCGCTTTCAATGGG<br>AAGGTATCGCGGTGTGGCCACATTTAGGTCACACGTTTAAATCT<br>ATGAAGAATCTGAATTCGATTATGACCGGTACGGCATACCCCGC<br>CCTTTGGGACCTGCACTATGACGCTAACGACGAATCTATGCACT<br>CTATGGCTGAAGCGTACACCCGTATTTATATTAATACTTGTCTGC<br>AGAACAAAGTAGAGGTCCTGCTTGGGATCATGGAAAAAGGCCA<br>GGTGGATGGTACCGTATATCATCTGAATCGCAGCTGCAAACTGA<br>TGAGTTTCCTGAACGTGGAAACGGCTGAAATTATTAAAGAGAA<br>GAACGGTCTTCCTTACGTCTCCATTGATGGCGATCAGACCGATC<br>CTCGCGTTTTTTCTCCGGCCCAGTTTGATACCCGTGTTCAGGCCC<br>TGGTTGAGATGATGGAGGCCAATATGGCGGCAGCGGAATAA |
| *lcdB*<br>**SEQ ID NO: 23** | ATGTCACGCGTGGAGGCAATCCTGTCGCAGCTGAAAGATGTCGC<br>CGCGAATCCGAAAAAAGCCATGGATGACTATAAAGCTGAAACA<br>GGTAAGGGCGCGGTTGGTATCATGCCGATCTACAGCCCCGAAG<br>AAATGGTACACGCCGCTGGCTATTTGCCGATGGGAATCTGGGGC<br>GCCCAGGGCAAAACGATTAGTAAAGCGCGCACCTATCTGCCTGC<br>TTTTGCCTGCAGCGTAATGCAGCAGGTTATGGAATTACAGTGCG<br>AGGGCGCGTATGATGACCTGTCCGCAGTTATTTTTAGCGTACCG<br>TGCGACACTCTCAAATGTCTTAGCCAGAAATGGAAAGGTACGTC<br>CCCAGTGATTGTATTTACGCATCCGCAGAACCGCGGATTAGAAG<br>CGGCGAACCAATTCTTGGTTACCGAGTATGAACTGGTAAAAGCA<br>CAACTGGAATCAGTTCTGGGTGTGAAAATTTCAAACGCCGCCCT<br>GGAAAATTCGATTGCAATTTATAACGAGAATCGTGCCGTGATGC<br>GTGAGTTCGTGAAAGTGGCAGCGGACTATCCTCAAGTCATTGAC<br>GCAGTGAGCCGCCACGCGGTTTTTAAAGCGCGCCAGTTTATGCT<br>TAAGGAAAAACATACCGCACTTGTGAAAGAACTGATCGCTGAG<br>ATTAAAGCAACGCCAGTCCAGCCGTGGGACGGAAAAAAGGTTG<br>TAGTGACGGGCATTCTGTTGGAACCGAATGAGTTATTAGATATC |

| Gene sequence | Description |
|---|---|
| | TTTAATGAGTTTAAGATCGCGATTGTTGATGATGATTTAGCGCA<br>GGAAAGCCGTCAGATCCGTGTTGACGTTCTGGACGGAGAAGGC<br>GGACCGCTCTACCGTATGGCTAAAGCGTGGCAGCAAATGTATGG<br>CTGCTCGCTGGCAACCGACACCAAGAAGGGTCGCGGCCGTATGT<br>TAATTAACAAAACGATTCAGACCGGTGCGGACGCTATCGTAGTT<br>GCAATGATGAAGTTTTGCGACCCAGAAGAATGGGATTATCCGGT<br>AATGTACCGTGAATTTGAAGAAAAAGGGGTCAAATCACTTATG<br>ATTGAGGTGGATCAGGAAGTATCGTCTTTCGAACAGATTAAAAC<br>CCGTCTGCAGTCATTCGTCGAAATGCTTTAA |
| *lcdC*<br>**SEQ ID NO: 24** | ATGTATACCTTGGGGATTGATGTCGGTTCTGCCTCTAGTAAAGC<br>GGTGATTCTGAAAGATGGAAAAGATATTGTCGCTGCCGAGGTTG<br>TCCAAGTCGGTACCGGCTCCTCGGGTCCCCAACGCGCACTGGAC<br>AAAGCCTTTGAAGTCTCTGGCTTAAAAAAGGAAGACATCAGCTA<br>CACAGTAGCTACGGGCTATGGGCGCTTCAATTTTAGCGACGCGG<br>ATAAACAGATTTCGGAAATTAGCTGTCATGCCAAAGGCATTTAT<br>TTCTTAGTACCAACTGCGCGCACTATTATTGACATTGGCGGCCA<br>AGATGCGAAAGCCATCCGCCTGGACGACAAGGGGGGGTATTAAG<br>CAATTCTTCATGAATGATAAATGCGCGGCGGGCACGGGGCGTTT<br>CCTGGAAGTCATGGCTCGCGTACTTGAAACCACCCTGGATGAAA<br>TGGCTGAACTGGATGAACAGGCGACTGACACCGCTCCCATTTCA<br>AGCACCTGCACGGTTTTCGCCGAAAGCGAAGTAATTAGCCAATT<br>GAGCAATGGTGTCTCACGCAACAACATCATTAAAGGTGTCCATC<br>TGAGCGTTGCGTCACGTGCGTGTGGTCTGGCGTATCGCGGCGGT<br>TTGGAGAAAGATGTTGTTATGACAGGTGGCGTGGCAAAAAATG<br>CAGGGGTGGTGCGCGCGGTGGCGGGCGTTCTGAAGACCGATGT<br>TATCGTTGCTCCGAATCCTCAGACGACCGGTGCACTGGGGGCAG<br>CGCTGTATGCTTATGAGGCCGCCCAGAAGAAGTA |
| *etfA*<br>**SEQ ID NO: 25** | ATGGCCTTCAATAGCGCAGATATTAATTCTTTCCGCGATATTTGG<br>GTGTTTTGTGAACAGCGTGAGGGCAAACTGATTAACACCGATTT<br>CGAATTAATTAGCGAAGGTCGTAAACTGGCTGACGAACGCGGA<br>AGCAAACTGGTTGGAATTTTGCTGGGGCACGAAGTTGAAGAAA<br>TCGCAAAAGAATTAGGCGGCTATGGTGCGGACAAGGTAATTGT<br>GTGCGATCATCCGGAACTTAAATTTTACACTACGGATGCTTATG<br>CCAAAGTTTTATGTGACGTCGTGATGGAAGAGAAACCGGAGGT<br>AATTTTGATCGGTGCCACCAACATTGGCCGTGATCTCGGACCGC<br>GTTGTGCTGCACGCTTGCACACGGGGCTGACGGCTGATTGCACG<br>CACCTGGATATTGATATGAATAAATATGTGGACTTTCTTAGCAC<br>CAGTAGCACCTTGGATATCTCGTCGATGACTTTCCCTATGGAAG<br>ATACAAACCTTAAAATGACGCGCCCTGCATTTGGCGGACATCTG<br>ATGGCAACGATCATTTGTCCACGCTTCCGTCCCTGTATGAGCAC<br>AGTGCGCCCCGGAGTGATGAAGAAAGCGGAGTTCTCGCAGGAG<br>ATGGCGCAAGCATGTCAAGTAGTGACCCGTCACGTAAATTTGTC<br>GGATGAAGACCTTAAAACTAAAGTAATTAATATCGTGAAGGAA<br>ACGAAAAAGATTGTGGATCTGATCGGCGCAGAAATTATTGTGTC<br>AGTTGGTCGTGGTATCTCGAAAGATGTCCAAGGTGGAATTGCAC<br>TGGCTGAAAAACTTGCGGACGCATTTGGTAACGGTGTCGTGGGC |

| Gene sequence | Description |
|---|---|
| | GGCTCGCGCGCAGTGATTGATTCCGGCTGGTTACCTGCGGATCA<br>TCAGGTTGGACAAACCGGTAAGACCGTGCACCCGAAAGTCTAC<br>GTGGCGCTGGGTATTAGTGGGGCTATCCAGCATAAGGCTGGGAT<br>GCAAGACTCTGAACTGATCATTGCCGTCAACAAAGACGAAACG<br>GCGCCTATCTTCGACTGCGCCGATTATGGCATCACCGGTGATTT<br>ATTTAAAATCGTACCGATGATGATCGACGCGATCAAAGAGGGT<br>AAAAACGCATGA |
| *acrB*<br>**SEQ ID NO: 26** | ATGCGCATCTATGTGTGTGTGAAACAAGTCCCAGATACGAGCGG<br>CAAGGTGGCCGTTAACCCTGATGGGACCCTTAACCGTGCCTCAA<br>TGGCAGCGATTATTAACCCGGACGATATGTCCGCGATCGAACAG<br>GCATTAAAACTGAAAGATGAAACCGGATGCCAGGTTACGGCGC<br>TTACGATGGGTCCTCCTCCTGCCGAGGGCATGTTGCGCGAAATT<br>ATTGCAATGGGGGCCGACGATGGTGTGCTGATTTCGGCCCGTGA<br>ATTTGGGGGGTCCGATACCTTCGCAACCAGTCAAATTATTAGCG<br>CGGCAATCCATAAATTAGGCTTAAGCAATGAAGACATGATCTTT<br>TGCGGTCGTCAGGCCATTGACGGTGATACGGCCCAAGTCGGCCC<br>TCAAATTGCCGAAAAACTGAGCATCCCACAGGTAACCTATGGCG<br>CAGGAATCAAAAAATCTGGTGATTTAGTGCTGGTGAAGCGTATG<br>TTGGAGGATGGTTATATGATGATCGAAGTCGAAACTCCATGTCT<br>GATTACCTGCATTCAGGATAAAGCGGTAAAACCACGTTACATGA<br>CTCTCAACGGTATTATGGAATGCTACTCCAAGCCGCTCCTCGTTC<br>TCGATTACGAAGCACTGAAAGATGAACCGCTGATCGAACTTGAT<br>ACCATTGGGCTTAAAGGCTCCCCGACGAATATCTTTAAATCGTT<br>TACGCCGCCTCAGAAAGGCGTTGGTGTCATGCTCCAAGGCACCG<br>ATAAGGAAAAAGTCGAGGATCTGGTGGATAAGCTGATGCAGAA<br>ACATGTCATCTAA |
| *acrC*<br>**SEQ ID NO: 27** | ATGTTCTTACTGAAGATTAAAAAAGAACGTATGAAACGCATGG<br>ACTTTAGTTTAACGCGTGAACAGGAGATGTTAAAAAAACTGGCG<br>CGTCAGTTTGCTGAGATCGAGCTGGAACCGGTGGCCGAAGAGA<br>TTGATCGTGAGCACGTTTTTCCTGCAGAAAACTTTAAGAAGATG<br>GCGGAAATTGGCTTAACCGGCATTGGTATCCCGAAAGAATTTGG<br>TGGCTCCGGTGGAGGCACCCTGGAGAAGGTCATTGCCGTGTCAG<br>AATTCGGCAAAAAGTGTATGGCCTCAGCTTCCATTTTAAGCATT<br>CATCTTATCGCGCCGCAGGCAATCTACAAATATGGGACCAAAGA<br>ACAGAAAGAGACGTACCTGCCGCGTCTTACCAAAGGTGGTGAA<br>CTGGGCGCCTTTGCGCTGACAGAACCAAACGCCGGAAGCGATG<br>CCGGCGCGGTAAAAACGACCGCGATTCTGGACAGCCAGACAAA<br>CGAGTACGTGCTGAATGGCACCAAATGCTTTATCAGCGGGGGCG<br>GGCGCGCGGGTGTTCTTGTAATTTTTGCGCTTACTGAACCGAAA<br>AAAGGTCTGAAAGGGATGAGCGCGATTATCGTGGAGAAAGGGA<br>CCCCGGGCTTCAGCATCGGCAAGGTGGAGAGCAAGATGGGGAT<br>CGCAGGTTCGGAAACCGCGGAACTTATCTTCGAAGATTGTCGCG<br>TTCCGGCTGCCAACCTTTTAGGTAAAGAAGGCAAAGGCTTTAAA<br>ATTGCTATGGAAGCCCTGGATGGCGCCCGTATTGGCGTGGGCGC<br>TCAAGCAATCGGAATTGCCGAGGGGGCGATCGACCTGAGTGTG<br>AAGTACGTTCACGAGCGCATTCAATTTGGTAAACCGATCGCGAA |

| Gene sequence | Description |
|---|---|
| | TCTGCAGGGAATTCAATGGTATATCGCGGATATGGCGACCAAAA<br>CCGCCGCGGCACGCGCACTTGTTGAGTTTGCAGCGTATCTTGAA<br>GACGCGGGTAAACCGTTCACAAAGGAATCTGCTATGTGCAAGCT<br>GAACGCCTCCGAAAACGCGCGTTTTGTGACAAATTTAGCTCTGC<br>AGATTCACGGGGGTTACGGTTATATGAAAGATTATCCGTTAGAG<br>CGTATGTATCGCGATGCTAAGATTACGGAAATTTACGAGGGGAC<br>ATCAGAAATCCATAAGGTGGTGATTGCGCGTGAAGTAATGAAA<br>CGCTAA |
| *thrA*$^{fbr}$<br>**SEQ ID NO: 28** | ATGCGAGTGTTGAAGTTCGGCGGTACATCAGTGGCAAATGCAG<br>AACGTTTTCTGCGTGTTGCCGATATTCTGGAAAGCAATGCCAGG<br>CAGGGGCAGGTGGCCACCGTCCTCTCTGCCCCCGCCAAAATCAC<br>CAACCACCTGGTGGCGATGATTGAAAAAACCATTAGCGGCCAG<br>GATGCTTTACCCAATATCAGCGATGCCGAACGTATTTTTGCCGA<br>ACTTTTGACGGGACTCGCCGCCGCCCAGCCGGGGTTCCCGCTGG<br>CGCAATTGAAAACTTTCGTCGATCAGGAATTTGCCCAAATAAAA<br>CATGTCCTGCATGGCATTAGTTTGTTGGGGCAGTGCCCGGATAG<br>CATCAACGCTGCGCTGATTTGCCGTGGCGAGAAAATGTCGATCG<br>CCATTATGGCCGGCGTATTAGAAGCGCGCGGTCACAACGTTACT<br>GTTATCGATCCGGTCGAAAAACTGCTGGCAGTGGGGCATTACCT<br>CGAATCTACCGTCGATATTGCTGAGTCCACCCGCCGTATTGCGG<br>CAAGCCGCATTCCGGCTGATCACATGGTGCTGATGGCAGGTTTC<br>ACCGCCGGTAATGAAAAAGGCGAACTGGTGGTGCTTGGACGCA<br>ACGGTTCCGACTACTCTGCTGCGGTGCTGGCTGCCTGTTTACGC<br>GCCGATTGTTGCGAGATTTGGACGGACGTTGACGGGGTCTATAC<br>CTGCGACCCGCGTCAGGTGCCCGATGCGAGGTTGTTGAAGTCGA<br>TGTCCTACCAGGAAGCGATGGAGCTTTCCTACTTCGGCGCTAAA<br>GTTCTTCACCCCCGCACCATTACCCCCATCGCCCAGTTCCAGATC<br>CCTTGCCTGATTAAAAATACCGGAAATCCTCAAGCACCAGGTAC<br>GCTCATTGGTGCCAGCCGTGATGAAGACGAATTACCGGTCAAGG<br>GCATTTCCAATCTGAATAACATGGCAATGTTCAGCGTTTCTGGT<br>CCGGGGATGAAAGGGATGGTCGGCATGGCGGCGCGCGTCTTTG<br>CAGCGATGTCACGCGCCCGTATTTCCGTGGTGCTGATTACGCAA<br>TCATCTTCCGAATACAGCATCAGTTTCTGCGTTCCACAAAGCGA<br>CTGTGTGCGAGCTGAACGGGCAATGCAGGAAGAGTTCTACCTG<br>GAACTGAAAGAAGGCTTACTGGAGCCGCTGGCAGTGACGGAAC<br>GGCTGGCCATTATCTCGGTGGTAGGTGATGGTATGCGCACCTTG<br>CGTGGGATCTCGGCGAAATTCTTTGCCGCACTGGCCCGCGCCAA<br>TATCAACATTGTCGCCATTGCTCAGAGATCTTCTGAACGCTCAA<br>TCTCTGTCGTGGTAAATAACGATGATGCGACCACTGGCGTGCGC<br>GTTACTCATCAGATGCTGTTCAATACCGATCAGGTTATCGAAGT<br>GTTTGTGATTGGCGTCGGTGGCGTTGGCGGTGCGCTGCTGGAGC<br>AACTGAAGCGTCAGCAAAGCTGGCTGAAGAATAAACATATCGA<br>CTTACGTGTCTGCGGTGTTGCCAACTCGAAGGCTCTGCTCACCA<br>ATGTACATGGCCTTAATCTGGAAAACTGGCAGGAAGAACTGGC<br>GCAAGCCAAAGAGCCGTTTAATCTCGGGCGCTTAATTCGCCTCG |

| Gene sequence | Description |
|---|---|
| | TGAAAGAATATCATCTGCTGAACCCGGTCATTGTTGACTGCACT<br>TCCAGCCAGGCAGTGGCGGATCAATATGCCGACTTCCTGCGCGA<br>AGGTTTCCACGTTGTCACGCCGAACAAAAAGGCCAACACCTCGT<br>CGATGGATTACTACCATCAGTTGCGTTATGCGGCGGAAAAATCG<br>CGGCGTAAATTCCTCTATGACACCAACGTTGGGGCTGGATTACC<br>GGTTATTGAGAACCTGCAAAATCTGCTCAATGCAGGTGATGAAT<br>TGATGAAGTTCTCCGGCATTCTTTCTGGTTCGCTTTCTTATATCTT<br>CGGCAAGTTAGACGAAGGCATGAGTTTCTCCGAGGCGACCACG<br>CTGGCGCGGGAAATGGGTTATACCGAACCGGACCCGCGAGATG<br>ATCTTTCTGGTATGGATGTGGCGCGTAAACTATTGATTCTCGCTC<br>GTGAAACGGGACGTGAACTGGAGCTGGCGGATATTGAAATTGA<br>ACCTGTGCTGCCCGCAGAGTTTAACGCCGAGGGTGATGTTGCCG<br>CTTTTATGGCGAATCTGTCACAACTCGACGATCTCTTTGCCGCGC<br>GCGTGGCGAAGGCCCGTGATGAAGGAAAAGTTTTGCGCTATGTT<br>GGCAATATTGATGAAGATGGCGTCTGCCGCGTGAAGATTGCCGA<br>AGTGGATGGTAATGATCCGCTGTTCAAAGTGAAAAATGGCGAA<br>AACGCCCTGGCCTTCTATAGCCACTATTATCAGCCGCTGCCGTT<br>GGTACTGCGCGGATATGGTGCGGGCAATGACGTTACAGCTGCCG<br>GTGTCTTTGCTGATCTGCTACGTACCCTCTCATGGAAGTTAGGA<br>GTCTGA |
| *thrB*<br>**SEQ ID NO: 29** | ATGGTTAAAGTTTATGCCCCGGCTTCCAGTGCCAATATGAGCGT<br>CGGGTTTGATGTGCTCGGGGCGGCGGTGACACCTGTTGATGGTG<br>CATTGCTCGGAGATGTAGTCACGGTTGAGGCGGCAGAGACATTC<br>AGTCTCAACAACCTCGGACGCTTTGCCGATAAGCTGCCGTCAGA<br>ACCACGGGAAAATATCGTTTATCAGTGCTGGGAGCGTTTTTGCC<br>AGGAACTGGGTAAGCAAATTCCAGTGGCGATGACCCTGGAAAA<br>GAATATGCCGATCGGTTCGGGCTTAGGCTCCAGTGCCTGTTCGG<br>TGGTCGCGGCGCTGATGGCGATGAATGAACACTGCGGCAAGCC<br>GCTTAATGACACTCGTTTGCTGGCTTTGATGGGCGAGCTGGAAG<br>GCCGTATCTCCGGCAGCATTCATTACGACAACGTGGCACCGTGT<br>TTTCTCGGTGGTATGCAGTTGATGATCGAAGAAAACGACATCAT<br>CAGCCAGCAAGTGCCAGGGTTTGATGAGTGGCTGTGGGTGCTGG<br>CGTATCCGGGGATTAAAGTCTCGACGGCAGAAGCCAGGGCTATT<br>TTACCGGCGCAGTATCGCCGCCAGGATTGCATTGCGCACGGGCG<br>ACATCTGGCAGGCTTCATTCACGCCTGCTATTCCCGTCAGCCTG<br>AGCTTGCCGCGAAGCTGATGAAAGATGTTATCGCTGAACCCTAC<br>CGTGAACGGTTACTGCCAGGCTTCCGGCAGGCGCGGCAGGCGG<br>TCGCGGAAATCGGCGCGGTAGCGAGCGGTATCTCCGGCTCCGGC<br>CCGACCTTGTTCGCTCTGTGTGACAAGCCGGAAACCGCCCAGCG<br>CGTTGCCGACTGGTTGGGTAAGAACTACCTGCAAAATCAGGAA<br>GGTTTTGTTCATATTTGCCGGCTGGATACGGCGGGCGCACGAGT<br>ACTGGAAAACTAA |

| Gene sequence | Description |
|---|---|
| *thrC*<br>**SEQ ID NO: 30** | ATGAAACTCTACAATCTGAAAGATCACAACGAGCAGGTCAGCTT<br>TGCGCAAGCCGTAACCCAGGGGTTGGGCAAAAATCAGGGGCTG<br>TTTTTTCCGCACGACCTGCCGGAATTCAGCCTGACTGAAATTGA<br>TGAGATGCTGAAGCTGGATTTTGTCACCCGCAGTGCGAAGATCC<br>TCTCGGCGTTTATTGGTGATGAAATCCCACAGGAAATCCTGGAA<br>GAGCGCGTGCGCGCGGCGTTTGCCTTCCCGGCTCCGGTCGCCAA<br>TGTTGAAAGCGATGTCGGTTGTCTGGAATTGTTCCACGGGCCAA<br>CGCTGGCATTTAAAGATTTCGGCGGTCGCTTTATGGCACAAATG<br>CTGACCCATATTGCGGGTGATAAGCCAGTGACCATTCTGACCGC<br>GACCTCCGGTGATACCGGAGCGGCAGTGGCTCATGCTTTCTACG<br>GTTTACCGAATGTGAAAGTGGTTATCCTCTATCCACGAGGCAAA<br>ATCAGTCCACTGCAAGAAAAACTGTTCTGTACATTGGGCGGCAA<br>TATCGAAACTGTTGCCATCGACGGCGATTTCGATGCCTGTCAGG<br>CGCTGGTGAAGCAGGCGTTTGATGATGAAGAACTGAAAGTGGC<br>GCTAGGGTTAAACTCGGCTAACTCGATTAACATCAGCCGTTTGC<br>TGGCGCAGATTTGCTACTACTTTGAAGCTGTTGCGCAGCTGCCG<br>CAGGAGACGCGCAACCAGCTGGTTGTCTCGGTGCCAAGCGGAA<br>ACTTCGGCGATTTGACGGCGGGTCTGCTGGCGAAGTCACTCGGT<br>CTGCCGGTGAAACGTTTTATTGCTGCGACCAACGTGAACGATAC<br>CGTGCCACGTTTCCTGCACGACGGTCAGTGGTCACCCAAAGCGA<br>CTCAGGCGACGTTATCCAACGCGATGGACGTGAGTCAGCCGAA<br>CAACTGGCCGCGTGTGGAAGAGTTGTTCCGCCGCAAAATCTGGC<br>AACTGAAAGAGCTGGGTTATGCAGCCGTGGATGATGAAACCAC<br>GCAACAGACAATGCGTGAGTTAAAAGAACTGGGCTACACTTCG<br>GAGCCGCACGCTGCCGTAGCTTATCGTGCGCTGCGTGATCAGTT<br>GAATCCAGGCGAATATGGCTTGTTCCTCGGCACCGCGCATCCGG<br>CGAAATTTAAAGAGAGCGTGGAAGCGATTCTCGGTGAAACGTT<br>GGATCTGCCAAAAGAGCTGGCAGAACGTGCTGATTTACCCTTGC<br>TTTCACATAATCTGCCCGCCGATTTTGCTGCGTTGCGTAAATTGA<br>TGATGAATCATCAGTAA |
| *ilvA*$^{fbr}$<br>**SEQ ID NO: 31** | ATGAGTGAAACATACGTGTCTGAGAAAAGTCCAGGAGTGATGG<br>CTAGCGGAGCGGAGCTGATTCGTGCCGCCGACATTCAAACGGC<br>GCAGGCACGAATTTCCTCCGTCATTGCACCAACTCCATTGCAGT<br>ATTGCCCTCGTCTTTCTGAGGAAACCGGAGCGGAAATCTACCTT<br>AAGCGTGAGGATCTGCAGGATGTTCGTTCCTACAAGATCCGCGG<br>TGCGCTGAACTCTGGAGCGCAGCTCACCCAAGAGCAGCGCGAT<br>GCAGGTATCGTTGCCGCATCTGCAGGTAACCATGCCCAGGGCGT<br>GGCCTATGTGTGCAAGTCCTTGGGCGTTCAGGGACGCATCTATG<br>TTCCTGTGCAGACTCCAAAGCAAAAGCGTGACCGCATCATGGTT<br>CACGGCGGAGAGTTTGTCTCCTTGGTGGTCACTGGCAATAACTT<br>CGACGAAGCATCGGCTGCAGCGCATGAAGATGCAGAGCGCACC<br>GGCGCAACGCTGATCGAGCCTTTCGATGCTCGCAACACCGTCAT<br>CGGTCAGGGCACCGTGGCTGCTGAGATCTTGTCGCAGCTGACTT<br>CCATGGGCAAGAGTGCAGATCACGTGATGGTTCCAGTCGGCGGT<br>GGCGGACTTCTTGCAGGTGTGGTCAGCTACATGGCTGATATGGC<br>ACCTCGCACTGCGATCGTTGGTATCGAACCAGCGGGAGCAGCAT |

| Gene sequence | Description |
|---|---|
| | CCATGCAGGCTGCATTGCACAATGGTGGACCAATCACTTTGGAG<br>ACTGTTGATCCCTTTGTGGACGGCGCAGCAGTCAAACGTGTCGG<br>AGATCTCAACTACACCATCGTGGAGAAGAACCAGGGTCGCGTG<br>CACATGATGAGCGCGACCGAGGGCGCTGTGTGTACTGAGATGCT<br>CGATCTTTACCAAAACGAAGGCATCATCGCGGAGCCTGCTGGCG<br>CGCTGTCTATCGCTGGGTTGAAGGAAATGTCCTTTGCACCTGGT<br>TCTGCAGTGGTGTGCATCATCTCTGGTGGCAACAACGATGTGCT<br>GCGTTATGCGGAAATCGCTGAGCGCTCCTTGGTGCACCGCGGTT<br>TGAAGCACTACTTCTTGGTGAACTTCCCGCAAAAGCCTGGTCAG<br>TTGCGTCACTTCCTGGAAGATATCCTGGGACCGGATGATGACAT<br>CACGCTGTTTGAGTACCTCAAGCGCAACAACCGTGAGACCGGTA<br>CTGCGTTGGTGGGTATTCACTTGAGTGAAGCATCAGGATTGGAT<br>TCTTTGCTGGAACGTATGGAGGAATCGGCAATTGATTCCCGTCG<br>CCTCGAGCCGGGCACCCCTGAGTACGAATACTTGACCTAA |
| *aceE*<br>**SEQ ID NO: 32** | ATGTCAGAACGTTTCCCAAATGACGTGGATCCGATCGAAACTCG<br>CGACTGGCTCCAGGCGATCGAATCGGTCATCCGTGAAGAAGGT<br>GTTGAGCGTGCTCAGTATCTGATCGACCAACTGCTTGCTGAAGC<br>CCGCAAAGGCGGTGTAAACGTAGCCGCAGGCACAGGTATCAGC<br>AACTACATCAACACCATCCCCGTTGAAGAACAACCGGAGTATCC<br>GGGTAATCTGGAACTGGAACGCCGTATTCGTTCAGCTATCCGCT<br>GGAACGCCATCATGACGGTGCTGCGTGCGTCGAAAAAAGACCT<br>CGAACTGGGCGGCCATATGGCGTCCTTCCAGTCTTCCGCAACCA<br>TTTATGATGTGTGCTTTAACCACTTCTTCCGTGCACGCAACGAGC<br>AGGATGGCGGCGACCTGGTTTACTTCCAGGGCCACATCTCCCCG<br>GGCGTGTACGCTCGTGCTTTCCTGGAAGGTCGTCTGACTCAGGA<br>GCAGCTGGATAACTTCCGTCAGGAAGTTCACGGCAATGGCCTCT<br>CTTCCTATCCGCACCCGAAACTGATGCCGGAATTCTGGCAGTTC<br>CCGACCGTATCTATGGGTCTGGGTCCGATTGGTGCTATTTACCA<br>GGCTAAATTCCTGAAATATCTGGAACACCGTGGCCTGAAAGATA<br>CCTCTAAACAAACCGTTTACGCGTTCCTCGGTGACGGTGAAATG<br>GACGAACCGGAATCCAAAGGTGCGATCACCATCGCTACCCGTG<br>AAAAACTGGATAACCTGGTCTTCGTTATCAACTGTAACCTGCAG<br>CGTCTTGACGGCCCGGTCACCGGTAACGGCAAGATCATCAACGA<br>ACTGGAAGGCATCTTCGAAGGTGCTGGCTGGAACGTGATCAAA<br>GTGATGTGGGGTAGCCGTTGGGATGAACTGCTGCGTAAGGATAC<br>CAGCGGTAAACTGATCCAGCTGATGAACGAAACCGTTGACGGC<br>GACTACCAGACCTTCAAATCGAAAGATGGTGCGTACGTTCGTGA<br>ACACTTCTTCGGTAAATATCCTGAAACCGCAGCACTGGTTGCAG<br>ACTGGACTGACGAGCAGATCTGGGCACTGAACCGTGGTGGTCA<br>CGATCCGAAGAAAATCTACGCTGCATTCAAGAAAGCGCAGGAA<br>ACCAAAGGCAAAGCGACAGTAATCCTTGCTCATACCATTAAAG<br>GTTACGGCATGGGCGACGCGGCTGAAGGTAAAAACATCGCGCA<br>CCAGGTTAAGAAAATGAACATGGACGGTGTGCGTCATATCCGC<br>GACCGTTTCAATGTGCCGGTGTCTGATGCAGATATCGAAAAACT<br>GCCGTACATCACCTTCCCGGAAGGTTCTGAAGAGCATACCTATC<br>TGCACGCTCAGCGTCAGAAACTGCACGGTTATCTGCCAAGCCGT |

| Gene sequence | Description |
|---|---|
| | CAGCCGAACTTCACCGAGAAGCTTGAGCTGCCGAGCCTGCAAG<br>ACTTCGGCGCGCTGTTGGAAGAGCAGAGCAAAGAGATCTCTAC<br>CACTATCGCTTTCGTTCGTGCTCTGAACGTGATGCTGAAGAACA<br>AGTCGATCAAAGATCGTCTGGTACCGATCATCGCCGACGAAGCG<br>CGTACTTTCGGTATGGAAGGTCTGTTCCGTCAGATTGGTATTTAC<br>AGCCCGAACGGTCAGCAGTACACCCCGCAGGACCGCGAGCAGG<br>TTGCTTACTATAAAGAAGACGAGAAAGGTCAGATTCTGCAGGA<br>AGGGATCAACGAGCTGGGCGCAGGTTGTTCCTGGCTGGCAGCG<br>GCGACCTCTTACAGCACCAACAATCTGCCGATGATCCCGTTCTA<br>CATCTATTACTCGATGTTCGGCTTCCAGCGTATTGGCGATCTGTG<br>CTGGGCGGCTGGCGACCAGCAAGCGCGTGGCTTCCTGATCGGCG<br>GTACTTCCGGTCGTACCACCCTGAACGGCGAAGGTCTGCAGCAC<br>GAAGATGGTCACAGCCACATTCAGTCGCTGACTATCCCGAACTG<br>TATCTCTTACGACCCGGCTTACGCTTACGAAGTTGCTGTCATCAT<br>GCATGACGGTCTGGAGCGTATGTACGGTGAAAAACAAGAGAAC<br>GTTTACTACTACATCACTACGCTGAACGAAAACTACCACATGCC<br>GGCAATGCCGGAAGGTGCTGAGGAAGGTATCCGTAAAGGTATC<br>TACAAACTCGAAACTATTGAAGGTAGCAAAGGTAAAGTTCAGC<br>TGCTCGGCTCCGGTTCTATCCTGCGTCACGTCCGTGAAGCAGCT<br>GAGATCCTGGCGAAAGATTACGGCGTAGGTTCTGACGTTTATAG<br>CGTGACCTCCTTCACCGAGCTGGCGCGTGATGGTCAGGATTGTG<br>AACGCTGGAACATGCTGCACCCGCTGGAAACTCCGCGCGTTCCG<br>TATATCGCTCAGGTGATGAACGACGCTCCGGCAGTGGCATCTAC<br>CGACTATATGAAACTGTTCGCTGAGCAGGTCCGTACTTACGTAC<br>CGGCTGACGACTACCGCGTACTGGGTACTGATGGCTTCGGTCGT<br>TCCGACAGCCGTGAGAACCTGCGTCACCACTTCGAAGTTGATGC<br>TTCTTATGTCGTGGTTGCGGCGCTGGGCGAACTGGCTAAACGTG<br>GCGAAATCGATAAGAAAGTGGTTGCTGACGCAATCGCCAAATT<br>CAACATCGATGCAGATAAAGTTAACCCGCGTCTGGCGTAA |
| *aceF*<br>**SEQ ID NO: 33** | ATGGCTATCGAAATCAAAGTACCGGACATCGGGGCTGATGAAG<br>TTGAAATCACCGAGATCCTGGTCAAAGTGGGCGACAAAGTTGA<br>AGCCGAACAGTCGCTGATCACCGTAGAAGGCGACAAAGCCTCT<br>ATGGAAGTTCCGTCTCCGCAGGCGGGTATCGTTAAAGAGATCAA<br>AGTCTCTGTTGGCGATAAAACCCAGACCGGCGCACTGATTATGA<br>TTTTCGATTCCGCCGACGGTGCAGCAGACGCTGCACCTGCTCAG<br>GCAGAAGAGAAGAAAGAAGCAGCTCCGGCAGCAGCACCAGCG<br>GCTGCGGCGGCAAAAGACGTTAACGTTCCGGATATCGGCAGCG<br>ACGAAGTTGAAGTGACCGAAATCCTGGTGAAAGTTGGCGATAA<br>AGTTGAAGCTGAACAGTCGCTGATCACCGTAGAAGGCGACAAG<br>GCTTCTATGGAAGTTCCGGCTCCGTTTGCTGGCACCGTGAAAGA<br>GATCAAAGTGAACGTGGGTGACAAAGTGTCTACCGGCTCGCTG<br>ATTATGGTCTTCGAAGTCGCGGGTGAAGCAGGCGCGGCAGCTCC<br>GGCCGCTAAACAGGAAGCAGCTCCGGCAGCGGCCCCTGCACCA<br>GCGGCTGGCGTGAAAGAAGTTAACGTTCCGGATATCGGCGGTG<br>ACGAAGTTGAAGTGACTGAAGTGATGGTGAAAGTGGGCGACAA<br>AGTTGCCGCTGAACAGTCACTGATCACCGTAGAAGGCGACAAA |

| Gene sequence | Description |
|---|---|
| | GCTTCTATGGAAGTTCCGGCGCCGTTTGCAGGCGTCGTGAAGGA<br>ACTGAAAGTCAACGTTGGCGATAAAGTGAAAACTGGCTCGCTG<br>ATTATGATCTTCGAAGTTGAAGGCGCAGCGCCTGCGGCAGCTCC<br>TGCGAAACAGGAAGCGGCAGCGCCGGCACCGGCAGCAAAAGCT<br>GAAGCCCCGGCAGCAGCACCAGCTGCGAAAGCGGAAGGCAAAT<br>CTGAATTTGCTGAAAACGACGCTTATGTTCACGCGACTCCGCTG<br>ATCCGCCGTCTGGCACGCGAGTTTGGTGTTAACCTTGCGAAAGT<br>GAAGGGCACTGGCCGTAAAGGTCGTATCCTGCGCGAAGACGTT<br>CAGGCTTACGTGAAAGAAGCTATCAAACGTGCAGAAGCAGCTC<br>CGGCAGCGACTGGCGGTGGTATCCCTGGCATGCTGCCGTGGCCG<br>AAGGTGGACTTCAGCAAGTTTGGTGAAATCGAAGAAGTGGAAC<br>TGGGCCGCATCCAGAAAATCTCTGGTGCGAACCTGAGCCGTAAC<br>TGGGTAATGATCCCGCATGTTACTCACTTCGACAAAACCGATAT<br>CACCGAGTTGGAAGCGTTCCGTAAACAGCAGAACGAAGAAGCG<br>GCGAAACGTAAGCTGGATGTGAAGATCACCCCGGTTGTCTTCAT<br>CATGAAAGCCGTTGCTGCAGCTCTTGAGCAGATGCCTCGCTTCA<br>ATAGTTCGCTGTCGGAAGACGGTCAGCGTCTGACCCTGAAGAAA<br>TACATCAACATCGGTGTGGCGGTGGATACCCCGAACGGTCTGGT<br>TGTTCCGGTATTCAAAGACGTCAACAAGAAAGGCATCATCGAGC<br>TGTCTCGCGAGCTGATGACTATTTCTAAGAAAGCGCGTGACGGT<br>AAGCTGACTGCGGGCGAAATGCAGGGCGGTTGCTTCACCATCTC<br>CAGCATCGGCGGCCTGGGTACTACCCACTTCGCGCCGATTGTGA<br>ACGCGCCGGAAGTGGCTATCCTCGGCGTTTCCAAGTCCGCGATG<br>GAGCCGGTGTGGAATGGTAAAGAGTTCGTGCCGCGTCTGATGCT<br>GCCGATTTCTCTCTCCTTCGACCACCGCGTGATCGACGGTGCTG<br>ATGGTGCCCGTTTCATTACCATCATTAACAACACGCTGTCTGAC<br>ATTCGCCGTCTGGTGATGTAA |
| *lpd*<br>**SEQ ID NO: 34** | ATGAGTACTGAAATCAAAACTCAGGTCGTGGTACTTGGGGCAG<br>GCCCCGCAGGTTACTCCGCTGCCTTCCGTTGCGCTGATTTAGGTC<br>TGGAAACCGTAATCGTAGAACGTTACAACACCCTTGGCGGTGTT<br>TGCCTGAACGTCGGCTGTATCCCTTCTAAAGCACTGCTGCACGT<br>AGCAAAAGTTATCGAAGAAGCCAAAGCGCTGGCTGAACACGGT<br>ATCGTCTTCGGCGAACCGAAAACCGATATCGACAAGATTCGTAC<br>CTGGAAAGAGAAAGTGATCAATCAGCTGACCGGTGGTCTGGCT<br>GGTATGGCGAAAGGCCGCAAAGTCAAAGTGGTCAACGGTCTGG<br>GTAAATTCACCGGGGCTAACACCCTGGAAGTTGAAGGTGAGAA<br>CGGCAAAACCGTGATCAACTTCGACAACGCGATCATTGCAGCG<br>GGTTCTCGCCCGATCCAACTGCCGTTTATTCCGCATGAAGATCC<br>GCGTATCTGGGACTCCACTGACGCGCTGGAACTGAAAGAAGTA<br>CCAGAACGCCTGCTGGTAATGGGTGGCGGTATCATCGGTCTGGA<br>AATGGGCACCGTTTACCACGCGCTGGGTTCACAGATTGACGTGG<br>TTGAAATGTTCGACCAGGTTATCCCGGCAGCTGACAAAGACATC<br>GTTAAAGTCTTCACCAAGCGTATCAGCAAGAAATTCAACCTGAT<br>GCTGGAAACCAAAGTTACCGCCGTTGAAGCGAAAGAAGACGGC<br>ATTTATGTGACGATGGAAGGCAAAAAAGCACCCGCTGAACCGC<br>AGCGTTACGACGCCGTGCTGGTAGCGATTGGTCGTGTGCCGAAC |

| Gene sequence | Description |
|---|---|
| | GGTAAAAACCTCGACGCAGGCAAAGCAGGCGTGGAAGTTGACG<br>ACCGTGGTTTCATCCGCGTTGACAAACAGCTGCGTACCAACGTA<br>CCGCACATCTTTGCTATCGGCGATATCGTCGGTCAACCGATGCT<br>GGCACACAAAGGTGTTCACGAAGGTCACGTTGCCGCTGAAGTTA<br>TCGCCGGTAAGAAACACTACTTCGATCCGAAAGTTATCCCGTCC<br>ATCGCCTATACCAAACCAGAAGTTGCATGGGTGGGTCTGACTGA<br>GAAAGAAGCGAAAGAGAAAGGCATCAGCTATGAAACCGCCACC<br>TTCCCGTGGGCTGCTTCTGGTCGTGCTATCGCTTCCGACTGCGCA<br>GACGGTATGACCAAGCTGATTTTCGACAAAGAATCTCACCGTGT<br>GATCGGTGGTGCGATTGTCGGTACTAACGGCGGCGAGCTGCTGG<br>GTGAAATCGGCCTGGCAATCGAAATGGGTTGTGATGCTGAAGA<br>CATCGCACTGACCATCCACGCGCACCCGACTCTGCACGAGTCTG<br>TGGGCCTGGCGGCAGAAGTGTTCGAAGGTAGCATTACCGACCTG<br>CCGAACCCGAAAGCGAAGAAGAAGTAA |
| *tesB*<br>**SEQ ID NO: 10** | ATGAGTCAGGCGCTAAAAAATTTACTGACATTGTTAAATCTGGA<br>AAAAATTGAGGAAGGACTCTTTCGCGGCCAGAGTGAAGATTTA<br>GGTTTACGCCAGGTGTTTGGCGGCCAGGTCGTGGGTCAGGCCTT<br>GTATGCTGCAAAAGAGACCGTCCCTGAAGAGCGGCTGGTACATT<br>CGTTTCACAGCTACTTTCTTCGCCCTGGCGATAGTAAGAAGCCG<br>ATTATTTATGATGTCGAAACGCTGCGTGACGGTAACAGCTTCAG<br>CGCCCGCCGGGTTGCTGCTATTCAAAACGGCAAACCGATTTTTT<br>ATATGACTGCCTCTTTCCAGGCACCAGAAGCGGGTTTCGAACAT<br>CAAAAAACAATGCCGTCCGCGCCAGCGCCTGATGGCCTCCCTTC<br>GGAAACGCAAATCGCCCAATCGCTGGCGCACCTGCTGCCGCCA<br>GTGCTGAAAGATAAATTCATCTGCGATCGTCCGCTGGAAGTCCG<br>TCCGGTGGAGTTTCATAACCCACTGAAAGGTCACGTCGCAGAAC<br>CACATCGTCAGGTGTGGATCCGCGCAAATGGTAGCGTGCCGGAT<br>GACCTGCGCGTTCATCAGTATCTGCTCGGTTACGCTTCTGATCTT<br>AACTTCCTGCCGGTAGCTCTACAGCCGCACGGCATCGGTTTTCT<br>CGAACCGGGGATTCAGATTGCCACCATTGACCATTCCATGTGGT<br>TCCATCGCCCGTTTAATTTGAATGAATGGCTGCTGTATAGCGTG<br>GAGAGCACCTCGGCGTCCAGCGCACGTGGCTTTGTGCGCGGTGA<br>GTTTTATACCCAAGACGGCGTACTGGTTGCCTCGACCGTTCAGG<br>AAGGGGTGATGCGTAATCACAATTAA |
| *acuI*<br>**SEQ ID NO: 35** | ATGCGTGCGGTACTGATCGAGAAGTCCGATGATACACAGTCCGT<br>CTCTGTCACCGAACTGGCTGAAGATCAACTGCCGGAAGGCGAC<br>GTTTTGGTAGATGTTGCTTATTCAACACTGAACTACAAAGACGC<br>CCTGGCAATTACCGGTAAAGCCCCCGTCGTTCGTCGTTTTCCGAT<br>GGTACCTGGAATCGACTTTACGGGTACCGTGGCCCAGTCTTCCC<br>ACGCCGACTTCAAGCCAGGTGATCGCGTAATCCTGAATGGTTGG<br>GGTGTGGGGGAAAAACATTGGGGCGGTTTAGCGGAGCGCGCTC<br>GCGTGCGCGGAGACTGGCTTGTTCCCTTGCCAGCCCCCCTGGAC<br>TTACGCCAAGCGGCCATGATCGGTACAGCAGGATACACGGCGA<br>TGTTGTGCGTTCTGGCGCTTGAACGTCACGGAGTGGTGCCGGGT<br>AATGGGGAAATCGTGGTGTCCGGTGCAGCAGGCGGCGTCGGCT<br>CCGTTGCGACGACCCTTCTTGCCGCTAAGGGCTATGAGGTAGCG |

| Gene sequence | Description |
|---|---|
| | GCAGTGACTGGACGTGCGTCCGAAGCAGAATATCTGCGCGGTTT<br>GGGGGCGGCGAGCGTAATTGATCGTAACGAATTAACGGGGAAG<br>GTACGCCCGCTGGGTCAGGAGCGTTGGGCTGGCGGGATTGACGT<br>GGCGGGATCAACCGTGCTTGCGAACATGCTTTCTATGATGAAGT<br>ATCGCGGGGTAGTCGCTGCGTGTGGCCTGGCCGCGGGCATGGAT<br>CTGCCCGCGTCTGTCGCGCCCTTTATTCTTCGTGGGATGACGCTG<br>GCAGGGGTGGATAGCGTTATGTGCCCAAAGACAGATCGTTTAGC<br>AGCGTGGGCCCGTTTGGCGTCAGATCTTGACCCTGCCAAGCTGG<br>AGGAGATGACTACAGAGTTGCCGTTTAGTGAAGTAATCGAGAC<br>AGCACCCAAATTCTTGGACGGGACGGTTCGTGGCCGCATTGTTA<br>TCCCCGTAACGCCCTAA |

**Table 5. Propionate Cassette Sequences Sleeping Beauty Operon**

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| *Sbm*<br>**SEQ ID NO: 36** | ATGTCTAACGTGCAGGAGTGGCAACAGCTTGCCAACAAGGAA<br>TTGAGCCGTCGGGAGAAAACTGTCGACTCGCTGGTTCATCAAA<br>CCGCGGAAGGGATCGCCATCAAGCCGCTGTATACCGAAGCCG<br>ATCTCGATAATCTGGAGGTGACAGGTACCCTTCCTGGTTTGCC<br>GCCCTACGTTCGTGGCCCGCGTGCCACTATGTATACCGCCCAA<br>CCGTGGACCATCCGTCAGTATGCTGGTTTTTCAACAGCAAAAG<br>AGTCCAACGCTTTTTATCGCCGTAACCTGGCCGCCGGGCAAAA<br>AGGTCTTTCCGTTGCGTTTGACCTTGCCACCCACCGTGGCTAC<br>GACTCCGATAACCCGCGCGTGGCGGGCGACGTCGGCAAAGCG<br>GGCGTCGCTATCGACACCGTGGAAGATATGAAAGTCCTGTTCG<br>ACCAGATCCCGCTGGATAAAATGTCGGTTTCGATGACCATGAA<br>TGGCGCAGTGCTACCAGTACTGGCGTTTTATATCGTCGCCGCA<br>GAAGAGCAAGGTGTTACACCTGATAAACTGACCGGCACCATT<br>CAAAACGATATTCTCAAAGAGTACCTCTGCCGCAACACCTATA<br>TTTACCCACCAAAACCGTCAATGCGCATTATCGCCGACATCAT<br>CGCCTGGTGTTCCGGCAACATGCCGCGATTTAATACCATCAGT<br>ATCAGCGGTTACCACATGGGTGAAGCGGGTGCCAACTGCGTG<br>CAGCAGGTAGCATTTACGCTCGCTGATGGGATTGAGTACATCA<br>AAGCAGCAATCTCTGCCGGACTGAAAATTGATGACTTCGCTCC<br>TCGCCTGTCGTTCTTCTTCGGCATCGGCATGGATCTGTTTATGA<br>ACGTCGCCATGTTGCGTGCGGCACGTTATTTATGGAGCGAAGC<br>GGTCAGTGGATTTGGCGCACAGGACCCGAAATCACTGGCGCT<br>GCGTACCCACTGCCAGACCTCAGGCTGGAGCCTGACTGAACA<br>GGATCCGTATAACAACGTTATCCGCACCACCATTGAAGCGCTG<br>GCTGCGACGCTGGGCGGTACTCAGTCACTGCATACCAACGCCT<br>TTGACGAAGCGCTTGGTTTGCCTACCGATTTCTCAGCACGCAT<br>TGCCCGCAACACCCAGATCATCATCCAGGAAGAATCAGAACT<br>CTGCCGCACCGTCGATCCACTGGCCGGATCCTATTACATTGAG<br>TCGCTGACCGATCAAATCGTCAAACAAGCCAGAGCTATTATCC<br>AACAGATCGACGAAGCCGGTGGCATGGCGAAAGCGATCGAAG<br>CAGGTCTGCCAAAACGAATGATCGAAGAGGCCTCAGCGCGCG<br>AACAGTCGCTGATCGACCAGGGCAAGCGTGTCATCGTTGGTGT |

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|---|---|
| | CAACAAGTACAAACTGGATCACGAAGACGAAACCGATGTACT<br>TGAGATCGACAACGTGATGGTGCGTAACGAGCAAATTGCTTC<br>GCTGGAACGCATTCGCGCCACCCGTGATGATGCCGCCGTAACC<br>GCCGCGTTGAACGCCCTGACTCACGCCGCACAGCATAACGAA<br>AACCTGCTGGCTGCCGCTGTTAATGCCGCTCGCGTTCGCGCCA<br>CCCTGGGTGAAATTTCCGATGCGCTGGAAGTCGCTTTCGACCG<br>TTATCTGGTGCCAAGCCAGTGTGTTACCGGCGTGATTGCGCAA<br>AGCTATCATCAGTCTGAGAAATCGGCCTCCGAGTTCGATGCCA<br>TTGTTGCGCAAACGGAGCAGTTCCTTGCCGACAATGGTCGTCG<br>CCCGCGCATTCTGATCGCTAAGATGGGCCAGGATGGACACGA<br>TCGCGGCGCGAAAGTGATCGCCAGCGCCTATTCCGATCTCGGT<br>TTCGACGTAGATTTAAGCCCGATGTTCTCTACACCTGAAGAGA<br>TCGCCCGCCTGGCCGTAGAAAACGACGTTCACGTAGTGGGCG<br>CATCCTCACTGGCTGCCGGTCATAAAACGCTGATCCCGGAACT<br>GGTCGAAGCGCTGAAAAAATGGGGACGCGAAGATATCTGCGT<br>GGTCGCGGGTGGCGTCATTCCGCCGCAGGATTACGCCTTCCTG<br>CAAGAGCGCGGCGTGGCGGCGATTTATGGTCCAGGTACACCT<br>ATGCTCGACAGTGTGCGCGACGTACTGAATCTGATAAGCCAGC<br>ATCATGATTAA |
| *ygfD*<br>**SEQ ID NO: 37** | ATGATTAATGAAGCCACGCTGGCAGAAAGTATTCGCCGCTTAC<br>GTCAGGGTGAGCGTGCCACACTCGCCCAGGCCATGACGCTGG<br>TGGAAAGCCGTCACCCGCGTCATCAGGCACTAAGTACGCAGC<br>TGCTTGATGCCATTATGCCGTACTGCGGTAACACCCTGCGACT<br>GGGCGTTACCGGCACCCCCGGCGCGGGGAAAAGTACCTTTCTT<br>GAGGCCTTTGGCATGTTGTTGATTCGAGAGGGATTAAAGGTCG<br>CGGTTATTGCGGTCGATCCCAGCAGCCCGGTCACTGGCGGTAG<br>CATTCTCGGGGATAAAACCCGCATGAATGACCTGGCGCGTGCC<br>GAAGCGGCGTTTATTCGCCCGGTACCATCCTCCGGTCATCTGG<br>GCGGTGCCAGTCAGCGAGCGCGGGAATTAATGCTGTTATGCG<br>AAGCAGCGGGTTATGACGTAGTGATTGTCGAAACGGTTGGCG<br>TCGGGCAGTCGGAAACAGAAGTCGCCCGCATGGTGGACTGTT<br>TTATCTCGTTGCAAATTGCCGGTGGCGGCGATGATCTGCAGGG<br>CATTAAAAAAGGGCTGATGGAAGTGGCTGATCTGATCGTTATC<br>AACAAAGACGATGGCGATAACCATACCAATGTCGCCATTGCC<br>CGGCATATGTACGAGAGTGCCCTGCATATTCTGCGACGTAAAT<br>ACGACGAATGGCAGCCACGGGTTCTGACTTGTAGCGCACTGG<br>AAAAACGTGGAATCGATGAGATCTGGCACGCCATCATCGACT<br>TCAAAACCGCGCTAACTGCCAGTGGTCGTTTACAACAAGTGCG<br>GCAACAACAATCGGTGGAATGGCTGCGTAAGCAGACCGAAGA<br>AGAAGTACTGAATCACCTGTTCGCGAATGAAGATTTCGATCGC<br>TATTACCGCCAGACGCTTTTAGCGGTCAAAAACAATACGCTCT<br>CACCGCGCACCGGCCTGCGGCAGCTCAGTGAATTTATCCAGAC<br>GCAATATTTTGATTAA |
| *ygfG*<br>**SEQ ID NO: 38** | ATGTCTTATCAGTATGTTAACGTTGTCACTATCAACAAAGTGG<br>CGGTCATTGAGTTTAACTATGGCCGAAAACTTAATGCCTTAAG<br>TAAAGTCTTTATTGATGATCTTATGCAGGCGTTAAGCGATCTC<br>AACCGGCCGGAAATTCGCTGTATCATTTTGCGCGCACCGAGTG<br>GATCCAAAGTCTTCTCCGCAGGTCACGATATTCACGAACTGCC |

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|---|---|
| | GTCTGGCGGTCGCGATCCGCTCTCCTATGATGATCCATTGCGT<br>CAAATCACCCGCATGATCCAAAAATTCCCGAAACCGATCATTT<br>CGATGGTGGAAGGTAGTGTTTGGGGTGGCGCATTTGAAATGAT<br>CATGAGTTCCGATCTGATCATCGCCGCCAGTACCTCAACCTTC<br>TCAATGACGCCTGTAAACCTCGGCGTCCCGTATAACCTGGTCG<br>GCATTCACAACCTGACCCGCGACGCGGGCTTCCACATTGTCAA<br>AGAGCTGATTTTTACCGCTTCGCCAATCACCGCCCAGCGCGCG<br>CTGGCTGTCGGCATCCTCAACCATGTTGTGGAAGTGGAAGAAC<br>TGGAAGATTTCACCTTACAAATGGCGCACCACATCTCTGAGAA<br>AGCGCCGTTAGCCATTGCCGTTATCAAAGAAGAGCTGCGTGTA<br>CTGGGCGAAGCACACACCATGAACTCCGATGAATTTGAACGT<br>ATTCAGGGGATGCGCCGCGCGGTGTATGACAGCGAAGATTAC<br>CAGGAAGGGATGAACGCTTTCCTCGAAAAACGTAAACCTAAT<br>TTCGTTGGTCATTAA |
| *ygfH*<br>**SEQ ID NO: 39** | ATGGAAACTCAGTGGACAAGGATGACCGCCAATGAAGCGGCA<br>GAAATTATCCAGCATAACGACATGGTGGCATTTAGCGGCTTTA<br>CCCCGGCGGGTTCGCCGAAAGCCCTACCCACCGCGATTGCCCG<br>CAGAGCTAACGAACAGCATGAGGCCAAAAAGCCGTATCAAAT<br>TCGCCTTCTGACGGGTGCGTCAATCAGCGCCGCCGCTGACGAT<br>GTACTTTCTGACGCCGATGCTGTTTCCTGGCGTGCGCCATATC<br>AAACATCGTCCGGTTTACGTAAAAAGATCAATCAGGGCGCGG<br>TGAGTTTCGTTGACCTGCATTTGAGCGAAGTGGCGCAAATGGT<br>CAATTACGGTTTCTTCGGCGACATTGATGTTGCCGTCATTGAA<br>GCATCGGCACTGGCACCGGATGGTCGAGTCTGGTTAACCAGC<br>GGGATCGGTAATGCGCCGACCTGGCTGCTGCGGGCGAAGAAA<br>GTGATCATTGAACTCAATCACTATCACGATCCGCGCGTTGCAG<br>AACTGGCGGATATTGTGATTCCTGGCGCGCCACCGCGGCGCAA<br>TAGCGTGTCGATCTTCCATGCAATGGATCGCGTCGGTACCCGC<br>TATGTGCAAATCGATCCGAAAAAGATTGTCGCCGTCGTGGAA<br>ACCAACTTGCCCGACGCCGGTAATATGCTGGATAAGCAAAAT<br>CCCATGTGCCAGCAGATTGCCGATAACGTGGTCACGTTCTTAT<br>TGCAGGAAATGGCGCATGGGCGTATTCCGCCGGAATTTCTGCC<br>GCTGCAAAGTGGCGTGGGCAATATCAATAATGCGGTAATGGC<br>GCGTCTGGGGGAAAACCCGGTAATTCCTCCGTTTATGATGTAT<br>TCGGAAGTGCTACAGGAATCGGTGGTGCATTTACTGGAAACC<br>GGCAAAATCAGCGGGGCCAGCGCCTCCAGCCTGACAATCTCG<br>GCCGATTCCCTGCGCAAGATTTACGACAATATGGATTACTTTG<br>CCAGCCGCATTGTGTTGCGTCCGCAGGAGATTTCCAATAACCC<br>GGAAATCATCCGTCGTCTGGGCGTCATCGCTCTGAACGTCGGC<br>CTGGAGTTTGATATTTACGGGCATGCCAACTCAACACACGTAG<br>CCGGGGTCGATCTGATGAACGGCATCGGCGGCAGCGGTGATT<br>TTGAACGCAACGCGTATCTGTCGATCTTTATGGCCCCGTCGAT<br>TGCTAAAGAAGGCAAGATCTCAACCGTCGTGCCAATGTGCAG<br>CCATGTTGATCACAGCGAACACAGCGTCAAAGTGATCATCACC<br>GAACAAGGGATCGCCGATCTGCGCGGTCTTTCCCCGCTTCAAC<br>GCGCCCGCACTATCATTGATAATTGTGCACATCCTATGTATCG<br>GGATTATCTGCATCGCTATCTGGAAAATGCGCCTGGCGGACAT<br>ATTCACCACGATCTTAGCCACGTCTTCGACTTACACCGTAATTT |

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|---|---|
| | AATTGCAACCGGCTCGATGCTGGGTTAA |

**Table 6. Sequences of Propionate Cassette from Propioni Bacteria**

| Description | Sequence |
|---|---|
| mutA<br>**SEQ ID NO: 40** | ATGAGCAGCACGGATCAGGGGACCAACCCCGCCGACACTGAC<br>GACCTCACTCCCACCACACTCAGTCTGGCCGGGGATTTCCCCA<br>AGGCCACTGAGGAGCAGTGGGAGCGCGAAGTTGAGAAGGTAT<br>TCAACCGTGGTCGTCCACCGGAGAAGCAGCTGACCTTCGCCGA<br>GTGTCTGAAGCGCCTGACGGTTCACACCGTCGATGGCATCGAC<br>ATCGTGCCGATGTACCGTCCGAAGGACGCGCCGAAGAAGCTG<br>GGTTACCCCGGCGTCACCCCCTTCACCCGCGGCACCACGGTGC<br>GCAACGGTGACATGGATGCCTGGGACGTGCGCGCCCTGCACG<br>AGGATCCCGACGAGAAGTTCACCCGCAAGGCGATCCTTGAAG<br>ACCTGGAGCGTGGCGTCACCTCCCTGTTGTTGCGCGTTGATCC<br>CGACGCGATCGCACCCGAGCACCTCGACGAGGTCCTCTCCGAC<br>GTCCTGCTGGAAATGACCAAGGTGGAGGTCTTCAGCCGCTACG<br>ACCAGGGTGCCGCCGCCGAGGCCTTGATGGGCGTCTACGAGC<br>GCTCCGACAAGCCGGCGAAGGACCTGGCCCTGAACCTGGGCC<br>TGGATCCCATCGGCTTCGCGGCCCTGCAGGGCACCGAGCCGG<br>ATCTGACCGTGCTCGGTGACTGGGTGCGCCGCCTGGCGAAGTT<br>CTCACCGGACTCGCGCGCCGTCACGATCGACGCGAACGTCTAC<br>CACAACGCCGGTGCCGGCGACGTGGCAGAGCTCGCTTGGGCA<br>CTGGCCACCGGCGCGGAGTACGTGCGCGCCCTGGTCGAACAG<br>GGCTTCAACGCCACAGAGGCCTTCGACACGATCAACTTCCGTG<br>TCACCGCCACCCACGACCAGTTCCTCACGATCGCCCGTCTTCG<br>CGCCCTGCGCGAGGCATGGGCCCGCATCGGCGAGGTCTTTGGC<br>GTGGACGAGGACAAGCGCGGCGCTCGCCAGAATGCGATCACC<br>AGTTGGCGTGAGCTCACCCGCGAAGACCCCTATGTCAACATCC<br>TTCGCGGTTCGATTGCCACCTTCTCCGCCTCCGTTGGCGGGGC<br>CGAGTCGATCACGACGCTGCCCTTCACCCAGGCCCTCGGCCTG<br>CCGGAGGACGACTTCCCGCTGCGCATCGCGCGCAACACGGGC<br>ATCGTGCTCGCCGAAGAGGTGAACATCGGCCGCGTCAACGAC<br>CCGGCCGGTGGCTCCTACTACGTCGAGTCGCTCACTCGCACCC<br>TGGCCGACGCTGCCTGGAAGGAATTCCAGGAGGTCGAGAAGC<br>TCGGTGGCATGTCGAAGGCGGTCATGACCGAGCACGTCACCA<br>AGGTGCTCGACGCCTGCAATGCCGAGCGCGCCAAGCGCCTGG<br>CCAACCGCAAGCAGCCGATCACCGCGGTCAGCGAGTTCCCGA<br>TGATCGGGGCCCGCAGCATCGAGACCAAGCCGTTCCCAACCG<br>CTCCGGCGCGCAAGGGCCTGGCCTGGCATCGCGATTCCGAGGT<br>GTTCGAGCAGCTGATGGATCGCTCCACCAGCGTCTCCGAGCGC<br>CCCAAGGTGTTCCTTGCCTGCCTGGGCACCCGTCGCGACTTCG<br>GTGGCCGCGAGGGCTTCTCCAGCCCGGTATGGCACATCGCCGG<br>TATCGACACCCCGCAGGTCGAAGGCGGCACCACCGCCGAGAT<br>CGTCGAGGCGTTCAAGAAGTCGGGCGCCCAGGTGGCCGATCT<br>CTGCTCGTCCGCCAAGATCTACGCGCAGCAGGGACTTGAGGTT<br>GCCAAGGCGCTCAAGGCCGCCGGCGCGAAGGCCCTGTATCTG<br>TCGGGCGCCTTCAAGGAGTTCGGCGATGACGCCGCCGAGGCC |

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| | GAGAAGCTGATCGACGGACGCCTGTACATGGGCATGGATGTC<br>GTCGACACCCTGTCCTCCACCCTTGATATCTTGGGAGTCGCGA<br>AGTGA |
| mutB<br>**SEQ ID NO: 41** | GTGAGCACTCTGCCCCGTTTTGATTCAGTTGACCTGGGCAATG<br>CCCCGGTTCCTGCTGATGCCGCACAGCGCTTCGAGGAGTTGGC<br>CGCCAAGGCCGGCACCGAAGAGGCGTGGGAGACGGCTGAGCA<br>GATTCCGGTTGGCACCCTGTTCAACGAAGACGTCTACAAGGAC<br>ATGGACTGGCTGGACACCTACGCCGGTATCCCGCCGTTCGTCC<br>ACGGCCCATATGCAACCATGTACGCGTTCCGTCCCTGGACGAT<br>TCGCCAGTACGCCGGCTTCTCCACGGCCAAGGAGTCCAACGCC<br>TTCTACCGCCGCAACCTTGCGGCGGGCCAGAAGGGCCTGTCGG<br>TTGCCTTCGACCTGCCCACCCACCGCGGCTACGACTCGGACAA<br>TCCCCGCGTCGCCGGTGACGTCGGCATGGCCGGGGTGGCCATC<br>GACTCCATCTATGACATGCGCGAGCTGTTCGCCGGCATTCCGC<br>TGGACCAGATGAGCGTGTCGATGACCATGAACGGCGCCGTGC<br>TGCCGATCCTGGCCCTCTATGTGGTGACCGCCGAGGAGCAGGG<br>CGTCAAGCCCGAGCAGCTCGCCGGGACGATCCAGAACGACAT<br>CCTCAAGGAGTTCATGGTTCGTAACACCTATATCTACCCGCCG<br>CAGCCGAGTATGCGAATCATCTCCGAGATCTTCGCCTACACGA<br>GTGCCAATATGCCGAAGTGGAATTCGATTTCCATTTCCGGCTA<br>CCACATGCAGGAAGCCGGCGCCACGGCCGACATCGAGATGGC<br>CTACACCCTGGCCGACGGTGTCGACTACATCCGCGCCGGCGAG<br>TCGGTGGGCCTCAATGTCGACCAGTTCGCGCCGCGTCTGTCCT<br>TCTTCTGGGGCATCGGCATGAACTTCTTCATGGAGGTTGCCAA<br>GCTGCGTGCCGCACGTATGTTGTGGGCCAAGCTGGTGCATCAG<br>TTCGGGCCGAAGAATCCGAAGTCGATGAGCCTGCGCACCCAC<br>TCGCAGACCTCCGGTTGGTCGCTGACCGCCCAGGACGTCTACA<br>ACAACGTCGTGCGTACCTGCATCGAGGCCATGGCCGCCACCCA<br>GGGCCATACCCAGTCGCTGCACACGAACTCGCTCGACGAGGC<br>CATTGCCCTACCGACCGATTTCAGCGCCCGCATCGCCCGTAAC<br>ACCCAGCTGTTCCTGCAGCAGGAATCGGGCACGACGCGCGTG<br>ATCGACCCGTGGAGCGGCTCGGCATACGTCGAGGAGCTCACC<br>TGGGACCTGGCCCGCAAGGCATGGGGCCACATCCAGGAGGTC<br>GAGAAGGTCGGCGGCATGGCCAAGGCCATCGAAAAGGGCATC<br>CCCAAGATGCGCATTGAGGAAGCCGCCGCCCGCACCCAGGCA<br>CGCATCGACTCCGGCCGTCAGCCGCTGATCGGCGTGAACAAGT<br>ACCGCCTGGAGCACGAGCCGCCGCTCGATGTGCTCAAGGTTG<br>ACAACTCCACGGTGCTCGCCGAGCAGAAGGCCAAGCTGGTCA<br>AGCTGCGCGCCGAGCGCGATCCCGAGAAGGTCAAGGCCGCCC<br>TCGACAAGATCACCTGGGCTGCCGCCAACCCCGACGACAAGG<br>ATCCGGATCGCAACCTGCTGAAGCTGTGCATCGACGCTGGCCG<br>CGCCATGGCGACGGTCGGCGAGATGAGCGACGCGCTCGAGAA<br>GGTCTTCGGACGCTACACCGCCCAGATTCGCACCATCTCCGGT<br>GTGTACTCGAAGGAAGTGAAGAACACGCCTGAGGTTGAGGAA<br>GCACGCGAGCTCGTTGAGGAATTCGAGCAGGCCGAGGGCCGT<br>CGTCCTCGCATCCTGCTGGCCAAGATGGGCCAGGACGGTCACG<br>ACCGTGGCCAGAAGGTCATCGCCACCGCCTATGCCGACCTCGG<br>TTTCGACGTCGACGTGGGCCCGCTGTTCCAGACCCCGGAGGAG |

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| | ACCGCACGTCAGGCCGTCGAGGCCGATGTGCACGTGGTGGGC<br>GTTTCGTCGCTCGCCGGCGGGCATCTGACGCTGGTTCCGGCCC<br>TGCGCAAGGAGCTGGACAAGCTCGGACGTCCCGACATCCTCA<br>TCACCGTGGGCGGCGTGATCCCTGAGCAGGACTTCGACGAGCT<br>GCGTAAGGACGGCGCCGTGGAGATCTACACCCCCGGCACCGT<br>CATTCCGGAGTCGGCGATCTCGCTGGTCAAGAAACTGCGGGCT<br>TCGCTCGATGCCTAG |
| GI:18042134<br>**SEQ ID NO: 42** | ATGAGTAATGAGGATCTTTTCATCTGTATCGATCACGTGGCAT<br>ATGCGTGCCCCGACGCCGACGAGGCTTCCAAGTACTACCAGG<br>AGACCTTCGGCTGGCATGAGCTCCACCGCGAGGAGAACCCGG<br>AGCAGGGAGTCGTCGAGATCATGATGGCCCCGGCTGCGAAGC<br>TGACCGAGCACATGACCCAGGTTCAGGTCATGGCCCCGCTCAA<br>CGACGAGTCGACCGTTGCCAAGTGGCTTGCCAAGCACAATGG<br>TCGCGCCGGACTGCACCACATGGCATGGCGTGTCGATGACATC<br>GACGCCGTCAGCGCCACCCTGCGCGAGCGCGGCGTGCAGCTG<br>CTGTATGACGAGCCCAAGCTCGGCACCGGCGGCAACCGCATC<br>AACTTCATGCATCCCAAGTCGGGCAAGGGCGTGCTCATCGAGC<br>TCACCCAGTACCCGAAGAACTGA |
| mmdA<br>**SEQ ID NO: 43** | ATGGCTGAAAACAACAATTTGAAGCTCGCCAGCACCATGGAA<br>GGTCGCGTGGAGCAGCTCGCAGAGCAGCGCCAGGTGATCGAA<br>GCCGGTGGCGGCGAACGTCGCGTCGAGAAGCAACATTCCCAG<br>GGTAAGCAGACCGCTCGTGAGCGCCTGAACAACCTGCTCGAT<br>CCCCATTCGTTCGACGAGGTCGGCGCTTTCCGCAAGCACCGCA<br>CCACGTTGTTCGGCATGGACAAGGCCGTCGTCCCGGCAGATGG<br>CGTGGTCACCGGCCGTGGCACCATCCTTGGTCGTCCCGTGCAC<br>GCCGCGTCCCAGGACTTCACGGTCATGGGTGGTTCGGCTGGCG<br>AGACGCAGTCCACGAAGGTCGTCGAGACGATGGAACAGGCGC<br>TGCTCACCGGCACGCCCTTCCTGTTCTTCTACGATTCGGGCGG<br>CGCCCGGATCCAGGAGGGCATCGACTCGCTGAGCGGTTACGG<br>CAAGATGTTCTTCGCCAACGTGAAGCTGTCGGGCGTCGTGCCG<br>CAGATCGCCATCATTGCCGGCCCCTGTGCCGGTGGCGCCTCGT<br>ATTCGCCGGCACTGACTGACTTCATCATCATGACCAAGAAGGC<br>CCATATGTTCATCACGGGCCCCCAGGTCATCAAGTCGGTCACC<br>GGCGAGGATGTCACCGCTGACGAACTCGGTGGCGCTGAGGCC<br>CATATGGCCATCTCGGGCAATATCCACTTCGTGGCCGAGGACG<br>ACGACGCCGCGGAGCTCATTGCCAAGAAGCTGCTGAGCTTCCT<br>TCCGCAGAACAACACTGAGGAAGCATCCTTCGTCAACCCGAA<br>CAATGACGTCAGCCCCAATACCGAGCTGCGCGACATCGTTCCG<br>ATTGACGGCAAGAAGGGCTATGACGTGCGCGATGTCATTGCC<br>AAGATCGTCGACTGGGGTGACTACCTCGAGGTCAAGGCCGGC<br>TATGCCACCAACCTCGTGACCGCCTTCGCCCGGGTCAATGGTC<br>GTTCGGTGGGCATCGTGGCCAATCAGCCGTCGGTGATGTCGGG<br>TTGCCTCGACATCAACGCCTCTGACAAGGCCGCCGAATTCGTG<br>AATTTCTGCGATTCGTTCAACATCCCGCTGGTGCAGCTGGTCG<br>ACGTGCCGGGCTTCCTGCCCGGCGTGCAGCAGGAGTACGGCG<br>GCATCATTCGCCATGGCGCGAAGATGCTGTACGCCTACTCCGA<br>GGCCACCGTGCCGAAGATCACCGTGGTGCTCCGCAAGGCCTA |

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| | CGGCGGCTCCTACCTGGCCATGTGCAACCGTGACCTTGGTGCC<br>GACGCCGTGTACGCCTGGCCCAGCGCCGAGATTGCGGTGATG<br>GGCGCCGAGGGTGCGGCAAATGTGATCTTCCGCAAGGAGATC<br>AAGGCTGCCGACGATCCCGACGCCATGCGCGCCGAGAAGATC<br>GAGGAGTACCAGAACGCGTTCAACACGCCGTACGTGGCCGCC<br>GCCCGCGGTCAGGTCGACGACGTGATTGACCCGGCTGATACCC<br>GTCGAAAGATTGCTTCCGCCCTGGAGATGTACGCCACCAAGCG<br>TCAGACCCGCCCGGCGAAGAAGCATGGAAACTTCCCCTGCTG<br>A |
| PFREUD_18870<br>**SEQ ID NO: 44** | ATGAGTCCGCGAGAAATTGAGGTTTCCGAGCCGCGCGAGGTT<br>GGTATCACCGAGCTCGTGCTGCGCGATGCCCATCAGAGCCTGA<br>TGGCCACACGAATGGCAATGGAAGACATGGTCGGCGCCTGTG<br>CAGACATTGATGCTGCCGGGTACTGGTCAGTGGAGTGTTGGGG<br>TGGTGCCACGTATGACTCGTGTATCCGCTTCCTCAACGAGGAT<br>CCTTGGGAGCGTCTGCGCACGTTCCGCAAGCTGATGCCCAACA<br>GCCGTCTCCAGATGCTGCTGCGTGGCCAGAACCTGCTGGGTTA<br>CCGCCACTACAACGACGAGGTCGTCGATCGCTTCGTCGACAAG<br>TCCGCTGAGAACGGCATGGACGTGTTCCGTGTCTTCGACGCCA<br>TGAATGATCCCCGCAACATGGCGCACGCCATGGCTGCCGTCAA<br>GAAGGCCGGCAAGCACGCGCAGGGCACCATTTGCTACACGAT<br>CAGCCCGGTCCACACCGTTGAGGGCTATGTCAAGCTTGCTGGT<br>CAGCTGCTCGACATGGGTGCTGATTCCATCGCCCTGAAGGACA<br>TGGCCGCCCTGCTCAAGCCGCAGCCGGCCTACGACATCATCAA<br>GGCCATCAAGGACACCTACGGCCAGAAGACGCAGATCAACCT<br>GCACTGCCACTCCACCACGGGTGTCACCGAGGTCTCCCTCATG<br>AAGGCCATCGAGGCCGGCGTCGACGTCGTCGACACCGCCATC<br>TCGTCCATGTCGCTCGGCCCGGGCCACAACCCCACCGAGTCGG<br>TTGCCGAGATGCTCGAGGGCACCGGGTACACCACCAACCTTG<br>ACTACGATCGCCTGCACAAGATCCGCGATCACTTCAAGGCCAT<br>CCGCCCGAAGTACAAGAAGTTCGAGTCGAAGACGCTTGTCGA<br>CACCTCGATCTTCAAGTCGCAGATCCCCGGCGGCATGCTCTCC<br>AACATGGAGTCGCAGCTGCGCGCCCAGGGCGCCGAGGACAAG<br>ATGGACGAGGTCATGGCAGAGGTGCCGCGCGTCCGCAAGGCC<br>GCCGGCTTCCCGCCCCTGGTCACCCCGTCCAGCCAGATCGTCG<br>GCACGCAGGCCGTGTTCAACGTGATGATGGGCGAGTACAAGA<br>GGATGACCGGCGAGTTCGCCGACATCATGCTCGGCTACTACGG<br>CGCCAGCCCGGCCGATCGCGATCCGAAGGTGGTCAAGTTGGC<br>CGAGGAGCAGTCCGGCAAGAAGCCGATCACCCAGCGCCCGGC<br>CGATCTGCTGCCCCCCGAGTGGGAGGAGCAGTCCAAGGAGGC<br>CGCGGCCCTCAAGGGCTTCAACGGCACCGACGAGGACGTGCT<br>CACCTATGCACTGTTCCCGCAGGTCGCTCCGGTCTTCTTCGAG<br>CATCGCGCCGAGGGCCCGCACAGCGTGGCTCTCACCGATGCCC<br>AGCTGAAGGCCGAGGCCGAGGGCGACGAGAAGTCGCTCGCCG<br>TGGCCGGTCCCGTCACCTACAACGTGAACGTGGGCGGAACCG<br>TCCGCGAAGTCACCGTTCAGCAGGCGTGA |
| Bccp<br>**SEQ ID NO: 45** | ATGAAACTGAAGGTAACAGTCAACGGCACTGCGTATGACGTT<br>GACGTTGACGTCGACAAGTCACACGAAAACCCGATGGGCACC<br>ATCCTGTTCGGCGGCGGCACCGGCGGCGCGCCGGCACCGCGC |

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| | GCAGCAGGTGGCGCAGGCGCCGGTAAGGCCGGAGAGGGCGA<br>GATTCCCGCTCCGCTGGCCGGCACCGTCTCCAAGATCCTCGTG<br>AAGGAGGGTGACACGGTCAAGGCTGGTCAGACCGTGCTCGTT<br>CTCGAGGCCATGAAGATGGAGACCGAGATCAACGCTCCCACC<br>GACGGCAAGGTCGAGAAGGTCCTTGTCAAGGAGCGTGACGCC<br>GTGCAGGGCGGTCAGGGTCTCATCAAGATCGGCTGA |

[0197] In some embodiments, the genetically engineered bacteria comprise one or more nucleic acid sequence(s) of **Table 4 (SEQ ID NO: 21- SEQ ID NO: 35, and SEQ ID NO: 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 one or more nucleic acid s sequence(s) of **Table 4 (SEQ ID NO: 21- SEQ ID NO: 35, and SEQ ID NO: 10**) 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 one or more nucleic acid sequence(s) of **Table 4 (SEQ ID NO: 21- SEQ ID NO: 35, and SEQ ID NO: 10**) or a functional fragment thereof, or a nucleic acid sequence that, but for the redundancy of the genetic code, encodes the same polypeptide as one or more nucleic acid sequence(s) of **Table 4 (SEQ ID NO: 21- SEQ ID NO: 35, and SEQ ID NO: 10**) or a functional fragment thereof.

[0198] In some embodiments, the genetically engineered bacteria comprise one or more nucleic acid sequence(s) of **Table 5 (SEQ ID NO: 36- SEQ ID NO: 39**) 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 one or more nucleic acid s sequence(s) of **Table 5 (SEQ ID NO: 36- SEQ ID NO: 39**) 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 one or more nucleic acid sequence(s) of **Table 5 (SEQ ID NO: 36- SEQ ID NO: 39**) or a functional fragment thereof, or a nucleic acid sequence that, but for the redundancy of the genetic code, encodes the same polypeptide as one or more nucleic acid sequence(s) of **Table 5 (SEQ ID NO: 36- SEQ ID NO: 39**) or a functional fragment thereof.

[0199] In some embodiments, the genetically engineered bacteria comprise one or more nucleic acid sequence(s) of **Table 6** (**SEQ ID NO: 40- SEQ ID NO: 45**) 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 one or more nucleic acid s sequence(s) of **Table 6** (**SEQ ID NO: 40- SEQ ID NO: 45**) 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 one or more nucleic acid sequence(s) of **Table 6** (**SEQ ID NO: 40- SEQ ID NO: 45**) or a functional fragment thereof, or a nucleic acid sequence that, but for the redundancy of the genetic code, encodes the same polypeptide as one or more nucleic acid sequence(s) of **Table 6** (**SEQ ID NO: 40- SEQ ID NO: 45**) or a functional fragment thereof.

[0200] **Table 7** lists exemplary polypeptide sequences, which may be encoded by the propionate production gene(s) or cattette(s) of the genetically engineered bacteria.

**Table 7. Polypeptide Sequences for Propionate Synthesis**

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| Pct **SEQ ID NO: 46** | MRKVPIITADEAAKLIKDGDTVTTSGFVGNAIPEALDRAVEKRFLETGE PKNITYVYCGSQGNRDGRGAEHFAHEGLLKRYIAGHWATVPALGKM AMENKMEAYNVSQGALCHLFRDIASHKPGVFTKVGIGTFIDPRNGGG KVNDITKEDIVELVEIKGQEYLFYPAFPIHVALIRGTYADESGNITFEKE VAPLEGTSVCQAVKNSGGIVVVQVERVVKAGTLDPRHVKVPGIYVDY VVVADPEDHQQSLDCEYDPALSGEHRRPEVVGEPLPLSAKKVIGRRGA IELEKDVAVNLGVGAPEYVASVADEEGIVDFMTLTAESGAIGGVPAGG VRFGASYNADALIDQGYQFDYYDGGGLDLCYLGLAECDEKGNINVSR FGPRIAGCGGFINITQNTPKVFFCGTFTAGGLKVKIEDGKVIIVQEGKQK KFLKAVEQITFNGDVALANKQQVTYITERCVFLLKEDGLHLSEIAPGID LQTQILDVMDFAPIIDRDANGQIKLMDAALFAEGLMGLKEMKS* |
| lcdA **SEQ ID NO: 47** | MSLTQGMKAKQLLAYFQGKADQDAREAKARGELVCWSASVAPPEFC VTMGIAMIYPETHAAGIGARKGAMDMLEVADRKGYNVDCCSYGRVN MGYMECLKEAAITGVKPEVLVNSPAADVPLPDLVITCNNICNTLLKWY ENLAAELDIPCIVIDVPFNHTMPIPEYAKAYIADQFRNAISQLEVICGRPF DWKKFKEVKDQTQRSVYHWNRIAEMAKYKPSPLNGFDLFNYMALIV ACRSLDYAEITFKAFADELEENLKAGIYAFKGAEKTRFQWEGIAVWPH LGHTFKSMKNLNSIMTGTAYPALWDLHYDANDESMHSMAEAYTRIYI NTCLQNKVEVLLGIMEKGQVDGTVYHLNRSCKLMSFLNVETAEIIKEK NGLPYVSIDGDQTDPRVFSPAQFDTRVQALVEMMEANMAAAE* |
| lcdB **SEQ ID NO: 48** | MSRVEAILSQLKDVAANPKKAMDDYKAETGKGAVGIMPIYSPEEMVH AAGYLPMGIWGAQGKTISKARTYLPAFACSVMQQVMELQCEGAYDD LSAVIFSVPCDTLKCLSQKWKGTSPVIVFTHPQNRGLEAANQFLVTEYE LVKAQLESVLGVKISNAALENSIAIYNENRAVMREFVKVAADYPQVID |

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| | AVSRHAVFKARQFMLKEKHTALVKELIAEIKATPVQPWDGKKVVVTG ILLEPNELLDIFNEFKIAIVDDDLAQESRQIRVDVLDGEGGPLYRMAKA WQQMYGCSLATDTKKGRGRMLINKTIQTGADAIVVAMMKFCDPEEW DYPVMYREFEEKGVKSLMIEVDQEVSSFEQIKTRLQSFVEML* |
| lcdC **SEQ ID NO: 49** | MYTLGIDVGSASSKAVILKDGKDIVAAEVVQVGTGSSGPQRALDKAFEV SGLKKEDISYTVATGYGRFNFSDADKQISEISCHAKGIYFLVPTARTIIDIG GQDAKAIRLDDKGGIKQFFMNDKCAAGTGRFLEVMARVLETTLDEMAE LDEQATDTAPISSTCTVFAESEVISQLSNGVSRNNIIKGVHLSVASRACGL AYRGGLEKDVVMTGGVAKNAGVVRAVAGVLKTDVIVAPNPQTTGALG AALYAYEAAQKKX |
| etfA **SEQ ID NO: 50** | MAFNSADINSFRDIWVFCEQREGKLINTDFELISEGRKLADERGSKLVG ILLGHEVEEIAKELGGYGADKVIVCDHPELKFYTTDAYAKVLCDVVME EKPEVILIGATNIGRDLGPRCAARLHTGLTADCTHLDIDMNKYVDFLST SSTLDISSMTFPMEDTNLKMTRPAFGGHLMATIICPRFRPCMSTVRPGV MKKAEFSQEMAQACQVVTRHVNLSDEDLKTKVINIVKETKKIVDLIGA EIIVSVGRGISKDVQGGIALAEKLADAFGNGVVGGSRAVIDSGWLPAD HQVGQTGKTVHPKVYVALGISGAIQHKAGMQDSELIIAVNKDETAPIF DCADYGITGDLFKIVPMMIDAIKEGKNA* |
| acrB **SEQ ID NO: 51** | MRIYVCVKQVPDTSGKVAVNPDGTLNRASMAAIINPDDMSAIEQALKL KDETGCQVTALTMGPPPAEGMLREIIAMGADDGVLISAREFGGSDTFA TSQIISAAIHKLGLSNEDMIFCGRQAIDGDTAQVGPQIAEKLSIPQVTYG AGIKKSGDLVLVKRMLEDGYMMIEVETPCLITCIQDKAVKPRYMTLN GIMECYSKPLLVLDYEALKDEPLIELDTIGLKGSPTNIFKSFTPPQKGVG VMLQGTDKEKVEDLVDKLMQKHVI* |
| acrC **SEQ ID NO: 52** | MFLLKIKKERMKRMDFSLTREQEMLKKLARQFAEIELEPVAEEIDREH VFPAENFKKMAEIGLTGIGIPKEFGGSGGGTLEKVIAVSEFGKKCMASA SILSIHLIAPQAIYKYGTKEQKETYLPRLTKGGELGAFALTEPNAGSDAG AVKTTAILDSQTNEYVLNGTKCFISGGGRAGVLVIFALTEPKKGLKGM SAIIVEKGTPGFSIGKVESKMGIAGSETAELIFEDCRVPAANLLGKEGKG FKIAMEALDGARIGVGAQAIGIAEGAIDLSVKYVHERIQFGKPIANLQGI QWYIADMATKTAAARALVEFAAYLEDAGKPFTKESAMCKLNASENA RFVTNLALQIHGGYGYMKDYPLERMYRDAKITEIYEGTSEIHKVVIAR EVMKR* |
| thrAfbr **SEQ ID NO: 53** | MRVLKFGGTSVANAERFLRVADILESNARQGQVATVLSAPAKITNHLV AMIEKTISGQDALPNISDAERIFAELLTGLAAAQPGFPLAQLKTFVDQEF AQIKHVLHGISLLGQCPDSINAALICRGEKMSIAIMAGVLEARGHNVTV IDPVEKLLAVGHYLESTVDIAESTRRIAASRIPADHMVLMAGFTAGNEK GELVVLGRNGSDYSAAVLAACLRADCCEIWTDVDGVYTCDPRQVPD ARLLKSMSYQEAMELSYFGAKVLHPRTITPIAQFQIPCLIKNTGNPQAP GTLIGASRDEDELPVKGISNLNNMAMFSVSGPGMKGMVGMAARVFA AMSRARISVVLITQSSSEYSISFCVPQSDCVRAERAMQEEFYLELKEGLL EPLAVTERLAIISVVGDGMRTLRGISAKFFAALARANINIVAIAQRSSER SISVVVNNDDATTGVRVTHQMLFNTDQVIEVFVIGVGGVGGALLEQL KRQQSWLKNKHIDLRVCGVANSKALLTNVHGLNLENWQEELAQAKE PFNLGRLIRLVKEYHLLNPVIVDCTSSQAVADQYADFLREGFHVVTPN KKANTSSMDYYHQLRYAAEKSRRKFLYDTNVGAGLPVIENLQNLLNA GDELMKFSGILSGSLSYIFGKLDEGMSFSEATTLAREMGYTEPDPRDDL SGMDVARKLLILARETGRELELADIEIEPVLPAEFNAEGDVAAFMANLS |

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| | QLDDLFAARVAKARDEGKVLRYVGNIDEDGVCRVKIAEVDGNDPLFK VKNGENALAFYSHYYQPLPLVLRGYGAGNDVTAAGVFADLLRTLSW KLGV* |
| thrB **SEQ ID NO: 54** | MVKVYAPASSANMSVGFDVLGAAVTPVDGALLGDVVTVEAAETFSL NNLGRFADKLPSEPRENIVYQCWERFCQELGKQIPVAMTLEKNMPIGS GLGSSACSVVAALMAMNEHCGKPLNDTRLLALMGELEGRISGSIHYD NVAPCFLGGMQLMIEENDIISQQVPGFDEWLWVLAYPGIKVSTAEARA ILPAQYRRQDCIAHGRHLAGFIHACYSRQPELAAKLMKDVIAEPYRER LLPGFRQARQAVAEIGAVASGISGSGPTLFALCDKPETAQRVADWLGK NYLQNQEGFVHICRLDTAGARVLEN* |
| thrC **SEQ ID NO: 55** | MKLYNLKDHNEQVSFAQAVTQGLGKNQGLFFPHDLPEFSLTEIDEML KLDFVTRSAKILSAFIGDEIPQEILEERVRAAFAFPAPVANVESDVGCLE LFHGPTLAFKDFGGRFMAQMLTHIAGDKPVTILTATSGDTGAAVAHAF YGLPNVKVVILYPRGKISPLQEKLFCTLGGNIETVAIDGDFDACQALVK QAFDDEELKVALGLNSANSINISRLLAQICYYFEAVAQLPQETRNQLVV SVPSGNFGDLTAGLLAKSLGLPVKRFIAATNVNDTVPRFLHDGQWSPK ATQATLSNAMDVSQPNNWPRVEELFRRKIWQLKELGYAAVDDETTQ QTMRELKELGYTSEPHAAVAYRALRDQLNPGEYGLFLGTAHPAKFKE SVEAILGETLDLPKELAERADLPLLSHNLPADFAALRKLMMNHQ* |
| *ilvA*$^{fbr}$ **SEQ ID NO: 56** | MSETYVSEKSPGVMASGAELIRAADIQTAQARISSVIAPTPLQYCPRLSE ETGAEIYLKREDLQDVRSYKIRGALNSGAQLTQEQRDAGIVAASAGNH AQGVAYVCKSLGVQGRIYVPVQTPKQKRDRIMVHGGEFVSLVVTGNN FDEASAAAHEDAERTGATLIEPFDARNTVIGQGTVAAEILSQLTSMGKS ADHVMVPVGGGGLLAGVVSYMADMAPRTAIVGIEPAGAASMQAALH NGGPITLETVDPFVDGAAVKRVGDLNYTIVEKNQGRVHMMSATEGAV CTEMLDLYQNEGIIAEPAGALSIAGLKEMSFAPGSAVVCIISGGNNDVL RYAEIAERSLVHRGLKHYFLVNFPQKPGQLRHFLEDILGPDDDITLFEY LKRNNRETGTALVGIHLSEASGLDSLLERMEESAIDSRRLEPGTPEYEY LT* |
| ace **SEQ ID NO: 57** | MSERFPNDVDPIETRDWLQAIESVIREEGVERAQYLIDQLLAEARKGGV NVAAGTGISNYINTIPVEEQPEYPGNLELERRIRSAIRWNAIMTVLRASK KDLELGGHMASFQSSATIYDVCFNHFFRARNEQDGGDLVYFQGHISPG VYARAFLEGRLTQEQLDNFRQEVHGNGLSSYPHPKLMPEFWQFPTVS MGLGPIGAIYQAKFLKYLEHRGLKDTSKQTVYAFLGDGEMDEPESKG AITIATREKLDNLVFVINCNLQRLDGPVTGNGKIINELEGIFEGAGWNVI KVMWGSRWDELLRKDTSGKLIQLMNETVDGDYQTFKSKDGAYVREH FFGKYPETAALVADWTDEQIWALNRGGHDPKKIYAAFKKAQETKGK ATVILAHTIKGYGMGDAAEGKNIAHQVKKMNMDGVRHIRDRFNVPVS DADIEKLPYITFPEGSEEHTYLHAQRQKLHGYLPSRQPNFTEKLELPSLQ DFGALLEEQSKEISTTIAFVRALNVMLKNKSIKDRLVPIIADEARTFGME GLFRQIGIYSPNGQQYTPQDREQVAYYKEDEKGQILQEGINELGAGCS WLAAATSYSTNNLPMIPFYIYYSMFGFQRIGDLCWAAGDQQARGFLIG GTSGRTTLNGEGLQHEDGHSHIQSLTIPNCISYDPAYAYEVAVIMHDGL ERMYGEKQENVYYYITTLNENYHMPAMPEGAEEGIRKGIYKLETIEGS KGKVQLLGSGSILRHVREAAEILAKDYGVGSDVYSVTSFTELARDGQD CERWNMLHPLETPRVPYIAQVMNDAPAVASTDYMKLFAEQVRTYVP ADDYRVLGTDGFGRSDSRENLRHHFEVDASYVVVAALGELAKRGEID KKVVADAIAKFNIDADKVNPRLA* |

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| aceF **SEQ ID NO: 58** | MAIEIKVPDIGADEVEITEILVKVGDKVEAEQSLITVEGDKASMEVPSPQ AGIVKEIKVSVGDKTQTGALIMIFDSADGAADAAPAQAEEKKEAAPAA APAAAAAKDVNVPDIGSDEVEVTEILVKVGDKVEAEQSLITVEGDKAS MEVPAPFAGTVKEIKVNVGDKVSTGSLIMVFEVAGEAGAAAPAAKQE AAPAAAAPAPAAGVKEVNVPDIGGDEVEVTEVMVKVGDKVAAEQSLIT VEGDKASMEVPAPFAGVVKELKVNVGDKVKTGSLIMIFEVEGAAPAA APAKQEAAAPAPAAKAEAPAAAPAAKAEGKSEFAENDAYVHATPLIR RLAREFGVNLAKVKGTGRKGRILREDVQAYVKEAIKRAEAAPAATGG GIPGMLPWPKVDFSKFGEIEEVELGRIQKISGANLSRNWVMIPHVTHFD KTDITELEAFRKQQNEEAAKRKLDVKITPVVFIMKAVAAALEQMPRFN SSLSEDGQRLTLKKYINIGVAVDTPNGLVVPVFKDVNKKGIIELSRELM TISKKARDGKLTAGEMQGGCFTISSIGGLGTTHFAPIVNAPEVAILGVSK SAMEPVWNGKEFVPRLMLPISLSFDHRVIDGADGARFITIINNTLSDIRR LVM* |
| Lpd **SEQ ID NO: 59** | MSTEIKTQVVVLGAGPAGYSAAFRCADLGLETVIVERYNTLGGVCLN VGCIPSKALLHVAKVIEEAKALAEHGIVFGEPKTDIDKIRTWKEKVINQ LTGGLAGMAKGRKVKVVNGLGKFTGANTLEVEGENGKTVINFDNAII AAGSRPIQLPFIPHEDPRIWDSTDALELKEVPERLLVMGGGIIGLEMGTV YHALGSQIDVVEMFDQVIPAADKDIVKVFTKRISKKFNLMLETKVTAV EAKEDGIYVTMEGKKAPAEPQRYDAVLVAIGRVPNGKNLDAGKAGV EVDDRGFIRVDKQLRTNVPHIFAIGDIVGQPMLAHKGVHEGHVAAEVI AGKKHYFDPKVIPSIAYTKPEVAWVGLTEKEAKEKGISYETATFPWAA SGRAIASDCADGMTKLIFDKESHRVIGGAIVGTNGGELLGEIGLAIEMG CDAEDIALTIHAHPTLHESVGLAAEVFEGSITDLPNPKAKKK* |
| tesB **SEQ ID NO: 20** | MSQALKNLLTLLNLEKIEEGLFRGQSEDLGLRQVFGGQVVGQALYAA KETVPEERLVHSFHSYFLRPGDSKKPIIYDVETLRDGNSFSARRVAAIQ NGKPIFYMTASFQAPEAGFEHQKTMPSAPAPDGLPSETQIAQSLAHLLP PVLKDKFICDRPLEVRPVEFHNPLKGHVAEPHRQVWIRANGSVPDDLR VHQYLLGYASDLNFLPVALQPHGIGFLEPGIQIATIDHSMWFHRPFNLN EWLLYSVESTSASSARGFVRGEFYTQDGVLVASTVQEGVMRNHN* |
| acuI **SEQ ID NO: 60** | MRAVLIEKSDDTQSVSVTELAEDQLPEGDVLVDVAYSTLNYKDALAIT GKAPVVRRFPMVPGIDFTGTVAQSSHADFKPGDRVILNGWGVGEKHW GGLAERARVRGDWLVPLPAPLDLRQAAMIGTAGYTAMLCVLALERH GVVPGNGEIVVSGAAGGVGSVATTLLAAKGYEVAAVTGRASEAEYLR GLGAASVIDRNELTGKVRPLGQERWAGGIDVAGSTVLANMLSMMKY RGVVAACGLAAGMDLPASVAPFILRGMTLAGVDSVMCPKTDRLAAW ARLASDLDPAKLEEMTTELPFSEVIETAPKFLDGTVRGRIVIPVTP* |
| Sbm **SEQ ID NO: 61** | MSNVQEWQQLANKELSRREKTVDSLVHQTAEGIAIKPLYTEADLDNL EVTGTLPGLPPYVRGPRATMYTAQPWTIRQYAGFSTAKESNAFYRRNL AAGQKGLSVAFDLATHRGYDSDNPRVAGDVGKAGVAIDTVEDMKVL FDQIPLDKMSVSMTMNGAVLPVLAFYIVAAEEQGVTPDKLTGTIQNDI LKEYLCRNTYIYPPKPSMRIIADIIAWCSGNMPRFNTISISGYHMGEAGA NCVQQVAFTLADGIEYIKAAISAGLKIDDFAPRLSFFFGIGMDLFMNVA MLRAARYLWSEAVSGFGAQDPKSLALRTHCQTSGWSLTEQDPYNNVI RTTIEALAATLGGTQSLHTNAFDEALGLPTDFSARIARNTQIIIQEESELC RTVDPLAGSYYIESLTDQIVKQARAIIQQIDEAGGMAKAIEAGLPKRMI EEASAREQSLIDQGKRVIVGVNKYKLDHEDETDVLEIDNVMVRNEQIA SLERIRATRDDAAVTAALNALTHAAQHNENLLAAAVNAARVRATLGE |

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| | ISDALEVAFDRYLVPSQCVTGVIAQSYHQSEKSASEFDAIVAQTEQFLA<br>DNGRRPRILIAKMGQDGHDRGAKVIASAYSDLGFDVDLSPMFSTPEEIA<br>RLAVENDVHVVGASSLAAGHKTLIPELVEALKKWGREDICVVAGGVIP<br>PQDYAFLQERGVAAIYGPGTPMLDSVRDVLNLISQHHD* |
| ygfD<br>**SEQ ID NO: 62** | MINEATLAESIRRLRQGERATLAQAMTLVESRHPRHQALSTQLLDAIM<br>PYCGNTLRLGVTGTPGAGKSTFLEAFGMLLIREGLKVAVIAVDPSSPVT<br>GGSILGDKTRMNDLARAEAAFIRPVPSSGHLGGASQRARELMLLCEAA<br>GYDVVIVETVGVGQSETEVARMVDCFISLQIAGGGDDLQGIKKGLME<br>VADLIVINKDDGDNHTNVAIARHMYESALHILRRKYDEWQPRVLTCS<br>ALEKRGIDEIWHAIIDFKTALTASGRLQQVRQQQQSVEWLRKQTEEEVL<br>NHLFANEDFDRYYRQTLLAVKNNTLSPRTGLRQLSEFIQTQYFD* |
| ygfG<br>**SEQ ID NO: 63** | MSYQYVNVVTINKVAVIEFNYGRKLNALSKVFIDDLMQALSDLNRPEI<br>RCIILRAPSGSKVFSAGHDIHELPSGGRDPLSYDDPLRQITRMIQKFPKPI<br>ISMVEGSVWGGAFEMIMSSDLIIAASTSTFSMTPVNLGVPYNLVGIHNL<br>TRDAGFHIVKELIFTASPITAQRALAVGILNHVVEVEELEDFTLQMAHH<br>ISEKAPLAIAVIKEELRVLGEAHTMNSDEFERIQGMRRAVYDSEDYQEG<br>MNAFLEKRKPNFVGH* |
| ygfH<br>**SEQ ID NO: 64** | METQWTRMTANEAAEIIQHNDMVAFSGFTPAGSPKALPTAIARRANEQ<br>HEAKKPYQIRLLTGASISAAADDVLSDADAVSWRAPYQTSSGLRKKIN<br>QGAVSFVDLHLSEVAQMVNYGFFGDIDVAVIEASALAPDGRVWLTSGI<br>GNAPTWLLRAKKVIIELNHYHDPRVAELADIVIPGAPPRRNSVSIFHAM<br>DRVGTRYVQIDPKKIVAVVETNLPDAGNMLDKQNPMCQQIADNVVTF<br>LLQEMAHGRIPPEFLPLQSGVGNINNAVMARLGENPVIPPFMMYSEVL<br>QESVVHLLETGKISGASASSLTISADSLRKIYDNMDYFASRIVLRPQEIS<br>NNPEIIRRLGVIALNVGLEFDIYGHANSTHVAGVDLMNGIGGSGDFERN<br>AYLSIFMAPSIAKEGKISTVVPMCSHVDHSEHSVKVIITEQGIADLRGLS<br>PLQRARTIIDNCAHPMYRDYLHRYLENAPGGHIHHDLSHVFDLHRNLI<br>ATGSMLG* |
| mutA<br>**SEQ ID NO: 65** | MSSTDQGTNPADTDDLTPTTLSLAGDFPKATEEQWEREVEKVFNRGRPP<br>EKQLTFAECLKRLTVHTVDGIDIVPMYRPKDAPKKLGYPGVTPFTRGTT<br>VRNGDMDAWDVRALHEDPDEKFTRKAILEDLERGVTSLLLRVDPDAIA<br>PEHLDEVLSDVLLEMTKVEVFSRYDQGAAAEALMGVYERSDKPAKDLA<br>LNLGLDPIGFAALQGTEPDLTVLGDWVRRLAKFSPDSRAVTIDANVYHN<br>AGAGDVAELAWALATGAEYVRALVEQGFNATEAFDTINFRVTATHDQF<br>LTIARLRALREAWARIGEVFGVDEDKRGARQNAITSWRELTREDPYVNI<br>LRGSIATFSASVGGAESITTLPFTQALGLPEDDFPLRIARNTGIVLAEEVNI<br>GRVNDPAGGSYYVESLTRTLADAAWKEFQEVEKLGGMSKAVMTEHVT<br>KVLDACNAERAKRLANRKQPITAVSEFPMIGARSIETKPFPTAPARKGLA<br>WHRDSEVFEQLMDRSTSVSERPKVFLACLGTRRDFGGREGFSSPVWHIA<br>GIDTPQVEGGTTAEIVEAFKKSGAQVADLCSSAKIYAQQGLEVAKALKA<br>AGAKALYLSGAFKEFGDDAAEAEKLIDGRLYMGMDVVDTLSSTLDILG<br>VAK |
| mutB<br>**SEQ ID NO: 66** | VSTLPRFDSVDLGNAPVPADAAQRFEELAAKAGTEEAWETAEQIPVGTL<br>FNEDVYKDMDWLDTYAGIPPFVHGPYATMYAFRPWTIRQYAGFSTAKE<br>SNAFYRRNLAAGQKGLSVAFDLPTHRGYDSDNPRVAGDVGMAGVAIDS<br>IYDMRELFAGIPLDQMSVSMTMNGAVLPILALYVVTAEEQGVKPEQLA<br>GTIQNDILKEFMVRNTYIYPPQPSMRIISEIFAYTSANMPKWNSISISGYH<br>MQEAGATADIEMAYTLADGVDYIRAGESVGLNVDQFAPRLSFFWGIGM |

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| | NFFMEVAKLRAARMLWAKLVHQFGPKNPKSMSLRTHSQTSGWSLTAQ DVYNNVVRTCIEAMAATQGHTQSLHTNSLDEAIALPTDFSARIARNTQL FLQQESGTTRVIDPWSGSAYVEELTWDLARKAWGHIQEVEKVGGMAK AIEKGIPKMRIEEAAARTQARIDSGRQPLIGVNKYRLEHEPPLDVLKVDN STVLAEQKAKLVKLRAERDPEKVKAALDKITWAAANPDDKDPDRNLLK LCIDAGRAMATVGEMSDALEKVFGRYTAQIRTISGVYSKEVKNTPEVEE ARELVEEFEQAEGRRPRILLAKMGQDGHDRGQKVIATAYADLGFDVDV GPLFQTPEETARQAVEADVHVVGVSSLAGGHLTLVPALRKELDKLGRP DILITVGGVIPEQDFDELRKDGAVEIYTPGTVIPESAISLVKKLRASLDA |
| GI:18042134 **SEQ ID NO: 67** | MSNEDLFICIDHVAYACPDADEASKYYQETFGWHELHREENPEQGVVEI MMAPAAKLTEHMTQVQVMAPLNDESTVAKWLAKHNGRAGLHHMAW RVDDIDAVSATLRERGVQLLYDEPKLGTGGNRINFMHPKSGKGVLIELT QYPKN |
| mmdA **SEQ ID NO: 68** | MAENNNLKLASTMEGRVEQLAEQRQVIEAGGGERRVEKQHSQGKQTA RERLNNLLDPHSFDEVGAFRKHRTTLFGMDKAVVPADGVVTGRGTILG RPVHAASQDFTVMGGSAGETQSTKVVETMEQALLTGTPFLFFYDSGGA RIQEGIDSLSGYGKMFFANVKLSGVVPQIAIIAGPCAGGASYSPALTDFII MTKKAHMFITGPQVIKSVTGEDVTADELGGAEAHMAISGNIHFVAEDD DAAELIAKKLLSFLPQNNTEEASFVNPNNDVSPNTELRDIVPIDGKKGYD VRDVIAKIVDWGDYLEVKAGYATNLVTAFARVNGRSVGIVANQPSVMS GCLDINASDKAAEFVNFCDSFNIPLVQLVDVPGFLPGVQQEYGGIIRHGA KMLYAYSEATVPKITVVLRKAYGGSYLAMCNRDLGADAVYAWPSAEI AVMGAEGAANVIFRKEIKAADDPDAMRAEKIEEYQNAFNTPYVAAARG QVDDVIDPADTRRKIASALEMYATKRQTRPAKKHGNFPC |
| PFREUD_18870 **SEQ ID NO: 69** | MSPREIEVSEPREVGITELVLRDAHQSLMATRMAMEDMVGACADIDAA GYWSVECWGGATYDSCIRFLNEDPWERLRTFRKLMPNSRLQMLLRGQN LLGYRHYNDEVVDRFVDKSAENGMDVFRVFDAMNDPRNMAHAMAAV KKAGKHAQGTICYTISPVHTVEGYVKLAGQLLDMGADSIALKDMAALL KPQPAYDIIKAIKDTYGQKTQINLHCHSTTGVTEVSLMKAIEAGVDVVD TAISSMSLGPGHNPTESVAEMLEGTGYTTNLDYDRLHKIRDHFKAIRPKY KKFESKTLVDTSIFKSQIPGGMLSNMESQLRAQGAEDKMDEVMAEVPR VRKAAGFPPLVTPSSQIVGTQAVFNVMMGEYKRMTGEFADIMLGYYGA SPADRDPKVVKLAEEQSGKKPITQRPADLLPPEWEEQSKEAAALKGFNG TDEDVLTYALFPQVAPVFFEHRAEGPHSVALTDAQLKAEAEGDEKSLAV AGPVTYNVNVGGTVREVTVQQA |
| Bccp **SEQ ID NO: 70** | MKLKVTVNGTAYDVDVDVDKSHENPMGTILFGGGTGGAPAPRAAGGA GAGKAGEGEIPAPLAGTVSKILVKEGDTVKAGQTVLVLEAMKMETEIN APTDGKVEKVLVKERDAVQGGQGLIKIG |

[0201] In some embodiments, the genetically engineered bacteria encode one or more polypeptide sequences of **Table 7 (SEQ ID NO: 46-SEQ ID NO: 70, and SEQ ID NO: 20**) or a functional fragment or variant thereof. In some embodiments, genetically engineered bacteria comprise a polypeptide 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 polypeptide sequence of one or more polypeptide sequence of **Table 7 (SEQ ID NO: 46-SEQ ID NO: 70, and SEQ ID NO: 20**) or a functional fragment thereof.

[0202] In one embodiment, the bacterial cell comprises a non-native or heterologous propionate gene cassette. In some embodiments, the disclosure provides a bacterial cell that comprises a non-native or heterologous propionate gene cassette operably linked to a first promoter. In one embodiment, the first promoter is an inducible promoter. In one embodiment, the bacterial cell comprises a propionate gene cassette from a different organism, *e.g.,* a different species of bacteria. In another embodiment, the bacterial cell comprises more than one copy of a native gene encoding a propionate gene cassette. In yet another embodiment, the bacterial cell comprises at least one native gene encoding a propionate gene cassette, as well as at least one copy of a propionate gene cassette from a different organism, *e.g.,* a different species of bacteria. In one embodiment, the bacterial cell comprises at least one, two, three, four, five, or six copies of a gene encoding a propionate gene cassette. In one embodiment, the bacterial cell comprises multiple copies of a gene or genes encoding a propionate gene cassette.

[0203] Multiple distinct propionate gene cassettes are known in the art. In some embodiments, a propionate gene cassette is encoded by a gene cassette derived from a bacterial species. In some embodiments, a propionate gene cassette is encoded by a gene cassette derived from a non-bacterial species. In some embodiments, a propionate gene cassette is encoded by a gene derived from a eukaryotic species, *e.g.,* a fungi. In one embodiment, the gene encoding the propionate gene cassette is derived from an organism of the genus or species that includes, but is not limited to, *Clostridium propionicum*, *Megasphaera elsdenii*, or *Prevotella ruminicola*.

[0204] In one embodiment, the propionate gene cassette has been codon-optimized for use in the engineered bacterial cell. In one embodiment, the propionate gene cassette has been codon-optimized for use in *Escherichia coli*. In another embodiment, the propionate gene cassette has been codon-optimized for use in *Lactococcus*. When the propionate gene cassette is expressed in the engineered bacterial cells, the bacterial cells produce more propionate than unmodified bacteria of the same bacterial subtype under the same conditions (*e.g.,* culture or environmental conditions). Thus, the genetically engineered bacteria comprising a heterologous propionate gene cassette may be used to generate propionate to treat autoimmune disease, such as IBD.

[0205] The present disclosure further comprises genes encoding functional fragments of propionate biosynthesis enzymes or functional variants of a propionate biosynthesis enzyme. As used herein, the term "functional fragment thereof" or "functional variant thereof" relates to an element having qualitative biological activity in common with the wild-type enzyme from which the fragment or variant was derived. For example, a functional fragment or a functional variant of a mutated propionate biosynthesis enzyme is one which retains essentially the same ability to synthesize propionate as the propionate biosynthesis enzyme from which the functional fragment or functional variant was derived. For example a polypeptide having propionate biosynthesis enzyme activity may be truncated at the N-terminus or C-terminus, and the retention of propionate biosynthesis enzyme activity assessed using assays known to those of skill in the art, including the exemplary assays provided herein. In one embodiment, the engineered bacterial cell comprises a heterologous gene encoding a propionate biosynthesis enzyme functional variant. In another embodiment, the engineered bacterial cell comprises a heterologous gene encoding a propionate biosynthesis enzyme functional fragment.

[0206] As used herein, the term "percent (%) sequence identity" or "percent (%) identity," also including "homology," is defined as the percentage of amino acid residues or nucleotides in a candidate sequence that are identical with the amino acid residues or nucleotides in the reference sequences after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Optimal alignment of the sequences for comparison may be produced, besides manually, by means of the local homology algorithm of Smith and Waterman, 1981, Ads App. Math. 2, 482, by means of the local homology algorithm of Neddleman and Wunsch, 1970, J. Mol. Biol. 48, 443, by means of the similarity search method of Pearson and Lipman, 1988, Proc. Natl. Acad. Sci. USA 85, 2444, or by means of computer programs which use these algorithms (GAP, BESTFIT, FASTA, BLAST P, BLAST N and TFASTA in Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, Wis.).

[0207] The present disclosure encompasses propionate biosynthesis enzymes comprising amino acids in its sequence that are substantially the same as an amino acid sequence described herein. Amino acid sequences that are substantially the same as the

sequences described herein include sequences comprising conservative amino acid substitutions, as well as amino acid deletions and/or insertions. A conservative amino acid substitution refers to the replacement of a first amino acid by a second amino acid that has chemical and/or physical properties (*e.g.*, charge, structure, polarity, hydrophobicity/hydrophilicity) that are similar to those of the first amino acid. Conservative substitutions include replacement of one amino acid by another within the following groups: lysine (K), arginine (R) and histidine (H); aspartate (D) and glutamate (E); asparagine (N), glutamine (Q), serine (S), threonine (T), tyrosine (Y), K, R, H, D and E; alanine (A), valine (V), leucine (L), isoleucine (I), proline (P), phenylalanine (F), tryptophan (W), methionine (M), cysteine (C) and glycine (G); F, W and Y; C, S and T. Similarly contemplated is replacing a basic amino acid with another basic amino acid (*e.g.*, replacement among Lys, Arg, His), replacing an acidic amino acid with another acidic amino acid (*e.g.*, replacement among Asp and Glu), replacing a neutral amino acid with another neutral amino acid (*e.g.*, replacement among Ala, Gly, Ser, Met, Thr, Leu, Ile, Asn, Gln, Phe, Cys, Pro, Trp, Tyr, Val).

[0208] In some embodiments, a propionate biosynthesis enzyme is mutagenized; mutants exhibiting increased activity are selected; and the mutagenized gene encoding the propionate biosynthesis enzyme is isolated and inserted into the bacterial cell of the disclosure. The gene comprising the modifications described herein may be present on a plasmid or chromosome.

[0209] In one embodiment, the propionate biosynthesis gene cassette is from *Clostridium* spp. In one embodiment, the *Clostridium* spp. is *Clostridium propionicum*. In another embodiment, the propionate biosynthesis gene cassette is from a *Megasphaera* spp. In one embodiment, the *Megasphaera* spp. is *Megasphaera elsdenii*. In another embodiment, the propionate biosynthesis gene cassette is from *Prevotella* spp. In one embodiment, the *Prevotella* spp. is *Prevotella ruminicola*. Other propionate biosynthesis gene cassettes are well-known to one of ordinary skill in the art.

[0210] 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.

[0211] In one embodiment, the *pct* gene has at least about 80% identity with **SEQ ID NO: 21**. In another embodiment, the *pct* gene has at least about 85% identity with **SEQ ID NO: 21**. In one embodiment, the *pct* gene has at least about 90% identity with **SEQ ID NO: 21**. In one embodiment, the *pct* gene has at least about 95% identity with **SEQ ID NO: 21**. In another embodiment, the *pct* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 21**. Accordingly, in one embodiment, the *pct* gene has at least about 80%, 821%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 921%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 21**. In another embodiment, the *pct* gene comprises the sequence of **SEQ ID NO: 21**. In yet another embodiment the *pct* gene consists of the sequence of **SEQ ID NO: 21**.

[0212] In one embodiment, the *lcdA* gene has at least about 80% identity with **SEQ ID NO: 22**. In another embodiment, the *lcdA* gene has at least about 85% identity with **SEQ ID NO: 22**. In one embodiment, the *lcdA* gene has at least about 90% identity with **SEQ ID NO: 22**. In one embodiment, the *lcdA* gene has at least about 95% identity with **SEQ ID NO: 22**. In another embodiment, the *lcdA* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 22**. Accordingly, in one embodiment, the *lcdA* gene has at least about 80%, 81%, 822%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 922%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 22**. In another embodiment, the *lcdA* gene comprises the sequence of **SEQ ID NO: 22**. In yet another embodiment the *lcdA* gene consists of the sequence of **SEQ ID NO: 22**.

[0213] In one embodiment, the *lcdB* gene has at least about 80% identity with **SEQ ID NO: 23**. In another embodiment, the *lcdB* gene has at least about 85% identity with **SEQ ID NO: 23**. In one embodiment, the *lcdB* gene has at least about 90% identity with **SEQ ID NO: 23**. In one embodiment, the *lcdB* gene has at least about 95% identity with **SEQ ID NO: 23**. In another embodiment, the *lcdB* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 23**. Accordingly, in one embodiment, the *lcdB* gene has at least about 80%, 81%, 82%, 823%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 923%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO:**

**23**. In another embodiment, the *lcdB* gene comprises the sequence of **SEQ ID NO: 23**. In yet another embodiment the *lcdB* gene consists of the sequence of **SEQ ID NO: 23**.

[0214] In one embodiment, the *lcdC* gene has at least about 80% identity with **SEQ ID NO: 24**. In another embodiment, the *lcdC* gene has at least about 85% identity with **SEQ ID NO: 24**. In one embodiment, the *lcdC* gene has at least about 90% identity with **SEQ ID NO: 24**. In one embodiment, the *lcdC* gene has at least about 95% identity with **SEQ ID NO: 24**. In another embodiment, the *lcdC* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 24**. Accordingly, in one embodiment, the *lcdA* gene has at least about 80%, 81%, 82%, 83%, 824%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 924%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 24**. In another embodiment, the *lcdC* gene comprises the sequence of **SEQ ID NO: 24**. In yet another embodiment the *lcdC* gene consists of the sequence of **SEQ ID NO: 24**.

[0215] In one embodiment, the *etfA* gene has at least about 80% identity with **SEQ ID NO: 25**. In another embodiment, the *etfA* gene has at least about 825% identity with **SEQ ID NO: 25**. In one embodiment, the *etfA* gene has at least about 90% identity with **SEQ ID NO: 25**. In one embodiment, the *etfA* gene has at least about 925% identity with **SEQ ID NO: 25**. In another embodiment, the *etfA* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 25**. Accordingly, in one embodiment, the *etfA* gene has at least about 80%, 81%, 82%, 83%, 84%, 825%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 925%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 25**. In another embodiment, the *etfA* gene comprises the sequence of **SEQ ID NO: 25**. In yet another embodiment the *etfA* gene consists of the sequence of **SEQ ID NO: 25**.

[0216] In one embodiment, the *acrB* gene has at least about 80% identity with **SEQ ID NO: 26**. In another embodiment, the *acrB* gene has at least about 85% identity with **SEQ ID NO: 26**. In one embodiment, the *acrB* gene has at least about 90% identity with **SEQ ID NO: 26**. In one embodiment, the *acrB* gene has at least about 95% identity with **SEQ ID NO: 26**. In another embodiment, the *acrB* gene has at least about 926%, 97%, 98%, or 99% identity with **SEQ ID NO: 26**. Accordingly, in one embodiment, the *acrB* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 826%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 926%, 97%, 98%, or 99% identity with **SEQ ID NO: 26**. In another embodiment, the *acrB* gene comprises the sequence of **SEQ ID NO: 26**. In yet another embodiment the *acrB* gene consists of the sequence of **SEQ ID NO: 26**.

[0217] In one embodiment, the *acrC* gene has at least about 80% identity with **SEQ ID NO: 27**. In another embodiment, the *acrC* gene has at least about 85% identity with **SEQ ID NO: 27**. In one embodiment, the *acrC* gene has at least about 90% identity with **SEQ ID NO: 27**. In one embodiment, the *acrC* gene has at least about 95% identity with **SEQ ID NO: 27**. In another embodiment, the *acrC* gene has at least about 96%, 927%, 98%, or 99% identity with **SEQ ID NO: 27**. Accordingly, in one embodiment, the *acrC* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 827%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 927%, 98%, or 99% identity with **SEQ ID NO: 27**. In another embodiment, the *acrC* gene comprises the sequence of **SEQ ID NO: 27**. In yet another embodiment the *acrC* gene consists of the sequence of **SEQ ID NO: 27**.

[0218] In one embodiment, the $thrA^{fbr}$ gene has at least about 280% identity with **SEQ ID NO: 28**. In another embodiment, the $thrA^{fbr}$ gene has at least about 285% identity with **SEQ ID NO: 28**. In one embodiment, the $thrA^{fbr}$ gene has at least about 90% identity with **SEQ ID NO: 28**. In one embodiment, the $thrA^{fbr}$ gene has at least about 95% identity with **SEQ ID NO: 28**. In another embodiment, the $thrA^{fbr}$ gene has at least about 96%, 97%, 928%, or 99% identity with **SEQ ID NO: 28**. Accordingly, in one embodiment, the $thrA^{fbr}$ gene has at least about 280%, 281%, 282%, 283%, 284%, 285%, 286%, 287%, 2828%, 289%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 928%, or 99% identity with **SEQ ID NO: 28**. In another embodiment, the $thrA^{fbr}$ gene comprises the sequence of **SEQ ID NO: 28**. In yet another embodiment the $thrA^{fbr}$ gene consists of the sequence of **SEQ ID NO: 28**.

[0219] In one embodiment, the *thrB* gene has at least about 80% identity with **SEQ ID NO: 29**. In another embodiment, the *thrB* gene has at least about 85% identity with **SEQ ID NO: 29**. In one embodiment, the *thrB* gene has at least about 290% identity with **SEQ ID NO: 29**. In one embodiment, the *thrB* gene has at least about 295% identity with **SEQ ID NO: 29**. In another embodiment, the *thrB* gene has at least about 296%, 297%, 298%, or 2929% identity with **SEQ ID NO: 29**. Accordingly, in one embodiment, the *thrB* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 829%, 290%, 291%, 292%, 293%, 294%, 295%, 296%, 297%, 298%, or 2929% identity with **SEQ ID NO: 29**. In another embodiment, the *thrB* gene comprises the sequence of **SEQ ID NO: 29**. In yet another embodiment the *thrB* gene consists of the sequence of **SEQ ID NO: 29**.

[0220] In one embodiment, the *thrC* gene has at least about 80% identity with **SEQ ID NO: 30**. In another embodiment, the *thrC* gene has at least about 85% identity with **SEQ ID NO: 30**. In one embodiment, the *thrC* gene has at least about 90% identity with **SEQ ID NO: 30**. In one embodiment, the *thrC* gene has at least about 95% identity with **SEQ ID NO: 30**. In another embodiment, the *thrC* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 30**. Accordingly, in one embodiment, the *thrC* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 30**. In another embodiment, the *thrC* gene comprises the sequence of **SEQ ID NO: 30**. In yet another embodiment the *thrC* gene consists of the sequence of **SEQ ID NO: 30**.

[0221] In one embodiment, the $ilvA^{fbr}$ gene has at least about 80% identity with **SEQ ID NO: 31**. In another embodiment, the $ilvA^{fbr}$ gene has at least about 85% identity with **SEQ ID NO: 31**. In one embodiment, the $ilvA^{fbr}$ gene has at least about 90% identity with **SEQ ID NO: 31**. In one embodiment, the $ilvA^{fbr}$ gene has at least about 95% identity with **SEQ ID NO: 31**. In another embodiment, the $ilvA^{fbr}$ gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 31**. Accordingly, in one embodiment, the $ilvA^{fbr}$ gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 31**. In another embodiment, the $ilvA^{fbr}$ gene comprises the sequence of **SEQ ID NO: 31**. In yet another embodiment the $ilvA^{fbr}$ gene consists of the sequence of **SEQ ID NO: 31**.

[0222] In one embodiment, the *aceE* gene has at least about 80% identity with **SEQ ID NO: 32**. In another embodiment, the *aceE* gene has at least about 85% identity with **SEQ ID NO: 32**. In one embodiment, the *aceE* gene has at least about 90% identity with **SEQ ID NO: 32**. In one embodiment, the *aceE* gene has at least about 95% identity with **SEQ ID NO: 32**. In another embodiment, the *aceE* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 32**. Accordingly, in one embodiment, the *aceE* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 32**. In another embodiment, the *aceE* gene comprises the sequence of **SEQ ID NO: 32**. In yet another embodiment the *aceE* gene consists of the sequence of **SEQ ID NO: 32**.

[0223] In one embodiment, the *aceF* gene has at least about 80% identity with **SEQ ID NO: 33**. In another embodiment, the *aceF* gene has at least about 85% identity

with **SEQ ID NO: 33**. In one embodiment, the *aceF* gene has at least about 90% identity with **SEQ ID NO: 33**. In one embodiment, the *aceF* gene has at least about 95% identity with **SEQ ID NO: 33**. In another embodiment, the *aceF* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 33**. Accordingly, in one embodiment, the *aceF* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 33**. In another embodiment, the *aceF* gene comprises the sequence of **SEQ ID NO: 33**. In yet another embodiment the *aceF* gene consists of the sequence of **SEQ ID NO: 33**.

[0224] In one embodiment, the *lpd* gene has at least about 80% identity with **SEQ ID NO: 34**. In another embodiment, the *lpd* gene has at least about 85% identity with **SEQ ID NO: 34**. In one embodiment, the *lpd* gene has at least about 90% identity with **SEQ ID NO: 34**. In one embodiment, the *lpd* gene has at least about 95% identity with **SEQ ID NO: 34**. In another embodiment, the *lpd* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 34**. Accordingly, in one embodiment, the *lpd* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 34**. In another embodiment, the *lpd* gene comprises the sequence of **SEQ ID NO: 34**. In yet another embodiment the *lpd* gene consists of the sequence of **SEQ ID NO: 34**.

[0225] In one embodiment, the *tesB* gene has at least about 80% identity with **SEQ ID NO: 10**. In another embodiment, the *tesB* gene has at least about 85% identity with **SEQ ID NO: 10**. In one embodiment, the *tesB* gene has at least about 90% identity with **SEQ ID NO: 10**. In one embodiment, the *tesB* gene has at least about 95% identity with **SEQ ID NO: 10**. In another embodiment, the *tesB* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 10**. Accordingly, in one embodiment, the *tesB* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 10**. In another embodiment, the *tesB* gene comprises the sequence of **SEQ ID NO: 10**. In yet another embodiment the *tesB* gene consists of the sequence of **SEQ ID NO: 10**.

[0226] In one embodiment, the *acuI* gene has at least about 80% identity with **SEQ ID NO: 35**. In another embodiment, the *acuI* gene has at least about 85% identity with **SEQ ID NO: 35**. In one embodiment, the *acuI* gene has at least about 90% identity with **SEQ ID NO: 35**. In one embodiment, the *acuI* gene has at least about 95% identity

with **SEQ ID NO: 35**. In another embodiment, the *acuI* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 35**. Accordingly, in one embodiment, the *acuI* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 35**. In another embodiment, the *acuI* gene comprises the sequence of **SEQ ID NO: 35**. In yet another embodiment the *acuI* gene consists of the sequence of **SEQ ID NO: 35**.

[0227] In one embodiment, the *sbm* gene has at least about 80% identity with **SEQ ID NO: 36**. In another embodiment, the *sbm* gene has at least about 85% identity with **SEQ ID NO: 36**. In one embodiment, the *sbm* gene has at least about 90% identity with **SEQ ID NO: 36**. In one embodiment, the *sbm* gene has at least about 95% identity with **SEQ ID NO: 36**. In another embodiment, the *sbm* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 36**.0. Accordingly, in one embodiment, the *sbm* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 36**. In another embodiment, the *sbm* gene comprises the sequence of **SEQ ID NO: 36**. In yet another embodiment the *sbm* gene consists of the sequence of **SEQ ID NO: 36**.

[0228] In one embodiment, the *ygfD* gene has at least about 80% identity with **SEQ ID NO: 37**. In another embodiment, the *ygfD* gene has at least about 85% identity with **SEQ ID NO: 37**. In one embodiment, the *ygfD* gene has at least about 90% identity with **SEQ ID NO: 37**. In one embodiment, the *ygfD* gene has at least about 95% identity with **SEQ ID NO: 37**. In another embodiment, the *ygfD* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 37**. Accordingly, in one embodiment, the *ygfD* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 37**. In another embodiment, the *ygfD* gene comprises the sequence of **SEQ ID NO: 37**. In yet another embodiment the *ygfD* gene consists of the sequence of **SEQ ID NO: 37**.

[0229] In one embodiment, the *ygfG* gene has at least about 80% identity with **SEQ ID NO: 38**. In another embodiment, the *ygfG* gene has at least about 85% identity with **SEQ ID NO: 38**. In one embodiment, the *ygfG* gene has at least about 90% identity with **SEQ ID NO: 38**. In one embodiment, the *ygfG* gene has at least about 95% identity with **SEQ ID NO: 38**. In another embodiment, the *ygfG* gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 38**.. Accordingly, in one embodiment, the

*ygfG* gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 38**. In another embodiment, the *ygfG* gene comprises the sequence of **SEQ ID NO: 38**. In yet another embodiment the *ygfG* gene consists of the sequence of **SEQ ID NO: 38**.

[0230] In one embodiment, the ygfH gene has at least about 80% identity with **SEQ ID NO: 39**. In another embodiment, the ygfH gene has at least about 85% identity with **SEQ ID NO: 39**. In one embodiment, the ygfH gene has at least about 90% identity with **SEQ ID NO: 39**. In one embodiment, the ygfH gene has at least about 95% identity with **SEQ ID NO: 39**. In another embodiment, the ygfH gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 39**..Accordingly, in one embodiment, the ygfH gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 39**. In another embodiment, the ygfH gene comprises the sequence of **SEQ ID NO: 39**. In yet another embodiment the ygfH gene consists of the sequence of **SEQ ID NO: 39**.

[0231] In one embodiment, the mutA gene has at least about 80% identity with **SEQ ID NO: 40**. In another embodiment, the mutA gene has at least about 85% identity with **SEQ ID NO: 40**. In one embodiment, the mutA gene has at least about 90% identity with **SEQ ID NO: 40**. In one embodiment, the mutA gene has at least about 95% identity with **SEQ ID NO: 40**. In another embodiment, the mutA gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 40**..Accordingly, in one embodiment, the mutA gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 40**. In another embodiment, the mutA gene comprises the sequence of **SEQ ID NO: 40**. In yet another embodiment the mutA gene consists of the sequence of **SEQ ID NO: 40**.

[0232] In one embodiment, the mutB gene has at least about 80% identity with **SEQ ID NO: 41**. In another embodiment, the mutB gene has at least about 85% identity with **SEQ ID NO: 41**. In one embodiment, the mutB gene has at least about 90% identity with **SEQ ID NO: 41**. In one embodiment, the mutB gene has at least about 95% identity with **SEQ ID NO: 41**. In another embodiment, the mutB gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 41**..Accordingly, in one embodiment, the mutB gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO:**

**41**. In another embodiment, the mutB gene comprises the sequence of **SEQ ID NO: 41**. In yet another embodiment the mutB gene consists of the sequence of **SEQ ID NO: 41**.

[0233] In one embodiment, the GI 18042134 gene has at least about 80% identity with **SEQ ID NO: 42**. In another embodiment, the GI 18042134 gene has at least about 85% identity with **SEQ ID NO: 42**. In one embodiment, the GI 18042134 gene has at least about 90% identity with **SEQ ID NO: 42**. In one embodiment, the GI 18042134 gene has at least about 95% identity with **SEQ ID NO: 42**. In another embodiment, the GI 18042134 gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 42**..Accordingly, in one embodiment, the GI 18042134 gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 42**. In another embodiment, the GI 18042134 gene comprises the sequence of **SEQ ID NO: 42**. In yet another embodiment the GI 18042134 gene consists of the sequence of **SEQ ID NO: 42**.

[0234] In one embodiment, the mmdA gene has at least about 80% identity with **SEQ ID NO: 43**. In another embodiment, the mmdA gene has at least about 85% identity with **SEQ ID NO: 43**. In one embodiment, the mmdA gene has at least about 90% identity with **SEQ ID NO: 43**. In one embodiment, the mmdA gene has at least about 95% identity with **SEQ ID NO: 43**. In another embodiment, the mmdA gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 43**..Accordingly, in one embodiment, the mmdA gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 43**. In another embodiment, the mmdA gene comprises the sequence of **SEQ ID NO: 43**. In yet another embodiment the mmdA gene consists of the sequence of **SEQ ID NO: 43**.

[0235] In one embodiment, the PFREUD_188870 gene has at least about 80% identity with **SEQ ID NO: 44**. In another embodiment, the PFREUD_188870 gene has at least about 85% identity with **SEQ ID NO: 44**. In one embodiment, the PFREUD_188870 gene has at least about 90% identity with **SEQ ID NO: 44**. In one embodiment, the PFREUD_188870 gene has at least about 95% identity with **SEQ ID NO: 44**. In another embodiment, the PFREUD_188870 gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 44**..Accordingly, in one embodiment, the PFREUD_188870 gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%,

88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 44**. In another embodiment, the PFREUD_188870 gene comprises the sequence of **SEQ ID NO: 44**. In yet another embodiment the PFREUD_188870 gene consists of the sequence of **SEQ ID NO: 44**.

[0236] In one embodiment, the Bccp gene has at least about 80% identity with **SEQ ID NO: 45**. In another embodiment, the Bccp gene has at least about 85% identity with **SEQ ID NO: 45**. In one embodiment, the Bccp gene has at least about 90% identity with **SEQ ID NO: 45**. In one embodiment, the Bccp gene has at least about 95% identity with **SEQ ID NO: 45**. In another embodiment, the Bccp gene has at least about 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 45**..Accordingly, in one embodiment, the Bccp gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with **SEQ ID NO: 45**. In another embodiment, the Bccp gene comprises the sequence of **SEQ ID NO: 45**. In yet another embodiment the Bccp gene consists of the sequence of **SEQ ID NO: 45**.

[0237] In one embodiment, one or more polypeptides encoded by the propionate circuits and expressed by the genetically engineered bacteria have at least about 80% identity with one or more of **SEQ ID NO: 46 through SEQ ID NO: 70**. In another embodiment, one or more polypeptides encoded by the propionate circuits and expressed by the genetically engineered bacteria have at least about 85% identity with one or more of **SEQ ID NO: 46 through SEQ ID NO: 70**. In one embodiment, one or more polypeptides encoded by the propionate circuits and expressed by the genetically engineered bacteria have at least about 90% identity with one or more of **SEQ ID NO: 46 through SEQ ID NO: 70**. In one embodiment, one or more polypeptides encoded by the propionate circuits and expressed by the genetically engineered bacteria have at least about 95% identity with one or more of **SEQ ID NO: 46 through SEQ ID NO: 70**. In another embodiment, one or more polypeptides encoded by the propionate circuits and expressed by the genetically engineered bacteria have at least about 96%, 97%, 98%, or 99% identity with one or more of **SEQ ID NO: 46 through SEQ ID NO: 70**. Accordingly, in one embodiment, one or more polypeptides encoded by the propionate circuits and expressed by the genetically engineered bacteria have at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with one or more of **SEQ ID NO: 46 through SEQ ID**

**NO: 70**. In another embodiment, one or more polypeptides encoded by the propionate circuits and expressed by the genetically engineered bacteria one or more polypeptides encoded by the propionate circuits and expressed by the genetically engineered bacteria comprise the sequence of one or more of **SEQ ID NO: 46 through SEQ ID NO: 70**. In yet another embodiment one or more polypeptides encoded by the propionate circuits and expressed by the genetically engineered bacteria consist of or or more of **SEQ ID NO: 46 through SEQ ID NO: 70**.

[0238] 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. In some embodiments, one or more of the propionate biosynthesis genes is a *R. sphaeroides* 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.

[0239] 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. 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. In some embodiments, the local production of propionate reduces food intake and improves gut barrier function and reduces inflammation In some embodiments, the genetically engineered bacteria are capable of expressing the propionate biosynthesis cassette and producing propionate in low-oxygen conditions, in the presence of certain molecules or metabolites, in the presence of molecules or metabolites associated with inflammation or an inflammatory response, or in the presence of some other metabolite that may or may not be present in the gut, such as arabinose.

[0240] In one embodiment, the propionate gene cassette is directly operably linked to a first promoter. In another embodiment, the propionate gene cassette is indirectly

operably linked to a first promoter. In one embodiment, the promoter is not operably linked with the propionate gene cassette in nature.

[0241] In some embodiments, the propionate gene cassette is expressed under the control of a constitutive promoter. In another embodiment, the propionate gene cassette is expressed under the control of an inducible promoter. In some embodiments, the propionate gene cassette is expressed under the control of a promoter that is directly or indirectly induced by exogenous environmental conditions. In one embodiment, the propionate gene cassette is expressed under the control of a promoter that is directly or indirectly induced by low-oxygen or anaerobic conditions, wherein expression of the propionate gene cassette is activated under low-oxygen or anaerobic environments, such as the environment of the mammalian gut. Inducible promoters are described in more detail *infra*.

[0242] The propionate gene cassette may be present on a plasmid or chromosome in the bacterial cell. In one embodiment, the propionate gene cassette is located on a plasmid in the bacterial cell. In another embodiment, the propionate gene cassette is located in the chromosome of the bacterial cell. In yet another embodiment, a native copy of the propionate gene cassette is located in the chromosome of the bacterial cell, and a propionate gene cassette from a different species of bacteria is located on a plasmid in the bacterial cell. In yet another embodiment, a native copy of the propionate gene cassette is located on a plasmid in the bacterial cell, and a propionate gene cassette from a different species of bacteria is located on a plasmid in the bacterial cell. In yet another embodiment, a native copy of the propionate gene cassette is located in the chromosome of the bacterial cell, and a propionate gene cassette from a different species of bacteria is located in the chromosome of the bacterial cell.

[0243] In some embodiments, the propionate gene cassette is expressed on a low-copy plasmid. In some embodiments, the propionate gene cassette is expressed on a high-copy plasmid. In some embodiments, the high-copy plasmid may be useful for increasing expression of propionate.

*Acetate*

[0244] 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.

*Tryptophan and Tryptophan Metabolism*

**Kynurenine**

[0245] 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.

[0246] 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, genes, or gene cassettes 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

**Tryptophan, Tryptophan Metabolism, and Tryptophan Metabolites**

*Tryptophan and the Kynurenine Pathway*

[0247] Tryptophan (TRP) is an essential amino acid that, after consumption, is either incorporated into proteins via new protein synthesis, or converted a number of biologically active metabolites with a number of differing roles in health and disease (Perez-De La Cruz *et al.*, 2007 Kynurenine Pathway and Disease: An Overview; CNS&Neurological Disorders -Drug Targets 2007, 6,398-410). Along one arm of tryptophan catabolism, trytophan is converted to the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) by tryptophan hydroxylase. Serotonin can further be converted into the hormone melatonin. A large share of tryptophan, however, is metabolized to a number of bioactive metabolites, collectively called kynurenines, along a second arm called the kynurenine pathway (KP). In the first step of catabolism, TRP is converted to Kynurenine, (KYN), which has well-documented immune suppressive functions in several types of immune cells, and has recently been shown to be an activating ligand for the arylcarbon receptor (AhR; also known as dioxin receptor). KYN was initially shown in the cancer setting as an endogenous AHR ligand in immune and tumor cells, acting both in an autocrine and paracrine manner, and promoting tumor cell survival. In the gut, kynurenine pathway metabolism is regulated by gut microbiota, which can regulate tryptophan availability for kynurenine pathway metabolism.

[0248] More recently, additional tryptophan metabolites, collectively termed "indoles", herein, including for example, indole-3 aldehyde, indole-3 acetate, indole-3 propoinic acid, indole, indole-3 acetaladehyde, indole-3acetonitrile, FICZ, etc. which are generated by the microbiota, some by the human host, some from the diet, which are also able to function as AhR agonists, see e.g., **Table 8 and FIG. 37** and elsewhere herein, and

Lama et al., Nat Med. 2016 Jun;22(6):598-605; CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands.

[0249] Ahr best known as a receptor for xenobiotics such as polycyclic aromatic hydrocarbons AhR is a ligand-dependent cytosolic transcription factor that is able to translocate to the cell nucleus after ligand binding. The in additiona to kynurenine, tryptophan metabolites L-kynurenine, 6-formylindolcarbazole (FICZ, a photoproduct of TRP), and KYNA are have recently been identified as endogenous AhR ligands mediating immunosuppressive functions. To induce transcription of AhR target genes in the nucleus, AhR partners with proteins such as AhR nuclear translocator (ARNT) or NF-κB subunit RelB. Studies on human cancer cells have shown that KYN activates the AhR-ARNT associated transcription of IL-6, which induced autocrine activation of IDO1 via STAT3. This AhR-IL-6-STAT3 loop is associated with a poor prognosis in lung cancer, supporting the idea that IDO/kynurenine-mediated immunosuppression enables the immune escape of tumor cells.

[0250] In the gut, tryptophan may also be transported across the epithelium by transport machinery comprising angiotensin I converting enzyme 2 (ACE2), and converted to kynurenine, where it functions in the suppression of T cell respononse and promotion of Treg cells.

[0251] The rate-limiting conversion of TRP to KYN may be mediated by either of two forms of indoleamine 2, 3-dioxygenase (IDO) or by tryptophan 2,3-dioxygenase (TDO). One characteristic of TRP metabolism is that the rate-limiting step of the catalysis from TRP to KYN is generated by both the hepatic enzyme tryptophan 2,3-dioxygenase (TDO) and the ubiquitous expressed enzyme IDO1. TDO is essential for homeostasis of TRP concentrations in organisms and has a lower affinity to TRP than IDO1. Its expression is activated mainly by increased plasma TRP concentrations but can also be activated by glucocorticoids and glucagon. The tryptophan kynurenine pathway is also expressed in a large number of microbiota, most prominently in Enterobacteriaceae, and kynurenine and metabolites may be synthesized in the gut (**FIG. 14** and Sci Transl Med. 2013 July 10; 5(193): 193ra91). In some embodiments, the genetically engineered bacteria comprise one or more heterologous bacterially derived genes from Enterobacteriaceae, e.g. whose gene products catalyze the conversion of TRP:KYN. Along one pathway, KYN may be further metabolized to another bioactive metabolite, kynurenic acid, (KYNA)

which can antagonize glutamate receptors and can also bind AHR and also GPCRs, e.g., GPR35, glutamate receptors, N-methyl D-aspartate (NMDA)-receptors, and others. Along a third pathway of the KP, KYN can be converted to anthranilic acid (AA) and further downstream quinolinic acid (QUIN), which is a glutamate receptor agonist and has a neurotoxic role.

[0252] Therefore, finding a means to upregulate and/or downregulate the levels of flux through the KP and to reset relative amounts and/or ratios of tryptophan and its various bioactive metabolites may be useful in the prevention, treatment and/or management of a number of diseases as described herein. The present disclosure describes compositions for modulating, regulating and fine tuning trypophan and tryptophan metabolite levels, e.g., in the serum or in the gastrointestinal system, through genetically engineered bacteria which comprise circuitry enabling the synthesis, bacterial uptake and catabolism of tryptophan and/or tryptophan metabolites. and provides methods for using these compositions in the treatment, management and/or prevention of a number of different diseases.

*Other Indole Tryptophan Metabolites*

[0253] In addition to kynurenine and KYNA, numerous compounds have been proposed as endogenous AHR ligands, many of which are generated through pathways involved in the metabolism of tryptophan and indole (Bittinger et al., 2003; Chung and Gadupudi, 2011) A large number of metabolites generated through the tryptophan indole pathway are generated by microbiota in the gut. For example, bacteria take up tryptophan, which can be converted to mono-substituted indole compounds, such as indole acetic acid (IAA) and tryptamine, and other compounds, which have been found to activate the AHR (Hubbard et al., 2015, Adaptation of the human aryl hydrocarbon receptor to sense microbiota-derived indoles; Nature Scientific Reoports 5:12689).

[0254] In the gastronintestinal tract, diet derived and bacterially AhR ligands promote IL-22 production by innate lymphoid cells, referred to as group 3 ILCs (Spits et al., 2013, Zelante et al., Tryptophan Catabolites from Microbiota Engage Aryl Hydrocarbon Receptor and Balance Mucosal Reactivity via Interleukin-22; Immunity 39, 372–385, August 22, 2013).

[0255] 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.

[0256] **Table 8** lists exemplary tryptophan metabolites which have been shown to bind to AhR and which can be produced by the genetically engineered bacteria of the disclosure.

**Table 8. Indole Tryptophan Metabolites**

| Origin | Compound |
|---|---|
| Exogenous | 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) |
| Dietary | Indole-3-carbinol (I3C) |
| Dietary | Indole-3-acetonitrile (I3ACN) |
| Dietary | 3.3'-Diindolylmethane (DIM) |
| Dietary | 2-(indol-3-ylmethyl)-3.3'-diindolylmethane (Ltr-1) |
| Dietary | Indolo(3,2-b)carbazole (ICZ) |
| Dietary | 2-(1'H-indole-3'-carbony)-thiazole-4-carboxylic acid methyl ester (ITE) |
| Microbial | Indole |
| Microbial | Indole-3-acetic acid (IAA) |
| Microbial | Indole-3-aldehyde (IAId) |
| Microbial | Tryptamine |
| Microbial | 3-methyl-indole (Skatole) |
| Yeast | Tryptanthrin |
| Microbial/Host Metabolism | Indigo |
| Microbial/Host Metabolism | Indirubin |
| Microbial/Host Metabolism | Indoxyl-3-sulfate (I3S) |
| Host Metabolism | Kynurenine (Kyn) |
| Host Metabolism | Kynurenic acid (KA) |
| Host Metabolism | Xanthurenic acid |
| Host Metabolism | Cinnabarinic acid (CA) |
| UV-Light Oxidation | 6-formylindolo(3,2-b)carbazole (FICZ) |

| | |
|---|---|
| Microbial metabolism | |

[0257] In addition, some indole metabolites may exert their effect through Pregnane X receptor (PXR), which is thought to play a key role as an essential regulator of intestinal barrier function. PXR-deficient (Nr1i2-/-) mice showed a distinctly ‘‘leaky’’gut physiology coupled with upregulation of the Toll-like receptor 4 (TLR4), a receptor well known for recognizing LPS and activating the innate immune system (Venkatesh et al., 2014 Symbiotic Bacterial Metabolites Regulate Gastrointestinal Barrier Function via the Xenobiotic Sensor PXR and Toll-like Receptor 4; Immunity 41, 296–310, August 21, 2014). In particular, indole 3-propionic acid (IPA), produced by microbiota in the gut, has been shown to be a ligand for PXR in vivo.

[0258] As a result of PXR agonism, indole levels e.g., produced by commensal bacteria, or by genetically engineered bacteria, may through the activation of PXR regulate and balance the levels of TLR4 expression to promote homeostasis and gut barrier health. Ie., low levels of IPA and/or PXR and an excess of TLR4 may lead to intestinaly barrier dysfunction, while increasing levels of IPA may promote PXR activation and TLR4 downregulation, and improved gut barrier health.

[0259] Although microbial degradation of tryptophan to indole-3-propionate has been shown in a numver of microorganisms (see, e.g., Elsden et al., The end products of the metabolism of aromatic amino acids by Clostridia, Arch Microbiol. 1976 Apr 1;107(3):283-8), to date, the bacterial entire biosynthetic pathway from tryptophan to IPA is unknown. In Clostridium sporogenes, tryptophan is catabolized via indole-3-pyruvate, indole-3-lactate, and indole-3-acrylate to indole-3-propionate (O’Neill and DeMoss, Tryptophan transaminase from Clostridium sporogenes, Arch Biochem Biophys. 1968 Sep 20;127(1):361-9). Two enzymes that have been purified from C. sporogenes are tryptophan transaminase and indole-3-lactate dehydrogenase (Jean and DeMoss, Indolelactate dehydrogenase from Clostridium sporogenes, Can J Microbiol. 1968 Apr;14(4):429-35). Lactococcus lactis, catabolizes tryptophan by an aminotransferase to indole-3-pyruvate. In Lactobacillus casei and Lactobacillus helveticus tryptophan is also catabolized to indole-3-lactate through successive transamination and dehydrogenation (see, e.g., Tryptophan catabolism by Lactobacillus casei and Lactobacillus helveticus

cheese flavor adjuncts Gummalla, S., Broadbent, J. R. J. Dairy Sci 82:2070-2077, and references therein).

[0260] L-tryptophan transaminase (e.g., EC 2.6.1.27, e.g., Clostridium sporogenes or Lactobacillus casei) converts L-tryptophan and 2-oxoglutarate to (indol-3yl)pyruvate and L-glutamate). Indole-3-lactate dehydrogenase (EC 1.1.1.110, e.g., Clostridium sporogenes orLactobacillus casei) converts (indol-3yl) pyruvate and NADH and H+ to indole-3 lactate and NAD+.

[0261] In some embodiments, the engineered bacteria comprises gene sequence(s) encoding one or more enzymes selected from tryptophan transaminase (e.g., from C. sporogenes) and/or indole-3-lactate dehydrogenase (e.g., from C. sporogenes), and/or indole-3-pyruvate aminotransferase (e.g., from Lactococcus lactis). In other embodiments, such enzymes encoded by the bacteria are from Lactobacillus casei and/or Lactobacillus helveticus.

[0262] In other embodiments, IPA producing circuits comprise enzymes depicted and described in **FIG. 44** and elsewhere herein.

[0263] In some embodiments, the bacteria comprise gene sequence for producing one or more tryptophan metabolites, e.g., “indoles”. In some embodiments, the bacteria comprise gene sequence for producing and indole selected from indole-3 aldehyde, indole-3 acetate, indole-3 propoinic acid, indole, indole-3 acetaladehyde, indole-3acetonitrile, FICZ. In some embodiments, the bacteria comprise gene sequence for producing an indole that functions as an AhR agonist, see e.g., **Table 8 and FIG. 37.**

[0264] In some embodiments, the genetically engineered bacteria comprise a circuit for the generation of IPA. In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding a tryptophan ammonia lyase and an indole-3-acrylate reductase (e.g., Tryptophan ammonia lyase (WAL) (*Rubrivivax benzoatilyticus*) and indole-3-acrylate reductase (*Clostridum botulinum*). In some embodiments the expression of the gene sequences is under the control of an inducible promoter. Exemplary inducible promoters which may control the expression of the IPA biosynthetic cassette include oxygen level-dependent promoters (*e.g.*, FNR-inducible promoter), promoters induced by inflammation or an inflammatory response (RNS, ROS promoters), and promoters induced by a metabolite that may or may not be naturally present (e.g., can be exogenously added) in the gut, e.g., arabinose and tetracycline.

[0265] In some embodiments, the bacteria comprise any one or more of the circuits described and depicted in Figures 39, 41A-H, 42A-E, 43A, 43B, 45A-E.

*Methoxyindole pathway, Serotonin and Melatonin*

[0266] The methoxyindole pathway leads to formation of serotonin (5-HT) and melatonin. Serotonin (5-hydroxytryptamine, 5-HT) is a biogenic amine synthesized in a two-step enzymatic reaction: First, enzymes encoded by one of two tryptophan hydroxylase genes (Tph1 or Tph2) catalyze the rate-limiting conversion of tryptophan to 5-hydroxytryptophan (5-HTP), thus allocating the bioactivity of serotonin into either the brain (Tph2) or the periphery (Tph1). Then, 5-HTP undergoes decarboxylation to serotonin. Intestinal serotonin (5-hydroxytryptamine, 5-HT) is released by enterochromaffin cells and neurons and is regulated via the serotonin re-uptake transporter (SERT). The SERT is located on epithelial cells and neurons in the intestine. In certain embodiments, the genetically engineered bacteria described herein may modulate serotonin levels in the intestine, *e.g.*, decrease serotonin levels.

[0267] 5-HT also functions a substrate for melatonin biosynthesis. The rate-limiting step of melatonin biosynthesis is 5-HT-N-acetylation resulting in the formation of N-acetyl-serotonin (NAS) with subsequent Omethylation into 5-methoxy-N-acetyltryptamine (melatonin). The deficient production of 5-HT, NAS, and melatonin contribute to depressed mood, disturbances of sleep and circadian rhythms. Melatonin acts as a neurohormone and is associated with the development of circadian rhythm and the sleep-wake cycle.

[0268] In certain embodiments, the genetically engineered bacteria influence 5-HT synthesis, release, and/or degradation. Gut microbiota are interconnected with serotonin signaling and care capable of increasing serotonin levels through host serotonin production (Jano *et al.*, Cell. 2015 Apr 9;161(2):264-76. doi: 10.1016/j.cell.2015.02.047. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis). In some embodiments, the genetically engineered bacteria may modulate the serotonin levels in the gut to ameliorate symptoms of inflammation. In some embodimetns, the genetically engineered bacteria take up serotonin from the environment, e.g., the gut. In a non limiting example, serotonin can be converted to melatonin by, e.g., , tryptophan hydroxylase (TPH), hydroxyl-O-methyltransferase (HIOMT), N-acetyltransferase (NAT), aromatic –

amino acid decarboxylase (AAAD). In some embodiments, the genetically engineered influence serotonin levels produced by the host.

[0269] In bacteria, melatonin is synthesized indirectly with tryptophan as an intermediate product of the shikimic acid pathway. In these cells, synthesis starts with d-erythrose-4-phosphate and phosphoenolpyruvate. In some embodiments the genetically engineered bacteria comprise an endogenous or exogenous cassette for the production of melatonin. As anon-limiting example, one pathway or cassette is described in Bochkov, Denis V.; Sysolyatin, Sergey V.; Kalashnikov, Alexander I.; Surmacheva, Irina A. (2011). "Shikimic acid: review of its analytical, isolation, and purification techniques from plant and microbial sources". Journal of Chemical Biology 5 (1): 5–17. doi:10.1007/s12154-011-0064-8.

**Exemplary Tryptophan and Tryptophan Metabolite Circuits**

*Decreasing Exogenous Tryptophan*

[0270] In some embodiments, the genetically engineered bacteria are capable of decreasing the level of tryptophan and/or the level of a tryptophan metabolite. In some embodiments, the engineered bacteria comprise gene sequence(s) for encoding one or more aromatic amino acid transporter(s). In one embodiment, the amino acid transporter is a tryptophan transporter. Tryptophan transporters may be expressed or modified in the recombinant bacteria described herein in order to enhance tryptophan transport into the cell. Specifically, when the tryptophan transporter is expressed in the recombinant bacterial cells described herein, the bacterial cells import more tryptophan into the cell when the transporter is expressed than unmodified bacteria of the same bacterial subtype under the same conditions. Thus, the genetically engineered bacteria comprising a heterologous gene encoding a tryptophan transporter which may be used to import tryptophan into the bacteria.

[0271] The uptake of tryptophan into bacterial cells is mediated by proteins well known to those of skill in the art. For example, three different tryptophan transporters, distinguishable on the basis of their affinity for tryptophan have been identified in E. coli (see, e.g., Yanofsky et al. (1991) J. Bacteriol. 173: 6009-17). The bacterial genes mtr, aroP, and tnaB encode tryptophan permeases responsible for tryptophan uptake in bacteria. High affinity permease, Mtr, is negatively regulated by the trp repressor and positively regulated by the TyR product (see, e.g., Yanofsky et al. (1991) J. Bacteriol. 173: 6009-17

and Heatwole et al. (1991) J. Bacteriol. 173: 3601-04), while AroP is negatively regulated by the tyR product (Chye et al. (1987) J. Bacteriol. 169:386-93).

[0272] In one embodiment, the at least one gene encoding a tryptophan transporter is a gene selected from the group consisting of mtr, aroP and tnaB. In one embodiment, the bacterial cell described herein has been genetically engineered to comprise at least one heterologous gene selected from the group consisting of mtr, aroP and tnaB. In one embodiment, the at least one gene encoding a tryptophan transporter is the Escherichia coli mtr gene. In one embodiment, the at least one gene encoding a tryptophan transporter is the Escherichia coli aroP gene. In one embodiment, the at least one gene encoding a tryptophan transporter is the Escherichia coli tnaB gene.

[0273] In some embodiments, the tryptophan transporter is encoded by a tryptophan transporter gene derived from a bacterial genus or species, including but not limited to, Escherichia, Corynebacterium, Escherichia coli, Saccharomyces cerevisiae or Corynebacterium glutamicum. In some embodiments, the bacterial species is Escherichia coli. In some embodiments, the bacterial species is Escherichia coli strain Nissle.

[0274] Assays for testing the activity of a tryptophan transporter, a functional variant of a tryptophan transporter, or a functional fragment of transporter of tryptophan are well known to one of ordinary skill in the art. For example, import of tryptophan may be determined using the methods as described in Shang et al. (2013) J. Bacteriol. 195:5334-42, the entire contents of each of which are expressly incorporated by reference herein.

[0275] In one embodiment, when the tryptophan transporter is expressed in the recombinant bacterial cells described herein, the bacterial cells import 10% more tryptophan into the bacterial cell when the transporter is expressed than unmodified bacteria of the same bacterial subtype under the same conditions. In another embodiment, when the tryptophan transporter is expressed in the recombinant bacterial cells described herein, the bacterial cells import 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% more tryptophan into the bacterial cell when the transporter is expressed than unmodified bacteria of the same bacterial subtype under the same conditions. In yet another embodiment, when the tryptophan transporter is expressed in the recombinant bacterial cells described herein, the bacterial cells import two-fold more tryptophan into the cell when the transporter is expressed than unmodified bacteria of the same bacterial subtype

under the same conditions. In yet another embodiment, when the tryptophan transporter is expressed in the recombinant bacterial cells described herein, the bacterial cells import three-fold, four-fold, five-fold, six-fold, seven-fold, eight-fold, nine-fold, ten-fold, fifteen-fold, twenty-fold, thirty-fold, fourty-fold, or fifty-fold, more tryptophan into the cell when the transporter is expressed than unmodified bacteria of the same bacterial subtype under the same conditions.

[0276] In addition to the tryptophan uptake transporters, in some embodiments, the genetically engineered bacteria further comprise a circuit for the production of tryptophan metabolites, as described herein, e.g., for the production of kynurenine, kynurenine metabolites, or indole tryptophan metabolites as shown in **Table 8.**

[0277] In some embodiments, the genetically engineered bacteria are capable of decreasing the level of tryptophan. In some embodiments, the engineered bacteria comprise one or more gene sequences for converting tryptophan to kynurenine. In some embodiments, the engineered bacteria comprise gene sequence(s) for encoding the enzyme indoleamine 2,3-dioxygenase (IDO-1). In some embodiments, the engineered bacteria comprise gene sequence(s) for encoding the enzyme tryptophan dioxygenase (TDO). In some embodiments, the engineered bacteria comprise gene sequence(s) for encoding the enzyme indoleamine 2,3-dioxygenase (IDO-1) and the enzyme tryptophan dioxygenase (TDO). In some embodiments, the genetically engineered bacteria comprise a gene cassette encoding Indoleamine 2, 3 dioxygenase (EC 1.13.11.52; producing N-formyl kynurenine from tryptophan) and Kynurenine formamidase (EC3.5.1.9) producing kynurenine from n-formylkynurenine). In some embodiments, the enzymes are bacterially derived, e.g., as described in Vujkovi-Cvijin et al. 2013.

[0278] In some embodiments, the genetically engineered bacteria are capable of decreasing the level of tryptophan, e.g., in combination with the production of indole metabolites, through expression of gene(s) and gene cassette(s) described herein.

*Increasing Kynurenine*

[0279] In some embodiments, the genetically engineered bacteria are capable of producing kynurenine.

[0280] In some embodiments, the genetically engineered bacteria are capable of decreasing the level of tryptophan. In some embodiments, the engineered bacteria comprises one or more gene sequences for converting tryptophan to kynurenine. In some

embodiments, the engineered bacteria comprises gene sequence(s) for encoding the enzyme indoleamine 2,3-dioxygenase (IDO-1). In some embodiments, the engineered bacteria comprises gene sequence(s) for encoding the enzyme tryptophan dioxygenase (TDO). In some embodiments, the engineered bacteria comprise on or more gene sequence(s) for encoding the enzyme indoleamine 2,3-dioxygenase (IDO-1) and the enzyme tryptophan dioxygenase (TDO). In some embodiments, the genetically engineered bacteria comprise a gene cassette encoding Indoleamine 2, 3 dioxygenase (EC 1.13.11.52; producing N-formyl kynurenine from tryptophan) and Kynurenine formamidase (EC3.5.1.9) producing kynurenine from n-formylkynurenine). In some embodiments, the enzymes are bacterially derived, e.g., as described in Vujkovi-Cvijin et al. 2013.

[0281] The genetically engineered bacteria may comprise any suitable gene for producing kynurenine. 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 also 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, as discussed in detail above. 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, in the presence of certain molecules or metabolites, in the presence of molecules or metabolites associated with inflammation or an inflammatory response, or in the presence of some other metabolite that may or may not be present in the gut, such as arabinose .

[0282] 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. 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, in the presence of certain molecules or metabolites, in the presence of molecules or metabolites associated with inflammation or an inflammatory response, or in the presence of some other metabolite that may or may not be present in the gut, such as arabinose.

[0283] In some embodiments, the genetically engineered bacteria comprise one or more gene(s) or gene cassette(s) for the consumption of tryptophan and production of kynurenine, which are bacterially derived. In some embodiments, the enzymes for TRP to KYN conversion are derived from one or more of Pseudomonas, Xanthomonas, Burkholderia, Stenotrophomonas, Shewanella, and Bacillus, and/or members of the families Rhodobacteraceae, Micrococcaceae, and Halomonadaceae, In some embodiments the enzymes are derived from the species listed in table S7 of Vujkovic-Cvijin et al. (Dysbiosis of the gut microbiota is associated with HIV diseaseprogression and tryptophan catabolism Sci Transl Med. 2013 July 10; 5(193): 193ra91), the contents of which is herein incorporated by reference in its entirety.

[0284] In some embodiments, the one or more genes for producing kynurenine are 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, in the presence of certain molecules or metabolites, in the presence of molecules or metabolites associated with inflammation or an inflammatory response, or in the presence of some other metabolite that may or may not be present in the gut, such as arabinose. 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. In some embodiments,

[0285] In some embodiments, the genetically engineered bacteria prevent the accumulation of post-kynurenine KP metabolites, e.g., neurotoxic metabolites, or diabetogenic metabolites. In some embodiments, the genetically engineered bacteria encode Kynureninase from Pseudomonas fluorescens.

[0286] In some embodiments, the genetically engineered bacteria comprising one or more gene(s) or gene cassette(s) can alter the TRP:KYN ratio, e.g. in the circulation. In some embodiments the TRP:KYN ratio is increased. In some embodiments, TRP:KYN ratio is decreased. In some embodiments, the genetically engineered bacteria the genetically engineered bacteria comprising one or more gene(s) or gene cassette(s) can alter the KYNA:QUIN ratio..

[0287] In some embodiments, the genetically engineered bacteria are capable of expressing any one or more of the described circuits in low-oxygen conditions, in the presence of disease or tissue specific molecules or metabolites, in the presence of molecules or metabolites associated with inflammation or an inflammatory response or immune suppression, or in the presence of some other metabolite that may or may not be present in the gut, such as arabinose. In some embodiments, any one or more of the described circuits are present on one or more plasmids (e.g., high copy or low copy) or are integrated into one or more sites in the bacterial chromosome. Also, in some embodiments, the genetically engineered bacteria are further capable of expressing any one or more of the described circuits and further comprise one or more of the following: (1) one or more auxotrophies, such as any auxotrophies known in the art and provided herein, e.g., thyA auxotrophy, (2) one or more kill switch circuits, such as any of the kill-switches described herein or otherwise known in the art, (3) one or more antibiotic resistance circuits, (4) one or more transporters for importing biological molecules or substrates, such any of the transporters described herein or otherwise known in the art, (5) one or more secretion circuits, such as any of the secretion circuits described herein and otherwise known in the art, and (6) combinations of one or more of such additional circuits.

*Increasing Tryptophan*

[0288] In some embodiments, the genetically engineered microorganisms of the present disclosure, are capable of producing tryptophan. Exemplary circuits for the production of tryptophan are shown in **FIG. 39, FIG. 45A** and **FIG. 45B**.

[0289] In some embodiments, the genetically engineered bacteria that produce tryptophan comprise one or more gene sequences encoding one or more enzymes of the tryptophan biosynthetic pathway. In some embodiments, the genetically engineered bacteria comprise a tryptophan operon. In some embodiments, the genetically engineered bacteria comprise the tryptophan operon of E. coli. (Yanofsky, RNA (2007), 13:1141-1154). In some embodiments, the genetically engineered bacteria comprise the tryptophan operon of B. subtilis. (Yanofsky, RNA (2007), 13:1141-1154). In some embodiments, the genetically engineered bacteria comprise sequence(s) encoding trypE, trypG-D, trypC-F, trypB, and trpA genes. In some embodiments, the genetically engineered bacteria comprise sequence(s) encoding trypE, trypG-D, trypC-F, trypB, and trpA genes from E. Coli. In some embodiments, the genetically engineered bacteria comprise sequence(s) encoding trypE, trypD, trypC, trypF, trypB, and trpA genes from B. subtilis.

[0290] Also, in any of these embodiments, the genetically engineered bacteria optionally comprise gene sequence(s) to produce the tryptophan precursor, chorismate. Thus, in some embodiments, the genetically engineered bacteria optionally comprise sequence(s) encoding aroG, aroF, aroH, aroB, aroD, aroE, aroK, and AroC. In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding one or more enzymes of the tryptophan biosynthetic pathway and one or more gene sequences encoding one or more enzymes of the chorismate biosynthetic pathway. In some embodiments, the genetically engineered bacteria comprise sequence(s) encoding trypE, trypG-D, trypC-F, trypB, and trpA genes from E. Coli and sequence(s) encoding aroG, aroF, aroH, aroB, aroD, aroE, aroK, and AroC genes. In some embodiments, the genetically engineered bacteria comprise sequence(s) encoding trypE, trypD, trypC, trypF, trypB, and trpA genes from B. subtilis and sequence(s) encoding aroG, aroF, aroH, aroB, aroD, aroE, aroK, and AroC genes.

[0291] In some embodiments, the genetically engineered bacteria comprise sequence(s) encoding either a wild type or a feedback resistant SerA gene (**Table 10**). Escherichia coli serA-encoded 3-phosphoglycerate (3PG) dehydrogenase catalyzes the first step of the major phosphorylated pathway of L-serine (Ser) biosynthesis. This step is an oxidation of 3PG to 3-phosphohydroxypyruvate (3PHP) with the concomitant reduction of NAD+ to NADH. As part of Tryptophan biosynthesis, E. coli uses one serine for each

tryptophan produced. As a result, by expressing serA, tryptophan production is improved (see, e.g., **FIG. 38**).

[0292] In any of these embodiments, AroG and TrpE are optionally replaced with feedback resistant versions to improve tryptophan production (**Table 10**).

[0293] In any of these embodiments, the tryptophan repressor (trpR) optionally may be deleted, mutated, or modified so as to diminish or obliterate its repressor function.

[0294] In any of these embodiments the tnaA gene (encoding a tryptophanase converting Trp into indole) optionally may be deleted to prevent tryptophan catabolism along this pathway and to further increase levels of tryptophan produced (**Table 10**).

[0295] The inner membrane protein YddG of Escherichia coli, encoded by the yddG gene, is a homologue of the known amino acid exporters RhtA and YdeD. Studies have shown that YddG is capable of exporting aromatic amino acids, including tryptophan. Thus, YddG can function as a tryptophan exporter or a tryptophan secretion system (or tryptophan secretion protein). Other aromatic amino acid exporters are described in Doroshenko *et al.*, FEMS Microbial Lett., 275:312-318 (2007). Thus, in some embodiments, the engineered bacteria optionally further comprise gene sequence(s) encoding YddG. In some embodiments, the engineered bacteria can over-express YddG. In some embodiments, the engineered bacteria optionally comprise one or more copies of yddG gene.

[0296] In some embodiments, the genetically engineered bacteria comprise a mechanism for metabolizing or degrading kyurenine, which, in some embodiments also results in the increased production of tryptophan. In some embodiments, the genetically engineered bacteria comprise sequence encoding the enzyme kynureninase. Kynureninase is produced to metabolize Kynurenine to Anthranilic acid in the cell. Schwarcz *et al.*, Nature Reviews Neuroscience, 13, 465-477; 2012; Chen & Guillemin, 2009; 2; 1-19; Intl. J. Tryptophan Res. Exemplary kynureninase sequences are provided herein below in **Table 11.** In some embodiments, the engineered microbe has a mechanism for importing (transporting) Kynurenine from the local environment into the cell. Thus, in some embodiments, the genetically engineered bacteria comprise gene sequence(s) encoding a kynureninase secreter. In some embodiments, the genetically engineered bacteria comprise one or more copies of *aroP*, *tnaB* or *mtr* gene.

[0297] In some embodiments, the genetically engineered bacteria comprise gene sequence(s) encoding enzymes of the tryptophan biosynthetic pathway and sequence

encoding kynureninase. In some embodiments, the genetically engineered bacteria comprise a tryptophan operon, for example that of E. coli. or B. subtilis, and sequence encoding kynureninase. In some embodiments, the genetically engineered bacteria comprise sequence(s) encoding trypE, trypG-D, trypC-F, trypB, and trpA genes, for example, from E. Coli and sequence encoding kyureninase. In some embodiments, the genetically engineered bacteria comprise sequence(s) encoding trypE, trypD, trypC, trypF, trypB, and trpA genes, for example from B. subtilis and sequence encoding kyureninase. In any of these embodiments, the tryptophan repressor (trpR) optionally may be deleted, mutated, or modified so as to diminish or obliterate its repressor function. Also, in any of these embodiments, the genetically engineered bacteria optionally comprise gene sequence(s) to produce the tryptophan precursor, Chorismate, for example, sequence(s) encoding aroG, aroF, aroH, aroB, aroD, aroE, aroK, and AroC. Thus, in some embodiments, the genetically engineered bacteria comprise sequence(s) encoding trypE, trypG-D, trypC-F, trypB, and trpA genes from E. Coli, sequence(s) encoding aroG, aroF, aroH, aroB, aroD, aroE, aroK, and AroC genes, and sequence encoding kyureninase. In some embodiments, the genetically engineered bacteria comprise sequence(s) encoding trypE, trypD, trypC, trypF, trypB, and trpA genes from B. subtilis, sequence(s) encoding aroG, aroF, aroH, aroB, aroD, aroE, aroK, and AroC genes, and sequence encoding kyureninase.

[0298] In some embodiments, the genetically engineered bacteria may optionally have a deletion or mutation in the endogenous *trpE*, rendering trpE non-functional. Accordingly, in one embodiment, the genetically engineered bacteria may comprise one or more gene(s) or gene cassette(s) encoding trpD, trpC, trpA, and trpD and kynureninase (see, e.g. **FIG. 18**). This deletion may prevent tryptophan production through the endogenous chorismate pathway, and may increase the production of tryptophan from kynurenine through kynureninase.

[0299] In some embodiments, the genetically engineered bacteria comprise sequence(s) encoding either a wild type or a feedback resistant SerA gene (**Table 10**) .

[0300] In any of these embodiments, AroG and TrpE are optionally replaced with feedback resistant versions to improve tryptophan production (**Table 10**).

[0301] In any of these embodiments, the tryptophan repressor (trpR) optionally may be deleted, mutated, or modified so as to diminish or obliterate its repressor function.

[0302] In any of these embodiments the tnaA gene (encoding a tryptophanase converting Trp into indole) optionally may be deleted to prevent tryptophan catabolism along this pathway and to further increase levels of tryptophan produced (**Table 10**).

[0303] In any of these embodiments, the genetically engineered bacterium may further comprise gene sequence for exporting or secreting tryptophan from the cell. Thus, in some embodiments, the engineered bacteria further comprise gene sequence(s) encoding YddG. In some embodiments, the engineered bacteria can over-express YddG, an aromatic amino acid exporter. In some embodiments, the engineered bacteria optionally comprise one or more copies of yddG gene. In any of these embodiments, the genetically engineered bacterium may further comprise gene sequence for importing or transporting kynurenine into the cell. Thus, in some embodiments, the genetically engineered bacteria comprise gene sequence(s) encoding a kynureninase secreter. In some embodiments, the genetically engineered bacteria comprise one or more copies of *aroP*, *tnaB* or *mtr* gene.

[0304] In some embodiments, the genetically engineered bacterium or genetically engineered microorganism comprises one or more genes for producing tryptophan and/or kynureninase, under the control of a promoter that is activated by low-oxygen conditions, by inflammatory conditions, such as any of the promoters activated by said conditions and described herein. In some embodiments, the genetically engineered bacteria expresses one or more genes for producing tryptophan and/or kynureninase, under the control of a cancer-specific promoter, a tissue-specific promoter, or a constitutive promoter, such as any of the promoters described herein. **Table 9** lists exemplary tryptophan synthesis cassettes encoded by the genetically engineered bacteria of the disclosure.

**Table 9. Tryptophan Synthesis Cassette Sequences**

| **Description** | **Sequence** |
|---|---|
| Tet-regulated Tryptophan operon **SEQ ID NO: 71** | taagacccactttcacatttaagttgtttttctaatccgcatatgatcaattcaaggccgaataagaaggctggctct<br>gcaccttggtgatcaaataattcgatagcttgtcgtaataatggcggcatactatcagtagtaggtgtttccctttct<br>tctttagcgacttgatgctcttgatcttccaatacgcaacctaaagtaaaatgccccacagcgctgagtgcatata<br>atgcattctctagtgaaaaaccttgttggcataaaaaggctaattgattttcgagagtttcatactgtttttctgtagg<br>ccgtgtacctaaatgtacttttgctccatcgcgatgacttagtaaagcacatctaaaacttttagcgttattacgtaa<br>aaaatcttgccagctttccccttctaaagggcaaaagtgagtatggtgcctatctaacatctcaatggctaaggcg<br>tcgagcaaagcccgcttatttttacatgccaatacaatgtaggctgctctacacctagcttctgggcgagtttacg<br>ggttgttaaaccttcgattccgacctcattaagcagctctaatgcgctgttaatcactttacttttatctaatctagaca<br>tcattaattcctaatttttgttgacactctatcattgatagagttattttaccactccctatcagtgatagagaaaagtg<br>aactctagaaataattttgtttaactttaagaaggagatatacatatgcaaacacaaaaaccgactctcgaactgct<br>aacctgcgaaggcgcttatcgcgacaacccgactgcgctttttcaccagttgtgtggggatcgtccggcaacg<br>ctgctgctggaatccgcagatatcgacagcaaagatgatttaaaaagcctgctgctggtagacagtgcgctgc<br>gcattacagcattaagtgacactgtcacaatccaggcgctttccggcaatggagaagccctgttgacactactg |

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|---|---|
| | gataacgccttgcctgcgggtgtggaaaatgaacaatcaccaaactgccgcgtactgcgcttcccgcctgtca<br>gtccactgctggatgaagacgcccgcttatgctccctttcggttttgacgctttccgcttattacagaatctgttga<br>atgtaccgaaggaagaacgagaagcaatgttcttcggcggcctgttctcttatgaccttgtggcgggatttgaaa<br>atttaccgcaactgtcagcggaaaatagctgccctgatttctgtttttatctcgctgaaacgctgatggtgattgac<br>catcagaaaaaaagcactcgtattcaggccagcctgtttgctccgaatgaagaagaaaaacaacgtctcactgc<br>tcgcctgaacgaactacgtcagcaactgaccgaagccgcgccgccgctgccggtggtttccgtgccgcatat<br>gcgttgtgaatgtaaccagagcgatgaagagttcggtggtgtagtgcgtttgttgcaaaaagcgattcgcgccg<br>gagaaattttccaggtggtgccatctcgccgtttctctctgccctgcccgtcaccgctggcagcctattacgtgct<br>gaaaaagagtaatcccagcccgtacatgtttttatgcaggataatgatttcaccctgtttggcgcgtcgccggaa<br>agttcgctcaagtatgacgccaccagccgccagattgagatttacccgattgccggaacacgtccacgcggtc<br>gtcgtgccgatggttcgctggacagagacctcgacagccgcatcgaactggagatgcgtaccgatcataaag<br>agctttctgaacatctgatgctggtggatctcgcccgtaatgacctggcacgcatttgcacacccggcagccgc<br>tacgtcgccgatctcaccaaagttgaccgttactcttacgtgatgcacctagtctcccgcgttgttggtgagctgc<br>gccacgatctcgacgccctgcacgcttaccgcgcctgtatgaatatggggacgttaagcggtgcaccgaaagt<br>acgcgctatgcagttaattgccgaagcagaaggtcgtcgacgcggcagctacggcggcgcggtaggttatttt<br>accgcgcatggcgatctcgacacctgcattgtgatccgctcggcgctggtggaaaacggtatcgccaccgtgc<br>aagccggtgctggcgtagtccttgattctgttccgcagtcggaagccgacgaaactcgtaataaagcccgcgc<br>tgtactgcgcgctattgccaccgcgcatcatgcacaggagacgttctaatggctgacattctgctgctcgataat<br>atcgactcttttacgtacaacctggcagatcagttgcgcagcaatggtcataacgtggtgatttaccgcaaccata<br>ttccggcgcagaccttaattgaacgcctggcgacgatgagcaatccggtgctgatgctttctcctggccccggt<br>gtgccgagcgaagccggttgtatgccggaactcctcacccgcttgcgtggcaagctgccaattattggcatttg<br>cctcggacatcaggcgattgtcgaagcttacgggggctatgtcggtcaggcgggcgaaattcttcacggtaaa<br>gcgtcgagcattgaacatgacggtcaggcgatgtttgccggattaacaaacccgctgccagtggcgcgttatc<br>actcgctggttggcagtaacattccggccggtttaaccatcaacgcccattttaatggcatggtgatggcggtgc<br>gtcacgatgcagatcgcgtttgtggattccagttccatccggaatccattcttactacccagggcgctcgcctgct<br>ggaacaaacgctggcctgggcgcagcagaaactagagccaaccaacacgctgcaaccgattctggaaaaa<br>ctgtatcaggcacagacgcttagccaacaagaaagccaccagctgttttcagcggtggtacgtggcgagctga<br>agccggaacaactggcggcggcgctggtgagcatgaaaattcgcggtgaacacccgaacgagatcgccgg<br>ggcagcaaccgcgctactggaaaacgccgcgccattcccgcgcccggattatctgtttgccgatatcgtcggt<br>actggcggtgacggcagcaacagcatcaatatttctaccgccagtgcgtttgtcgccgcggcctgcgggctga<br>aagtggcgaaacacggcaaccgtagcgtctccagtaaatccggctcgtcggatctgctggcggcgttcggtat<br>taatcttgatatgaacgccgataaatcgcgccaggcgctggatgagttaggcgtctgtttcctctttgcgccgaa<br>gtatcacaccggattccgccatgcgatgccggttcgccagcaactgaaaacccgcactctgttcaacgtgctg<br>ggaccattgattaacccggcgcatccgccgctggcgctaattggtgtttatagtccggaactggtgctgccgatt<br>gccgaaaccttgcgcgtgctggggtatcaacgcgcggcagtggtgcacagcggcgggatggatgaagtttc<br>attacacgcgccgacaatcgttgccgaactacatgacggcgaaattaagagctatcaattgaccgctgaagatt<br>ttggcctgacaccctaccaccaggagcaattggcaggcggaacaccggaagaaaaccgtgacattttaacac<br>gcttgttacaaggtaaaggcgacgccgcccatgaagcagccgtcgcggcgaatgtcgccatgttaatgcgcct<br>gcatggccatgaagatctgcaagccaatgcgcaaaccgttcttgaggtactgcgcagtggttccgcttacgaca<br>gagtcaccgcactggcggcacgagggtaaatgatgcaaaccgttttagcgaaaatcgtcgcagacaaggcg<br>atttgggtagaaacccgcaaagagcagcaaccgctggccagttttcagaatgaggttcagccgagcacgcga<br>catttttatgatgcacttcagggcgcacgcacggcgtttattctggagtgtaaaaaagcgtcgccgtcaaaaggc<br>gtgatccgtgatgatttcgatccggcacgcattgccgccatttataaacattacgcttcggcaatttcagtgctgac<br>tgatgagaaatattttcaggggagctttgatttcctccccatcgtcagccaaatcgccccgcagccgattttatgta<br>aagacttcattatcgatccttaccagatctatctggcgcgctattaccaggccgatgcctgcttattaatgctttcag<br>tactggatgacgaacaatatcgccagcttgcagccgtcgcccacagtctggagatgggtgtgctgaccgaagt<br>cagtaatgaagaggaactggagcgcgccattgcattgggggcaaaggtcgttggcatcaacaaccgcgatct<br>gcgcgatttgtcgattgatctcaaccgtacccgcgagcttgcgccgaaactggggcacaacgtgacggtaatc |

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| | agcgaatccggcatcaatacttacgctcaggtgcgcgagttaagccacttcgctaacggctttctgattggttcg<br>gcgttgatggcccatgacgatttgaacgccgccgtgcgtcgggtgttgctgggtgagaataaagtatgtggcct<br>gacacgtgggcaagatgctaaagcagcttatgacgcgggcgcgatttacggtgggttgatttttgttgcgacat<br>caccgcgttgcgtcaacgttgaacaggcgcaggaagtgatggctgcagcaccgttgcagtatgttggcgtgtt<br>ccgcaatcacgatattgccgatgtggcggacaaagctaaggtgttatcgctggcggcagtgcaactgcatggt<br>aatgaagatcagctgtatatcgacaatctgcgtgaggctctgccagcacacgtcgccatctggaaggctttaag<br>tgtcggtgaaactcttcccgcgcgcgattttcagcacatcgataaatatgtattcgacaacggtcagggcggga<br>gcggacaacgtttcgactggtcactattaaatggtcaatcgcttggcaacgttctgctggcgggggggcttaggc<br>gcagataactgcgtggaagcggcacaaaccggctgcgccgggcttgattttaattctgctgtagagtcgcaac<br>cgggtatcaaagacgcacgtcttttggcctcggttttccagacgctgcgcgcatattaaggaaaggaacaatga<br>caacattacttaacccctattttggtgagtttggcggcatgtacgtgccacaaatcctgatgcctgctctgcgcca<br>gctggaagaagcttttgtcagcgcgcaaaaagatcctgaatttcaggctcagttcaacgacctgctgaaaaact<br>atgccgggcgtccaaccgcgctgaccaaatgccagaacattacagccgggacgaacaccacgctgtatctga<br>agcgcgaagatttgctgcacggcggcgcgcataaaactaaccaggtgctcggtcaggctttactggcgaagc<br>ggatgggtaaaactgaaattattgccgaaaccggtgccggtcagcatggcgtggcgtcggcccttgccagcg<br>ccctgctcggcctgaaatgccgaatttatatgggtgccaaagacgttgaacgccagtcgcccaacgttttccgg<br>atgcgcttaatgggtgcggaagtgatcccggtacatagcggttccgcgaccctgaaagatgcctgtaatgagg<br>cgctacgcgactggtccggcagttatgaaaccgcgcactatatgctgggtaccgcagctggcccgcatcctta<br>cccgaccattgtgcgtgagtttcagcggatgattggcgaagaaacgaaagcgcagattctggaaagagaagg<br>tcgcctgccggatgccgttatcgcctgtgttggcggtggttcgaatgccatcggtatgtttgcagatttcatcaac<br>gaaaccgacgtcggcctgattggtgtggagcctggcggccacggtatcgaaactggcgagcacggcgcacc<br>gttaaaacatggtcgcgtgggcatctatttcggtatgaaagcgccgatgatgcaaaccgaagacgggcaaatt<br>gaagagtcttactccatttctgccgggctggatttcccgtccgtcggcccgcaacatgcgtatctcaacagcact<br>ggacgcgctgattacgtgtctattaccgacgatgaagccctggaagcctttaaaacgctttgcctgcatgaagg<br>gatcatcccggcgctggaatcctcccacgccctggcccatgcgctgaaaatgatgcgcgaaaatccggaaaa<br>agagcagctactggtggttaacctttccggtcgcggcgataaagacatcttcaccgttcacgatattttgaaagc<br>acgaggggaaatctgatggaacgctacgaatctctgtttgcccagttgaaggagcgcaaagaaggcgcattc<br>gttcctttcgtcaccctcggtgatccgggcattgagcagtcgttgaaaattatcgatacgctaattgaagccggtg<br>ctgacgcgctggagttaggcatccccttctccgacccactggcggatggcccgacgattcaaaacgccacact<br>gcgtgcttttgcggcgggagtaaccccggcgcagtgctttgagatgctggcactcattcgccagaagcacccg<br>accattcccatcggcctttgatgtatgccaacctggtgtttaacaaaggcattgatgagttttatgccgagtgcga<br>gaaagtcggcgtcgattcggtgctggttgccgatgtgcccgtggaagagtccgcgcccttccgccaggccgc<br>gttgcgtcataatgtcgcacctatctttatttgcccgccgaatgccgacgatgatttgctgcgccagatagcctctt<br>acggtcgtggttacacctatttgctgtcgcgagcgggcgtgaccggcgcagaaaaccgcgccgcgttacccc<br>tcaatcatctggttgcgaagctgaaagagtacaacgctgcgcctccattgcagggatttggtatttccgccccgg<br>atcaggtaaaagccgcgattgatgcaggagctgcgggcgcgatttctggttcggccatcgttaaaatcatcgag<br>caacatattaatgagccagagaaaatgctggcggcactgaaagcttttgtacaaccgatgaaagcggcgacgc<br>gcagttaatacgcatggcatggatgaCCGATGGTAGTGTGGGGTCTCCCCATGCG<br>AGAGTAGGGAACTGCCAGGCATCAAATAAAACGAAAGGCTCAGT<br>CGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGC<br>TCTCCTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGTTGC<br>GAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGCCATAAA<br>CTGCCAGGCATCAAATTAAGCAGAAGGCCATCCTGACGGATGGCC<br>TTTTTGCGTGGCCAGTGCCAAGCTTGCATGCGTGC |
| Tet repressor **SEQ ID NO:72** | taagacccactttcacatttaagttgtttttctaatccgcatatgatcaattcaaggccgaataagaaggctggctct<br>gcaccttggtgatcaaataattcgatagcttgtcgtaataatggcggcatactatcagtagtaggtgtttccctttct<br>tctttagcgacttgatgctcttgatcttccaatacgcaacctaaagtaaaatgccccacagcgctgagtgcatata<br>atgcattctctagtgaaaaaccttgttggcataaaaaggctaattgattttcgagagtttcatactgtttttctgtagg |

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| | ccgtgtacctaaatgtacttttgctccatcgcgatgacttagtaaagcacatctaaaacttttagcgttattacgtaa aaaatcttgccagctttccccttctaaagggcaaaagtgagtatggtgcctatctaacatctcaatggctaaggcg tcgagcaaagcccgcttatttttacatgccaatacaatgtaggctgctctacacctagcttctgggcgagtttacg ggttgttaaaccttcgattccgacctcattaagcagctctaatgcgctgttaatcactttacttttatctaatctagaca t |
| tetR/tetA promoters and RBS and leader region **SEQ ID NO 73:** | cattaattcctaatttttgttgacactctatcattgatagagttattttaccactccctatcagtgatagagaaaagtga actctagaaataatttttgtttaactttaagaaggagatatacat |
| trpE **SEQ ID NO: 74** | atgcaaacacaaaaaccgactctcgaactgctaacctgcgaaggcgcttatcgcgacaacccgactgcgctttt tcaccagttgtgtggggatcgtccggcaacgctgctgctggaatccgcagatatcgacagcaaagatgatttaa aaagcctgctgctggtagacagtgcgctgcgcattacagcattaagtgacactgtcacaatccaggcgctttcc ggcaatggagaagccctgttgacactactggataacgccttgcctgcgggtgtggaaaatgaacaatcaccaa actgccgcgtactgcgcttcccgcctgtcagtccactgctggatgaagacgcccgcttatgctccctttcggtttt tgacgctttccgcttattacagaatctgttgaatgtaccgaaggaagaacgagaagcaatgttcttcggcggcct gttctcttatgaccttgtggcgggatttgaaaatttaccgcaactgtcagcggaaaatagctgccctgatttctgttt ttatctcgctgaaacgctgatggtgattgaccatcagaaaaaaagcactcgtattcaggccagcctgtttgctcc gaatgaagaagaaaaacaacgtctcactgctcgcctgaacgaactacgtcagcaactgaccgaagccgcgc cgccgctgccggtggtttccgtgccgcatatgcgttgtgaatgtaaccagagcgatgaagagttcggtggtgta gtgcgtttgttgcaaaaagcgattcgcgccggagaaattttccaggtggtgccatctcgccgtttctctctgccct gcccgtcaccgctggcagcctattacgtgctgaaaaagagtaatcccagcccgtacatgttttttatgcaggata atgatttcaccctgtttggcgcgtcgccggaaagttcgctcaagtatgacgccaccagccgccagattgagattt acccgattgccggaacacgtccacgcggtcgtcgtgccgatggttcgctggacagagacctcgacagccgc atcgaactggagatgcgtaccgatcataaagagctttctgaacatctgatgctggtggatctcgcccgtaatgac ctggcacgcatttgcacacccggcagccgctacgtcgccgatctcaccaaagttgaccgttactcttacgtgat gcacctagtctcccgcgttgttggtgagctgcgccacgatctcgacgccctgcacgcttaccgcgcctgtatga atatggggacgttaagcggtgcaccgaaagtacgcgctatgcagttaattgccgaagcagaaggtcgtcgac gcggcagctacggcggcgcggtaggttattttaccgcgcatggcgatctcgacacctgcattgtgatccgctc ggcgctggtggaaaacggtatcgccaccgtgcaagccggtgctggcgtagtccttgattctgttccgcagtcg gaagccgacgaaactcgtaataaagcccgcgctgtactgcgcgctattgccaccgcgcatcatgcacaggag acgttcta |
| TrpE **SEQ ID NO: 75** | MQTQKPTLELLTCEGAYRDNPTALFHQLCGDRPATLLLESADIDSKD DLKSLLLVDSALRITALSDTVTIQALSGNGEALLTLLDNALPAGVENE QSPNCRVLRFPPVSPLLDEDARLCSLSVFDAFRLLQNLLNVPKEEREA MFFGGLFSYDLVAGFENLPQLSAENSCPDFCFYLAETLMVIDHQKKST RIQASLFAPNEEEKQRLTARLNELRQQLTEAAPPLPVVSVPHMRCECN QSDEEFGGVVRLLQKAIRAGEIFQVVPSRRFSLPCPSPLAAYYVLKKS NPSPYMFFMQDNDFTLFGASPESSLKYDATSRQIEIYPIAGTRPRGRRA DGSLDRDLDSRIELEMRTDHKELSEHLMLVDLARNDLARICTPGSRY VADLTKVDRYSYVMHLVSRVVGELRHDLDALHAYRACMNMGTLSG APKVRAMQLIAEAEGRRRGSYGGAVGYFTAHGDLDTCIVIRSALVEN GIATVQAGAGVVLDSVPQSEADETRNKARAVLRAIATAHHAQETF |
| trpD **SEQ ID NO: 76** | atggctgacattctgctgctcgataatatcgactcttttacgtacaacctggcagatcagttgcgcagcaatggtc ataacgtggtgatttaccgcaaccatattccggcgcagaccttaattgaacgcctggcgacgatgagcaatccg gtgctgatgctttctcctggccccggtgtgccgagcgaagccggttgtatgccggaactcctcacccgcttgcg tggcaagctgccaattattggcatttgcctcggacatcaggcgattgtcgaagcttacgggggctatgtcggtca |

| | |
|---|---|
| | ggcgggcgaaattcttcacggtaaagcgtcgagcattgaacatgacggtcaggcgatgtttgccggattaaca<br>aacccgctgccagtggcgcgttatcactcgctggttggcagtaacattccggccggtttaaccatcaacgccca<br>ttttaatggcatggtgatggcggtgcgtcacgatgcagatcgcgtttgtggattccagttccatccggaatccatt<br>cttactacccagggcgctcgcctgctggaacaaacgctggcctgggcgcagcagaaactagagccaaccaa<br>cacgctgcaaccgattctggaaaaactgtatcaggcacagacgcttagccaacaagaaagccaccagctgttt<br>tcagcggtggtacgtggcgagctgaagccggaacaactggcggcggcgctggtgagcatgaaaattcgcgg<br>tgaacacccgaacgagatcgccggggcagcaaccgcgctactggaaaacgccgcgccattcccgcgcccg<br>gattatctgtttgccgatatcgtcggtactggcggtgacggcagcaacagcatcaatatttctaccgccagtgcg<br>tttgtcgccgcggcctgcgggctgaaagtggcgaaacacggcaaccgtagcgtctccagtaaatccggctcg<br>tcggatctgctggcggcgttcggtattaatcttgatatgaacgccgataaatcgcgccaggcgctggatgagtta<br>ggcgtctgtttcctctttgcgccgaagtatcacaccggattccgccatgcgatgccggttcgccagcaactgaa<br>aacccgcactctgttcaacgtgctgggaccattgattaacccggcgcatccgccgctggcgctaattggtgttta<br>tagtccggaactggtgctgccgattgccgaaaccttgcgcgtgctggggtatcaacgcgcggcagtggtgca<br>cagcggcgggatggatgaagtttcattacacgcgccgacaatcgttgccgaactacatgacggcgaaattaag<br>agctatcaattgaccgctgaagattttggcctgacaccctaccaccaggagcaattggcaggcggaacaccgg<br>aagaaaaccgtgacattttaacacgcttgttacaaggtaaaggcgacgccgcccatgaagcagccgtcgcgg<br>cgaatgtcgccatgttaatgcgcctgcatggccatgaagatctgcaagccaatgcgcaaaccgttcttgaggta<br>ctgcgcagtggttccgcttacgacagagtcaccgcactggcggcacgagggtaa |
| TrpD<br>**SEQ ID NO: 77** | MADILLLDNIDSFTYNLADQLRSNGHNVVIYRNHIPAQTLIERLATMS<br>NPVLMLSPGPGVPSEAGCMPELLTRLRGKLPIIGICLGHQAIVEAYGG<br>YVGQAGEILHGKASSIEHDGQAMFAGLTNPLPVARYHSLVGSNIPAG<br>LTINAHFNGMVMAVRHDADRVCGFQFHPESILTTQGARLLEQTLAW<br>AQQKLEPTNTLQPILEKLYQAQTLSQQESHQLFSAVVRGELKPEQLAA<br>ALVSMKIRGEHPNEIAGAATALLENAAPFPRPDYLFADIVGTGGDGSN<br>SINISTASAFVAAACGLKVAKHGNRSVSSKSGSSDLLAAFGINLDMNA<br>DKSRQALDELGVCFLFAPKYHTGFRHAMPVRQQLKTRTLFNVLGPLI<br>NPAHPPLALIGVYSPELVLPIAETLRVLGYQRAAVVHSGGMDEVSLH<br>APTIVAELHDGEIKSYQLTAEDFGLTPYHQEQLAGGTPEENRDILTRLL<br>QGKGDAAHEAAVAANVAMLMRLHGHEDLQANAQTVLEVLRSGSA<br>YDRVTALAARG |
| trpC<br>**SEQ ID NO: 78** | atgcaaaccgttttagcgaaaatcgtcgcagacaaggcgatttgggtagaaacccgcaaagagcagcaaccg<br>ctggccagttttcagaatgaggttcagccgagcacgcgacatttttatgatgcacttcagggcgcacgcacggc<br>gtttattctggagtgtaaaaaagcgtcgccgtcaaaaggcgtgatccgtgatgatttcgatccggcacgcattgc<br>cgccatttataaacattacgcttcggcaatttcagtgctgactgatgagaaatattttcaggggagctttgatttcct<br>ccccatcgtcagccaaatcgccccgcagccgattttatgtaaagacttcattatcgatccttaccagatctatctg<br>gcgcgctattaccaggccgatgcctgcttattaatgctttcagtactggatgacgaacaatatcgccagcttgca<br>gccgtcgcccacagtctggagatgggtgtgctgaccgaagtcagtaatgaagaggaactggagcgcgccatt<br>gcattgggggcaaaggtcgttggcatcaacaaccgcgatctgcgcgatttgtcgattgatctcaaccgtacccg<br>cgagcttgcgccgaaactggggcacaacgtgacggtaatcagcgaatccggcatcaatacttacgctcaggt<br>gcgcgagttaagccacttcgctaacggctttctgattggttcggcgttgatggcccatgacgatttgaacgccgc<br>cgtgcgtcgggtgttgctgggtgagaataaagtatgtggcctgacacgtgggcaagatgctaaagcagcttat<br>gacgcgggcgcgatttacggtgggttgatttttgttgcgacatcaccgcgttgcgtcaacgttgaacaggcgca<br>ggaagtgatggctgcagcaccgttgcagtatgttggcgtgttccgcaatcacgatattgccgatgtggcggaca<br>aagctaaggtgttatcgctggcggcagtgcaactgcatggtaatgaagatcagctgtatatcgacaatctgcgt<br>gaggctctgccagcacacgtcgccatctggaaggctttaagtgtcggtgaaactcttcccgcgcgcgattttca<br>gcacatcgataaatatgtattcgacaacggtcagggcgggagcggacaacgtttcgactggtcactattaaatg<br>gtcaatcgcttggcaacgttctgctggcgggggggcttaggcgcagataactgcgtggaagcggcacaaaccg |

| | |
|---|---|
| | gctgcgccgggcttgattttaattctgctgtagagtcgcaaccgggtatcaaagacgcacgtcttttggcctcggtttccagacgctgcgcgcatattaa |
| TrpC **SEQ ID NO: 79** | MQTVLAKIVADKAIWVETRKEQQPLASFQNEVQPSTRHFYDALQGARTAFILECKKASPSKGVIRDDFDPARIAAIYKHYASAISVLTDEKYFQGSFDFLPIVSQIAPQPILCKDFIIDPYQIYLARYYQADACLLMLSVLDDEQYRQLAAVAHSLEMGVLTEVSNEEELERAIALGAKVVGINNRDLRDLSIDLNRTRELAPKLGHNVTVISESGINTYAQVRELSHFANGFLIGSALMAHDDLNAAVRRVLLGENKVCGLTRGQDAKAAYDAGAIYGGLIFVATSPRCVNVEQAQEVMAAAPLQYVGVFRNHDIADVADKAKVLSLAAVQLHGNEDQLYIDNLREALPAHVAIWKALSVGETLPARDFQHIDKYVFDNGQGGSGQRFDWSLLNGQSLGNVLLAGGLGADNCVEAAQTGCAGLDFNSAVESQPGIKDARLLASVFQTLRAY |
| trpB **SEQ ID NO: 80** | atgacaacattacttaacccctattttggtgagtttggcggcatgtacgtgccacaaatcctgatgcctgctctgccgccagctggaagaagcttttgtcagcgcgcaaaaagatcctgaatttcaggctcagttcaacgacctgctgaaaaactatgccgggcgtccaaccgcgctgaccaaatgccagaacattacagccgggacgaacaccacgctgtatctgaagcgcgaagatttgctgcacggcggcgcgcataaaactaaccaggtgctcggtcaggctttactggcgaagcggatgggtaaaactgaaattattgccgaaaccggtgccggtcagcatggcgtggcgtcggcccttgccagcgccctgctcggcctgaaatgccgaatttatatgggtgccaaagacgttgaacgccagtcgcccaacgttttccggatgcgcttaatgggtgcggaagtgatcccggtacatagcggttccgcgaccctgaaagatgcctgtaatgaggcgctacgcgactggtccggcagttatgaaaccgcgcactatatgctgggtaccgcagctggcccgcatccttacccgaccattgtgcgtgagtttcagcggatgattggcgaagaaacgaaagcgcagattctggaaagagaaggtcgcctgccggatgccgttatcgcctgtgttggcggtggttcgaatgccatcggtatgtttgcagatttcatcaacgaaaccgacgtcggcctgattggtgtggagcctggcggccacggtatcgaaactggcgagcacggcgcaccgttaaaacatggtcgcgtgggcatctatttcggtatgaaagcgccgatgatgcaaaccgaagacgggcaaattgaagagtcttactccatttctgccgggctggatttcccgtccgtcggcccgcaacatgcgtatctcaacagcactggacgcgctgattacgtgtctattaccgacgatgaagccctggaagcctttaaaacgctttgcctgcatgaagggatcatcccggcgctggaatcctcccacgccctggcccatgcgctgaaaatgatgcgcgaaaatccggaaaaagagcagctactggtggttaacctttccggtcgcggcgataaagacatcttcaccgttcacgatattttgaaagcacgaggggaaatctga |
| TrpB **SEQ ID NO: 81** | MTTLLNPYFGEFGGMYVPQILMPALRQLEEAFVSAQKDPEFQAQFNDLLKNYAGRPTALTKCQNITAGTNTTLYLKREDLLHGGAHKTNQVLGQALLAKRMGKTEIIAETGAGQHGVASALASALLGLKCRIYMGAKDVERQSPNVFRMRLMGAEVIPVHSGSATLKDACNEALRDWSGSYETAHYMLGTAAGPHPYPTIVREFQRMIGEETKAQILEREGRLPDAVIACVGGGSNAIGMFADFINETDVGLIGVEPGGHGIETGEHGAPLKHGRVGIYFGMKAPMMQTEDGQIEESYSISAGLDFPSVGPQHAYLNSTGRADYVSITDDEALEAFKTLCLHEGIIPALESSHALAHALKMMRENPEKEQLLVVNLSGRGDKDIFTVHDILKARGEI |
| trpA **SEQ ID NO: 82** | atggaacgctacgaatctctgtttgcccagttgaaggagcgcaaagaaggcgcattcgttcctttcgtcaccctcggtgatccgggcattgagcagtcgttgaaaattatcgatacgctaattgaagccggtgctgacgcgctggagttaggcatccccttctccgacccactggcggatggcccgacgattcaaaacgccacactgcgtgcttttgcggcgggagtaaccccggcgcagtgctttgagatgctggcactcattcgccagaagcacccgaccattcccatcggccttttgatgtatgccaacctggtgtttaacaaaggcattgatgagttttatgccgagtgcgagaaagtcggcgtcgattcggtgctggttgccgatgtgcccgtggaagagtccgcgcccttccgccaggccgcgttgcgtcataatgtcgcacctatctttatttgcccgccgaatgccgacgatgatttgctgcgccagatagcctcttacggtcgtggttacacctatttgctgtcgcgagcgggcgtgaccggcgcagaaaaccgcgccgcgttacccctcaatcatctggttgcgaagctgaaagagtacaacgctgcgcctccattgcagggatttggtatttccgccccggatcaggtaaaagccgcgattgatgcaggagctgcgggcgcgatttctggttcggccatcgttaaaatcatcgagcaacatattaatgagc |

| | |
|---|---|
| | cagagaaaatgctggcggcactgaaagcttttgtacaaccgatgaaagcggcgacgcgcagttaa |
| TrpA **SEQ ID NO: 83** | MERYESLFAQLKERKEGAFVPFVTLGDPGIEQSLKIIDTLIEAGADALE LGIPFSDPLADGPTIQNATLRAFAAGVTPAQCFEMLALIRQKHPTIPIGL LMYANLVFNKGIDEFYAECEKVGVDSVLVADVPVEESAPFRQAALR HNVAPIFICPPNADDDLLRQIASYGRGYTYLLSRAGVTGAENRAALPL NHLVAKLKEYNAAPPLQGFGISAPDQVKAAIDAGAAGAISGSAIVKII EQHINEPEKMLAALKAFVQPMKAATRS |

[0305] In some embodiments, the genetically engineered bacteria comprise one or more nucleic acid sequence of **Table 9** 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 one or more nucleic acid sequence of **Table 9** 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 one or more nucleic acid sequence of **Table 9** or a functional fragment thereof, or a nucleic acid sequence that, but for the redundancy of the genetic code, encodes the same polypeptide as one or more nucleic acid sequence of **Table 9** or a functional fragment thereof.

[0306] In one embodiment, one or more polypeptides and/or polynucleotides encoded and expressed by the genetically engineered bacteria have at least about 80% identity with one or more of **SEQ ID NO: 71 through SEQ ID NO: 83**. In one embodiment, one or more polypeptides and/or polynucleotides encoded and expressed by the genetically engineered bacteria have at least about 85% identity with one or more of **SEQ ID NO: 71 through SEQ ID NO: 83**. In one embodiment, one or more polypeptides and/or polynucleotides encoded and expressed by the genetically engineered bacteria have at least about 90% identity with one or more of **SEQ ID NO: 71 through SEQ ID NO: 83**. In one embodiment, one or more polypeptides and/or polynucleotides encoded and expressed by the genetically engineered bacteria have at least about 95% identity with one or more of **SEQ ID NO: 71 through SEQ ID NO: 83**. In one embodiment, one or more polypeptides and/or polynucleotides encoded and expressed by the genetically engineered bacteria have have at least about 96%, 97%, 98%, or 99% identity with one or more of **SEQ ID NO: 71 through SEQ ID NO: 83**. Accordingly, in one embodiment, one or more polypeptides and/or polynucleotides expressed by the genetically engineered bacteria have at least about 80%, 81%, 82%, 83%, 84%, 85%,

86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with one or more of **SEQ ID NO: 71 through SEQ ID NO: 83**. In another embodiment, one or more polynucleotides and/or polypeptides encoded and expressed by the genetically engineered bacteria comprise the sequence of one or more of **SEQ ID NO: 71 through SEQ ID NO: 83**. In another embodiment, one or more polynucleotides and/or polypeptides encoded and expressed by the genetically engineered bacteria consist of the sequence of one or more of **SEQ ID NO: 71 through SEQ ID NO: 83**.

[0307] **Table 10** depicts exemplary polypeptide sequences feedback resistant AroG and TrpE. **Table 10** also depicts an exemplary TnaA (tryptophanase from E. coli) sequence. IN some embodiments, the sequence is encoded in circuits for tryptophan catabolism to indole; in other embodimetns, the sequence is deleted from the E coli chromosome to increase levels of tryptophan.

**Table 10. Feedback resistant AroG and TrpE and tryptophanase sequences**

| **Description** | **Sequence** |
|---|---|
| AroGfbr: feedback resistant 2-dehydro-3-deoxyphosphoheptonate aldolase from E. coli<br><br>**SEQ ID NO: 84** | MNYQNDDLRIKEIKELLPPVALLEKFPATENAANTVAHARKAI HKILKGNDDRLLVVIGPCSIHDPVAAKEYATRLLTLREELQDE LEIVMRVYFEKPRTTVGWKGLINDPHMDNSFQINDGLRIARK LLLDINDSGLPAAGEFLDMITLQYLADLMSWGAIGARTTESQ VHRELASGLSCPVGFKNGTDGTIKVAIDAINAAGAPHCFLSVT KWGHSAIVNTSGNGDCHIILRGGKEPNYSAKHVAEVKEGLNK AGLPAQVMIDFSHANSSKQFKKQMDVCTDVCQQIAGGEKAII GVMVESHLVEGNQSLESGEPLAYGKSITDACIGWDDTDALLR QLASAVKARRG |
| TrpEfbr: feedback resistant anthranilate synthase component I from E. coli<br><br>**SEQ ID NO: 85** | MQTQKPTLELLTCEGAYRDNPTALFHQLCGDRPATLLLEFADI DSKDDLKSLLLVDSALRITALSDTVTIQALSGNGEALLTLLDN ALPAGVENEQSPNCRVLRFPPVSPLLDEDARLCSLSVFDAFRL LQNLLNVPKEEREAMFFGGLFSYDLVAGFENLPQLSAENSCP DFCFYLAETLMVIDHQKKSTRIQASLFAPNEEEKQRLTARLNE LRQQLTEAAPPLPVVSVPHMRCECNQSDEEFGGVVRLLQKAI RAGEIFQVVPSRRFSLPCPSPLAAYYVLKKSNPSPYMFFMQDN DFTLFGASPESSLKYDATSRQIEIYPIAGTRPRGRRADGSLDRD LDSRIELEMRTDHKELSEHLMLVDLARNDLARICTPGSRYVA DLTKVDRYSYVMHLVSRVVGELRHDLDALHAYRACMNMGT LSGAPKVRAMQLIAEAEGRRRGSYGGAVGYFTAHGDLDTCIV IRSALVENGIATVQAGAGVVLDSVPQSEADETRNKARAVLRA IATAHHAQETF |

| | |
|---|---|
| SerA: 2-oxoglutarate reductase from E. coli Nissle<br><br>**SEQ ID NO: 86** | MAKVSLEKDKIKFLLVEGVHQKALESLRAAGYTNIEFHKGAL<br>DDEQLKESIRDAHFIGLRSRTHLTEDVINAAEKLVAIGCFCIGT<br>NQVDLDAAAKRGIPVFNAPFSNTRSVAELVIGELLLLLRGVPE<br>ANAKAHRGVWNKLAAGSFEARGKKLGIIGYGHIGTQLGILAE<br>SLGMYVYFYDIENKLPLGNATQVQHLSDLLNMSDVVSLHVPE<br>NPSTKNMMGAKEISLMKPGSLLINASRGTVVDIPALCDALASK<br>HLAGAAIDVFPTEPATNSDPFTSPLCEFDNVLLTPHIGGSTQEA<br>QENIGLEVAGKLIKYSDNGSTLSAVNFPEVSLPLHGGRRLMHI<br>HENRPGVLTALNKIFAEQGVNIAAQYLQTSAQMGYVVIDIEA<br>DEDVAEKALQAMKAIPGTIRARLLY |
| SerAfbr: feedback resistant 2-oxoglutarate reductase from E. coli Nissle<br><br>**SEQ ID NO: 87** | MAKVSLEKDKIKFLLVEGVHQKALESLRAAGYTNIEFHKGAL<br>DDEQLKESIRDAHFIGLRSRTHLTEDVINAAEKLVAIGCFCIGT<br>NQVDLDAAAKRGIPVFNAPFSNTRSVAELVIGELLLLLRGVPE<br>ANAKAHRGVWNKLAAGSFEARGKKLGIIGYGHIGTQLGILAE<br>SLGMYVYFYDIENKLPLGNATQVQHLSDLLNMSDVVSLHVPE<br>NPSTKNMMGAKEISLMKPGSLLINASRGTVVDIPALCDALASK<br>HLAGAAIDVFPTEPATNSDPFTSPLCEFDNVLLTPHIGGSTQEA<br>QENIGLEVAGKLIKYSDNGSTLSAVNFPEVSLPLHGGRRLMHI<br>AEARPGVLTALNKIFAEQGVNIAAQYLQTSAQMGYVVIDIEA<br>DEDVAEKALQAMKAIPGTIRARLLY |
| TnaA: tryptophanase from E. coli<br><br>**SEQ ID NO: 88** | MENFKHLPEPFRIRVIEPVKRTTRAYREEAIIKSGMNPFLLDSE<br>DVFIDLLTDSGTGAVTQSMQAAMMRGDEAYSGSRSYYALAE<br>SVKNIFGYQYTIPTHQGRGAEQIYIPVLIKKREQEKGLDRSKM<br>VAFSNYFFDTTQGHSQINGCTVRNVYIKEAFDTGVRYDFKGN<br>FDLEGLERGIEEVGPNNVPYIVATITSNSAGGQPVSLANLKVM<br>YSIAKKYDIPVVMDSARFAENAYFIKQREAEYKDWTIEQITRE<br>TYKYADMLAMSAKKDAMVPMGGLLCMKDDSFFDVYTECRT<br>LCVVQEGFPTYGGLEGGAMERLAVGLYDGMNLDWLAYRIA<br>QVQYLVDGLEEIGVVCQQAGGHAAFVDAGKLLPHIPADQFPA<br>QALACELYKVAGIRAVEIGSFLLGRDPKTGKQLPCPAELLRLTI<br>PRATYTQTHMDFIIEAFKHVKENAANIKGLTFTYEPKVLRHFT<br>AKLKEV |

[0308] In one embodiment, one or more polypeptides and/or polynucleotides encoded and expressed by the genetically engineered bacteria have at least about 80% identity with one or more of **SEQ ID NO: 84 through SEQ ID NO: 87**. In one embodiment, one or more polypeptides and/or polynucleotides encoded and expressed by the genetically engineered bacteria have at least about 85% identity with one or more of **SEQ ID NO: 84 through SEQ ID NO: 87**. In one embodiment, one or more polypeptides and/or polynucleotides encoded and expressed by the genetically engineered bacteria have at least about 90% identity with one or more of **SEQ ID NO: 84 through SEQ ID NO: 87**. In one embodiment, one or more polypeptides and/or polynucleotides

encoded and expressed by the genetically engineered bacteria have at least about 95% identity with one or more of **SEQ ID NO: 84 through SEQ ID NO: 87**. In one embodiment, one or more polypeptides and/or polynucleotides encoded and expressed by the genetically engineered bacteria have have at least about 96%, 97%, 98%, or 99% identity with one or more of **SEQ ID NO: 84 through SEQ ID NO: 87**. Accordingly, in one embodiment, one or more polypeptides and/or polynucleotides expressed by the genetically engineered bacteria have at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with one or more of **SEQ ID NO: 84 through SEQ ID NO: 87**. In another embodiment, one or more polynucleotides and/or polypeptides encoded and expressed by the genetically engineered bacteria comprise the sequence of one or more of **SEQ ID NO: 84 through SEQ ID NO: 87**. In another embodiment, one or more polynucleotides and/or polypeptides encoded and expressed by the genetically engineered bacteria consist of the sequence of one or more of **SEQ ID NO: 84 through SEQ ID NO: 87**.

[0309] **Table 11** lists exemplary genes encoding kynureninase which are encoded by the genetically engineered bacteria of the disclosure in certain embodiments.

**Table 11. Kynureninase protein sequences**

| Description | ID | Sequence |
|---|---|---|
| Pseudomonas kynureninase **SEQ ID NO: 89** | P83788 | MTTRNDCLALDAQDSLAPLRQQFALPEGVIYLDGNS LGARPVAALARAQAVIAEEWGNGLIRSWNSAGWRD LSERLGNRLATLIGARDGEVVVTDTTSINLFKVLSAA LRVQATRSPERRVIVTETSNFPTDLYIAEGLADMLQQ GYTLRLVDSPEELPQAIDQDTAVVMLTHVNYKTGYM HDMQALTALSHECGALAIWDLAHSAGAVPVDLHQA GADYAIGCTYKYLNGGPGSQAFVWVSPQLCDLVPQP LSGWFGHSRQFAMEPRYEPSNGIARYLCGTQPITSLA MVECGLDVFAQTDMASLRRKSLALTDLFIELVEQRC AAHELTLVTPREHAKRGSHVSFEHPEGYAVIQALIDR GVIGDYREPRIMRFGFTPLYTTFTEVWDAVQILGEILD RKTWAQAQFQVRHSVT* |
| Human **SEQ ID NO: 90** | Q16719 | MEPSSLELPADTVQRIAAELKCHPTDERVALHLDEED KLRHFRECFYIPKIQDLPPVDLSLVNKDENAIYFLGNS LGLQPKMVKTYLEEELDKWAKIAAYGHEVGKRPWI TGDESIVGLMKDIVGANEKEIALMNALTVNLHLLML SFFKPTPKRYKILLEAKAFPSDHYAIESQLQLHGLNIE ESMRMIKPREGEETLRIEDILEVIEKEGDSIAVILFSGV HFYTGQHFNIPAITKAGQAKGCYVGFDLAHAVGNVE LYLHDWGVDFACWCSYKYLNAGAGGIAGAFIHEKH AHTIKPALVGWFGHELSTRFKMDNKLQLIPGVCGFRI SNPPILLVCSLHASLEIFKQATMKALRKKSVLLTGYLE |

| | | |
|---|---|---|
| | | YLIKHNYGKDKAATKKPVVNIITPSHVEERGCQLTITF SVPNKDVFQELEKRGVVCDKRNPNGIRVAPVPLYNS FHDVYKFTNLLTSILDSAETKN* |
| Shewanella **SEQ ID NO: 91** | Q8E973 | MLLNVKQDFCLAGPGYLLNHSVGRPLKSTEQALKQA FFAPWQESGREPWGQWLGVIDNFTAALASLFNGQPQ DFCPQVNLSSALTKIVMSLDRLTRDLTRNGGAVVLM SEIDFPSMGFALKKALPASCELRFIPKSLDVTDPNVW DAHICDDVDLVFVSHAYSNTGQQAPLAQIISLARERG CLSLVDVAQSAGILPLDLAKLQPDFMIGSSVKWLCSG PGAAYLWVNPAILPECQPQDVGWFSHENPFEFDIHDF RYHPTALRFWGGTPSIAPYAIAAHSIEYFANIGSQVM REHNLQLMEPVVQALDNELVSPQEVDKRSGTIILQFG ERQPQILAALAAANISVDTRSLGIRVSPHIYNDEADIA RLLGVIKANR* |

* designates the position of the stop codon

[0310] In one embodiment, one or more polypeptides and/or polynucleotides encoded and expressed by the genetically engineered bacteria have at least about 80% identity with one or more of SEQ ID NO: 89 through SEQ ID NO: 91. In one embodiment, one or more polypeptides and/or polynucleotides encoded and expressed by the genetically engineered bacteria have at least about 85% identity with one or more of SEQ ID NO: 89 through SEQ ID NO: 91. In one embodiment, one or more polypeptides and/or polynucleotides encoded and expressed by the genetically engineered bacteria have at least about 90% identity with one or more of SEQ ID NO: 89 through SEQ ID NO: 91. In one embodiment, one or more polypeptides and/or polynucleotides encoded and expressed by the genetically engineered bacteria have at least about 95% identity with one or more of SEQ ID NO: 89 through SEQ ID NO: 91. In one embodiment, one or more polypeptides and/or polynucleotides encoded and expressed by the genetically engineered bacteria have have at least about 96%, 97%, 98%, or 99% identity with one or more of SEQ ID NO: 89 through SEQ ID NO: 91. Accordingly, in one embodiment, one or more polypeptides and/or polynucleotides expressed by the genetically engineered bacteria have at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with one or more of SEQ ID NO: 89 through SEQ ID NO: 91. In another embodiment, one or more polynucleotides and/or polypeptides encoded and expressed by the genetically engineered bacteria comprise the sequence of one or more of SEQ ID NO: 89 through SEQ ID NO: 91. In another embodiment, one or more polynucleotides and/or polypeptides encoded and expressed by

the genetically engineered bacteria consist of the sequence of one or more of SEQ ID NO: 89 through SEQ ID NO: 91.

[0311] **Table 12** lists exemplary codon-optimized kynureninase cassette sequences.

**Table 12. Selected codon-optimized kynureninase cassette sequences**

| **Kynureninase protein sequences** | **Kynureninase protein sequences** |
|---|---|
| Ptet-kynU(Pseudomonas)<br>**SEQ ID NO: 92** | **atctaatctagacatcattaattcctaattttgttgacactctatcattgatagagttatttta ccactccctatcagtgatagagaaaagtgaa**ttatataaaagtgggaggtgcccgaatgacg acccgaaatgattgcctagcgttggatgcacaggacagtctggctccgctgcgccaacaatttg cgctgccggagggtgtgatatacctggatggcaattcgctgggcgcacgtccggtagctgcgc tggctcgcgcgcaggctgtgatcgcagaagaatggggcaacgggttgatccgttcatggaact ctgcgggctggcgtgatctgtctgaacgcctgggtaatcgcctggctaccctgattggtgcgcg cgatggggaagtagttgttactgataccacctcgattaatctgtttaaagtgctgtcagcggcgct gcgcgtgcaagctacccgtagcccggagcgccgtgttatcgtgactgagacctcgaatttcccg accgacctgtatattgcggaagggttggcggatatgctgcaacaaggttacactctgcgtttggt ggattcaccggaagagctgccacaggctatagatcaggacaccgcggtggtgatgctgacgc acgtaaattataaaaccggttatatgcacgacatgcaggctctgaccgcgttgagccacgagtgt ggggctctggcgatttgggatctggcgcactctgctggcgctgtgccggtggacctgcaccaa gcgggcgcggactatgcgattggctgcacgtacaaatacctgaatggcggcccgggttcgcaa gcgtttgtttgggtttcgccgcaactgtgcgacctggtaccgcagccgctgtctggttggttcggc catagtcgccaattcgcgatggagccgcgctacgaaccttctaacggcattgctcgctatctgtg cggcactcagcctattactagcttggctatggtggagtgcggcctggatgtgtttgcgcagacgg atatggcttcgctgcgccgtaaaagtctggcgctgactgatctgttcatcgagctggttgaacaac gctgcgctgcacacgaactgaccctggttactccacgtgaacacgcgaaacgcggctctcacg tgtcttttgaacaccccgagggttacgctgttattcaagctctgattgatcgtggcgtgatcggcga ttaccgtgagccacgtattatgcgtttcggtttcactcctctgtatactacttttacggaagtttggga tgcagtacaaatcctgggcgaaatcctggatcgtaagacttgggcgcaggctcagtttcaggtg cgccactctgttacttaa*aaataaaacgaaaggctcagtcgaaagactgggcctttcgttttat ctgttg*

| | |
|---|---|
| Ptet-kynU(Human)<br>**SEQ ID NO: 93** | **atctaatctagacatcattaattcctaattttgttgacactctatcattgatagagttatttta ccactccctatcagtgatagagaaaagtgaa**tatcaagacacgaggaggtaagattatgga gccttcatctttagaactgccagcggacacggtgcagcgcatcgcggcggaactgaagtgcca tccgactgatgagcgtgtggcgctgcatctggacgaagaagataaactgcgccactttcgtgaa tgtttttatattcctaaaattcaagacttgccgccggtagatttgagtctcgttaacaaagatgaaaa cgcgatctactttctgggcaactctctgggtctgcaaccaaaaatggttaaaacgtacctggagg aagaactggataaatgggcaaaaatcgcggcttatggtcacgaagtgggcaagcgtccttggat tactggcgacgagtctattgtgggtttgatgaaagatattgtgggcgcgaatgaaaaggaaattg cactgatgaatgctctgaccgttaatctgcacctgctgatgctgtctttttttaaaccgaccccgaaa cgctacaaaatactgctggaagcgaaagcgtttccgtcggatcactatgctatagaaagtcaact gcagttgcatggtctgaatatcgaggaatctatgcgcatgattaaaccgcgtgagggtgaagaa acgctgcgtattgaagacattctggaagttattgaaaaagaaggtgattctatcgcagttatactgt ttctggcgtgcacttttatacaggtcagcacttcaatatcccggcaatcactaaagcggggcagg caaaaggctgctatgttggttttgacctggcgcatgcagtggggaatgttgaactgtatctgcacg attggggcgttgatttcgcgtgttggtgtagctacaaatatctgaacgctggcgcgggtggcattg ctggcgcttttattcacgaaaaacacgcgcacaccattaaaccggctctggttggctggttcggtc atgagctgagtactcgctttaaaatggataacaaactgcaattgattccgggtgtttgcggcttccg tatcagcaatccgccgattctgctggtttgcagcctgcacgctagtctggaaatctttaagcaggc gactatgaaagcgctgcgcaaaaaatctgtgctgctgaccggctatctggagtatctgatcaaac acaattatggcaaagataaagctgcaactaaaaaaccggtagtgaacattatcacccccctcacac gtggaggagcgcggttgtcagctgactattactttcagtgtacctaataaagatgtgttccaggaa ctggaaaaacgcggcgttgtttgtgataaacgtaacccgaatggtattcgcgtggctcctgtgcc gctgtacaattcattccacgatgtttataaattcaccaacctgctgacttctattctcgacagtgctga gactaaaaattaa*aaataaaacgaaaggctcagtcgaaagactgggcctttcgttttatctgtt g* |
| ptet-kynU(Shewanella)<br>**SEQ ID NO: 94** | **atctaatctagacatcattaattcctaattttgttgacactctatcattgatagagttatttta ccactccctatcagtgatagagaaaagtgaa**tggttcaccaccacaaggagggattatgctg ctgaatgtaaaacaggacttttgcctggcaggcccgggctacctgctgaatcactcggttggcc gtccgctgaaatcaactgagcaagcgctgaaacaagcatttttgctccgtggcaagagagcgg tcgtgaaccgtggggccagtggctgggtgttattgataatttcactgctgcgctggcatctctgttt aatggtcaaccgcaggatttttgtccgcaggttaacctgagcagcgcgctgactaaaattgtgat gtcactggatcgtctgactcgcgatctgacccgcaatggcggtgctgttgtgctgatgtctgaaat cgatttcccatctatgggcttcgcgttgaaaaaagcgctgccagcgagctgcgaactgcgttttat cccgaaaagtctggacgtgactgatccgaacgtatgggatgcacacatctgtgatgatgtagac ctggtttttgtgtctcacgcctatagtaatacgggccaacaggctccgctggcgcaaatcatctct ctggcgcgtgaacgtggctgcctgtcactggtggatgtagcgcaatcagcggggattttgccgc tggatctggcgaaactgcaaccggacttcatgatcggcagttcggttaaatggctgtgctcgggc cctggtgcggcatatctgtgggttaatccggcgattctgccggaatgtcagccgcaggatgtgg gctggttttcacatgagaatccctttgaattcgacatccacgatttccgctaccacccgactgcact gcgcttttggggtggtacgccgtcgatcgcgccttatgcgatcgcggcgcactcgatcgaatatt ttgccaatatcggctcgcaagtgatgcgtgaacacaacctgcaactgatggaaccggtggttca ggcgctggacaatgaactggtgagcccgcaggaagtggataaacgctcaggcactattattctg caattcggtgaacgtcaaccgcaaattctggcggctctggctgcggcgaacatttcggtggaca ctcgttctttggggattcgtgttagtccgcacatttataatgatgaggcggacattgcgcgcctgct gggtgtgatcaaagcaaatcgctaaaaataaaacgaaaggctcagtcgaaagactgggcctttc gttttatctgttg |

The ptet-promoter is in bold, designed Ribosome binding site is underlined, codon-optimized protein coding sequence is in plain text, and the terminator is in italics.

[0312] In some embodiments, the genetically engineered bacteria comprise one or more nucleic acid sequence of **Table 12** 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 one or more nucleic acid sequence of **Table 12** 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 one or more nucleic acid sequence of **Table 12** or a functional fragment thereof, or a nucleic acid sequence that, but for the redundancy of the genetic code, encodes the same polypeptide as one or more nucleic acid sequence of **Table 12** or a functional fragment thereof.

[0313] In one embodiment, one or more polynucleotides encoded and expressed by the genetically engineered bacteria have at least about 80% identity with one or more of **SEQ ID NO: 92 through SEQ ID NO: 94**. In one embodiment, one or more polynucleotides encoded and expressed by the genetically engineered bacteria have at least about 85% identity with one or more of **SEQ ID NO: 92 through SEQ ID NO: 94**. In one embodiment, one or more polynucleotides encoded and expressed by the genetically engineered bacteria have at least about 90% identity with one or more of **SEQ ID NO: 92 through SEQ ID NO: 94**. In one embodiment, one or more polynucleotides encoded and expressed by the genetically engineered bacteria have at least about 95% identity with one or more of **SEQ ID NO: 92 through SEQ ID NO: 94**. In one embodiment, one or more polynucleotides encoded and expressed by the genetically engineered bacteria have have at least about 96%, 97%, 98%, or 99% identity with one or more of **SEQ ID NO: 92 through SEQ ID NO: 94**. Accordingly, in one embodiment, one or more polynucleotides expressed by the genetically engineered bacteria have at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with one or more of **SEQ ID NO: 92 through SEQ ID NO: 94**. In another embodiment, one or more polynucleotides encoded and expressed by the genetically engineered bacteria comprise the sequence of one or more of **SEQ ID NO: 92 through SEQ ID NO: 94**. In another embodiment, one or more polynucleotides encoded and

expressed by the genetically engineered bacteria consists of the sequence of one or more of **SEQ ID NO: 92 through SEQ ID NO: 94**.

[0314] The genetically engineered bacteria may comprise any suitable gene for producing kynureninase. In some embodiments, the gene for producing kynureninase is modified and/or mutated, *e.g.*, to enhance stability, increase kynureninase production. In some embodiments, the engineered bacteria also 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, as discussed in detail above. In some embodiments, the genetically engineered bacteria are capable of producing kynureninase under inducing conditions, *e.g.*, under a condition(s) associated with inflammation. In some embodiments, the genetically engineered bacteria are capable of producing kynureninase in low-oxygen conditions, in the presence of certain molecules or metabolites, in the presence of molecules or metabolites associated with inflammation or an inflammatory response, or in the presence of some other metabolite that may or may not be present in the gut, such as arabinose.

[0315] The genetically engineered bacteria may comprise any suitable gene for producing kynureninase. In some embodiments, the gene for producing kynureninase is modified and/or mutated, *e.g.*, to enhance stability, increase kynureninase production. In some embodiments, the engineered bacteria also 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, as discussed in detail above. In some embodiments, the genetically engineered bacteria are capable of producing kynureninase under inducing conditions, *e.g.*, under a condition(s) associated with inflammation. In some embodiments, the genetically engineered bacteria are capable of producing kynureninase in low-oxygen conditions. In some embodiments, the genetically engineered bacteria are capable of producing kynureninase in the presence of certain molecules or metabolites, in the presence of molecules or metabolites associated with inflammation or an inflammatory response, or in the presence of some other metabolite that may or may not be present in the gut, such as arabinose.

*Producing Kynurenic Acid*

[0316] 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 or genes for producing kynurenic acid. In some embodiments, the engineered bacteria comprise gene sequence(s) encoding one or more kynurenine-oxoglutarate transaminases (also referred to as kynurenine aminotransferases (*e.g.*, KAT I, II, III)).

[0317] In some embodiments, the gene or genes for producing kynurenic acid is modified and/or mutated, *e.g.*, to enhance stability, increase kynurenic acid production 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, in the presence of certain molecules or metabolites, in the presence of molecules or metabolites associated with inflammation or an inflammatory response, or in the presence of some other metabolite that may or may not be present in the gut, such as arabinose.

[0318] In some embodiments, the genetically engineered bacteria comprise one or more gene(s) or gene cassette(s) for the consumption of tryptophan and production of kynurenic acid, which are bacterially derived. In some embodiments, the enzymes for producing kynureic acid are derived from one or more of Pseudomonas, Xanthomonas, Burkholderia, Stenotrophomonas, Shewanella, and Bacillus, and/or members of the families Rhodobacteraceae, Micrococcaceae, and Halomonadaceae, In some embodiments the enzymes are derived from the species listed in table S7 of Vujkovic-Cvijin *et al.* (Dysbiosis of the gut microbiota is associated with HIV diseaseprogression and tryptophan

catabolism Sci Transl Med. 2013 July 10; 5(193): 193ra91), the contents of which is herein incorporated by reference in its entirety.

[0319] In some embodiments, the genetically engineered bacteria comprise gene sequence(s) encoding one or more tryptophan transporters and gene sequence(s) encoding kynureninase. In some embodiments, the genetically engineered bacteria comprise gene sequence(s) encoding one or more tryptophan transporters and gene sequence(s) encoding one or more kynurenine-oxoglutarate transaminases (kynurenine aminotransferases). In some embodiments, the genetically engineered bacteria comprise gene sequence(s) encoding one or more tryptophan transporters, gene sequence(s) encoding kynureninase, and gene sequence(s) encoding one or more kynurenine-oxoglutarate transaminases (kynurenine aminotransferases). In some embodiments, the genetically engineered bacteria comprise gene sequence(s) encoding kynureninase and gene sequence(s) encoding one or more kynurenine aminotransferases.

[0320] In some embodiments, the one or more genes for producing kynurenic acid are modified and/or mutated, *e.g.*, to enhance stability, increase kynurenic acid production 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 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, in the presence of certain molecules or metabolites , in the presence of molecules or metabolites associated with inflammation or an inflammatory response, or in the presence of some other metabolite that may or may not be present in the gut, such as arabinose.

[0321] In some embodiments, the genetically engineered bacteria are capable of expressing any one or more of the described circuits in low-oxygen conditions, in the presence of disease or tissue specific molecules or metabolites, in the presence of molecules or metabolites associated with inflammation or an inflammatory response or immune suppression or in the presence of some other metabolite that may or may not be present in the gut, such as arabinose. In some embodiments, any one or more of the described circuits are present on one or more plasmids (*e.g.*, high copy or low copy) or are

integrated into one or more sites in the bacterial chromosome. Also, in some embodiments, the genetically engineered bacteria are further capable of expressing any one or more of the described circuits and further comprise one or more of the following: (1) one or more auxotrophies, such as any auxotrophies known in the art and provided herein, *e.g.*, thyA auxotrophy, (2) one or more kill switch circuits, such as any of the kill-switches described herein or otherwise known in the art, (3) one or more antibiotic resistance circuits, (4) one or more transporters for importing biological molecules or substrates, such any of the transporters described herein or otherwise known in the art, (5) one or more secretion circuits, such as any of the secretion circuits described herein and otherwise known in the art, and (6) combinations of one or more of such additional circuits.

*Producing Indole Tryptophan Metabolites and Tryptamine*

**Tryptamine**

[0322] In some embodiments the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more tryptophan catabolism enzymes, produce tryptamine from tryptophan. The monoamine alkaloid, tryptamine, is derived from the direct decarboxylation of tryptophan. Tryptophan is converted to indole-3-acetic acid (IAA) via the enzymes tryptophan monooxygenase (*IaaM*) and indole-3- acetamide hydrolase (*IaaH*), which constitute the indole-3-acetamide (IAM) pathway, see eg., **FIG. 36B, FIG. 37A and FIG. 37B**.

[0323] A non-limiting example of such as strain is shown in **FIG. 41.** In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more Tryptophan decarboxylase(s). e.g., from *Catharanthus roseus*. In one embodiment the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more Tryptophan decarboxylase(s). e.g., from *Catharanthus roseus*. In one embodiment the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more Tryptophan decarboxylase(s) e.g., from Ruminococcus Gnavus.

Another non-limiting example of such as strain is shown in **FIG. 45C.** In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode tdc from Catharanthus roseus.

[0324] In some embodiments, the genetically engineered bacteria which produce tryptamine from tryptophan also optionally comprise one or more gene sequence(s) comprising one or more enzymes for tryptophan production, and gene deletions/or mutations as depicted and described in **FIG. 39, FIG. 45A and/or FIG. 45B** and described elsewhere herein. In some embodiments, the genetically engineered bacteria which produce tryptamine from tryptophan also optionally comprise one or more gene sequence(s) which encode one or more transporter(s) as described herein, through which tryptophan can be imported. Optionally, In some embodiments, the genetically engineered bacteria which produce tryptamine from tryptophan also optionally comprise one or more gene sequence(s) which encode an exporter as described herein, which can export tryptophan or any of its metabolites.

**Indole-3-acetaldehyde and FICZ**

In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more tryptophan catabolism enzymes, which produce indole-3-acetaldehyde and FICZ from tryptophan. Exemplary gene cassettes for the production of produce indole-3-acetaldehyde and FICZ from tryptophan are shown in **FIG. 41B**.

In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode aro9 ( L-tryptophan aminotransferase). In one embodiment, the (L-tryptophan aminotransferase is from S. cerevisiae. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode ipdC (Indole-3-pyruvate decarboxylase, e.g., from Enterobacter cloacae). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode aro9 and ipdC. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode aspC (aspartate aminotransferase. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode aspC from E. coli. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode aspC and ipdC. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode taa1 (L-tryptophan-pyruvate aminotransferase, In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode taa1 from Arabidopsis thaliana. In one embodiment, the genetically engineered bacteria comprise one or more

gene sequence(s) which encode taa1 and ipdC. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode staO (L-tryptophan oxidase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode staO from streptomyces sp. TP-A0274. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode staO and ipdC. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode trpDH (Tryptophan dehydrogenase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode trpDH from Nostoc punctiforme NIES-2108. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode trpDH and ipdC. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more of aro9 or aspC or taa1 or staO or trpDH. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more of aro9 or aspC or taa1 or staO or trpDH and ipdC.

Further exemplary gene cassettes for the production of produce indole-3-acetaldehyde and FICZ from tryptophan are shown in **FIG. 41C.** In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode tdc (Tryptophan decarboxylase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode tdc from Catharanthus roseus. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode tynA (Monoamine oxidase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode tynA from E. coli. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode tdc and tynA.

In any of these embodiments, the genetically engineered bacteria which produce produce indole-3-acetaldehyde and FICZ also optionally comprise one or more gene sequence(s) comprising one or more enzymes for tryptophan production, and gene deletions/or mutations as depicted and described in **FIG. 39, FIG. 45A and/or FIG. 45B** and described elsewhere herein. In some embodiments, the genetically engineered bacteria which produce indole-3-acetaldehyde and FICZ also optionally comprise one or more gene sequence(s) which encode one or more transporter(s) as described herein, through

which tryptophan can be imported. Optionally, in some embodiments, the genetically engineered bacteria which produce indole-3-acetaldehyde and FICZ also optionally comprise one or more gene sequence(s) which encode an exporter as described herein, which can export tryptophan or any of its metabolites.

**Indole-3-acetonitrile**

In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more tryptophan catabolism enzymes, which produce indole-3-acetonitrile from tryptophan. A non-limiting example of such gene sequence(s) which allow in which the genetically engineered bacteria to produce indole-3-acetonitrile from tryptophan is depicted in **FIG. 41D.**

In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp79B2 (tryptophan N-monooxygenase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp79B2 from Arabidopsis thaliana. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp71a13 (indoleacetaldoxime dehydratase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp71a13 from Arabidopis thaliana. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp79B2 and cyp71a13.

In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp79B3 (tryptophan N-monooxygenase) In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp79B3 from Arabidopsis thaliana. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp79B3 and cyp71a13. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp79B3, cyp79B2 and cyp71a13.

In any of these embodiments, the genetically engineered bacteria which produce indole-3-acetonitrile from tryptophan also optionally comprise one or more gene sequence(s) comprising one or more enzymes for tryptophan production, and gene deletions/or mutations as depicted and described in **FIG. 39, FIG. 45A and/or FIG. 45B** and described elsewhere herein. In some embodiments, the genetically engineered bacteria

which produce indole-3-acetonitrile from tryptophan also optionally comprise one or more gene sequence(s) which encode one or more transporter(s) as described herein, through which tryptophan can be imported. Optionally, in some embodiments, the genetically engineered bacteria which produce indole-3-acetonitrile from tryptophan also optionally comprise one or more gene sequence(s) which encode an exporter as described herein, which can export tryptophan or any of its metabolites.

**Kynurenine**

In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more tryptophan catabolism enzymes, which produce kynurenine from tryptophan. Non-limiting example of such gene sequence(s) are shown **FIG. 41E** and described elsewhere herein. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode IDO1(indoleamine 2,3-dioxygenase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode IDO1 from homo sapiens. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode TDO2 (tryptophan 2,3-dioxygenase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode TDO2 from homo sapiens. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode BNA2 (indoleamine 2,3-dioxygenase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode BNA2 from S. cerevisiae). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode Afmid: Kynurenine formamidase. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode Afmid: Kynurenine formamidase from mouse. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode Afmid in combination with one or more of ido1 and/or tdo2 and/or bna2. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode Afmid in combination with ido1. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode BNA2 in combination with tdo2. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode Afmid in combination with bna2.In

one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode BNA3 (kynurenine--oxoglutarate transaminase. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode BNA3 from S. cerevisae. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode BNA2 in combination with one or more of ido1 and/or tdo2 and/or bna2. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode BNA2 in combination with ido1. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode BNA2 in combination with tdo2. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode BNA2 in combination with bna2.In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more of ido1 and/or tdo2 and/or bna2.In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more of afmid and/or bna3. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more of ido1 and/or tdo2 and/or bna2, in combination with one or more of afmid and/or bna3.

In any of these embodiments, the genetically engineered bacteria which produce kynurenine from tryptophan also optionally comprise one or more gene sequence(s) comprising one or more enzymes for tryptophan production, and gene deletions/or mutations as depicted and described in **FIG. 39, FIG. 45A and/or FIG. 45B** and described elsewhere herein. In some embodiments, the genetically engineered bacteria which produce kynurenine from tryptophan also optionally comprise one or more gene sequence(s) which encode one or more transporter(s) as described herein, through which tryptophan can be imported. Optionally, in some embodiments, the genetically engineered bacteria which produce kynurenine from tryptophan also optionally comprise one or more gene sequence(s) which encode an exporter as described herein, which can export tryptophan or any of its metabolites.

**Kynureninic acid**

In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more tryptophan catabolism enzymes, which produce

kynureninic acid from tryptophan. Non-limiting example of such gene sequence(s) are shown **FIG. 41F** and described elsewhere herein. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode IDO1(indoleamine 2,3-dioxygenase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode IDO1 from homo sapiens. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode TDO2 (tryptophan 2,3-dioxygenase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode TDO2 from homo sapiens. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode BNA2 (indoleamine 2,3-dioxygenase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode BNA2 from S. cerevisiae). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode Afmid: Kynurenine formamidase. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode Afmid: Kynurenine formamidase from mouse. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode Afmid in combination with one or more of ido1 and/or tdo2 and/or bna2. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode Afmid in combination with ido1. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode BNA2 in combination with tdo2. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode Afmid in combination with bna2. In one embodiment, the genetically engineered bacteria further comprise one or more gene sequence(s) which encode cclb1 and/or cclb2 and/or aadat and/or got2.In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode BNA3 (kynurenine--oxoglutarate transaminase. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode BNA3 from S. cerevisae. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode BNA2 in combination with one or more of ido1 and/or tdo2 and/or bna2. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode BNA2 in combination with ido1. In one embodiment, the genetically engineered bacteria comprise

one or more gene sequence(s) which encode BNA2 in combination with tdo2. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode BNA2 in combination with bna2. In one embodiment, the genetically engineered bacteria further comprise one or more gene sequence(s) which encode cclb1 and/or cclb2 and/or aadat and/or got2.In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more of ido1 and/or tdo2 and/or bna2.

In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more of afmid and/or bna3.In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more of ido1 and/or tdo2 and/or bna2, in combination with one or more of afmid and/or bna3.In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode GOT2 (Aspartate aminotransferase, mitochondrial). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode GOT2 from homo sapiens.In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode AADAT (Kynurenine/alpha-aminoadipate aminotransferase, mitochondrial). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode AADAT from homo sapiens. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode CCLB1 (Kynurenine--oxoglutarate transaminase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode CCLB1 from homo sapiens). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode CCLB2 (kynurenine--oxoglutarate transaminase 3) In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode CCLB2 from homo sapiens.In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cclb1 and/or cclb2 and/or aadat and/or got2.In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more of ido1 and/or tdo2 and/or bna2, in combination with one or more of afmid and/or bna3, and in combination with one or more of . cclb1 and/or cclb2 and/or aadat and/or got2.

In any of these embodiments, the genetically engineered bacteria which produce kynurenic acid from tryptophan also optionally comprise one or more gene sequence(s) comprising one or more enzymes for tryptophan production, and gene deletions/or mutations as depicted and described in **FIG. 39, FIG. 45A and/or FIG. 45B** and described elsewhere herein. In some embodiments, the genetically engineered bacteria which produce kynurenic acid from tryptophan also optionally comprise one or more gene sequence(s) which encode one or more transporter(s) as described herein, through which tryptophan can be imported. Optionally, in some embodiments, the genetically engineered bacteria which produce kynurenic acid from tryptophan also optionally comprise one or more gene sequence(s) which encode an exporter as described herein, which can export tryptophan or any of its metabolites.

**Indole**

In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more tryptophan catabolism enzymes, which produce indole from tryptophan. Non-limiting example of such gene sequence(s) are shown **FIG. 41G** and described elsewhere herein. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode tnaA (tryptophanase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode tnaA from E. coli.

In any of these embodiments, the genetically engineered bacteria which produce indole from tryptophan also optionally comprise one or more gene sequence(s) comprising one or more enzymes for tryptophan production, and gene deletions/or mutations as depicted and described in **FIG. 39, FIG. 45A and/or FIG. 45B** and described elsewhere herein. In some embodiments, the genetically engineered bacteria which produce indole from tryptophan also optionally comprise one or more gene sequence(s) which encode one or more transporter(s) as described herein, through which tryptophan can be imported. Optionally, in some embodiments, the genetically engineered bacteria which produce indole from tryptophan also optionally comprise one or more gene sequence(s) which encode an exporter as described herein, which can export tryptophan or any of its metabolites.

**Other indole metabolites**

In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more tryptophan catabolism enzymes, which produce indole-3-carbinol, indole-3-aldehyde, 3,3' diindolylmethane (DIM), indolo(3,2-b) carbazole (ICZ) from indole glucosinolate taken up through the diet. Non-limiting example of such gene sequence(s) are shown **FIG. 41G** and described elsewhere herein. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode pne2 (myrosinase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode pne2from Arabidopsis thaliana.

In any of these embodiments, the genetically engineered bacteria also optionally comprise one or more gene sequence(s) comprising one or more enzymes for tryptophan production, and gene deletions/or mutations as depicted and described in **FIG. 39, FIG. 45A and/or FIG. 45B** and described elsewhere herein. In some embodiments, the genetically engineered bacteria also optionally comprise one or more gene sequence(s) which encode one or more transporter(s) as described herein, through which tryptophan can be imported. Optionally, in some embodiments, the genetically engineered bacteria also optionally comprise one or more gene sequence(s) which encode an exporter as described herein, which can export tryptophan or any of its metabolites.

**Indole acetic acid**

In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more tryptophan catabolism enzymes, which produce indole-3-acetic acid.

Non-limiting example of such gene sequence(s) are shown in **FIG. 42A, FIG. 42B, FIG. 42C, FIG. 42D, and FIG. 42E.**

In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode aro9 ( L-tryptophan aminotransferase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode aro9 from S. cerevisae). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode aspC (aspartate aminotransferase), In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode aspC from E. coli. In one embodiment, the genetically engineered bacteria

comprise one or more gene sequence(s) which encode taa1 (L-tryptophan-pyruvate aminotransferase. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode taa1 from Arabidopsis thaliana). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode staO (L-tryptophan oxidase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode staO from streptomyces sp. TP-A0274). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode trpDH (Tryptophan dehydrogenase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode trpDH from Nostoc punctiforme NIES-2108). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode iad1 (Indole-3-acetaldehyde dehydrogenase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode iad1 from Ustilago maydis. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode AAO1 (Indole-3-acetaldehyde oxidase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode AAO1 from Arabidopsis thaliana. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode ipdC (Indole-3-pyruvate decarboxylase, e.g., from Enterobacter cloacae). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode ipdC (Indole-3-pyruvate decarboxylase, e.g., from Enterobacter cloacae) in combination with one or more sequences encoding enzymes selected from aro9 and/or aspC and/or taa1 and/or staO and/or trpDH. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode ipdC (Indole-3-pyruvate decarboxylase, e.g., from Enterobacter cloacae) in combination with one or more sequences encoding enzymes selected from iad1 and/or aao1.In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode ipdC (Indole-3-pyruvate decarboxylase, e.g., from Enterobacter cloacae) in combination with one or more sequences encoding enzymes selected from aro9 and/or aspC and/or taa1 and/or staO and in combination with one or more sequences encoding enzymes selected from iad1 and/or aao1 (see, e.g., **FIG. 42A)**.

Another non-limiting example of gene sequence(s) for the production of acetic acid are shown in **FIG. 42B.** In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode tdc (Tryptophan decarboxylase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode tdc from Catharanthus roseus). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode tynA (Monoamine oxidase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode tynA from E. coli). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode iad1 (Indole-3-acetaldehyde dehydrogenase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode iad1 from Ustilago maydis). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode AAO1 (Indole-3-acetaldehyde oxidase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode AAO1 from Arabidopsis thaliana). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode tdc and tynA. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode tdc and one or more sequence(s) selected from iad1 and/or aao1. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode tynA and one or more sequence(s) selected from iad1 and/or aao1. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode tdc and tynA and one or more sequence(s) selected from iad1 and/or aao1.

Another non-limiting example of gene sequence(s) for the production of acetic acid are shown in **FIG. 45D.** In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode trpDH (Tryptophan dehydrogenase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode trpDH from Nostoc punctiforme NIES-2108. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode ipdC (Indole-3-pyruvate decarboxylase, e.g., from Enterobacter cloacae). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode iad1 (Indole-3-acetaldehyde dehydrogenase). In one embodiment, the

genetically engineered bacteria comprise one or more gene sequence(s) which encode iad1 from Ustilago maydis. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more of trpDH and/or ipdC and/or iad1. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more of trpDH and ipdC and iad1.

Another non-limiting example of gene sequence(s) for the production of acetic acid are shown in **FIG. 42C.**In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode yuc2 (indole-3-pyruvate monooxygenase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode yuc2 from Enterobacter cloacae. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode aro9 (L-tryptophan aminotransferase). In one embodiment, the (L-tryptophan aminotransferase is from S. cerevisiae. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode aro9 and yuc2. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode aspC (aspartate aminotransferase. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode aspC from E. coli. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode aspC and yuc2. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode taa1 (L-tryptophan-pyruvate aminotransferase, In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode taa1 from Arabidopsis thaliana. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode taa1 and yuc2.In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode staO (L-tryptophan oxidase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode staO from streptomyces sp. TP-A0274. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode staO and yuc2. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode trpDH (Tryptophan dehydrogenase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode trpDH from Nostoc punctiforme NIES-2108. In one embodiment, the genetically engineered bacteria

comprise one or more gene sequence(s) which encode trpDH and yuc2.. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more of aro9 or aspC or taa1 or staO or trpDH. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more of aro9 or aspC or taa1 or staO or trpDH and yuc2.

Another non-limiting example of gene sequence(s) for the production of acetic acid are shown in **FIG. 42D.** In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode IaaM (Tryptophan 2-monooxygenase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode IaaM from Pseudomonas savastanoi). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode iaaH (Indoleacetamide hydrolase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode iaaH from Pseudomonas savastanoi). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode IaaM and iaaH.

Another non-limiting example of gene sequence(s) for the production of acetic acid are shown in **FIG. 42E.** In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp71a13 (indoleacetaldoxime dehydratase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp71a13 from Arabidopis thaliana. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode nit1 (Nitrilase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode nit1 from Arabidopsis thaliana. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode iaaH (Indoleacetamide hydrolase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode iaaH from Pseudomonas savastanoi).In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp79B2 (tryptophan N-monooxygenase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp79B2 from Arabidopsis thaliana. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp79B2 and cyp71a13. In one embodiment, the genetically engineered bacteria comprise one or more

gene sequence(s) which encode cyp79B2 from Arabidopsis thaliana. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp79B2 and nit1 and/or iaaH. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp79B3 (tryptophan N-monooxygenase). In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp79B3 from Arabidopsis thaliana. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp79B3 and cyp71a13. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp79B3 and cyp71a13 and nit1 and/or iaaH. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp79B3, cyp79B2 and cyp71a13. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp79B3, cyp79B2 and cyp71a13, and nit1 and/or iaaH. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp79B3 from Arabidopsis thaliana. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp79B3 and cyp71a13 and nit1 and iaaH. In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode cyp79B3, cyp79B2 and cyp71a13 and nit1 and iaaH.

In any of these embodiments, the genetically engineered bacteria which produce indole acetic acid also optionally comprise one or more gene sequence(s) comprising one or more enzymes for tryptophan production, and gene deletions/or mutations as depicted and described in **FIG. 39, FIG. 45A and/or FIG. 45B** and described elsewhere herein. In some embodiments, the genetically engineered bacteria which produce indole acetic acid also optionally comprise one or more gene sequence(s) which encode one or more transporter(s) as described herein, through which tryptophan can be imported. Optionally, in some embodiments, the genetically engineered bacteria which produce indole acetic acid also optionally comprise one or more gene sequence(s) which encode an exporter as described herein, which can export tryptophan or any of its metabolites.

**Indole-3-propionic acid (IPA)**

In one embodiment, the genetically engineered bacteria comprise one or more gene sequence(s) which encode one or more tryptophan catabolism enzymes, which produce

indole-3-propionic acid from tryptophan. **FIG. 43A and FIG 43B** depict schematics fexemplary circuits for the production of indole-3-propionic acid.

In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding tryptophan ammonia lyase. In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding tryptophan ammonia lyase from *Rubrivivax benzoatilyticus*. In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding indole-3-acrylate reductase. In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding indole-3-acrylate reductase from *Clostridum botulinum*. In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding a tryptophan ammonia lyase and an indole-3-acrylate reductase.

**FIG. 45E** depicts another non-limiting example of an indole-3-propionate-producing strain. In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding trpDH (Tryptophan dehydrogenase). In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding trpDH from Nostoc punctiforme NIES-2108. In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding fldA (indole-3-propionyl-CoA:indole-3-lactate CoA transferase). In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding fldA from Clostridium sporogenes. In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding fldB and fldC (indole-3-lactate dehydratase). In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding fldB and fldC Clostridium sporogenes. In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding fldD (indole-3-acrylyl-CoA reductase). In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding fldD from Clostridium sporogenes. In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding AcuI (acrylyl-CoA reductase). In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding AcuI from Rhodobacter sphaeroides. In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding *fldH1* (3-lactate dehydrogenase 1). In some embodiments, the genetically engineered bacteria comprise one or more gene

sequences encoding *fldH1* from Clostridium sporogenes,. In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding *fldH2 (*indole-3-lactate dehydrogenase 2). In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding *fldH2* from Clostridium sporogenes). In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding trpDH and/or fldA and/or fldB and/or flD and/or fldH1. In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding trpDH and/or fldA and/or fldB and/or flD and/or fldH2. In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding trpDH and/or fldA and/or fldB and/or acul and/or fldH1. In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding trpDH and/or fldA and/or fldB and/or acul and/or fldH2. In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding trpDH and fldA and fldB and flD and fldH1. In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding trpDH and fldA and fldB and flD and fldH2. In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding trpDH and fldA and fldB and acul and fldH1. In some embodiments, the genetically engineered bacteria comprise one or more gene sequences encoding trpDH and fldA and fldB and acul and fldH2.

In any of these embodiments, the genetically engineered bacteria which produce indole-3-propionic acid also optionally comprise one or more gene sequence(s) comprising one or more enzymes for tryptophan production, and gene deletions/or mutations as depicted and described in **FIG. 39, FIG. 45A and/or FIG. 45B** and described elsewhere herein. In some embodiments, the genetically engineered bacteria which produce indole-3-propionic acid also optionally comprise one or more gene sequence(s) which encode one or more transporter(s) as described herein, through which tryptophan can be imported. Optionally, in some embodiments, the genetically engineered bacteria which produce indole-3-propionic acid also optionally comprise one or more gene sequence(s) which encode an exporter as described herein, which can export tryptophan or any of its metabolites.

In certain embodiments, the genetically engineered bacteria comprise one or more gene sequence(s) encoding one or more enzymes for the production of tryptophan metabolites.

In some embodiments, the genetically engineered bacteria comprise one or more gene

sequence(s) encoding 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 different tryptophan metabolites. In certain embodiments the bacteria comprise one or more gene sequence(s) encoding one or more enzymes for the production of tryptophan metabolites selected from tryptamine and/or indole-3 acetaladehyde, indole-3acetonitrile, kynurenine, kynurenic acid, indole, indole acetic acid FICZ, indole-3-propionic acid.

[0325] In in any of these embodiments the expression of the gene sequences for the production of the indole and other tryptophan metabolites, including, but not limited to, tryptamine and/or indole-3 acetaladehyde, indole-3acetonitrile, kynurenine, kynurenic acid, indole, indole acetic acid FICZ, indole-3-propionic acid is under the control of an inducible promoter. Exemplary inducible promoters which may control the expression of the biosynthetic cassettes include oxygen level-dependent promoters (*e.g.*, FNR-inducible promoter), promoters induced by inflammation or an inflammatory response (RNS, ROS promoters), and promoters induced by a metabolite that may or may not be naturally present (e.g., can be exogenously added) in the gut, e.g., arabinose and tetracycline.

[0326] Exemplary circuits for the production of indole metabolites/derivatives are shown in **FIG. 41A through FIG. 41H, FIG. 42A through FIG. 42E, and FIG. 43A though FIG 43B, and FIG. 45A thrugh FIG. 45E.**

[0327]

**Table 13. Non-limiting examples of Sequences for Tryptophan to tryptamine conversion**

| **Description** | **Sequence** |
|---|---|
| Tryptophan Decarboxylase (EC 4.1.1.28) Chain A, Ruminococcus Gnavus **Tryptophan Decarboxylase** Rumg na_01526 (alpha-fmt)<br><br>**SEQ ID NO: 95** | MSQVIKKKRNTFMIGTEYILNSTQLEEAIKSFVHDFCAEKHEIH DQPVVVEAKEHQEDKIKQIKIPEKGRPVNEVVSEMMNEVYRY RGDANHPRFFSFVPGPASSVSWLGDIMTSAYNIHAGGSKLAP MVNCIEQEVLKWLAKQVGFTENPGGVFVSGGSMANITALTA ARDNKLTDINLHLGTAYISDQTHSSVAKGLRIIGITDSRIRRIPT NSHFQMDTTKLEEAIETDKKSGYIPFVVIGTAGTTNTGSIDPLT EISALCKKHDMWFHIDGAYGASVLLSPKYKSLLTGTGLADSIS WDAHKWLFQTYGCAMVLVKDIRNLFHSFHVNPEYLKDLEN DIDNVNTWDIGMELTRPARGLKLWLTLQVLGSDLIGSAIEHG FQLAVWAEEALNPKKDWEIVSPAQMAMINFRYAPKDLTKEE QDILNEKISHRILESGYAAIFTTVLNGKTVLRICAIHPEATQED MQHTIDLLDQYGREIYTEMKKa |
| Tryptophan Decarboxylase (EC 4.1.1.28) Chain A, | ATGAGTCAAGTGATTAAGAAGAAACGTAACACCTTTATGA TCGGAACGGAGTACATTCTTAACAGTACACAATTGGAGGA AGCGATTAAATCATTCGTACATGATTTCTGCGCAGAGAAGC |

| | |
|---|---|
| Ruminococcus Gnavus **Tryptophan Decarboxylase** Rumg na_01526 (alpha-fmt); codon optimized for the expression in E. coli<br><br>**SEQ ID NO: 96** | ATGAGATCCATGATCAACCTGTGGTAGTAGAAGCTAAAGA<br>ACATCAGGAGGACAAAATCAAACAAATCAAAAATCCCGGAA<br>AAGGGACGTCCTGTAAATGAAGTCGTTTCTGAGATGATGA<br>ATGAAGTGTATCGCTACCGCGGAGACGCCAACCATCCTCG<br>CTTTTTTTCTTTTGTGCCCGGACCTGCAAGCAGTGTGTCGTG<br>GTTGGGGGATATTATGACGTCCGCCTACAATATTCATGCTG<br>GAGGCTCAAAGCTGGCACCGATGGTTAACTGCATTGAGCA<br>GGAAGTTCTGAAGTGGTTAGCAAAGCAAGTGGGGTTCACA<br>GAAAATCCAGGTGGCGTATTTGTGTCGGGCGGTTCAATGG<br>CGAATATTACGGCACTTACTGCGGCTCGTGACAATAAACTG<br>ACCGACATTAACCTTCATTTGGGAACTGCTTATATTAGTGA<br>CCAGACTCATAGTTCAGTTGCGAAAGGATTACGCATTATTG<br>GAATCACTGACAGTCGCATCCGTCGCATTCCCACTAACTCC<br>CACTTCCAGATGGATACCACCAAGCTGGAGGAAGCCATCG<br>AGACCGACAAGAAGTCTGGCTACATTCCGTTCGTCGTTATC<br>GGAACAGCAGGTACCACCAACACTGGTTCGATTGACCCCC<br>TGACAGAAATCTCTGCGTTATGTAAGAAGCATGACATGTG<br>GTTTCATATCGACGGAGCGTATGGAGCTAGTGTTCTGCTGT<br>CACCTAAGTACAAGAGCCTTCTTACCGGAACCGGCTTGGCT<br>GACAGTATTTCGTGGGATGCTCATAAATGGTTGTTCCAAAC<br>GTACGGCTGTGCAATGGTACTTGTCAAAGATATCCGTAATT<br>TATTCCACTCTTTTCATGTGAATCCCGAGTATCTTAAGGAT<br>CTGGAAAAACGACATCGATAACGTTAATACATGGGACATCG<br>GCATGGAGCTGACGCGCCCTGCACGCGGTCTTAAATTGTG<br>GCTTACTTTACAGGTCCTTGGATCTGACTTGATTGGGAGTG<br>CCATTGAACACGGTTTCCAGCTGGCAGTTTGGGCTGAGGA<br>AGCATTGAATCCAAAGAAAGACTGGGAGATCGTTTCTCCA<br>GCTCAGATGGCTATGATTAATTTCCGTTATGCCCCTAAGGA<br>TTTAACCAAAGAGGAACAGGATATTCTGAATGAAAAGATC<br>TCCCACCGCATTTTAGAGAGCGGATACGCTGCAATTTTCAC<br>TACTGTATTAAACGGCAAGACCGTTTTACGCATCTGTGCAA<br>TTCACCCGGAGGCAACTCAAGAGGATATGCAACACACAAT<br>CGACTTATTAGACCAATACGGTCGTGAAATCTATACCGAG<br>ATGAAGAAAGCG |

[0328] In some embodiments, the genetically engineered bacteria comprise one or more nucleic acid sequence of **Table 13** 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 one or more nucleic acid sequence of **Table 13** 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 one or more nucleic acid sequence of **Table 13** or a functional fragment thereof, or a nucleic acid sequence that, but for the redundancy of the genetic code, encodes the same polypeptide as one or more nucleic acid sequence of **Table 13** or a functional fragment thereof.

[0329] In one embodiment, the Tryptophan Decarboxylase gene has at least about 80% identity with the entire sequence of **SEQ ID NO: 95 or SEQ ID NO: 96:** . In another embodiment, the Tryptophan Decarboxylase gene has at least about 85% identity with the entire sequence of **SEQ ID NO: 95 or SEQ ID NO: 96**. In one embodiment, the Tryptophan Decarboxylase gene has at least about 90% identity with the entire sequence of **SEQ ID NO: 95 or SEQ ID NO: 96**. In one embodiment, the Tryptophan Decarboxylase gene has at least about 95% identity with the entire sequence of **SEQ ID NO: 95 or SEQ ID NO: 96**. In another embodiment, the Tryptophan Decarboxylase gene has at least about 96%, 97%, 98%, or 99% identity with the entire sequence of **SEQ ID NO: 95 or SEQ ID NO: 96**. Accordingly, in one embodiment, the Tryptophan Decarboxylase gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with the entire sequence of **SEQ ID NO: 95 or SEQ ID NO: 96**. In another embodiment, the Tryptophan Decarboxylase gene comprises the sequence of **SEQ ID NO: 95 or SEQ ID NO: 96**. In yet another embodiment the Tryptophan Decarboxylase gene consists of the sequence of **SEQ ID NO: 95 or SEQ ID NO: 96**.

[0330] In some embodiments, the genetically engineered bacteria comprise one or more gene cassettes which convert tryptophan to Indole-3-aldehyde and Indole Acetic Acid, e.g., via a tryptophan aminotransferase cassette. A non-limiting example of such a tryptophan aminotransferase expressed by the genetically engineered bacteria is in **Table 14**. In some embodiments, the genetically engineered bacteria take up tryptophan through an endogenous or exogenous transporter, and further produce Indole-3-aldehyde and Indole Acetic Acid from tryptophan. In some embodiments, the genetically engineered bacteria optionally comprise a tryptophan and/or indole metabolite exporter.

**Table 14. Exemplary tryptophan aminotransferase sequences**

| **Description** | **Sequence** |
|---|---|
| **Trp aminotransferase** | MTATTISIETVPQAPAAGTKTNGTSGKYNPRTYLSDRAKVTEI DGSDAGRPNPDTFPFNSITLNLKPPLGLPESSNNMPVSITIEDPD |

| | |
|---|---|
| **(EC 2.6.1.27);** tryptophan aminotransferase [Cryptococcus deuterogattii R265] **SEQ ID NO: 97** | LATALQYAPSAGIPKLREWLADLQAHVHERPRGDYAISVGSG SQDLMFKGFQAVLNPGDPVLLETPMYSGVLPALRILKADYAE VDVDDQGLSAKNLEKVLSEWPADKKRPRVLYTSPIGSNPSGC SASKERKLEVLKVCKKYDVLIFEDDPYYYLAQELIPSYFALEK QVYPEGGHVVRFDSFSKLLSAGMRLGFATGPKEILHAIDVSTA GANLHTSAVSQGVALRLMQYWGIEGFLAHGRAVAKLYTERR AQFEATAHKYLDGLATWVSPVAGMFLWIDLRPAGIEDSYELI RHEALAKGVLGVPGMAFYPTGRKSSHVRVSFSIVDLEDESDL GFQRLAEAIKDKRKALGLA |
| **Trp aminotransferase (EC 2.6.1.27);** tryptophan aminotransferase [Cryptococcus deuterogattii R265], codon optimized for expression in E. coli **SEQ ID NO: 98** | ATGACGGCAACTACAATTTCTATTGAGACCGTACCTCAGGC CCCGGCGGCGGGGACCAAAACTAATGGGACTTCAGGAAAA TACAACCCCCGCACTTACCTGTCCGACCGCGCCAAAGTCAC TGAGATTGATGGATCTGACGCCGGTCGCCCCAATCCCGATA CTTTCCCATTTAACTCGATTACCTTAAATTTGAAACCACCTT TAGGCTTGCCCGAGAGTTCAAATAACATGCCGGTCTCTATC ACGATTGAAGACCCCGATTTAGCGACGGCCTTACAATATG CACCTAGCGCCGGTATTCCTAAGCTGCGCGAATGGCTGGCT GACTTACAAGCTCACGTTCATGAGCGCCCCCGTGGCGATTA TGCCATCTCGGTCGGGTCGGGGTCACAGGATTTGATGTTTA AGGGCTTCCAAGCTGTCTTGAATCCAGGTGATCCAGTCCTT CTGGAAACCCCAATGTATTCAGGTGTTCTGCCAGCGCTGCG CATTCTGAAGGCGGATTATGCAGAAGTTGATGTAGACGAC CAGGGGTTATCTGCTAAAAACCTTGAAAAAGTTTTATCAGA GTGGCCCGCAGATAAGAAGCGTCCTCGTGTCCTGTATACGT CGCCAATCGGCTCCAATCCTTCCGGATGTTCAGCATCCAAG GAACGCAAGTTAGAGGTACTGAAAGTCTGTAAGAAGTACG ATGTGCTGATCTTCGAAGACGATCCGTATTATTACCTTGCT CAAGAGCTTATTCCATCCTATTTTGCGTTGGAAAAACAAGT TTATCCGGAGGGTGGGCACGTTGTACGCTTTGACTCATTTA GTAAATTGCTTTCTGCTGGGATGCGCTTGGGATTTGCTACA GGGCCGAAGGAAATTCTTCATGCGATTGACGTCAGTACAG CAGGCGCAAATTTACATACTTCAGCGGTCTCTCAAGGTGTC GCTCTTCGCCTGATGCAGTATTGGGGGATCGAGGGATTCCT TGCACATGGCCGCGCGGTGGCCAAACTTTACACGGAGCGC CGCGCTCAGTTCGAGGCAACCGCACATAAGTACCTGGACG GGCTGGCCACTTGGGTATCTCCCGTAGCGGGAATGTTTTTA TGGATCGATCTTCGTCCAGCAGGAATCGAAGATTCTTACGA ATTAATTCGCCATGAAGCATTAGCCAAAGGCGTTTTAGGCG TTCCAGGGATGGCGTTTTATCCGACAGGCCGTAAGTCTTCC CATGTTCGTGTCAGTTTCAGTATCGTCGACCTGGAAGACGA ATCTGACCTTGGTTTTCAACGCCTGGCTGAAGCTATTAAGG ATAAACGCAAGGCTTTAGGGCTGGCT |

[0331] In some embodiments, the genetically engineered bacteria comprise one or more nucleic acid sequence of **Table 14** 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 one or more nucleic acid sequence of **Table 14** 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 one or more nucleic acid sequence of **Table 14** or a functional fragment thereof, or a nucleic acid sequence that, but for the redundancy of the genetic code, encodes the same polypeptide as one or more nucleic acid sequence of **Table 14** or a functional fragment thereof.

[0332] In one embodiment, the Trp aminotransferase gene has at least about 80% identity with the entire sequence of **SEQ ID NO: 97 or SEQ ID NO: 98**. In another embodiment, the Trp aminotransferase gene has at least about 85% identity with the entire sequence of **SEQ ID NO: 97 or SEQ ID NO: 98**. In one embodiment, the Trp aminotransferase gene has at least about 90% identity with the entire sequence of **SEQ ID NO: 97 or SEQ ID NO: 98**. In one embodiment, the Trp aminotransferase gene has at least about 95% identity with the entire sequence of **SEQ ID NO: 97 or SEQ ID NO: 98**. In another embodiment, the Trp aminotransferase gene has at least about 96%, 97%, 98%, or 99% identity with the entire sequence of **SEQ ID NO: 97 or SEQ ID NO: 98**. Accordingly, in one embodiment, the Trp aminotransferase gene has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with the entire sequence of **SEQ ID NO: 97 or SEQ ID NO: 98**. In another embodiment, the Trp aminotransferase gene comprises the sequence of **SEQ ID NO: 97 or SEQ ID NO: 98**. In yet another embodiment the Trp aminotransferase gene consists of the sequence of **SEQ ID NO: 97 or SEQ ID NO: 98.**

[0333] The genetically engineered bacteria may comprise any suitable gene for producing Indole-3-aldehyde and/or Indole Acetic Acidand/or Tryptamine. In some embodiments, the gene for producing kynurenine is modified and/or mutated, *e.g.*, to enhance stability, increase Indole-3-aldehyde and/or Indole Acetic Acidand/or Tryptamine production, and/or increase anti-inflammatory potency under inducing conditions. In some embodiments, the engineered bacteria also 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, as discussed in detail above. In some embodiments, the engineered bacteria also have enhanced export of a indole tryptophan metabolite , *e.g.*,

comprise an exporter or other mechanism for increasing the uptake of tryptophan into the bacterial cell, as discussed in detail above. In some embodiments, the genetically engineered bacteria are capable of producing Indole-3-aldehyde and/or Indole Acetic Acidand/or Tryptamine 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, in the presence of certain molecules or metabolites, in the presence of molecules or metabolites associated with inflammation or an inflammatory response, or in the presence of some other metabolite that may or may not be present in the gut, such as arabinose.

[0334] **Table 15** comprises polypeptide sequences of such enzymes which are encoded by the genetically engineered bacteria of the disclosure.

**Table 15. Tryptophan Pathway Catabolic Enzymes**

| **Description** | **Sequence** |
|---|---|
| TDC: Tryptophan decarboxylase from Catharanthus roseus **SEQ ID NO: 99** | MGSIDSTNVAMSNSPVGEFKPLEAEEFRKQAHRMVDFIADYY KNVETYPVLSEVEPGYLRKRIPETAPYLPEPLDDIMKDIQKDII PGMTNWMSPNFYAFFPATVSSAAFLGEMLSTALNSVGFTWV SSPAATELEMIVMDWLAQILKLPKSFMFSGTGGGVIQNTTSES ILCTIIAARERALEKLGPDSIGKLVCYGSDQTHTMFPKTCKLA GIYPNNIRLIPTTVETDFGISPQVLRKMVEDDVAAGYVPLFLC ATLGTTSTTATDPVDSLSEIANEFGIWIHVDAAYAGSACICPEF RHYLDGIERVDSLSLSPHKWLLAYLDCTCLWVKQPHLLLRAL TTNPEYLKNKQSDLDKVVDFKNWQIATGRKFRSLKLWLILRS YGVVNLQSHIRSDVAMGKMFEEWVRSDSRFEIVVPRNFSLVC FRLKPDVSSLHVEEVNKKLLDMLNSTGRVYMTHTIVGGIYML RLAVGSSLTEEHHVRRVWDLIQKLTDDLLKEA |
| TYNA: Monoamine oxidase from E. coli **SEQ ID NO: 100** | MGSPSLYSARKTTLALAVALSFAWQAPVFAHGGEAHMVPM DKTLKEFGADVQWDDYAQLFTLIKDGAYVKVKPGAQTAIVN GQPLALQVPVVMKDNKAWVSDTFINDVFQSGLDQTFQVEKR PHPLNALTADEIKQAVEIVKASADFKPNTRFTEISLLPPDKEAV WAFALENKPVDQPRKADVIMLDGKHIIEAVVDLQNNKLLSW QPIKDAHGMVLLDDFASVQNIINNSEEFAAAVKKRGITDAKK VITTPLTVGYFDGKDGLKQDARLLKVISYLDVGDGNYWAHPI ENLVAVVDLEQKKIVKIEEGPVVPVPMTARPFDGRDRVAPAV KPMQIIEPEGKNYTITGDMIHWRNWDFHLSMNSRVGPMISTV TYNDNGTKRKVMYEGSLGGMIVPYGDPDIGWYFKAYLDSGD YGMGTLTSPIARGKDAPSNAVLLNETIADYTGVPMEIPRAIAV FERYAGPEYKHQEMGQPNVSTERRELVVRWISTVGNYDYIFD WIFHENGTIGIDAGATGIEAVKGVKAKTMHDETAKDDTRYGT LIDHNIVGTTHQHIYNFRLDLDVDGENNSLVAMDPVVKPNTA GGPRTSTMQVNQYNIGNEQDAAQKFDPGTIRLLSNPNKENRM |

| | |
|---|---|
| | GNPVSYQIIPYAGGTHPVAKGAQFAPDEWIYHRLSFMDKQLW VTRYHPGERFPEGKYPNRSTHDTGLGQYSKDNESLDNTDAV VWMTTGTTHVARAEEWPIMPTEWVHTLLKPWNFFDETPTLG ALKKDK |
| AAO1: Indole-3-acetaldehyde oxidase from Arabidopsis thaliana **SEQ ID NO: 101** | MGEKAIDEDKVEAMKSSKTSLVFAINGQRFELELSSIDPSTTL VDFLRNKTPFKSVKLGCGEGGCGACVVLLSKYDPLLEKVDEF TISSCLTLLCSIDGCSITTSDGLGNSRVGFHAVHERIAGFHATQ CGFCTPGMSVSMFSALLNADKSHPPPRSGFSNLTAVEAEKAV SGNLCRCTGYRPLVDACKSFAADVDIEDLGFNAFCKKGENRD EVLRRLPCYDHTSSHVCTFPEFLKKEIKNDMSLHSRKYRWSSP VSVSELQGLLEVENGLSVKLVAGNTSTGYYKEEKERKYERFI DIRKIPEFTMVRSDEKGVELGACVTISKAIEVLREEKNVSVLA KIATHMEKIANRFVRNTGTIGGNIMMAQRKQFPSDLATILVA AQATVKIMTSSSSQEQFTLEEFLQQPPLDAKSLLLSLEIPSWHS AKKNGSSEDSILLFETYRAAPRPLGNALAFLNAAFSAEVTEAL DGIVVNDCQLVFGAYGTKHAHRAKKVEEFLTGKVISDEVLM EAISLLKDEIVPDKGTSNPGYRSSLAVTFLFEFFGSLTKKNAKT TNGWLNGGCKEIGFDQNVESLKPEAMLSSAQQIVENQEHSPV GKGITKAGACLQASGEAVYVDDIPAPENCLYGAFIYSTMPLA RIKGIRFKQNRVPEGVLGIITYKDIPKGGQNIGTNGFFTSDLLF AEEVTHCAGQIIAFLVADSQKHADIAANLVVIDYDTKDLKPPI LSLEEAVENFSLFEVPPPLRGYPVGDITKGMDEAEHKILGSKIS FGSQYFFYMETQTALAVPDEDNCMVVYSSTQTPEFVHQTIAG CLGVPENNVRVITRRVGGGFGGKAVKSMPVAAACALAASK MQRPVRTYVNRKTDMITTGGRHPMKVTYSVGFKSNGKITAL DVEVLLDAGLTEDISPLMPKGIQGALMKYDWGALSFNVKVC KTNTVSRTALRAPGDVQGSYIGEAIIEKVASYLSVDVDEIRKV NLHTYESLRLFHSAKAGEFSEYTLPLLWDRIDEFSGFNKRRKV VEEFNASNKWRKRGISRVPAVYAVNMRSTPGRVSVLGDGSIV VEVQGIEIGQGLWTKVKQMAAYSLGLIQCGTTSDELLKKIRVI QSDTLSMVQGSMTAGSTTSEASSEAVRICCDGLVERLLPVKT ALVEQTGGPVTWDSLISQAYQQSINMSVSSKYMPDSTGEYLN YGIAASEVEVNVLTGETTILRTDIIYDCGKSLNPAVDLGQIEGA FVQGLGFFMLEEFLMNSDGLVVTDSTWTYKIPTVDTIPRQFN VEILNSGQHKNRVLSSKASGEPPLLLAASVHCAVRAAVKEAR KQILSWNSNKQGTDMYFELPVPATMPIVKEFCGLDVVEKYLE WKIQQRKNV |
| ARO9: L-tryptophan aminotransferase from S. cerevisae **SEQ ID NO: 102** | MTAGSAPPVDYTSLKKNFQPFLSRRVENRSLKSFWDASDISD DVIELAGGMPNERFFPIESMDLKISKVPFNDNPKWHNSFTTAH LDLGSPSELPIARSFQYAETKGLPPLLHFVKDFVSRINRPAFSD ETESNWDVILSGGSNDSMFKVFETICDESTTVMIEEFTFTPAM SNVEATGAKVIPIKMNLTFDRESQGIDVEYLTQLLDNWSTGP YKDLNKPRVLYTIATGQNPTGMSVPQWKREKIYQLAQRHDF LIVEDDPYGYLYFPSYNPQEPLENPYHSSDLTTERYLNDFLMK SFLTLDTDARVIRLETFSKIFAPGLRLSFIVANKFLLQKILDLAD ITTRAPSGTSQAIVYSTIKAMAESNLSSSLSMKEAMFEGWIRW IMQIASKYNHRKNLTLKALYETESYQAGQFTVMEPSAGMFIII KINWGNFDRPDDLPQQMDILDKFLLKNGVKVVLGYKMAVCP |

| | |
|---|---|
| | NYSKQNSDFLRLTIAYARDDDQLIEASKRIGSGIKEFFDNYKS |
| aspC: aspartate aminotransferase from E. coli **SEQ ID NO: 103** | MFENITAAPADPILGLADLFRADERPGKINLGIGVYKDETGKT PVLTSVKKAEQYLLENETTKNYLGIDGIPEFGRCTQELLFGKG SALINDKRARTAQTPGGTGALRVAADFLAKNTSVKRVWVSN PSWPNHKSVFNSAGLEVREYAYYDAENHTLDFDALINSLNEA QAGDVVLFHGCCHNPTGIDPTLEQWQTLAQLSVEKGWLPLF DFAYQGFARGLEEDAEGLRAFAAMHKELIVASSYSKNFGLYN ERVGACTLVAADSETVDRAFSQMKAAIRANYSNPPAHGASV VATILSNDALRAIWEQELTDMRQRIQRMRQLFVNTLQEKGAN RDFSFIIKQNGMFSFSGLTKEQVLRLREEFGVYAVASGRVNVA GMTPDNMAPLCEAIVAVL |
| TAA1: L-tryptophan-pyruvate aminotransferase from Arabidopsis thaliana **SEQ ID NO: 104** | MVKLENSRKPEKISNKNIPMSDFVVNLDHGDPTAYEEYWRK MGDRCTVTIRGCDLMSYFSDMTNLCWFLEPELEDAIKDLHGV VGNAATEDRYIVVGTGSTQLCQAAVHALSSLARSQPVSVVA AAPFYSTYVEETTYVRSGMYKWEGDAWGFDKKGPYIELVTS PNNPDGTIRETVVNRPDDDEAKVIHDFAYYWPHYTPITRRQD HDIMLFTFSKITGHAGSRIGWALVKDKEVAKKMVEYIIVNSIG VSKESQVRTAKILNVLKETCKSESESENFFKYGREMMKNRWE KLREVVKESDAFTLPKYPEAFCNYFGKSLESYPAFAWLGTKE ETDLVSELRRHKVMSRAGERCGSDKKHVRVSMLSREDVFNV FLERLANMKLIKSIDL |
| STAO: L-tryptophan oxidase from streptomyces sp. TP-A0274 **SEQ ID NO: 105** | MTAPLQDSDGPDDAIGGPKQVTVIGAGIAGLVTAYELERLGH HVQIIEGSDDIGGRIHTHRFSGAGGPGPFAEMGAMRIPAGHRL TMHYIAELGLQNQVREFRTLFSDDAAYLPSSAGYLRVREAHD TLVDEFATGLPSAHYRQDTLLFGAWLDASIRAIAPRQFYDGL HNDIGVELLNLVDDIDLTPYRCGTARNRIDLHALFADHPRVR ASCPPRLERFLDDVLDETSSSIVRLKDGMDELPRRLASRIRGKI SLGQEVTGIDVHDDTVTLTVRQGLRTVTRTCDYVVCTIPFTVL RTLRLTGFDQDKLDIVHETKYWPATKIAFHCREPFWEKDGIS GGASFTGGHVRQTYYPPAEGDPALGAVLLASYTIGPDAEALA RMDEAERDALVAKELSVMHPELRRPGMVLAVAGRDWGARR WSRGAATVRWGQEAALREAERRECARPQKGLFFAGEHCSSK PAWIEGAIESAIDAAHEIEWYEPRASRVFAASRLSRSDRSA |
| ipdC: Indole-3-pyruvate decarboxylase from Enterobacter cloacae **SEQ ID NO: 106** | MRTPYCVADYLLDRLTDCGADHLFGVPGDYNLQFLDHVIDS PDICWVGCANELNASYAADGYARCKGFAALLTTFGVGELSA MNGIAGSYAEHVPVLHIVGAPGTAAQQRGELLHHTLGDGEFR HFYHMSEPITVAQAVLTEQNACYEIDRVLTTMLRERRPGYLM LPADVAKKAATPPVNALTHKQAHADSACLKAFRDAAENKLA MSKRTALLADFLVLRHGLKHALQKWVKEVPMAHATMLMG KGIFDERQAGFYGTYSGSASTGAVKEAIEGADTVLCVGTRFT DTLTAGFTHQLTPAQTIEVQPHAARVGDVWFTGIPMNQAIET LVELCKQHVHAGLMSSSSGAIPFPQPDGSLTQENFWRTLQTFI RPGDIILADQGTSAFGAIDLRLPADVNFIVQPLWGSIGYTLAA AFGAQTACPNRRVIVLTGDGAAQLTIQELGSMLRDKQHPIILV LNNEGYTVERAIHGAEQRYNDIALWNWTHIPQALSLDPQSEC WRVSEAEQLADVLEKVAHHERLSLIEVMLPKADIPPLLGALT KALEACNNA |

| | |
|---|---|
| IAD1: Indole-3-acetaldehyde dehydrogenase from Ustilago maydis **SEQ ID NO: 107** | MPTLNLDLPNGIKSTIQADLFINNKFVPALDGKTFATINPSTGK EIGQVAEASAKDVDLAVKAAREAFETTWGENTPGDARGRLLI KLAELVEANIDELAAIESLDNGKAFSIAKSFDVAAVAANLRY YGGWADKNHGKVMEVDTKRLNYTRHEPIGVCGQIIPWNFPL LMFAWKLGPALATGNTIVLKTAEQTPLSAIKMCELIVEAGFPP GVVNVISGFGPVAGAAISQHMDIDKIAFTGSTLVGRNIMKAA ASTNLKKVTLELGGKSPNIIFKDADLDQAVRWSAFGIMFNHG QCCCAGSRVYVEESIYDAFMEKMTAHCKALQVGDPFSANTF QGPQVSQLQYDRIMEYIESGKKDANLALGGVRKGNEGYFIEP TIFTDVPHDAKIAKEEIFGPVVVVSKFKDEKDLIRIANDSIYGL AAAVFSRDISRAIETAHKLKAGTVWVNCYNQLIPQVPFGGYK ASGIGRELGEYALSNYTNIKAVHVNLSQPAPI |
| YUC2: indole-3-pyruvate monoxygenase from Arabidopsis thaliana **SEQ ID NO: 108** | MEFVTETLGKRIHDPYVEETRCLMIPGPIIVGSGPSGLATAACL KSRDIPSLILERSTCIASLWQHKTYDRLRLHLPKDFCELPLMPF PSSYPTYPTKQQFVQYLESYAEHFDLKPVFNQTVEEAKFDRR CGLWRVRTTGGKKDETMEYVSRWLVVATGENAEEVMPEID GIPDFGGPILHTSSYKSGEIFSEKKILVVGCGNSGMEVCLDLCN FNALPSLVVRDSVHVLPQEMLGISTFGISTSLLKWFPVHVVDR FLLRMSRLVLGDTDRLGLVRPKLGPLERKIKCGKTPVLDVGT LAKIRSGHIKVYPELKRVMHYSAEFVDGRVDNFDAIILATGY KSNVPMWLKGVNMFSEKDGFPHKPFPNGWKGESGLYAVGF TKLGLLGAAIDAKKIAEDIEVQRHFLPLARPQHC |
| IaaM: Tryptophan 2-monooxygenase from Pseudomonas savastanoi **SEQ ID NO: 109** | MYDHFNSPSIDILYDYGPFLKKCEMTGGIGSYSAGTPTPRVAI VGAGISGLVAATELLRAGVKDVVLYESRDRIGGRVWSQVFD QTRPRYIAEMGAMRFPPSATGLFHYLKKFGISTSTTFPDPGVV DTELHYRGKRYHWPAGKKPPELFRRVYEGWQSLLSEGYLLE GGSLVAPLDITAMLKSGRLEEAAIAWQGWLNVFRDCSFYNAI VCIFTGRHPPGGDRWARPEDFELFGSLGIGSGGFLPVFQAGFT EILRMVINGYQSDQRLIPDGISSLAARLADQSFDGKALRDRVC FSRVGRISREAEKIIIQTEAGEQRVFDRVIVTSSNRAMQMIHCL TDSESFLSRDVARAVRETHLTGSSKLFILTRTKFWIKNKLPTTI QSDGLVRGVYCLDYQPDEPEGHGVVLLSYTWEDDAQKMLA MPDKKTRCQVLVDDLAAIHPTFASYLLPVDGDYERYVLHHD WLTDPHSAGAFKLNYPGEDVYSQRLFFQPMTANSPNKDTGL YLAGCSCSFAGGWIEGAVQTALNSACAVLRSTGGQLSKGNPL DCINASYRY |
| iaaH: Indoleacetamide hydrolase from Pseudomonas savastanoi **SEQ ID NO: 110** | MHEIITLESLCQALADGEIAAAELRERALDTEARLARLNCFIRE GDAVSQFGEADHAMKGTPLWGMPVSFKDNICVRGLPLTAGT RGMSGFVSDQDAAIVSQLRALGAVVAGKNNMHELSFGVTSI NPHWGTVGNPVAPGYCAGGSSGGSAAAVASGIVPLSVGTDT GGSIRIPAAFCGITGFRPTTGRWSTAGIIPVSHTKDCVGLLTRT AGDAGFLYGLLSGKQQSFPLSRTAPCRIGLPVSMWSDLDGEV ERACVNALSLLRKTGFEFIEIDDADIVELNQTLTFTVPLYEFFA DLAQSLLSLGWKHGIHHIFAQVDDANVKGIINHHLGEGAIKP AHYLSSLQNGELLKRKMDELFARHNIELLGYPTVPCRVPHLD HADRPEFFSQAIRNTDLASNAMLPSITIPVGPEGRLPVGLSFDA LRGRDALLLSRVSAIEQVLGFVRKVLPHTT |

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| TrpDH: Tryptophan dehydrogenase from Nostoc punctiforme NIES-2108 **SEQ ID NO: 111** | MLLFETVREMGHEQVLFCHSKNPEIKAIIAIHDTTLGPAMGAT RILPYINEEAALKDALRLSRGMTYKAACANIPAGGGKAVIIAN PENKTDDLLRAYGRFVDSLNGRFITGQDVNITPDDVRTISQET KYVVGVSEKSGGPAPITSLGVFLGIKAAVESRWQSKRLDGMK VAVQGLGNVGKNLCRHLHEHDVQLFVSDVDPIKAEEVKRLF GATVVEPTEIYSLDVDIFAPCALGGILNSHTIPFLQASIIAGAAN NQLENEQLHSQMLAKKGILYSPDYVINAGGLINVYNEMIGYD EEKAFKQVHNIYDTLLAIFEIAKEQGVTTNDAARRLAEDRINN SKRSKSKAIAA |
| CYP79B2: tryptophan N-monooxygenase from Arabidopsis thaliana **SEQ ID NO: 112** | MNTFTSNSSDLTTTATETSSFSTLYLLSTLQAFVAITLVMLLKK LMTDPNKKKPYLPPGPTGWPIIGMIPTMLKSRPVFRWLHSIMK QLNTEIACVKLGNTHVITVTCPKIAREILKQQDALFASRPLTY AQKILSNGYKTCVITPFGDQFKKMRKVVMTELVCPARHRWL HQKRSEENDHLTAWVYNMVKNSGSVDFRFMTRHYCGNAIK KLMFGTRTFSKNTAPDGGPTVEDVEHMEAMFEALGFTFAFCI SDYLPMLTGLDLNGHEKIMRESSAIMDKYHDPIIDERIKMWR EGKRTQIEDFLDIFISIKDEQGNPLLTADEIKPTIKELVMAAPDN PSNAVEWAMAEMVNKPEILRKAMEEIDRVVGKERLVQESDIP KLNYVKAILREAFRLHPVAAFNLPHVALSDTTVAGYHIPKGS QVLLSRYGLGRNPKVWADPLCFKPERHLNECSEVTLTENDLR FISFSTGKRGCAAPALGTALTTMMLARLLQGFTWKLPENETR VELMESSHDMFLAKPLVMVGDLRLPEHLYPTVK |
| CYP79B3: tryptophan N-monooxygenase from Arabidopsis thaliana **SEQ ID NO: 113** | MDTLASNSSDLTTKSSLGMSSFTNMYLLTTLQALAALCFLMI LNKIKSSSRNKKLHPLPPGPTGFPIVGMIPAMLKNRPVFRWLH SLMKELNTEIACVRLGNTHVIPVTCPKIAREIFKQQDALFASRP LTYAQKILSNGYKTCVITPFGEQFKKMRKVIMTEIVCPARHR WLHDNRAEETDHLTAWLYNMVKNSEPVDLRFVTRHYCGNA IKRLMFGTRTFSEKTEADGGPTLEDIEHMDAMFEGLGFTFAFC ISDYLPMLTGLDLNGHEKIMRESSAIMDKYHDPIIDERIKMWR EGKRTQIEDFLDIFISIKDEAGQPLLTADEIKPTIKELVMAAPDN PSNAVEWAIAEMINKPEILHKAMEEIDRVVGKERFVQESDIPK LNYVKAIIREAFRLHPVAAFNLPHVALSDTTVAGYHIPKGSQV LLSRYGLGRNPKVWSDPLSFKPERHLNECSEVTLTENDLRFIS FSTGKRGCAAPALGTAITTMMLARLLQGFKWKLAGSETRVE LMESSHDMFLSKPLVLVGELRLSEDLYPMVK |
| CYP71A13: indoleacetaldoxime dehydratase from Arabidopis thaliana **SEQ ID NO: 114** | MSNIQEMEMILSISLCLTTLITLLLLRRFLKRTATKVNLPPSPW RLPVIGNLHQLSLHPHRSLRSLSLRYGPLMLLHFGRVPILVVSS GEAAQEVLKTHDHKFANRPRSKAVHGLMNGGRDVVFAPYG EYWRQMKSVCILNLLTNKMVESFEKVREDEVNAMIEKLEKA SSSSSSENLSELFITLPSDVTSRVALGRKHSEDETARDLKKRVR QIMELLGEFPIGEYVPILAWIDGIRGFNNKIKEVSRGFSDLMDK VVQEHLEASNDKADFVDILLSIEKDKNSGFQVQRNDIKFMILD MFIGGTSTTSTLLEWTMTELIRSPKSMKKLQDEIRSTIRPHGSY IKEKEVENMKYLKAVIKEVLRLHPSLPMILPRLLSEDVKVKGY NIAAGTEVIINAWAIQRDTAIWGPDAEEFKPERHLDSGLDYHG KNLNYIPFGSGRRICPGINLALGLAEVTVANLVGRFDWRVEA GPNGDQPDLTEAIGIDVCRKFPLIAFPSSVV |

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| PEN2: myrosinase from Arabidopsis thaliana **SEQ ID NO: 115** | MAHLQRTFPTEMSKGRASFPKGFLFGTASSSYQYEGAVNEGA RGQSVWDHFSNRFPHRISDSSDGNVAVDFYHRYKEDIKRMK DINMDSFRLSIAWPRVLPYGKRDRGVSEEGIKFYNDVIDELLA NEITPLVTIFHWDIPQDLEDEYGGFLSEQIIDDFRDYASLCFERF GDRVSLWCTMNEPWVYSVAGYDTGRKAPGRCSKYVNGASV AGMSGYEAYIVSHNMLLAHAEAVEVFRKCDHIKNGQIGIAHN PLWYEPYDPSDPDDVEGCNRAMDFMLGWHQHPTACGDYPE TMKKSVGDRLPSFTPEQSKKLIGSCDYVGINYYSSLFVKSIKH VDPTQPTWRTDQGVDWMKTNIDGKQIAKQGGSEWSFTYPTG LRNILKYVKKTYGNPPILITENGYGEVAEQSQSLYMYNPSIDT ERLEYIEGHIHAIHQAIHEDGVRVEGYYVWSLLDNFEWNSGY GVRYGLYYIDYKDGLRRYPKMSALWLKEFLRFDQEDDSSTS KKEEKKESYGKQLLHSVQDSQFVHSIKDSGALPAVLGSLFVV SATVGTSLFFKGANN |
| Nit1: Nitrilase from Arabidopsis thaliana **SEQ ID NO: 116** | MSSTKDMSTVQNATPFNGVAPSTTVRVTIVQSSTVYNDTPATI DKAEKYIVEAASKGAELVLFPEGFIGGYPRGFRFGLAVGVHN EEGRDEFRKYHASAIHVPGPEVARLADVARKNHVYLVMGAI EKEGYTLYCTVLFFSPQGQFLGKHRKLMPTSLERCIWGQGDG STIPVYDTPIGKLGAAICWENRMPLYRTALYAKGIELYCAPTA DGSKEWQSSMLHIAIEGGCFVLSACQFCQRKHFPDHPDYLFT DWYDDKEHDSIVSQGGSVIISPLGQVLAGPNFESEGLVTADID LGDIARAKLYFDSVGHYSRPDVLHLTVNEHPRKSVTFVTKVE KAEDDSNK |
| IDO1: indoleamine 2,3-dioxygenase from homo sapiens **SEQ ID NO: 117** | MAHAMENSWTISKEYHIDEEVGFALPNPQENLPDFYNDWMFI AKHLPDLIESGQLRERVEKLNMLSIDHLTDHKSQRLARLVLG CITMAYVWGKGHGDVRKVLPRNIAVPYCQLSKKLELPPILVY ADCVLANWKKKDPNKPLTYENMDVLFSFRDGDCSKGFFLVS LLVEIAAASAIKVIPTVFKAMQMQERDTLLKALLEIASCLEKA LQVFHQIHDHVNPKAFFSVLRIYLSGWKGNPQLSDGLVYEGF WEDPKEFAGGSAGQSSVFQCFDVLLGIQQTAGGGHAAQFLQ DMRRYMPPAHRNFLCSLESNPSVREFVLSKGDAGLREAYDA CVKALVSLRSYHLQIVTKYILIPASQQPKENKTSEDPSKLEAK GTGGTDLMNFLKTVRSTTEKSLLKEG |
| TDO2: tryptophan 2,3-dioxygenase from homo sapiens **SEQ ID NO: 118** | MSGCPFLGNNFGYTFKKLPVEGSEEDKSQTGVNRASKGGLIY GNYLHLEKVLNAQELQSETKGNKIHDEHLFIITHQAYELWFK QILWELDSVREIFQNGHVRDERNMLKVVSRMHRVSVILKLLV QQFSILETMTALDFNDFREYLSPASGFQSLQFRLLENKIGVLQ NMRVPYNRRHYRDNFKGEENELLLKSEQEKTLLELVEAWLE RTPGLEPHGFNFWGKLEKNITRGLEEEFIRIQAKEESEEKEEQV AEFQKQKEVLLSLFDEKRHEHLLSKGERRLSYRALQGALMIY FYREEPRFQVPFQLLTSLMDIDSLMTKWRYNHVCMVHRMLG SKAGTGGSSGYHYLRSTVSDRYKVFVDLFNLSTYLIPRHWIPK MNPTIHKFLYTAEYCDSSYFSSDESD |
| BNA2: indoleamine 2,3-dioxygenase from S. cerevisiae **SEQ ID NO: 119** | MNNTSITGPQVLHRTKMRPLPVLEKYCISPHHGFLDDRLPLTR LSSKKYMKWEEIVADLPSLLQEDNKVRSVIDGLDVLDLDETIL GDVRELRRAYSILGFMAHAYIWASGTPRDVLPECIARPLLETA HILGVPPLATYSSLVLWNFKVTDECKKTETGCLDLENITTINTF TGTVDESWFYLVSVRFEKIGSACLNHGLQILRAIRSGDKGDA |

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| | NVIDGLEGLAATIERLSKALMEMELKCEPNVFYFKIRPFLAGW TNMSHMGLPQGVRYGAEGQYRIFSGGSNAQSSLIQTLDILLG VKHTANAAHSSQGDSKINYLDEMKKYMPREHREFLYHLESV CNIREYVSRNASNRALQEAYGRCISMLKIFRDNHIQIVTKYIIL PSNSKQHGSNKPNVLSPIEPNTKASGCLGHKVASSKTIGTGGT RLMPFLKQCRDETVATADIKNEDKN |
| Afmid: Kynurenine formamidase from mouse **SEQ ID NO: 120** | MAFPSLSAGQNPWRNLSSEELEKQYSPSRWVIHTKPEEVVGN FVQIGSQATQKARATRRNQLDVPYGDGEGEKLDIYFPDEDSK AFPLFLFLHGGYWQSGSKDDSAFMVNPLTAQGIVVVIVAYDI APKGTLDQMVDQVTRSVVFLQRRYPSNEGIYLCGHSAGAHL AAMVLLARWTKHGVTPNLQGFLLVSGIYDLEPLIATSQNDPL RMTLEDAQRNSPQRHLDVVPAQPVAPACPVLVLVGQHDSPE FHRQSKEFYETLLRVGWKASFQQLRGVDHFDIIENLTREDDV LTQIILKTVFQKL |
| BNA3: kynurenine--oxoglutarate transaminase from S. cerevisae **SEQ ID NO: 121** | MKQRFIRQFTNLMSTSRPKVVANKYFTSNTAKDVWSLTNEA AAKAANNSKNQGRELINLGQGFFSYSPPQFAIKEAQKALDIPM VNQYSPTRGRPSLINSLIKLYSPIYNTELKAENVTVTTGANEGI LSCLMGLLNAGDEVIVFEPFFDQYIPNIELCGGKVVYVPINPPK ELDQRNTRGEEWTIDFEQFEKAITSKTKAVIINTPHNPIGKVFT REELTTLGNICVKHNVVIISDEVYEHLYFTDSFTRIATLSPEIGQ LTLTVGSAGKSFAATGWRIGWVLSLNAELLSYAAKAHTRICF ASPSPLQEACANSINDALKIGYFEKMRQEYINKFKIFTSIFDEL GLPYTAPEGTYFVLVDFSKVKIPEDYPYPEEILNKGKDFRISH WLINELGVVAIPPTEFYIKEHEKAAENLLRFAVCKDDAYLEN AVERLKLLKDYL |
| GOT2: Aspartate aminotransferase, mitochondrial from homo sapiens **SEQ ID NO: 122** | MALLHSGRVLPGIAAAFHPGLAAAAASARASSWWTHVEMGPP DPILGVTEAFKRDTNSKKMNLGVGAYRDDNGKPYVLPSVRK AEAQIAAKNLDKEYLPIGGLAEFCKASAELALGENSEVLKSG RFVTVQTISGTGALRIGASFLQRFFKFSRDVFLPKPTWGNHTPI FRDAGMQLQGYRYYDPKTCGFDFTGAVEDISKIPEQSVLLLH ACAHNPTGVDPRPEQWKEIATVVKKRNLFAFFDMAYQGFAS GDGDKDAWAVRHFIEQGINVCLCQSYAKNMGLYGERVGAFT MVCKDADEAKRVESQLKILIRPMYSNPPLNGARIAAAILNTPD LRKQWLQEVKVMADRIIGMRTQLVSNLKKEGSTHNWQHITD QIGMFCFTGLKPEQVERLIKEFSIYMTKDGRISVAGVTSSNVG YLAHAIHQVTK |
| AADAT: Kynurenine/alpha-aminoadipate aminotransferase, mitochondrial **SEQ ID NO: 123** | MNYARFITAASAARNPSPIRTMTDILSRGPKSMISLAGGLPNP NMFPFKTAVITVENGKTIQFGEEMMKRALQYSPSAGIPELLSW LKQLQIKLHNPPTIHYPPSQGQMDLCVTSGSQQGLCKVFEMII NPGDNVLLDEPAYSGTLQSLHPLGCNIINVASDESGIVPDSLR DILSRWKPEDAKNPQKNTPKFLYTVPNGNNPTGNSLTSERKK EIYELARKYDFLIIEDDPYYFLQFNKFRVPTFLSMDVDGRVIRA DSFSKIISSGLRIGFLTGPKPLIERVILHIQVSTLHPSTFNQLMIS QLLHEWGEEGFMAHVDRVIDFYSNQKDAILAAADKWLTGLA EWHVPAAGMFLWIKVKGINDVKELIEEKAVKMGVLMLPGN AFYVDSSAPSPYLRASFSSASPEQMDVAFQVLAQLIKESL |
| CCLB1: Kynurenine--oxoglutarate | MAKQLQARRLDGIDYNPWVEFVKLASEHDVVNLGQGFPDFP PPDFAVEAFQHAVSGDFMLNQYTKTFGYPPLTKILASFFGELL |

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| transaminase 1 from homo sapiens **SEQ ID NO: 124** | GQEIDPLRNVLVTVGGYGALFTAFQALVDEGDEVIIEPFFDC YEPMTMMAGGRPVFVSLKPGPIQNGELGSSSNWQLDPMELA GKFTSRTKALVLNTPNNPLGKVFSREELELVASLCQQHDVVCI TDEVYQWMVYDGHQHISIASLPGMWERTLTIGSAGKTFSATG WKVGWVLGPDHIMKHLRTVHQNSVFHCPTQSQAAVAESFER EQLLFRQPSSYFVQFPQAMQRCRDHMIRSLQSVGLKPIIPQGS YFLITDISDFKRKMPDLPGAVDEPYDRRFVKWMIKNKGLVAI PVSIFYSVPHQKHFDHYIRFCFVKDEATLQAMDEKLRKWKVE L |
| CCLB2: kynurenine--oxoglutarate transaminase 3 from homo sapiens **SEQ ID NO: 125** | MFLAQRSLCSLSGRAKFLKTISSSKILGFSTSAKMSLKFTNAKR IEGLDSNVWIEFTKLAADPSVVNLGQGFPDISPPTYVKEELSKI AAIDSLNQYTRGFGHPSLVKALSYLYEKLYQKQIDSNKEILVT VGAYGSLFNTIQALIDEGDEVILIVPFYDCYEPMVRMAGATPV FIPLRSKPVYGKRWSSSDWTLDPQELESKFNSKTKAIILNTPHN PLGKVYNREELQVIADLCIKYDTLCISDEVYEWLVYSGNKHL KIATFPGMWERTITIGSAGKTFSVTGWKLGWSIGPNHLIKHLQ TVQQNTIYTCATPLQEALAQAFWIDIKRMDDPECYFNSLPKEL EVKRDRMVRLLESVGLKPIVPDGGYFIIADVSLLDPDLSDMK NNEPYDYKFVKWMTKHKKLSAIPVSAFCNSETKSQFEKFVRF CFIKKDSTLDAAEEIIKAWSVQKS |
| TnaA: tryptophanase from E. coli **SEQ ID NO: 126** | MENFKHLPEPFRIRVIEPVKRTTRAYREEAIIKSGMNPFLLDSE DVFIDLLTDSGTGAVTQSMQAAMMRGDEAYSGSRSYYALAE SVKNIFGYQYTIPTHQGRGAEQIYIPVLIKKREQEKGLDRSKM VAFSNYFFDTTQGHSQINGCTVRNVYIKEAFDTGVRYDFKGN FDLEGLERGIEEVGPNNVPYIVATITSNSAGGQPVSLANLKAM YSIAKKYDIPVVMDSARFAENAYFIKQREAEYKDWTIEQITRE TYKYADMLAMSAKKDAMVPMGGLLCMKDDSFFDVYTECRT LCVVQEGFPTYGGLEGGAMERLAVGLYDGMNLDWLAYRIA QVQYLVDGLEEIGVVCQQAGGHAAFVDAGKLLPHIPADQFP AQALACELYKVAGIRAVEIGSFLLGRDPKTGKQLPCPAELLRL TIPRATYTQTHMDFIIEAFKHVKENAANIKGLTFTYEPKVLRH FTAKLKEV |

[0335] In one embodiment, the tryptophan pathway catabolic enzyme has at least about 80% identity with the entire sequence of one or more of **SEQ ID NO: 99 through SEQ ID NO: 126**. In another embodiment, the tryptophan pathway catabolic enzyme has at least about 85% identity with the entire sequence of one or more **SEQ ID NO: 99 through SEQ ID NO: 126**. In one embodiment, the tryptophan pathway catabolic enzyme has at least about 90% identity with the entire sequence of one or more **SEQ ID NO: 99 through SEQ ID NO: 126**. In one embodiment, the tryptophan pathway catabolic enzyme has at least about 95% identity with the entire sequence of one or more **SEQ ID NO: 99 through SEQ ID NO: 126**. In another embodiment, the tryptophan pathway catabolic enzyme has at least about 96%, 97%, 98%, or 99% identity with the

entire sequence of one or more **SEQ ID NO: 99 through SEQ ID NO: 126**. Accordingly, in one embodiment, the tryptophan pathway catabolic enzyme has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with the entire sequence of one or more **SEQ ID NO: 99 through SEQ ID NO: 126**. In another embodiment, the tryptophan pathway catabolic enzyme comprises the sequence of one or more **SEQ ID NO: 99 through SEQ ID NO: 126**. In yet another embodiment the tryptophan pathway catabolic enzyme consists of the sequence of one or more **SEQ ID NO: 99 through SEQ ID NO: 126**.

[0336] In some embodiments, the genetically engineered bacteria comprise a gene cassette for the production of tryptamine from tryptophan. In some embodiments, the genetically engineered bacteria take up tryptophan through an endogenous or exogenous transporter as described above herein. In som embodiments the bacteria further produce tryptamine from tryptophan. In some embodiments, the genetically engineered bacteria optionally comprise a tryptamine exporter. In some embodiments the genetically engineered bacteria comprise an exporter of one or more indole metabolites, in order to increase the export of indole metabolites produced.

[0337] **Indole 3-propionic acid (IPA)**

[0338] In some embodiments, the genetically engineered bacteria comprise at least one genetic circuit for the producton of indole-3-propionate (IPA). In some embodiments, the indole-3-propionate-producing strain optionally produces tryptophan from a chorismate precursor, and the strain optionally comprises additional circuits for tryptophan production and/or tryptophan uptake/transport s described herein. Additionally the genetically engineered bacteria comprise a circuit, comprising trpDH (Tryptophan dehydrogenase, e.g., from Nostoc punctiforme NIES-2108, which produces (indol-3yl)pyruvate from tryptophan), fldA (indole-3-propionyl-CoA:indole-3-lactate CoA transferase, e.g., from Clostridium sporogenes, which converts converts indole-3-lactate and indol-3-propionyl-CoA to indole-3-propionic acid and indole-3-lactate-CoA), fldB and fldC (indole-3-lactate dehydratase e.g., from Clostridium sporogenes, which converts indole-3-lactate-CoA to indole-3-acrylyl-CoA) fldD and/or AcuI: (indole-3-acrylyl-CoA reductase, e.g., from Clostridium sporogenes and/or acrylyl-CoA reductase, e.g., from Rhodobacter sphaeroides, which convert indole-3-acrylyl-CoA to indole-3-propionyl-CoA). The circuits further comprise *fldH1 and/or fldH2 (*indole-3-lactate dehydrogenase 1

and/or 2, e.g., from Clostridium sporogenes), which converts (indol-3-yl)pyruvate into indole-3-lactate) (see, e.g., **FIG. 44)**.

[0339] **Table 16** depicts non-limiting examples of contemplated polypeptide sequences, which are encoded by the indole-3-propionate producing bacteria.

**Table 16. Non-limiting Examples of Sequences for indole-3-propionate Production**

| Description | Sequence |
|---|---|
| FldA: indole-3-propionyl-CoA:indole-3-lactate CoA transferase from Clostridium sporogenes<br><br>**SEQ ID NO:127** | MENNTNMFSGVKVIELANFIAAPAAGRFFADGGAEVIKIESPA GDPLRYTAPSEGRPLSQEENTTYDLENANKKAIVLNLKSEKGK KILHEMLAEADILLTNWRTKALVKQGLDYETLKEKYPKLVFA QITGYGEKGPDKDLPGFDYTAFFARGGVSGTLYEKGTVPPNV VPGLGDHQAGMFLAAGMAGALYKAKTTGQGDKVTVSLMHS AMYGLGIMIQAAQYKDHGLVYPINRNETPNPFIVSYKSKDDYF VQVCMPPYDVFYDRFMTALGREDLVGDERYNKIENLKDGRA KEVYSIIEQQMVTKTKDEWDKIFRDADIPFAIAQTWEDLLEDE QAWANDYLYKMKYPTGNERALVRLPVFFKEAGLPEYNQSPQI AENTVEVLKEMGYTEQEIEELEKDKDIMVRKEK |
| FldB: subunit of indole-3-lactate dehydratase from Clostridium sporogenes<br><br>**SEQ ID NO:128** | MSDRNKEVKEKKAKHYLREITAKHYKEALEAKERGEKVGWC ASNFPQEIATTLGVKVVYPENHAAAVAARGNGQNMCEHAEA MGFSNDVCGYARVNLAVMDIGHSEDQPIPMPDFVLCCNNICN QMIKWYEHIAKTLDIPMILIDIPYNTENTVSQDRIKYIRAQFDD AIKQLEEITGKKWDENKFEEVMKISQESAKQWLRAASYAKYK PSPFSGFDLFNHMAVAVCARGTQEAADAFKMLADEYEENVKT GKSTYRGEEKQRILFEGIACWPYLRHKLTKLSEYGMNVTATV YAEAFGVIYENMDELMAAYNKVPNSISFENALKMRLNAVTST NTEGAVIHINRSCKLWSGFLYELARRLEKETGIPVVSFDGDQA DPRNFSEAQYDTRIQGLNEVMVAKKEAE |
| FldC: subunit of indole-3-lactate dehydratase from Clostridium sporogenes<br><br>**SEQ ID NO:129** | MSNSDKFFNDFKDIVENPKKYIMKHMEQTGQKAIGCMPLYTP EELVLAAGMFPVGVWGSNTELSKAKTYFPAFICSILQTTLENA LNGEYDMLSGMMITNYCDSLKCMGQNFKLTVENIEFIPVTVPQ NRKMEAGKEFLKSQYKMNIEQLEKISGNKITDESLEKAIEIYDE HRKVMNDFSMLASKYPGIITPTKRNYVMKSAYYMDKKEHTE KVRQLMDEIKAIEPKPFEGKRVITTGIIADSEDLLKILEENNIAIV GDDIAHESRQYRTLTPEANTPMDRLAEQFANRECSTLYDPEKK RGQYIVEMAKERKADGIIFFMTKFCDPEEYDYPQMKKDFEEA GIPHVLIETDMQMKNYEQARTAIQAFSETL |
| FldD: indole-3-acrylyl-CoA reductase from Clostridium sporogenes<br><br>**SEQ ID NO:130** | MFFTEQHELIRKLARDFAEQEIEPIADEVDKTAEFPKEIVKKMA QNGFFGIKMPKEYGGAGADNRAYVTIMEEISRASGVAGIYLSS PNSLLGTPFLLVGTDEQKEKYLKPMIRGEKTLAFALTEPGAGS DAGALATTAREEGDYYILNGRKTFITGAPISDNIIVFAKTDMSK GTKGITTFIVDSKQEGVSFGKPEDKMGMIGCPTSDIILENVKVH KSDILGEVNKGFITAMKTLSVGRIGVASQALGIAQAAVDEAVK YAKQRKQFNRPIAKFQAIQFKLANMETKLNAAKLLVYNAAYK MDCGEKADKEASMAKYFAAESAIQIVNDALQIHGGYGYIKDY KIERLYRDVRVIAIYEGTSEVQQMVIASNLLK |
| FldH1: indole-3- | MKILAYCVRPDEVDSFKKFSEKYGHTVDLIPDSFGPNVAHLAK |

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| lactate dehydrogenase from Clostridium sporogenes<br><br>**SEQ ID NO: 131** | GYDGISILGNDTCNREALEKIKDCGIKYLATRTAGVNNIDFDA AKEFGINVANVPAYSPNSVSEFTIGLALSLTRKIPFALKRVELN NFALGGLIGVELRNLTLGVIGTGRIGLKVIEGFSGFGMKKMIGY DIFENEEAKKYIEYKSLDEVFKEADIITLHAPLTDDNYHMIGKE SIAKMKDGVFIINAARGALIDSEALIEGLKSGKIAGAALDSYEY EQGVFHNNKMNEIMQDDTLERLKSFPNVVITPHLGFYTDEAVS NMVEITLMNLQEFELKGTCKNQRVCK |
| FldH2: indole-3-lactate dehydrogenase from Clostridium sporogenes<br><br>**SEQ ID NO: 132** | MKILMYSVREHEKPAIKKWLEANPGVQIDLCNNALSEDTVCK AKEYDGIAIQQTNSIGGKAVYSTLKEYGIKQIASRTAGVDMIDL KMASDSNILVTNVPAYSPNAIAELAVTHTMNLLRNIKTLNKRI AYGDYRWSADLIAREVRSVTVGVVGTGKIGRTSAKLFKGLGA NVIGYDAYPDKKLEENNLLTYKESLEDLLREADVVTLHTPLLE STKYMINKNNLKYMKPDAFIVNTGRGGIINTEDLIEALEQNKIA GAALDTFENEGLFLNKVVDPTKLPDSQLDKLLKMDQVLITHH VGFFTTTAVQNIVDTSLDSVVEVLKTNNSVNKVN |
| AcuI: acrylyl-CoA reductase from Rhodobacter sphaeroides<br><br>**SEQ ID NO: 133** | MRAVLIEKSDDTQSVSVTELAEDQLPEGDVLVDVAYSTLNYK DALAITGKAPVVRRFPMVPGIDFTGTVAQSSHADFKPGDRVIL NGWGVGEKHWGGLAERARVRGDWLVPLPAPLDLRQAAMIG TAGYTAMLCVLALERHGVVPGNGEIVVSGAAGGVGSVATTLL AAKGYEVAAVTGRASEAEYLRGLGAASVIDRNELTGKVRPLG QERWAGGIDVAGSTVLANMLSMMKYRGVVAACGLAAGMDL PASVAPFILRGMTLAGVDSVMCPKTDRLAAWARLASDLDPAK LEEMTTELPFSEVIETAPKFLDGTVRGRIVIPVTP |

[0340] In one embodiment, the tryptophan pathway catabolic enzyme has at least about 80% identity with the entire sequence of one or more of **SEQ ID NO: 127 through SEQ ID NO: 133**. In another embodiment, the tryptophan pathway catabolic enzyme has at least about 85% identity with the entire sequence of one or more **SEQ ID NO: 127 through SEQ ID NO: 133**. In one embodiment, the tryptophan pathway catabolic enzyme has at least about 90% identity with the entire sequence of one or more **SEQ ID NO: 127 through SEQ ID NO: 133**. In one embodiment, the tryptophan pathway catabolic enzyme has at least about 95% identity with the entire sequence of one or more **SEQ ID NO: 127 through SEQ ID NO: 133**. In another embodiment, the tryptophan pathway catabolic enzyme has at least about 96%, 97%, 98%, or 99% identity with the entire sequence of one or more **SEQ ID NO: 127 through SEQ ID NO: 133**. Accordingly, in one embodiment, the tryptophan pathway catabolic enzyme has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with the entire sequence of one or more **SEQ ID NO: 127 through SEQ ID NO: 133**. In another embodiment, the tryptophan pathway catabolic enzyme comprises the sequence of one or more **SEQ ID NO: 127 through SEQ**

**ID NO: 133**. In yet another embodiment the tryptophan pathway catabolic enzyme consists of the sequence of one or more **SEQ ID NO: 127 through SEQ ID NO: 133.**

[0341] In some embodiments, the genetically engineered bacteria comprise a gene cassette for the production of one or more indole pathway metabolites described herein from tryptophan or a tryptophan metabolite. In some embodiments, the genetically engineered bacteria take up tryptophan through an endogenous or exogenous transporter as described above herein. In some embodiments, the genetically engineered bacteria additionally produce tryptophan and/or chorismate through any of the pathways described herein, e.g. **FIG. 39, FIG45A and FIG. 45B.** In some embodiments the genetically engineered bacteria comprise an exporter of one or more indole metabolites, in order to increase the export of indole metabolites produced.

**[01]** In some embodiments, the genetically engineered bacteria are capable of expressing any one or more of the described circuits in low-oxygen conditions, in the presence of disease or tissue specific molecules or metabolites, in the presence of molecules or metabolites associated with inflammation or an inflammatory response or immune suppression or in the presence of some other metabolite that may or may not be present in the gut, such as arabinose or tetracycline. In some embodiments, any one or more of the described circuits are present on one or more plasmids (*e.g.*, high copy or low copy) or are integrated into one or more sites in the bacterial chromosome. In some embodiments, the tryptophan synthesis and/or tryptophan catabolism cassette(s) is under control of an inducible promoter. Exemplary inducible promoters which may control the expression of the al teast one sequence(s) include oxygen level-dependent promoters (*e.g.*, FNR-inducible promoter), promoters induced by inflammation or an inflammatory response (RNS, ROS promoters), and promoters induced by a metabolite that may or may not be naturally present (e.g., can be exogenously added) in the gut, e.g., arabinose and tetracycline.

[0342] Also, in some embodiments, the genetically engineered bacteria are further capable of expressing any one or more of the described circuits and further comprise one or more of the following: (1) one or more auxotrophies, such as any auxotrophies known in the art and provided herein, *e.g.*, thyA auxotrophy, (2) one or more kill switch circuits, such as any of the kill-switches described herein or otherwise known in the art, (3) one or more antibiotic resistance circuits, (4) one or more transporters for importing biological

molecules or substrates, such any of the transporters described herein or otherwise known in the art, (5) one or more exporters for exporting biological molecules or substrates, such any of the exporters described herein or otherwise known in the art, (6) one or more secretion circuits, such as any of the secretion circuits described herein and otherwise known in the art, and (7) combinations of one or more of such additional circuits.

*Tryptophan Repressor (TrpR)*

[0343] In any of these embodiments, the tryptophan repressor (trpR) optionally may be deleted, mutated, or modified so as to diminish or obliterate its repressor function. Also, in any of these embodiments, the genetically engineered bacteria optionally comprise gene sequence(s) to produce the tryptophan precursor, Chorismate, *e.g.*, sequence(s) encoding aroG, aroF, aroH, aroB, aroD, aroE, aroK, and AroC.

*Tryptophan and Tryptophan Metabolite Transport*

[0344] Metabolite transporters may further be expressed or modified in the genetically engineered bacteria of the invention in order to enhance tryptophan or KP metabolite transport into the cell.

[0345] The inner membrane protein YddG of *E. coli*, encoded by the *yddG* gene, is a homologue of the known amino acid exporters RhtA and YdeD. Studies have shown that YddG is capable of exporting aromatic amino acids, including tryptophan. Thus, YddG can function as a tryptophan exporter or a tryptophan secretion system (or tryptophan secretion protein). Other aromatic amino acid exporters are described in Doroshenko *et al.*, FEMS Microbiol. Lett., 275:312-318 (2007). Thus, in some embodiments, the engineered bacteria optionally further comprise gene sequence(s) encoding YddG. In some embodiments, the engineered bacteria can over-express YddG. In some embodiments, the engineered bacteria optionally comprise one or more copies of yddG gene.

[0346] In some embodiments, the engineered microbe has a mechanism for importing (transporting) Kynurenine from the local environment into the cell. Thus, in some embodiments, the genetically engineered bacteria comprise gene sequence(s) encoding a kynureninase secreter. In some embodiments, the genetically engineered bacteria comprise one or more copies of *aroP, tnaB* or *mtr* gene.

[0347] In some embodiments the genetically engineered bacteria comprise a transporter to facilitate uptake of tryptophan into the cell. Three permeases, Mtr, TnaB, and AroP, are involved in the uptake of L-tryptophan in Escherichia coli. In some

embodiments, the genetically engineered bacteria comprise one or more copies of one or more of Mtr, TnaB, and AroP.

[0348] In some embodiments, the genetically engineered bacteria of the invention also comprise multiple copies of the the transporter gene. In some embodiments, the genetically engineered bacteria of the invention also comprise a transporte gene from a different bacterial species. In some embodiments, the genetically engineered bacteria of the invention comprise multiple copies of a transporter gene from a different bacterial species. In some embodiments, the native transporter gene in the genetically engineered bacteria of the invention is not modified. In some embodiments, the genetically engineered bacteria of the invention comprise a transporter gene that is controlled by its native promoter, an inducible promoter, or a promoter that is stronger than the native promoter, *e.g.*, a GlnRS promoter, a P(Bla) promoter, or a constitutive promoter.

[0349] In some embodiments, the native transporter gene in the genetically engineered bacteria is not modified, and one or more additional copies of the native transporter gene are inserted into the genome under the control of the same inducible promoter that controls expression of the payload, *e.g.*, a FNR promoter, or a different inducible promoter than the one that controls expression of the payload or a constitutive promoter. In alternate embodiments, the native transporter gene is not modified, and a copy of a non-native transporter gene from a different bacterial species is inserted into the genome under the control of the same inducible promoter that controls expression of the payload, *e.g.*, a FNR promoter, or a different inducible promoter than the one that controls expression of the payload or a constitutive promoter.

[0350] In some embodiments, the native transporter gene in the genetically engineered bacteria is not modified, and one or more additional copies of the native transporter gene are present in the bacteria on a plasmid and under the control of the same inducible promoter that controls expression of the payload *e.g.*, a FNR promoter, or a different inducible promoter than the one that controls expression of the payload or a constitutive promoter. In alternate embodiments, the native transporter gene is not modified, and a copy of a non-native transporter gene from a different bacterial species is present in the bacteria on a plasmid and under the control of the same inducible promoter that controls expression of the payload , *e.g.*, a FNR promoter, or a different inducible promoter than the one that controls expression of the payload or a constitutive promoter.

[0351] In some embodiments, the native transporter gene is mutagenized, the mutants exhibiting increased ammonia transport are selected, and the mutagenized transporter gene is isolated and inserted into the genetically engineered bacteria. In some embodiments, the native transporter gene is mutagenized, mutants exhibiting increased ammonia transport are selected, and those mutants are used to produce the bacteria of the invention. The transporter modifications described herein may be present on a plasmid or chromosome.

[0352] In some embodiments, the genetically engineered bacterium is *E. coli* Nissle, and the native transporter gene in *E. coli* Nissle is not modified; one or more additional copies the native *E. coli* Nissle transporter genes are inserted into the *E. coli* Nissle genome under the control of the same inducible promoter that controls expression of the payload *e.g.*, a FNR promoter, or a different inducible promoter than the one that controls expression of the payload or a constitutive promoter. In an alternate embodiment, the native *transporter* gene in *E. coli* Nissle is not modified, and a copy of a non-native *transporter* gene from a different bacterium, *e.g.*, *Lactobacillus plantarum,* is inserted into the *E. coli* Nissle genome under the control of the same inducible promoter that controls expression of the payload, *e.g.*, a FNR promoter, or a different inducible promoter than the one that controls expression of the payload or a constitutive promoter.

[0353] In some embodiments, the genetically engineered bacterium is *E. coli* Nissle, and the native *transporter* gene in *E. coli* Nissle is not modified; one or more additional copies the native *E. coli* Nissle *transporter* genes are present in the bacterium on a plasmid and under the control of the same inducible promoter that controls expression of the payload , *e.g.*, a FNR promoter, or a different inducible promoter than the one that controls expression of the payload, or a constitutive promoter. In an alternate embodiment, the native *transporter* gene in *E. coli* Nissle is not modified, and a copy of a non-native *transporter* gene from a different bacterium, *e.g.*, *Lactobacillus plantarum,* are present in the bacterium on a plasmid and under the control of the same inducible promoter that controls expression of the payload, *e.g.*, a FNR promoter, or a different inducible promoter than the one that controls expression of the payload, or a constitutive promoter.

<u>Secreted Polypeptides</u>

*IL-10*

[0354] 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. Anti-inflammatory properties of human IL-10 include down-regulation of pro-inflammatory cytokines, inhibition of antigen presentation on dendritic cells or suppression of major histocompatibility complex expression. 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: 134** 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.

**Table 17. IL-10 (SEQ ID NO: 134):**

| |
|---|
| ATGAGCCCCGGACAGGGAACTCAAAGCGAGAACAGCTGCACACATTTTCCAGGTAATCTTCCAAAT |
| ATGCTTCGTGACTTGCGTGACGCTTTCTCTCGCGTGAAAACCTTTTTTCAGATGAAGGATCAGTTA |
| GATAATCTGCTGCTGAAAGAATCGCTTCTTGAGGACTTCAAGGGATATCTGGGATGTCAGGCGTTA |
| TCTGAGATGATTCAGTTTTATTTGGAAGAAGTTATGCCCCAGGCTGAGAATCAAGACCCTGACATC |
| AAAGCGCATGTGAATAGCCTGGGCGAGAATCTGAAGACACTGCGCCTGCGTCTTCGCCGCTGTCAC |
| CGTTTTCTGCCTTGCGAAAATAAGAGTAAGGCCGTTGAGCAAGTGAAAAATGCTTTCAACAAGTTA |

CAAGAAAAAGGGATTTACAAAGCTATGTCTGAGTTTGACATTTTCATTAATTACATTGAGGCCTAC
ATGACTATGAAGATTCGCAAT

[0355] Wild type IL-10 (wtIL-10) is a domain swapped dimer whose structural integrity depends on the dimerization of two peptide chains. wtIL-10 was converted to a monomeric isomer by inserting 6 amino acids into the loop connecting the swapped secondary structural elements (see, e.g., Josephson, K. et al. Design and analysis of an engineered human interleukin-10 monomer. J. Biol. Chem. 275, 13552–13557 (2000), and Yoon, S. I. et al. Epstein-Barr Virus IL-10 Engages IL-10R1 by a Two-step Mechanism Leading to Altered Signaling Properties. J. Biol. Chem. 287, 26586–26595 (2012). Monomoerized IL-10 therefore comnprises a small linker which deviates from the wild-type human IL-10 sequence. This linker causes the IL10 to become active as a monomer rather than a dimer (see, e.g., Josephson, K. et al. Design and analysis of an engineered human interleukin-10 monomer. J. Biol. Chem. 275, 13552–13557 (2000), and Yoon, S. I. et al. Epstein-Barr Virus IL-10 Engages IL-10R1 by a Two-step Mechanism Leading to Altered Signaling Properties. J. Biol. Chem. 287, 26586–26595 (2012)).

[0356] Secretion of a monomeric protein may have advantages, avoiding the extra step of dimerization in the periplasmic space. Moreover, there is more flexibility in the selection of appropriate secretion systems. For example, the tat-dependent secretion system secretes polypeptides in a folded fashion. Dimers cannot fold correctly without the formation of disulfide bonds. Disulfide bonds, however, cannot form in the reducing intracellular environment and require the oxidizing environment of the periplasm to form. Therefore, the tat-dependent system may no be appropriate for the secretion of proteins which require dimerization to function properly.

[0357] In some embodiments, the genetically engineered bacteria of the invention are capable of producing monomerized human IL-10. In some embodiments, the genetically engineered bacteria are capable of producing monomerized 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 monomerized IL-10 in low-oxygen conditions. In some embodiments, the genetically engineered bacteria comprise a nucleic acid sequence that encodes monomerized IL-10. In some embodiments, the genetically engineered bacteria comprise a nucleic acid sequence

comprising **SEQ ID NO: 198** 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: 198** or a functional fragment thereof. In some embodiments, the genetically engineered bacteria comprise a sequence which encodes the polypeptide encoded by **SEQ ID NO: 198** or a fragment or functiona variant thereof. In some embodiments, the monomerized IL-10 expressed by the bacteria stimulates IL-10R1 and IL-10R2 and initiates signal transduction. Signaling includes Stat signaling, e.g. through the phosphorylation of Tyr705 and/or Ser727.

[0358] In some embodiments, the genetically engineered bacteria of the invention are capable of producing viral IL-10. Exemplary viral IL-10 homologues encoded by the bacteria include human cytomegalo- (HCMV) and Epstein-Barr virus (EBV) IL-10. Apart from its anti-inflammatory effects, human IL-10 also possesses pro-inflammatory activity, e.g., stimulation of B-cell maturation and proliferation of natural killer cells (Foerster et al., Secretory expression of biologically active human Herpes virus interleukin-10 analogues in Escherichia coli via a modified Sec-dependent transporter construct, BMC Biotechnol. 2013; 13: 82, and references therein). In contrast, viral IL-10 homologues share many biological activities of hIL-10 but, due to selective pressure during virus evolution and the need to escape the host immune system, also display unique traits, including increased stability and lack of immunostimulatory functions (Foerster et al, and references therein). As such, viral counterparts may be useful and possibly more effective than hIL-10 with respect to anti-inflammatory and/or immune suppressing effects.

[0359] In some embodiments, the genetically engineered bacteria are capable of producing viral 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 viral IL-10 in low-oxygen conditions. In some embodiments, the genetically engineered bacteria comprise a nucleic acid sequence that encodes viral IL-10. In some embodiments, the genetically engineered bacteria comprise a nucleic acid sequence comprising **SEQ ID NO: 193 and/or SEQ ID NO: 194** 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: 193**

**and/or SEQ ID NO: 194** or a functional fragment thereof. In some embodiments, the viral d IL-10 expressed by the bacteria stimulates IL-10R1 and IL-10R2 and initiates signal transduction. Signaling includes Stat signaling, e.g. through the phosphorylation of Tyr705 and/or Ser727.

*IL-2*

[0360] 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: 135** 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: 135** 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.

**Table 18. SEQ ID NO: 135**

| **SEQ ID NO: 135** |
|---|
| MAPTSSSTKK TQLQLEHLLL DLQMILNGIN NYKNPKLTRM |
| LTFKFYMPKK ATELKHLQCL EEELKPLEEV LNLAQSKNFH |
| LRPRDLISNI NVIVLELKGS ETTFMCEYAD ETATIVEFLN |
| RWITFCQSII STLT |

*IL-22*

[0361] 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: 136** 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: 136** 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.

**Table 19. SEQ ID NO: 136**

| SEQ ID NO: 136 |
|---|
| MAALQKSVSS FLMGTLATSC LLLLALLVQG GAAAPISSHC RLDKSNFQQP YITNRTFMLA KEASLADNNT DVRLIGEKLF HGVSMSERCY LMKQVLNFTL EEVLFPQSDR FQPYMQEVVP FLARLSNRLS TCHIEGDDLH IQRNVQKLKD TVKKLGESGE IKAIGELDLL FMSLRNACI |

*IL-27*

**[01]** 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: 137** 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: 137** 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.

**Table 20. SEQ ID NO: 137**

| SEQ ID NO: 137 |
|---|
| 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 |

*SOD*

**[02]** 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: 138** 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: 138** 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.

**Table 21. SEQ ID NO: 138**

| SEQ ID NO: 138 |
|---|
| MATKAVCVLK GDGPVQGIIN FEQKESNGPV KVWGSIKGLT EGLHGFHVHE FGDNTAGCTS AGPHFNPLSR KHGGPKDEER HVGDLGNVTA DKDGVADVSI EDSVISLSGD HCIIGRTLVV HEKADDLGKG GNEESTKTGN AGSRLACGVI GIAQ |

*GLP2*

[0362] 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: 139** 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: 139** 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.

**Table 22. SEQ ID NO: 139 GLP-2**

| SEQ ID NO: 139 |
|---|
| HADGSFSDEMNTILDNLAARDFINWLIQTKITD |

[0363] In some embodiments, the genetically engineered bacteria are capable of producing GLP-2 analogs, including but not limited to, Gattex and teduglutide. Teduglutide is a protease resistan analog of GLP-2. It is made up of 33 amino acids and differs from GLP-2 by one amino acid (alanine is substituted by glycine). The significance of this substitution is that teduglutide is longer acting than endogenous GLP-2 as it is more resistant to proteolysis from dipeptidyl peptidase-4.

**Table 23. SEQ ID NO: 140 Teduglutide**

| SEQ ID NO: 140 |
|---|
| HGDGSFSDEMNTILDNLAARDFINWLIQTKITD |

[0364] In some embodiments, the genetically engineered bacteria comprise a nucleic acid sequence encoding **SEQ ID NO: 140** 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: 140** or a functional fragment thereof. In some embodiments, the genetically engineered bacteria are capable of producing Teduglutide under inducing conditions, e.g., under a condition(s) associated with inflammation. In some embodiments, the genetically engineered bacteria are capable of producing Teduglutide in low-oxygen conditions.

*IL-19, IL-20, and/or IL-24*

[0365] 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.

Inhibition of pro-inflammatory molecules

[0366] 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 24**. In some embodiments, the genetically engineered bacteria are capable of inhibiting, removing, degrading, and/or metabolizing one or more inflammatory molecules.

**Table 24**

| 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-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 | Clostridium difficile, F. prausnitzii, Bifidobacterium, Subdoligranulum, Lactobacillus | Detoxification of xenobiotics; indicate gut microbial composition and activity; utilize polyphenols. Urinary hippuric acid may be a biomarker of hypertension and 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, | Bifidobacterium, Roseburia, Lactobacillus, | Impact intestinal permeability, activate intestinebrain- liver neural axis to regulate glucose homeostasis; LPS |

| | | |
|---|---|---|
| phosphatidylcholines, phosphoethanolamines, triglycerides | Klebsiella, Enterobacter, Citrobacter, Clostridium | 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. |

[0367] 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., $PGE_2$.

RNAi, scFV, other mechanisms

[0368] 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.

[0369] 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).

[0370] 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.

[0371] 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.

[0372] 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.

Generation of Bacterial Strains with Enhance Ability to Transport Amino Acids

[0373] Due to their ease of culture, short generation times, very high population densities and small genomes, microbes can be evolved to unique phenotypes in abbreviated timescales. Adaptive laboratory evolution (ALE) is the process of passaging microbes under selective pressure to evolve a strain with a preferred phenotype. Most commonly, this is applied to increase utilization of carbon/energy sources or adapting a strain to environmental stresses (*e.g.*, temperature, pH), whereby mutant strains more capable of growth on the carbon substrate or under stress will outcompete the less adapted strains in the population and will eventually come to dominate the population.

[0374] This same process can be extended to any essential metabolite by creating an auxotroph. An auxotroph is a strain incapable of synthesizing an essential metabolite and must therefore have the metabolite provided in the media to grow. In this scenario, by making an auxotroph and passaging it on decreasing amounts of the metabolite, the resulting dominant strains should be more capable of obtaining and incorporating this essential metabolite.

[0375] For example, if the biosynthetic pathway for producing an amino acid is disrupted a strain capable of high-affinity capture of said amino acid can be evolved via ALE. First, the strain is grown in varying concentrations of the auxotrophic amino acid, until a minimum concentration to support growth is established. The strain is then passaged at that concentration, and diluted into lowering concentrations of the amino acid at regular intervals. Over time, cells that are most competitive for the amino acid – at growth-limiting concentrations – will come to dominate the population. These strains will likely have mutations in their amino acid-transporters resulting in increased ability to import the essential and limiting amino acid.

[0376] Similarly, by using an auxotroph that cannot use an upstream metabolite to form an amino acid, a strain can be evolved that not only can more efficiently import the upstream metabolite, but also convert the metabolite into the essential downstream metabolite. These strains will also evolve mutations to increase import of the upstream metabolite, but may also contain mutations which increase expression or reaction kinetics of downstream enzymes, or that reduce competitive substrate utilization pathways.

[0377] A metabolite innate to the microbe can be made essential via mutational auxotrophy and selection applied with growth-limiting supplementation of the endogenous metabolite. However, phenotypes capable of consuming non-native compounds can be

evolved by tying their consumption to the production of an essential compound. For example, if a gene from a different organism is isolated which can produce an essential compound or a precursor to an essential compound this gene can be recombinantly introduced and expressed in the heterologous host. This new host strain will now have the ability to synthesize an essential nutrient from a previously non-metabolizable substrate.

[0378] Hereby, a similar ALE process can be applied by creating an auxotroph incapable of converting an immediately downstream metabolite and selecting in growth-limiting amounts of the non-native compound with concurrent expression of the recombinant enzyme. This will result in mutations in the transport of the non-native substrate, expression and activity of the heterologous enzyme and expression and activity of downstream native enzymes. It should be emphasized that the key requirement in this process is the ability to tether the consumption of the non-native metabolite to the production of a metabolite essential to growth.

[0379] Once the basis of the selection mechanism is established and minimum levels of supplementation have been established, the actual ALE experimentation can proceed. Throughout this process several parameters must be vigilantly monitored. It is important that the cultures are maintained in an exponential growth phase and not allowed to reach saturation/stationary phase. This means that growth rates must be check during each passaging and subsequent dilutions adjusted accordingly. If growth rate improves to such a degree that dilutions become large, then the concentration of auxotrophic supplementation should be decreased such that growth rate is slowed, selection pressure is increased and dilutions are not so severe as to heavily bias subpopulations during passaging. In addition, at regular intervals cells should be diluted, grown on solid media and individual clones tested to confirm growth rate phenotypes observed in the ALE cultures.

[0380] Predicting when to halt the stop the ALE experiment also requires vigilance. As the success of directing evolution is tied directly to the number of mutations "screened" throughout the experiment and mutations are generally a function of errors during DNA replication, the cumulative cell divisions (CCD) acts as a proxy for total mutants which have been screened. Previous studies have shown that beneficial phenotypes for growth on different carbon sources can be isolated in about 1011.2 CCD1. This rate can be accelerated by the addition of chemical mutagens to the cultures – such as

N-methyl-N-nitro-N-nitrosoguanidine (NTG) – which causes increased DNA replication errors. However, when continued passaging leads to marginal or no improvement in growth rate the population has converged to some fitness maximum and the ALE experiment can be halted.

[0381] At the conclusion of the ALE experiment, the cells should be diluted, isolated on solid media and assayed for growth phenotypes matching that of the culture flask. Best performers from those selected are then prepped for genomic DNA and sent for whole genome sequencing. Sequencing with reveal mutations occurring around the genome capable of providing improved phenotypes, but will also contain silent mutations (those which provide no benefit but do not detract from desired phenotype). In cultures evolved in the presence of NTG or other chemical mutagen, there will be significantly more silent, background mutations. If satisfied with the best performing strain in its current state, the user can proceed to application with that strain. Otherwise the contributing mutations can be deconvoluted from the evolved strain by reintroducing the mutations to the parent strain by genome engineering techniques. See Lee, D.-H., Feist, A. M., Barrett, C. L. & Palsson, B. Ø. Cumulative Number of Cell Divisions as a Meaningful Timescale for Adaptive Laboratory Evolution of Escherichia coli. PLoS ONE 6, e26172 (2011).

[0382] Similar methods can be used to generate E.Coli Nissle mutants that consume or import tryptophan.

Inducible regulatory regions

FNR-dependent regulation

[0383] In some embodiments, the genetically engineered bacteria comprise a promoter that is directly or indirectly induced by exogenous environmental conditions. In certain embodiments, the bacterial cell comprises one or more gene sequence(s) for producing the payload(s). As used herein the term “payload” refers to one or more e.g. anti-inflammation and/or gut barrier function enhancer molecule(s), including but not limited to, butyrate, propionate, acetate, IL10, IL-2, IL-22, IL-27, IL-20, IL-24, IL-19, SOD, GLP2, and/or tryptophan and/or its metabolites. In some embodiments the payload is expressed under the control of the fumarate and nitrate reductase regulator (FNR) promoter. In certain embodiments, the bacterial cell comprises one or more gene sequence(s) for producing the payload(s), e.g., an anti-inflammation and/or gut barrier

function enhancer molecule, which is expressed under the control of the fumarate and nitrate reductase regulator (FNR) promoter. In certain embodiments, the bacterial cell comprises one or more gene sequence(s) for producing the payload(s) which 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 transcriptional regulator, thereby driving production of the therapeutic molecule(s.). In certain embodiments, the genetically engineered bacteria comprise one or more gene sequence(s) 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. 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.

[0384] FNR responsive promoters include, but are not limited to, the FNR responsive promoters listed in the chart, below. Underlined sequences are predicted ribosome binding sites, and bolded sequences are restriction sites used for cloning.

**Table 25. FNR Promoter Sequences**

| FNR Responsive Promoter | Sequence |
|---|---|
| **SEQ ID NO: 141** | GTCAGCATAACACCCTGACCTCTCATTAATTGTTCATGCCGGGCGGCACTATCGTCGTCCGGCCT TTTCCTCTCTTACTCTGCTACGTACATCTATTTCTATAAATCCGTTCAATTTGTCTGTTTTTTGCACA AACATGAAATATCAGACAATTCCGTGACTTAAGAAAATTTATACAAATCAGCAATATACCCCTTA AGGAGTATATAAAGGTGAATTTGATTTACATCAATAAGCGGGGTTGCTGAATCGTTAAGGTAGG CGGTAATAGAAAAGAAATCGAGGCAAAA |
| **SEQ ID NO: 142** | ATTTCCTCTCATCCCATCCGGGGTGAGAGTCTTTTCCCCCGACTTATGGCTCATGCATGCATCAAA AAAGATGTGAGCTTGATCAAAAACAAAAAATATTTCACTCGACAGGAGTATTTATATTGCGCCCG TTACGTGGGCTTCGACTGTAAATCAGAAAGGAGAAAACACCT |
| **SEQ ID NO: 143** | GTCAGCATAACACCCTGACCTCTCATTAATTGTTCATGCCGGGCGGCACTATCGTCGTCCGGCCT TTTCCTCTCTTACTCTGCTACGTACATCTATTTCTATAAATCCGTTCAATTTGTCTGTTTTTTGCACA AACATGAAATATCAGACAATTCCGTGACTTAAGAAAATTTATACAAATCAGCAATATACCCCTTA AGGAGTATATAAAGGTGAATTTGATTTACATCAATAAGCGGGGTTGCTGAATCGTTAA**GGATCC** CTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACAT |

| | |
|---|---|
| **SEQ ID NO: 144** | CATTTCCTCTCATCCCATCCGGGGTGAGAGTCTTTTCCCCCGACTTATGGCTCATGCATGCATCAA AAAAGATGTGAGCTTGATCAAAAACAAAAAATATTTCACTCGACAGGAGTATTTATATTGCGCCC **GGATCC**CTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACAT |
| **SEQ ID NO: 145** | AGTTGTTCTTATTGGTGGTGTTGCTTTATGGTTGCATCGTAGTAAATGGTTGTAACAAAAGCAAT TTTTCCGGCTGTCTGTATACAAAAACGCCGTAAAGTTTGAGCGAAGTCAATAAACTCTCTACCCA TTCAGGGCAATATCTCTCTT**GGATCC**CTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATA CAT |

**[02]** In one embodiment, the FNR responsive promoter comprises **SEQ ID NO: 141**. In another embodiment, the FNR responsive promoter comprises **SEQ ID NO: 142**. In another embodiment, the FNR responsive promoter comprises **SEQ ID NO: 143**. In another embodiment, the FNR responsive promoter comprises **SEQ ID NO: 144**. In yet another embodiment, the FNR responsive promoter comprises **SEQ ID NO: 145**. Additional FNR responsive promoters are shown below.

**Table 26. FNR Promoter sequences**

| FNR-responsive regulatory region | 12345678901234567890123456789012345678901234567890 |
|---|---|
| SEQ ID NO: 146 | ATCCCCATCACTCTTGATGGAGATCAATTCCCCAAGCTGCTAGAGCGTTA CCTTGCCCTTAAACATTAGCAATGTCGATTTATCAGAGGGCCGACAGGCT CCCACAGGAGAAAACCG |
| SEQ ID NO: 147 | CTCTTGATCGTTATCAATTCCCACGCTGTTTCAGAGCGTTACCTTGCCCT TAAACATTAGCAATGTCGATTTATCAGAGGGCCGACAGGCTCCCACAGGA GAAAACCG |
| *nirB1* SEQ ID NO: 148 | GTCAGCATAACACCCTGACCTCTCATTAATTGTTCATGCCGGGCGGCACT ATCGTCGTCCGGCCTTTTCCTCTCTTACTCTGCTACGTACATCTATTTCT ATAAATCCGTTCAATTTGTCTGTTTTTTGCACAAACATGAAATATCAGAC AATTCCGTGACTTAAGAAAATTTATACAAATCAGCAATATACCCCTTAAG GAGTATATAAAGGTGAATTTGATTTACATCAATAAGCGGGGTTGCTGAAT CGTTAAGGTAGGCGGTAATAGAAAAGAAATCGAGGCAAAA |
| *nirB2* SEQ ID NO: 149 | CGGCCCGATCGTTGAACATAGCGGTCCGCAGGCGGCACTGCTTACAGCAA ACGGTCTGTACGCTGTCGTCTTTGTGATGTGCTTCCTGTTAGGTTTCGTC AGCCGTCACCGTCAGCATAACACCCTGACCTCTCATTAATTGCTCATGCC GGACGGCACTATCGTCGTCCGGCCTTTTCCTCTCTTCCCCCGCTACGTGC ATCTATTTCTATAAACCCGCTCATTTTGTCTATTTTTTTGCACAAACATGA AATATCAGACAATTCCGTGACTTAAGAAAATTTATACAAATCAGCAATAT ACCCATTAAGGAGTATATAAAGGTGAATTTGATTTACATCAATAAGCGGG GTTGCTGAATCGTTAAGGTAGGCGGTAATAGAAAAGAAATCGAGGCAAAA atgtttgtttaactttaagaaggagatatacat |

| | |
|---|---|
| *nirB3* SEQ ID NO: 150 | GTCAGCATAACACCCTGACCTCTCATTAATTGCTCATGCCGGACGGCACT ATCGTCGTCCGGCCTTTTCCTCTCTTCCCCCGCTACGTGCATCTATTTCT ATAAACCCGCTCATTTTGTCTATTTTTTGCACAAACATGAAATATCAGAC AATTCCGTGACTTAAGAAAATTTATACAAATCAGCAATATACCCATTAAG GAGTATATAAAGGTGAATTTGATTTACATCAATAAGCGGGGTTGCTGAAT CGTTAAGGTAGGCGGTAATAGAAAAGAAATCGAGGCAAAA |
| *ydfZ* SEQ ID NO: 151 | ATTTCCTCTCATCCCATCCGGGGTGAGAGTCTTTTCCCCCGACTTATGGC TCATGCATGCATCAAAAAAGATGTGAGCTTGATCAAAAACAAAAAATATT TCACTCGACAGGAGTATTTATATTGCGCCCGTTACGTGGGCTTCGACTGT AAATCAGAAAGGAGAAAACACCT |
| *nirB+RBS* SEQ ID NO: 152 | GTCAGCATAACACCCTGACCTCTCATTAATTGTTCATGCCGGGCGGCACT ATCGTCGTCCGGCCTTTTCCTCTCTTACTCTGCTACGTACATCTATTTCT ATAAATCCGTTCAATTTGTCTGTTTTTTGCACAAACATGAAATATCAGAC AATTCCGTGACTTAAGAAAATTTATACAAATCAGCAATATACCCCTTAAG GAGTATATAAAGGTGAATTTGATTTACATCAATAAGCGGGGTTGCTGAAT CGTTAA**GGATCC**CTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATA TACAT |
| *ydfZ+RBS* SEQ ID NO: 153 | CATTTCCTCTCATCCCATCCGGGGTGAGAGTCTTTTCCCCCGACTTATGG CTCATGCATGCATCAAAAAAGATGTGAGCTTGATCAAAAACAAAAAATAT TTCACTCGACAGGAGTATTTATATTGCGCCC**GGATCC**CTCTAGAAATAAT TTTGTTTAACTTTAAGAAGGAGATATACAT |
| *fnrS1* SEQ ID NO: 154 | AGTTGTTCTTATTGGTGGTGTTGCTTTATGGTTGCATCGTAGTAAATGGT TGTAACAAAAGCAATTTTTCCGGCTGTCTGTATACAAAAACGCCGTAAAG TTTGAGCGAAGTCAATAAACTCTCTACCCATTCAGGGCAATATCTCTCTT **GGATCC**CTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACAT |
| *fnrS2* SEQ ID NO: 155 | AGTTGTTCTTATTGGTGGTGTTGCTTTATGGTTGCATCGTAGTAAATGGT TGTAACAAAAGCAATTTTTCCGGCTGTCTGTATACAAAAACGCCGCAAAG TTTGAGCGAAGTCAATAAACTCTCTACCCATTCAGGGCAATATCTCTCTT GGATCCAAAGTGAACTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGA TATACAT |

| | |
|---|---|
| *nirB+crp* SEQ ID NO: 156 | TCGTCTTTGTGATGTGCTTCCTGTTAGGTTTCGTCAGCCGTCACCGTCAG CATAACACCCTGACCTCTCATTAATTGCTCATGCCGGACGGCACTATCGT CGTCCGGCCTTTTCCTCTCTTCCCCCGCTACGTGCATCTATTTCTATAAA CCCGCTCATTTTGTCTATTTTTTGCACAAACATGAAATATCAGACAATTC CGTGACTTAAGAAAATTTATACAAATCAGCAATATACCCATTAAGGAGTA TATAAAGGTGAATTTGATTTACATCAATAAGCGGGGTTGCTGAATCGTTA AGGTAGaaatgtgatctagttcacatttGCGGTAATAGAAAAGAAATCGA GGCAAAA*atgtttgtttaactttaagaaggagatatacat* |
| *fnrS+crp* SEQ ID NO: 157 | AGTTGTTCTTATTGGTGGTGTTGCTTTATGGTTGCATCGTAGTAAATGGT TGTAACAAAAGCAATTTTTCCGGCTGTCTGTATACAAAAACGCCGCAAAG TTTGAGCGAAGTCAATAAACTCTCTACCCATTCAGGGCAATATCTCTCaa atgtgatctagttcacattt*tttgtttaactttaagaaggagatatacat* |

[0385] 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. 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 26**. In some embodiments, the genetically engineered bacteria of the invention comprise one or more of**: SEQ ID NO: 146, SEQ ID NO: 147**, *nirB1* promoter (**SEQ ID NO: 148**), *nirB2* promoter (**SEQ ID NO: 149**), *nirB3* promoter (**SEQ ID NO: 150**), *ydfZ* promoter (**SEQ ID NO: 151**), *nirB* promoter fused to a strong ribosome binding site (**SEQ ID NO: 152**), *ydfZ* promoter fused to a strong ribosome binding site (**SEQ ID NO: 153**), *fnrS*, an anaerobically induced small RNA gene (*fnrS1* promoter **SEQ ID NO: 154** or *fnrS2* promoter **SEQ ID NO: 155**), *nirB* promoter fused to a *crp* binding site (**SEQ ID NO: 156**), and *fnrS* fused to a *crp* binding site (**SEQ ID NO: 157**). 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: 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, or 157,** or a functional fragment thereof.

[0386] In some embodiments, multiple distinct FNR nucleic acid sequences are inserted in the genetically engineered bacteria. In alternate embodiments, the genetically engineered bacteria comprise one or more gene sequence(s) for producing the payload(s) which are expressed under the control of an alternate oxygen level-dependent promoter, *e.g.*, DNR (Trunk *et al.*, 2010) or ANR (Ray *et al.*, 1997). In these embodiments, expression of the payload is particularly activated in a low-oxygen or anaerobic environment, such as in the gut. In one embodiment, the mammalian gut is a human mammalian gut.

[0387] In other embodiments, the one or more gene sequence(s) for producing an anti-inflammation and/or gut barrier function enhancer molecule are 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 one or more gene sequence(s) for producing an anti-inflammation and/or gut barrier function enhancer molecule are 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*.

[0388] 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 transcriptional regulator and/or promoter increases the transcription of genes operably linked to said promoter, *e.g.*, one or more gene sequence(s) for producing the payload(s) in a low-oxygen or anaerobic environment, as compared to the native gene(s) and promoter in the bacteria under the same conditions. In certain embodiments, the non-native oxygen-level dependent transcriptional regulator is an FNR protein from *N. gonorrhoeae (see, e.g.,* Isabella *et al.*, 2011). In some embodiments, the corresponding wild-type transcriptional regulator is left intact and retains wild-type activity. In alternate embodiments, the corresponding wild-type transcriptional regulator is deleted or mutated to reduce or eliminate wild-type activity.

[0389] In some embodiments, the genetically engineered bacteria comprise a wild-type oxygen-level dependent transcriptional regulator, *e.g.*, FNR, ANR, or DNR, and corresponding promoter that is mutated relative to the wild-type promoter from bacteria of the same subtype. The mutated promoter enhances binding to the wild-type transcriptional regulator and increases the transcription of genes operably linked to said promoter, 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.*, FNR, ANR, or DNR promoter, and corresponding transcriptional regulator that is mutated relative to the wild-type transcriptional regulator from bacteria of the same subtype. The mutated transcriptional regulator enhances binding to the wild-type promoter and increases the transcription of genes operably linked to said promoter in a low-oxygen or anaerobic environment, as compared to the wild-type transcriptional regulator under the same conditions. In certain embodiments, the mutant oxygen-level dependent transcriptional regulator is an 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 transcriptional regulator 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.

[0390] In some embodiments, the bacterial cells disclosed herein comprise multiple copies of the endogenous gene encoding the oxygen level-sensing transcriptional regulator, *e.g.*, the *FNR* gene. In some embodiments, the gene encoding the oxygen level-sensing transcriptional regulator is present on a plasmid. In some embodiments, the gene encoding the oxygen level-sensing transcriptional regulator and the one or more gene sequence(s) for producing the payload(s) are present on different plasmids. In some embodiments, the gene encoding the oxygen level-sensing transcriptional regulator and one or more gene sequence(s) for producing the payload(s) are present on different plasmids. In some embodiments, the gene encoding the oxygen level-sensing transcriptional regulator and the one or more gene sequence(s) for producing the payload(s) are present on the same plasmid.

[0391] In some embodiments, the gene encoding the oxygen level-sensing transcriptional regulator is present on a chromosome. In some embodiments, the gene encoding the oxygen level-sensing transcriptional regulator and the one or more gene sequence(s) for producing the payload(s) are present on different chromosomes. In some embodiments, the gene encoding the oxygen level-sensing transcriptional regulator and the one or more gene sequence(s)for producing the payload(s) are present on the same chromosome.

[0392] In some instances, it may be advantageous to express the oxygen level-sensing transcriptional regulator under the control of an inducible promoter in order to enhance expression stability. In some embodiments, expression of the transcriptional regulator is controlled by a different promoter than the promoter that controls expression of the one or more gene sequence(s) for producing the payload(s). In some embodiments, expression of the transcriptional regulator is controlled by the same promoter that controls expression of the one or more gene sequence(s) for producing the payload(s). In some embodiments, the transcriptional regulator and the payload(s) are divergently transcribed from a promoter region.

[0393] 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.

[0394] 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.

[0395] 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.

[0396] 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).

[0397] 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 one more more payload(s), e.g., one or more anti-inflammation and/or gut barrier enhancer molecule(s) 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.

[0398] 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.

[0399] 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

[0400] In some embodiments, the genetically engineered bacteria comprise one or more gene sequence(s) for producing one or more payload(s) which are expressed under

the control of an inducible promoter. In some embodiments, the genetically engineered bacterium that expresses one or more gene sequence(s) for producing the payload(s) are under the control of a promoter that is activated by inflammatory conditions. In one embodiment, the one or more gene sequence(s) for producing the payload(s) are expressed under the control of an inflammatory-dependent promoter that is activated in inflammatory environments, *e.g.*, a reactive nitrogen species or RNS promoter.

[0401] 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 includes, but is not limited to, nitric oxide (NO•), peroxynitrite or peroxynitrite anion (ONOO-), nitrogen dioxide (•NO2), dinitrogen trioxide (N2O3), peroxynitrous acid (ONOOH), and nitroperoxycarbonate (ONOOCO2-) (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.

[0402] 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 one or more gene sequence(s) for producing the payload(s). 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. Thus, RNS induces expression of the gene or gene sequences.

[0403] 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 one or more gene sequence(s) for producing the payload(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 genes.

[0404] 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 sequences. Thus, RNS represses expression of the gene or gene sequences.

[0405] 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 27**.

**Table 27. 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* |

[0406] 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 one or more gene sequence(s) for producing the payload(s), thus controlling expression of the payload(s) relative to RNS levels. For example, the tunable regulatory region is a RNS-inducible regulatory region, and the payload is any of the payloads described herein; 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 payload(s). Subsequently, when inflammation is ameliorated, RNS levels are reduced, and production of the payload(s) is decreased or eliminated.

[0407] 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 genes. 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.

[0408] 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 one or more gene sequence(s) for producing the payload(s). 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 gene, gene(s), or gene cassettes and producing the payload(s).

[0409] 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; **Table 1**). 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 gene or genes and producing one or more payload(s). In some embodiments, the DNR is Pseudomonas aeruginosa DNR.

[0410] 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.

[0411] 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 genes. 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 gene, gene(s), or gene cassettes for producing the payload(s) and producing the payload(s).

[0412] 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.

[0413] 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.

[0414] In these embodiments, the genetically engineered bacteria may comprise a two repressor activation regulatory circuit, which is used to express one or more payload(s). The two repressor activation regulatory circuit comprises a first RNS-sensing repressor and a second repressor, which is operatively linked to one or more gene sequence(s)for producing the payload(s). 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 genes. 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 one or more gene sequence(s) for producing the payload(s) are expressed.

[0415] 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).

[0416] 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.

[0417] 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.

[0418] 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.

[0419] 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.

[0420] 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 payload(s) 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 payload(s) 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 payload(s) in the presence of RNS.

[0421] 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, 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.

[0422] In some embodiments, any of the gene(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 a payload(s) may be integrated into the bacterial chromosome. Having multiple copies of the gene or gen(s) integrated into the chromosome allows for greater production of the payload(s) and also permits fine-tuning of the level of expression. Alternatively, different circuits described herein, such as any of the secretion or exporter 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.

[0423] 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).

[0424] 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.

[0425] 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

[0426] In some embodiments, the genetically engineered bacteria comprise gene, gene(s), or gene cassettes for producing the payload(s) that is expressed under the control of an inducible promoter. In some embodiments, the genetically engineered bacterium that expresses a payload(s) under the control of a promoter that is activated by conditions of cellular damage. In one embodiment, the one or more gene sequence(s) for producing the payload(s) is expressed under the control of a cellular damaged-dependent promoter that is activated in environments in which there is cellular or tissue damage, *e.g.*, a reactive oxygen species or ROS promoter.

[0427] 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 includes, but is not limited to, hydrogen peroxide (H2O2), organic peroxide (ROOH), hydroxyl ion (OH-), hydroxyl radical (•OH), superoxide or superoxide anion (•O2-), singlet oxygen (1O2), ozone (O3), carbonate radical, peroxide or peroxyl radical (•O2-2), 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).

[0428] 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 one or more gene sequence(s) for producing the payload(s). 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 sequences. Thus, ROS induces expression of the gene or genes.

[0429] 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 one or more gene sequence(s) for producing the payload(s). 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.

[0430] 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 sequences. 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 sequences. Thus, ROS represses expression of the gene or gene sequence(s).

[0431] 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 28**.

**Table 28. 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<br>NaOCl | *ohrA* |
| SoxR | $\bullet O_2^-$<br>NO•<br>(also capable of sensing $H_2O_2$) | *soxS* |
| RosR | $H_2O_2$ | *rbtT; tnp16a; rluC1; tnp5a; mscL; tnp2d; phoD; tnp15b; pstA; tnp5b;* |

| ROS-sensing transcription factor: | Primarily capable of sensing: | Examples of responsive genes, promoters, and/or regulatory regions: |
|---|---|---|
| | | *xylC; gabD1; rluC2; cgtS9; azlC; narKGHJI; rosR* |

[0432] In some embodiments, the genetically engineered bacteria 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 one or more payloads, thus controlling expression of the payload(s) 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 gene sequence for the payload(s) thereby producing the payload(s). Subsequently, when inflammation is ameliorated, ROS levels are reduced, and production of the payload(s) is decreased or eliminated.

[0433] 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.

[0434] 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 H2O2 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 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 one or more gene sequence(s) for producing the payload(s). In the presence of ROS, *e.g.*, H2O2, an OxyR transcription factor senses ROS and activates to the oxyS regulatory region, thereby driving expression of the operatively linked payload(s) and producing the payload(s). 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.

[0435] 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 H2O2. 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. In the presence of ROS, the SoxR transcription factor senses ROS and activates the soxS regulatory region, thereby driving expression of the operatively linked gene, gene(s), or gene cassettes for producing the payload(s) and producing the payload(s).

[0436] 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.

[0437] 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 H2O2 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. 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 gene, gene(s), or gene cassettes for producing the payload(s) and producing the payload(s).

[0438] 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).

[0439] 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 H2O2" (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.. In the presence of ROS, *e.g.*, H2O2, a RosR transcription factor senses ROS and no longer binds to the cgtS9 regulatory region, thereby derepressing the operatively linked gene, gene(s), or gene cassettes for producing the payload(s) and producing the payload(s).

[0440] 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.

[0441] 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.

[0442] 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 H2O2" (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**).

[0443] In these embodiments, the genetically engineered bacteria may comprise a two repressor activation regulatory circuit, which is used to express an amino acid catabolism enzyme. 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.*, or more payload(s), . 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.*, an amino acid catabolism enzyme. 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.*, one or more anti-inflammation and/or gut barrier enhancer molecule(s). In the presence of PerR binding (which occurs in the presence of ROS), tetR expression is repressed, and the gene or gene cassette is expressed.

[0444] 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

# DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 3
CONTENANT LES PAGES 1 À 241

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

# JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 3
CONTAINING PAGES 1 TO 241

NOTE: For additional volumes, please contact the Canadian Patent Office

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# Claims

1. A bacterium comprising at least one gene or gene cassette encoding one or more non-native gut barrier function enhancer molecule(s) or biosynthetic pathway(s) for producing a gut barrier function enhancer molecule, wherein at least one of the gut barrier enhancer molecule(s) is selected from an aryl hydrocarbon receptor (AHR) agonist, Pregnane X receptor (PXR) agonist, histone deacetylase (HDAC) inhibitor, GPR41 activator, GPR43 activator, and GPR109A, activator and wherein the at least one gene or gene cassette is operably linked to a directly or indirectly inducible promoter that is not associated with the gene or gene cassette in nature and is induced by exogenous environmental conditions found in a mammalian gut.

2. The bacterium of claim 1, wherein the bacterium further comprises at least one gene or gene cassette encoding one or more non-native anti-inflammation molecule(s).

3. The bacterium of claim 1 or claim 2, wherein the promoter is induced under low-oxygen or anaerobic conditions.

4. The bacterium of claim 3, wherein the promoter is a FNR-responsive promoter, an ANR-responsive promoter, or a DNR-responsive promoter.

5. The bacterium of claim 4, wherein the promoter is a FNR-responsive promoter.

6. The bacterium of claim 1 or claim 2, wherein the promoter is induced by the presence of reactive nitrogen species.

7. The bacterium of claim 6, wherein the promoter is a NsrR-responsive promoter, NorR-responsive promoter, or a DNR-responsive promoter.

8. The bacterium of claim 1 or claim 2, wherein the promoter is induced by the presence of reactive oxygen species.

9. The bacterium of claim 8, wherein the promoter is a OxyR-responsive promoter, PerR-responsive promoter, OhrR-responsive promoter, SoxR-responsive promoter, or a RosR-responsive promoter.

10. The bacterium of any one of claims 1-9, wherein the gene and/or gene cassette is located on a chromosome in the bacterium.

11. The bacterium of any one of claims 1-9, wherein the at least one gene and/or gene cassette is located on a plasmid in the bacterium.

12. The bacterium of any one of claims 1-11, wherein the aryl hydrocarbon receptor (AHR) agonist is selected from indole, indole-3 aldehyde, indole-3 propionic acid, indole-3 acetate, kynurenine, 6-formylindolcarbazole, and kynurenic acid.

13. The bacterium of any one of claims 1-11, wherein the Pregnane X receptor (PXR) agonist is indole 3-propionic acid (IPA).

14. The bacterium of any one of claims 1-11, wherein the histone deacetylase (HDAC) inhibitor is butyrate.

15. The bacterium of any one of claims 1-11, wherein the GPR41 activator is selected from butyrate, propionate, and acetate.

16. The bacterium of any one of claims 1-11, wherein the GPR43 activator is selected from butyrate, propionate, and acetate.

17. The bacterium of any one of claims 1-11, wherein the GPR109A activator is selected from butyrate, kynurenic acid, and niacin.

18. The bacterium of any one of claims 1-17, wherein the anti-inflammation and/or gut barrier enhancer molecule is selected from a short-chain fatty acid, propionate, butyrate, acetate, IL-10 (human), IL-10 (viral), IL-2, IL-19, IL-20, IL-22, IL-24, IL-27, TGF-ß2, TGF-ß1, GLP-2, elafin, trefoil factor, superoxide dismutase (SOD), GLP-2, GLP-2

analog, GLP-1, N-acylphosphatidylethanolamines (NAPEs), melatonin, tryptophan, tryptophan metabolite, $PGD_2$, kynurenic acid, indole, and indole metabolite.

19. The bacterium of claim 18, wherein the anti-inflammation and/or gut barrier enhancer molecule is a short-chain fatty acid.

20. The bacterium of claim 19, wherein the anti-inflammation and/or gut barrier enhancer molecule is selected from propionate, butyrate, and acetate.

21. The bacterium of claim 18, wherein the anti-inflammation and/or gut barrier enhancer molecule is selected from IL-10, IL-22, and GLP-2.

22. The bacterium of claim 19, further comprising an anti-inflammation and/or gut barrier enhancer molecule selected from IL-10, IL-22, and GLP-2.

23. The bacterium of any one of claims 1-17, wherein 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.

24. The bacterium of claim 23, further comprising one or more anti-inflammation and/or gut barrier enhancer molecule(s) selected from butyrate, propionate, acetate, IL-10, IL-22, and GLP-2.

25. The bacterium of any one of claims 1-24, wherein the bacterium has one or more native genes that have been mutated or deleted, wherein the native genes are selected from lpp, pal, tolA, nlpl, degP, and ompT.

26. The bacterium of any one of claims 1-25, wherein the anti-inflammation and/or gut barrier enhancer molecule comprises an N-terminal secretion tag.

27. The bacterium of claim 26, wherein the N-terminal secretion tag facilitates sec-dependent secretion of the anti-inflammation and/or gut barrier enhancer molecule.

28. The bacterium of claim 26, wherein the N-terminal secretion tag facilitates tat-dependent secretion of the anti-inflammation and/or gut barrier enhancer molecule.

29. The bacterium of claim 27, wherein the N-terminal secretion tag is selected from PhoA, OmpF, OmpA, and CvaC.

30. The bacterium of claim 28, wherein the N-terminal secretion tag is selected from TorA, FdnG, and DmsA.

31. The bacterium of any of claims 1-24, wherein the gene(s) or gene cassette(s) encoding the anti-inflammation and/or gut barrier enhancer molecule(s) comprises a 5'-UTR sequence derived from the native fliC gene.

32. The bacterium of claim 31, wherein the gene(s) or gene cassette(s) encoding the anti-inflammation and/or gut barrier enhancer molecule(s) further comprise gene sequence encoding at least the first 20 amino acids of FliC.

33. The bacterium of any one of claims 1-32, wherein the bacterium is a probiotic bacterium.

34. The bacterium of claim 33, wherein the bacterium is selected from the group consisting of *Bacteroides, Bifidobacterium, Clostridium, Escherichia, Lactobacillus*, and *Lactococcus*.

35. The bacterium of claim 34, wherein the bacterium is *Escherichia coli* strain Nissle.

36. The bacterium of any one of claims 1-35, wherein the bacterium is an auxotroph in a gene that is complemented when the bacterium is present in a mammalian gut.

37. The bacterium of claim 36, wherein the bacterium is an auxotroph in diaminopimelic acid or an enzyme in the thymine biosynthetic pathway.

38. A pharmaceutically acceptable composition comprising the bacterium of any one of claims 1-37; and a pharmaceutically acceptable carrier.

39. The composition of claim 38 formulated for oral or rectal administration.

40. A method of treating or preventing an autoimmune disorder, comprising the step of administering to a patient in need thereof, the composition of any one of claims 38 or 39.

41. A method of treating a disease or condition associated with gut inflammation and/or compromised gut barrier function comprising the step of administering to a patient in need thereof, the composition of any one of claims 38 or 39.

42. The method of claim 40, wherein 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,

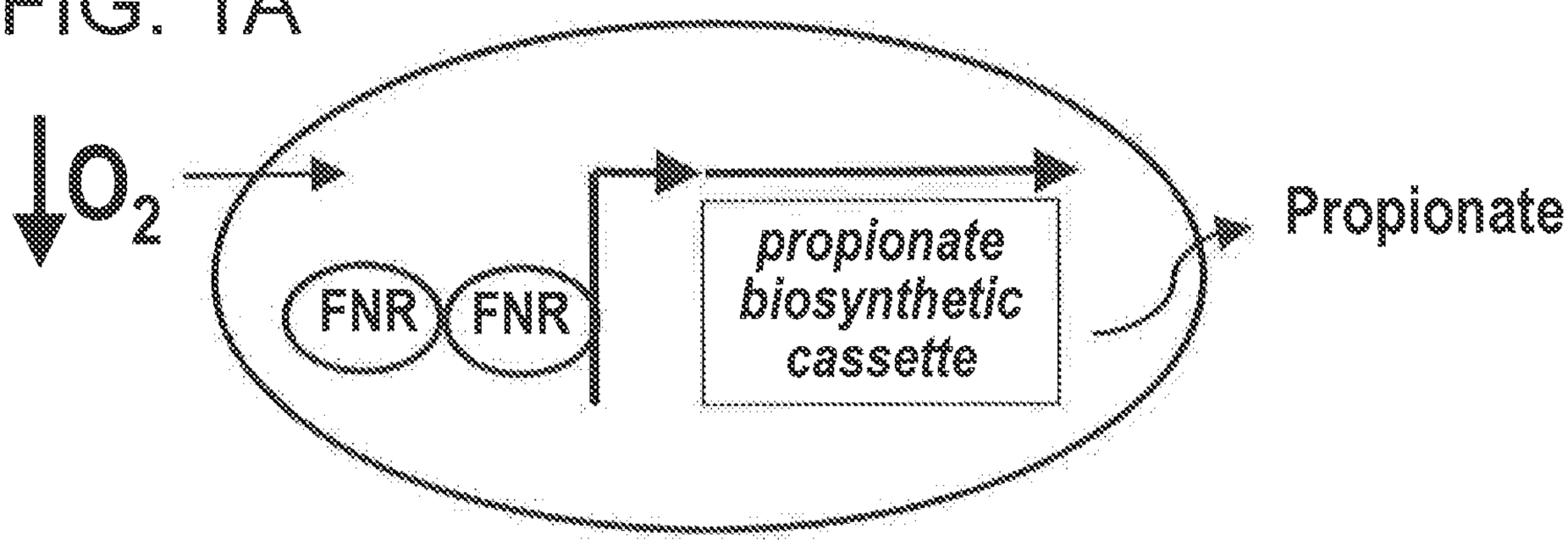
Dermatomyositis, Devic's disease (neuromyelitis optica), Discoid lupus, Dressler's syndrome, Endometriosis, Eosinophilic esophagitis, Eosinophilic fasciitis, Erythema nodosum, Experimental allergic encephalomyelitis, Evans syndrome, Fibrosing alveolitis, Giant cell arteritis (temporal arteritis), Giant 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.

43. The method of claim 42, wherein 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.

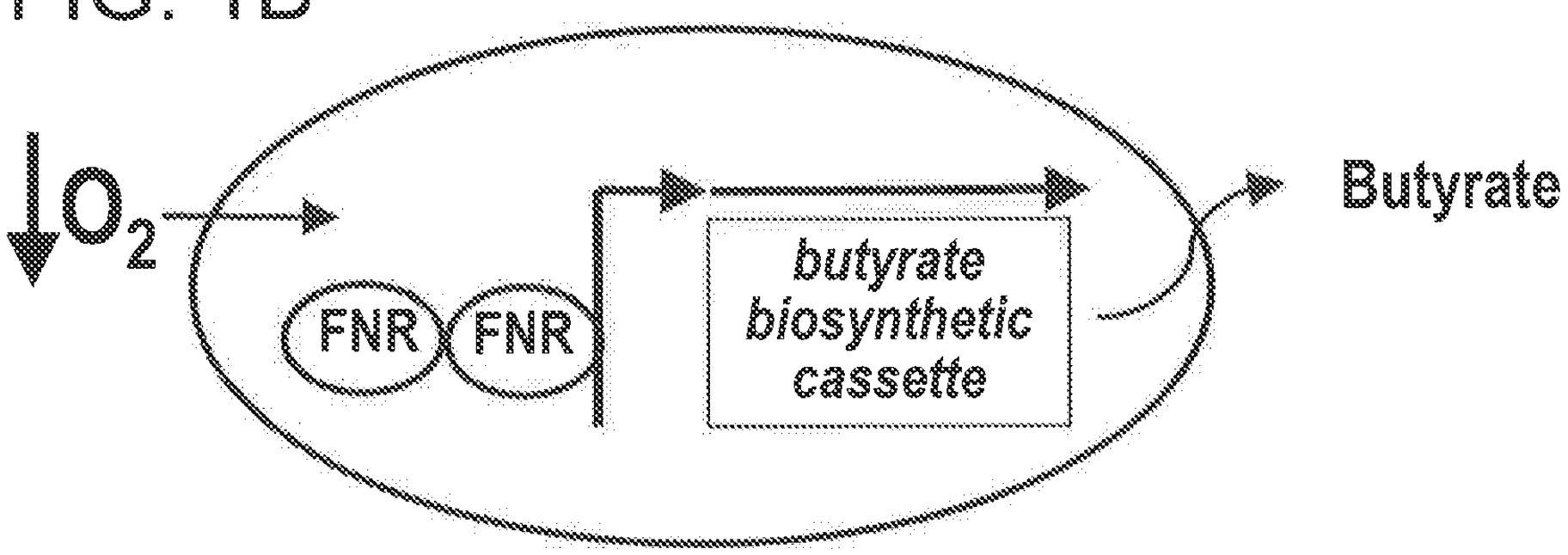
44. The method of claim 41, wherein the disease or condition is selected from an inflammatory bowel disease, including Crohn's disease and ulcerative colitis, and a diarrheal disease.

FIG. 1A



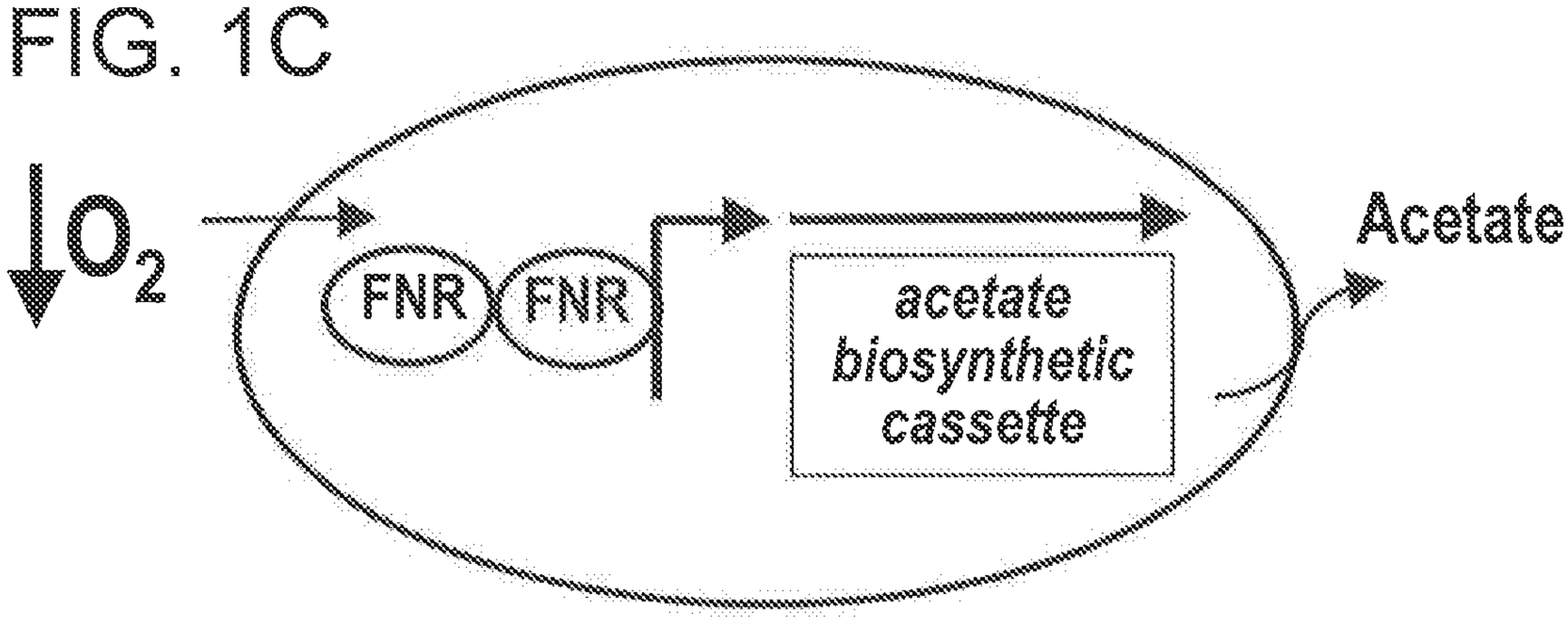

*E. Coli* Nissle 1917

FIG. 1B



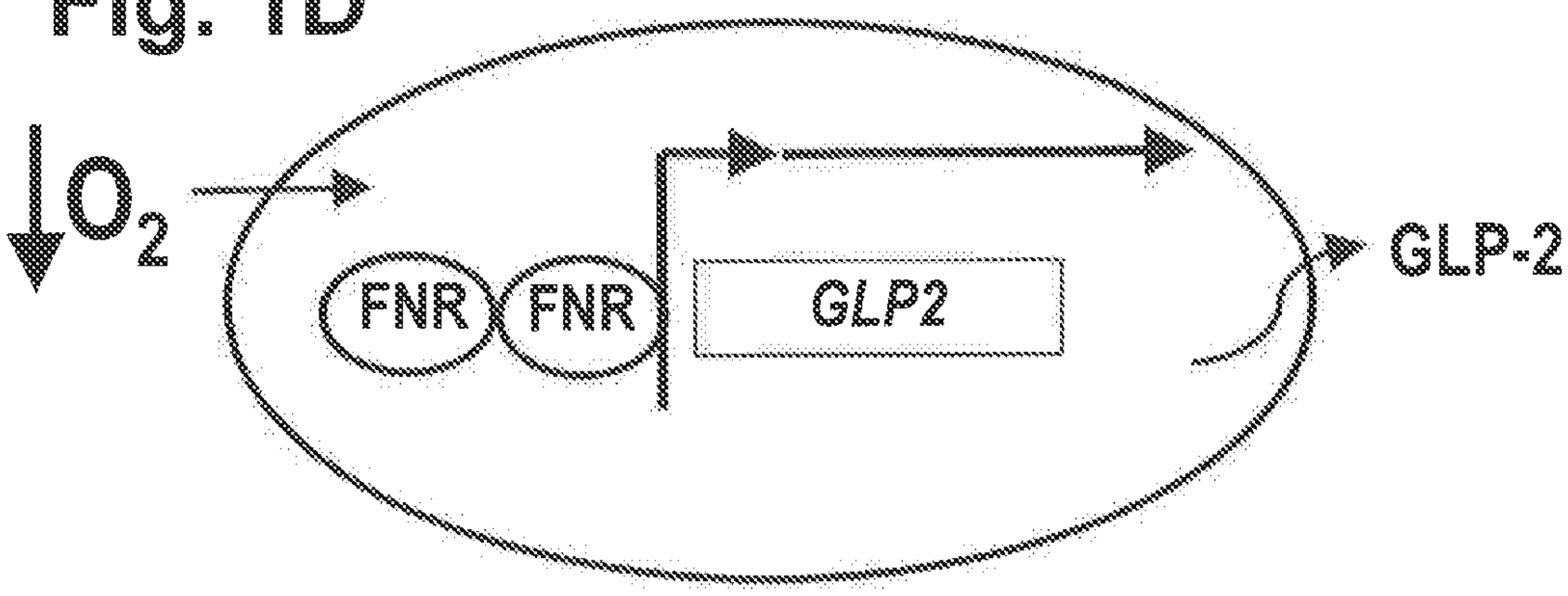

*E. Coli* Nissle 1917

FIG. 1C



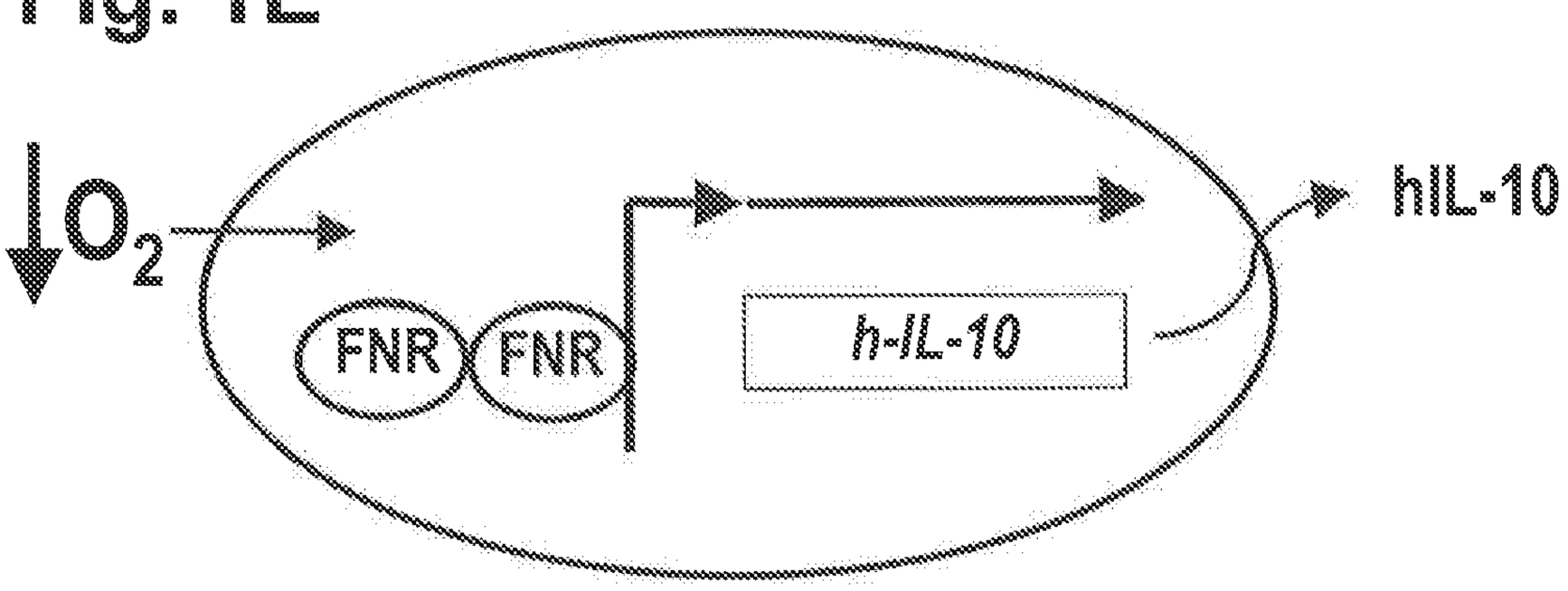

*E. Coli* Nissle 1917

Fig. 1D



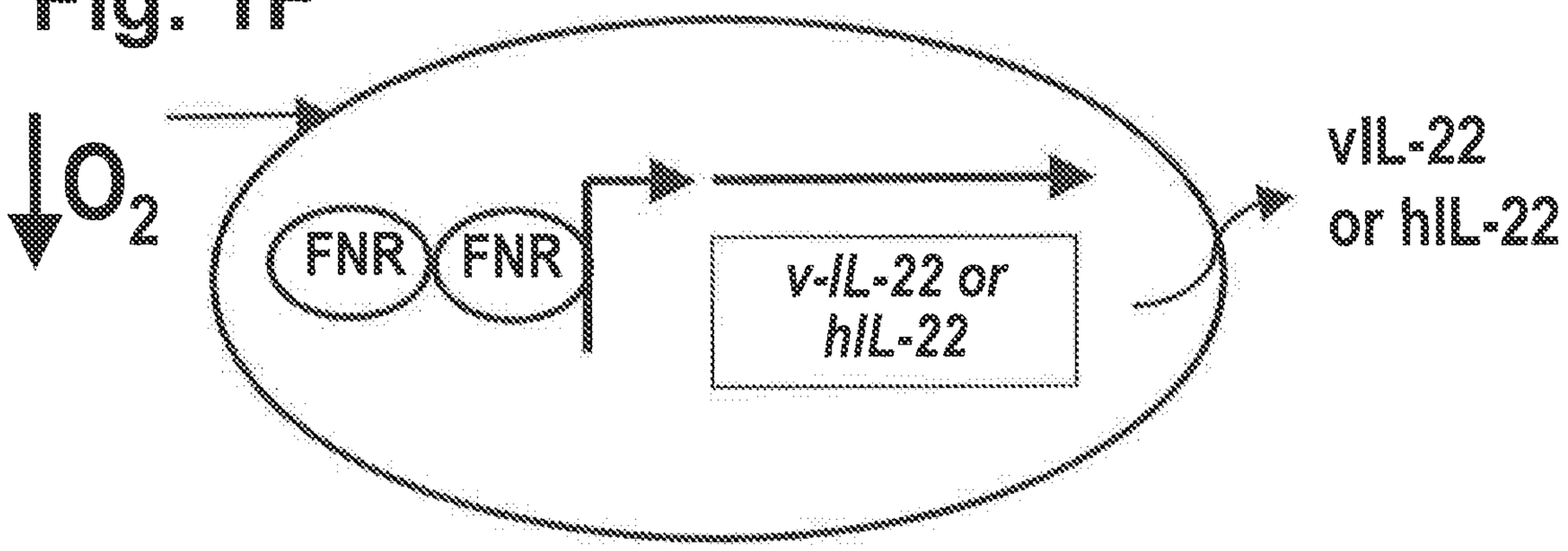

*E. Coli* Nissle 1917

Fig. 1E




*E. Coli* Nissle 1917

Fig. 1F




*E. Coli* Nissle 1917

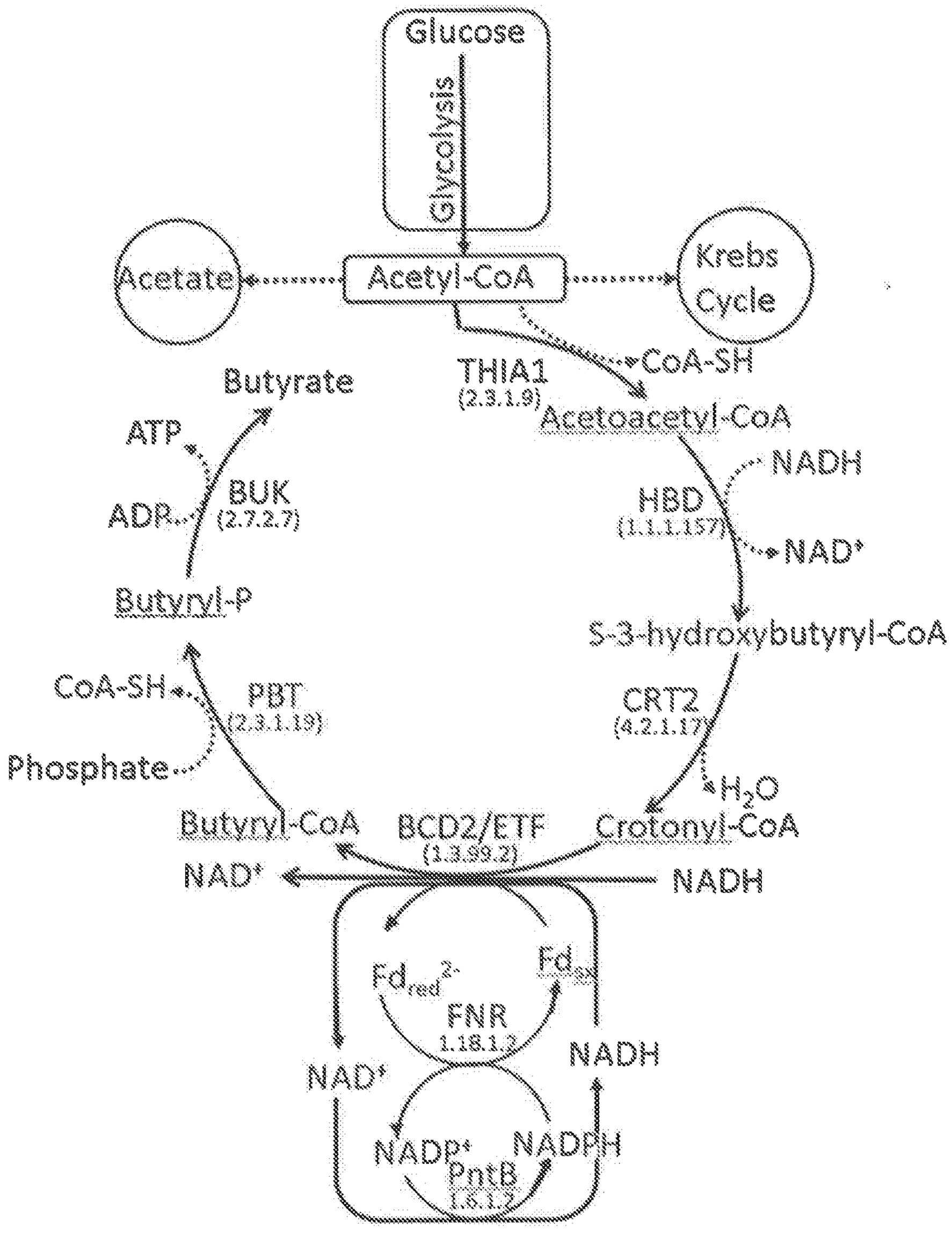

Glucose
Glycolysis
Acetate
Acetyl-CoA
Krebs Cycle
THIA1 (2.3.1.9)
CoA-SH
Acetoacetyl-CoA
Butyrate
ATP
ADR
BUK (2.7.2.7)
NADH
HBD (1.1.1.157)
NAD+
Butyryl-P
S-3-hydroxybutyryl-CoA
CoA-SH
PBT (2.3.1.19)
CRT2 (4.2.1.17)
Phosphate
H2O
Butyryl-CoA
BCD2/ETF (1.3.99.2)
Crotonyl-CoA
NAD+
NADH
Fdred2-
Fdox
FNR 1.18.1.3
NADH
NAD+
NADP+
NADPH
PntB 1.6.1.2

FIG. 2A

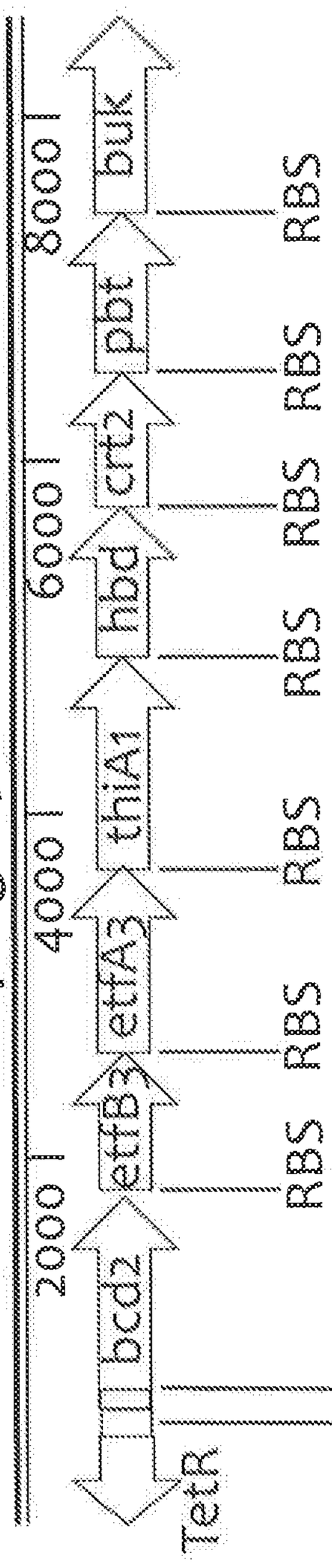

pLogic031
2000 bp
tetR/tetA promoters
TetR
bcd2
RBS and leader region
RBS
etfB2
etfA3
thiA1
4000 bp
hbd
crt2
bukpbt
6000 bp
8000 bp

FIG. 2B

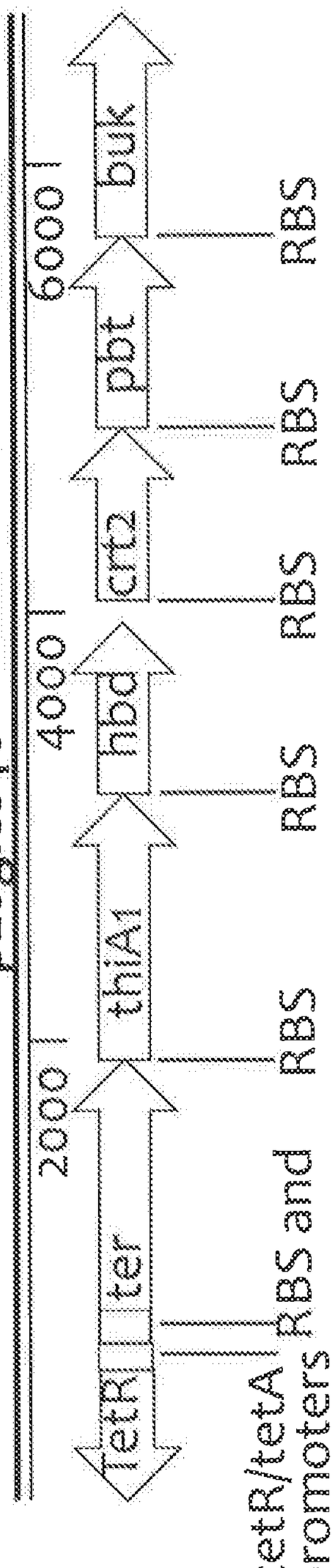

pLogic046
2000 bp
tetR/tetA promoters
TetR
ter
RBS and leader region
RBS
thiA1
4000 bp
hbd
crt2
pbt
bukRBS
6000 bp

FIG. 2C

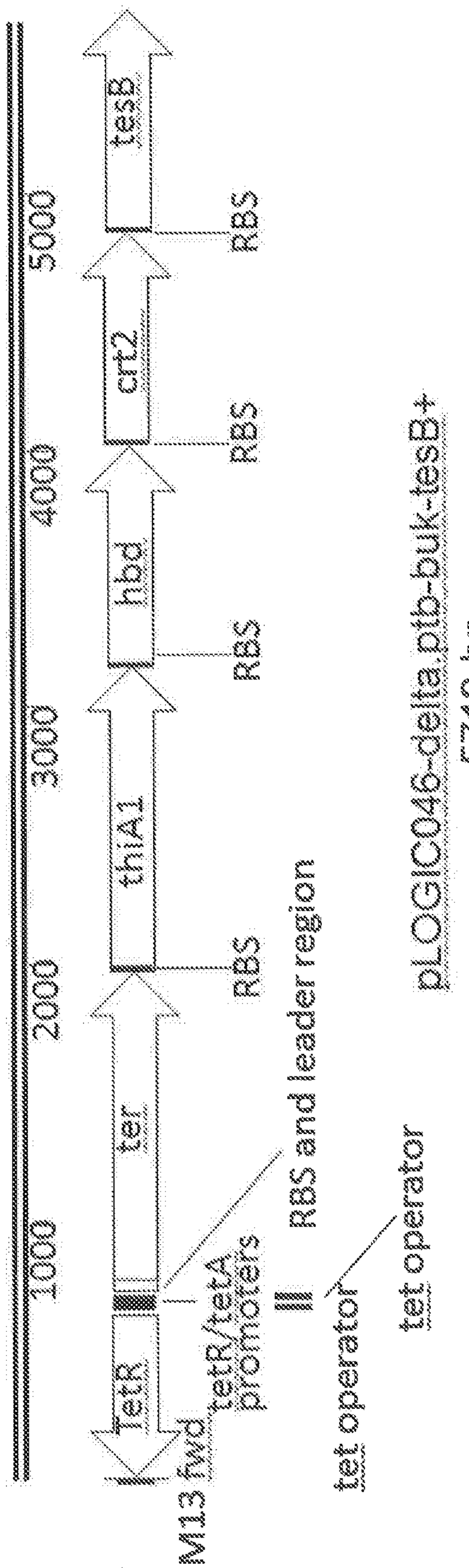

5000
4000
3000
2000
1000
M13 fwd
tetR
PtetR/tetA promoters
RBS and leader region
tet operator
tet operator
ter
thiA1
RBS
hbd
RBS
crt2
RBS
tesB
RBS
pLOGI046-delta.ptb-buk-tesB+
5719 bp

FIG. 2D

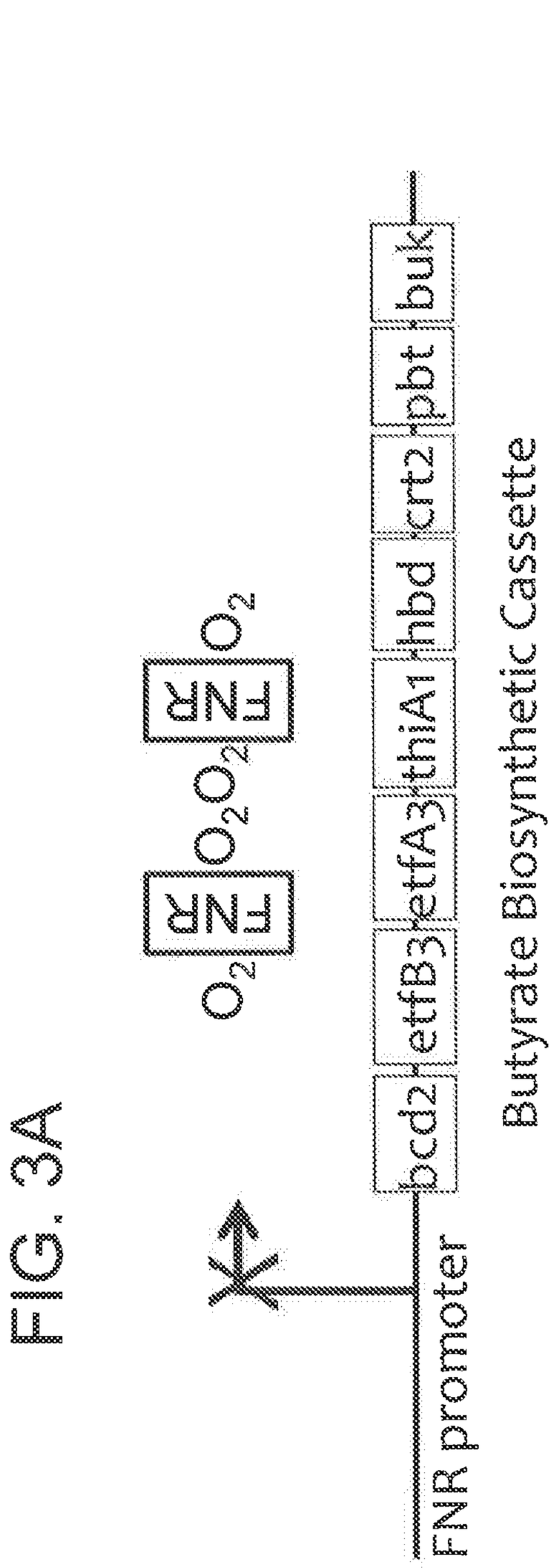

FNR promoter
bcd2
etfB3
etfA3
thiA1
hbd
crt2
pbt
buk
Butyrate Biosynthetic Cassette
$O_2$
$O_2$
$O_2$
$O_2$

FIG. 3A

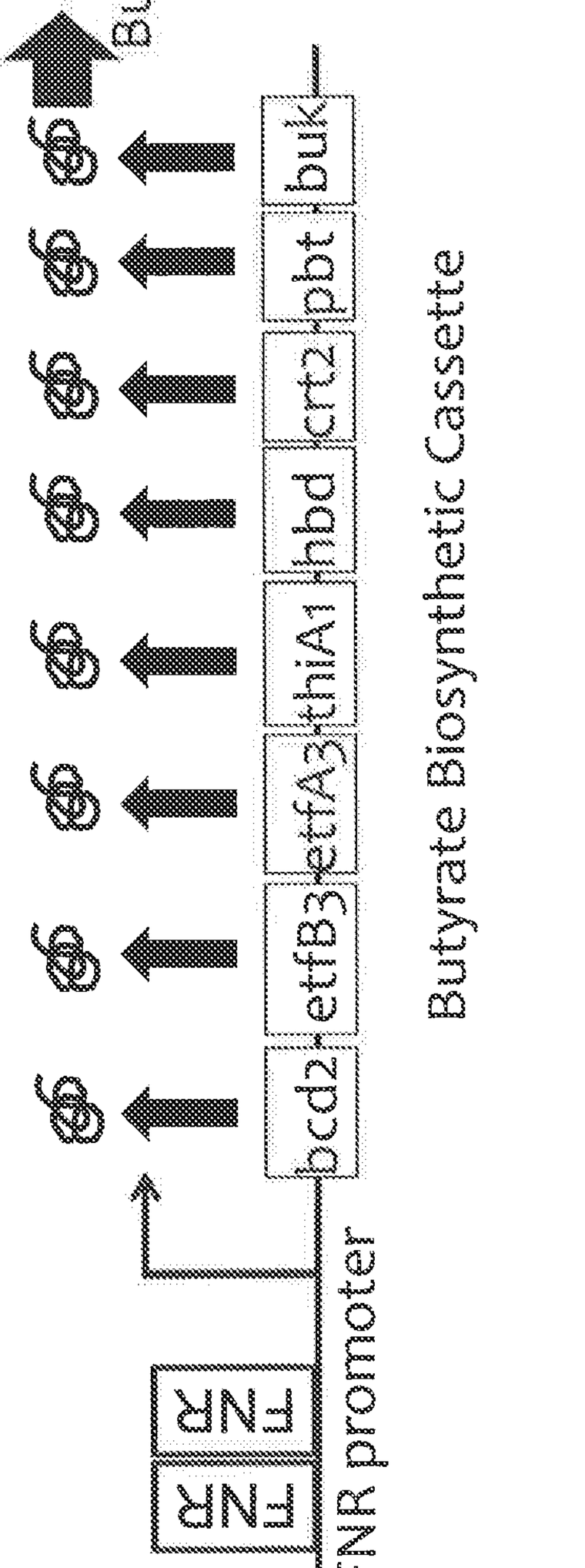
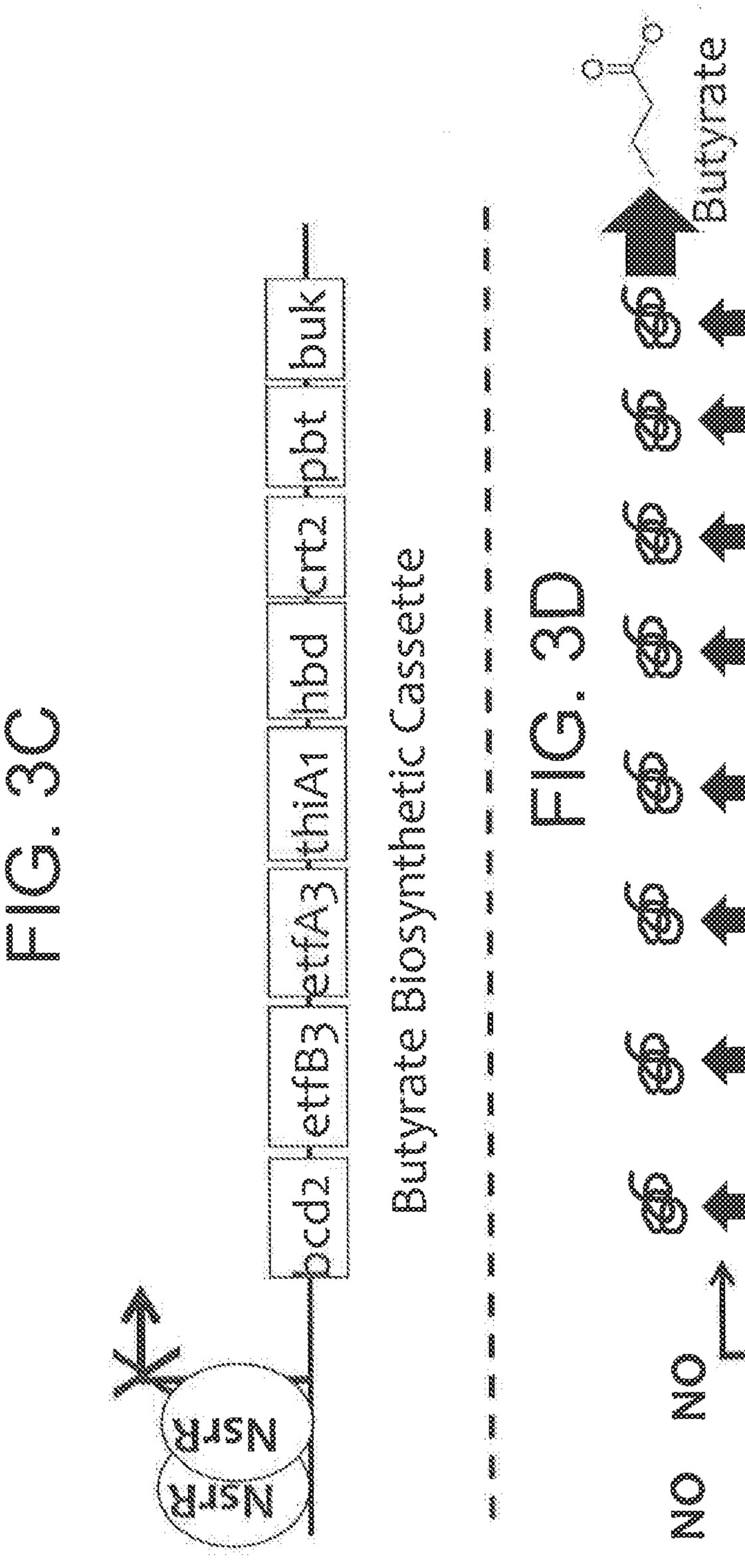

FNR promoter
FNR
FNR
bcd2
etfB3
etfA3
thiA1
hbd
crt2
pbt
buk
Butyrate Biosynthetic Cassette

FIG. 3B


FIG. 3C
Butyrate Biosynthetic Cassette
pcd2
etfB3
etfA3
bhbA1
hbd
crt2
pbt
buk
NSTR
NO
Butyrate Biosynthetic Cassette
FIG. 3D
Butyrate

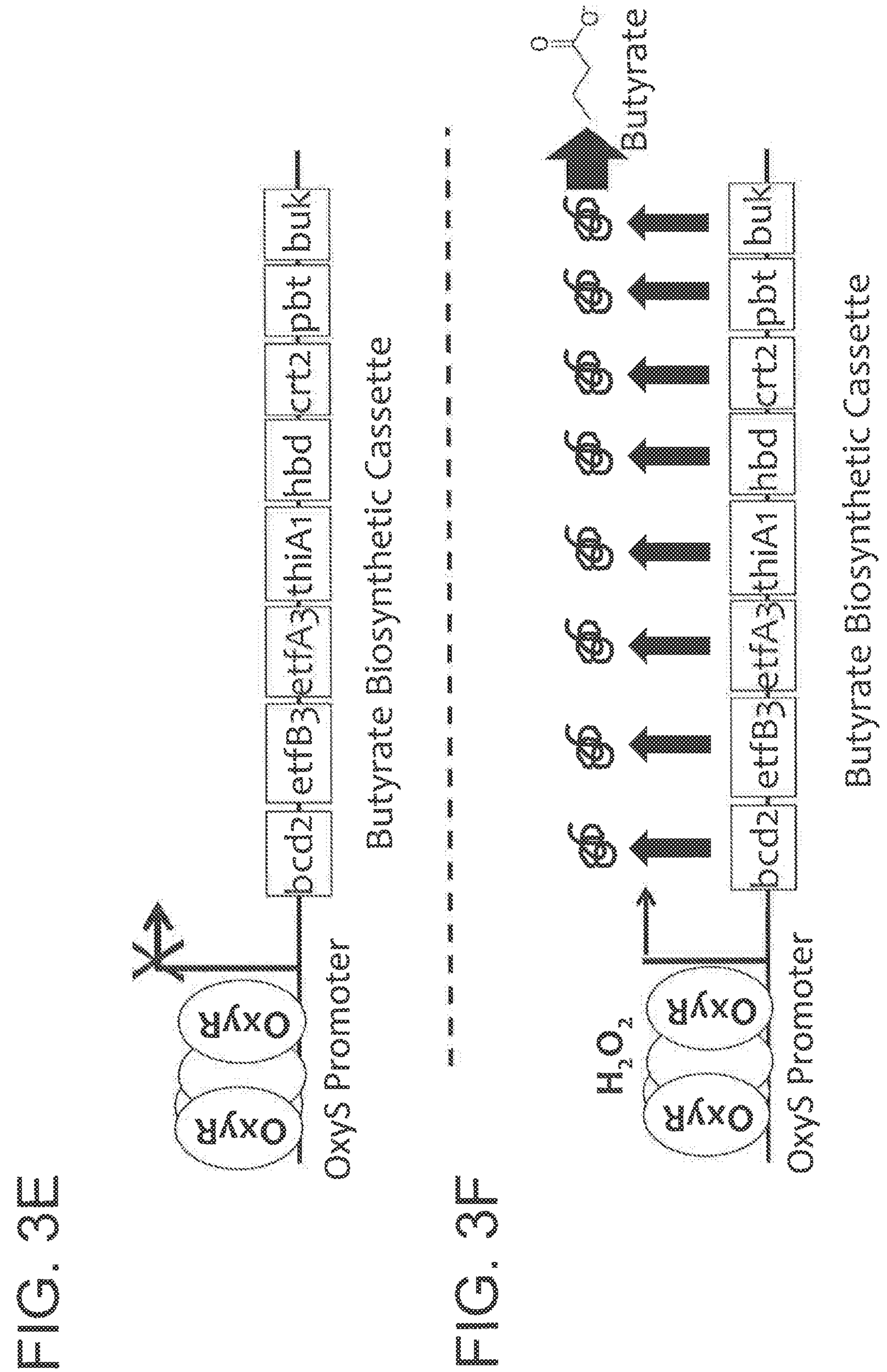

Butyrate
Butyrate Biosynthetic Cassette
bcd2 etfB3 etfA3 thiA1 hbd crt2 pbt
OxyS Promoter
OxyR
OxyR
$H_2O_2$

FIG. 3

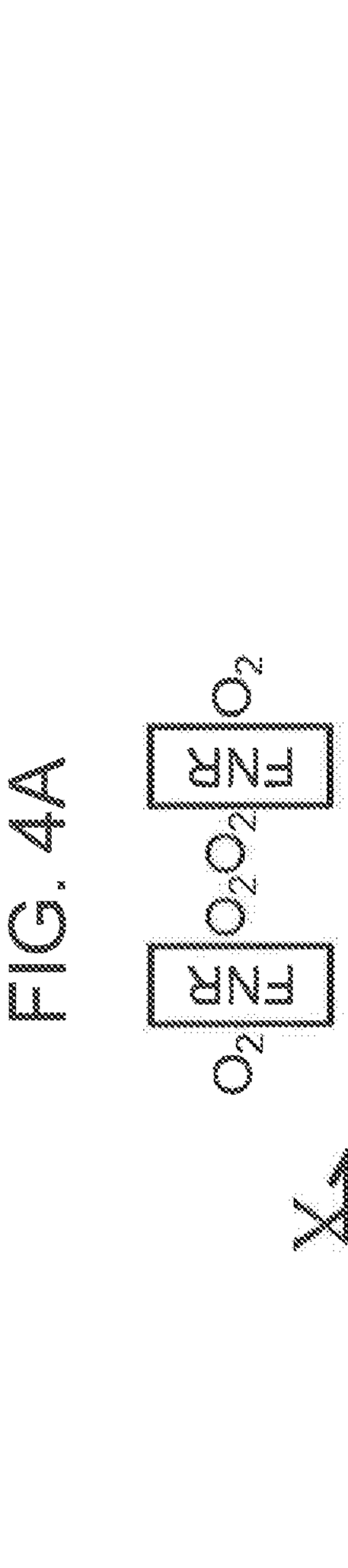

FNR
FNR
$O_2$
$O_2$
$O_2$
$O_2$
FNR promoter
ter
thiA1
hbd
crt2
pbt
buk
Butyrate Biosynthetic Cassette

FIG. 4A

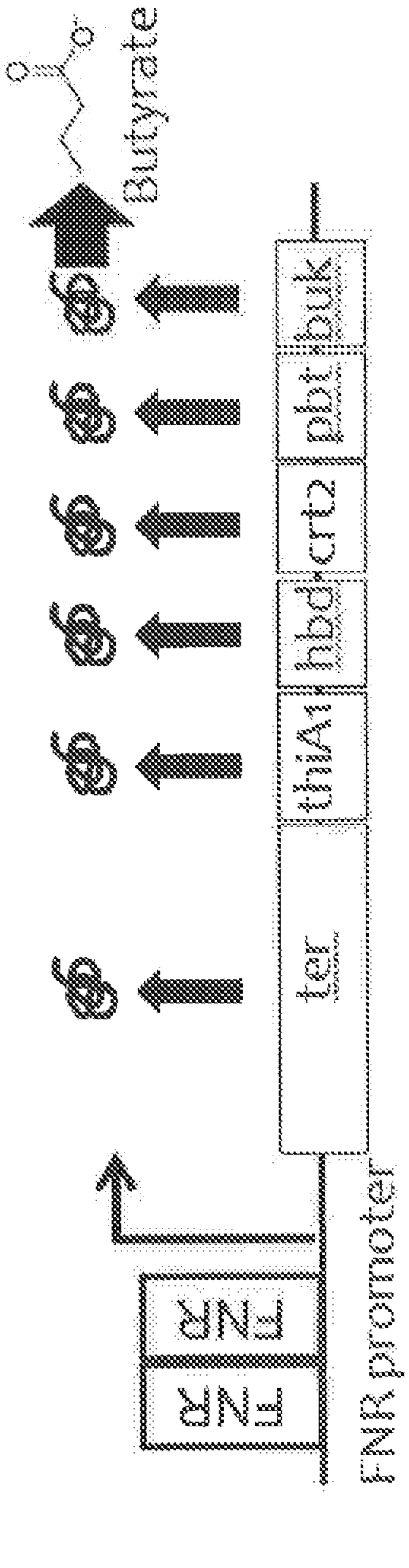

butyrate
FNR
FNR
FNR promoter
ter
thiA1
hbd
crt2
pbt
buk
Butyrate Biosynthetic Cassette

FIG. 4B


thiA1
hbd
crt2
pbt
buk
ter
Butyrate Biosynthetic Cassette
NSfR
NSfR

FIG. 4C

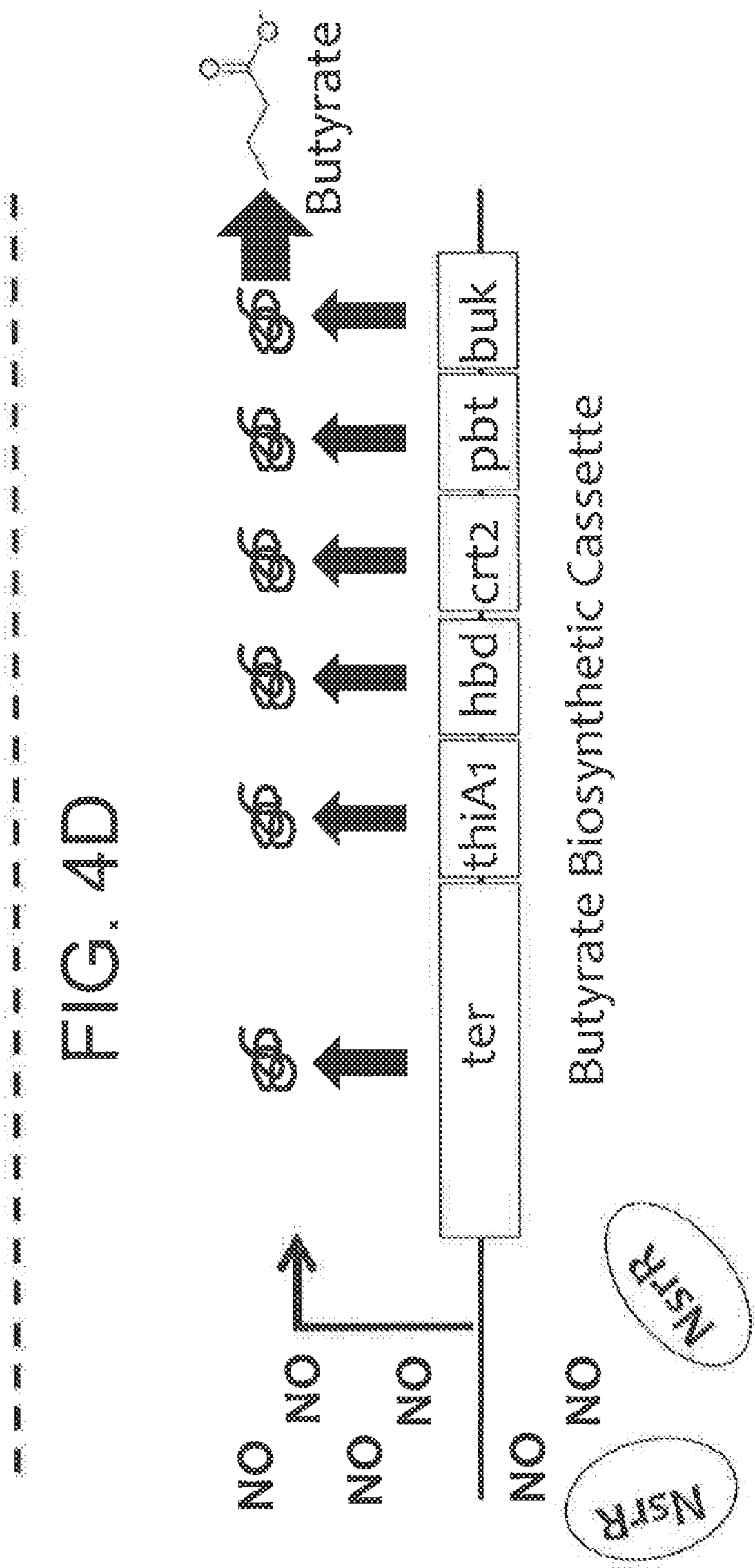

thiA1
hbd
crt2
pbt
buk
ter
Butyrate Biosynthetic Cassette
NO
NSfR
NSfR

FIG. 4D

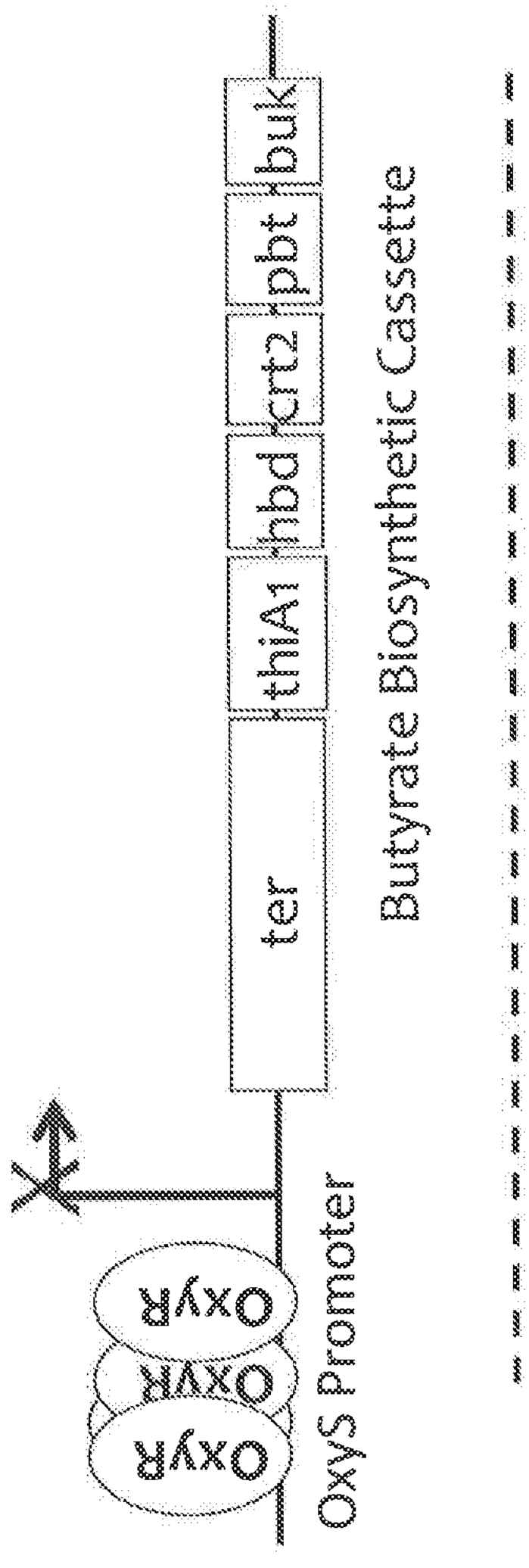

Butyrate Biosynthetic Cassette
ter
thiA1
hbd
crt2
pbt
buk
OxyS Promoter
OxyR
OxyR
OxyR

FIG. 4E

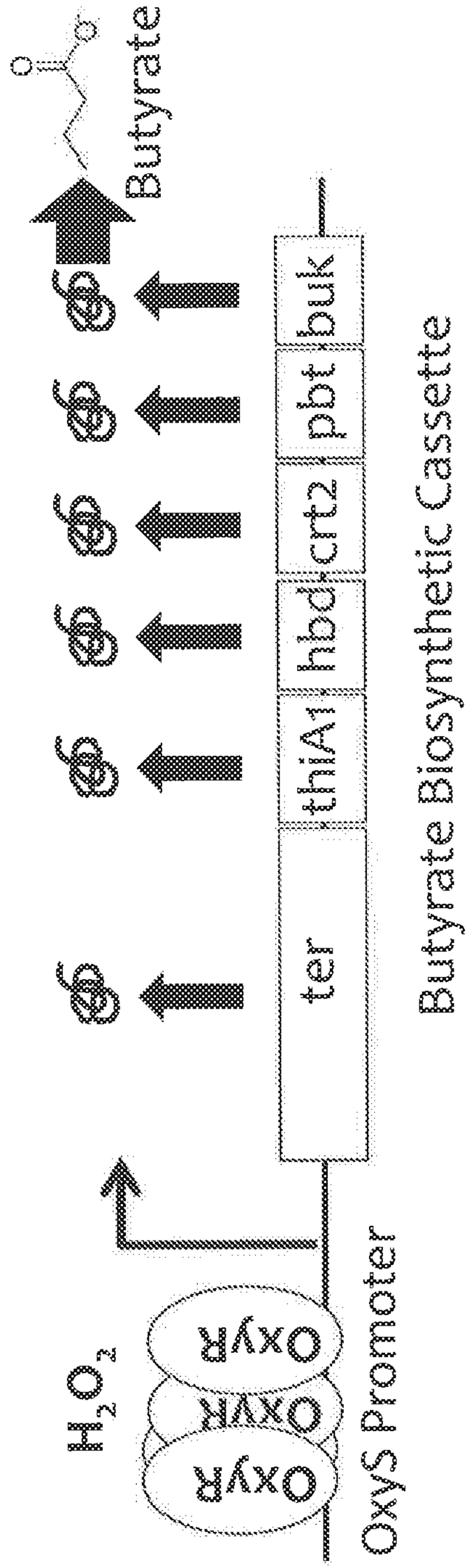

Butyrate
Butyrate Biosynthetic Cassette
ter
thiA1
hbd
crt2
pbt
buk
OxyS Promoter
$H_2O_2$
OxyR
OxyR
OxyR

FIG. 4F

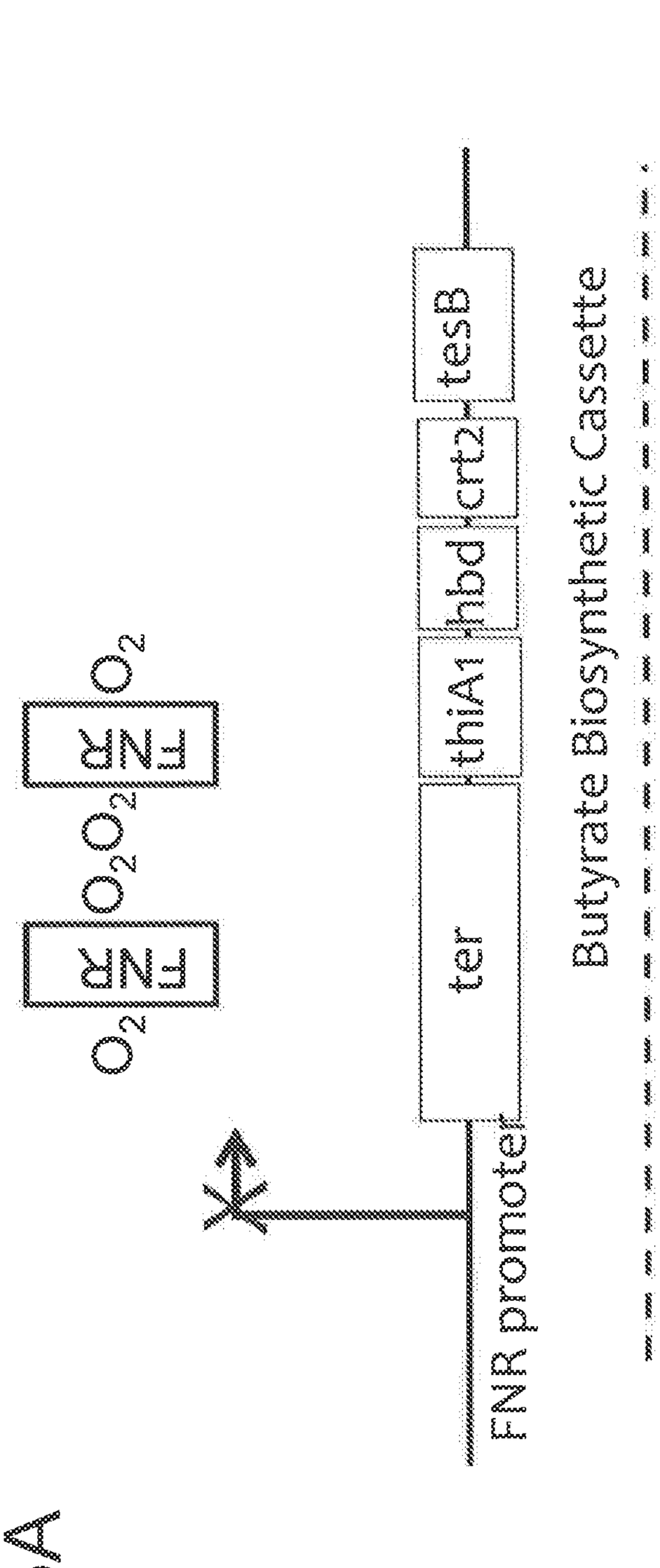

FNR
$O_2$
$O_2$
FNR
$O_2$
$O_2$
FNR
FNR promoter
ter
tesB
hbd,crt2
thiA1
Butyrate Biosynthetic Cassette

FIG. 5A

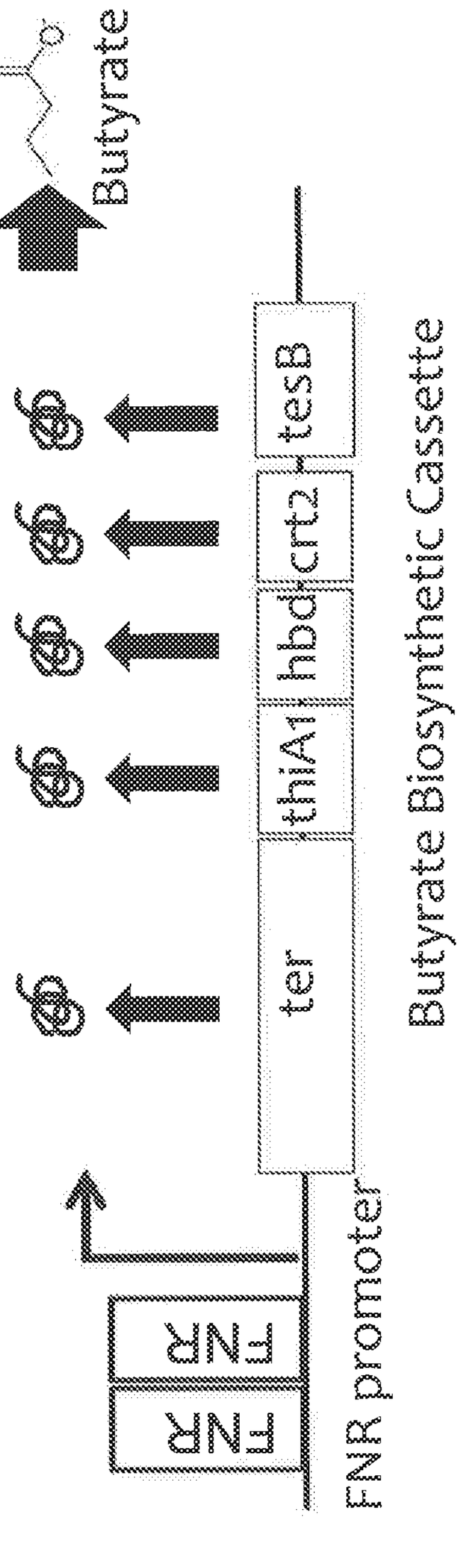

Butyrate
FNR
FNR
FNR promoter
ter
tesB
hbd,crt2
thiA1
Butyrate Biosynthetic Cassette

FIG. 5B

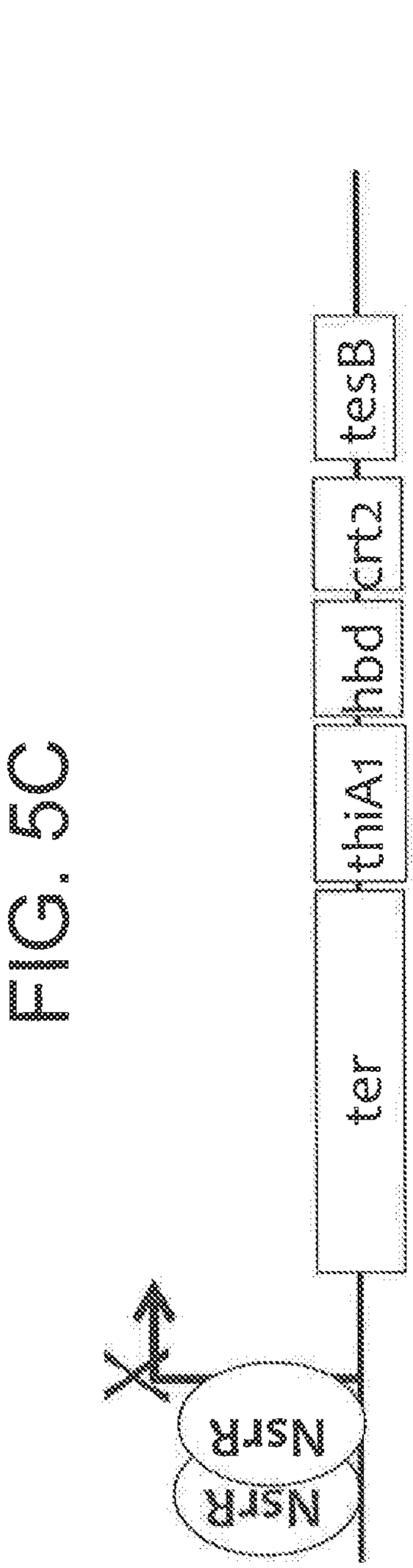
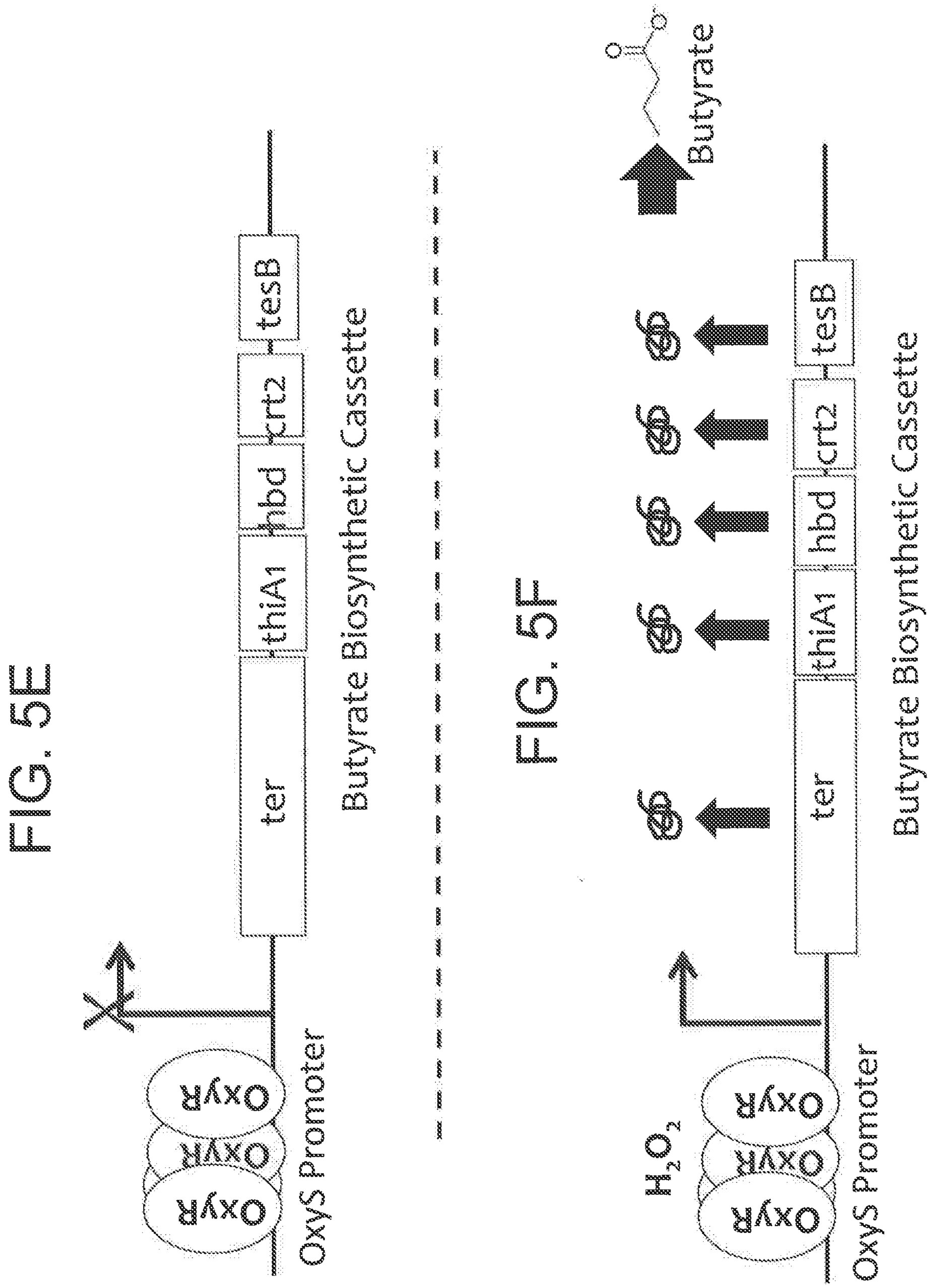

Butyrate Biosynthetic Cassette
thiA1
hbd
crt2
tesB
ter
NSFR
NSFR

FIG. 5C


Butyrate
Butyrate Biosynthetic Cassette
thiA1
hbd
crt2
tesB
ter
NO
NO
NO
NO
NO
NO
NSFR
NSFR

FIG. 5D


Butyrate
tesB
crt2
hbd
thiA1
ter
Butyrate Biosynthetic Cassette
OxyS Promoter
OxyR
OxyR
OxyR
FIG. 5E
FIG. 5F

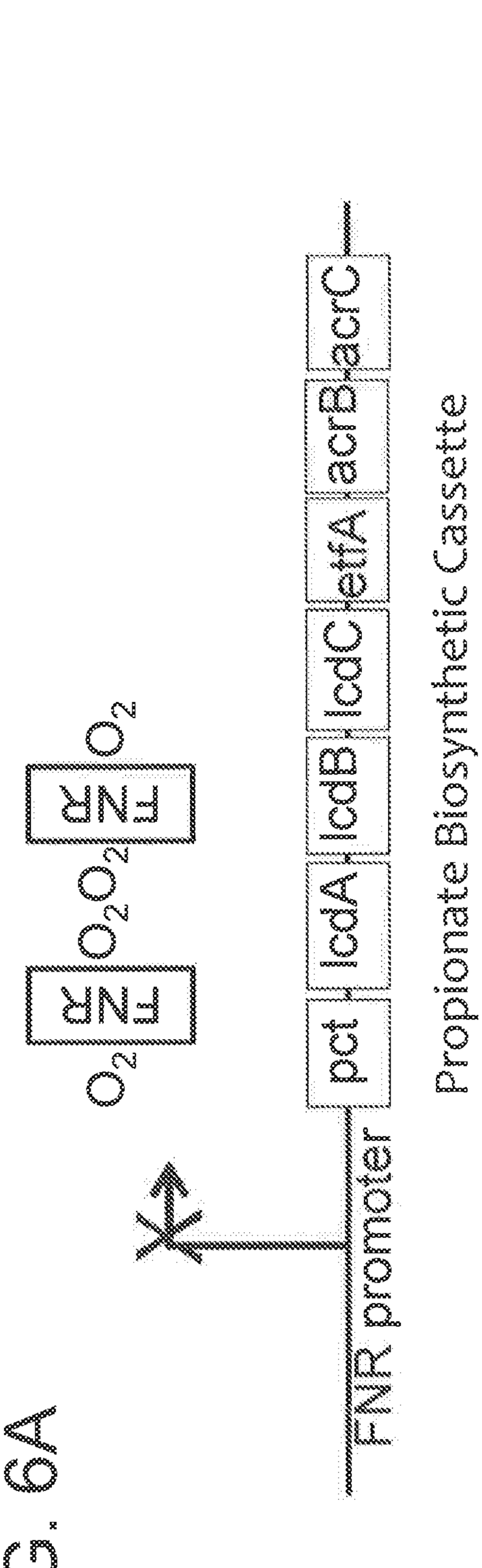

FNR
$O_2$
FNR
$O_2$ $O_2$
$O_2$
pct
lcdA
lcdB
lcdC
etfA
acrB
acrC
Propionate Biosynthetic Cassette
FNR promoter

FIG. 6A

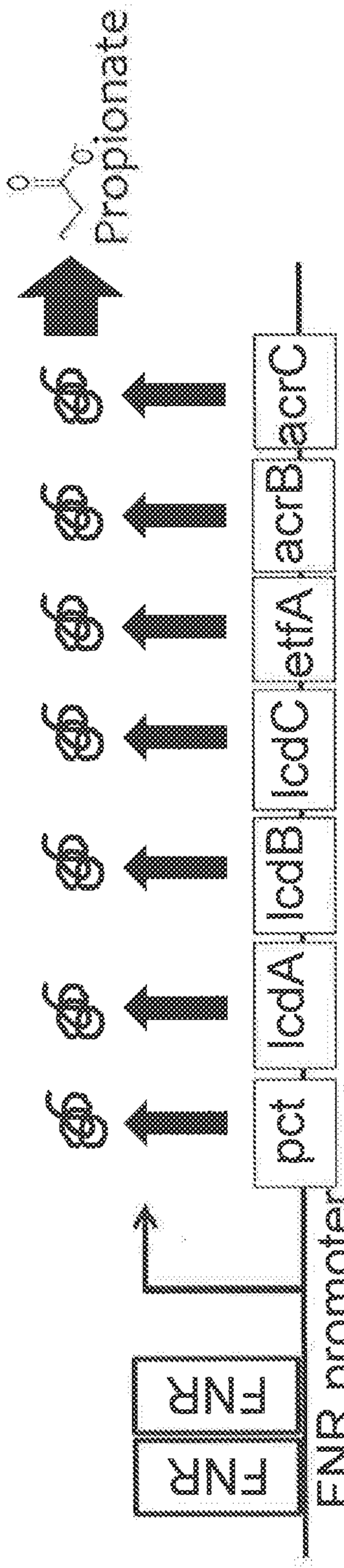

Propionate
FNR
FNR
pct
lcdA
lcdB
lcdC
etfA
acrB
acrC
Propionate Biosynthetic Cassette
FNR promoter

FIG. 6B

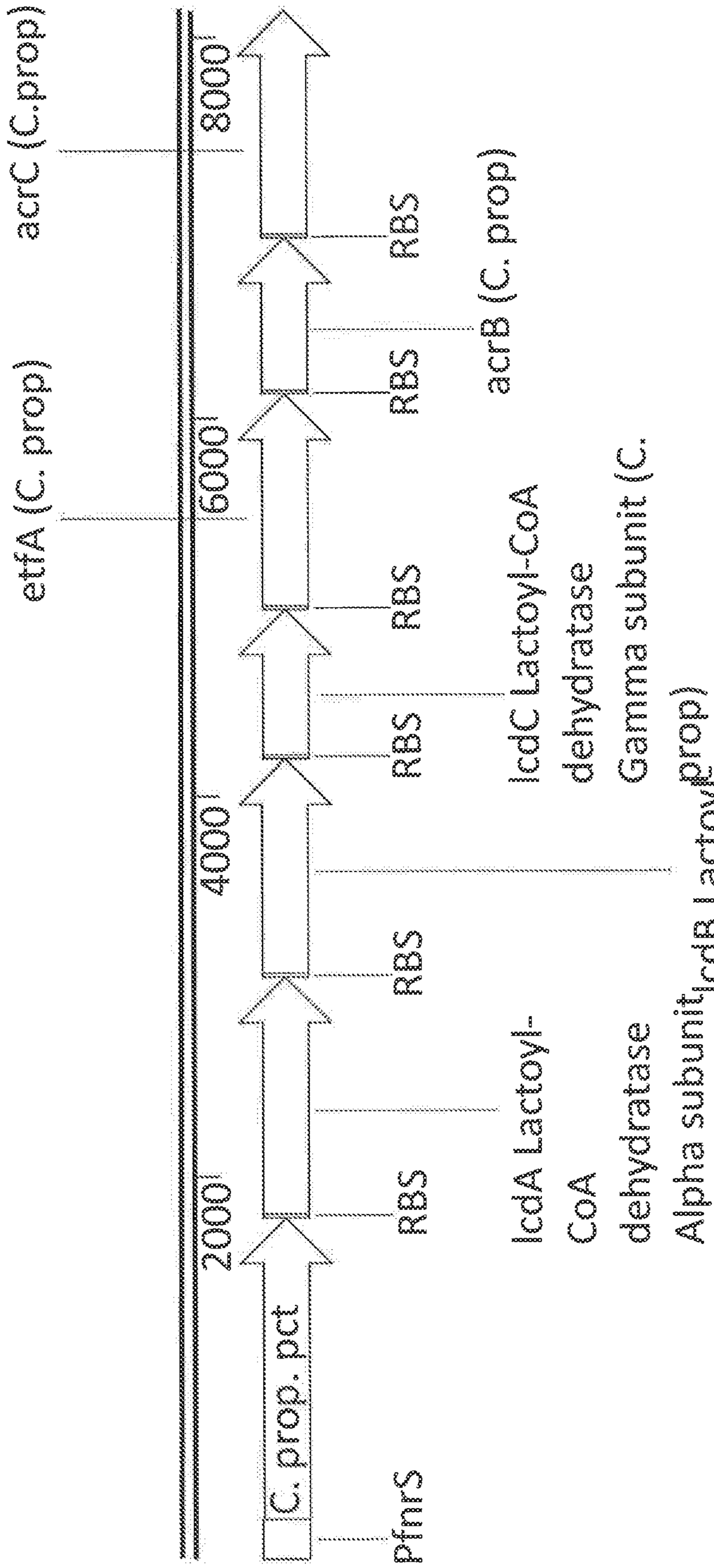

pfnrS
C. prop. pct
2000
RBS
IcdA Lactoyl-
CoA
dehydratase
Alpha subunit
(C. prop)
4000
RBS
IcdB Lactoyl-CoA
dehydratase
Beta subunit
(C. prop)
6000
RBS
IcdC Lactoyl-CoA
dehydratase
Gamma subunit (C.
prop)
8000
RBS
acrB (C. prop)
RBS
acrC (C. prop)
etfA (C. prop)

FIG. 7

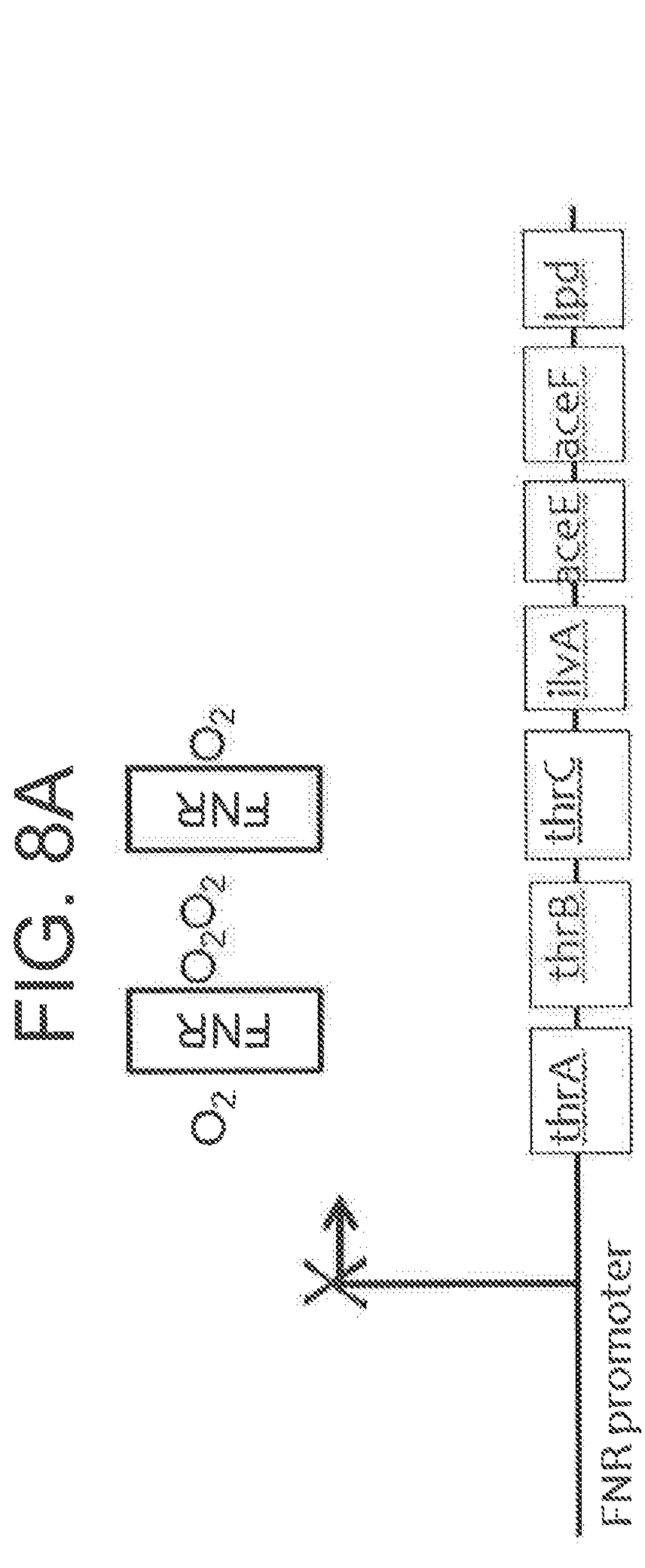
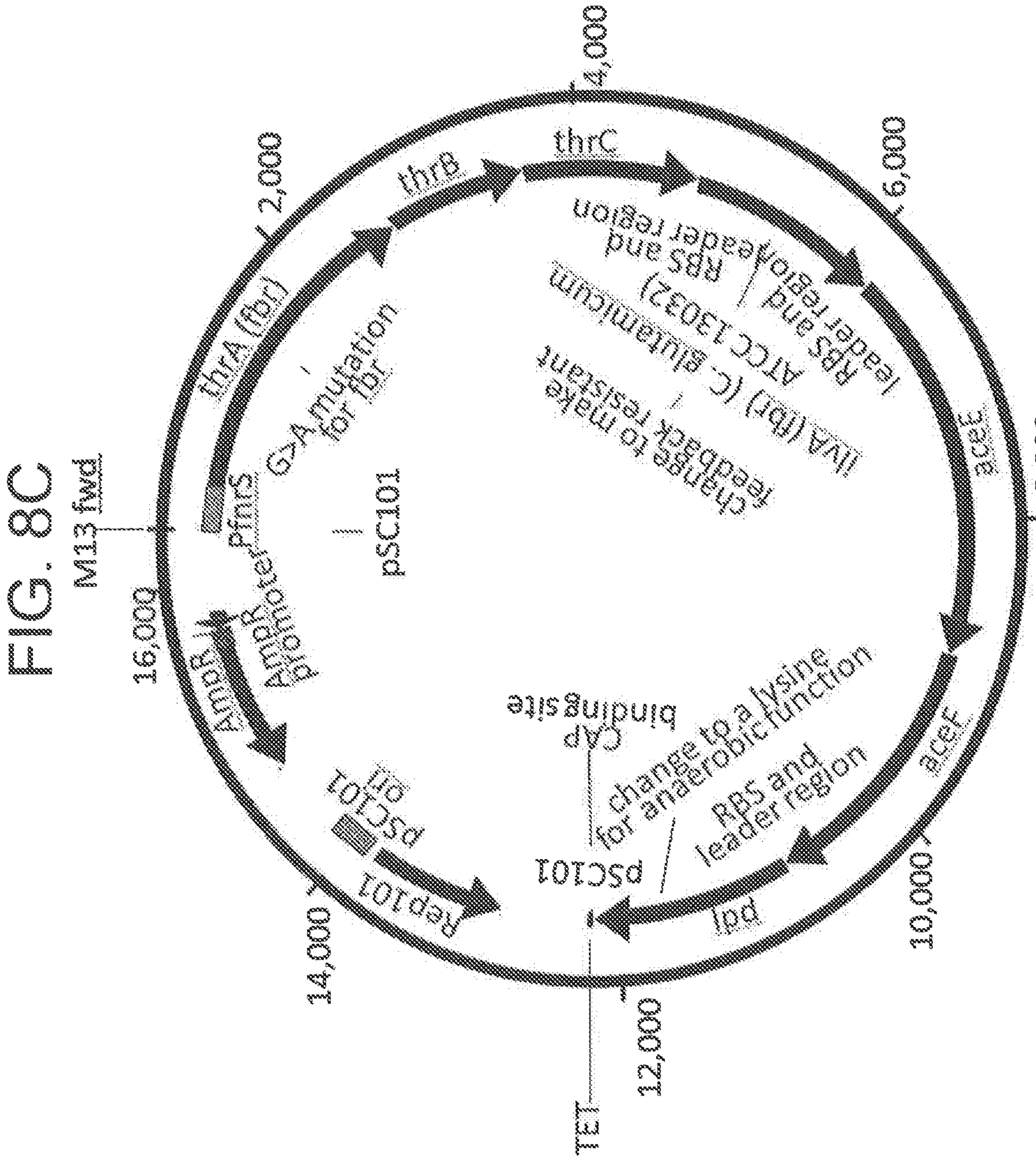

FNR promoter
thrA
thrB
thrC
Propionate Biosynthetic Cassette
FNR promoter
thrA
thrB
thrC
Propionate Biosynthetic Cassette
FNR
FNR
FNR
FNR
FNR
FNR
O2
O2
O2
O2
O2
O2

FIG. 8A

FIG. 8B

FIG. 8C


M13 fwd
Pfmrs
thrA (fbr)
2,000
thrB
thrC
6,000
8,000
RBS and leader region
G>A mutation for fbr
pSC101
ori
Amp R
Amp promoter
16,000
Amp R
Ap promoter
CAP binding site
change to a lysine for anaerobic function
RBS and leader region
acrF
pSC101 ori
rep101
14,000
pSC101
lpd
10,000
12,000
M13 rev

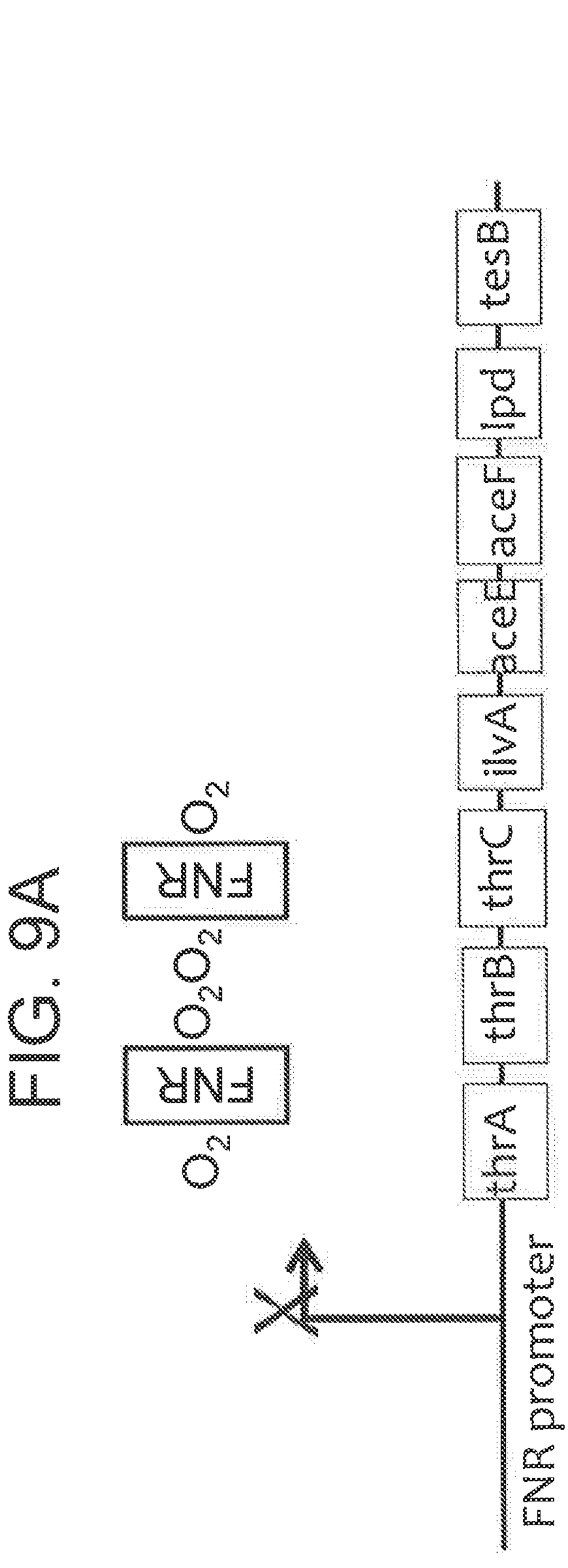

FNR
FNR
$O_2$
$O_2$
$O_2$
FNR promoter
thrA
thrB
thrC
tesB
ldhA
aceE
aceF
ldh
Propionate Biosynthetic Cassette

FIG. 9A

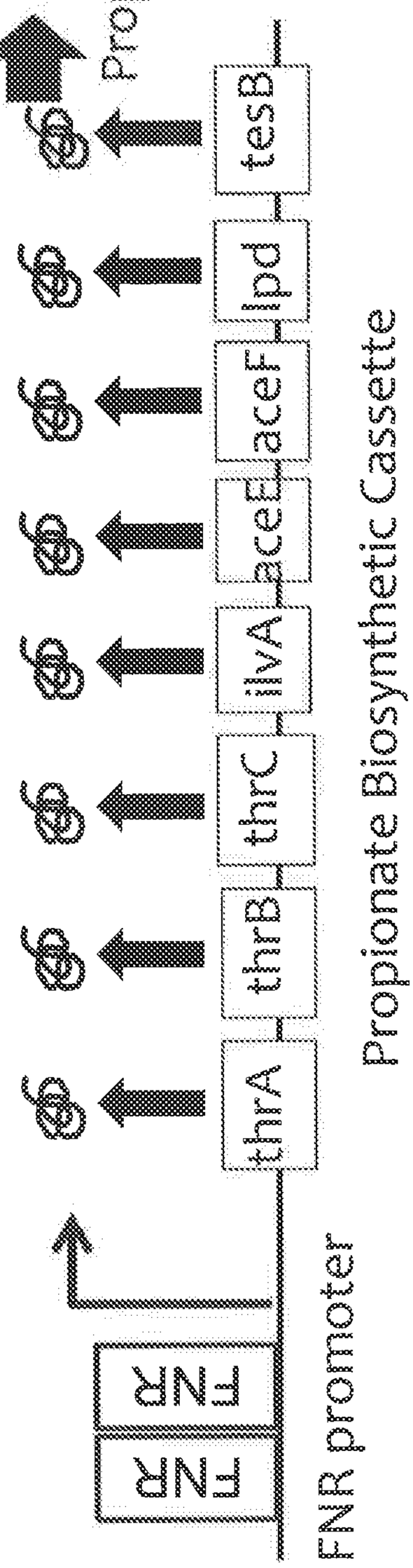

FNR
FNR
FNR promoter
thrA
thrB
thrC
tesB
ldhA
aceE
aceF
ldh
Propionate Biosynthetic Cassette
Propionate

FIG. 9B

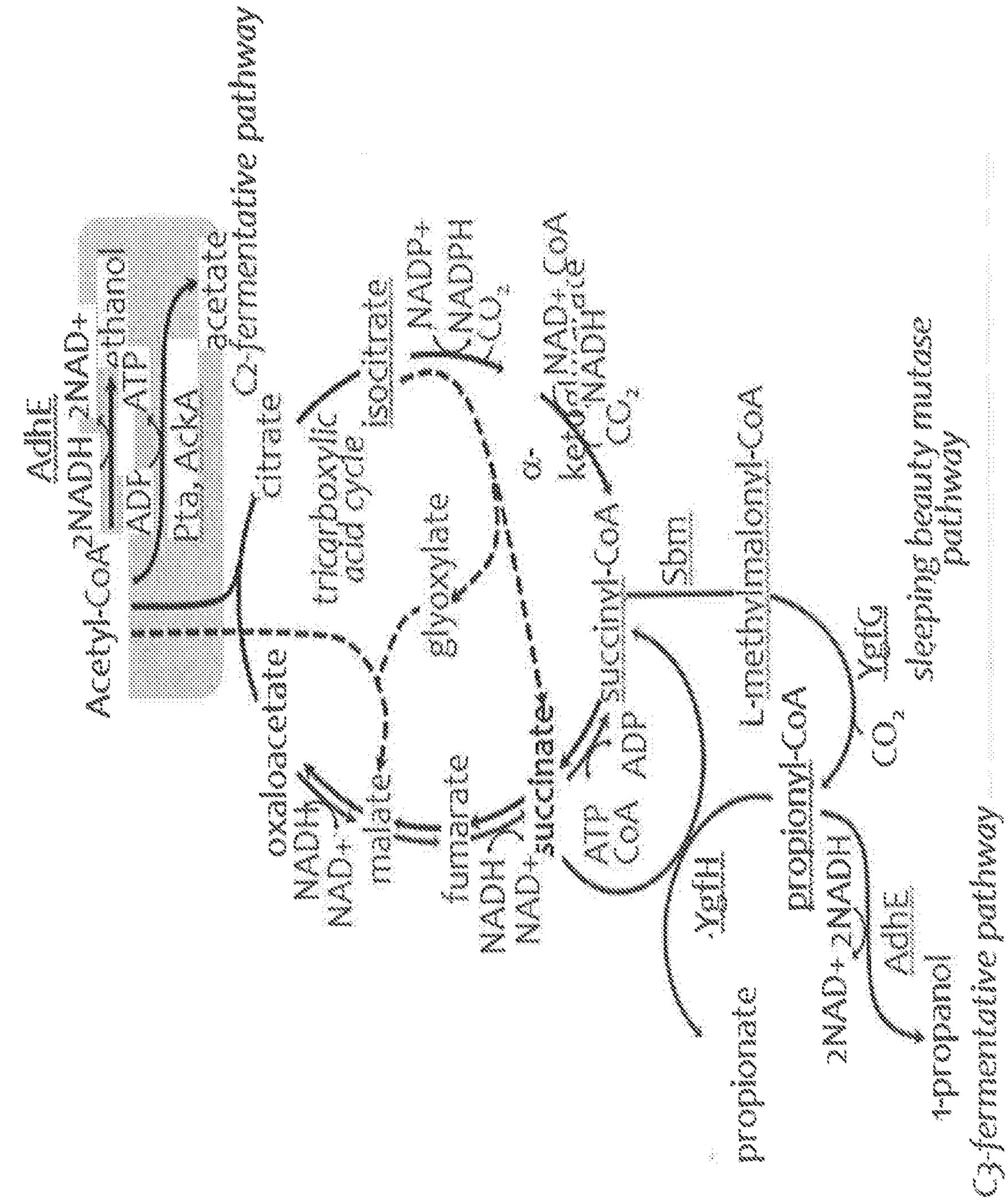

AdhE
Acetyl-CoA 2NADH 2NAD+
ethanol
ATP
ADP
Pta, AckA
acetate
C2-fermentative pathway
citrate
tricarboxylic acid cycle
isocitrate
NADP+
NADPH
$CO_2$
glyoxylate
α-
ketoglutarate
NAD+ CoA
NADH
$CO_2$
succinyl-CoA
Sbm
methylmalonyl-CoA
oxaloacetate
NADH
NAD+
malate
fumarate
NADH
NAD+
succinate
ATP
CoA
ADP
succinate
L-methylmalonyl-CoA
YgfH
propionyl-CoA
YgfG
$CO_2$
sleeping beauty mutase pathway
propionate
2NAD+ 2NADH
AdhE
1-propanol
C3-fermentative pathway

FIG. 10A

FIG. 10B

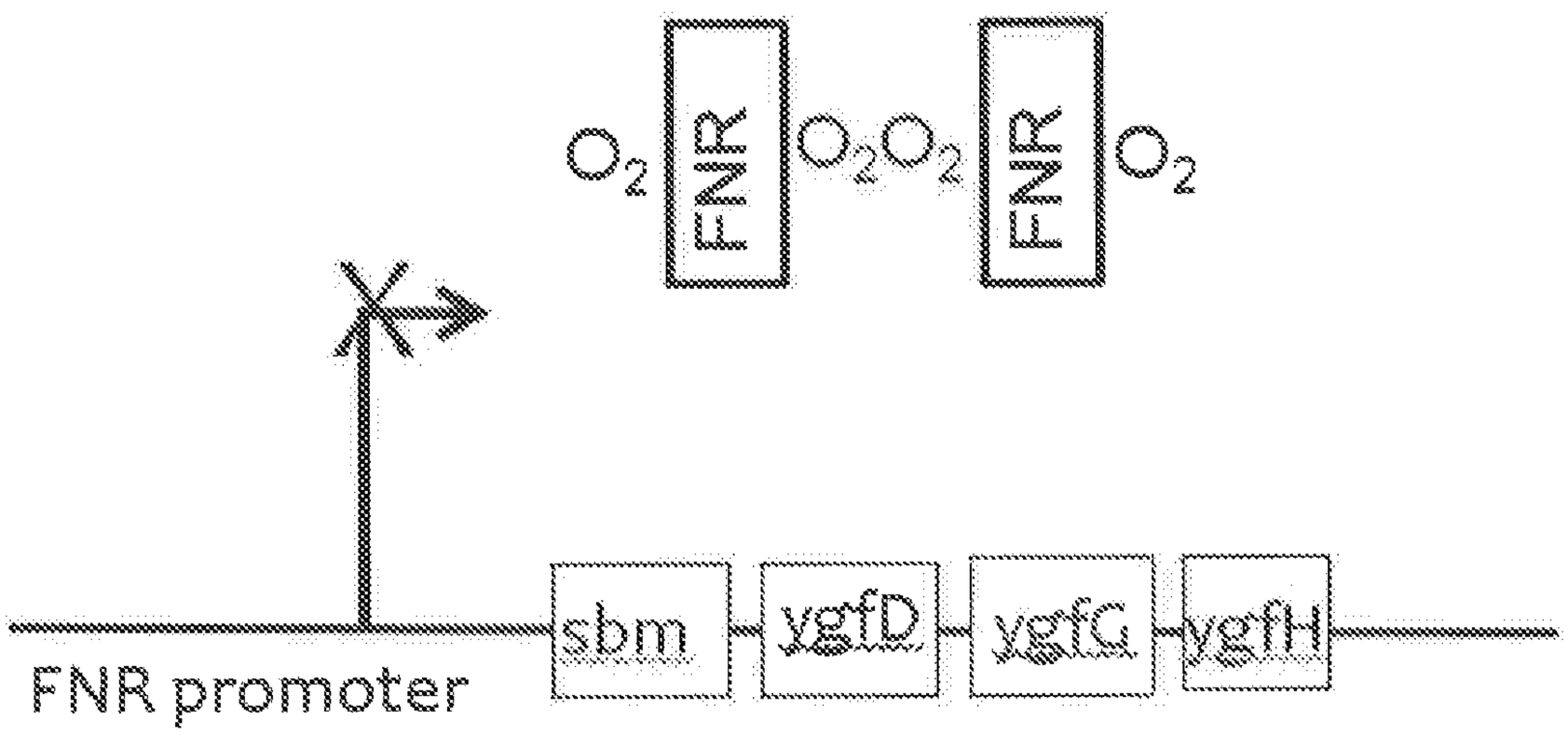
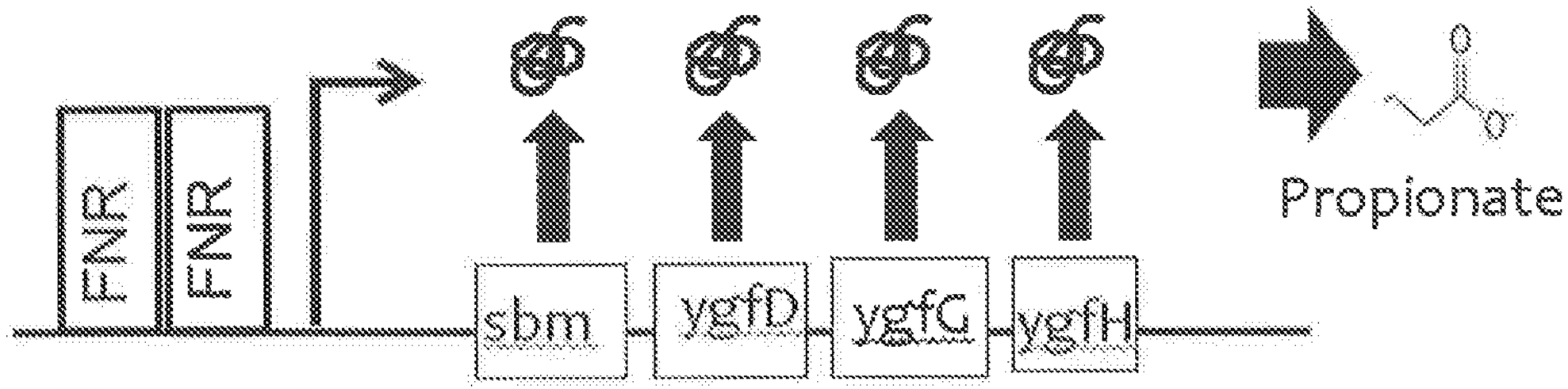



FIG. 10C



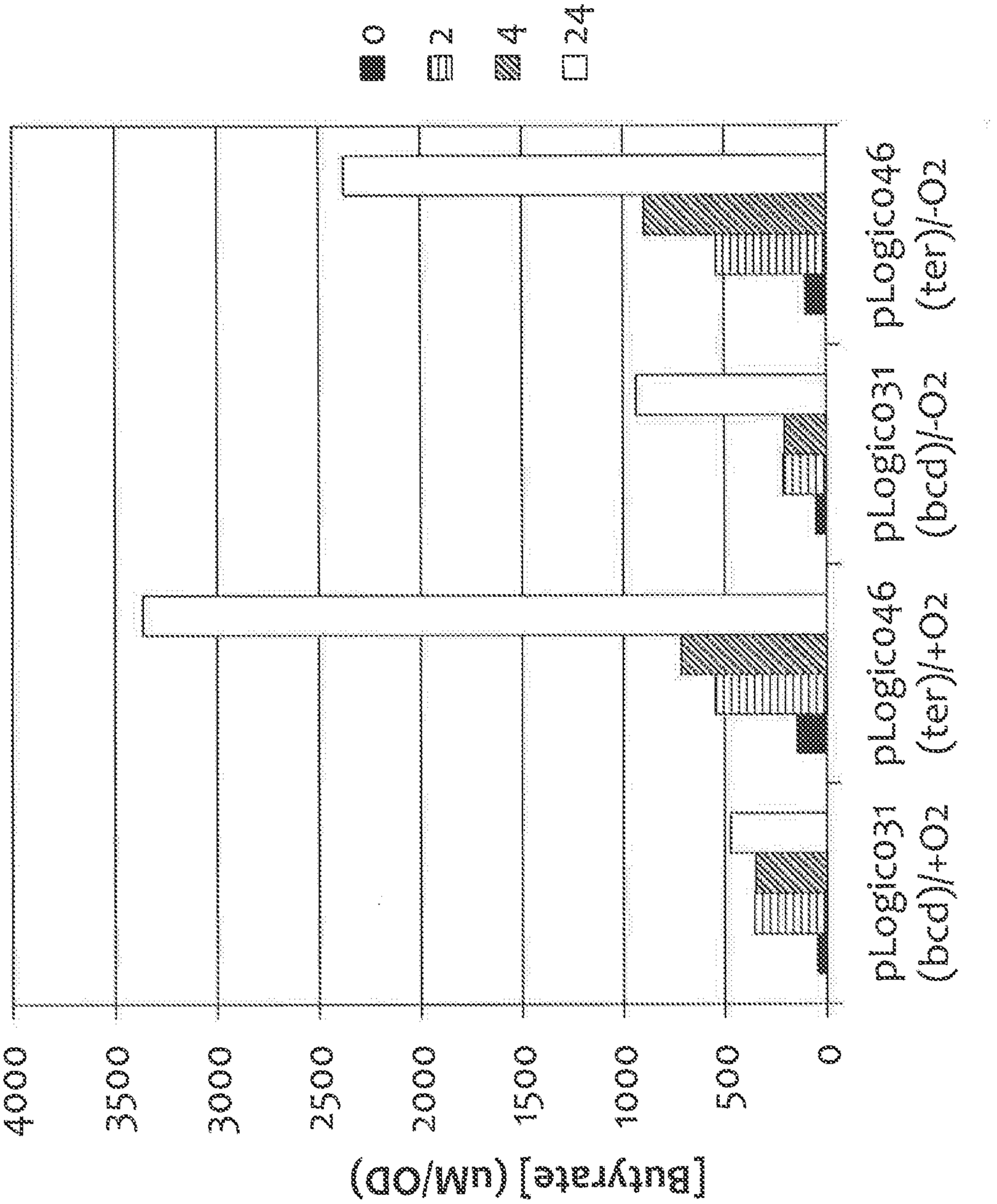

0
2
4
24
4000
3500
3000
2500
2000
1500
1000
500
0
[Butyrate] (uM/OD)
pLogic031 (bcd)/+O2
pLogic046 (ter)/+O2
pLogic031 (bcd)/-O2
pLogic046 (ter)/-O2

FIG. 11

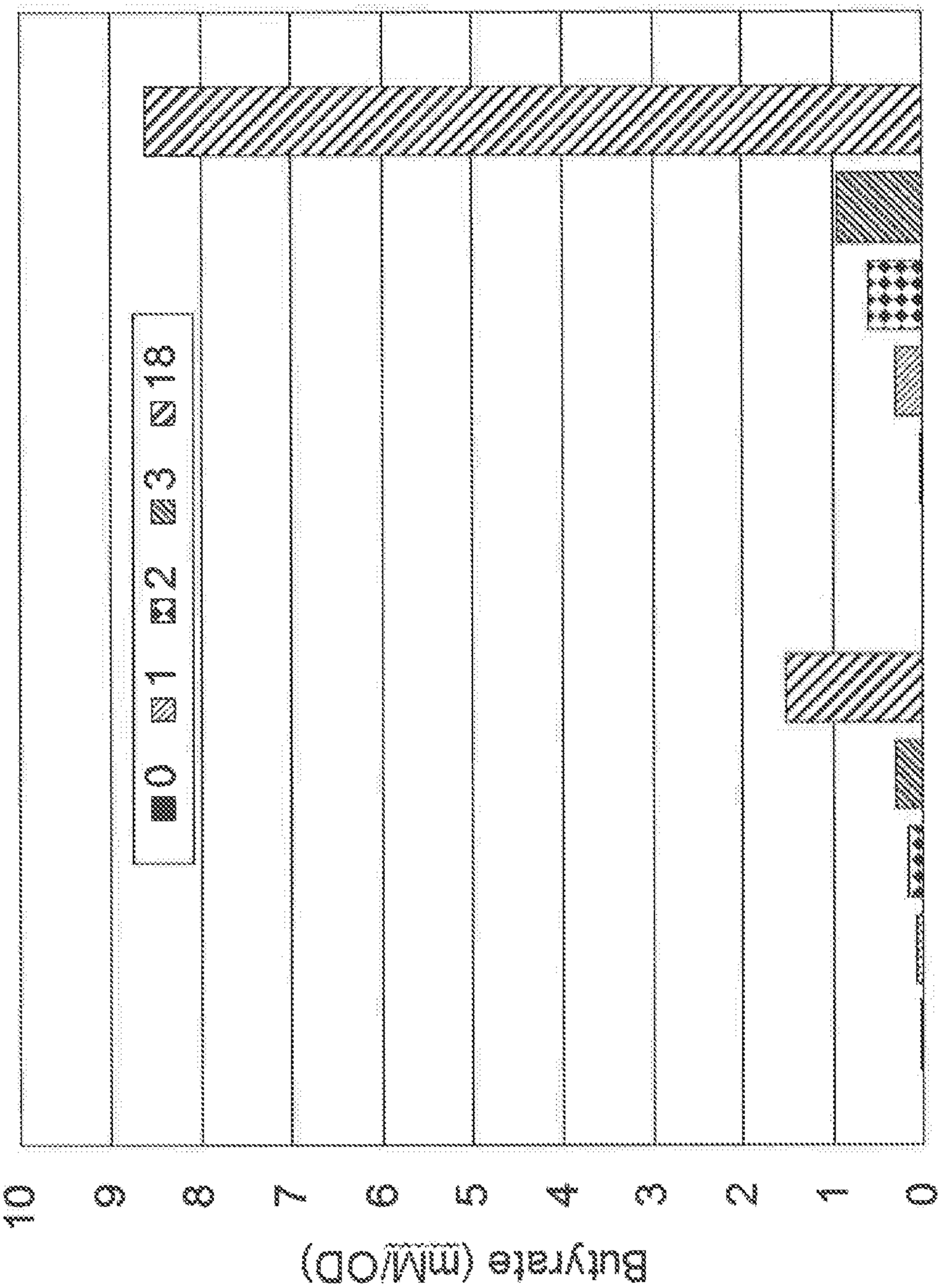

0
1
2
3
18
pLOGIC046C
pLOGIC046C-delta.ptb-buk/tesB+
Time (h)
Butyrate (mM/OD)

FIG. 12

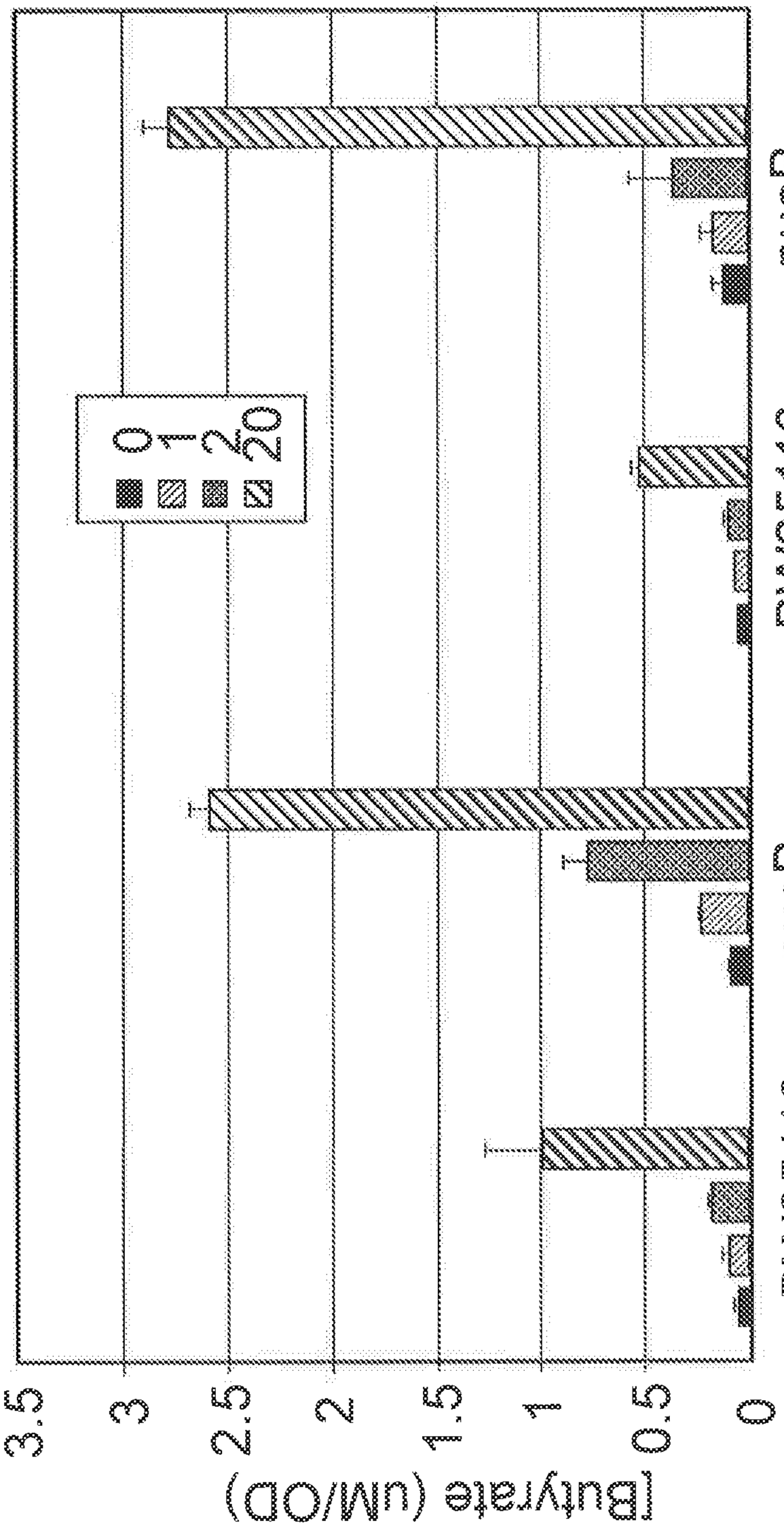

0
1
20
20
BW25113 pLogic046
nuoB pLogic046
pLogic031 - pBR322/bcd-butyrate cassette
pLogic046 - pBR322/ter-butyrate cassette
BW25113 pLogic031
nuoB pLogic031
3.5
3
2.5
2
1.5
1
0.5
0
[Butyrate (uM/OD)

FIG. 13

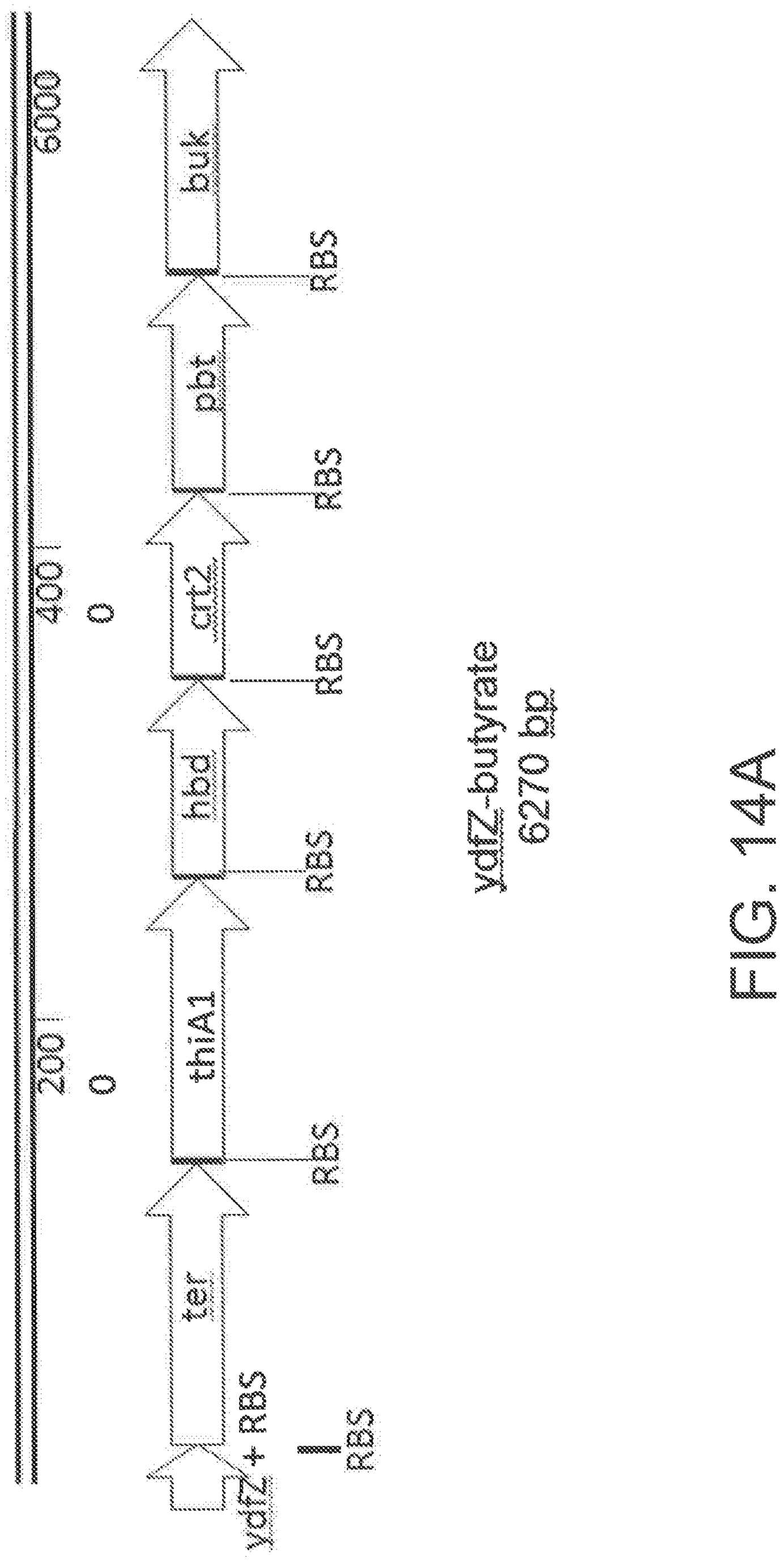

6000
4000
0
2000
0
2000
0
ydfZ-butyrate
6270 bp
RBS
pbt
RBS
crt2
RBS
hbd
RBS
thiA1
RBS
ter
ydfZ + RBS
RBS

FIG. 14A

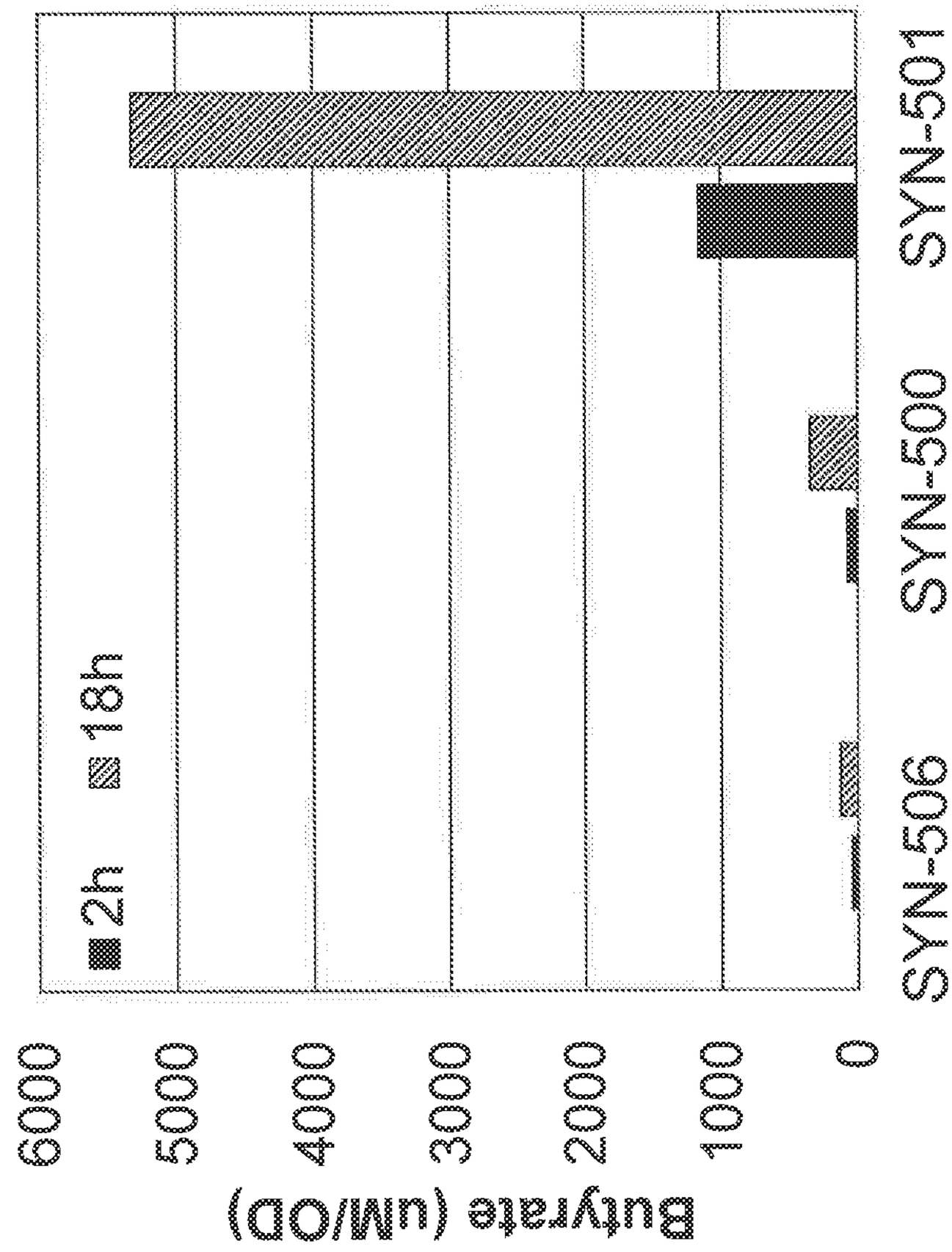

Butyrate (uM/OD)
6000
5000
4000
3000
2000
1000
0
2h
18h
SYN-500
SYN-501
SYN-506

FIG. 14B

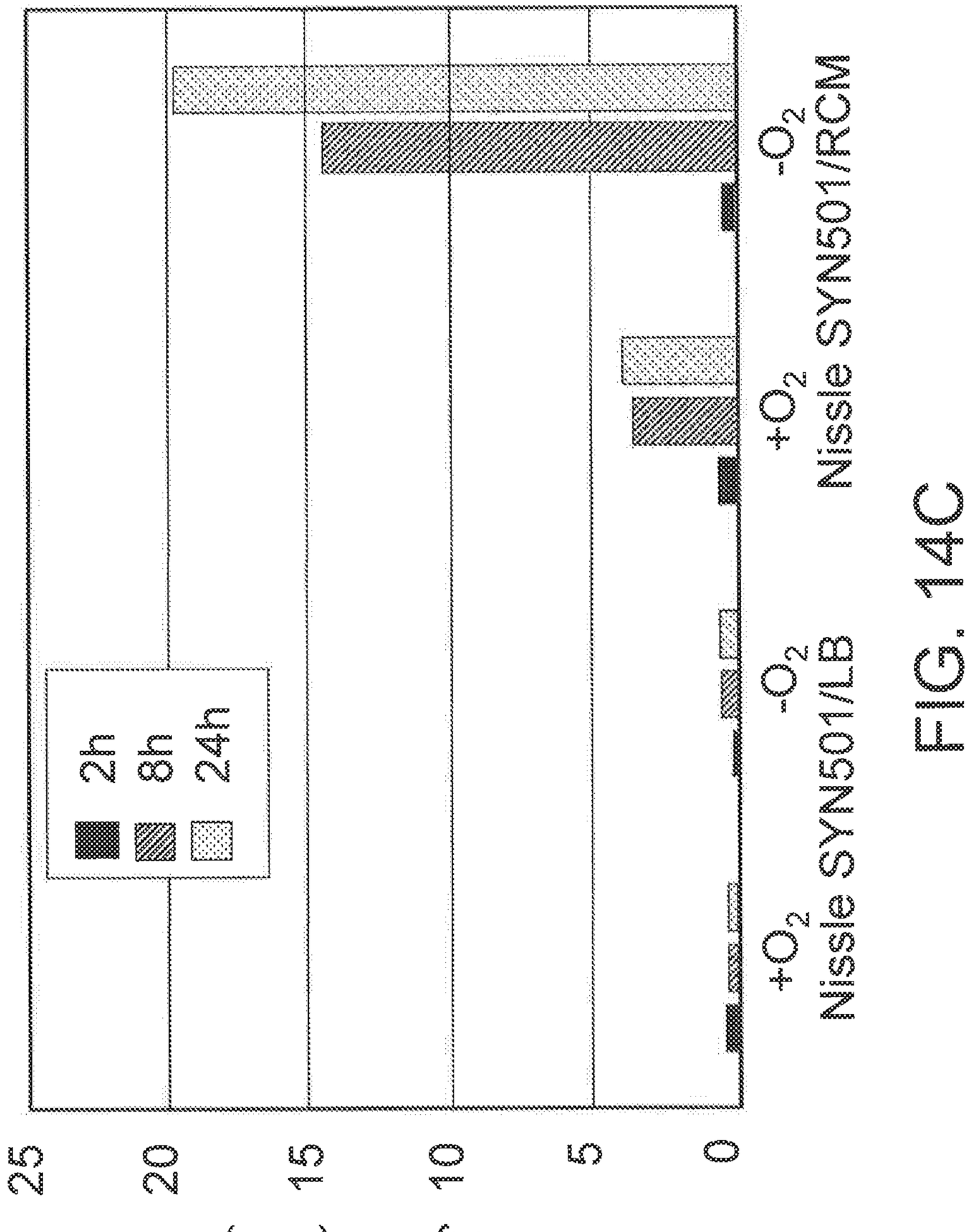

+O2
Nisle SYN501/RCM
-O2
+O2
Nisle SYN501/LB
-O2
2h
8h
24h
5
0
5
0
Butyrate (mM)

FIG. 14C

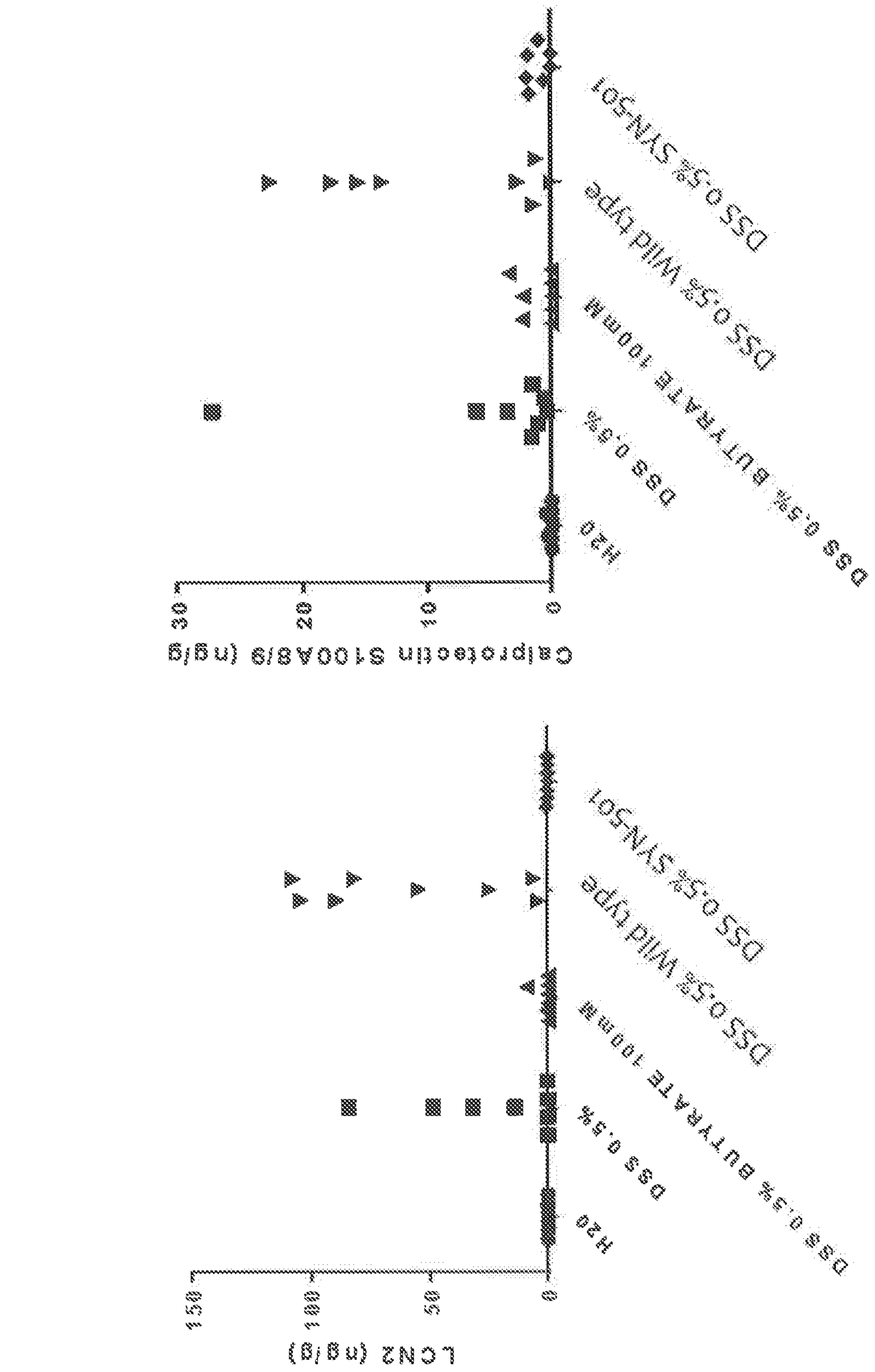

Calprotectin S100A8/9 (ng/g)
0
10
20
30
H2O
DSS 0.5%
DSS 0.5% BUTYRATE 100mM
DSS 0.5% Vivid-type
DSS 0.5% Vivid-type 100mM
LCN2 (ng/g)
0
500
1000
1500

FIG. 14

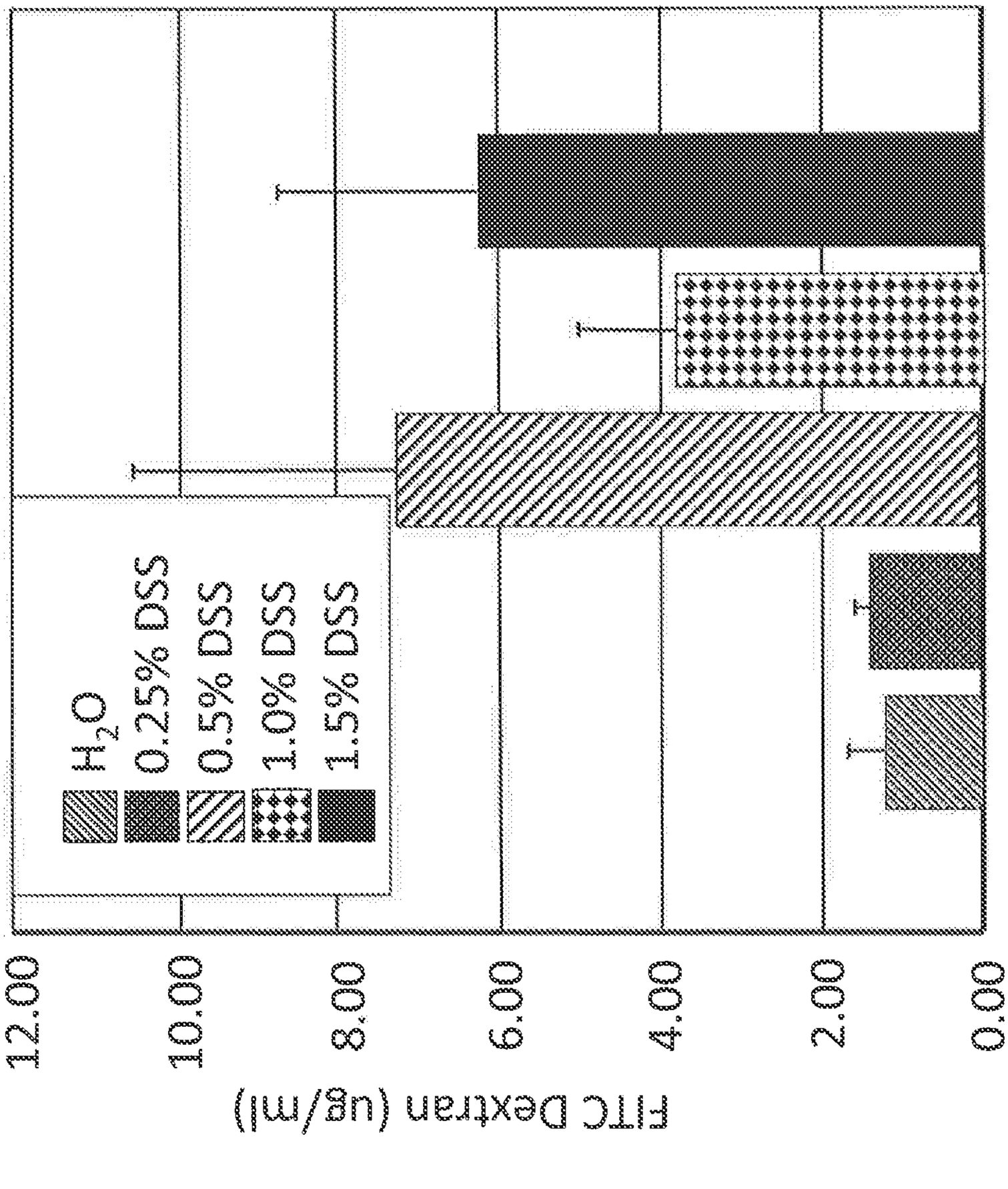

12.00
10.00
8.00
6.00
4.00
2.00
0.00
FITC Dextran (ug/ml)
H2O
0.25% DSS
0.5% DSS
1.0% DSS
1.5% DSS

FIG. 15

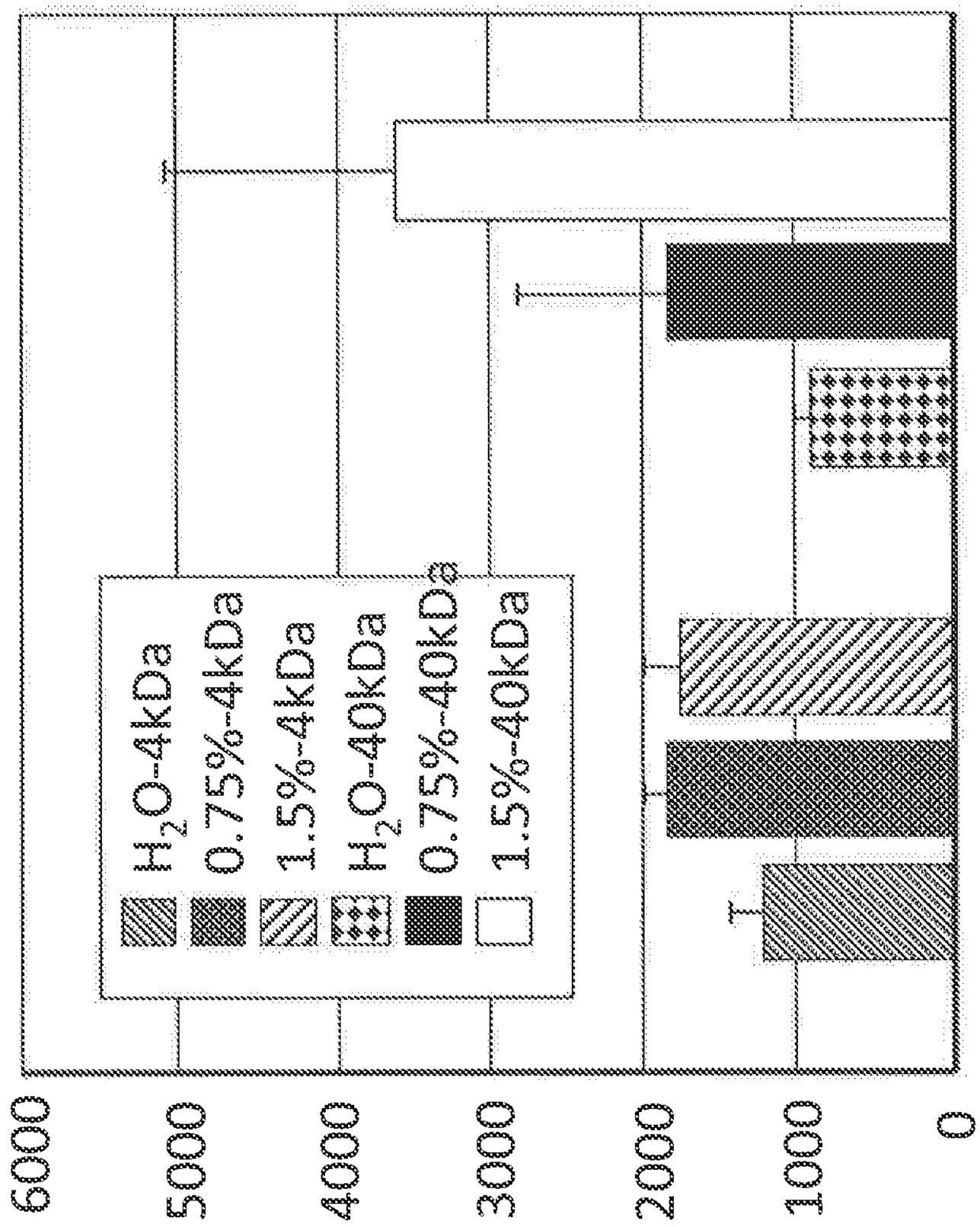

FITC Dextran (ug/ml)
6000
5000
4000
3000
2000
1000
0
H2O-4kDa
0.75%-4kDa
1.5%-4kDa
H2O-40kDa
0.75%-40kDa
1.5%-40kDa

FIG. 16

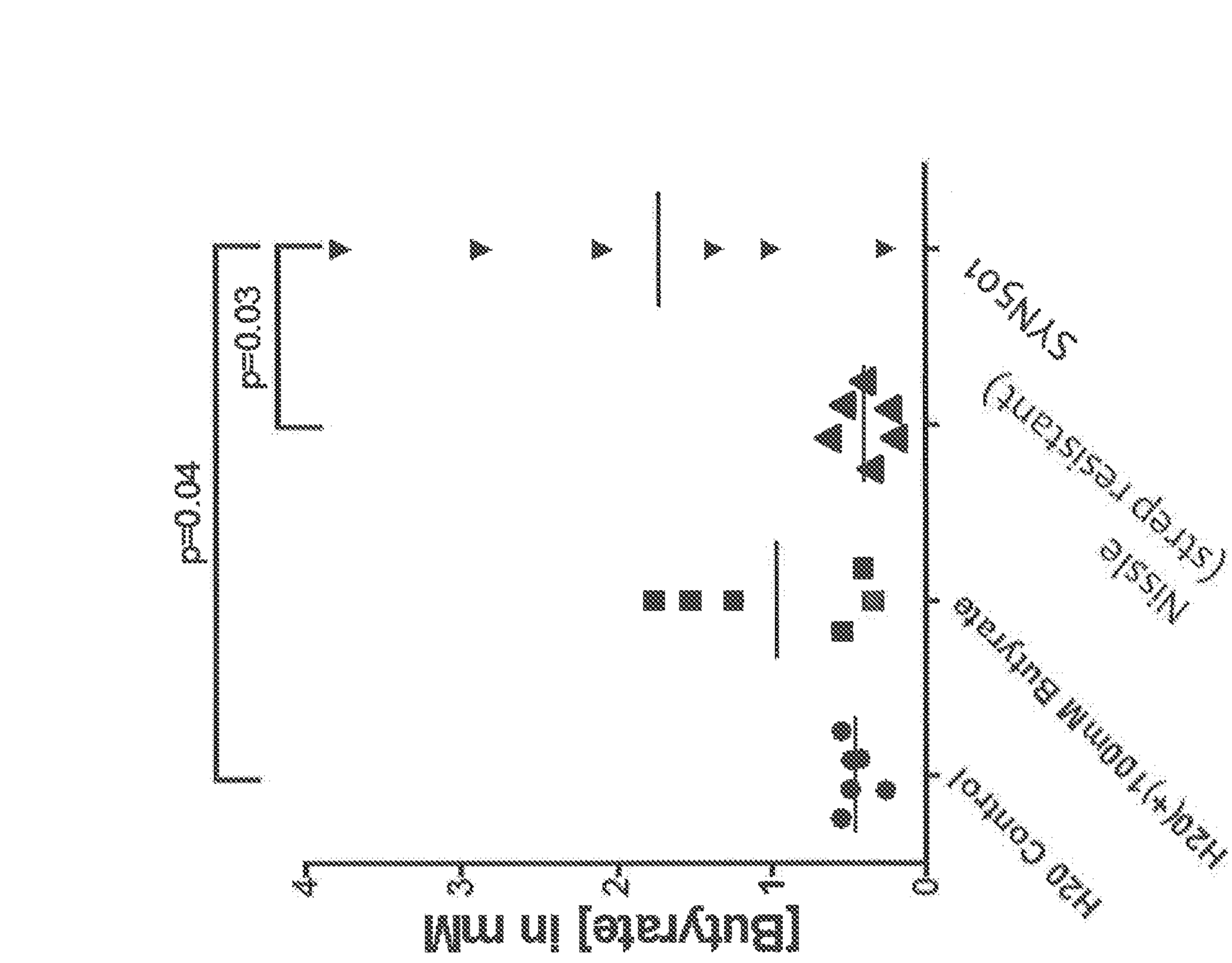

[Butyrate] in mM
0
1
2
3
4
p=0.04
p=0.03
H2O Control
H2O+Butyrate
H2O+Butyrate+Bumetanide
NKCC1 Inhibitor
(Bumetanide)
siNKCC1

FIG. 17

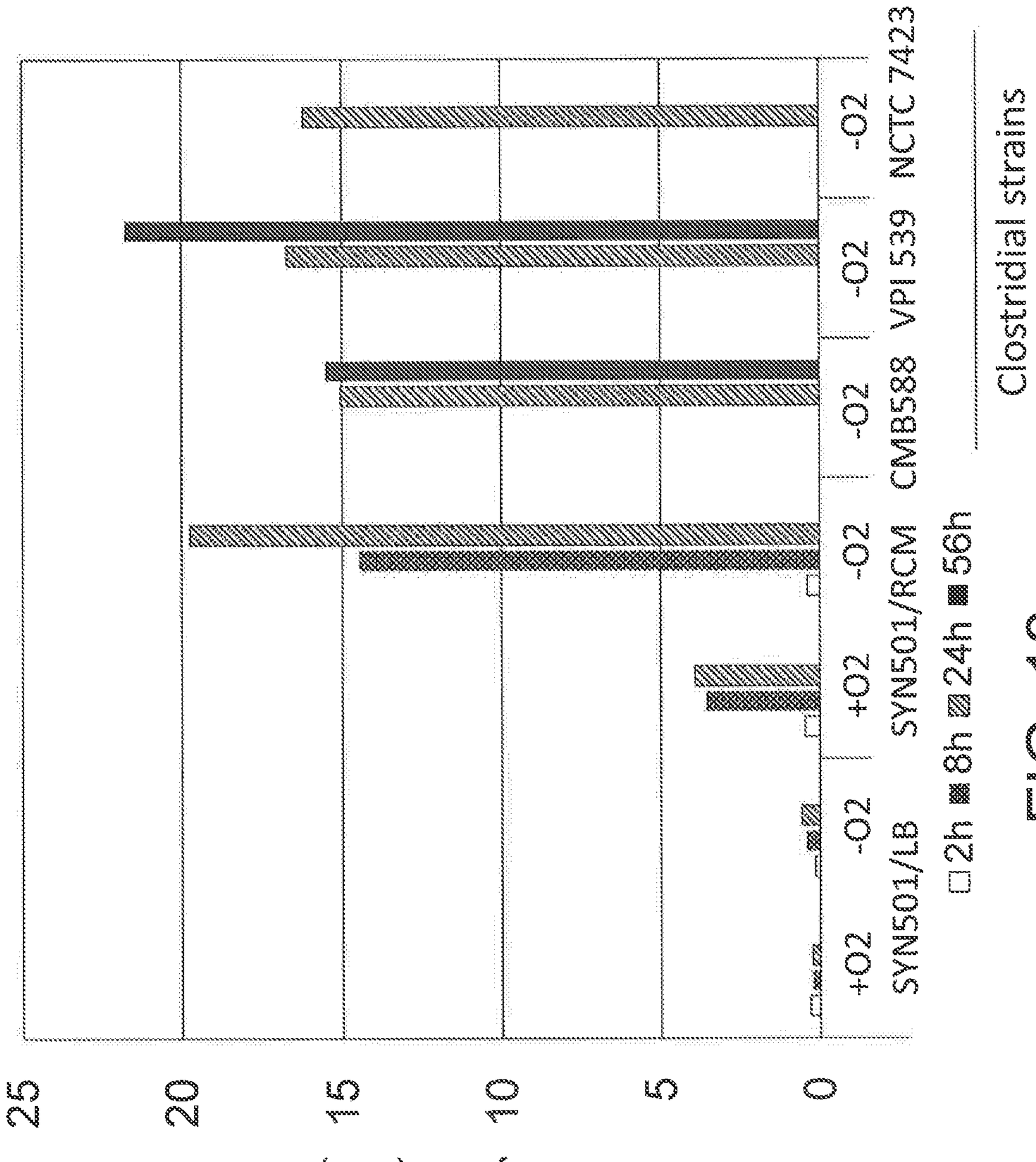

Butyrate (mM)
5
0
5
0
SYN501/LB
SYN501/RCM
+O2
-O2
+O2
-O2
CMB588
VPI 539
NCTC 742
-O2
-O2
-O2
Clostridial strains
2h 8h 24h 56h

FIG. 18

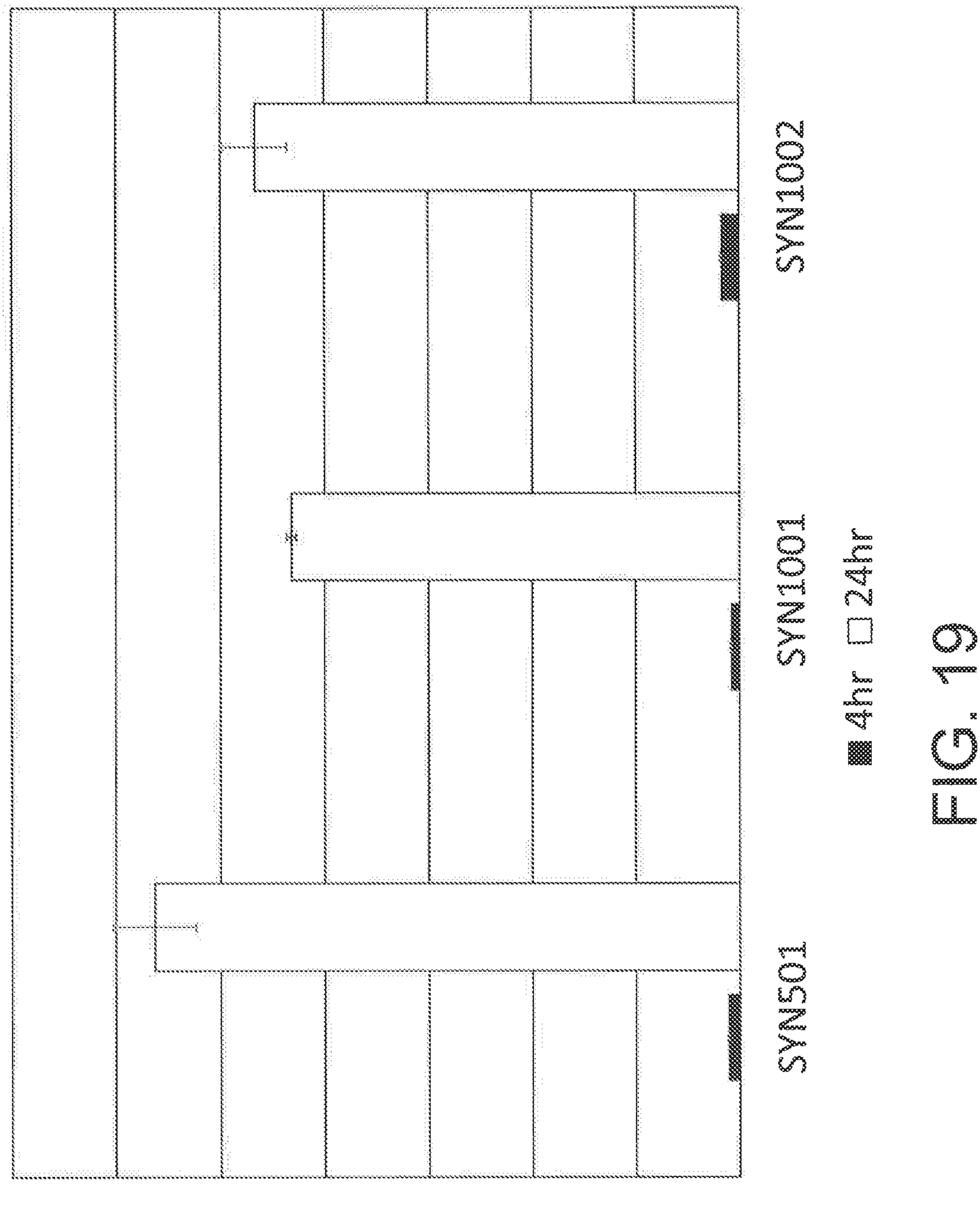



FIG. 19

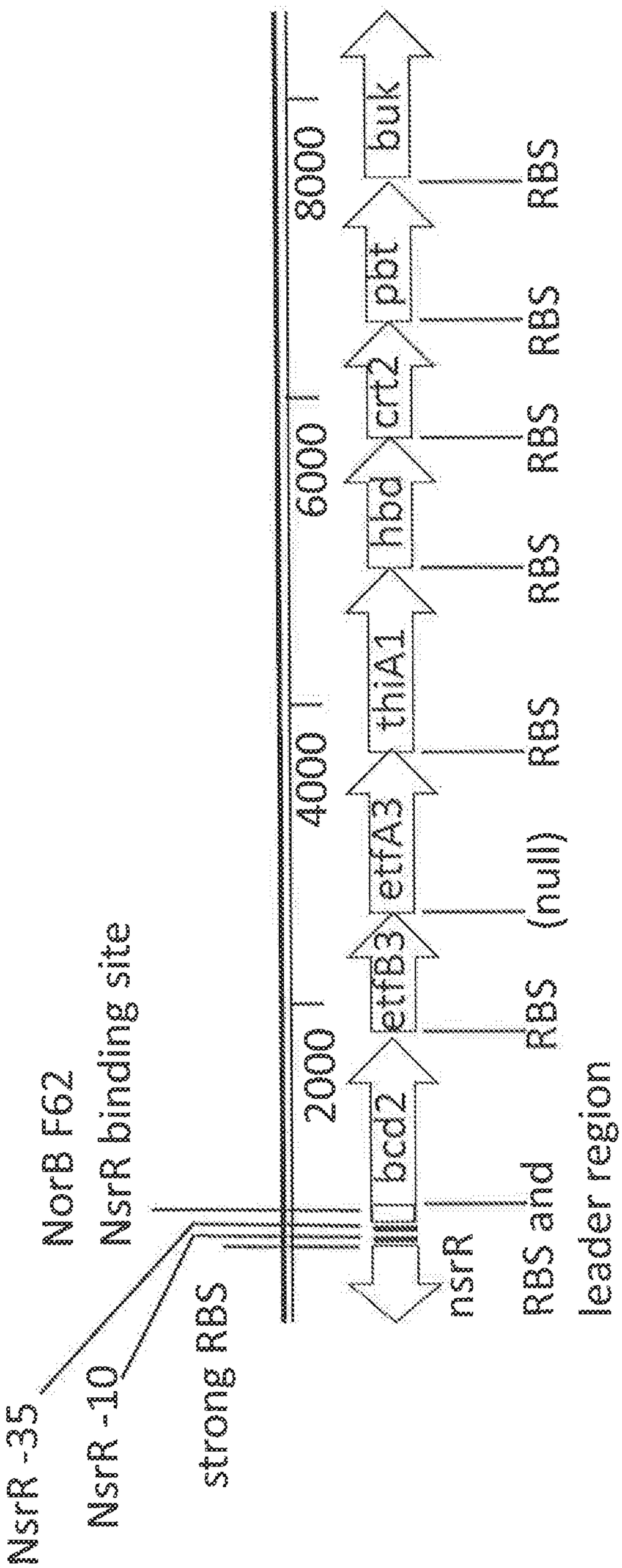

pLogic031-NsrR
8575 bp
8000
6000
4000
2000
buk
RBS
ptb
crt2
hbd
thiA1
etfA3
etfB3
bcd2
RBS and
leader region
nsrR
NsrR binding site
NorB E62
NsrR -35
NsrR -10
strong RBS

FIG. 20A

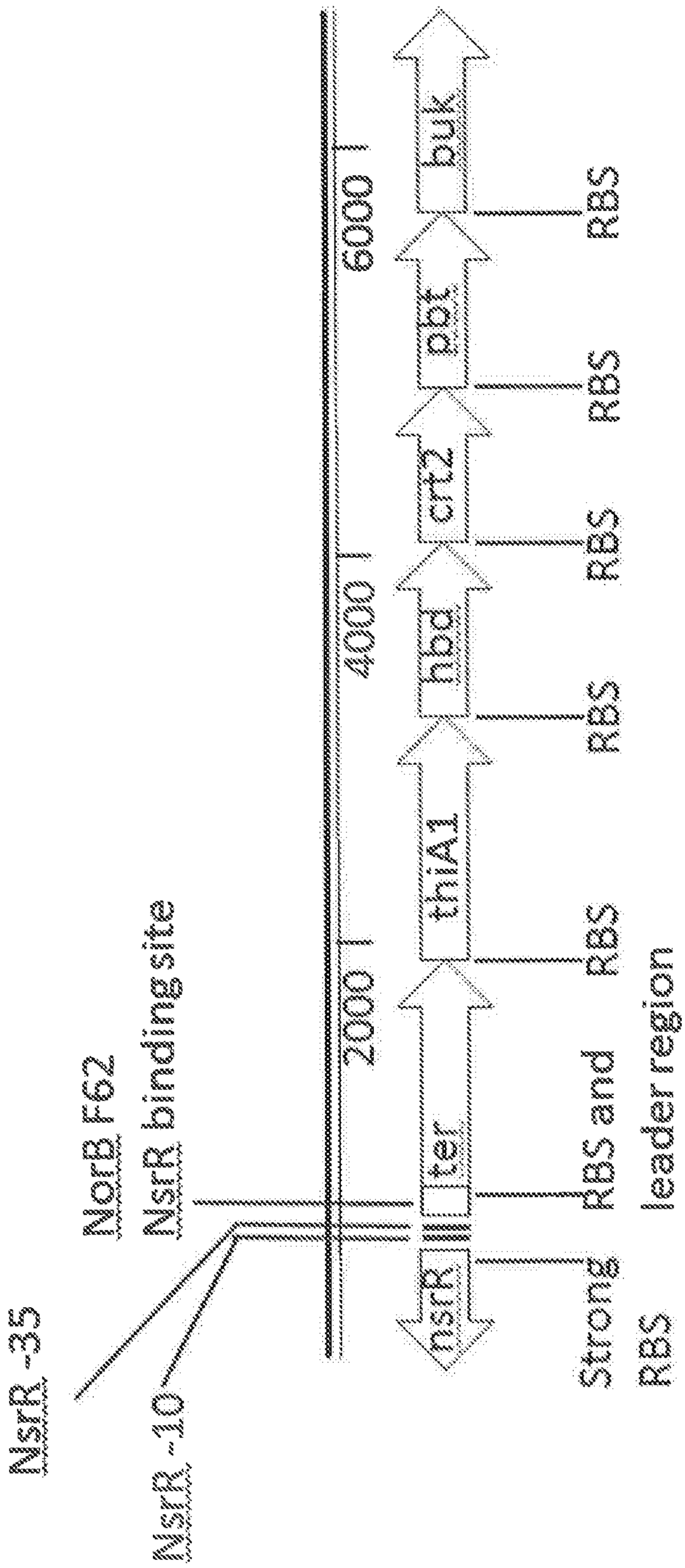

pBAD-T7-NsrR
-35
-10
NorB F62
NsrR binding site
6000
4000
2000
nsrR
ter
RBS and
RBS
thiA1
RBS
RBS
leader region
cnt2
RBS
abf
RBS
hbd
RBS
bcr
RBS
buk
RBS
pBAD-T7-NsrR-NorB F62-Nsr R(2)-thiA1-ter-cnt2-abf-hbd-bcr-buk
6787 bp

FIG. 20B

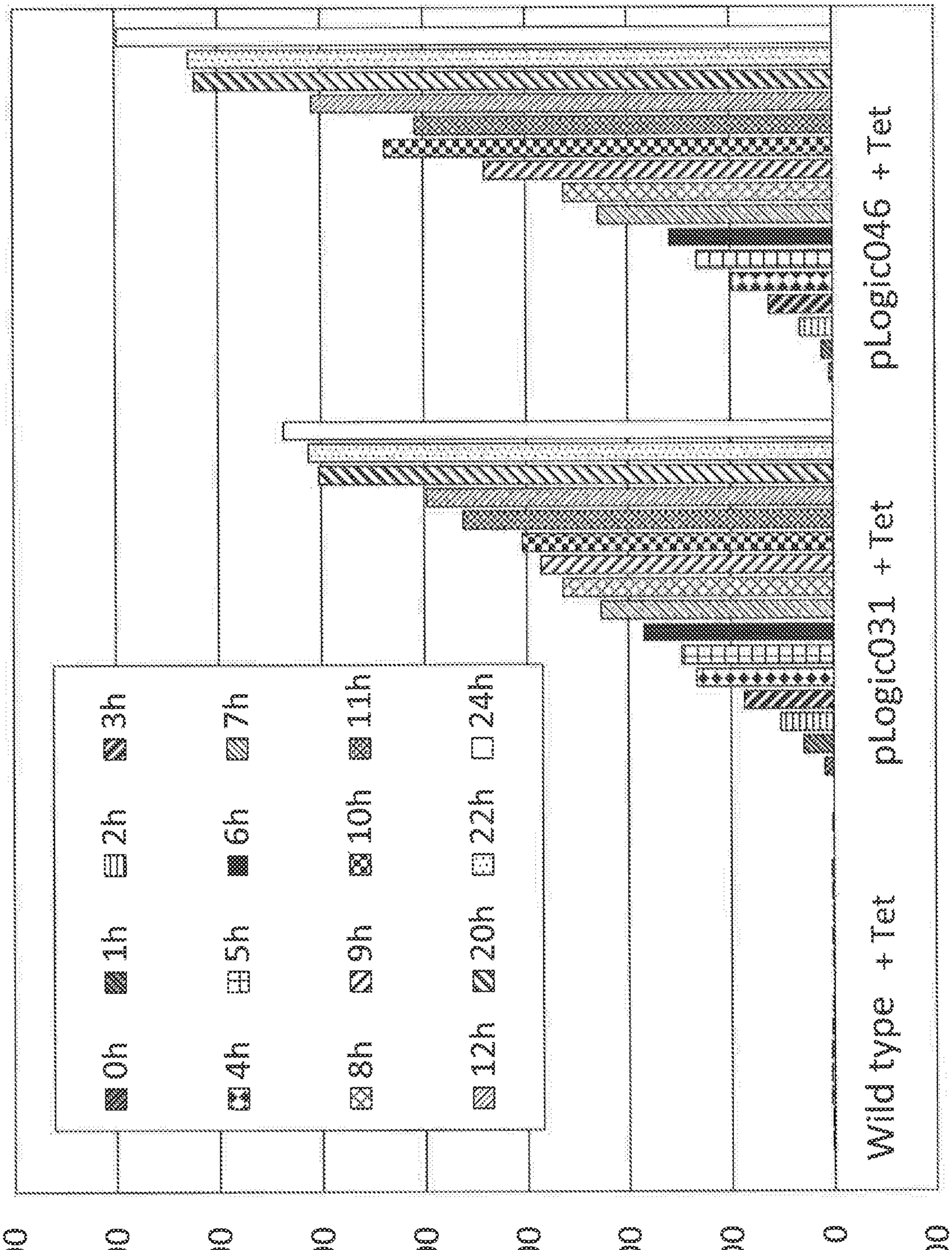

Butyrate (uM/OD)
1600
1400
1200
1000
800
600
400
200
0
-200
Wild type + Tet
pLogic031 + Tet
pLogic046 + Tet
0h
1h
2h
3h
4h
5h
6h
7h
8h
9h
10h
11h
12h
20h
22h
24h

FIG. 21

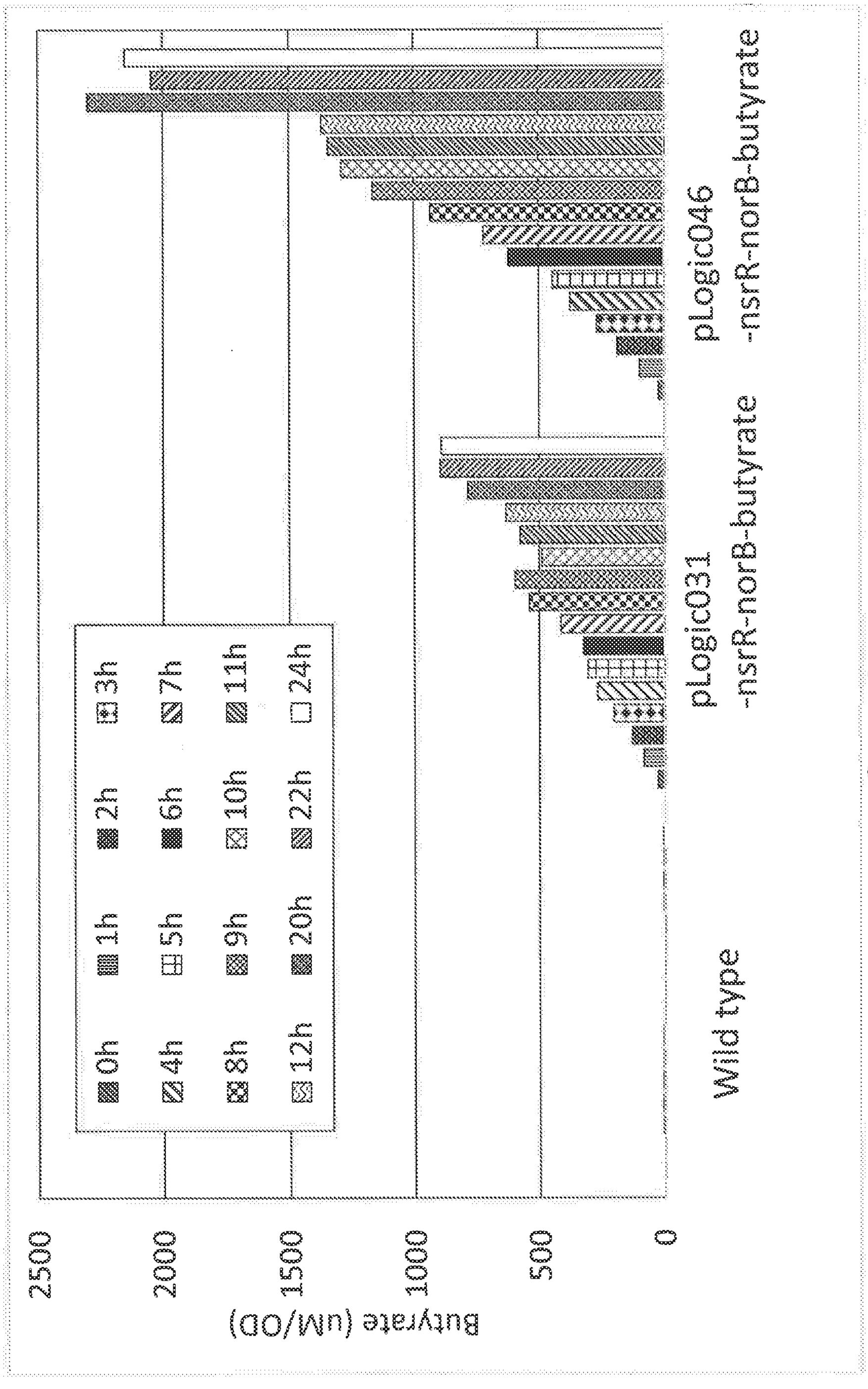

Butyrate (uM/OD)
2500
2000
1500
1000
500
0
Wild type
-nsrR-norB-butyrate
plogic031
plogic046
-nsrR-norB-b
0h
1h
2h
3h
4h
5h
6h
7h
8h
9h
10h
11h
12h
20h
22h
24h

FIG. 22

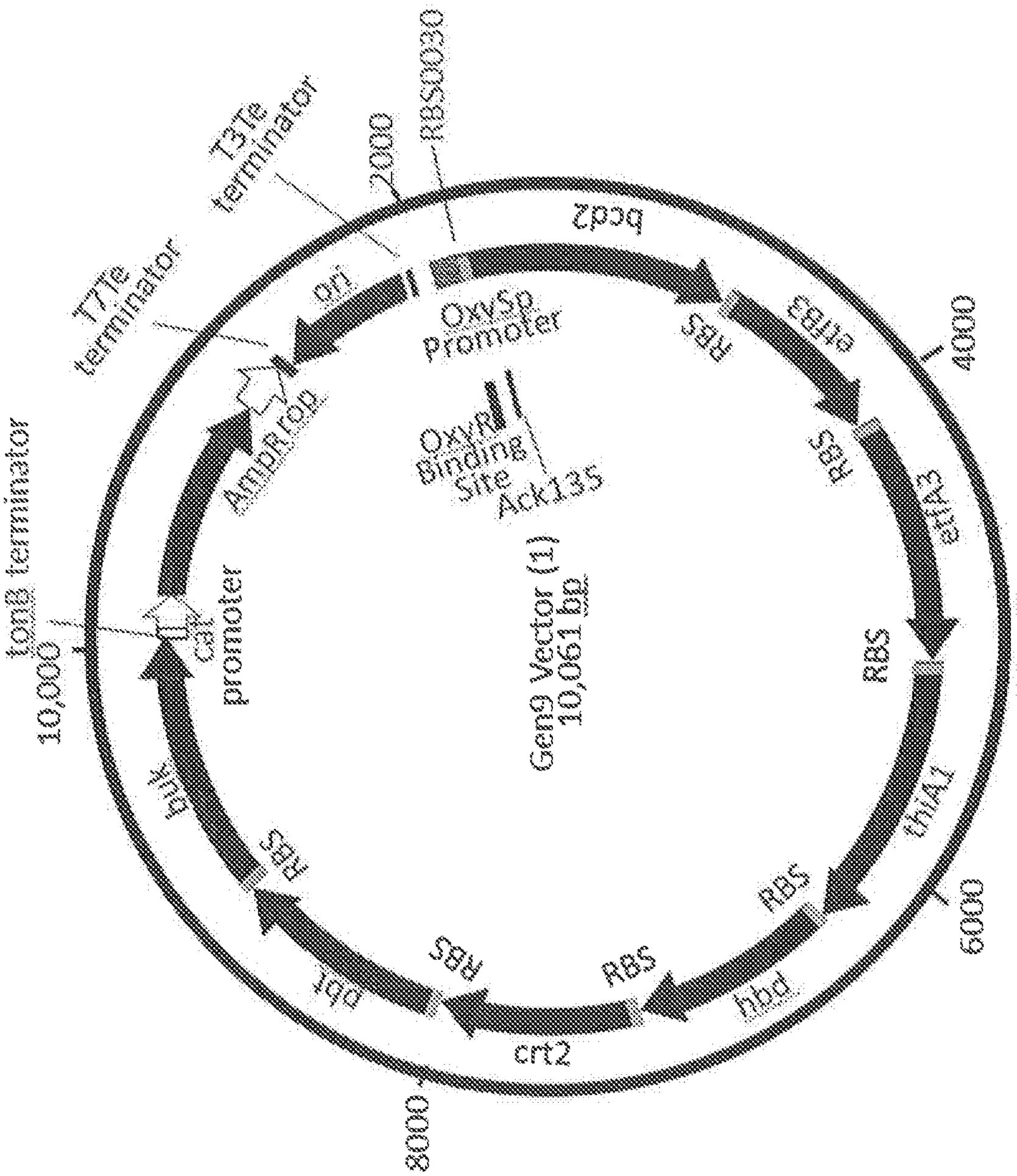

T3Te terminator
2000
RBS00
bcd2
T7Te terminator
ori
OxvSp promoter
SapR
sapA
4000
AmpR-op
OxyR Binding Site Ack135
(1)
SapR
thlA2
tomB terminator
10,000
1
cat
promoter
Gen9 Vector (1
10,061 bp
RBS
thlA1
ampR
RBS
6000
bcd
RBS
RBS
hbd
crt2
8000

FIG. 23

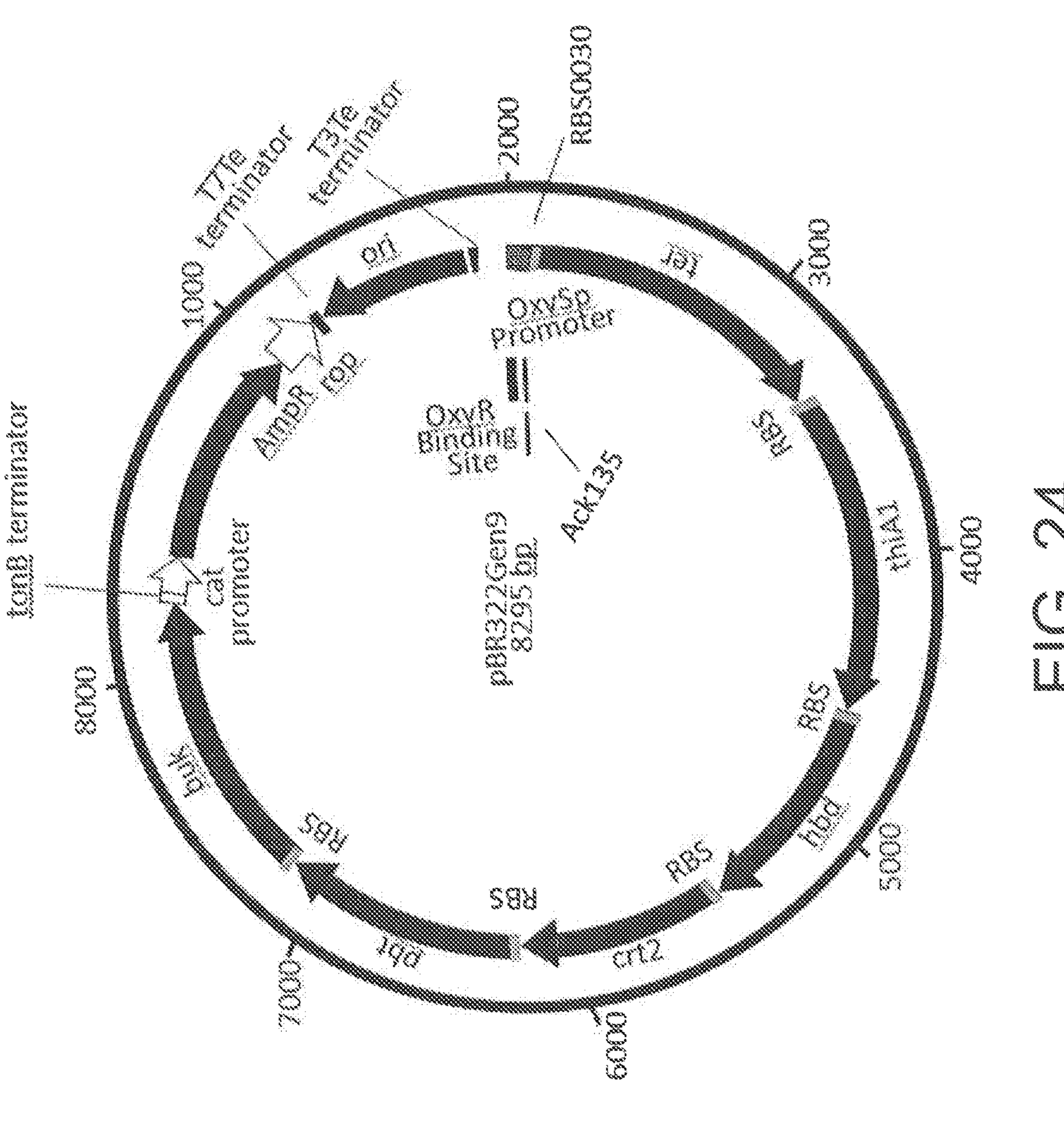

T7Te terminator
T3Te terminator
1000
2000
RBS0030
3000
ori
rep
OxyS promoter
AmpR rep
AmpR
OxyR Binding Site
Ack135
pBR322Gen9 8295 bp
sacB
tomB terminator
cat promoter
cat
8000
ampR
RBS
RBS
RBS
RBS
RBS
thlA1
hbd
crt2
bgl
7000
6000
5000
4000

FIG. 24

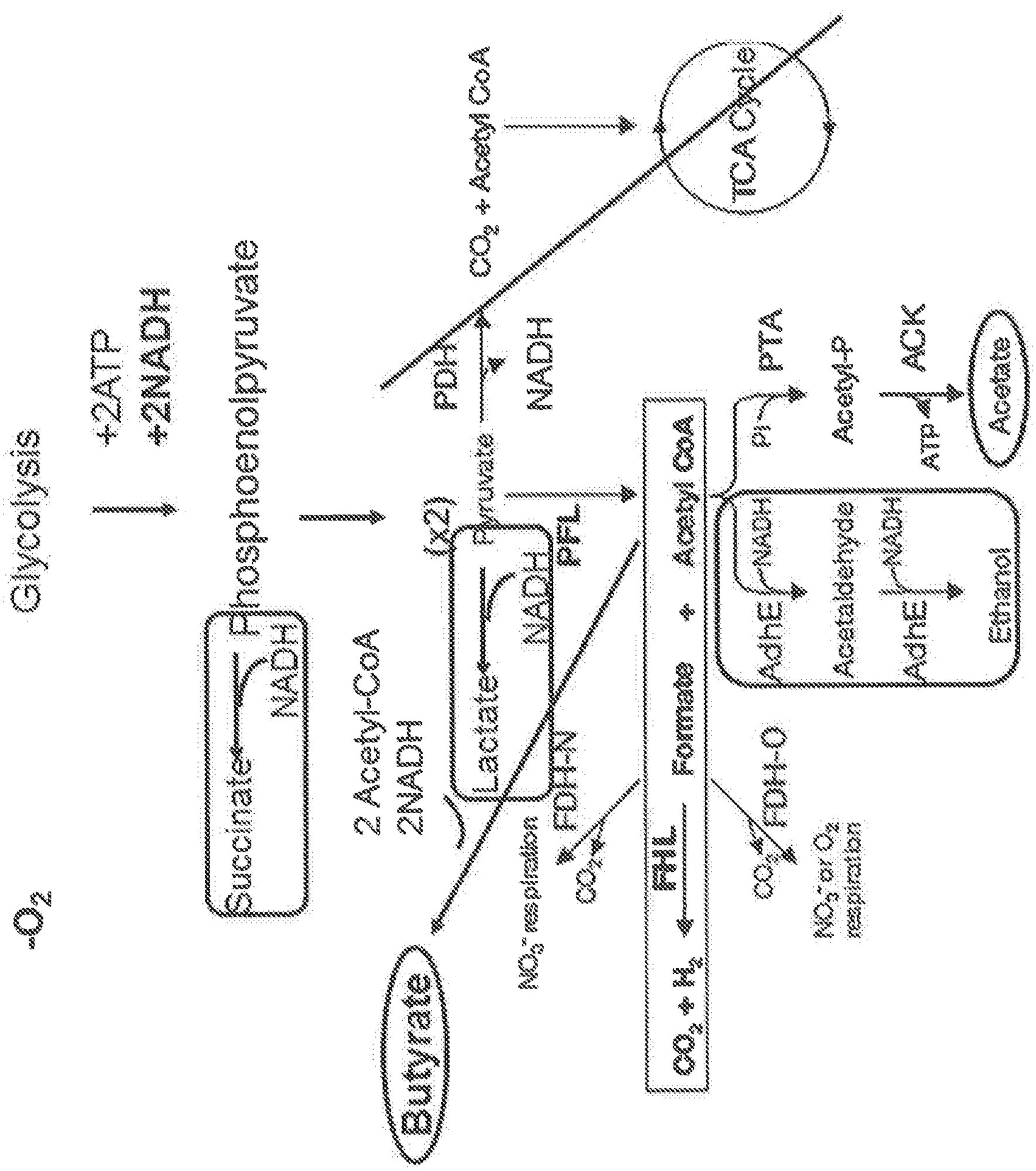

Glycolysis
+2ATP
+2NADH
Phosphoenolpyruvate
PDH
$CO_2$ + Acetyl CoA
TCA Cycle
NADH
(x2)
Pyruvate
PFL
Acetyl CoA
Succinate
NADH
2 Acetyl-CoA
2NADH
Lactate
Butyrate
$NO_3^-$ respiration
FDH-N
$CO_2$
RNL
$CO_2 + H_2$
Formate
$CO_2$
FDH-O
$NO_3^-$ or $O_2$ respiration
$O_2$
Pi
PTA
Acetyl-P
ATP
ACK
Acetate
AdhE
(NADH)
Acetaldehyde
AdhE
(NADH)
Ethanol

FIG. 25

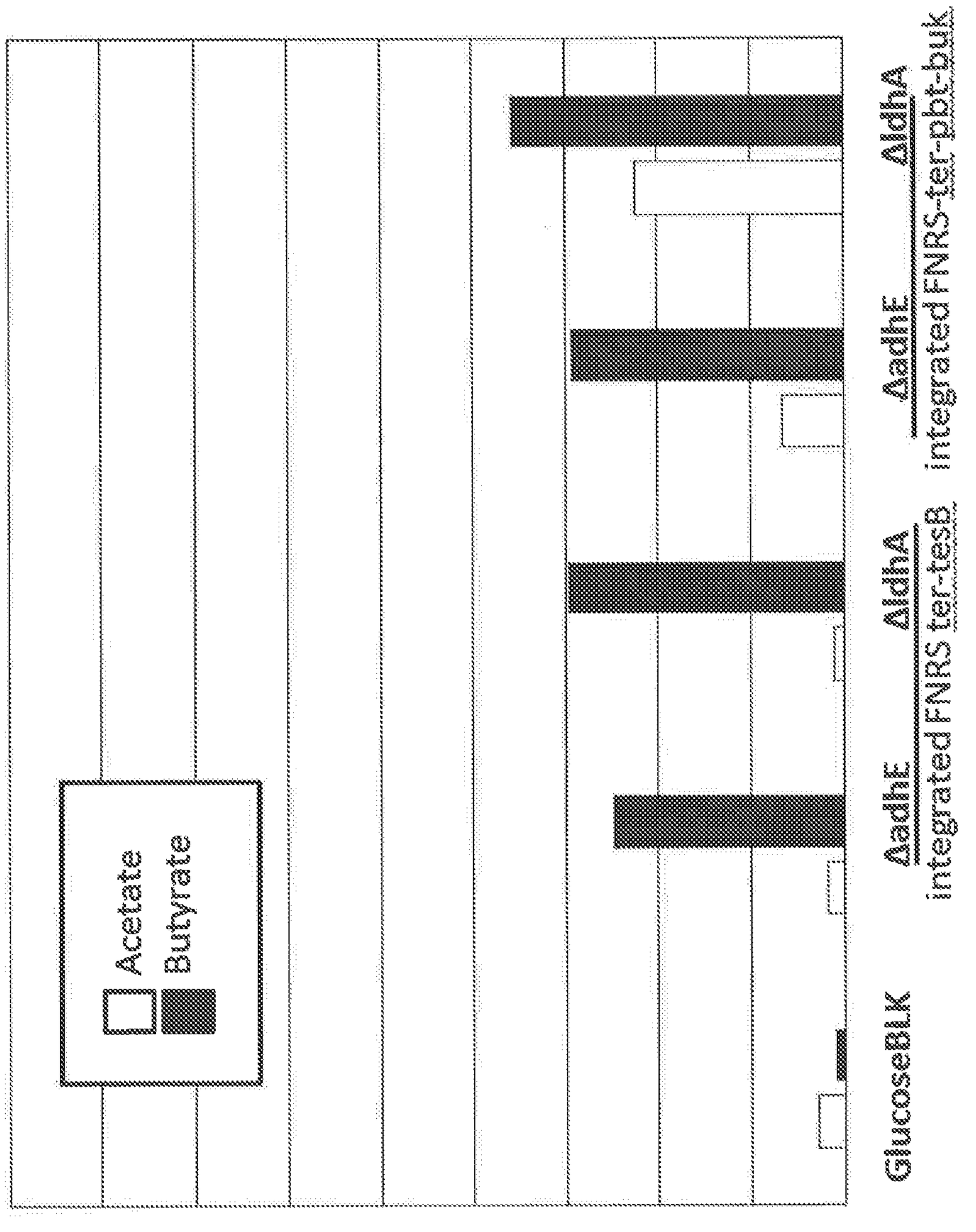

Acetate
Butyrate
GlucoseBLK
ΔadhE
integrated FNRS-ter-pbt-buk
ΔadhA
integrated FNRS ter-tesB
ΔldhA
integrated FNRS-ter-pbt-buk
ΔadhE
ΔldhA
integrated FNRS ter-tesB
0
1
2
3
4
5
6
7
8
9
Acids (mM)

FIG. 26A

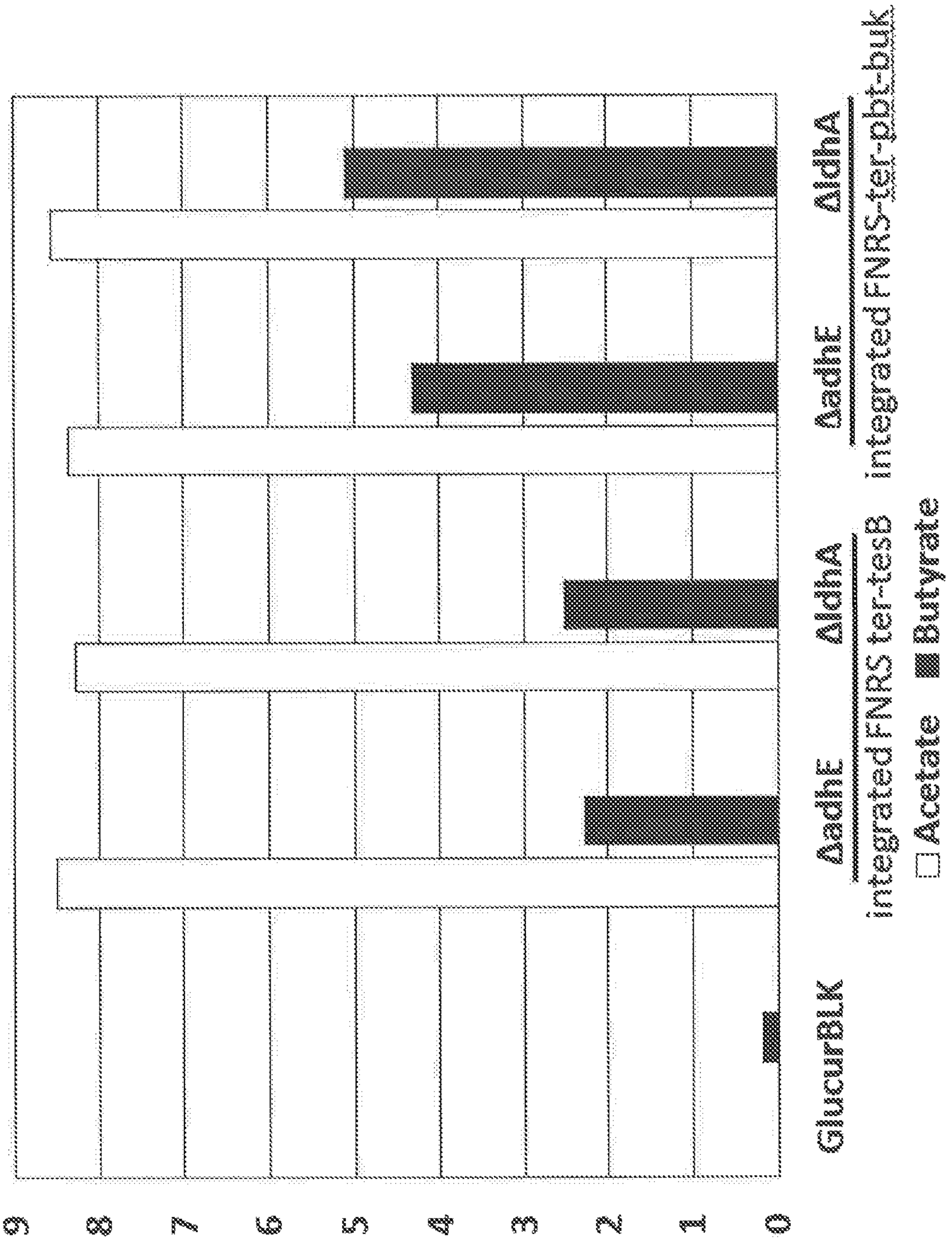

AldhA
integrated FNRS ter-tesB
AldhA
integrated FNRS-ter-pbt-buk
Butyrate
Acetate
GlucurBLK
ΔadhE
ΔadhE
ΔadhE
0
1
2
3
4
5
6
7
8
9
Acids (mM)

FIG. 26B

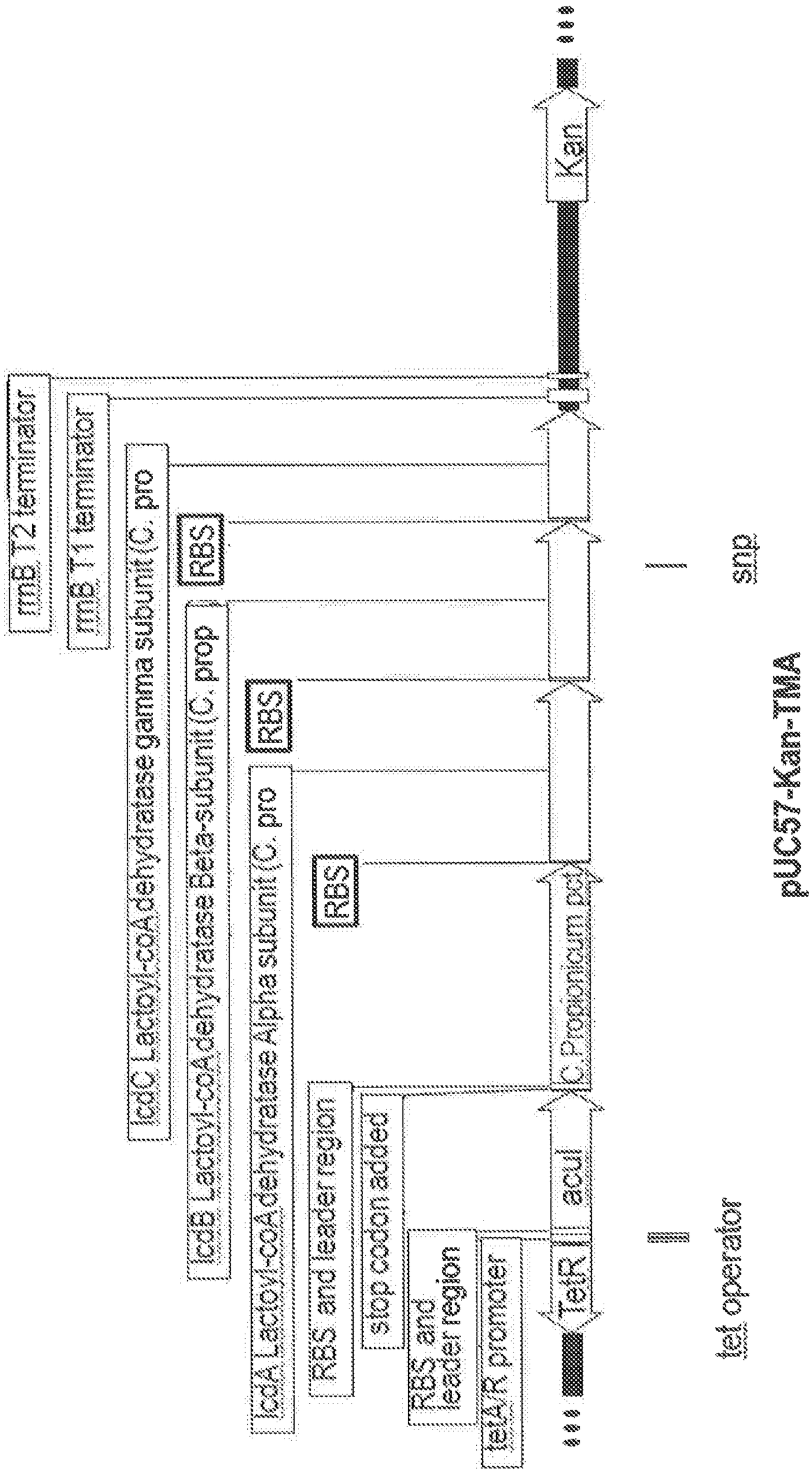

Kan
rrnB T2 terminator
rrnB T1 terminator
lcdC Lactoyl-coA dehydratase gamma subunit(C. pro
RBS
lcdB Lactoyl-coA dehydratase Beta-subunit(C. pro
RBS
lcdA Lactoyl-coA dehydratase Alpha subunit(C. pro
RBS
C.Proionicum pdu
lcdB Lactoyl-coA dehydratase Beta-subunit(C. pro
lcdA Lactoyl-coA dehydratase Alpha subunit(C. pro
RBS and leader region
stop codon added
RBS and leader region
tetA/R promoter
acul
TetR
tet operator
snp
pUC57-Kan-TMA
9509 bp

FIG. 27

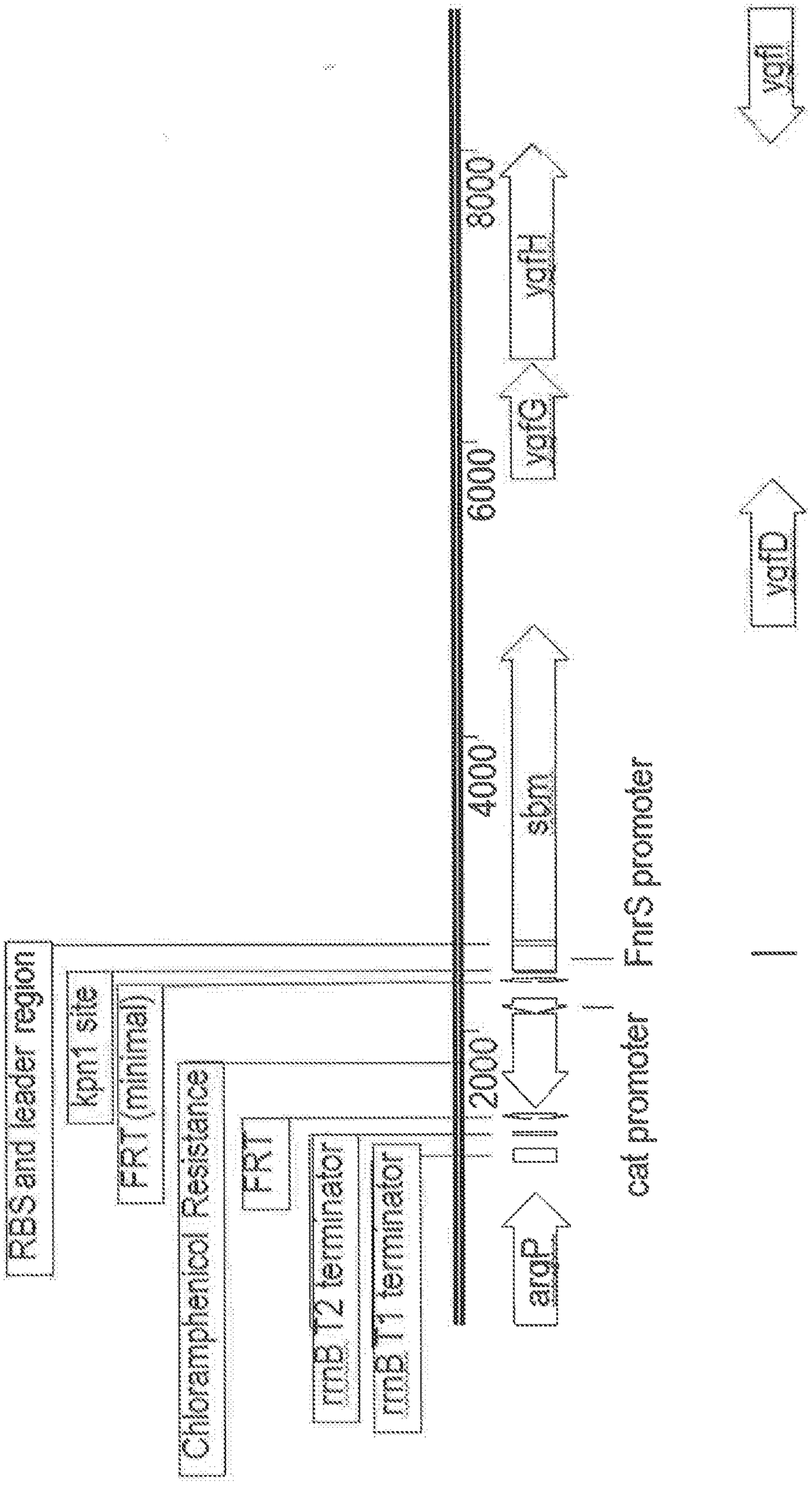

8000
KanR
6000
bla
4000
sbm
2000
araP
cat promoter
FnrS promoter
FNR binding site
RBS and leader region
kpn1 site
FRT (minimal)
Chloramphenicol Resistance
FRT
rrnB T2 terminator
rrnB T1 terminator

FIG. 28

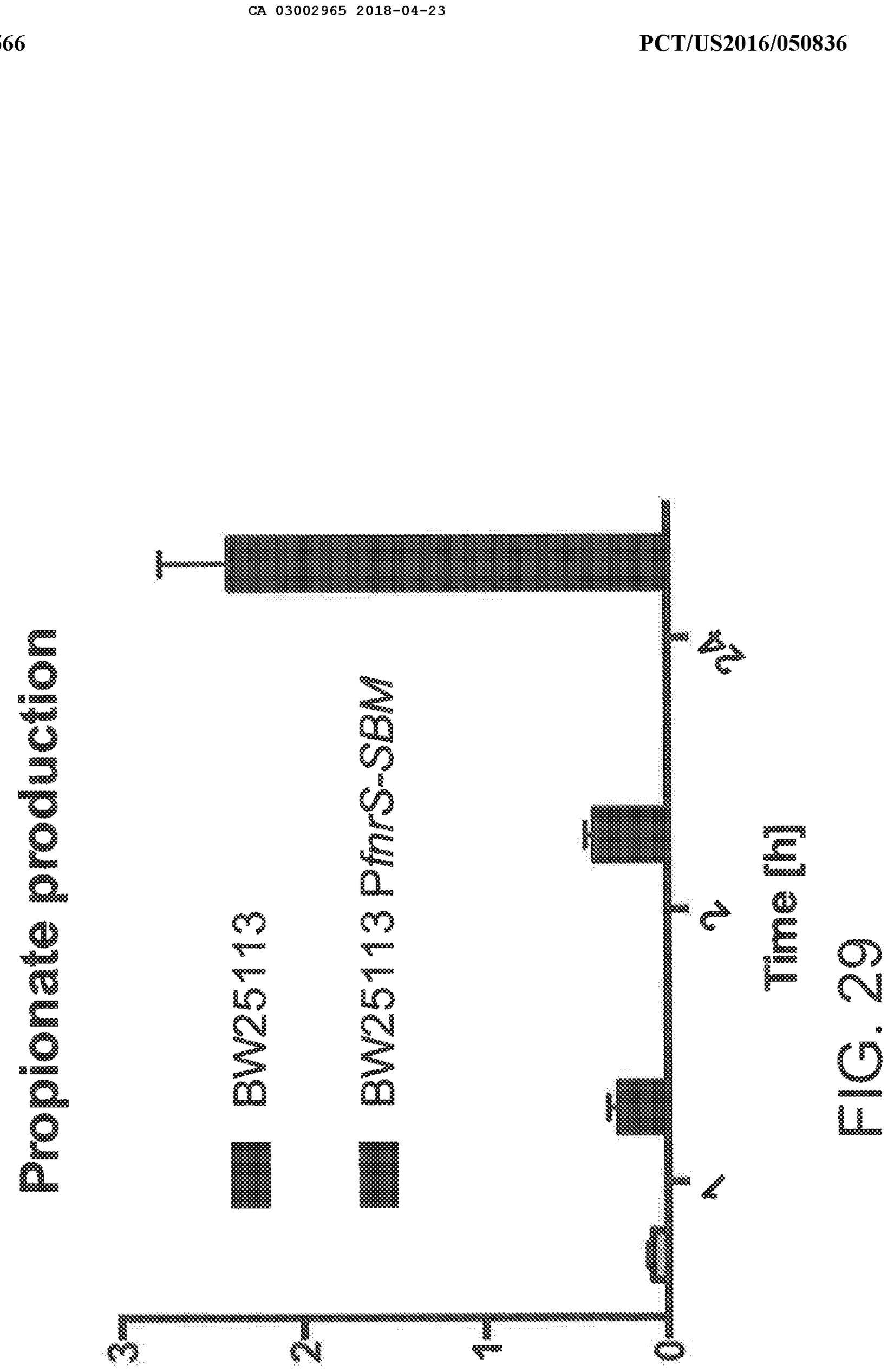

Propionate production
BW25113 PfrS-SBM
BW25113
propionate (mM)
0
1
2
3
Time [h]
24
7

FIG. 29

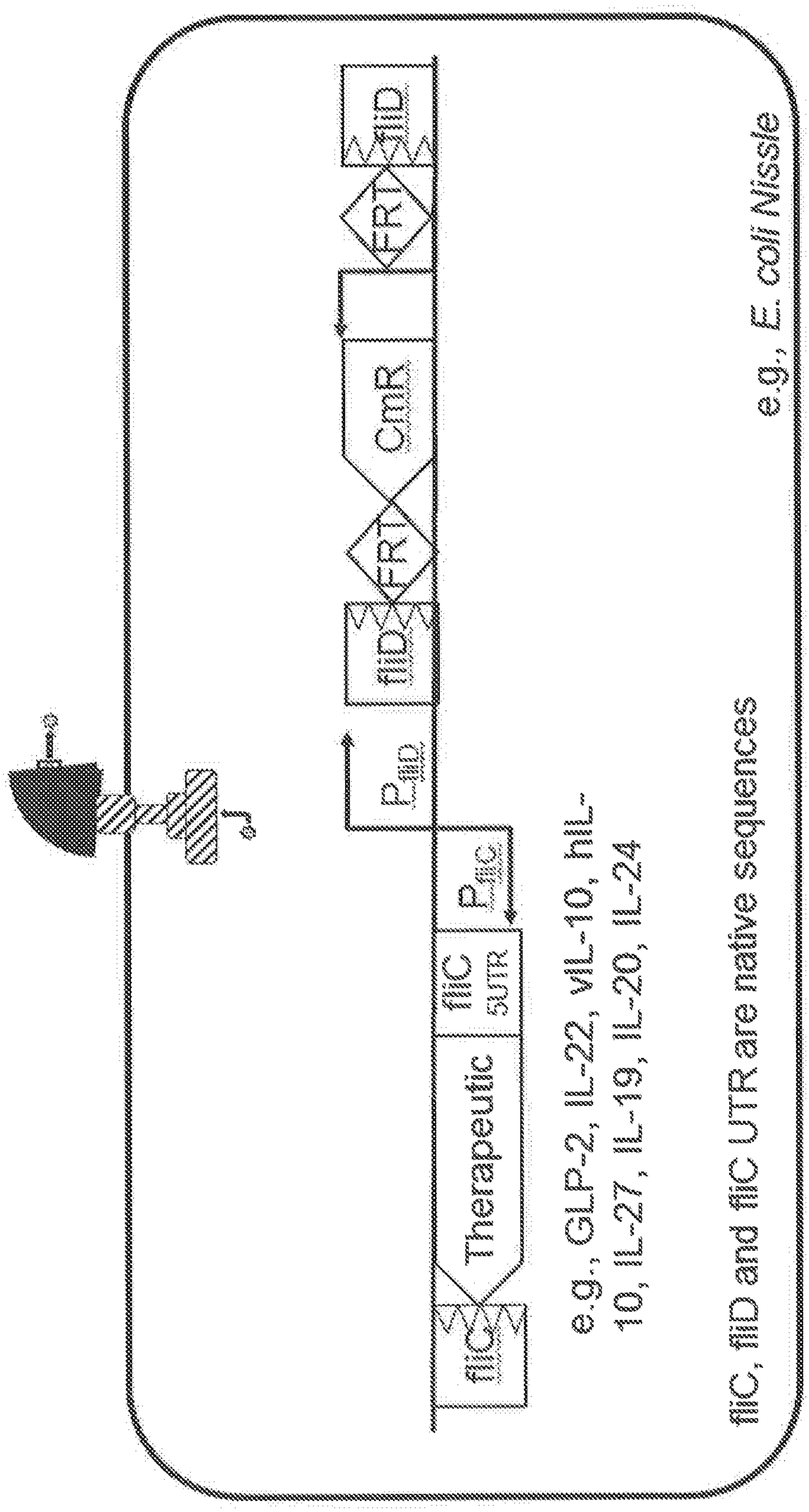

fliD
fliC CMR
fliD ERT
fliC ERT
fliD
fliC 5UTR
Therapeutic
fliC
e.g., E. coli Nissle
e.g., GLP-2, IL-22, vIL-10, hIL-10, IL-27, IL-19, IL-20, IL-24
fliC, fliD and fliC UTR are native sequences

FIG. 30A

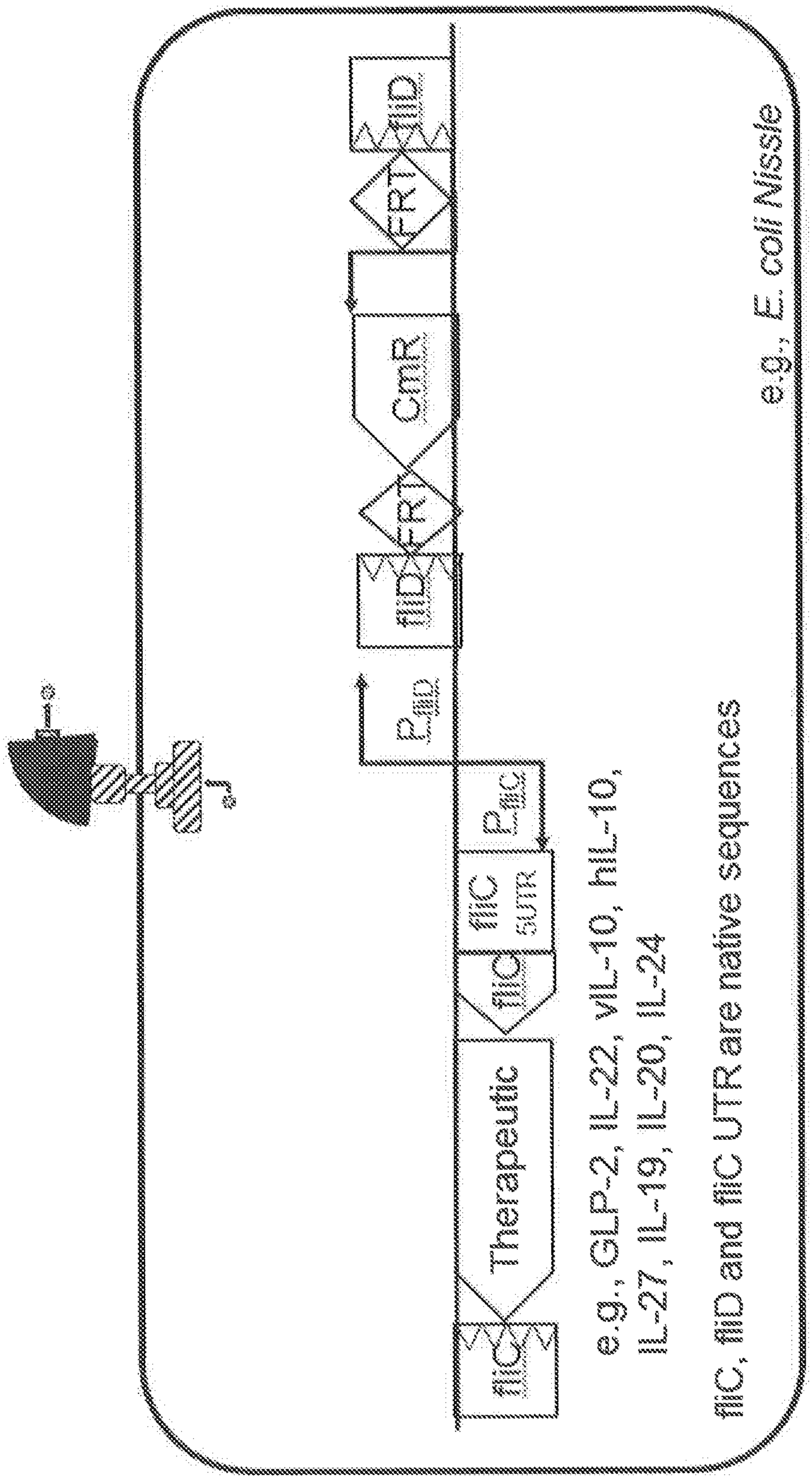

fliD
FRT
Cmr
FRT
fliD
Therapeutic
fliC
fliC 5UTR
e.g., GLP-2, IL-22, vIL-10, hIL-10,
IL-27, IL-19, IL-20, IL-24
fliC, fliD and fliC UTR are native sequences
e.g., E. coli Nissle

FIG. 30B

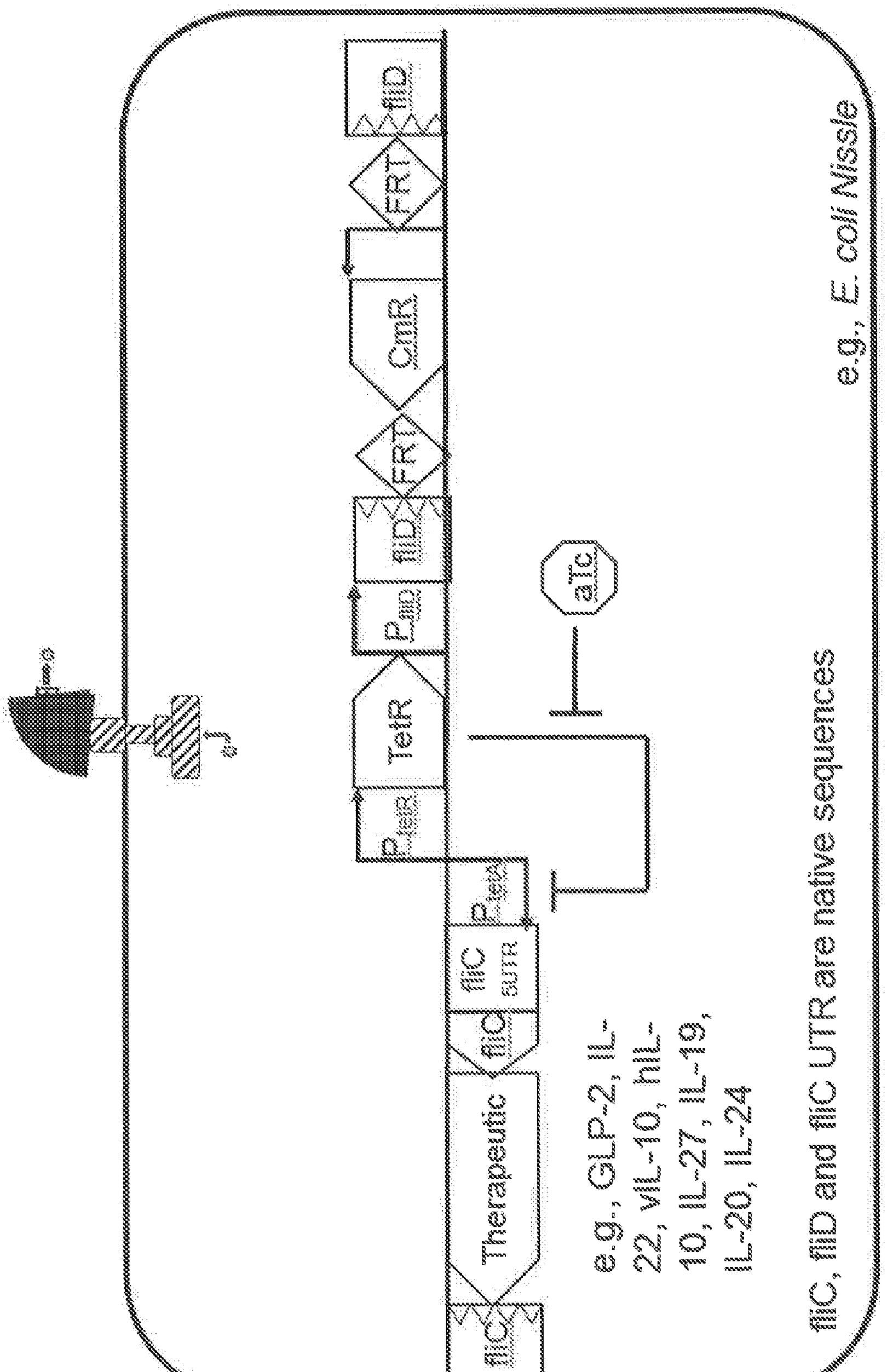

fliD
fliT
P_fliD
fliD
P_fliC
fliC 5'UTR
Therapeutic
fliC
fliD
fliC
P_TetR
TetR
P_fliD
fliD
FRT
CmR
FRT
atc
e.g., GLP-2, IL-22, hIL-10, hIL-10, IL-27, IL-19, IL-20, IL-24
e.g., E. coli Nissle
fliC, fliD and fliC UTR are native sequences

FIG. 30C

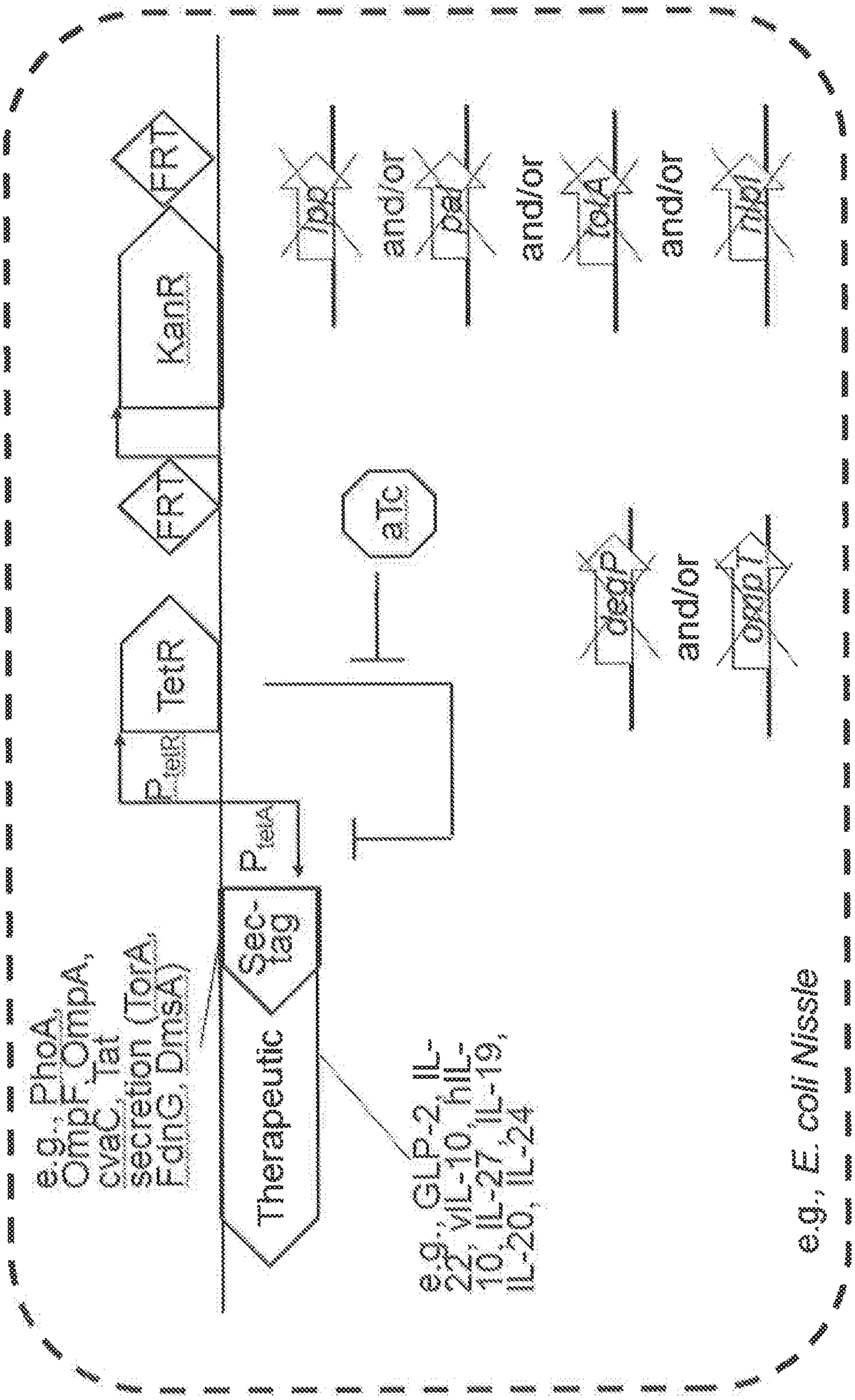

disrupted outer membrane
e.g., PhoA, OmpF, OmpA, cyaC, pat secretion (torA, fdnG, dmsA)
Therapeutic
Sec/ Tag
GLP-2, IL-6, IL-10, hIL-12, IL-27, IL-19, IL-20, IL-24
aTc
PtetR
TetR
PtetA
FliAT
KanR
FRT
lacI
and/or
cat
tetA
and/or
rbsD
and/or
dapA
and/or
ompT
e.g., E. coli Nissle

FIG. 31A

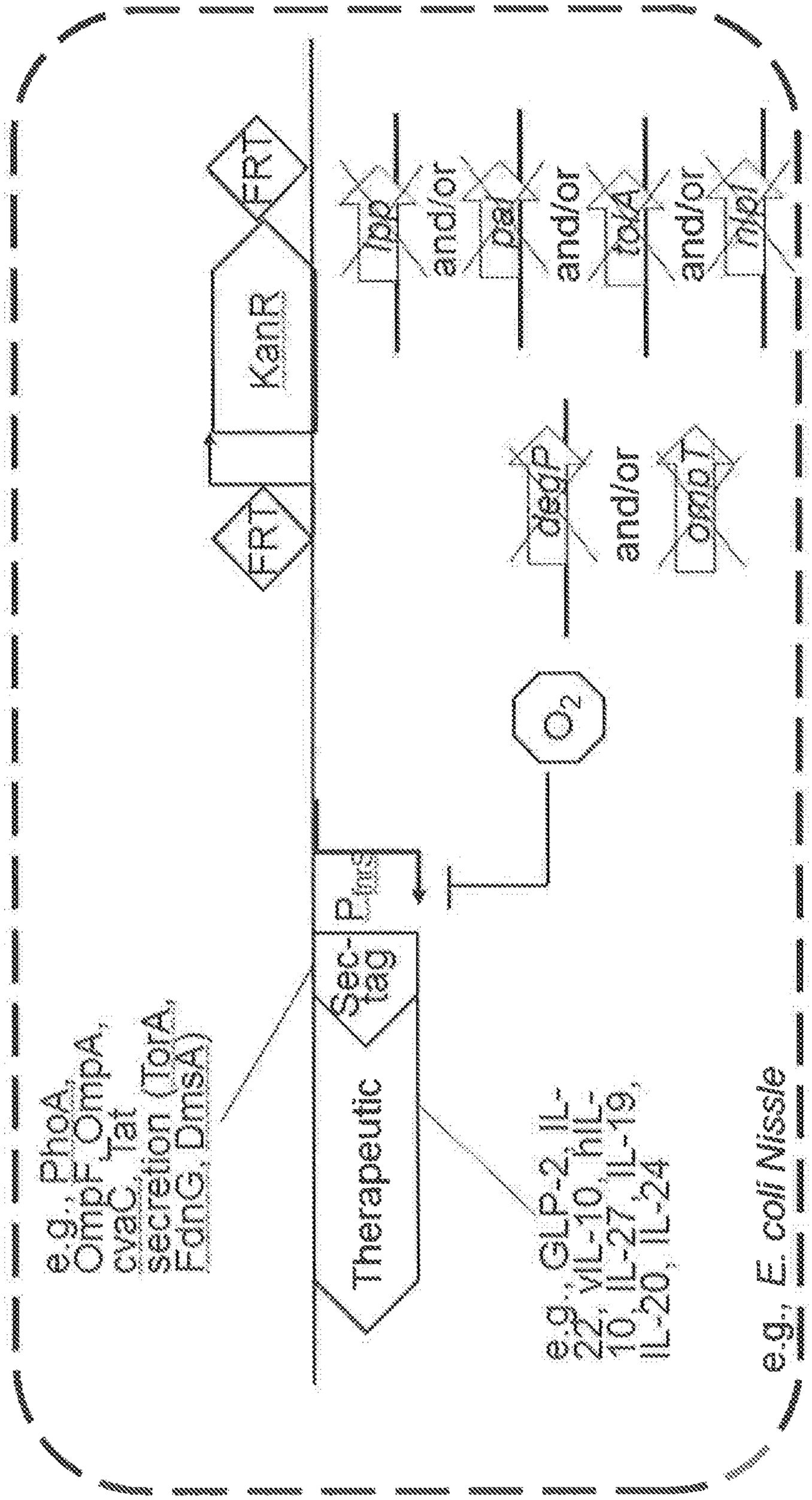

FRT
KanR
FRT
lpp
and/or
pal
and/or
tolA
and/or
mal
degP
and/or
ompT
O2
Sec/Tat
SRP
Therapeutic
e.g., phoA, ompF, OmpA, cvaC, tat secretion (TorA, sufG, DmsA)
e.g., GLP-2, IL-22, vIL-10, hIL-10, IL-27, IL-19, IL-20, IL-24
e.g., E. coli Nissle

FIG. 31B

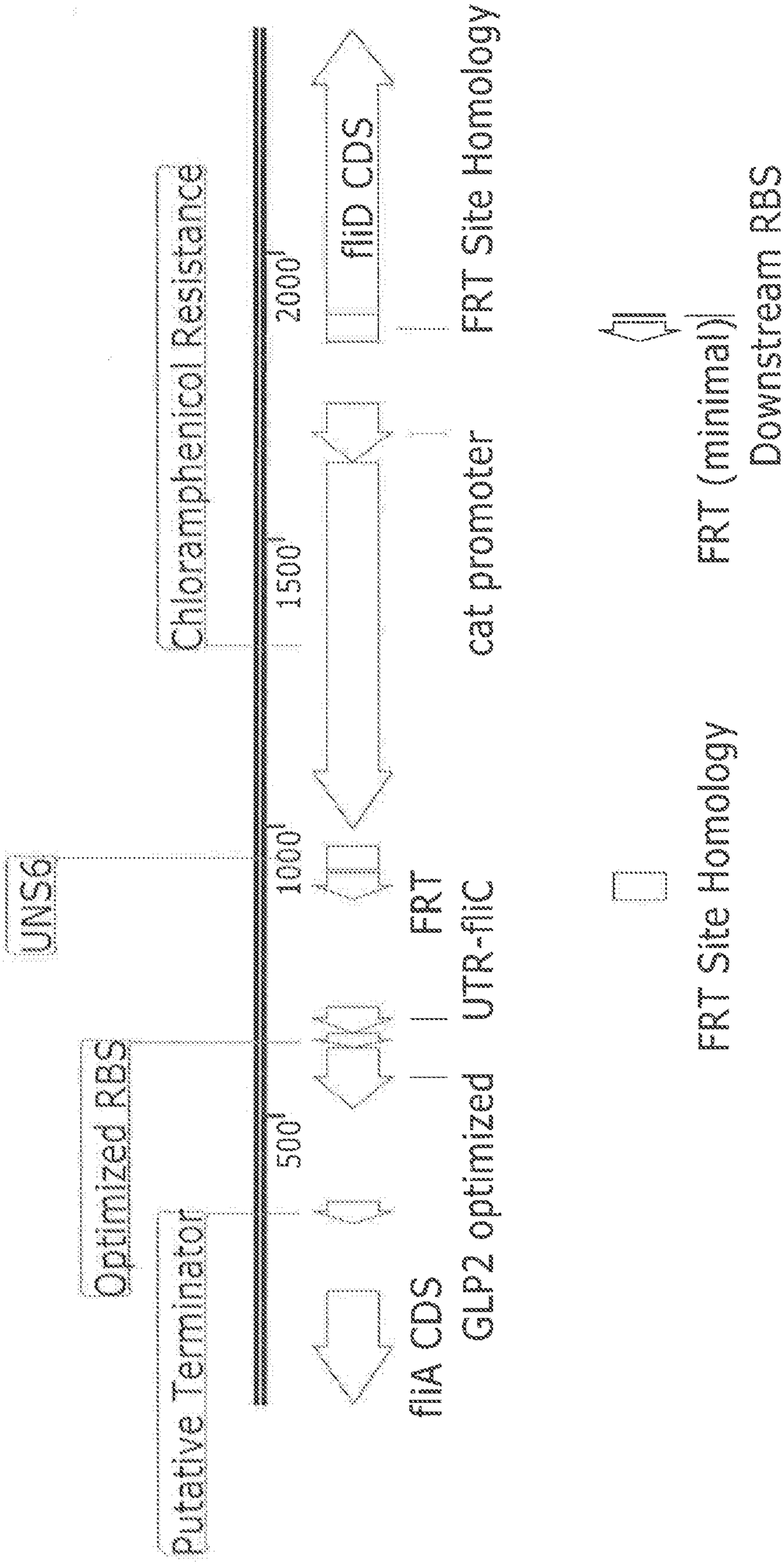

Chloramphenicol Resistance
1500
2000
fliD CDS
FRT Site Homology
cat promoter
FRT
UNS6
1000
Optimized RBS
500
UTR-fliC
putative Terminator
fliA CDS
GLP2 optimized
FRT (minimal)
Downstream RBS
FRT Site Homology

FIG. 32A

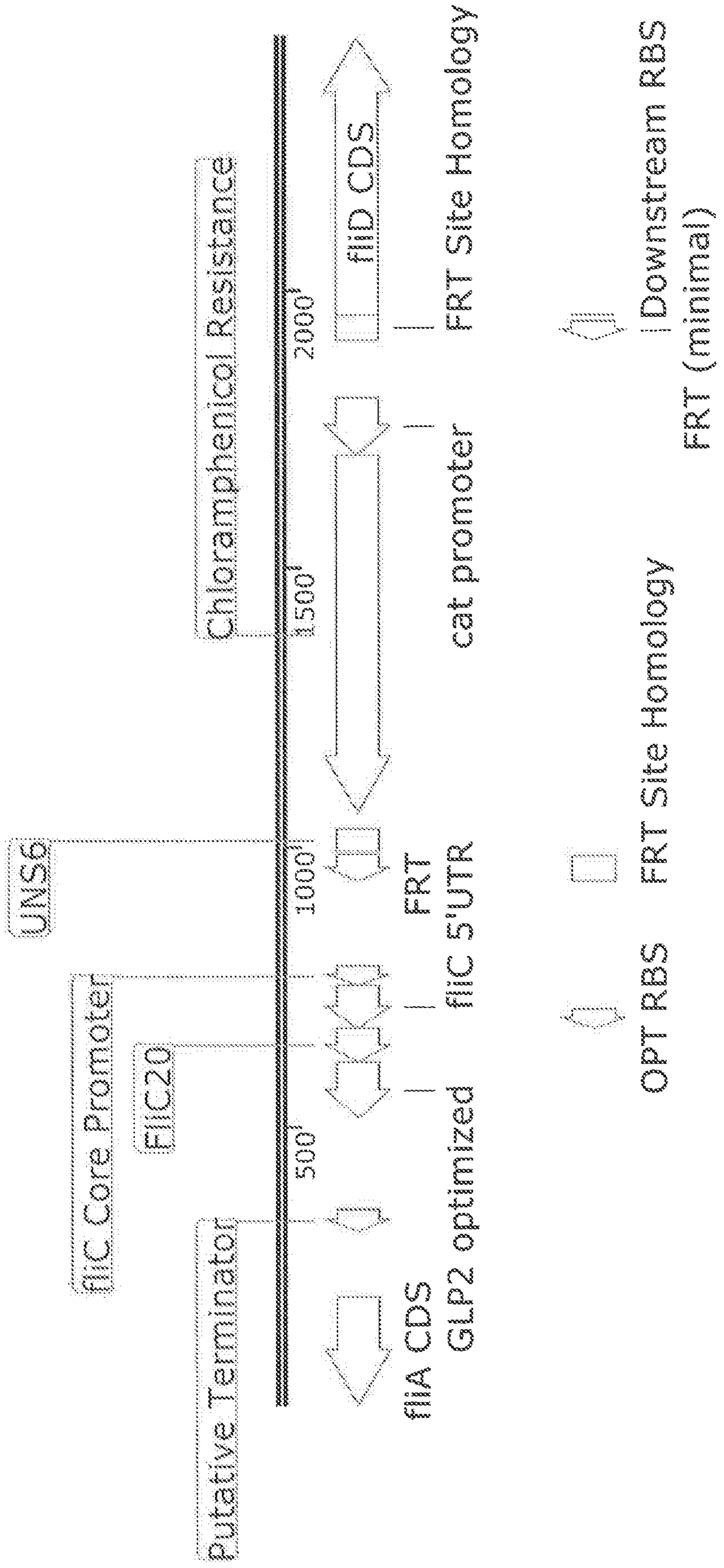

Chloramphenicol Resistance
1500'
2000'
fliD CDS
FRT Site Homology
cat promoter
Downstream RBS
FRT (minimal)
UMS6
1000'
FRT
FLIC20
500'
fliC 5'UTR
optimized
OPT RBS
FRT Site Homology
fliC Core Promoter
Putative Terminator
fliA CDS
GLP2 op

FIG. 32B

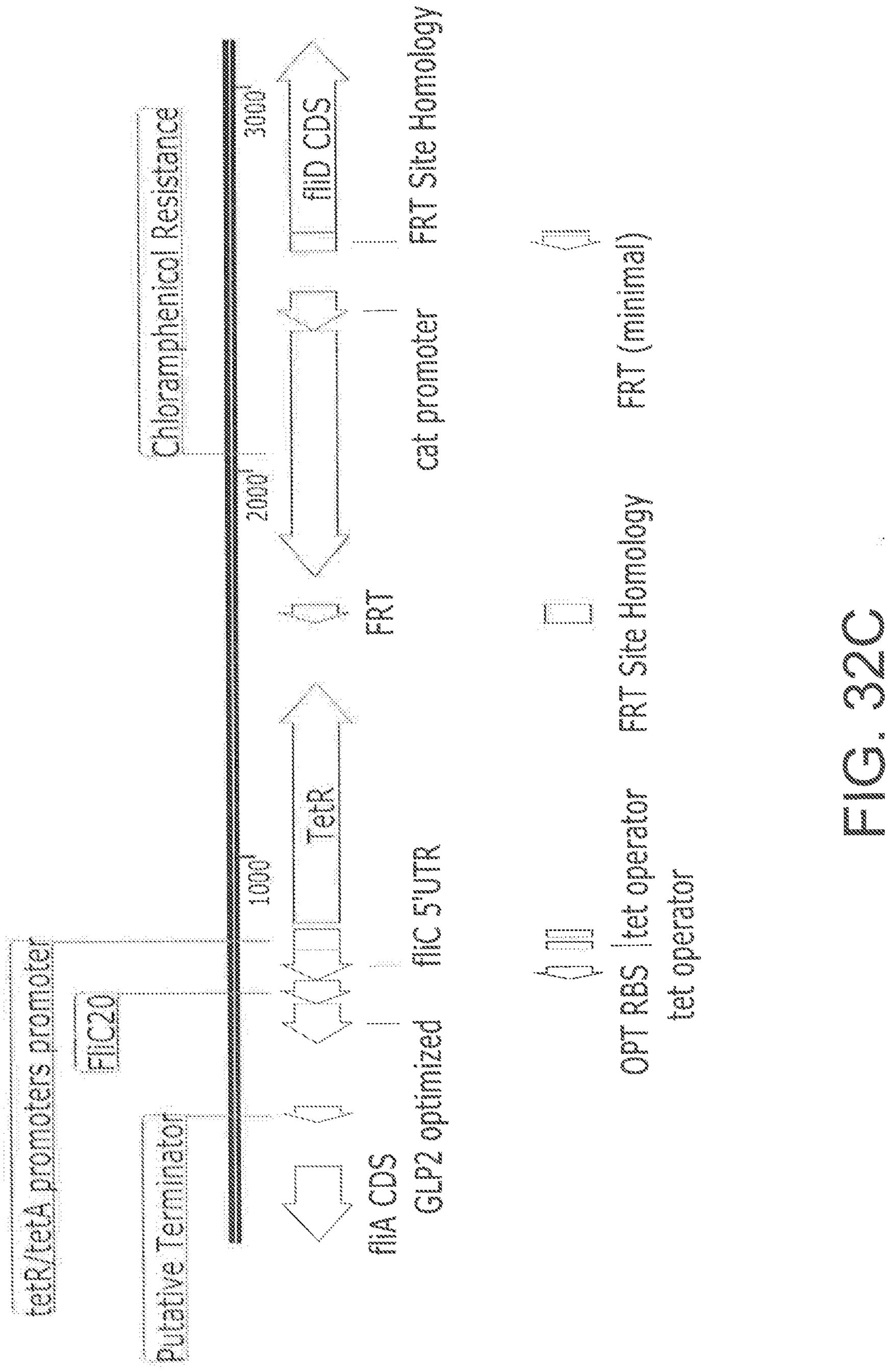

Chloramphenicol Resistance
3000
fliD CDS
FRT Site Homology
2000
cat promoter
FRT (minimal)
FRT
fliC 5'UTR
1000
TetR
OPT RBS tet operator
FRT Site Homology
tet operator
tetR/tetA promoters
fliC20
Putative Terminator
fliA CDS
GLP2 optimized

FIG. 32C

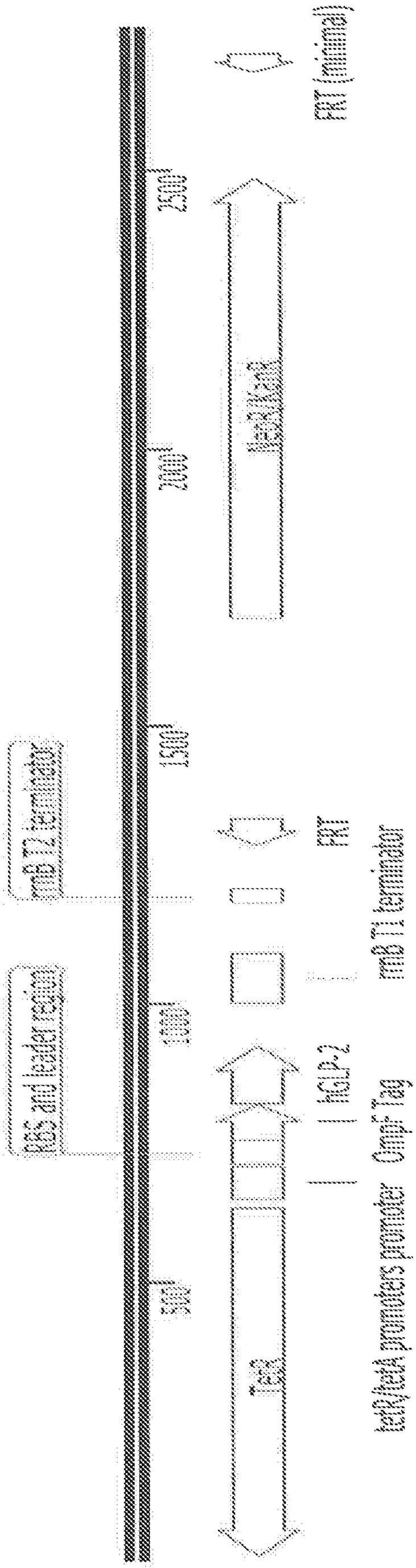

EBV trimer
2500
2000
1500
1000
500
RBS and leader region
mB7.1Z terminator
RBS and leader region
mB7.1 terminator
RBS number
Comp Tag
tetO/hetA promoters
Term

FIG. 32D

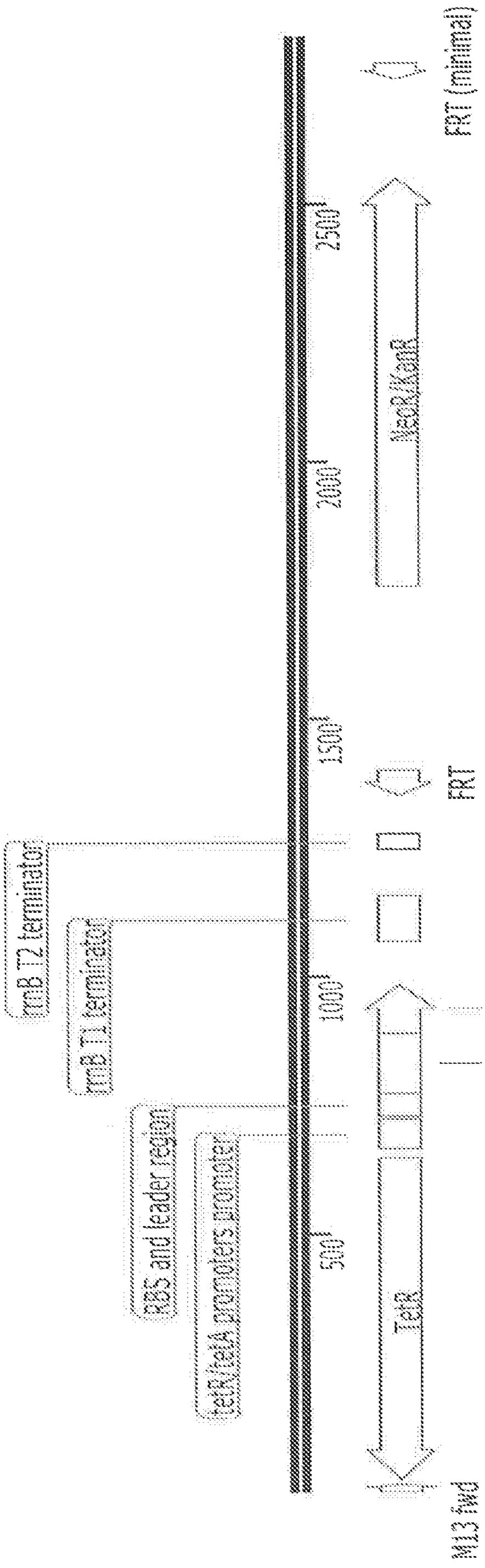

rrnB T2 terminator
rrnB T1 terminator
rbs and leader region
tetR/tetA promoters promoter
Tat-export (TorA)
Tet regulated GLP2
FRT
FRT (minimal)
M13 fwd
TetR
NeoR/KanR
500
1000
1500
2000
2500

FIG. 32E

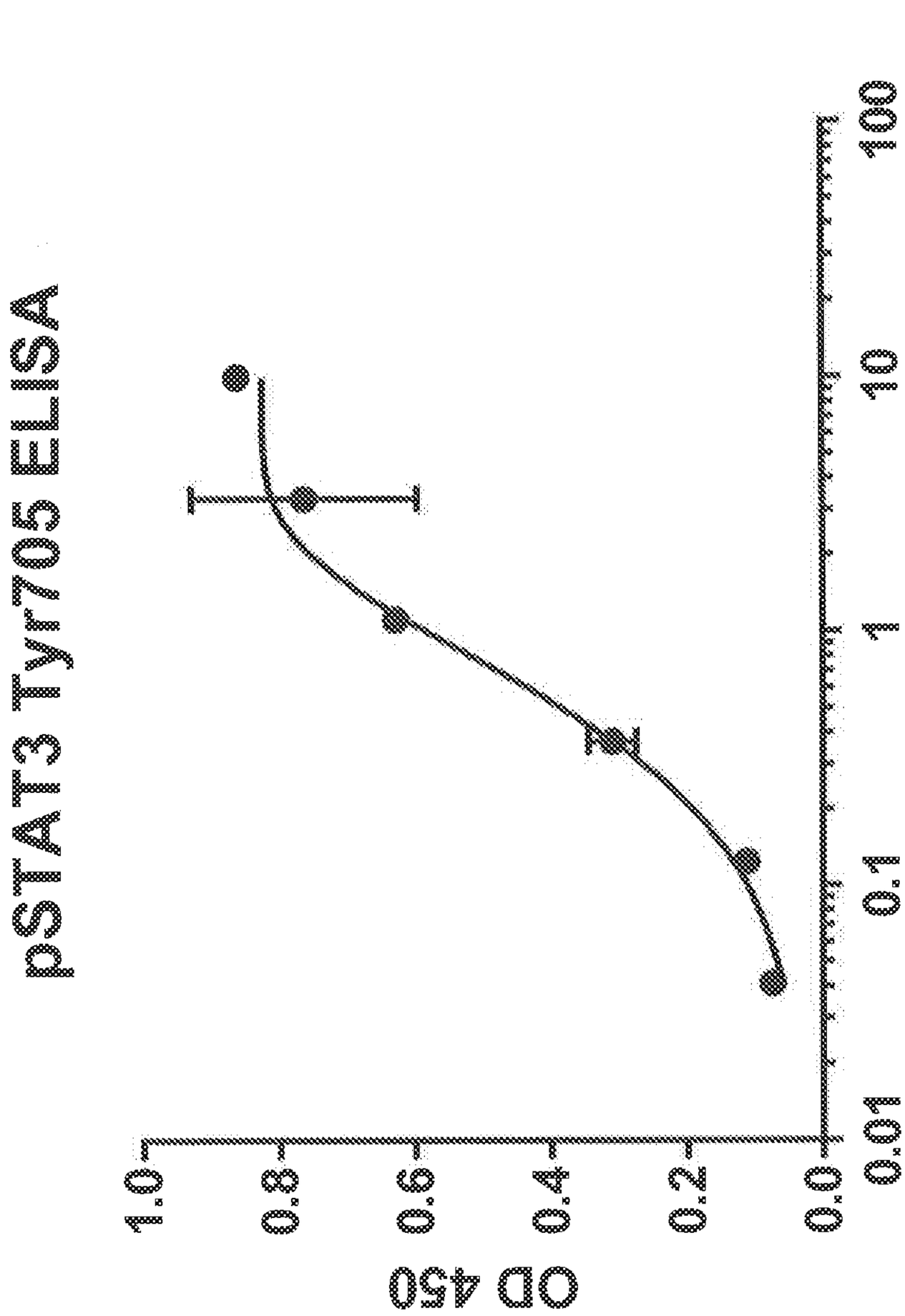

pSTAT3 Tyr705 ELISA
OD 450
1.0
0.8
0.6
0.4
0.2
0.0
0.01
0.1
1
10
100
IL22 (ng/ml)

FIG. 33A

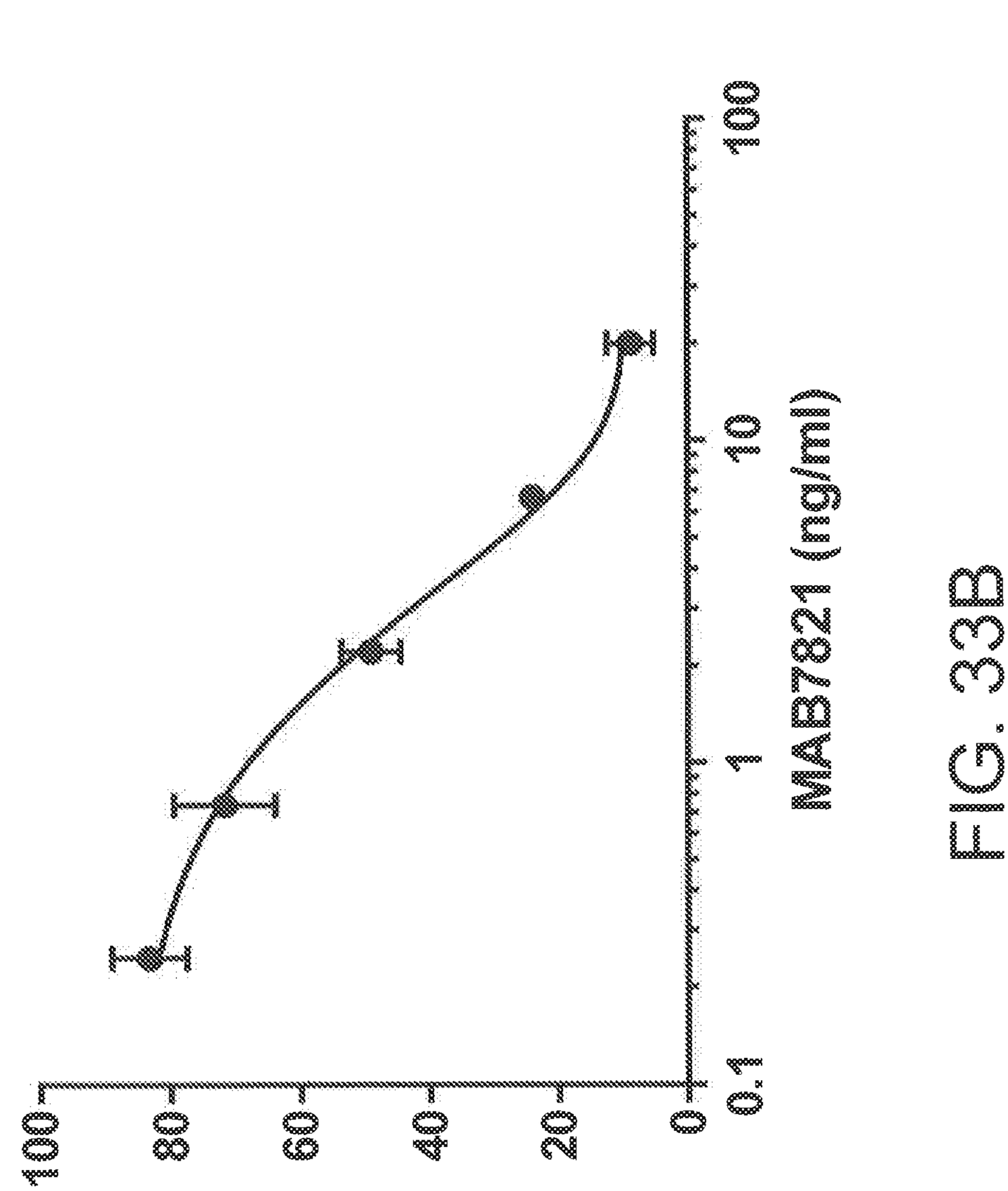

pSTAT3 Tyr705 ELISA
% Competition
100
80
60
40
20
0
0.1
1
10
100
MAB7821 (ng/ml)

FIG. 33B

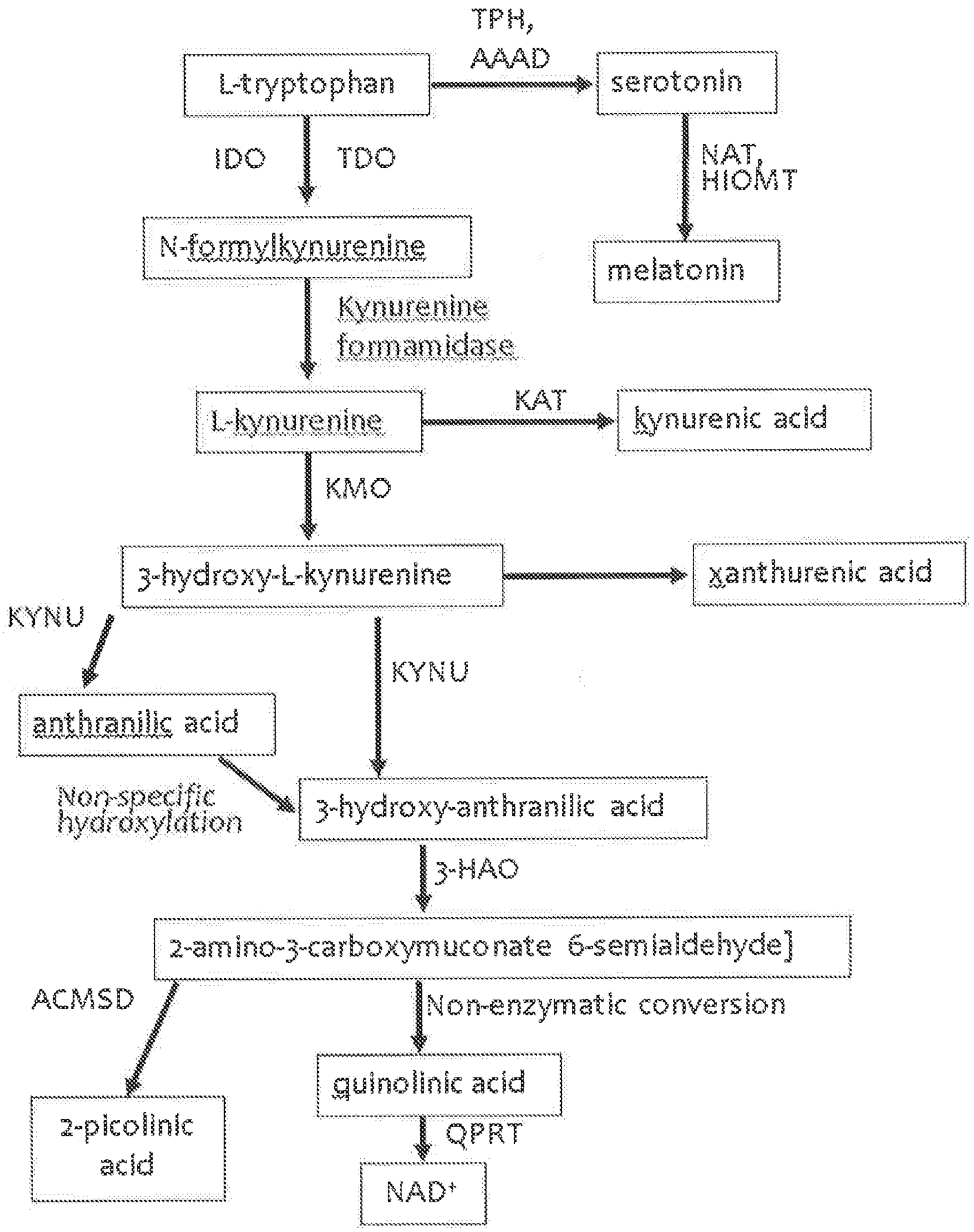

TPH,
AAAD
L-tryptophan
serotonin
IDO
TDO
NAT,
HIOMT
N-formylkynurenine
melatonin
Kynurenine
formamidase
L-kynurenine
KAT
kynurenic acid
KMO
3-hydroxy-L-kynurenine
xanthurenic acid
KYNU
KYNU
anthranillc acid
Non-specific
hydroxylation
3-hydroxy-anthranillc acid
3-HAO
2-amino-3-carboxymuconate 6-semialdehyde]
ACMSD
Non-enzymatic conversion
2-plcollinlc
acid
qulnollnlc acid
QPRT
NAD+

FIG. 34

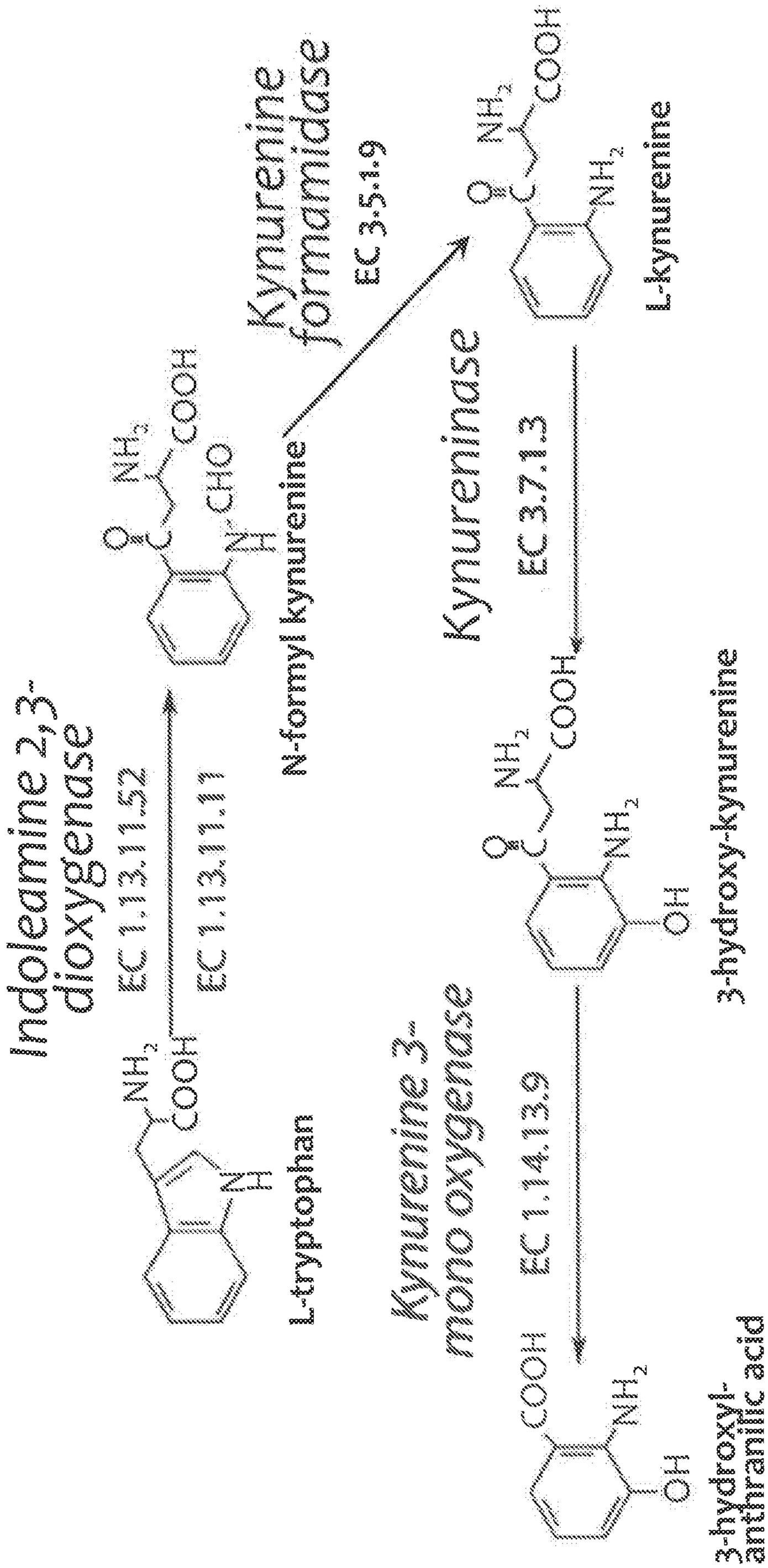
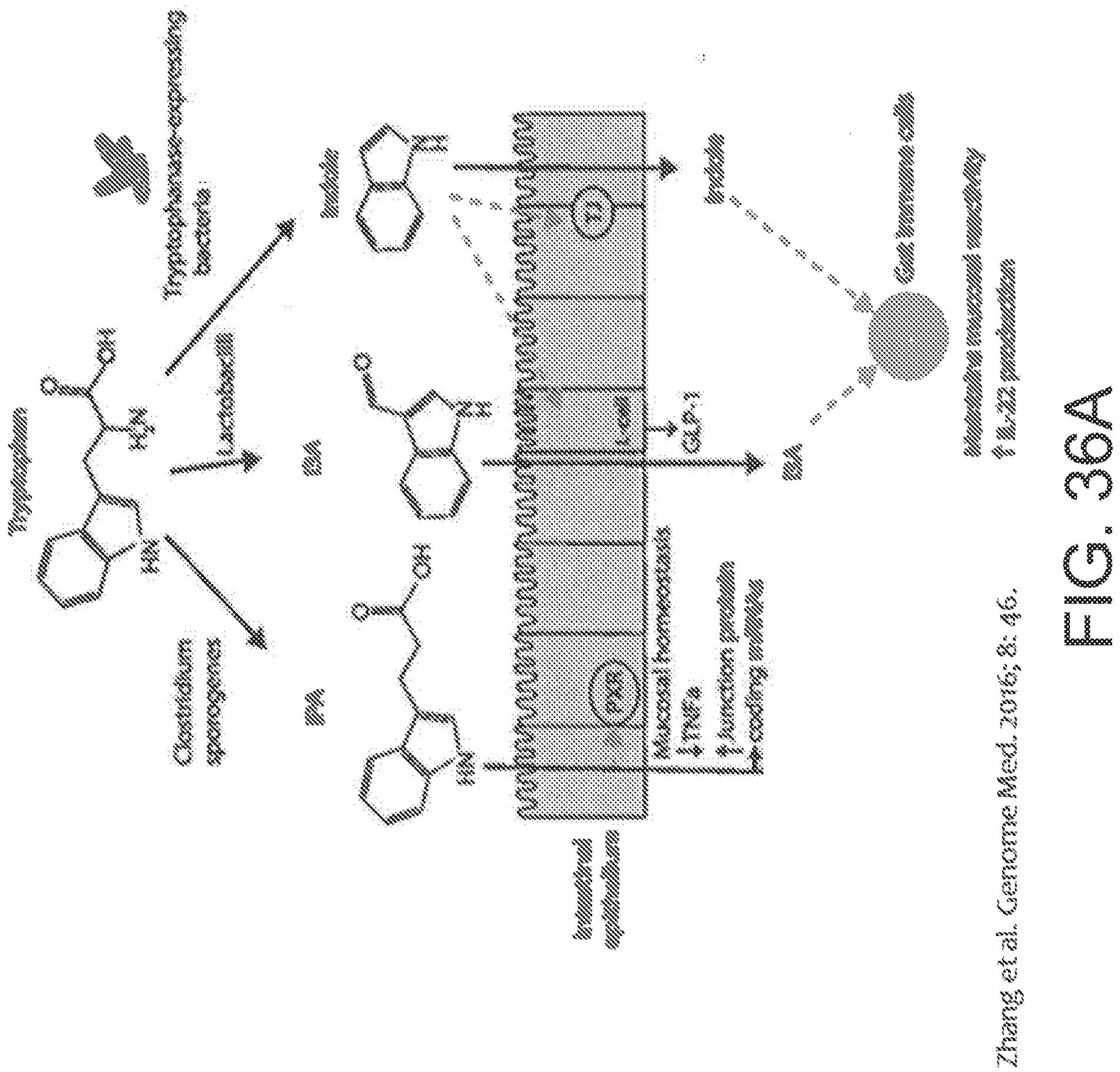

Indoleamine 2,3-dioxygenase
EC 1.13.11.52
L-tryptophan
EC 1.13.11.11
N-formyl kynurenine
Kynurenine formamidase
EC 3.5.1.9
L-kynurenine
Kynureninase
EC 3.7.1.3
Kynurenine 3-monooxygenase
EC 1.14.13.9
3-hydroxy-kynurenine
3-hydroxyl-anthranilic acid

FIG. 35


tryptophan
tryptophan
Clostridium sporogenes
indole
indole
indole
indole
Mucosal homeostasis
↓TNFα
↑Junctional proteins
↑colonic mucus
Gut lumen
Host
Host
Host

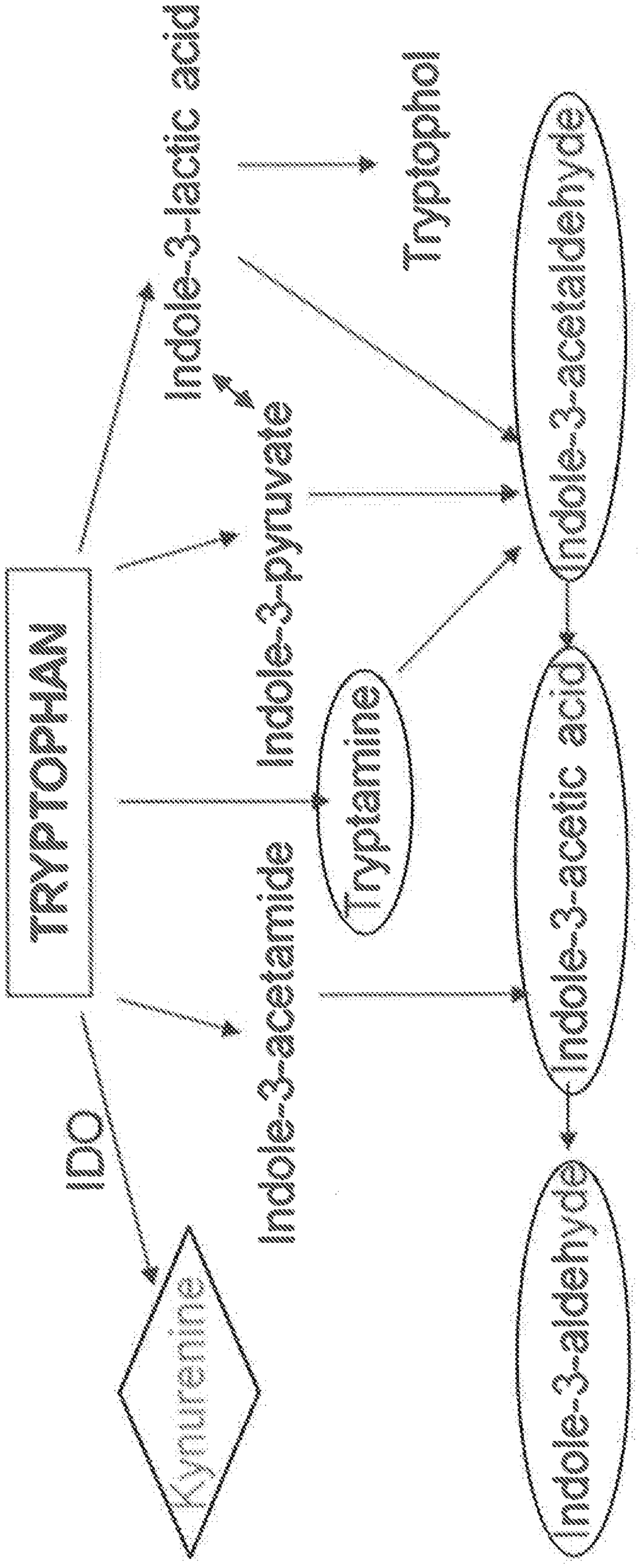

TRYPTOPHAN
IDO
kynurenine
indole-3-acetamide
indole-3-pyruvate
tryptamine
indole-3-lactic acid
tryptophol
indole-3-acetaldehyde
indole-3-acetic acid
indole-3-ethanol

FIG. 36B

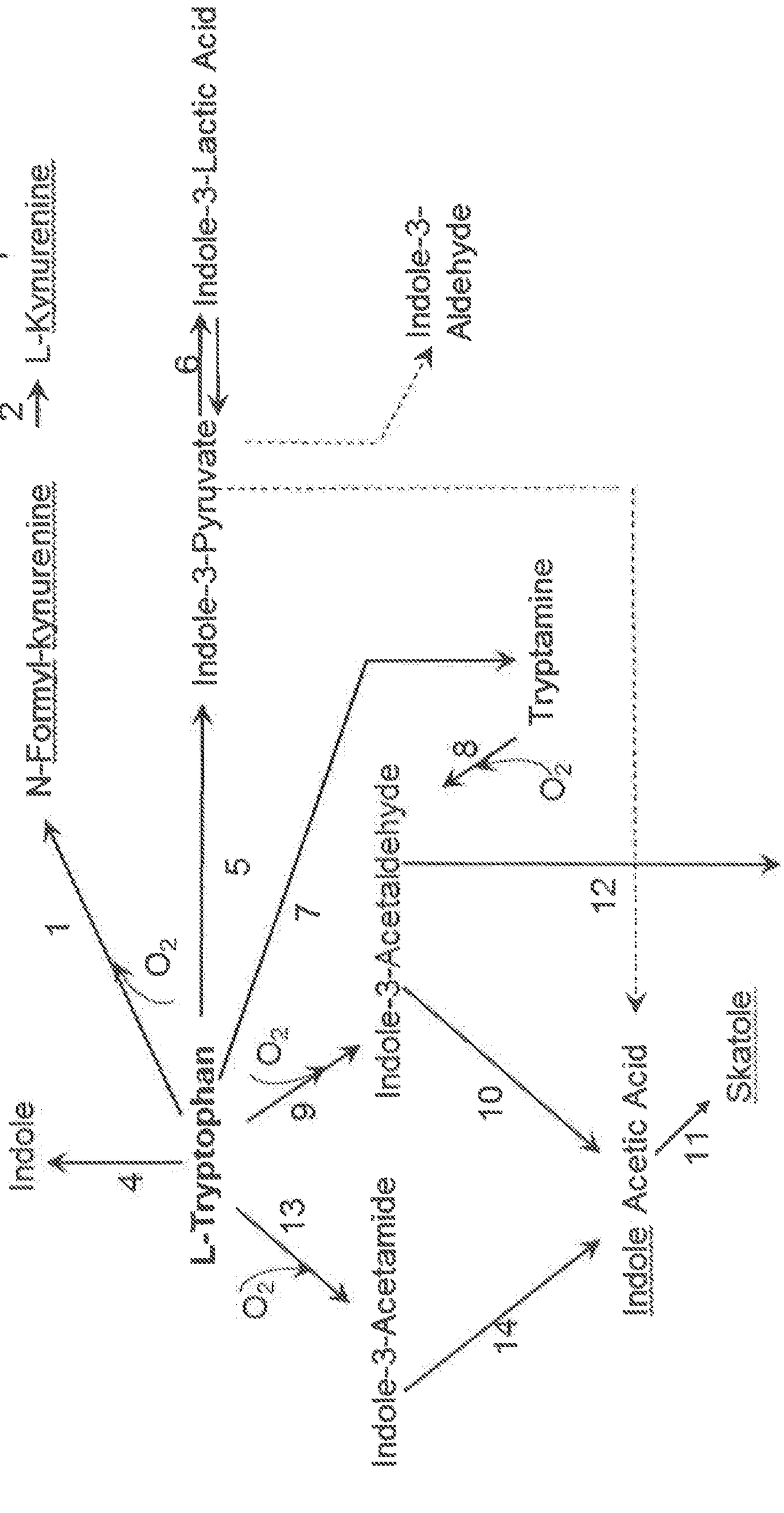

Anthranilate
3
2
L-Kynurenine
N-Formyl-kynurenine
Indole-3-Pyruvate
6
Indole-3-L
Indole-3-
Aldehyde
Tryptamine
1
$O_2$
5
7
$O_2$
9
8
$O_2$
Indole-3-Acetaldehyde
12
Indole-3-Ethanol
10
Indole Acetic Acid
11
skatole
L-Tryptophan
Indole
4
$O_2$
13
Indole-3-Acetamide
14

FIG. 37A

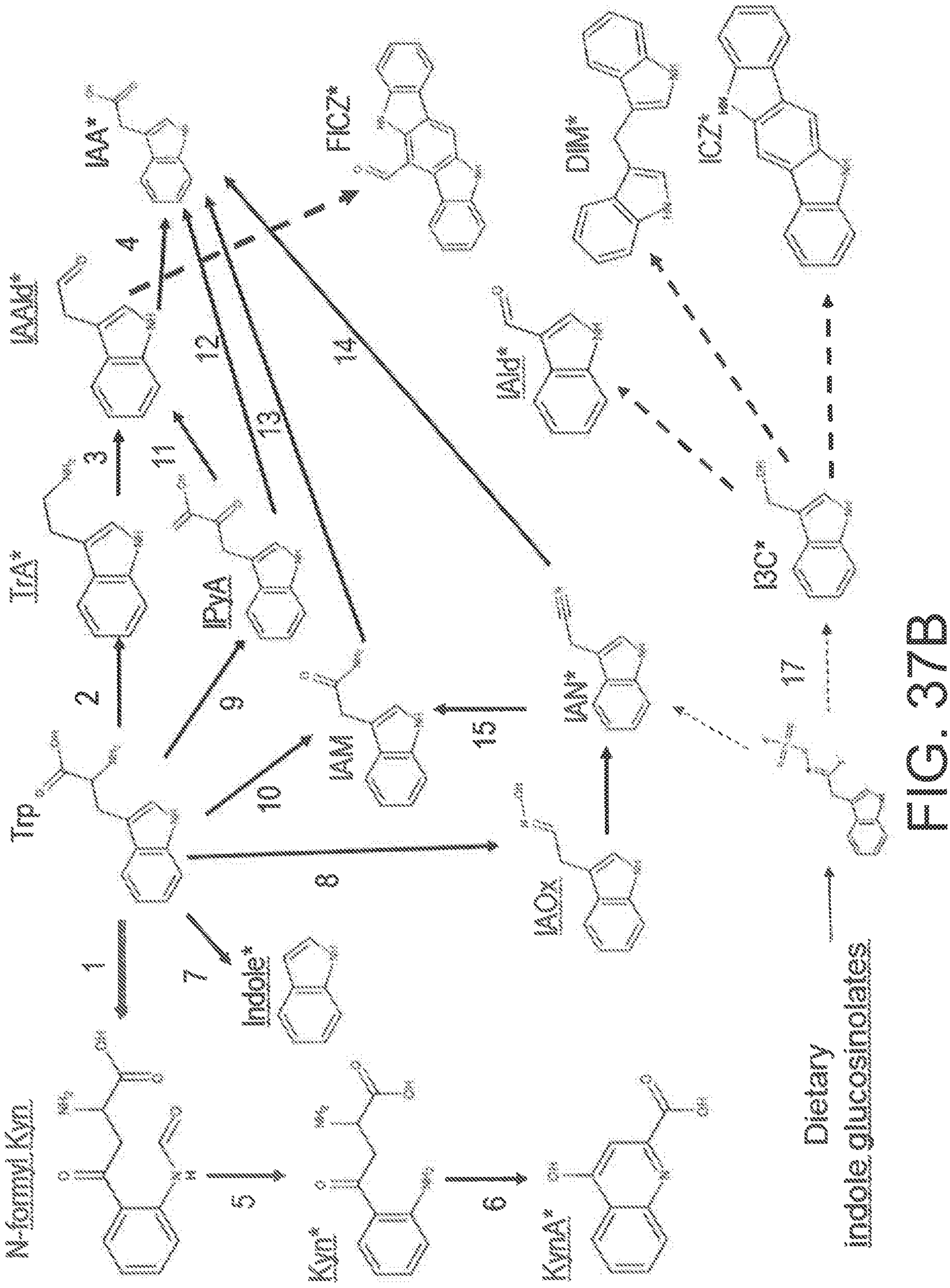

IAA*
FICZ*
DIM*
ICZ*
IAMk*
IAMd*
IGC*
Trp
IPyA*
IAM*
IAld*
IAOx*
Indole*
N-formyl Kyn
Kyn*
KynA*
Dietary indole glucosinolates
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17

FIG. 37B

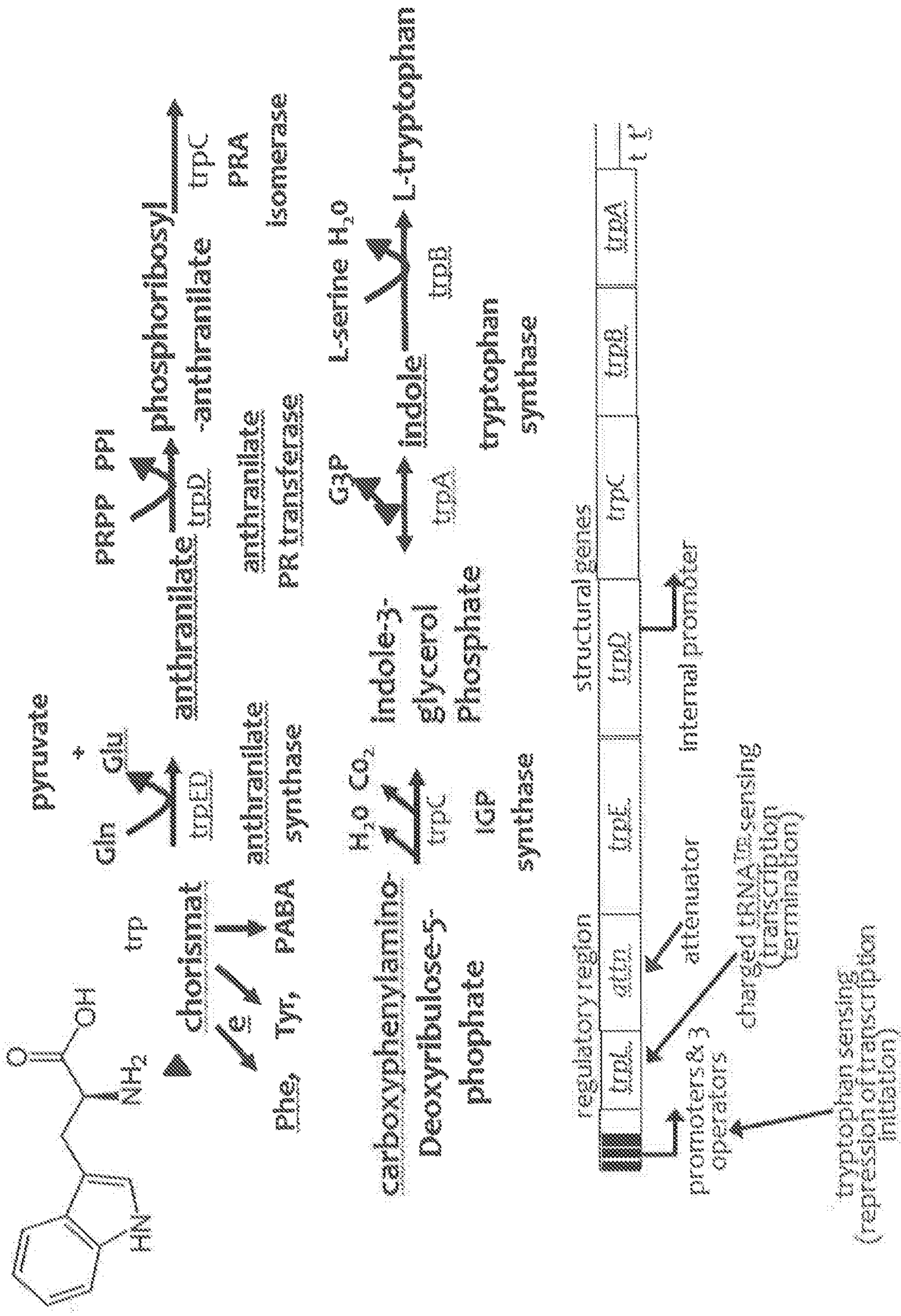

PRPP PPi
phosphoribosyl
anthranilate trpD
anthranilate
pr transferase
G3P
indole
tryptophan
synthase
L-serine H2O
L-tryptophan
trpC
pra
isomerase
trpB
trpA
pyruvate
Gln Glu
trpED
anthranilate
synthase
chorismat
Phe, Tyr, PABA
carboxyphenylamino-
Deoxyribulose-5-
phosphate
H2o CO2
indole-3-
glycero
Phosphate
synthase
IGP
trp
trp
trpE
trpG
trpD
trpC
trpB
trpA
regulatory region
structural genes
trpL
attm
attenuator
internal pro
promoters &
operators
charged tRNATrp-sensing
(transcription
termination)
tryptophan sensing
(translation
termination)
(repr

FIG. 38

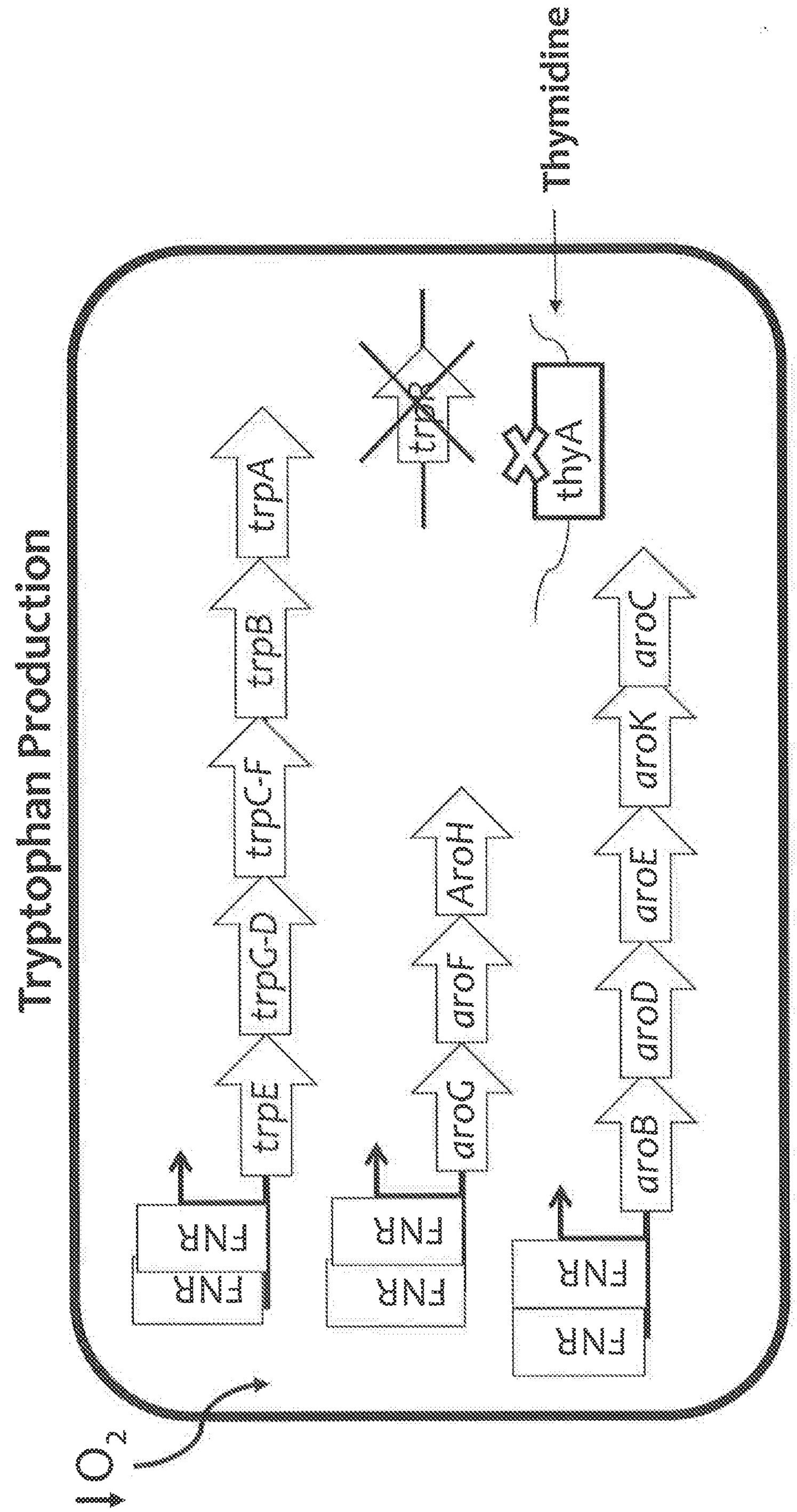

Thymidine
thyA
trpE
trpG-D
trpC-F
trpB
trpA
aroG
aroF
AroH
aroB
aroD
aroE
aroK
aroC
FNR
FNR
FNR
FNR
Tryptophan Production
e.g., E. coli Nissle
$O_2$

FIG. 39

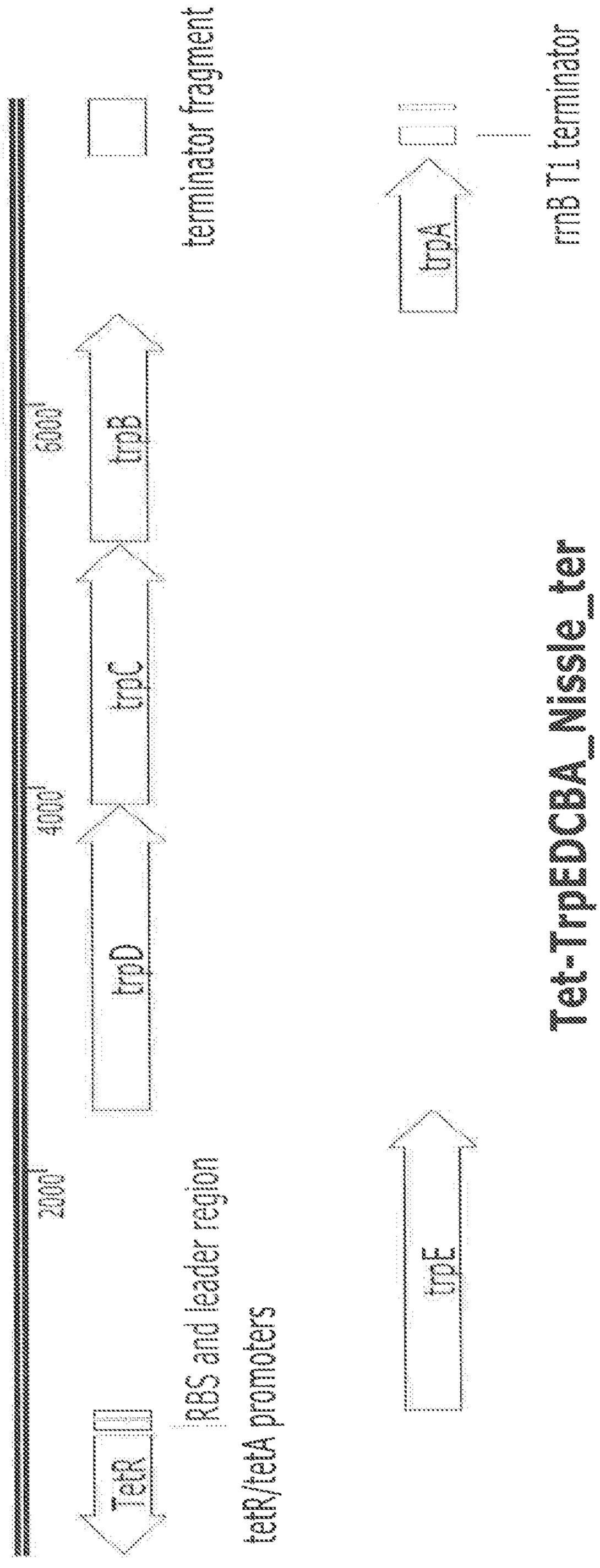

terminator fragment
rrnB T1 terminator
tetA
tetR
RBS and leader region
tetR/tetA promoters
tetE
tetD
tetC
tetB
2000
4000
6000
Tet-TrEDCBA_Nisle_ter
7592 bp

FIG. 40

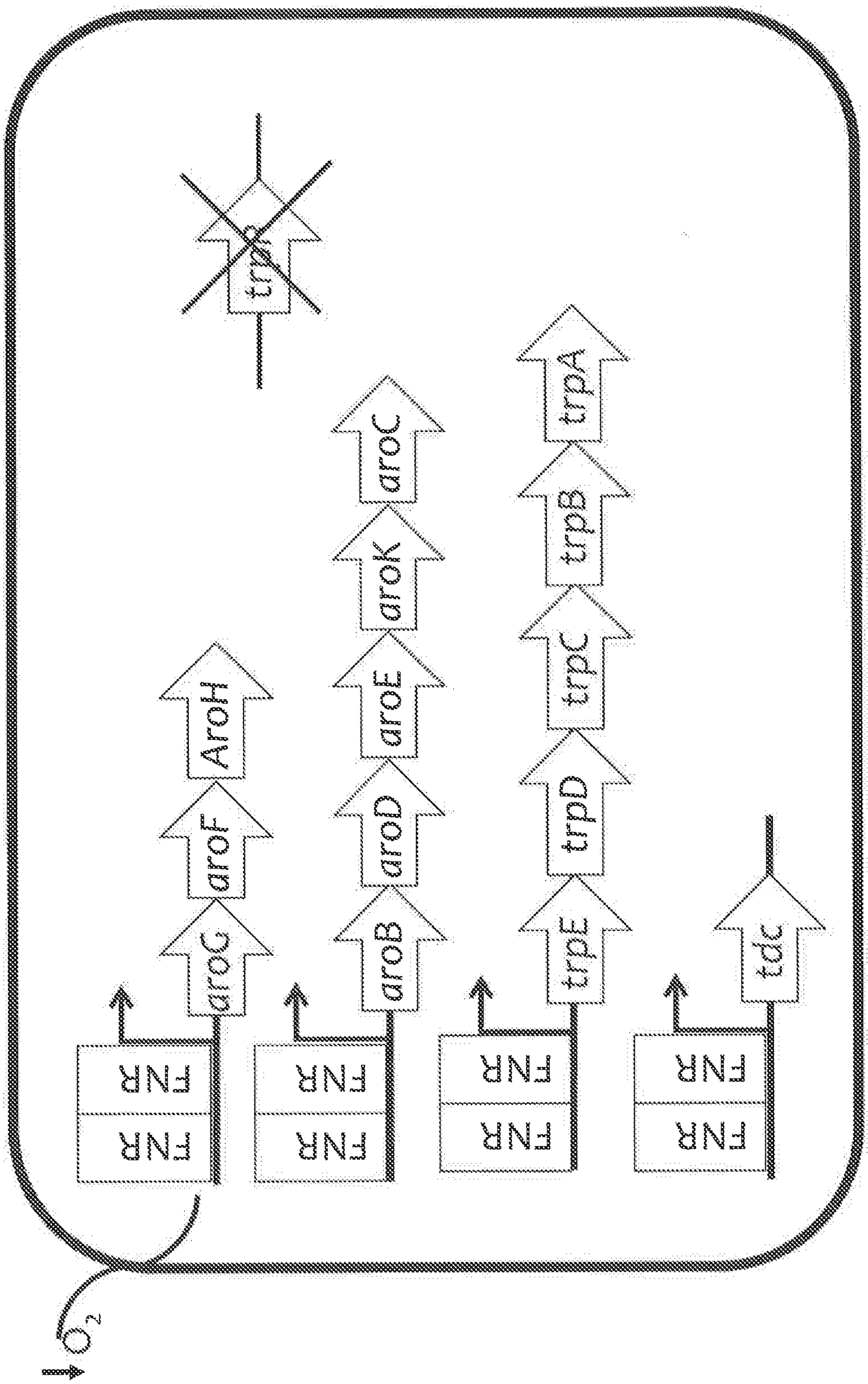

e.g., E. Coli Nissle
trpA
trpB
trpC
trpD
trpE
tdc
aroG
aroF
AroH
aroB
aroD
aroE
aroK
aroC
PTNR
PTNR
PTNR
PTNR
PTNR
PTNR
PTNR
PTNR
O2

FIG. 41A

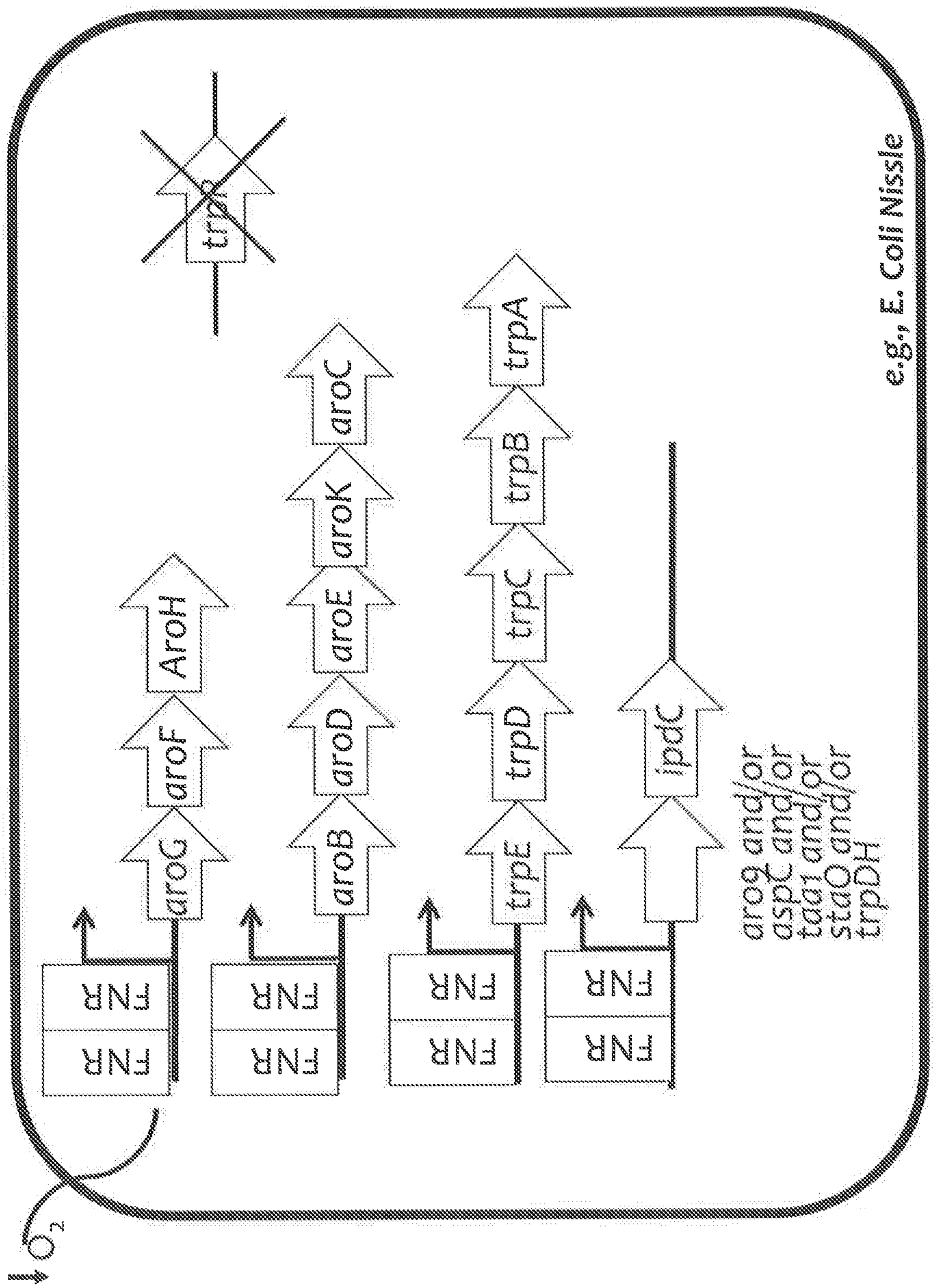

e.g., E. Coli Nissle
trpA
trpB
trpC
trpD
trpE
ipdC
aroG and/or
aspC and/or
tyrA and/or
shiO and/or
trpDH
aroF
aroH
aroB
aroD
aroE
aroC
aroK
trpR
trpR
trpR
trpR
trpR
trpR
trpR
trpR
trpR
trpR
trpR
trpR
6
2

FIG. 41B

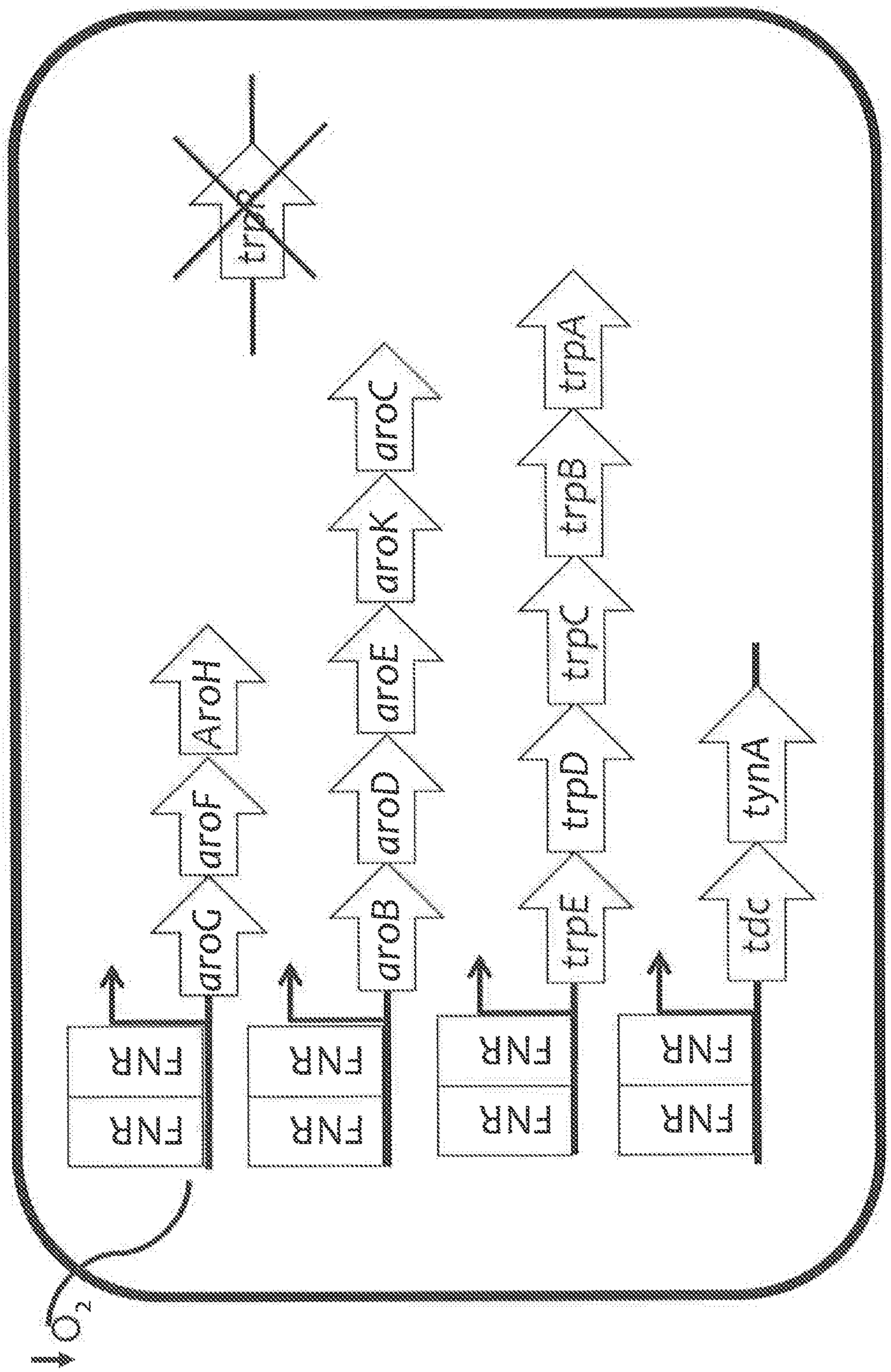

e.g., E. Coli Nissle
trpR
aroK
aroC
trpB
trpA
aroH
aroF
aroG
aroE
aroD
aroB
trpC
trpD
trpE
tynA
tdc
TF
NR
$O_2$

FIG. 41C

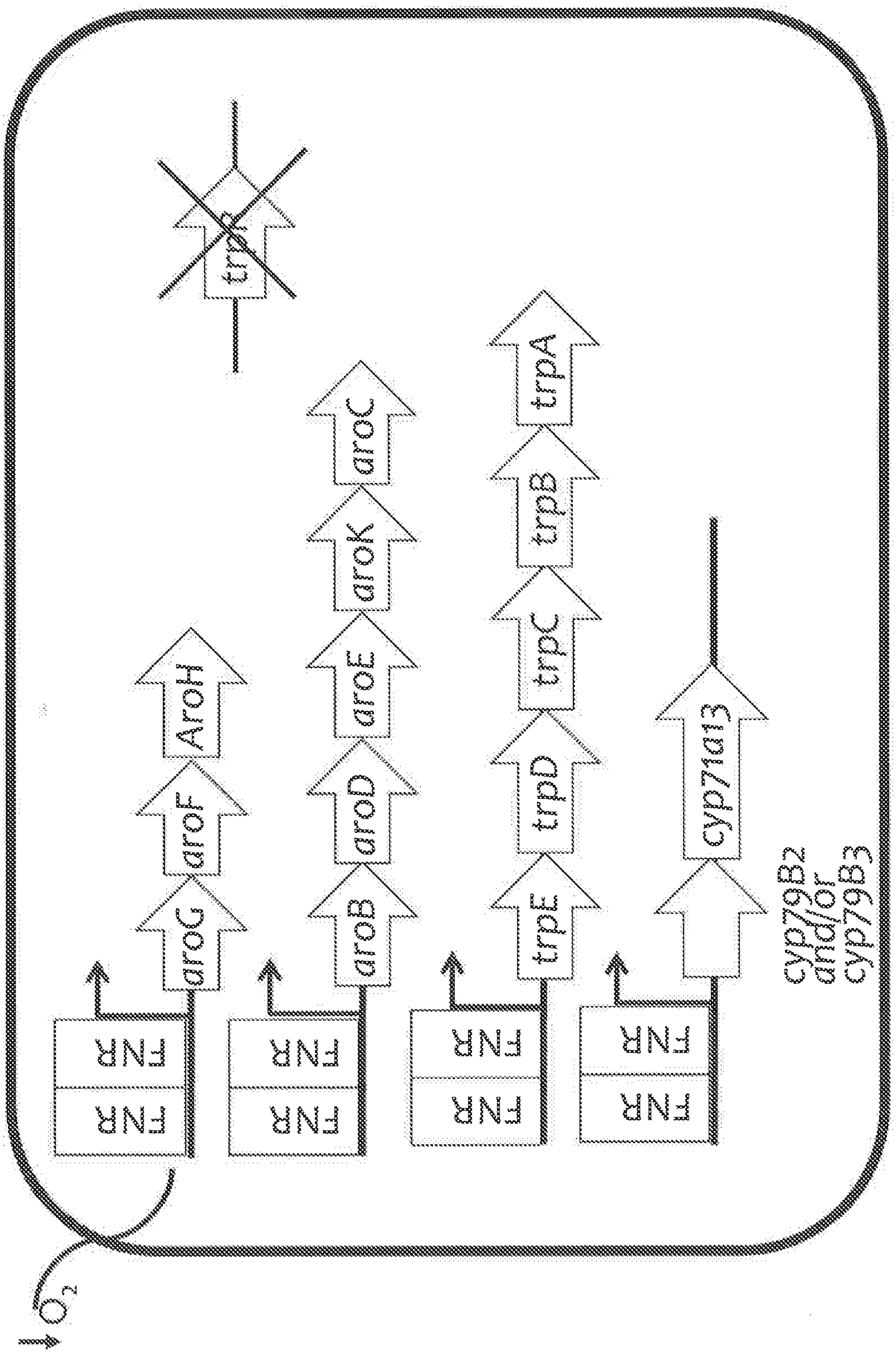

e.g., E. Coli Nissle
trpA
trpB
trpC
trpD
trpE
aroG
aroF
AroH
aroB
aroD
aroE
aroK
aroC
trpR
cyp79B2
and/or
cyp79B3
cyp71a13
O2

FIG. 41D

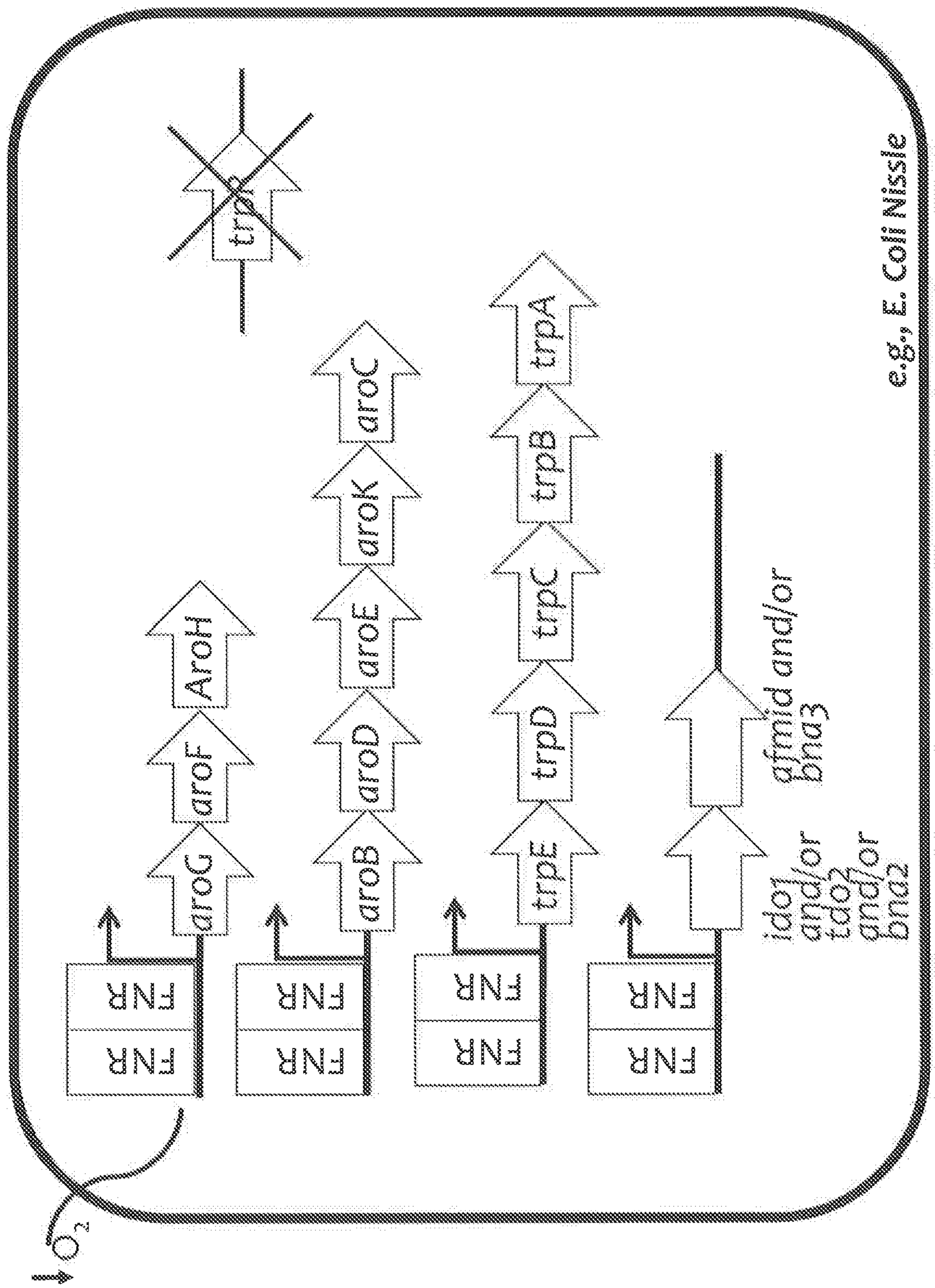

e.g., E. coli Nissle
idoT and/or tpgT and/or bna2
atmid and/or bna3
trpC
trpD
trpE
trpB
trpA
aroG
aroF
aroH
aroB
aroD
aroE
aroK
aroC
trpF
TNR
TNR
TNR
TNR
TNR
TNR
TNR
TNR
5'
3'

FIG. 1E

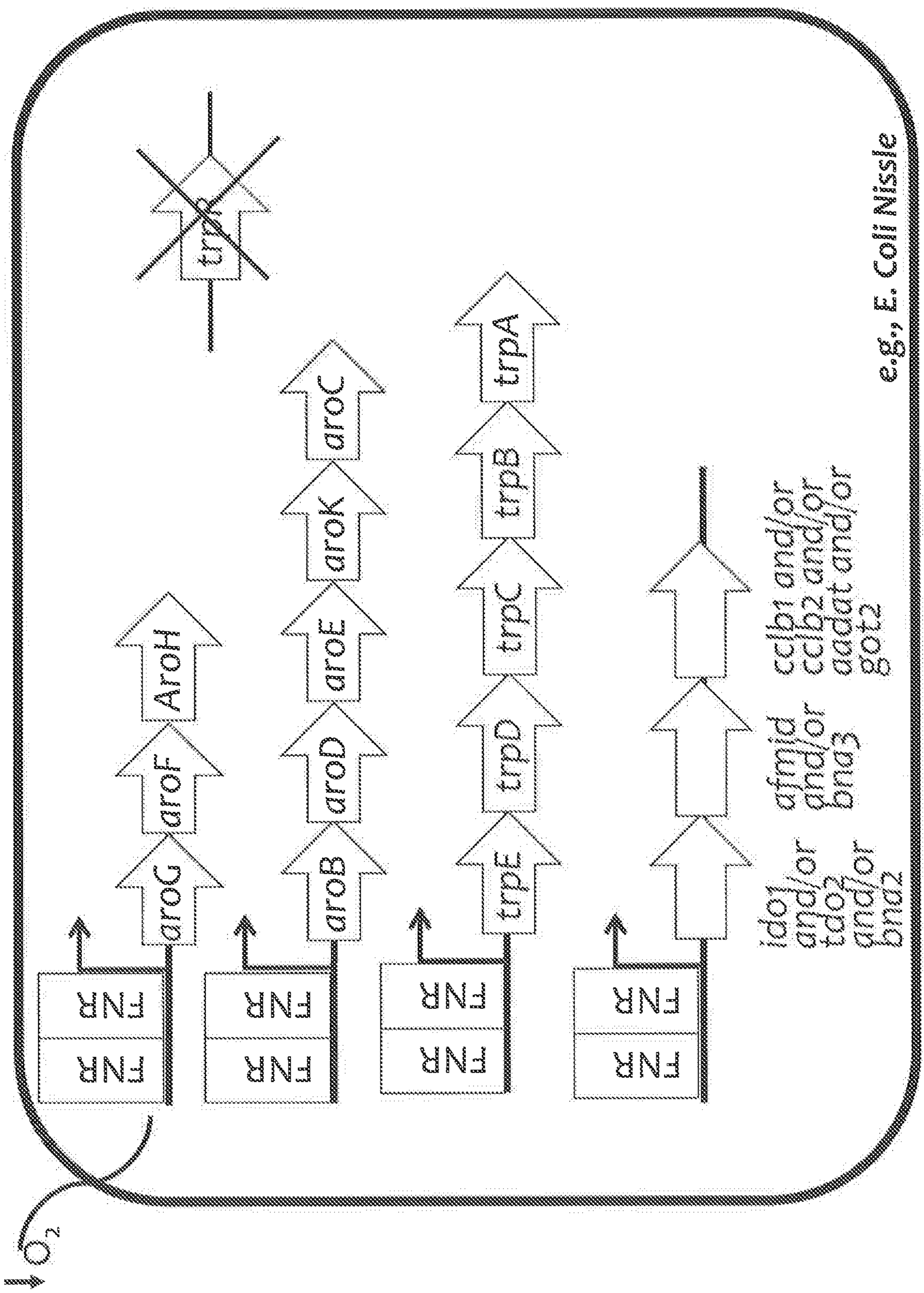

e.g., E. Coli Nissle
idi and/or idi2 and/or idi3
afmid and/or ana2 and/or ana3
cslb1 and/or cslb2 and/or aadat and/or so2
aroG
aroF
AroH
aroB
aroD
aroE
trpE
trpD
trpC
arok
aroC
trpB
trpA
trpR
O2

FIG. 41F

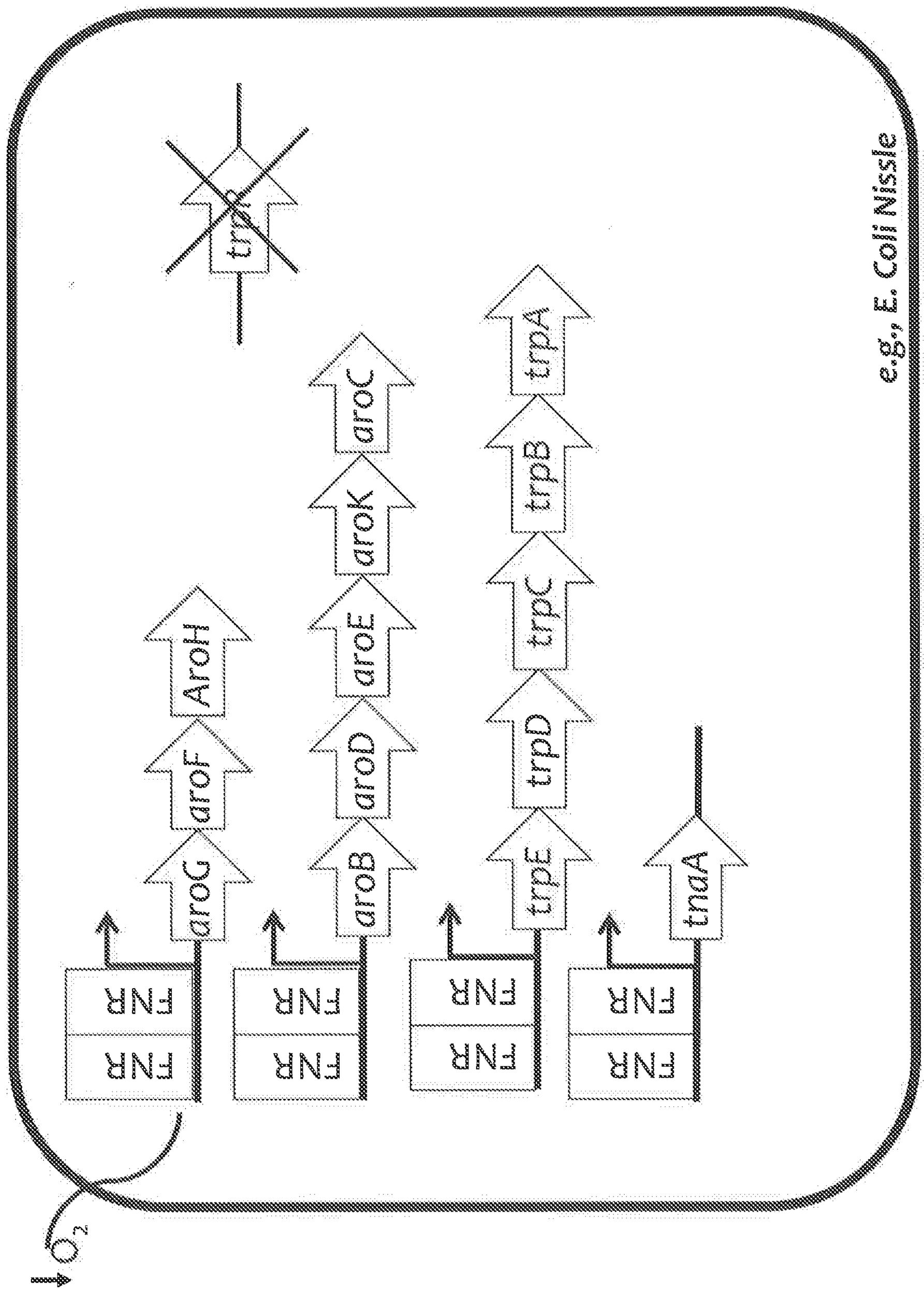

e.g., E. Coli Nissle
trpR
aroK
aroC
trpB
trpA
aroG
aroF
AroH
aroB
aroD
aroE
trpE
trpD
trpC
tnaA
TTR
TTR
TTR
TTR
TTR
TTR
TTR
TTR
2

FIG. 41G

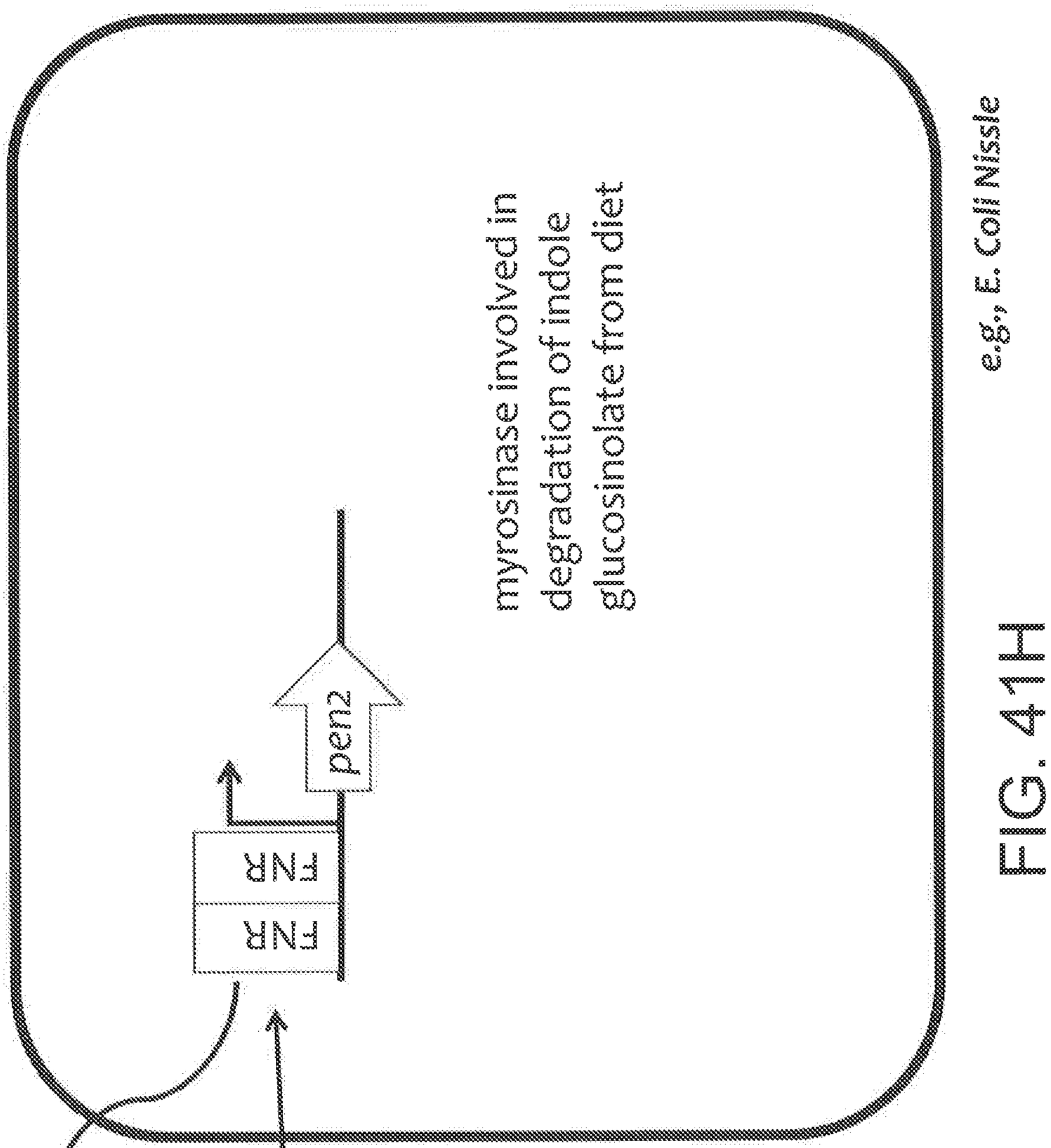

Dietary indole glucosinolate (glucobrassicin)
$O_2$
pen2
FNR
FNR
myrosinase involved in degradation of indole glucosinolate from diet
e.g., E. Coli Nissle

FIG. 41H

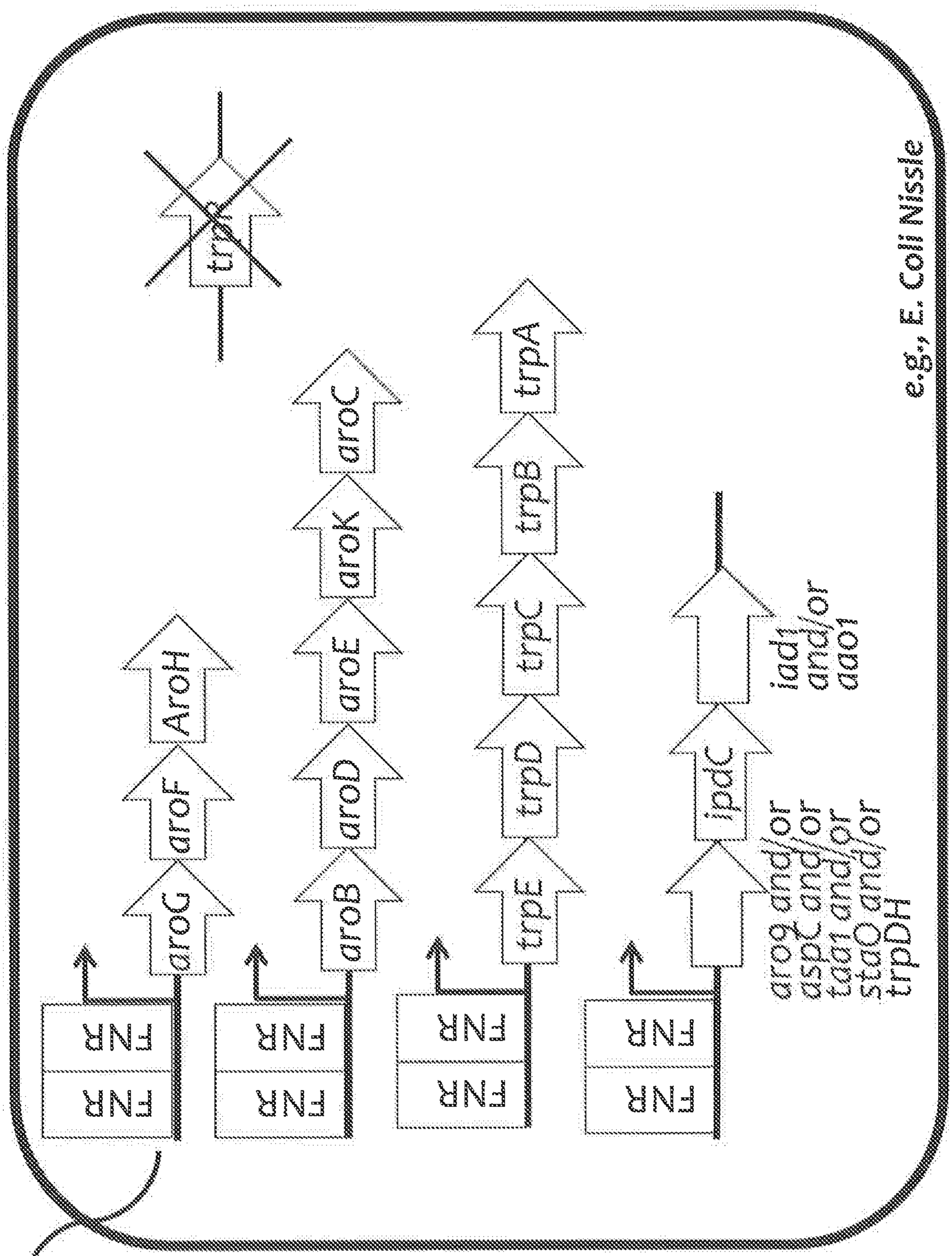

e.g., E. Coli Nissle
trpA
trpB
trpA
aroK
aroC
aroG
aroF
AroH
aroB
aroD
aroE
trpE
trpD
trpC
ipdC
iad1 and/or aao1
aroG and/or aspC and/or taa1 and/or staO and/or trpDH
TF NR
TF NR
TF NR
TF NR
$O_2$

FIG. 42A

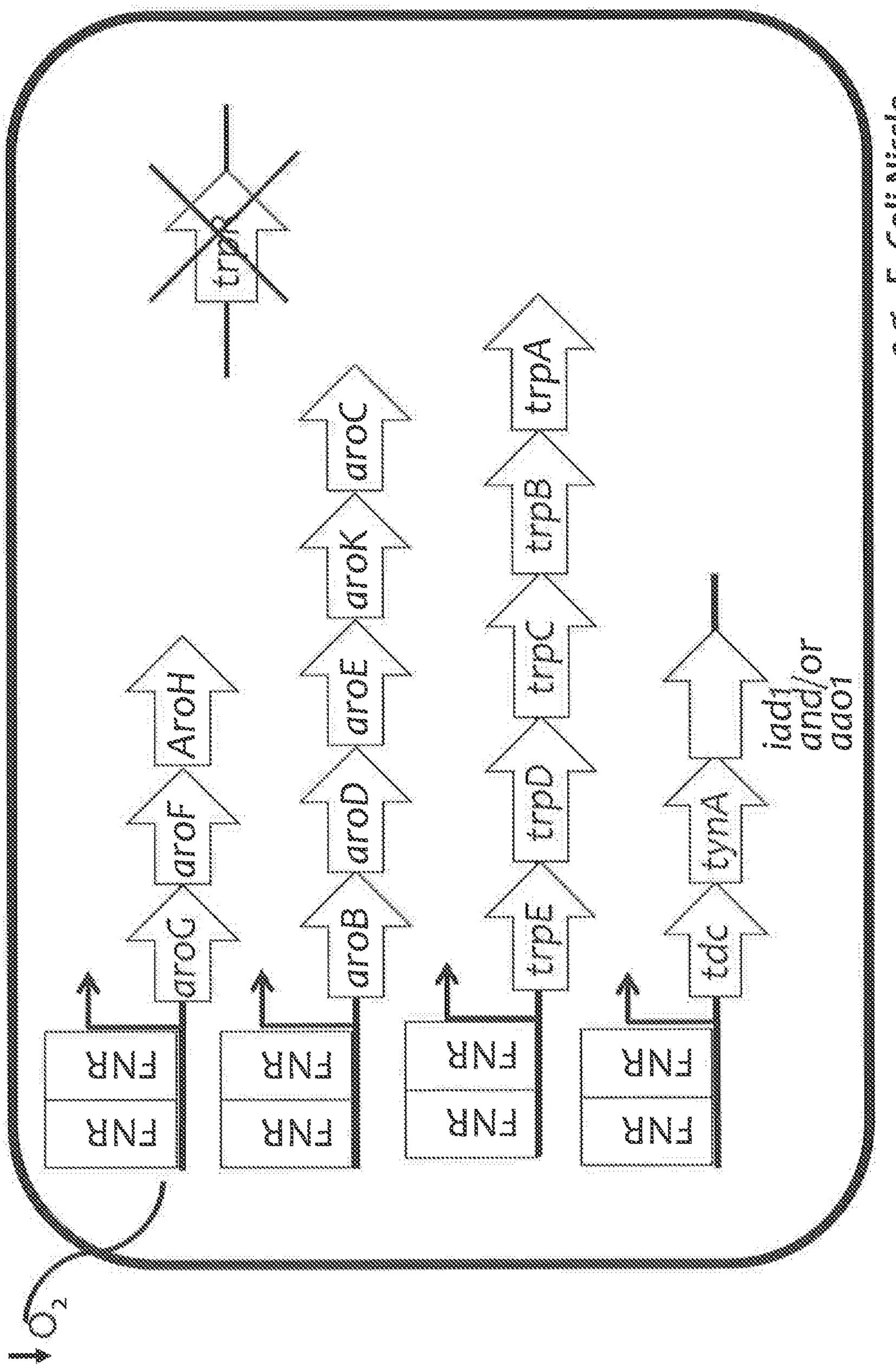

e.g., E. coli Nissle
trpR
aroK
aroC
aroG
aroF
AroH
aroB
aroD
aroE
trpE
trpD
trpC
trpB
trpA
tdc
tynA
iadI
and/or
aaoT
O2

FIG. 42B

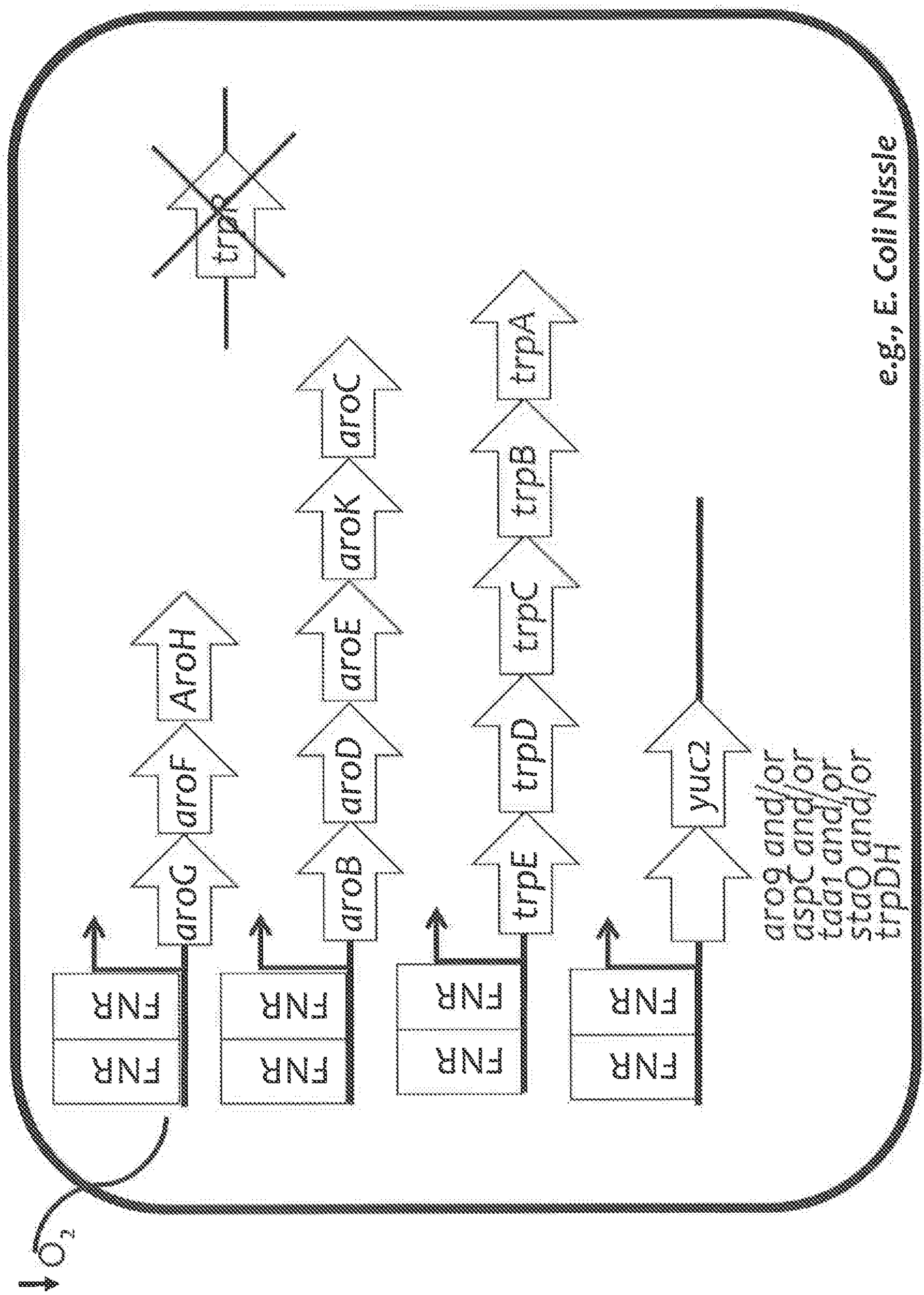

e.g., E. coli Nissle
trpR
aroK
aroC
aroE
aroH
aroF
aroG
aroD
aroB
trpC
trpB
trpA
trpD
trpE
yuc2
TNR
TNR
TNR
TNR
TNR
TNR
TNR
TNR
aroG and/or
aspC and/or
taaI and/or
staO and/or
trpDH
O2

FIG. 42C

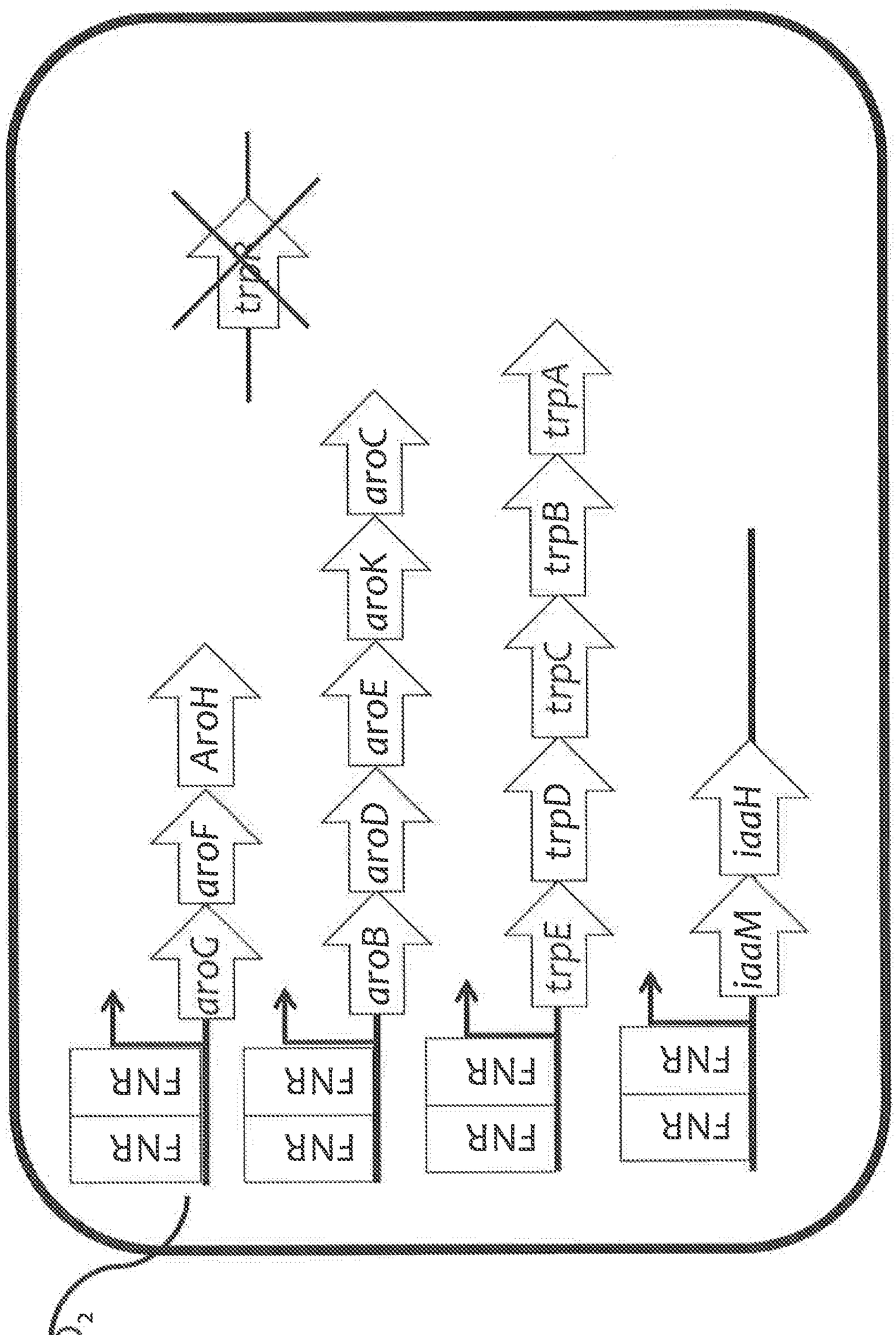

e.g., E. coli Nissle
trpR
aroK
aroC
trpB
trpA
aroG
aroF
AroH
aroB
aroD
aroE
trpE
trpD
trpC
iaaM
iaaH
R
N
L
O2

FIG. 42D

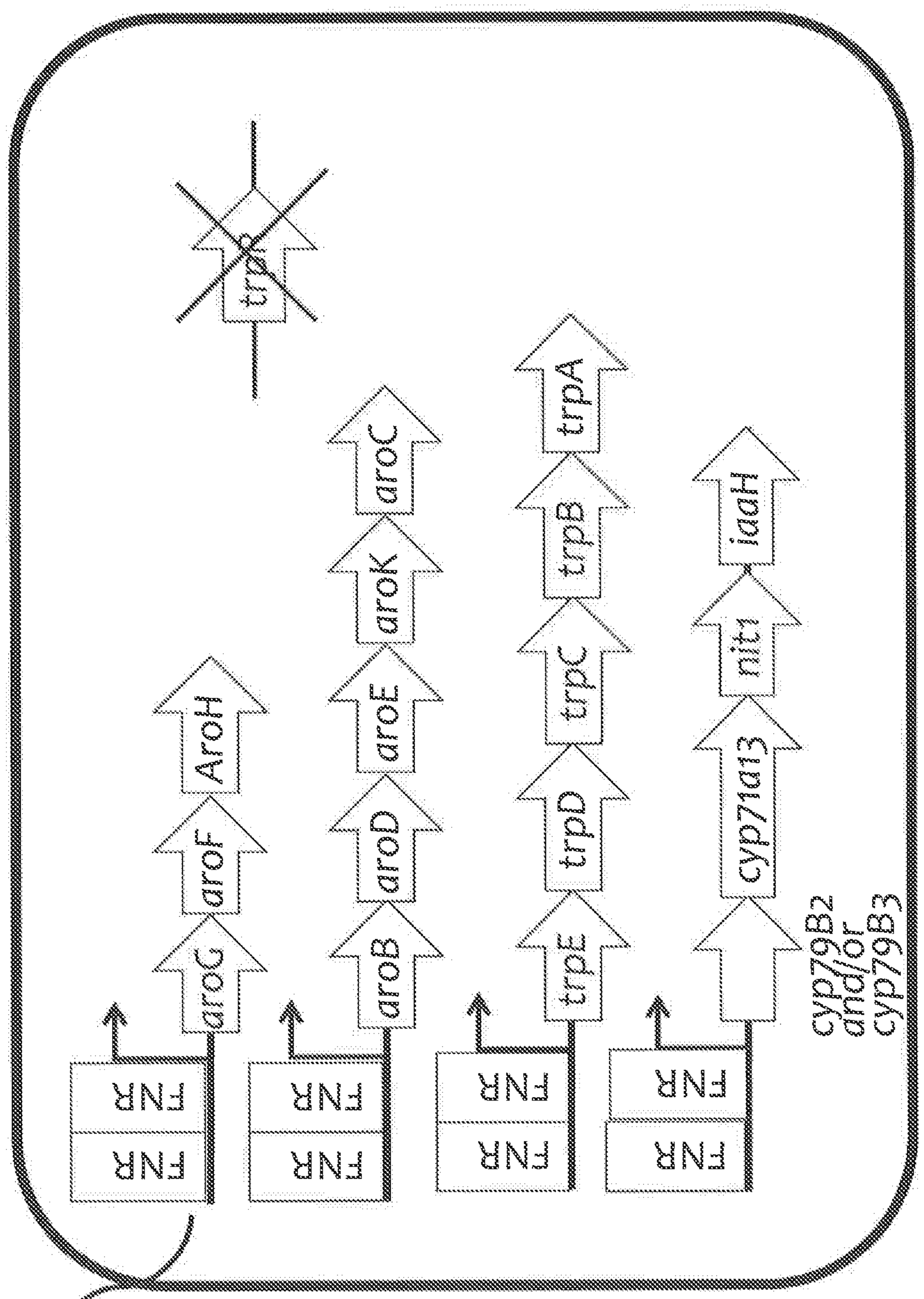
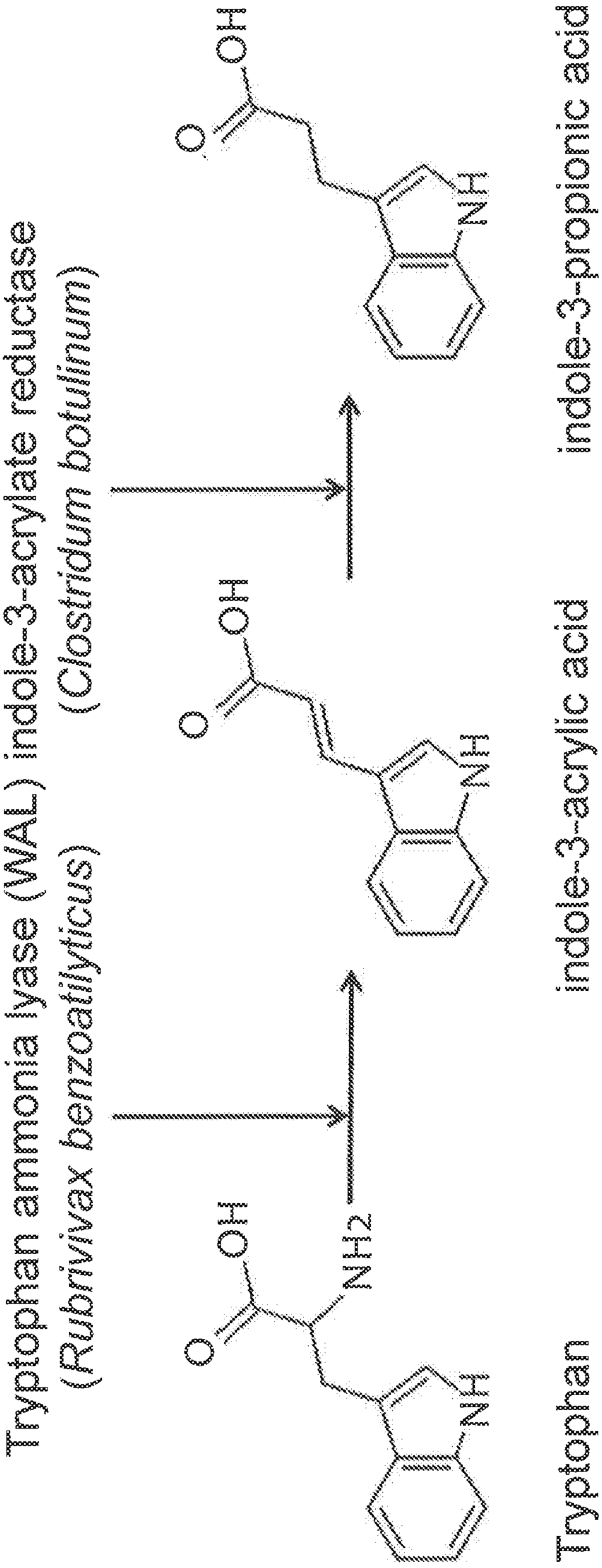

e.g., E. Coli Nissle
trpA
trpB
trpC
trpD
trpE
aroG
aroF
aroH
aroB
aroD
aroE
aroK
aroC
nit1
nit2
nit3
iaaH
cyp71a1
cyp79B2
and/or
cyp79B3
TNR
TNR
TNR
TNR
TNR
TNR
TNR
TNR
62

FIG. 42E


Tryptophan
(Rubrivax benzoatilyticus)
Tryptophan ammonia lyase (WAL)
indole-3-acrylate reductase
(Clostridium botulinum)
indole-3-propionic acid
indole-3-acrylic acid
indole-3-propionic acid
FIG. 43A

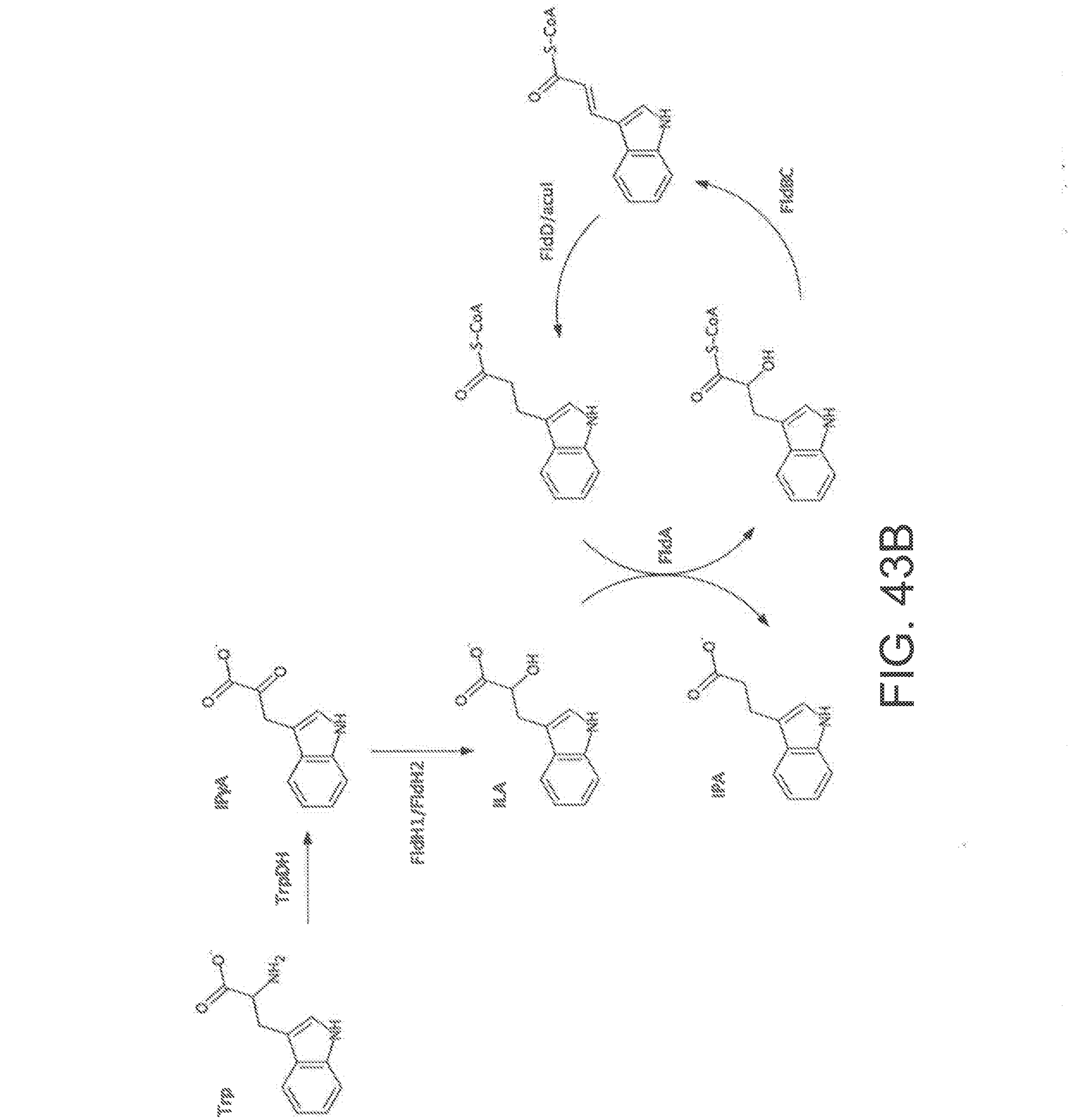



FIG. 43B

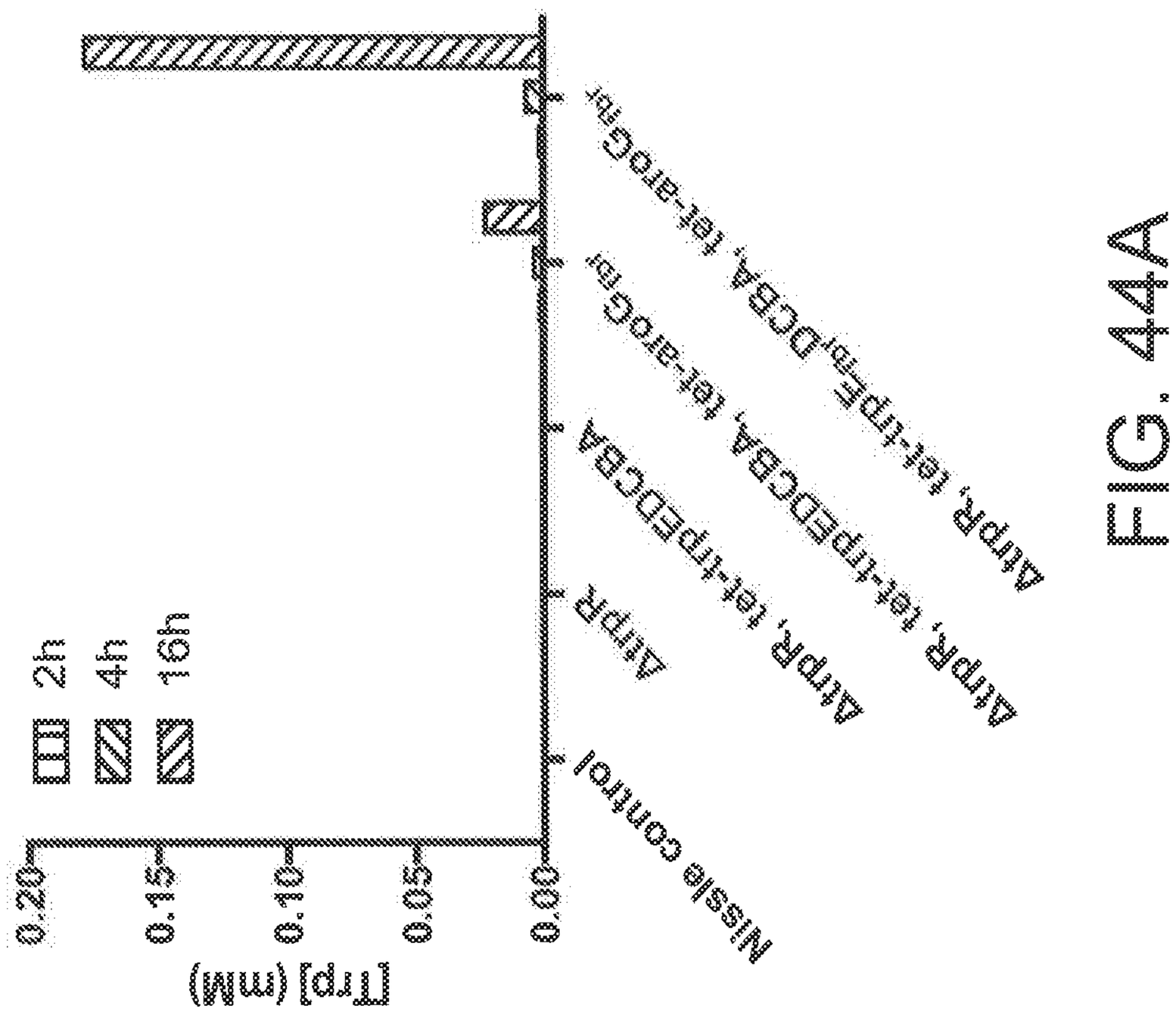

0.20
0.15
0.10
0.05
0.00
[Trp] (mM)
2h
4h
16h
Naïve control
ΔtnpR
ΔtnpR, tet-tnpEDCBA
ΔtnpR, tet-tnpEDCBA, tet-atoGpt
ΔtnpR, tet-tnpEDCBA, tet-atoGpt

FIG. 44A

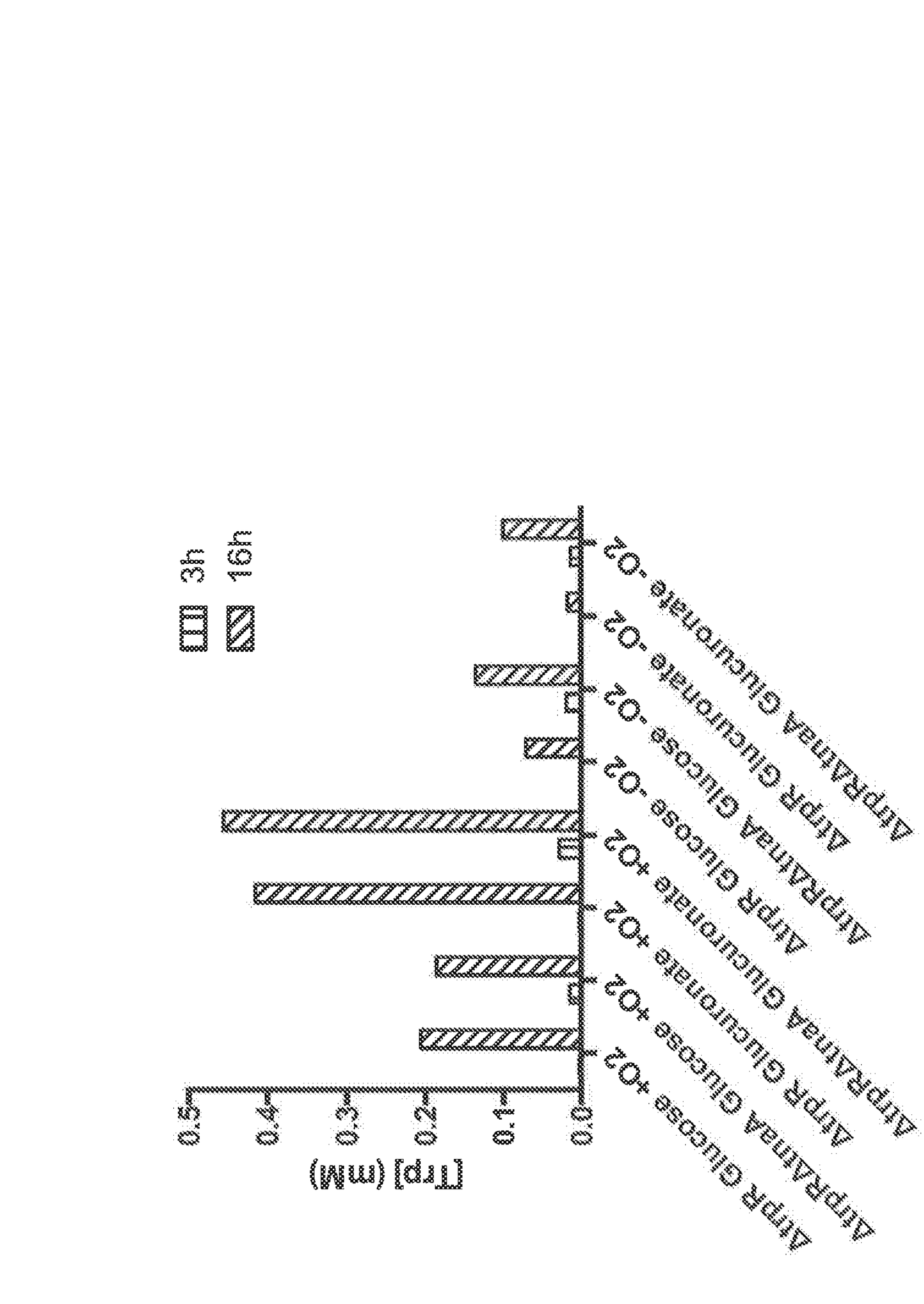

3h
16h
0.5
0.4
0.3
0.2
0.1
0.0
[Trp] (mM)
Ampa Glucose +O2
Ampa Glucose -O2
Kainate Glucose +O2
Kainate Glucose -O2
Nmda Glucose +O2
Nmda Glucose -O2
Atpa Glucose +O2
Atpa Glucose -O2
Dhpg Glucose +O2
Dhpg Glucose -O2
Ampa Galactose +O2
Ampa Galactose -O2
Kainate Galactose +O2
Kainate Galactose -O2
Nmda Galactose +O2
Nmda Galactose -O2
Atpa Galactose +O2
Atpa Galactose -O2
Dhpg Galactose +O2
Dhpg Galactose -O2

FIG. 4B

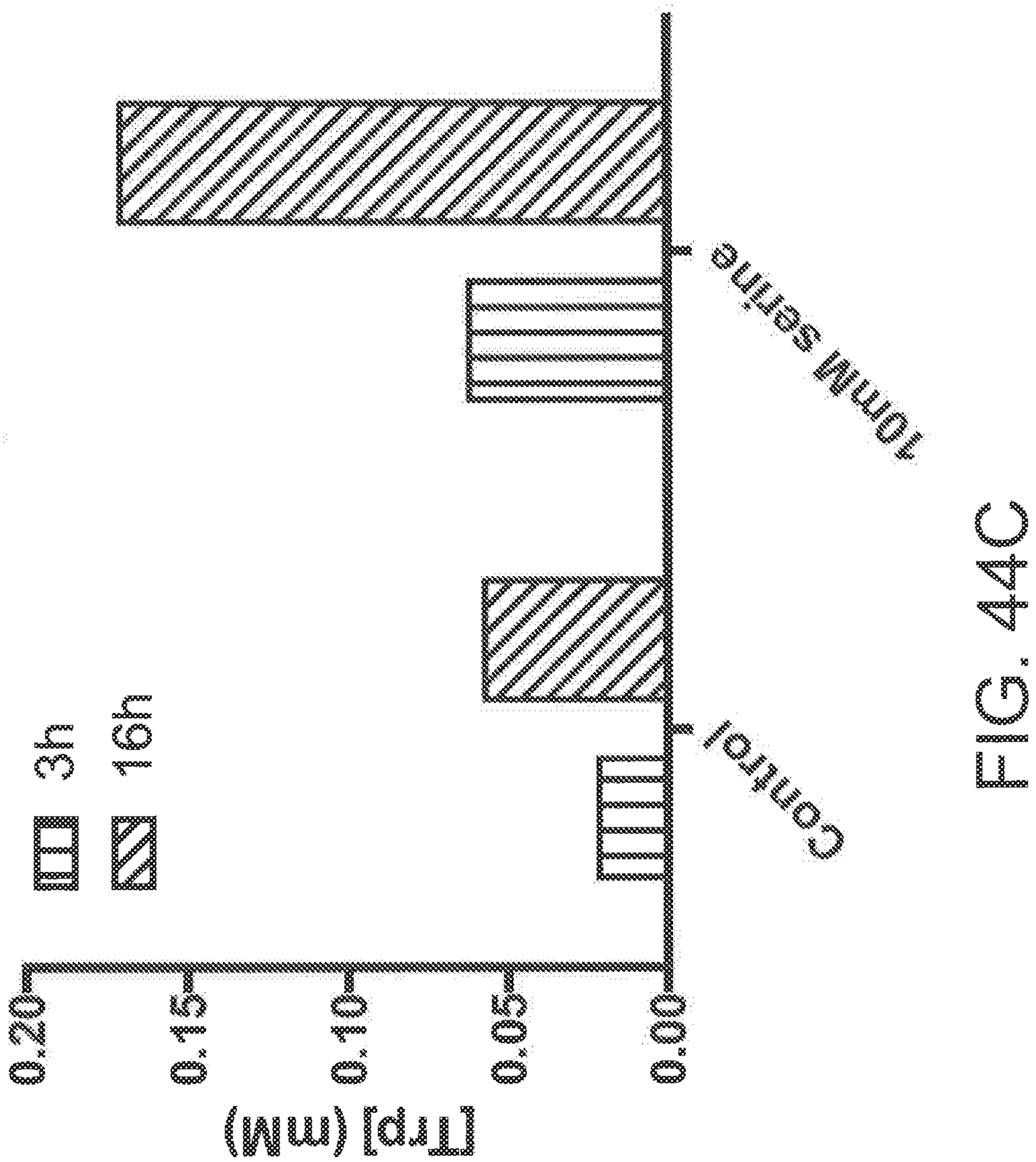



FIG. 44C

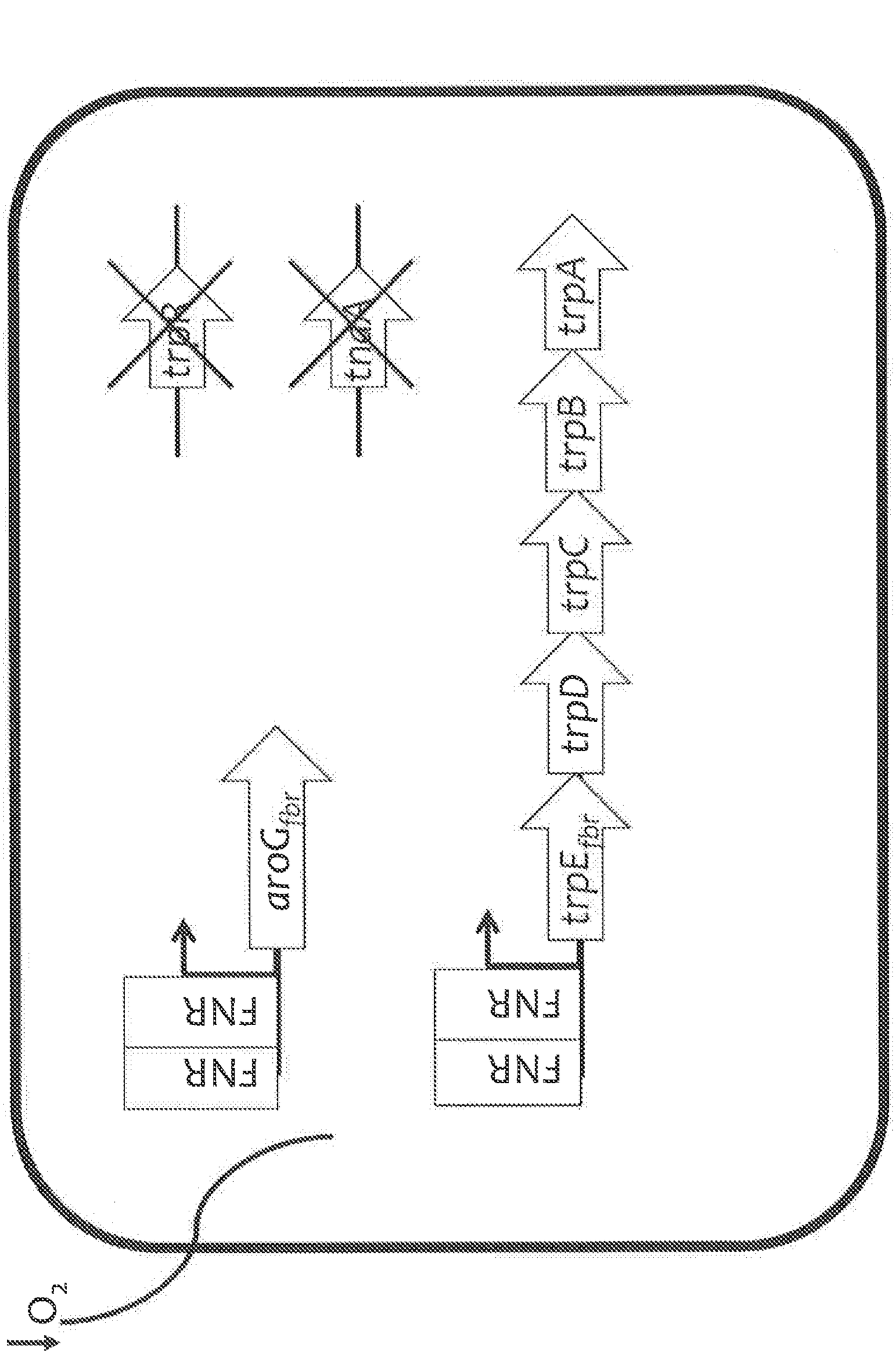
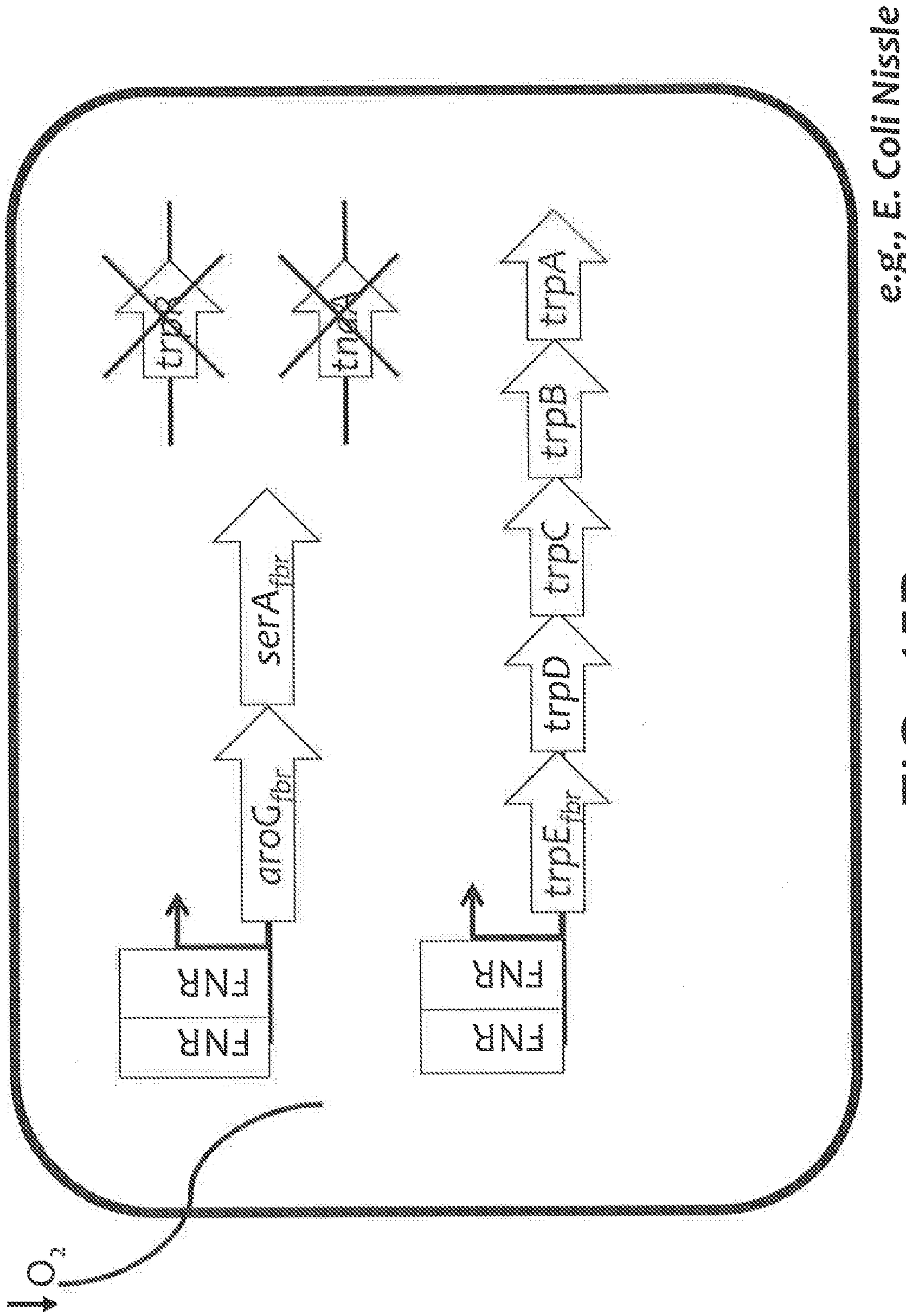

e.g., E. Coli Native trpE
trpA
trpB
trpC
trpD
trpE_fbr
FNR
FNR
aroG_fbr
FNR
FNR
O2

FIG. 45A


e.g., E. Coli NRRL-11442
trpR
tnaA
serA$_{fbr}$
aroG$_{fbr}$
trpE$_{fbr}$
trpD
trpC
trpB
trpA
FIG. 45B

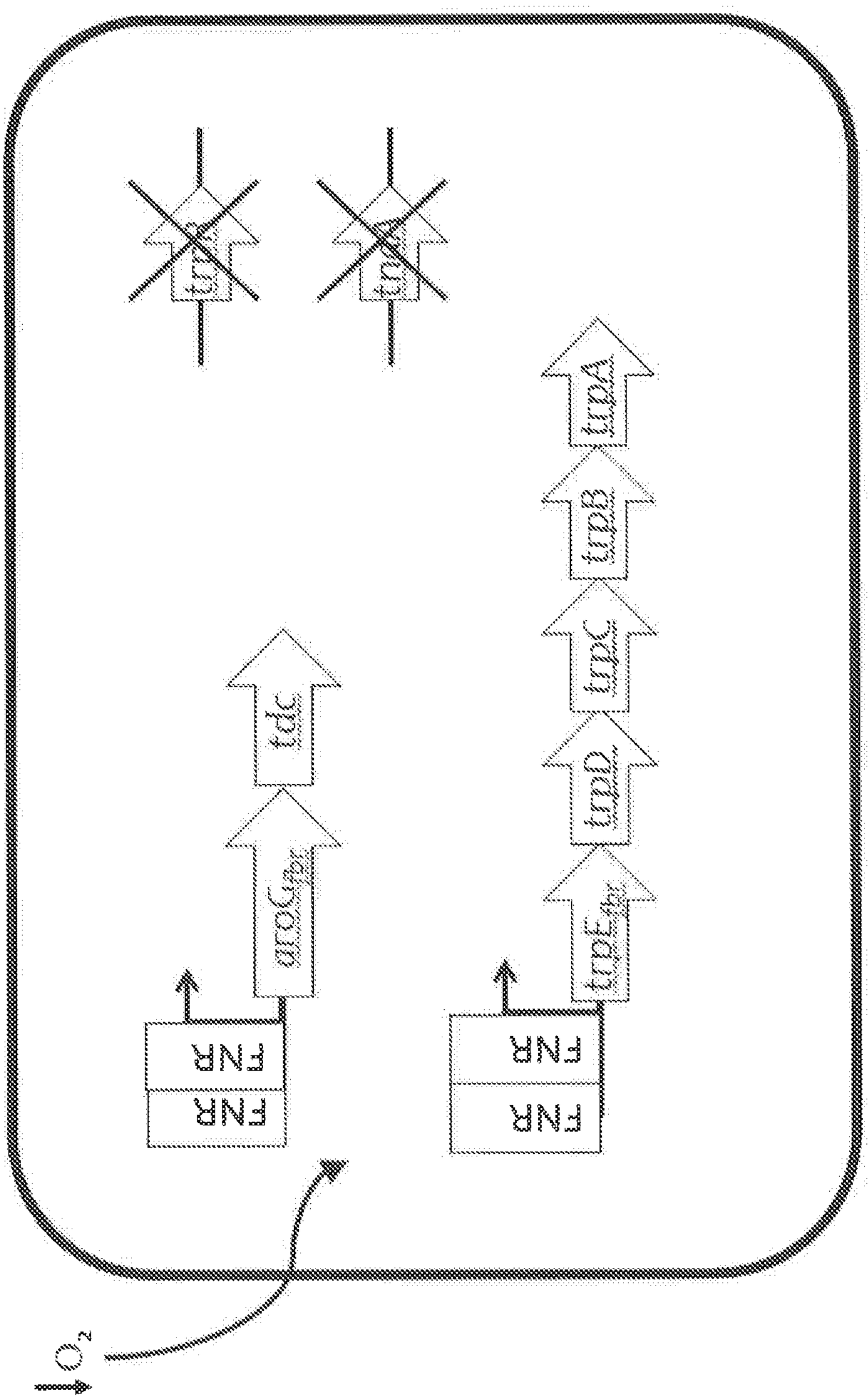

e.g., E. coli Nissle
tpre
tmp
tps
tpE_thr
tpD
tpC_thr
tdc
FNR
FNR
FNR
FNR
+Sp
+Sp
S'

FIG. 45C

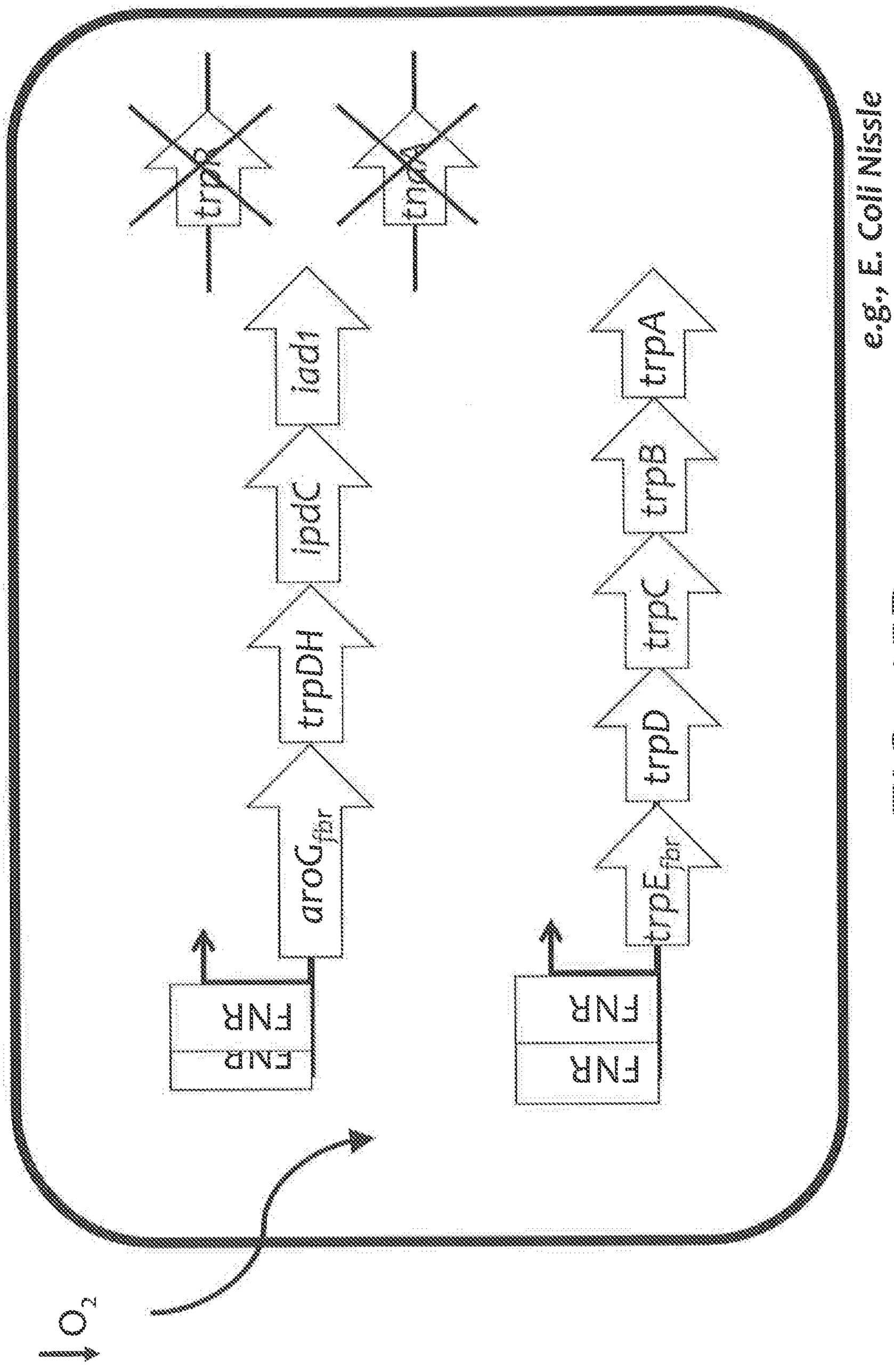

tyrR
tyrR
aroH
ipdC
iad1
trpE
trpB
trpA
trpC
trpD
aroG
TNR
TNR
TNR
TNR
e.g., E. Coli Nissle
$O_2$

FIG. 45D

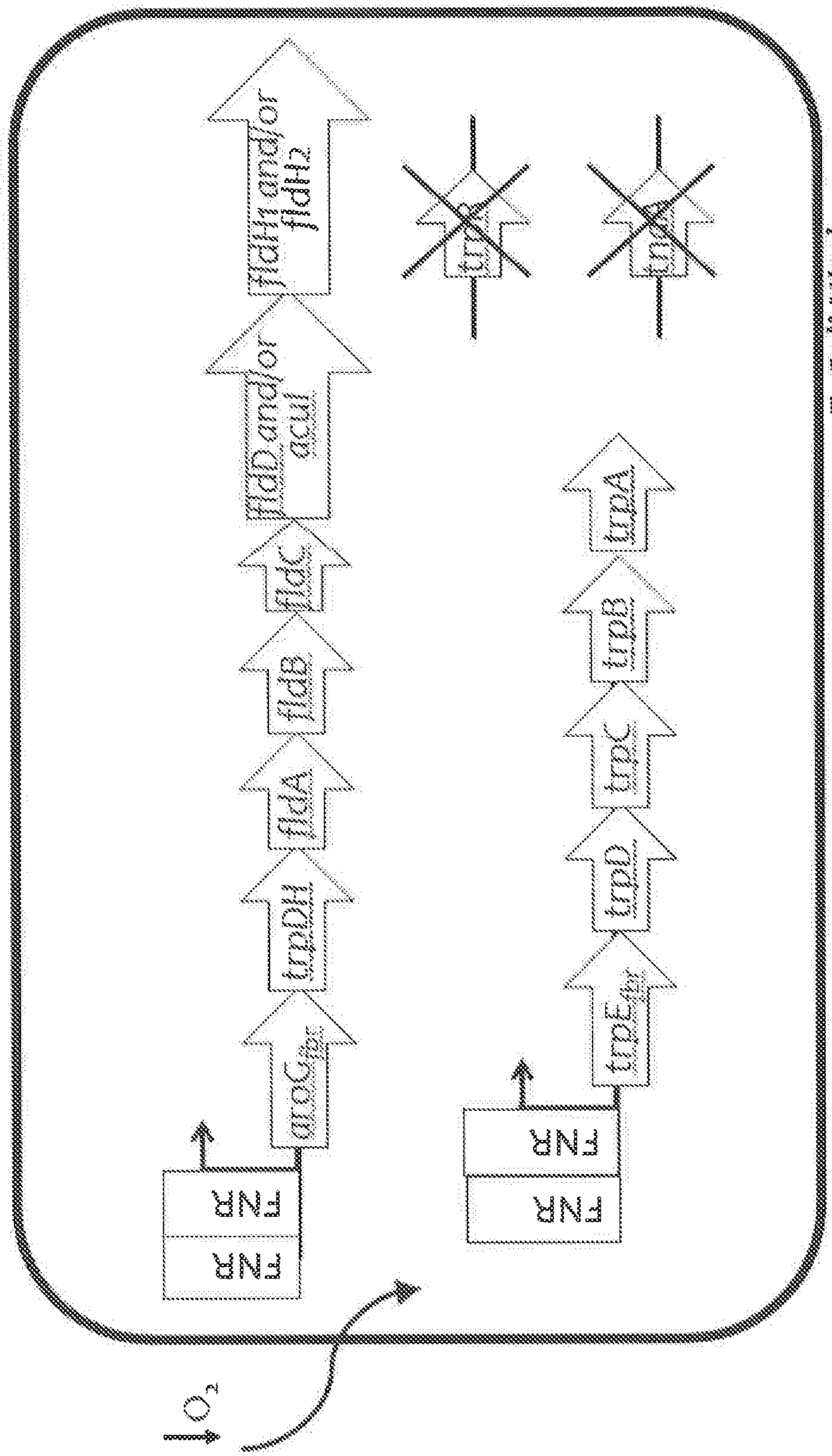

O2
FNR
FNR
aroGfbr
tpnDH
fldA
fldB
fldC
fldD and/or acul
fldH and/or fldH2
tnaA
tnaA
FNR
FNR
tpnEfbr
tpnD
tpnC
tnaA
tnaB
e.g., E. Coli Nissle

FIG. 45E

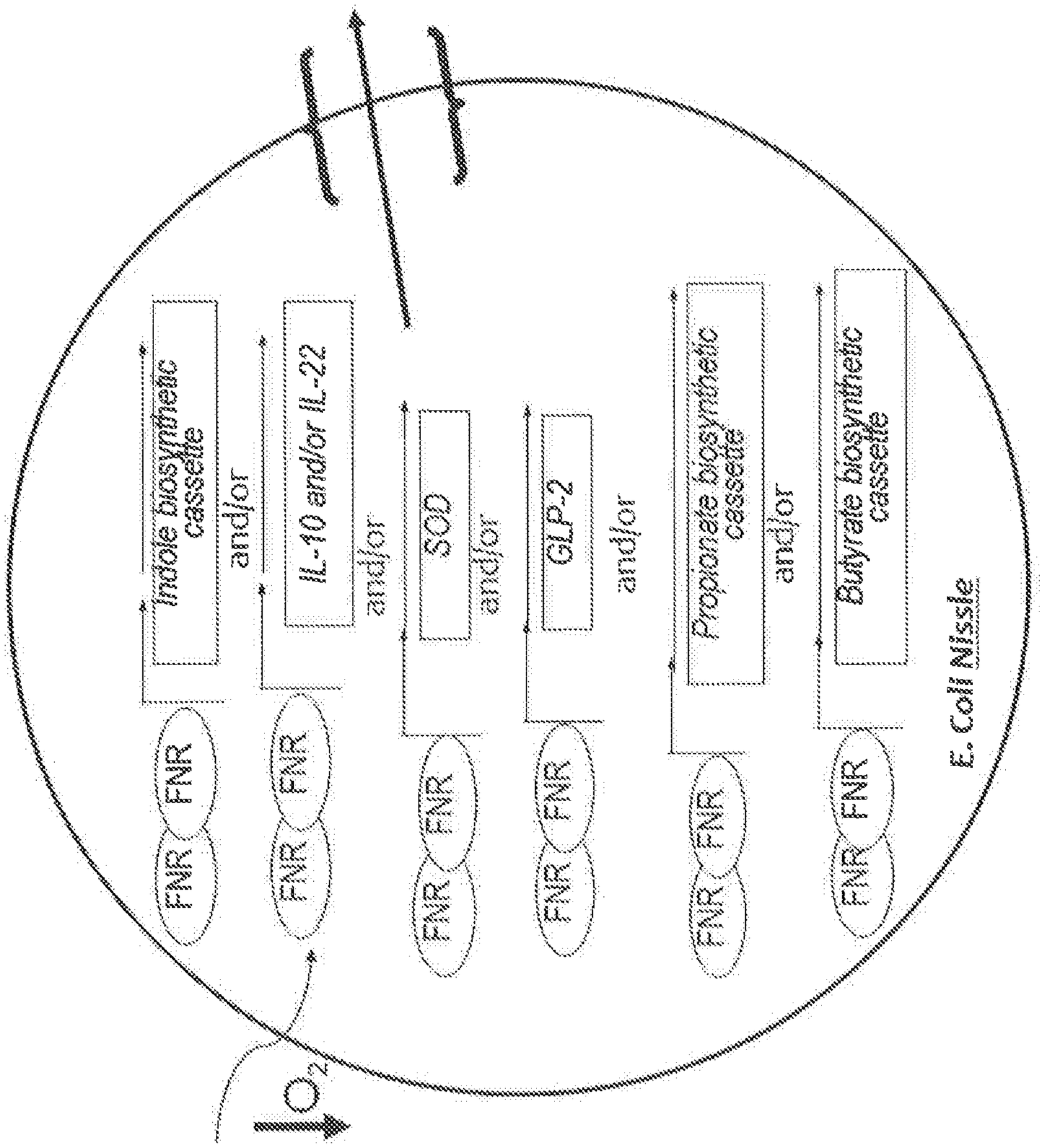

E. Coli Missle
Indole biosynthetic cassette
and/or
IL-10 and/or IL-22
and/or
SOD
and/or
GLP-2
and/or
Propionate biosynthetic cassette
and/or
Butyrate biosynthetic cassette
FNR
O2

FIG. 46A

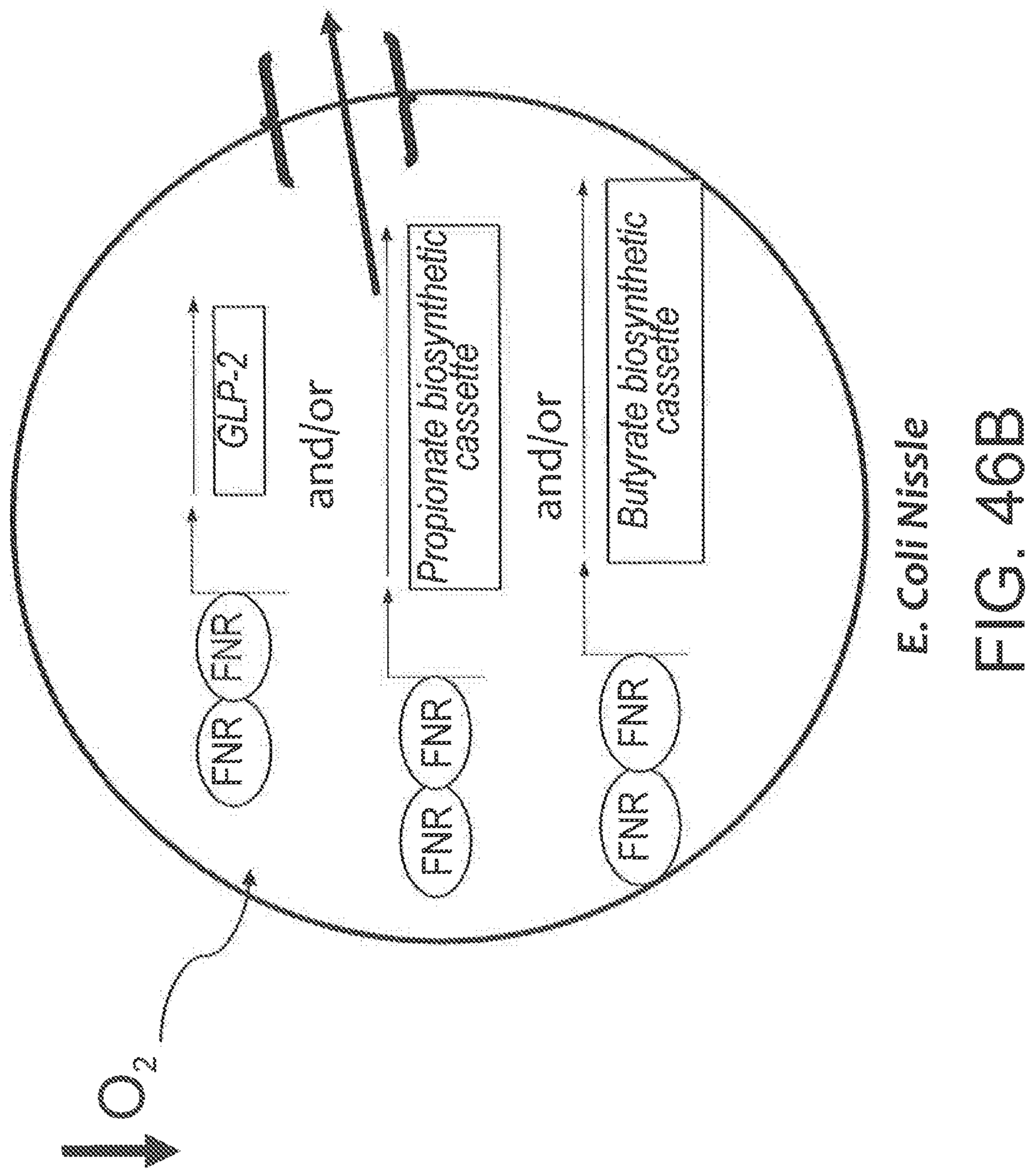

GLP-2
and/or
Propionate biosynthetic cassette
and/or
Butyrate biosynthetic cassette
FNR
FNR
FNR
FNR
FNR
FNR
E. coli Nissle
O2

FIG. 46B

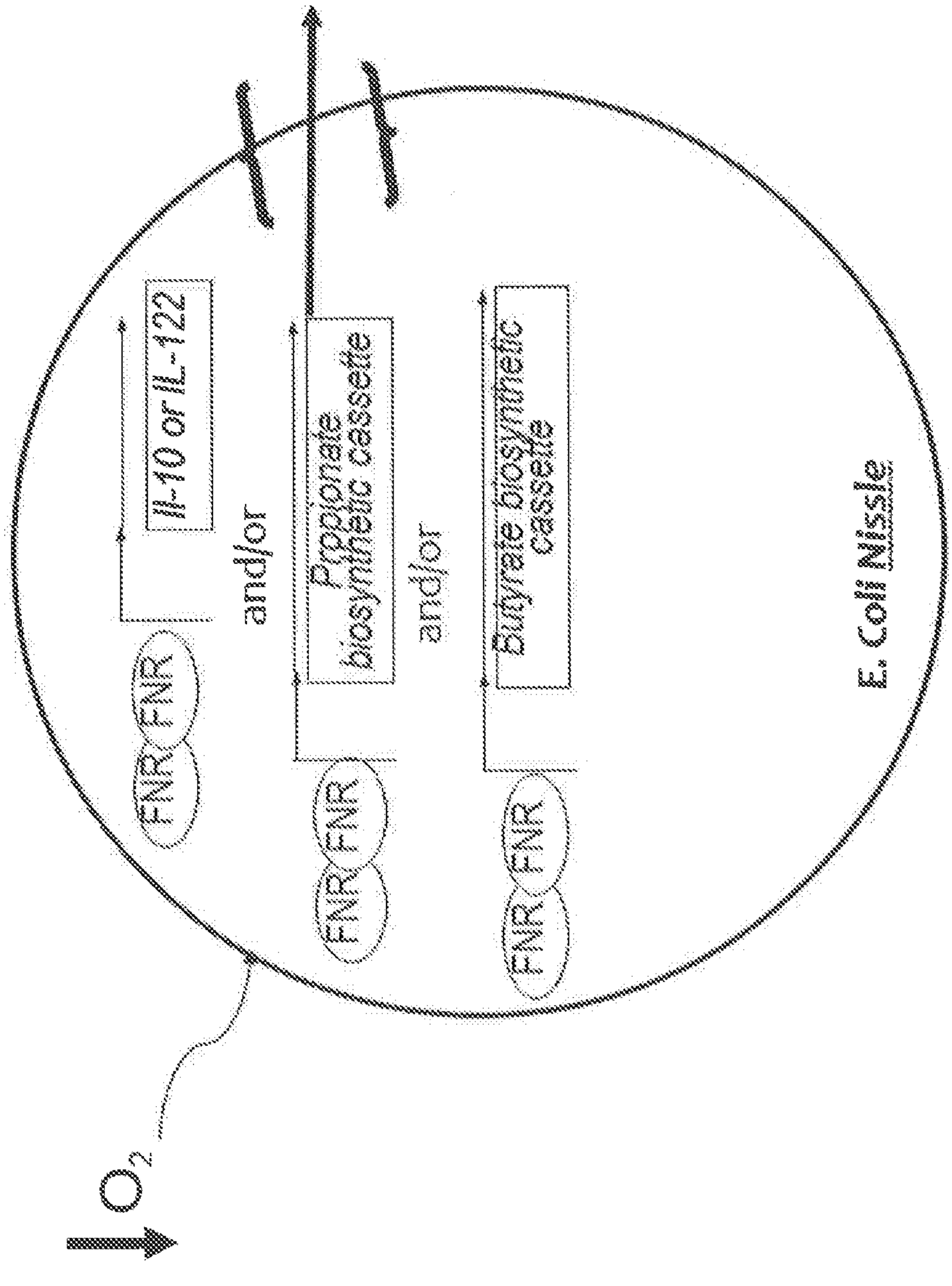

E. Coli Nissle
Butyrate biosynthetic cassette
Propionate biosynthetic cassette
IL-10 or IL-22
and/or
FNR FNR
FNR FNR
FNR FNR
O2

FIG. 46C

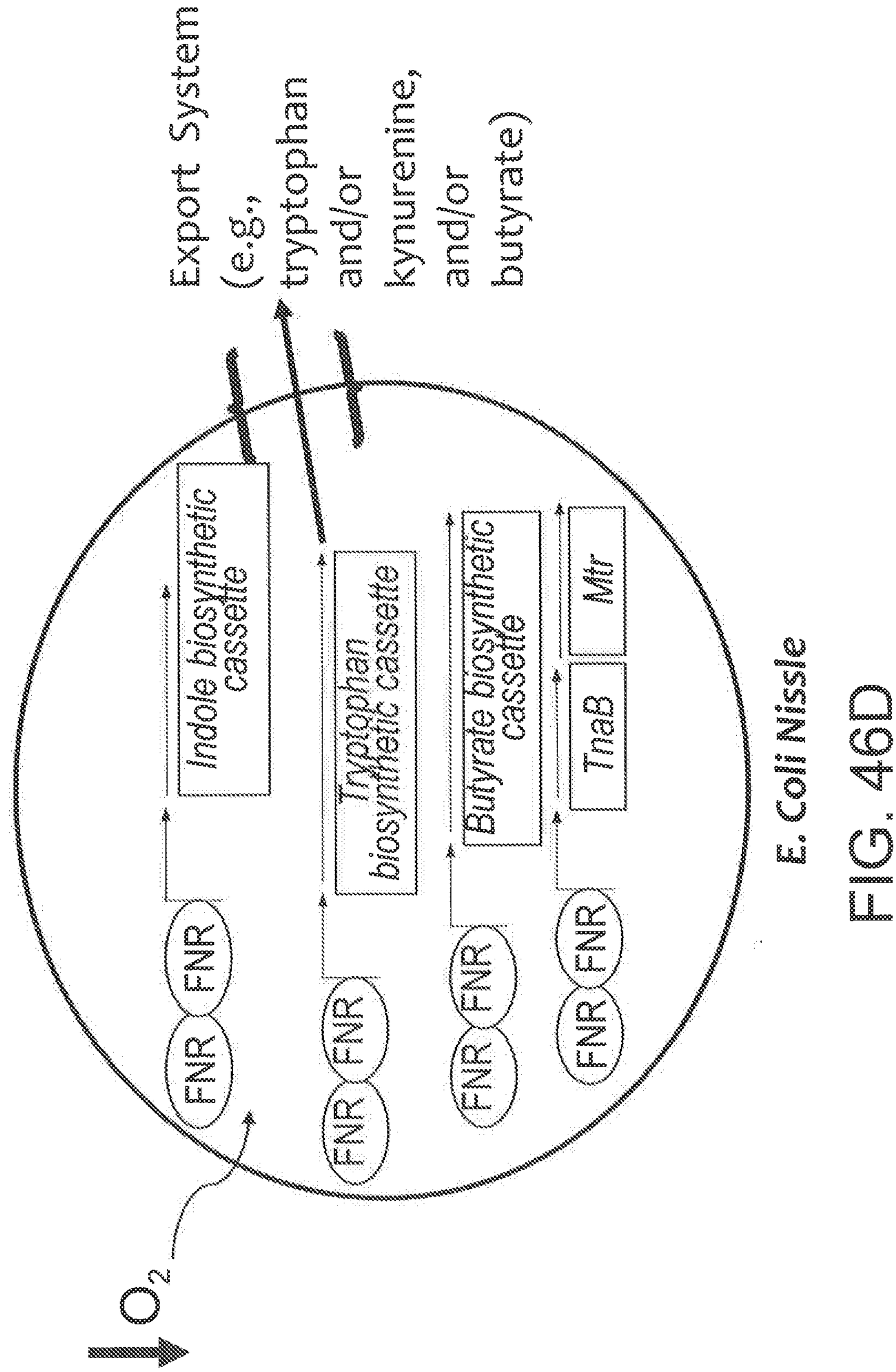

Export System
(e.g.;
tryptophan
and/or
kynurenine,
and/or
butyrate)
Indole biosynthetic cassette
Tryptophan biosynthetic cassette
Butyrate biosynthetic cassette
TnaB
Mtr
FNR
E. Coli Nissle
O2

FIG. 46D

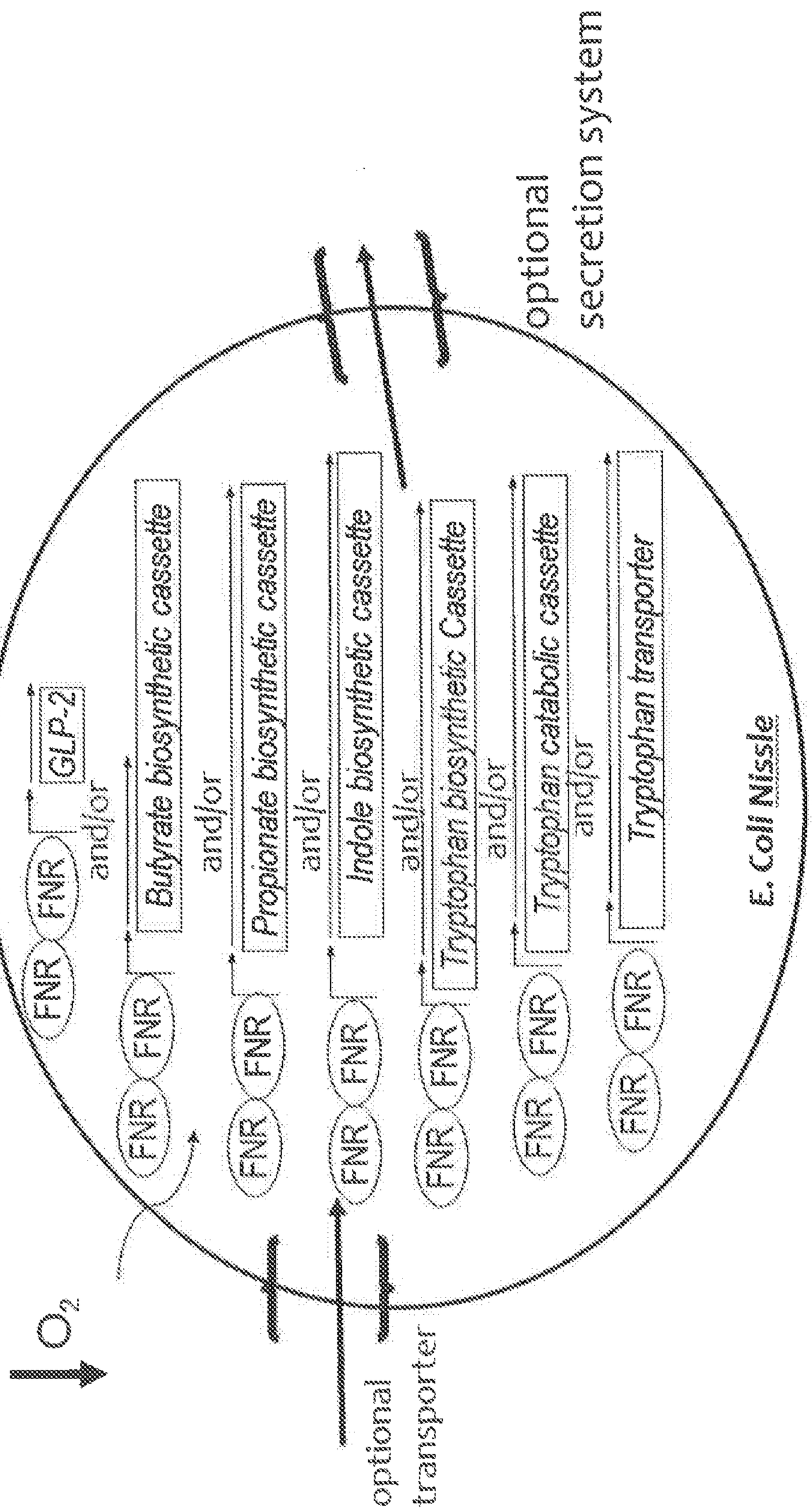

system
optional
secretion
FNR
GLP-2
and/or
Butyrate biosynthetic cassette
Propionate biosynthetic cassette
Indole biosynthetic cassette
Tryptophan biosynthetic Cassette
Tryptophan catabolic cassette
Tryptophan transporter
E. coli Nissle
$O_2$
optional
transporter

FIG. 46E

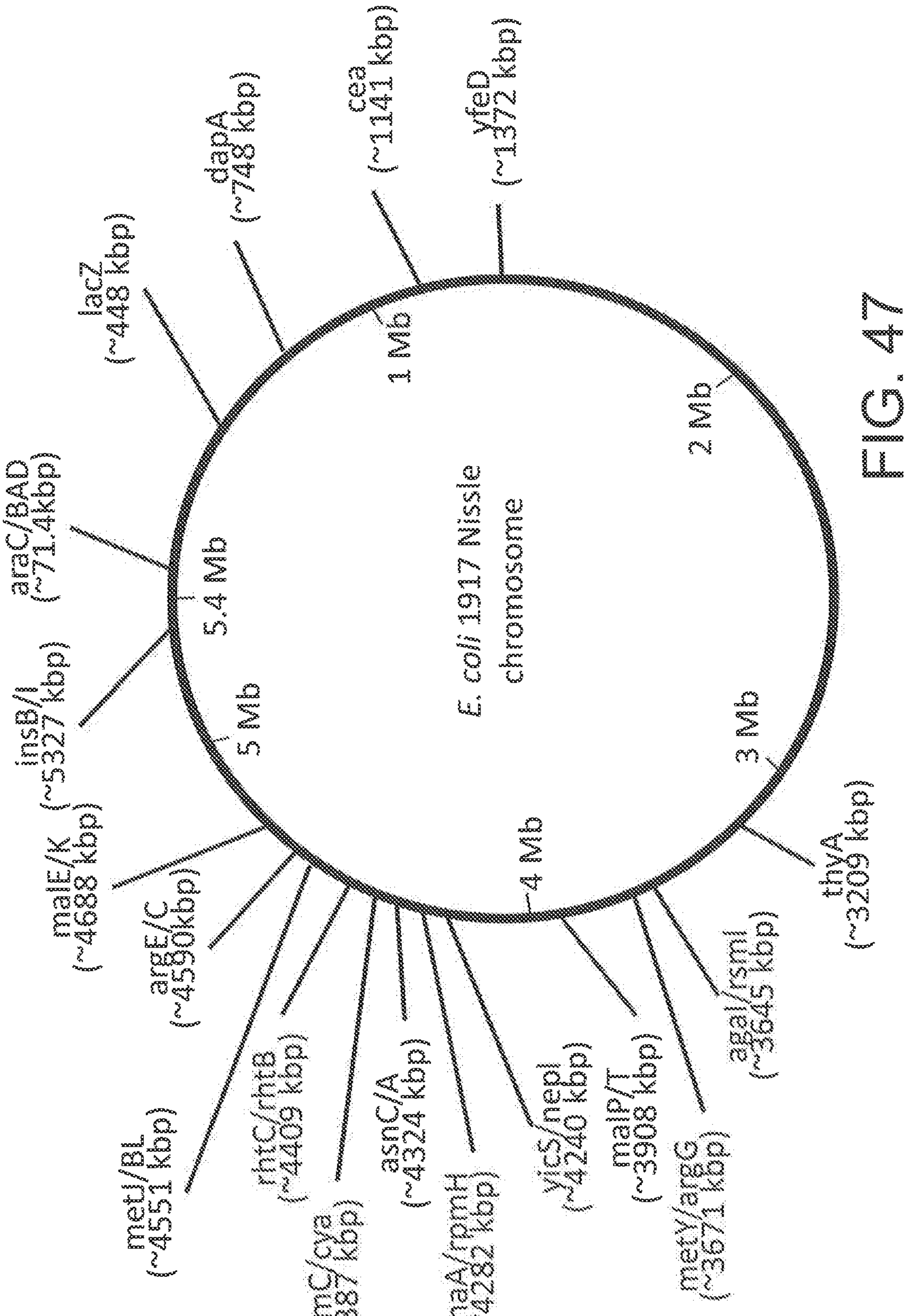

dapA
(~748 kbp)
cea
(~1141 kbp)
yfeD
(~1372 kbp)
lacZ
(~448 kbp)
araC/BAD
(~71.4kbp)
1 Mb
2 Mb
5.4 Mb
1917 Nissle
chromosome
E. coli
insB/I
(~5327 kbp)
malE/K
(~4688 kbp)
5 Mb
argE/C
(~4590kbp)
4 Mb
rhtC/rhtB
(~4409 kbp)
asnC/A
(~4324 kbp)
ycjS/nepI
(~4240 kbp)
malP/T
(~3908 kbp)
aaaI/rsmI
(~3645 kbp)
3 Mb
thyA
(~3209 kbp)
metA/I
(~4511
hemC/cya
(~4387 kba)
dnaA/rnpH
(~4282 kbp)
matA/ariA
(~3974

FIG. 47

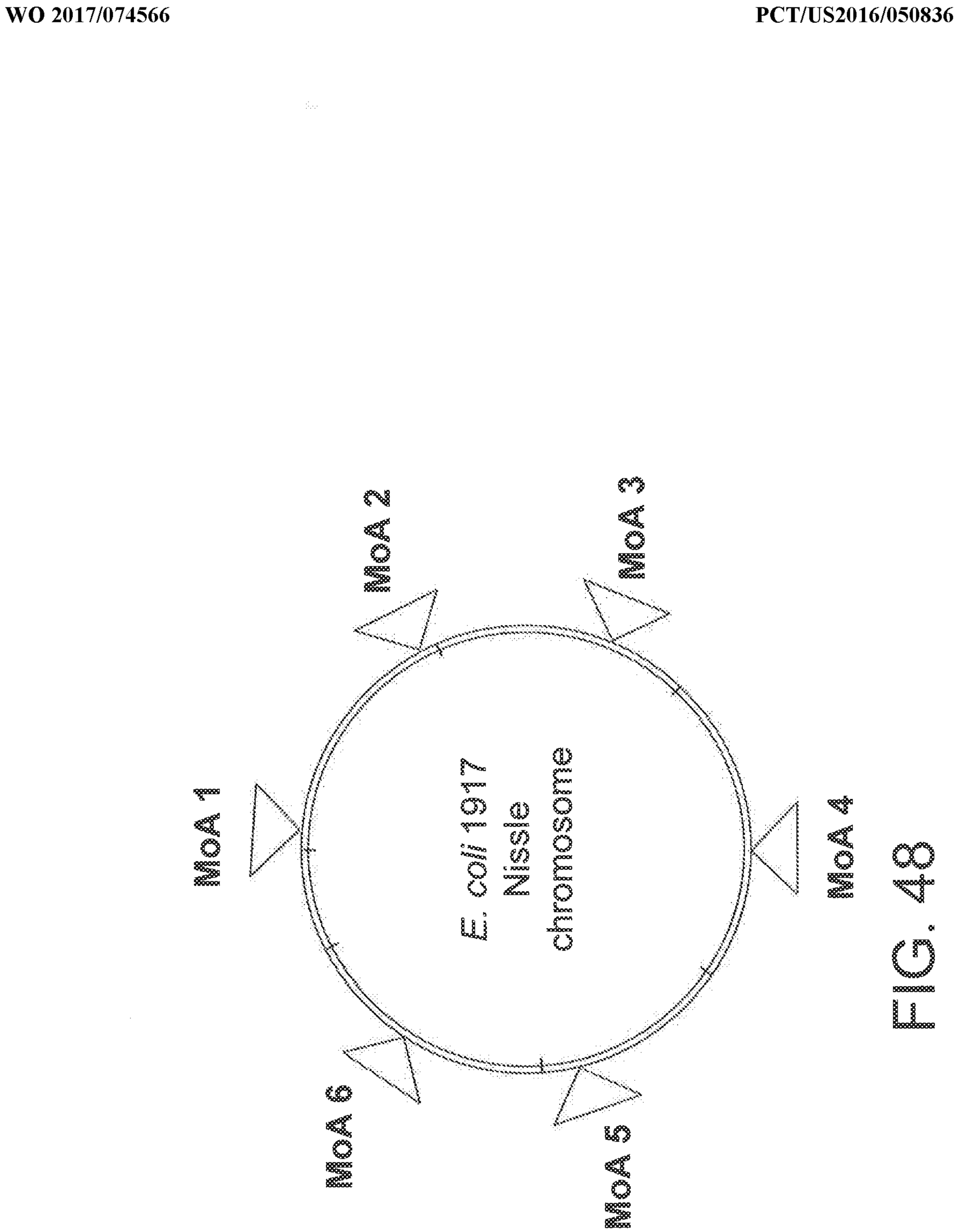



FIG. 48

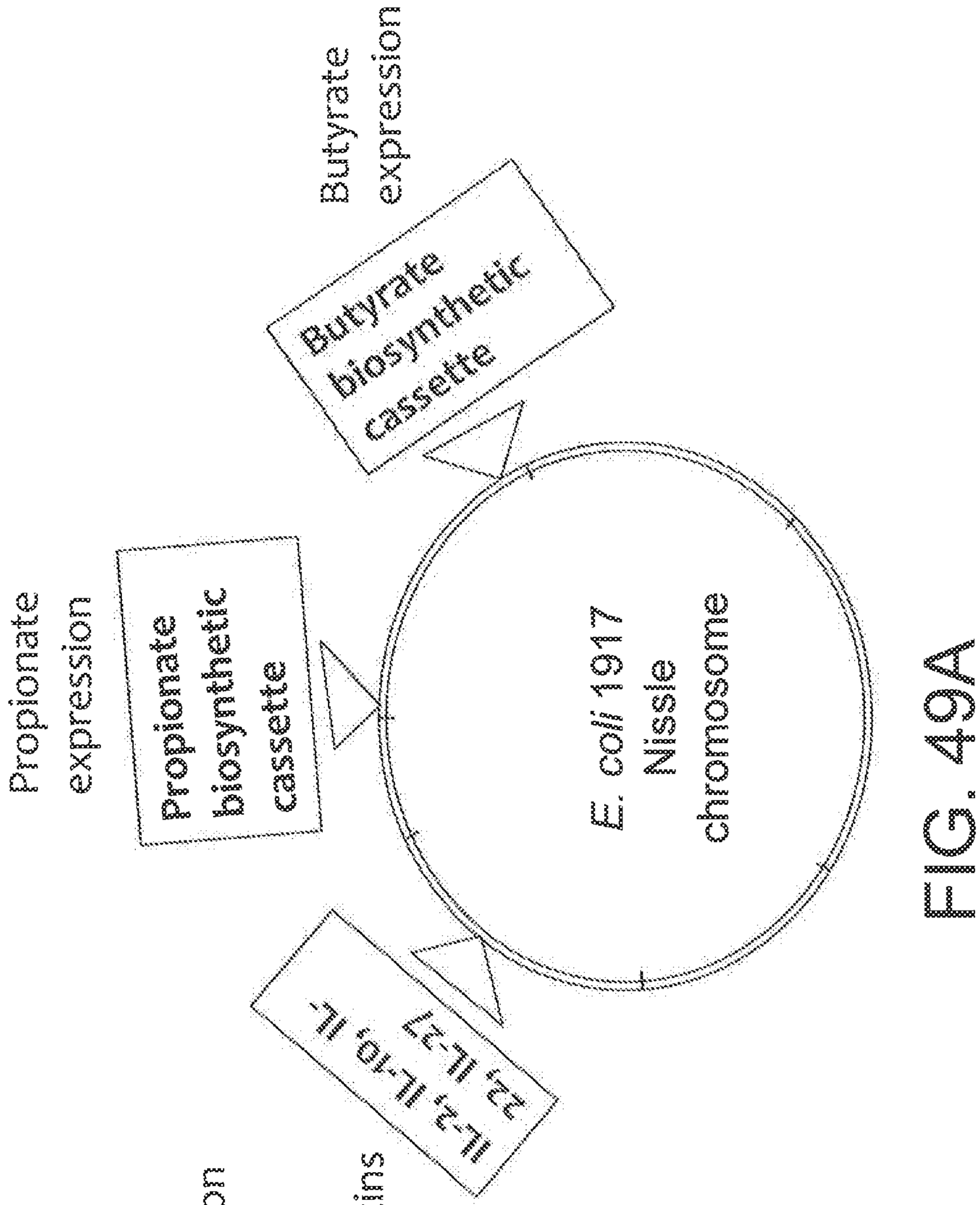

Butyrate expression
Butyrate biosynthetic cassette
Propionate expression
Propionate biosynthetic cassete
E. coli 1917 Nissle chromosome
Expression of IBD relevant interleukins

FIG. 49A

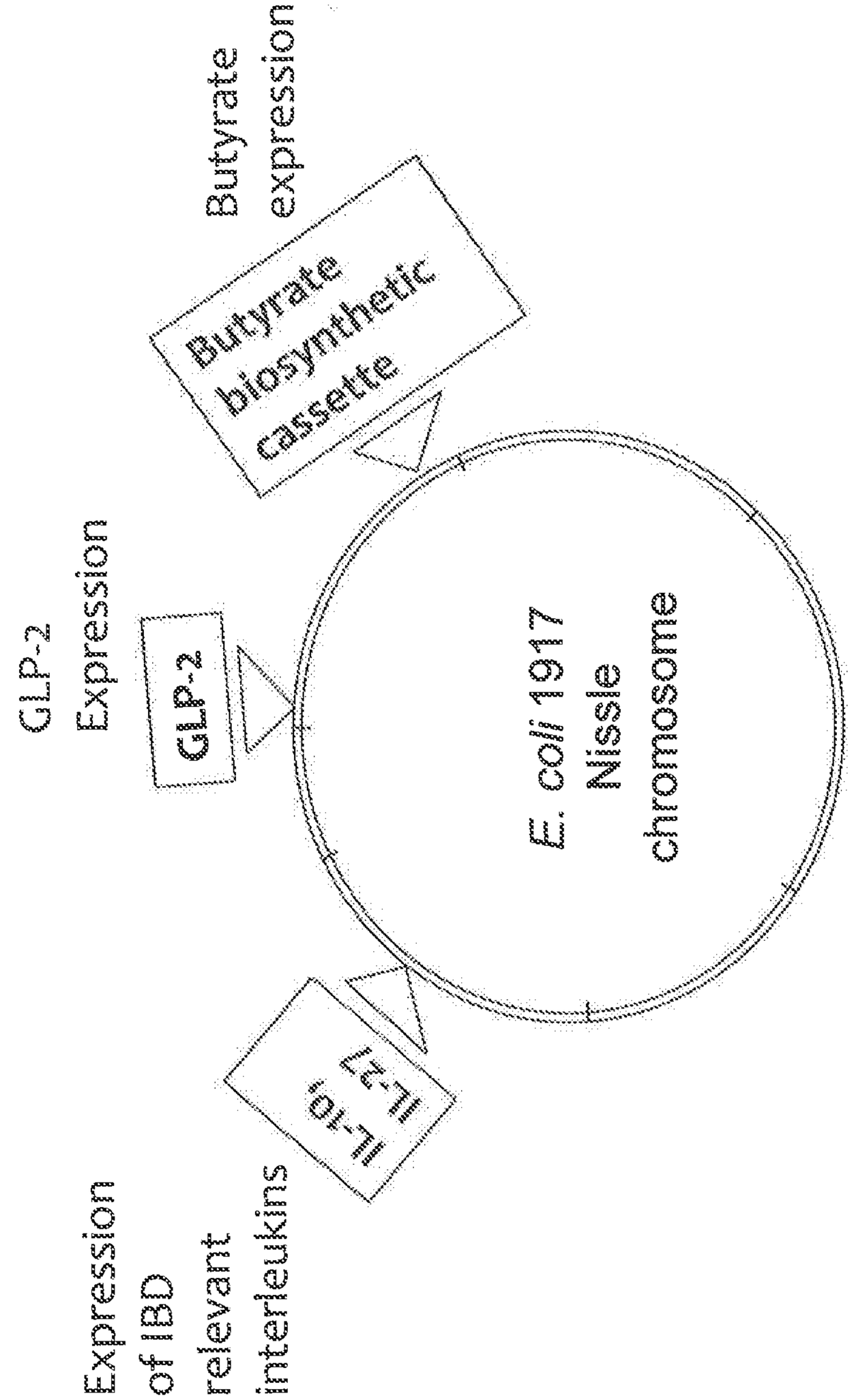

Butyrate expression
Butyrate biosynthetic cassette
GLP-2 Expression
GLP-2
E. coli 1917 Nissle chromosome
Expression of IBD relevant interleukins

FIG. 49B

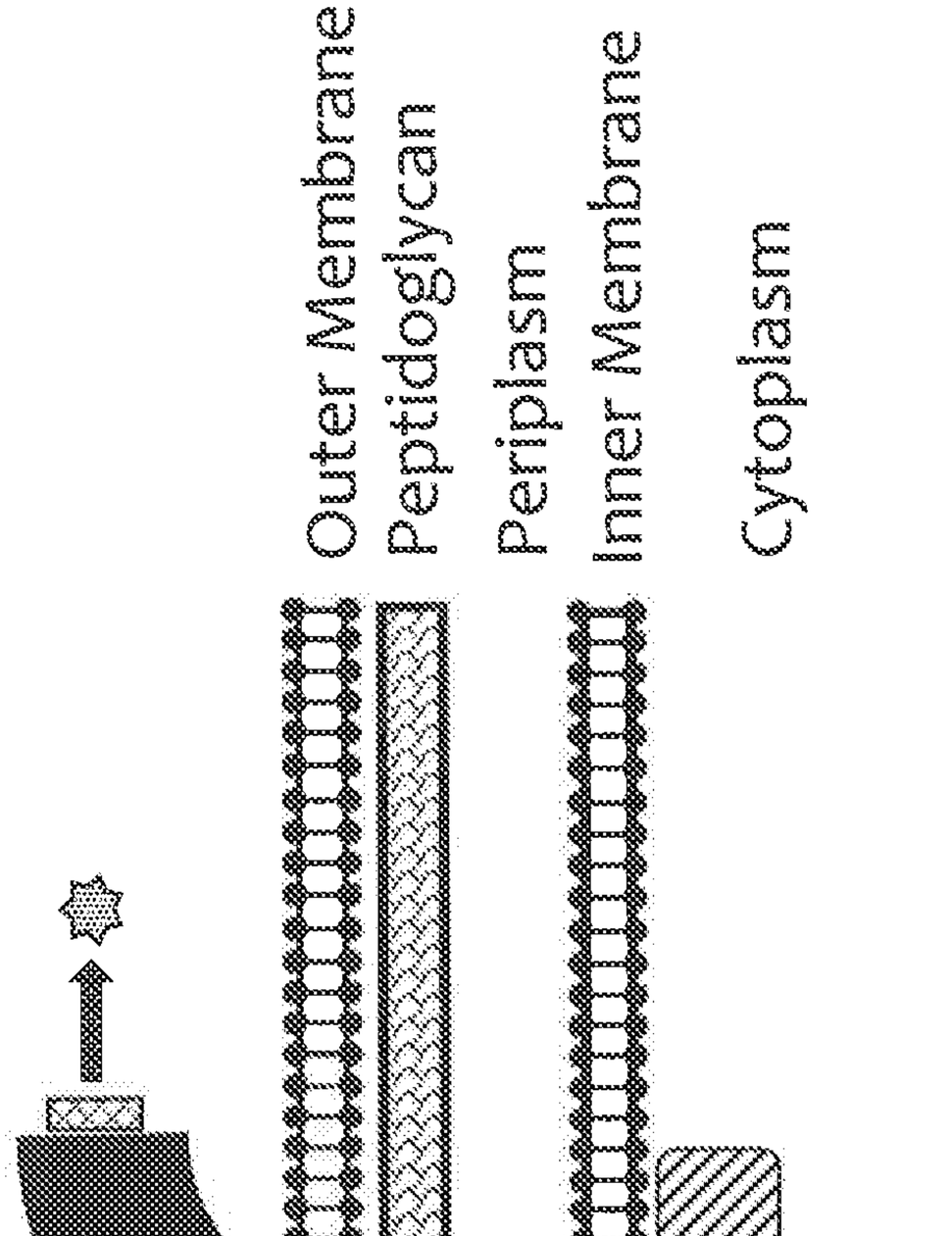

Outer Membrane
Peptidoglycan
Periplasm
Inner Membrane
Cytoplasm

FIG. 50

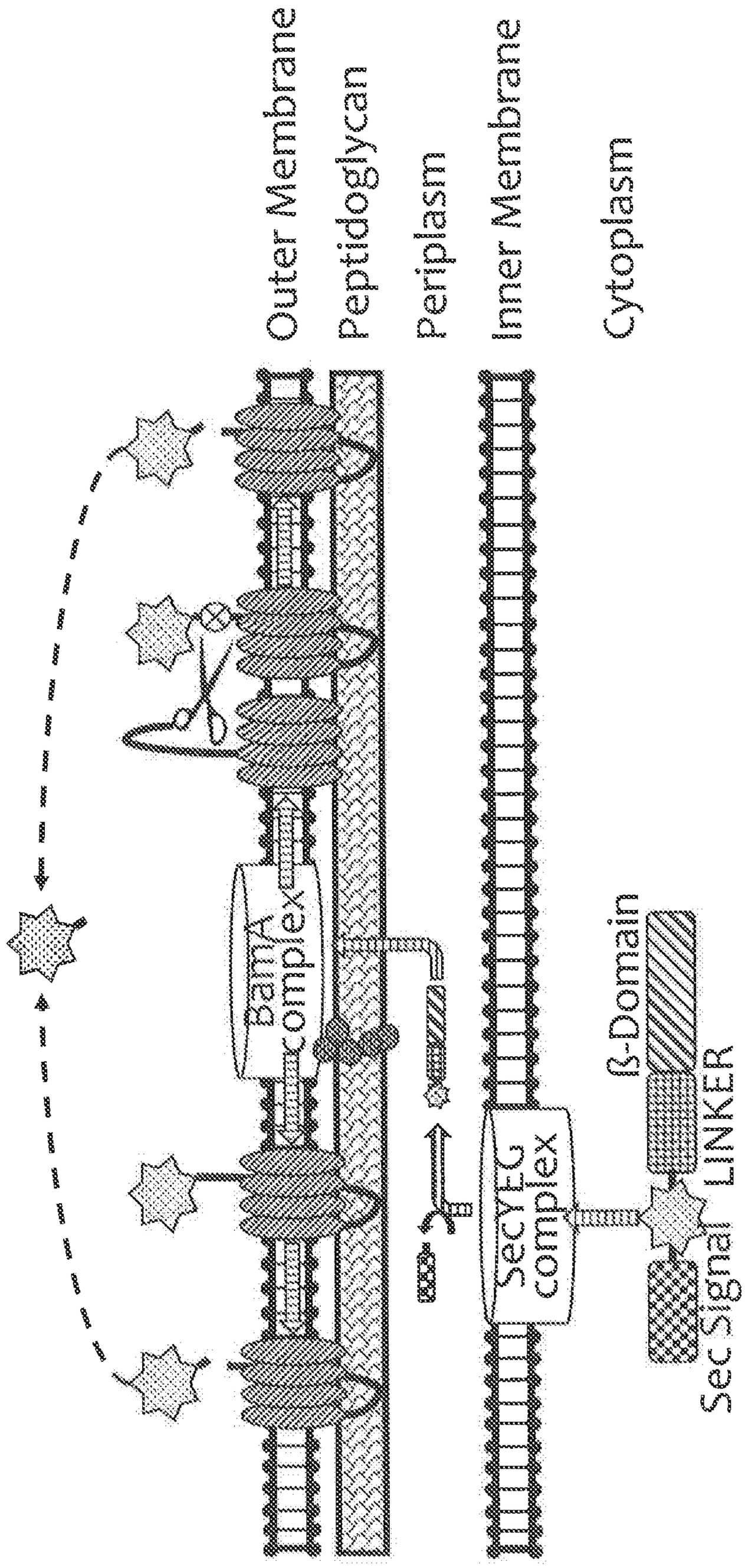

Outer Membrane
Peptidoglycan
Periplasm
Inner Membrane
Cytoplasm
BamA complex
SecYEG complex
&-Domain
LINKER
Sec signal

FIG. 51

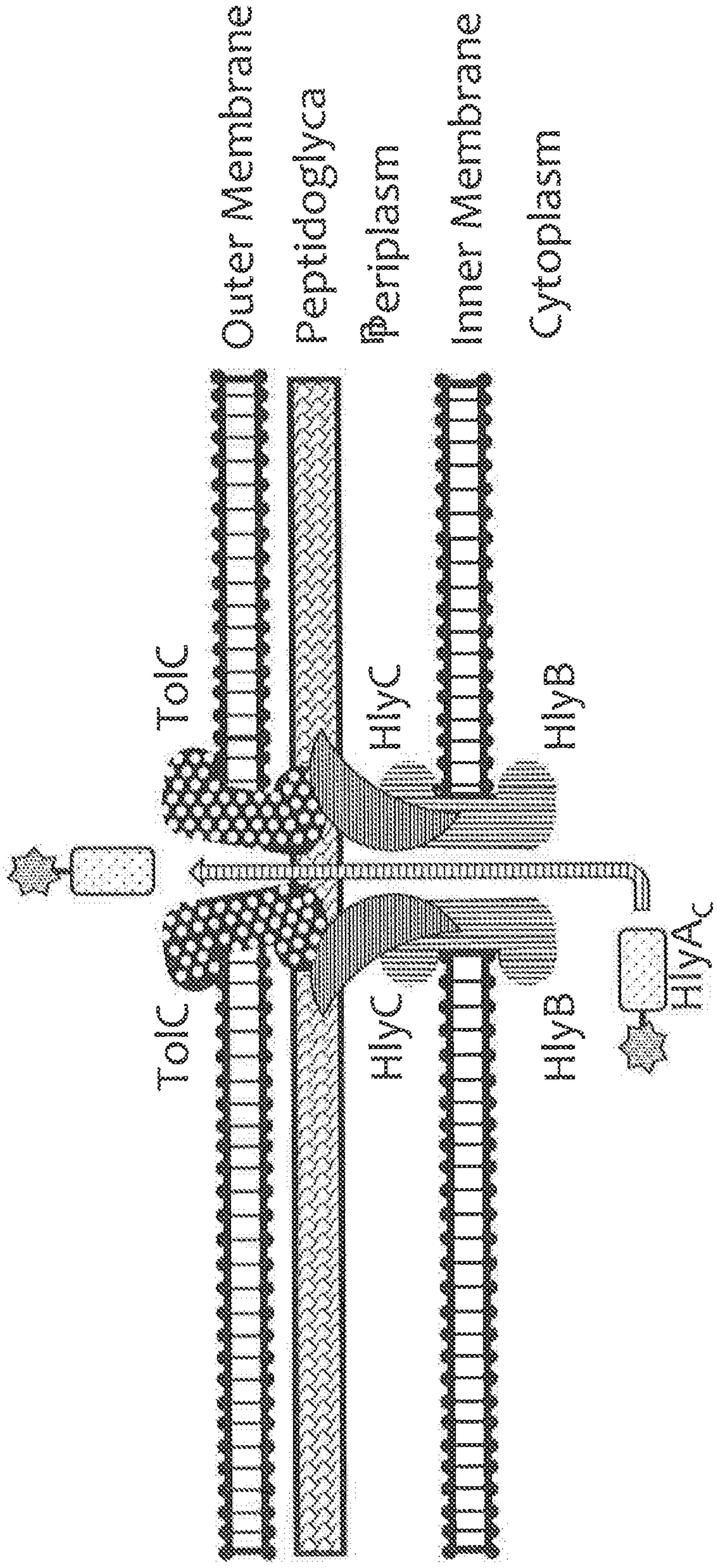

Outer Membrane
Peptidoglycan
Periplasm
Inner Membrane
Cytoplasm
TolC
HlyC
HlyB
TolC
HlyC
HlyB
HlyA

FIG. 52

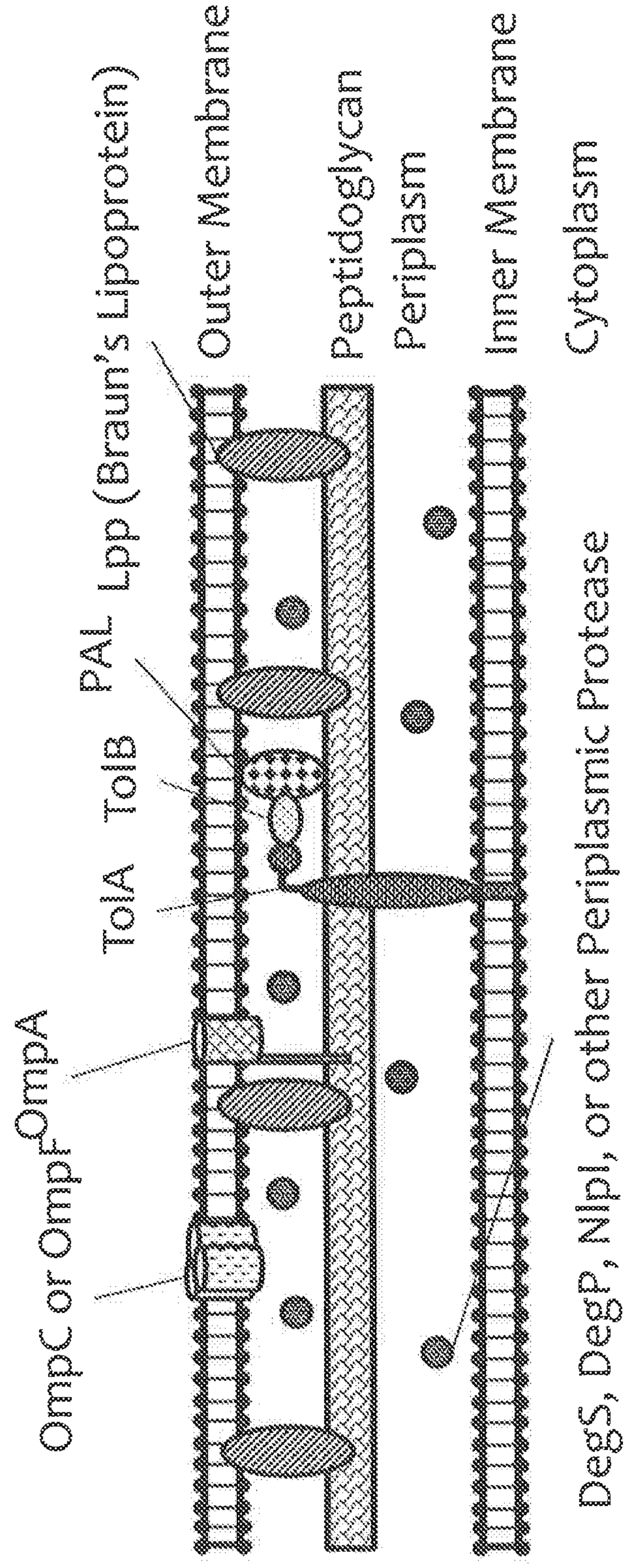

OmpC or OmpF OmpA
ToIA ToIB
PAL Lpp (Braun's Lipoprotein)
DegS, DegP, NlpI, or other Periplasmic Protease
Outer Membrane
Peptidoglycan
Periplasm
Inner Membrane
Cytoplasm

FIG. 53

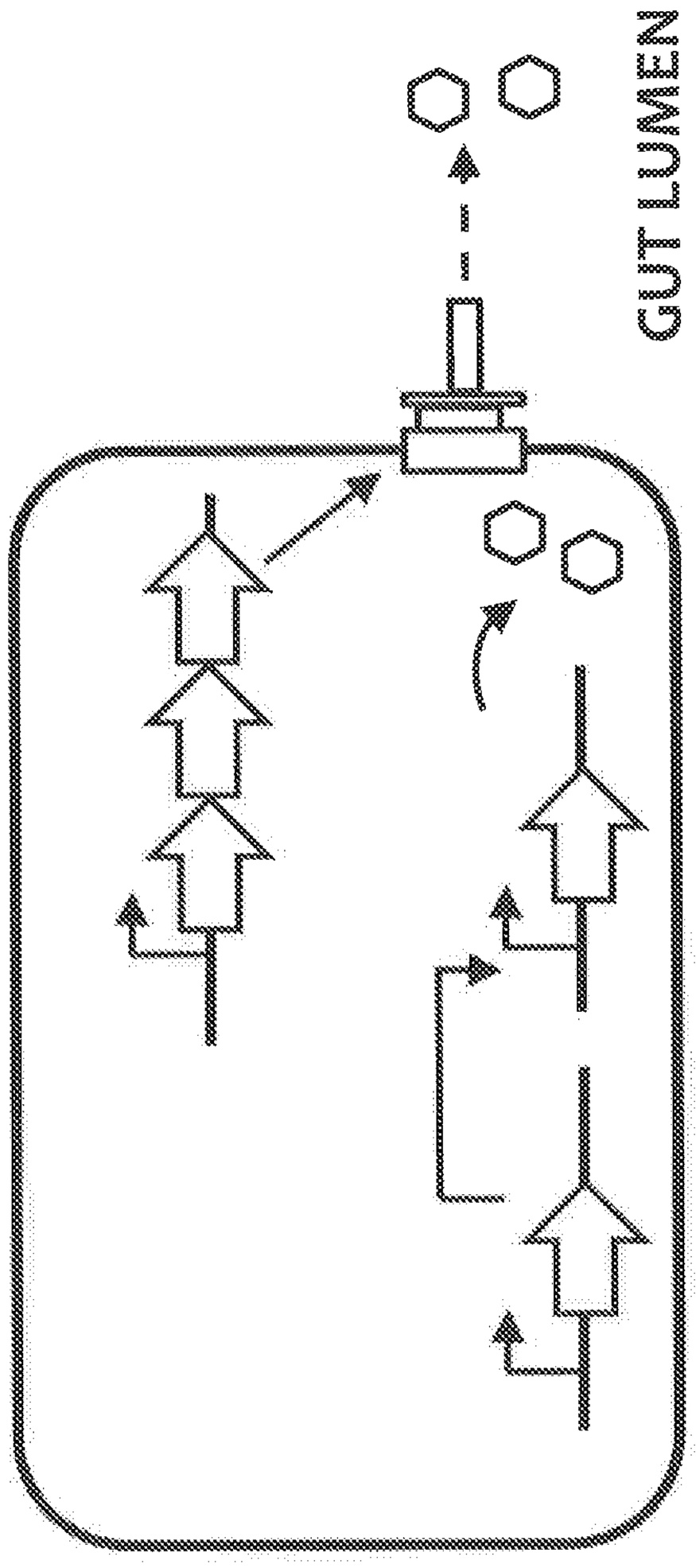



FIG. 54

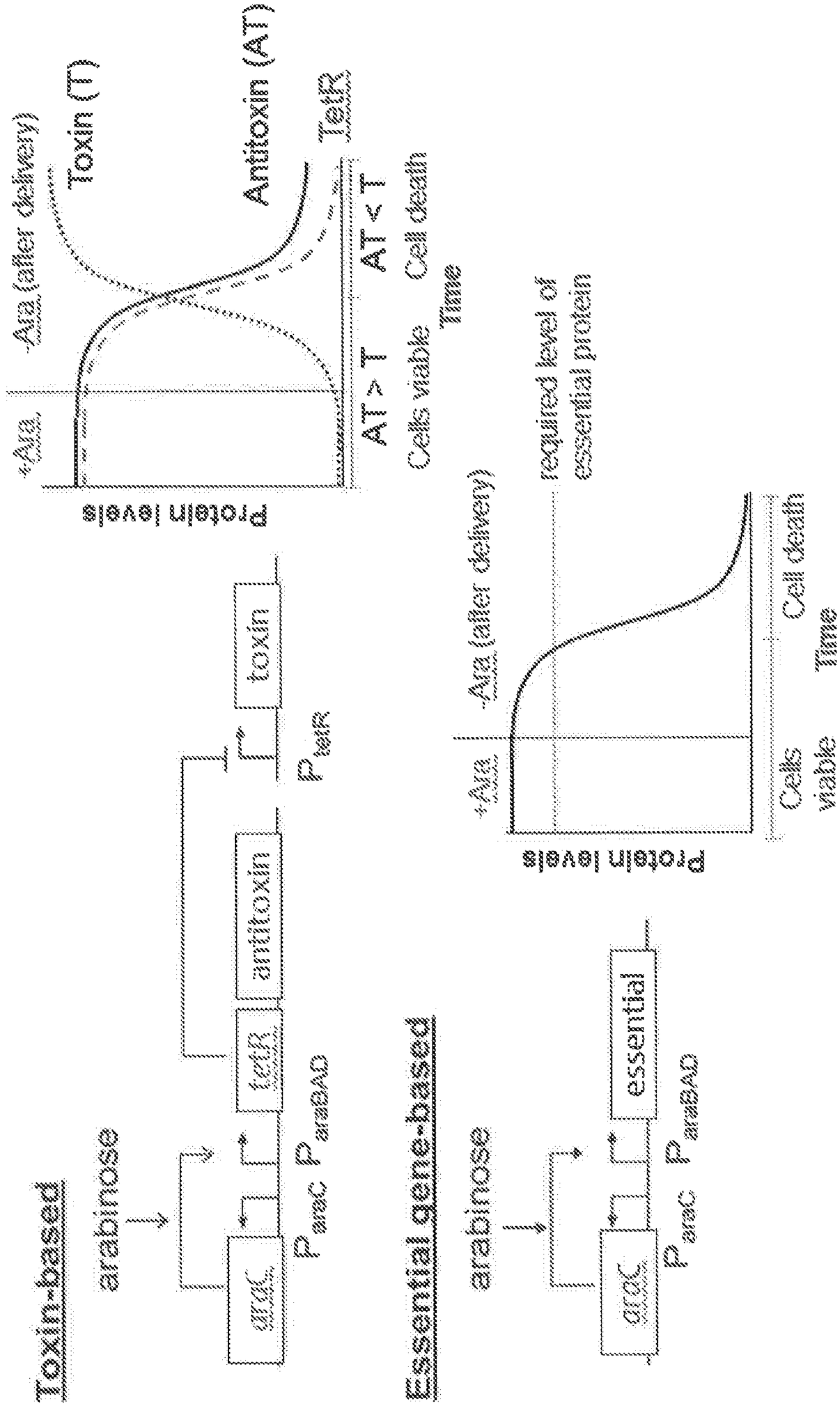

Toxin-based
arabinose
araC
$P_{araBAD}$
tetR
antitoxin
$P_{tet}$
$P_{araC}$
Essential gene-based
arabinose
araC
$P_{araBAD}$
essential
$P_{araC}$
Time
Cell death
Cells viable
AT > T
AT < T
Antitoxin (AT)
Toxin (T)
Ara (after delivery)
+Ara
-Ara
Protein levels
Time
Cell death
Cells viable
required level of
essential protein
Ara (after delivery)
+Ara
-Ara
Protein levels

FIG. 55A

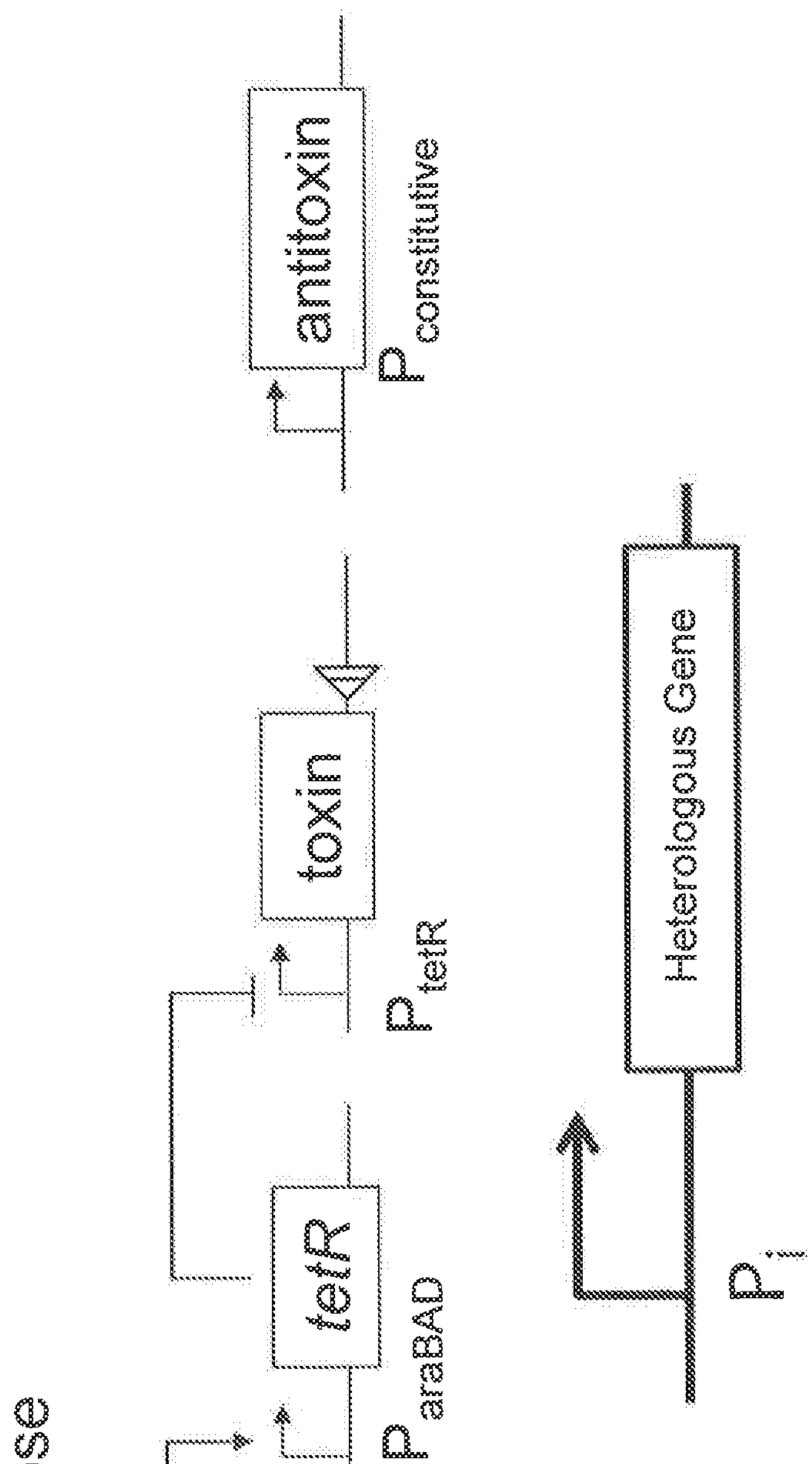

arabinose
araC
$P_{araC}$
tetR
$P_{araBAD}$
toxin
$P_{tetR}$
antitoxin
$P_{constitutive}$
Heterologous Gene
$P_{...}$

FIG. 55B

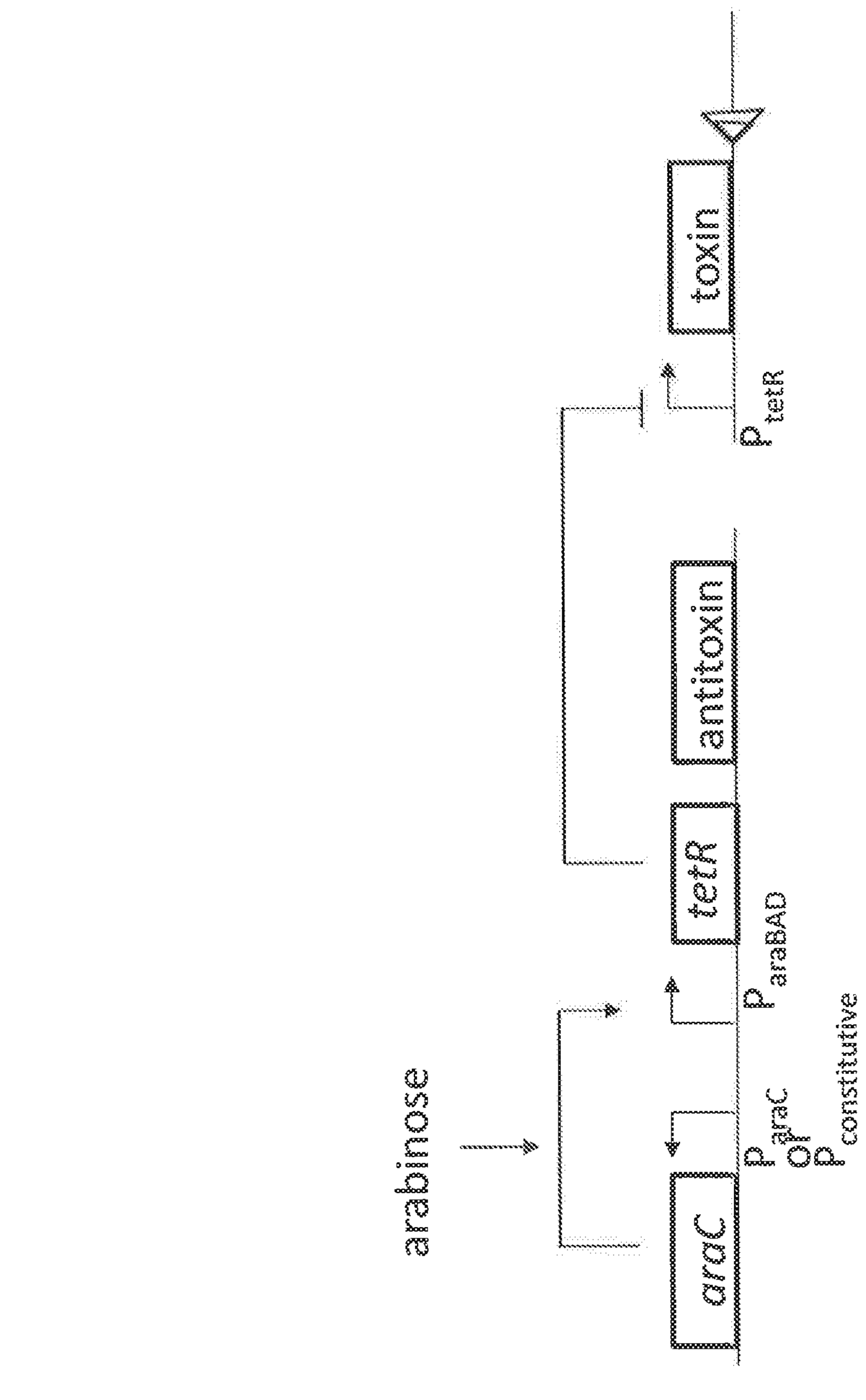

arabinose
araC
$p_{araBAD}$
tetR
$p_{constitutive}$
antitoxin
$p_{tetR}$
toxin

FIG. 55C

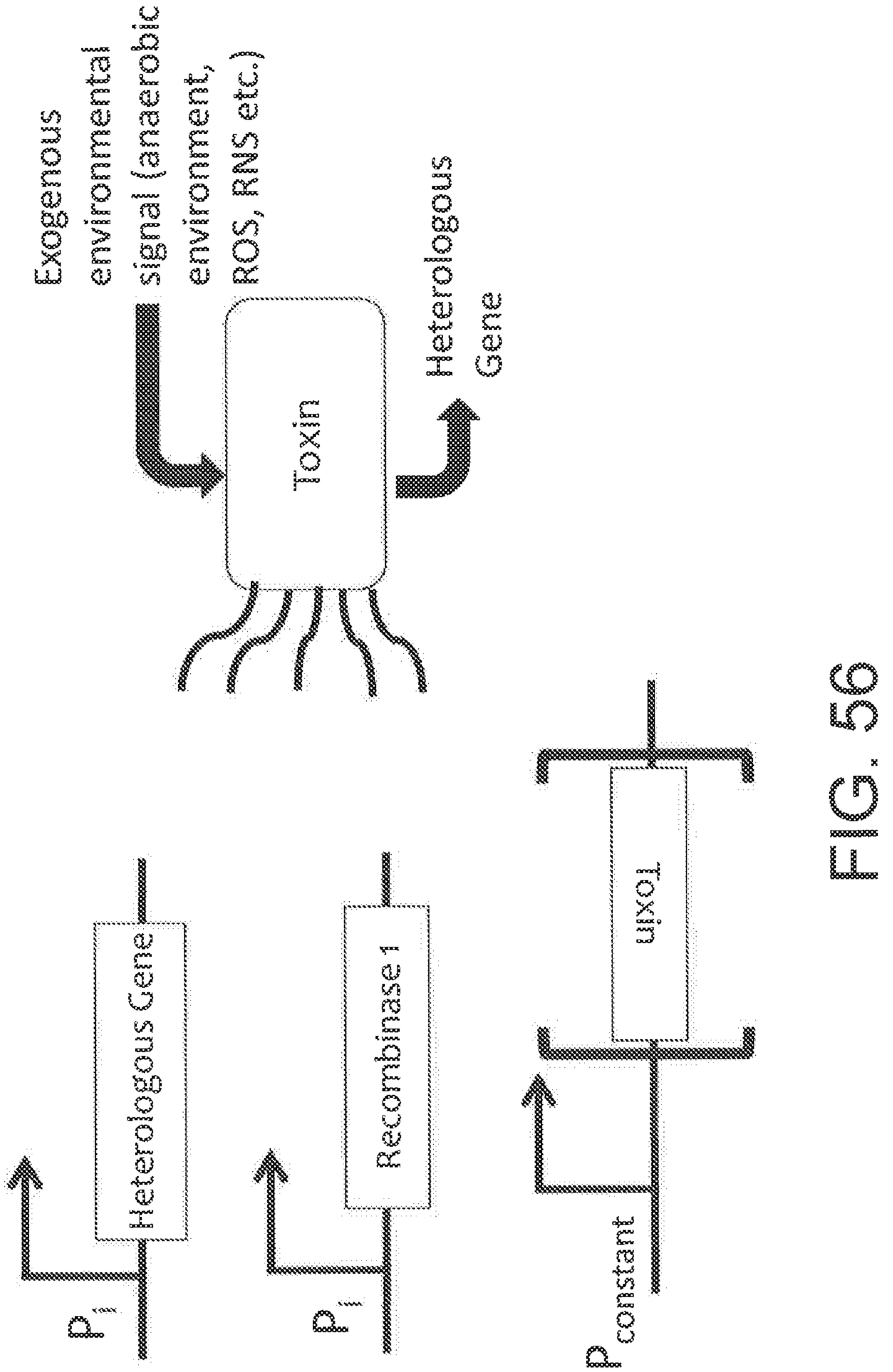

Exogenous
environmental
signal (anaerobic
environment, low T, pH,
ROS, RNS etc.)
Toxin
Heterologous
Gene
Heterologous Gene
Recombinase 1
Toxin
P constant

FIG. 56

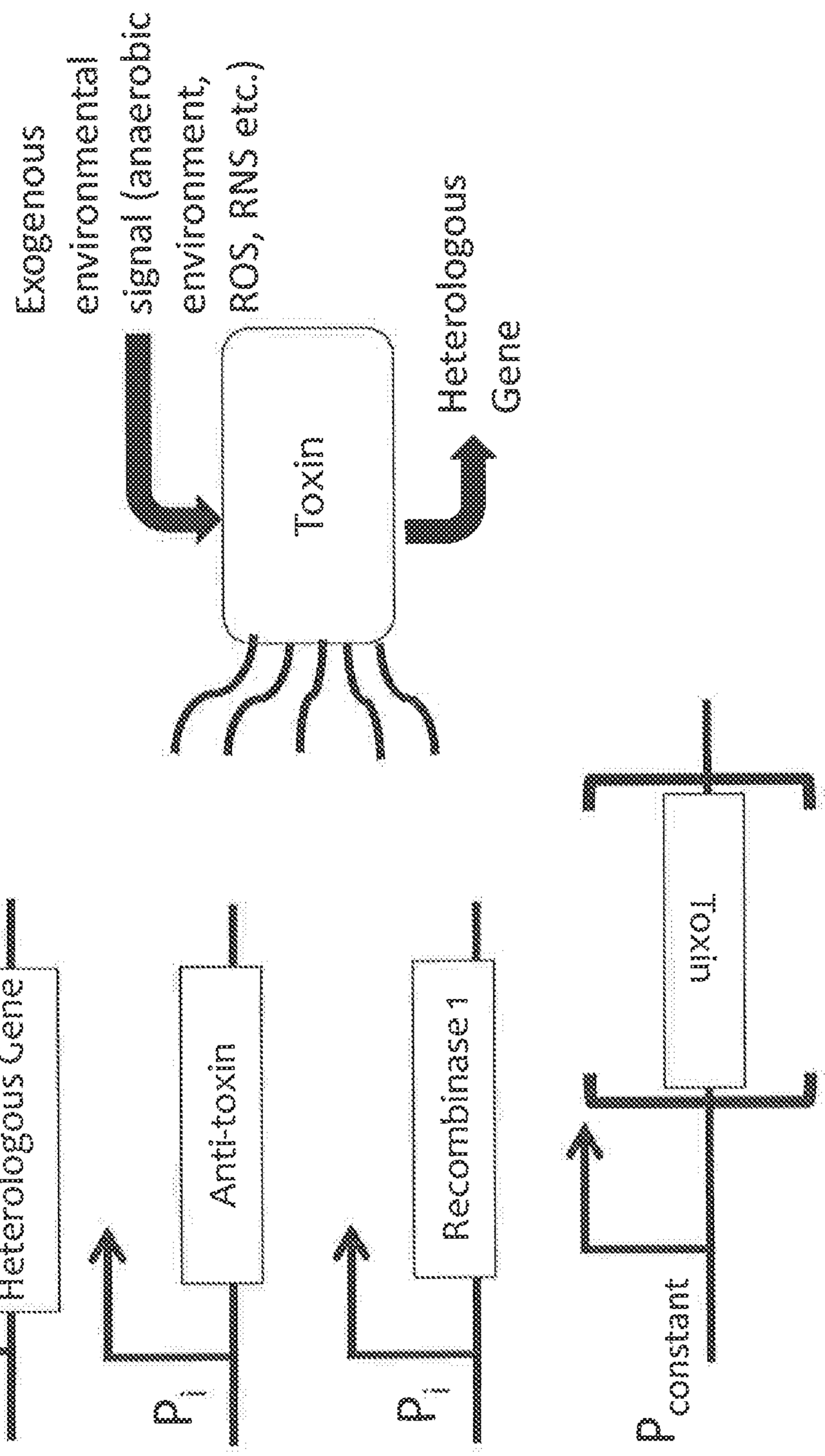

Exogenous environmental signal (anaerobic environment, ROS, RNS etc.)
Toxin
Heterologous Gene
Heterologous Gene
Anti-toxin
Recombinase1
Toxin
Constant
$P_{constant}$
$P_{1}$
$P_{2}$
$P_{3}$

FIG. 57

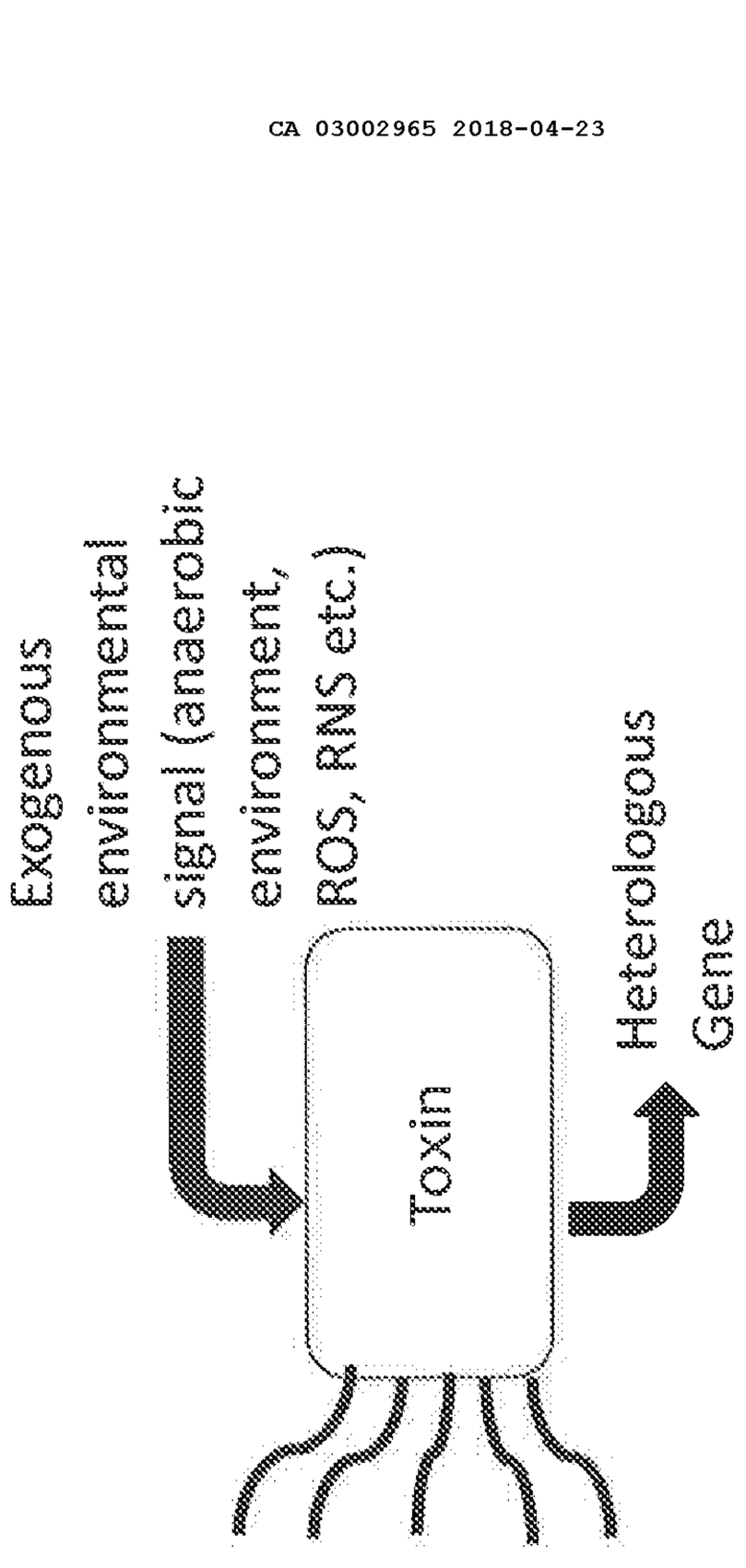

Exogenous environmental signal (anaerobic environment, ROS, RNS etc.)
Toxin
Heterologous Gene
Heterologous Gene
Recombinase 1
P constant
Xis 1
Xis 2
X1
Essential 1
X2
Essential 2

FIG. 58

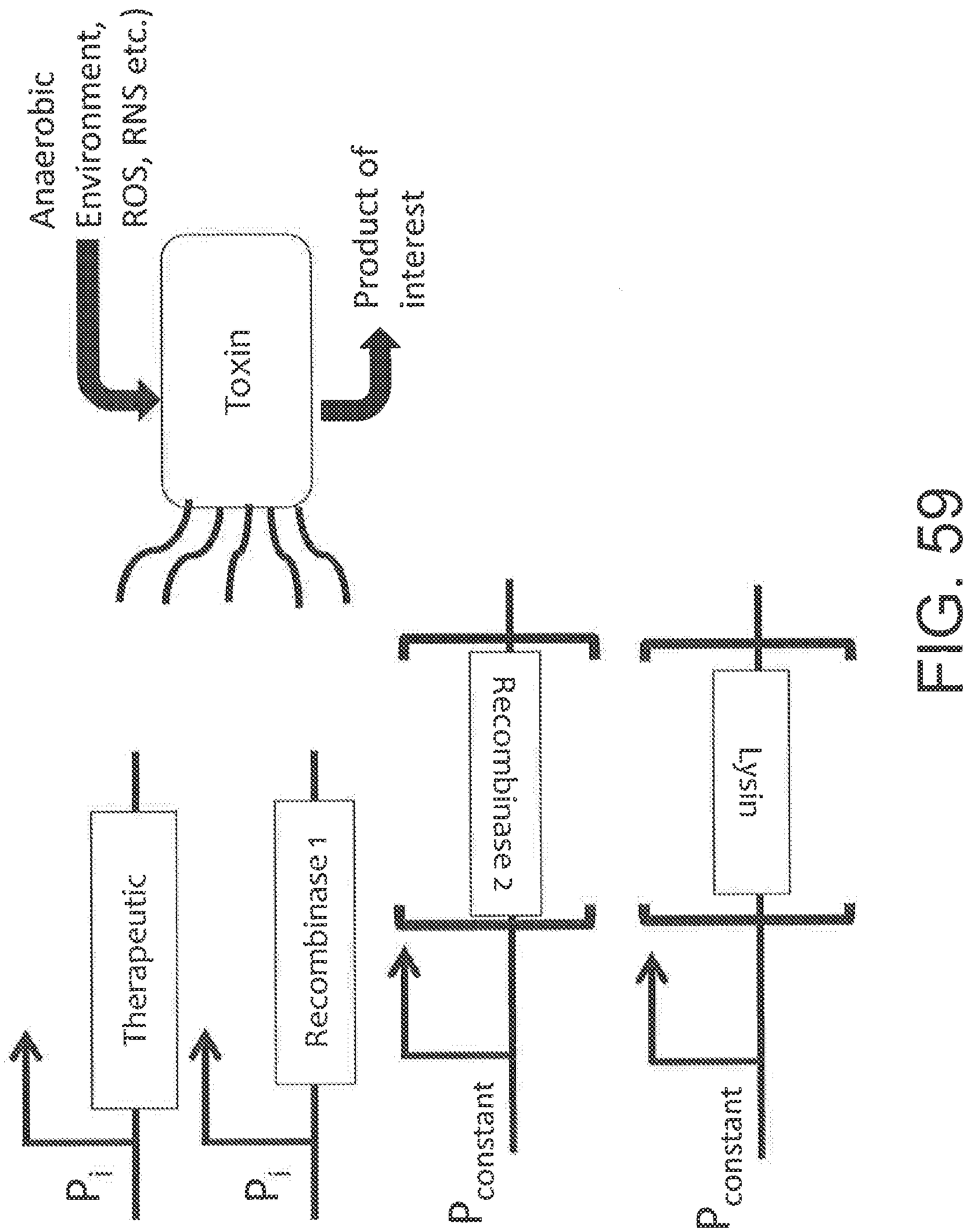

Anaerobic
Environment
ROS, RNS etc
Toxin
Product of
interest
Therapeutic
Recombinase 1
Recombinase 2
Lysin
Pconstant
Pconstant
Pconstant
Pconstant

FIG. 5G

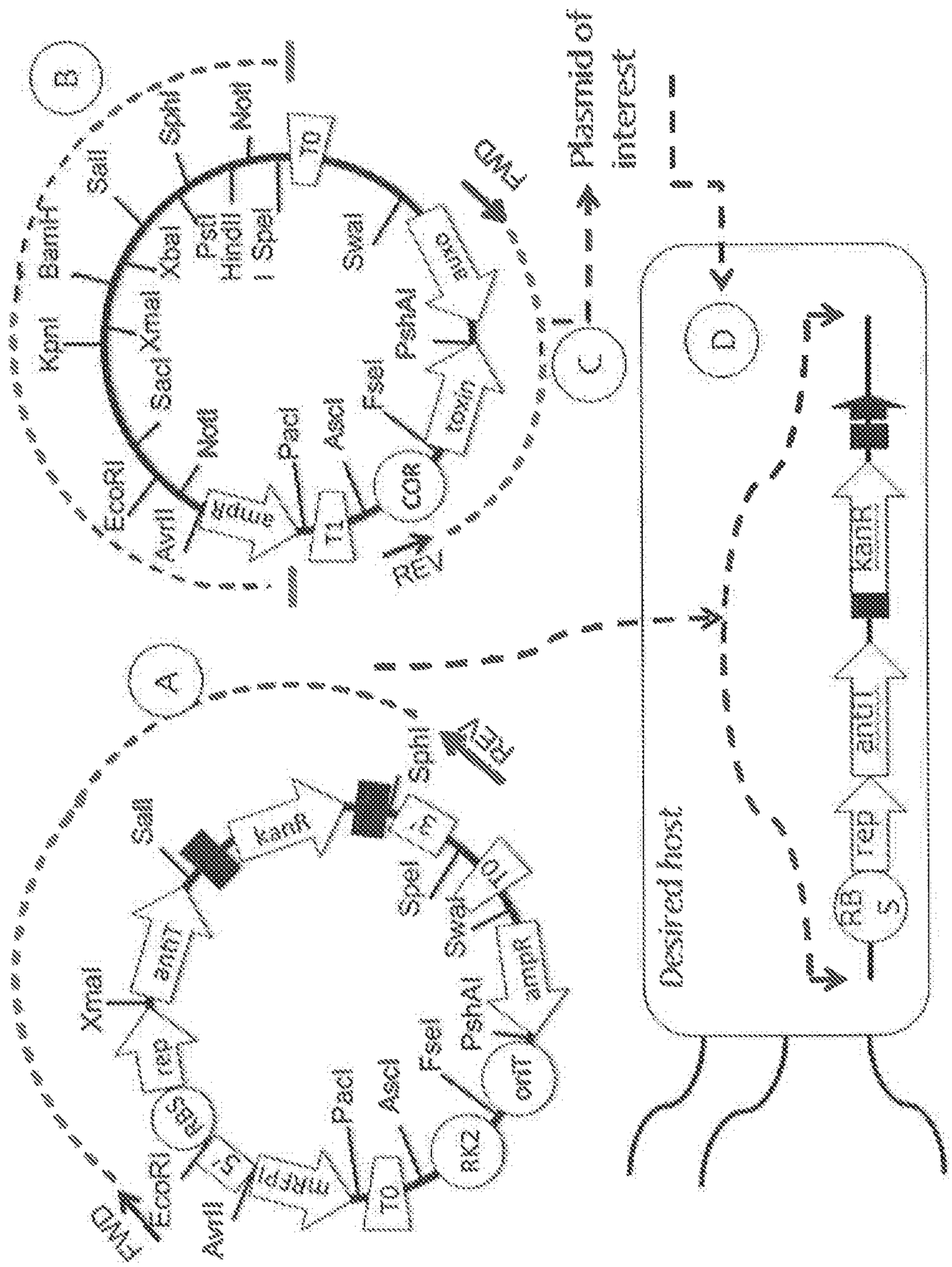

Plasmid of interest
CmR
REV
ori
T1
AscI
PacI
NotI
EcoRI
AvrII
XmaI
SacI
KpnI
BamHI
SalI
XbaI
PstI
HindIII
SphI
SpeI
T0
SwaI
PshAI
FseI
toxin
antir
KanR
Desired host
Rep
amp
RB
5
RK2
ori
T0
T1
T2

FIG. 60

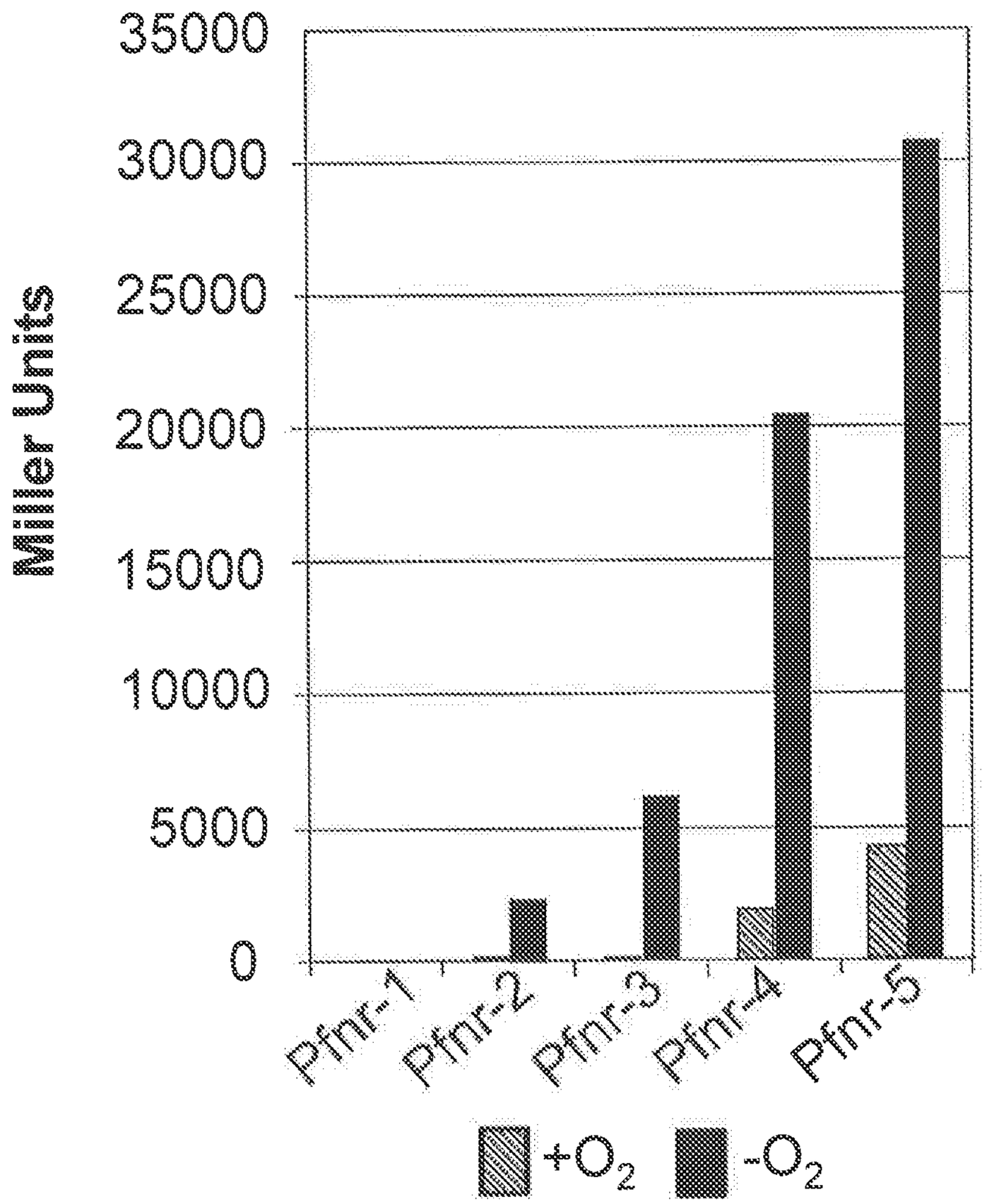

35000
30000
25000
20000
15000
10000
5000
0
Miller Units
Pfnr-1
Pfnr-2
Pfnr-3
Pfnr-4
Pfnr-5
+O2
-O2

FIG. 61

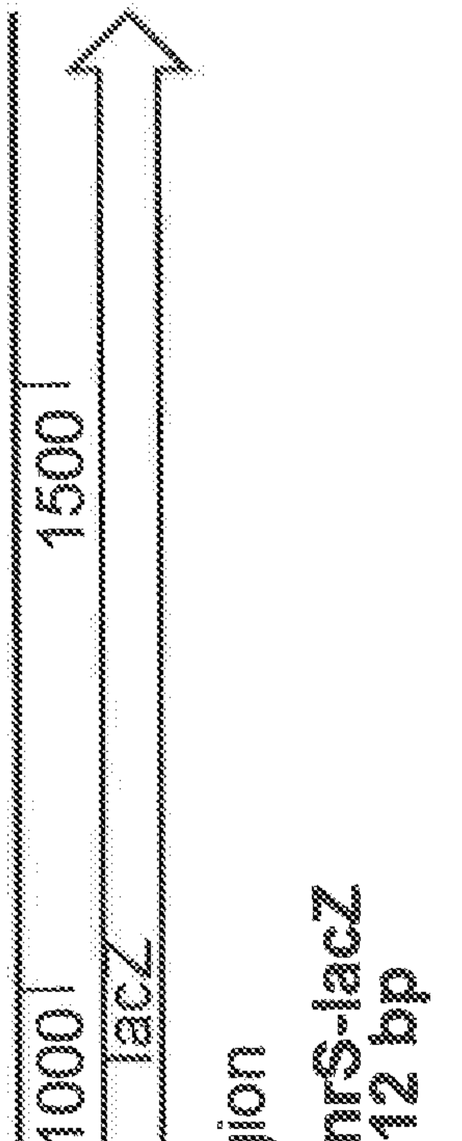



FIG. 62A

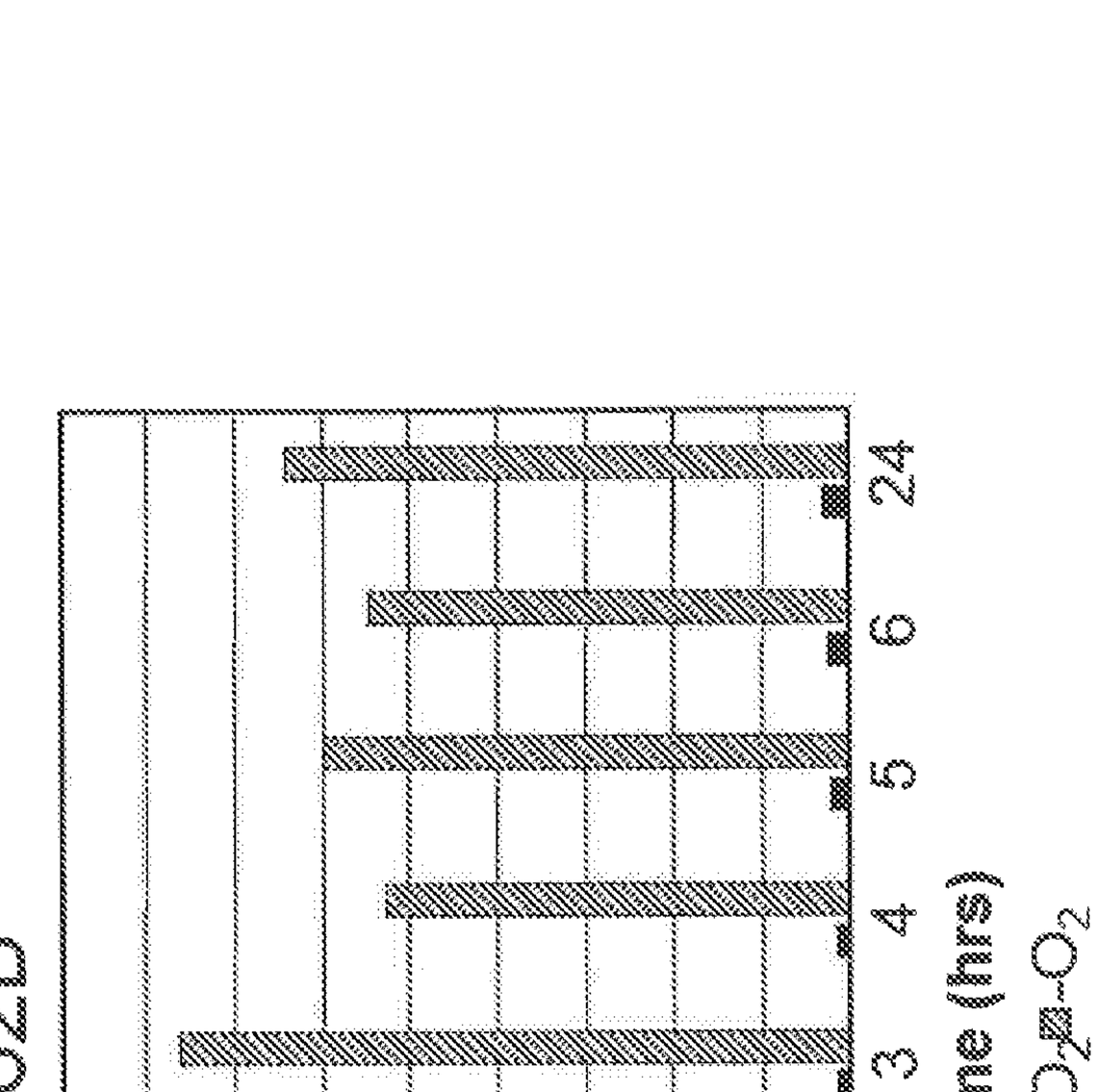



FIG. 62B

OD600
0
3
8
13
18
23
28
Time (hrs)
#1 ENRS-lacZ+O2
#2 ENRS-lacZ+O2
#1 ENRS-lacZ+O2
#2 ENRS-lacZ+O2

FIG. 62C

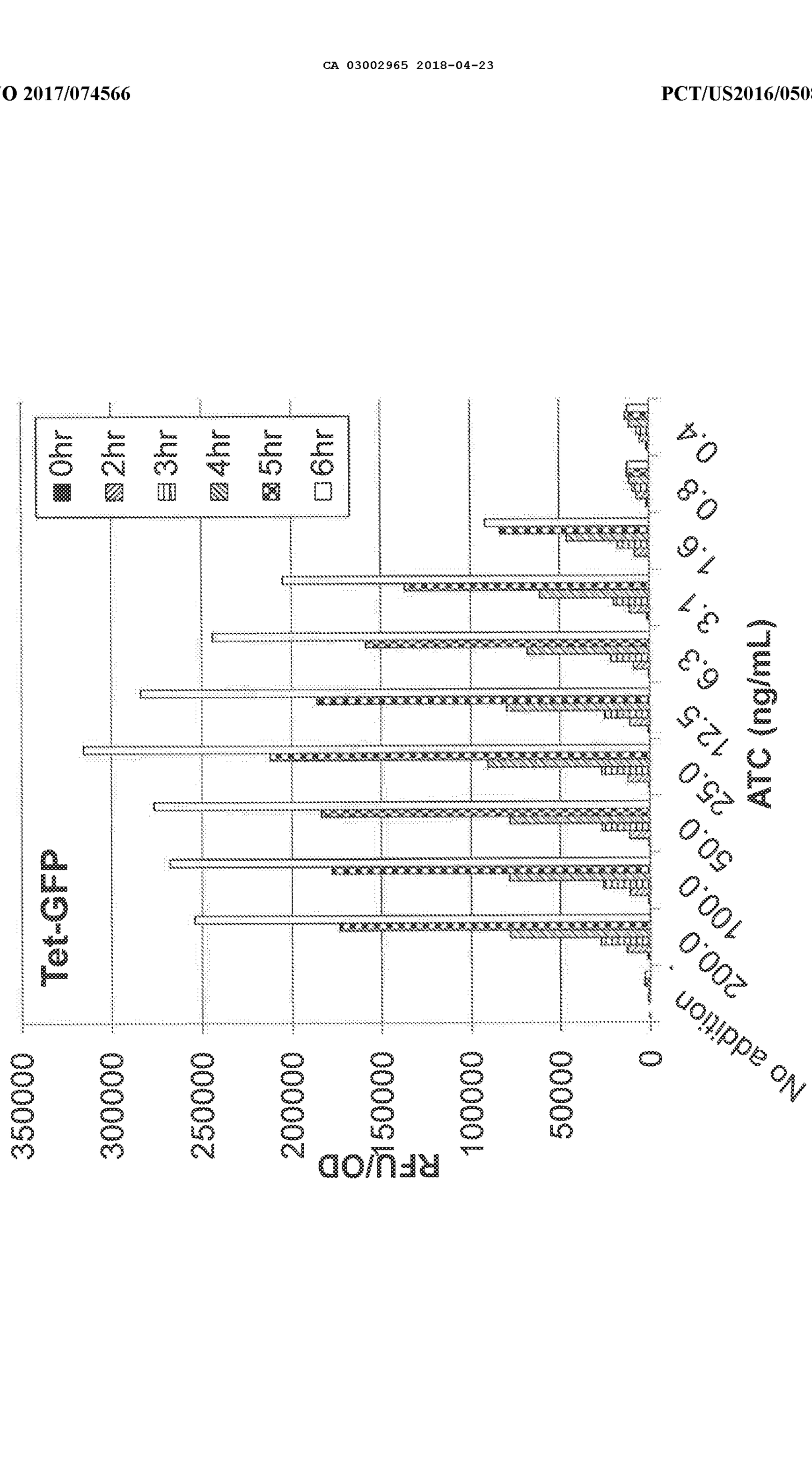

0hr
2hr
3hr
4hr
5hr
6hr
Tet-GFP
RFU/OD
350000
300000
250000
200000
150000
100000
50000
0
No addition
200.0
100.0
50.0
25.0
12.5
6.3
3.1
1.6
0.8
0.4
ATC (ng/mL)

FIG. 3A

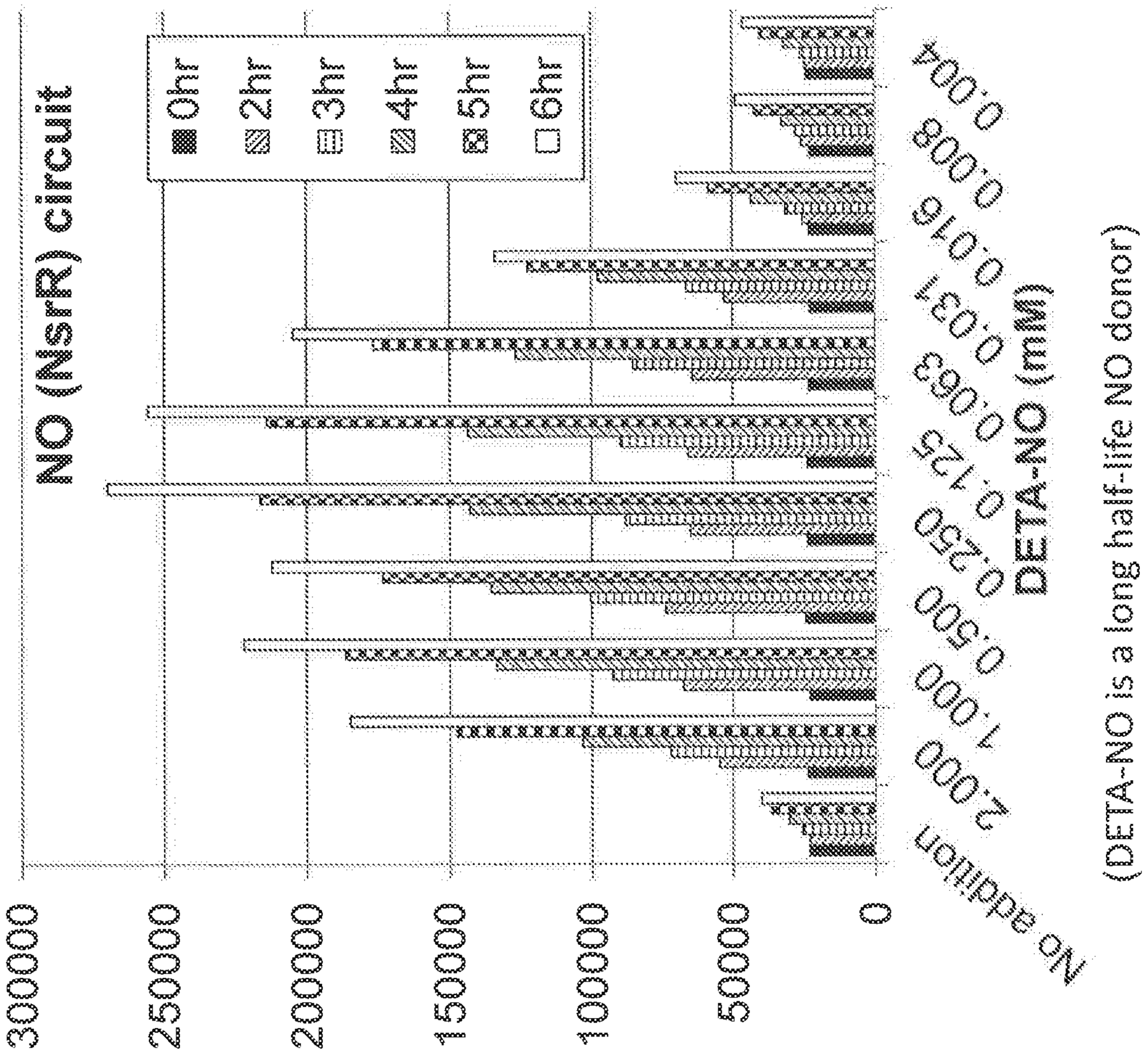

NO (NsrR) circuit
0hr
2hr
3hr
4hr
5hr
6hr
RFU/OD
300000
250000
200000
150000
100000
50000
0
DETA-NO (mM)
0.0
400.0
800.0
1.600
3.200
6.400
12.800
25.600
51.200
102.400
204.800
409.600
819.200
(DETA-NO is a long half-life NO donor)
No additional DETA-NO

FIG. 3B

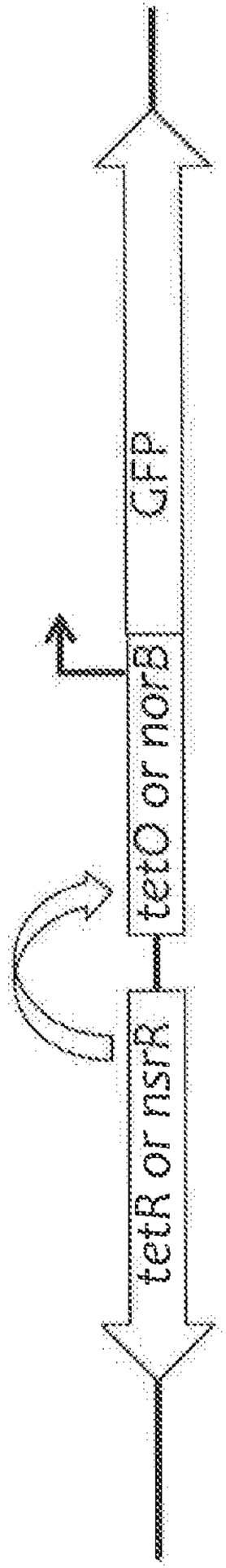



FIG. 63C

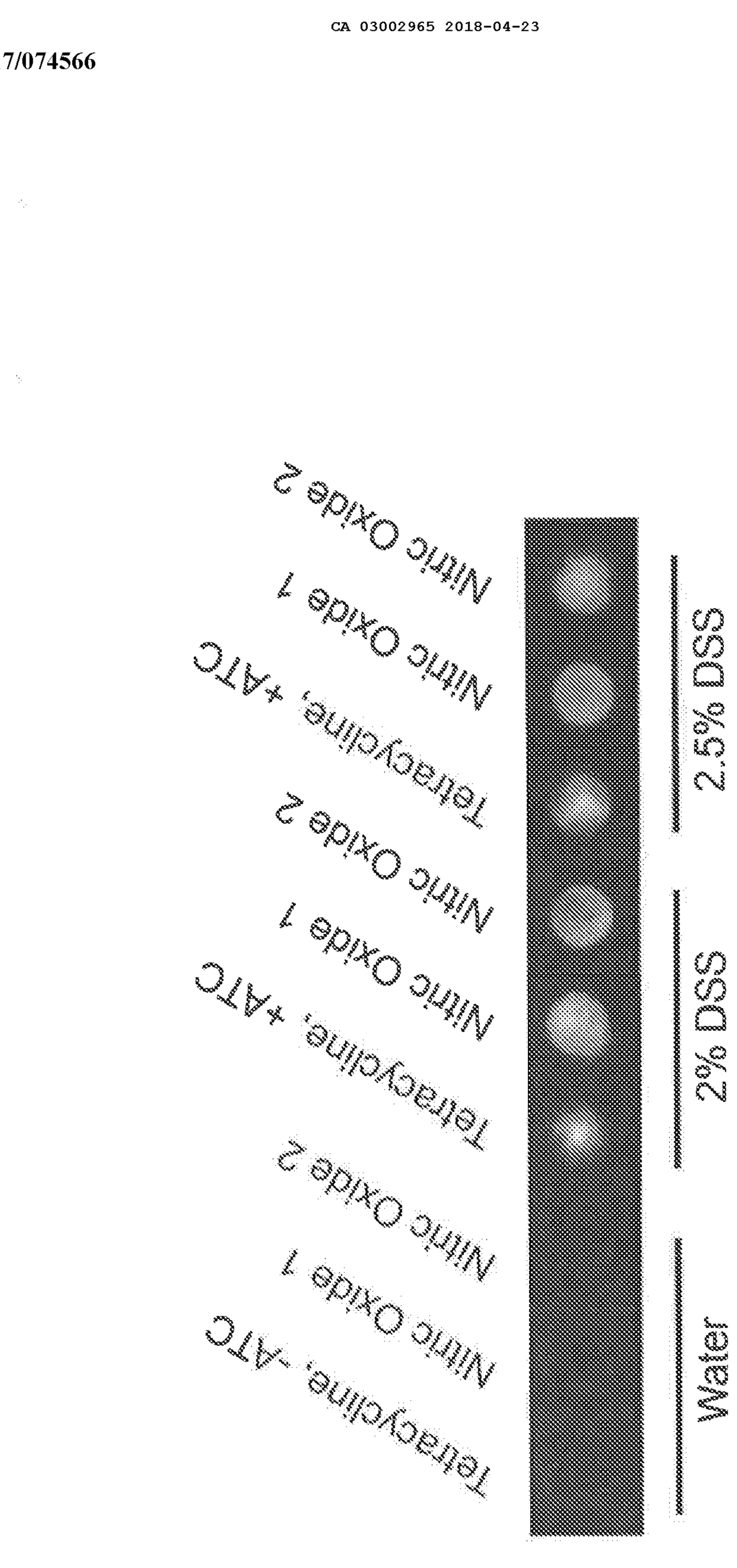

2.5% DSS
2% DSS
Water
Nitric Oxide 2
Nitric Oxide 1
Tetracycline, +ATC
Nitric Oxide 2
Nitric Oxide 1
Tetracycline, +ATC
Nitric Oxide 2
Nitric Oxide 1
Tetracycline, -ATC

FIG. 63D

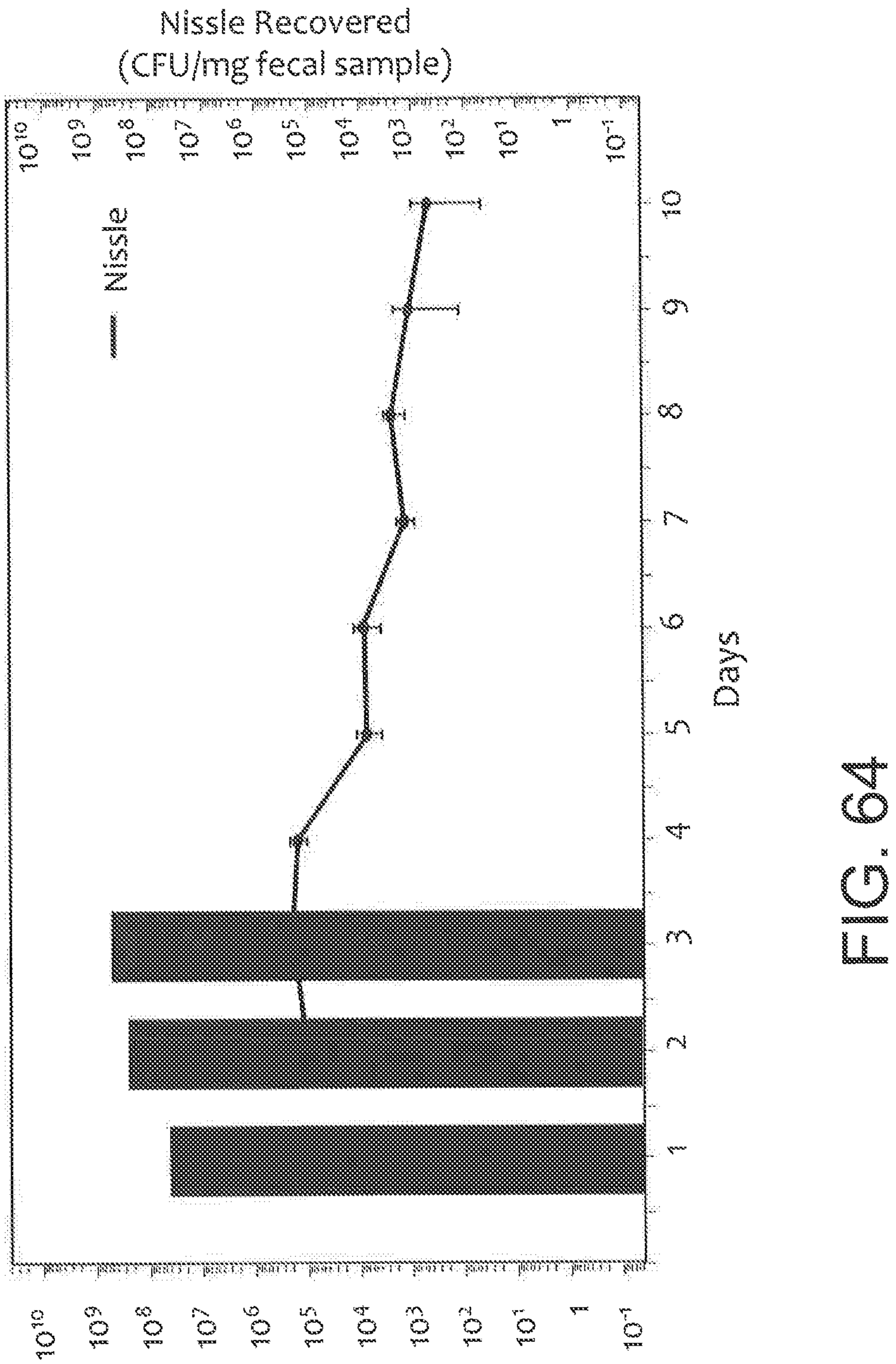

Nissle Recovered
(CFU/mg fecal sample)
$10^{10}$
$10^{9}$
$10^{8}$
$10^{7}$
$10^{6}$
$10^{5}$
$10^{4}$
$10^{3}$
$10^{2}$
$10^{1}$
1
$10^{-1}$
Nissle
0
10
9
8
7
6
5
4
3
2
1
Days
Nissle Administered (CFU/mL)
$10^{10}$
$10^{9}$
$10^{8}$
$10^{7}$
$10^{6}$
$10^{5}$
$10^{4}$
$10^{3}$
$10^{2}$
$10^{1}$
1
$10^{-1}$

FIG. 64

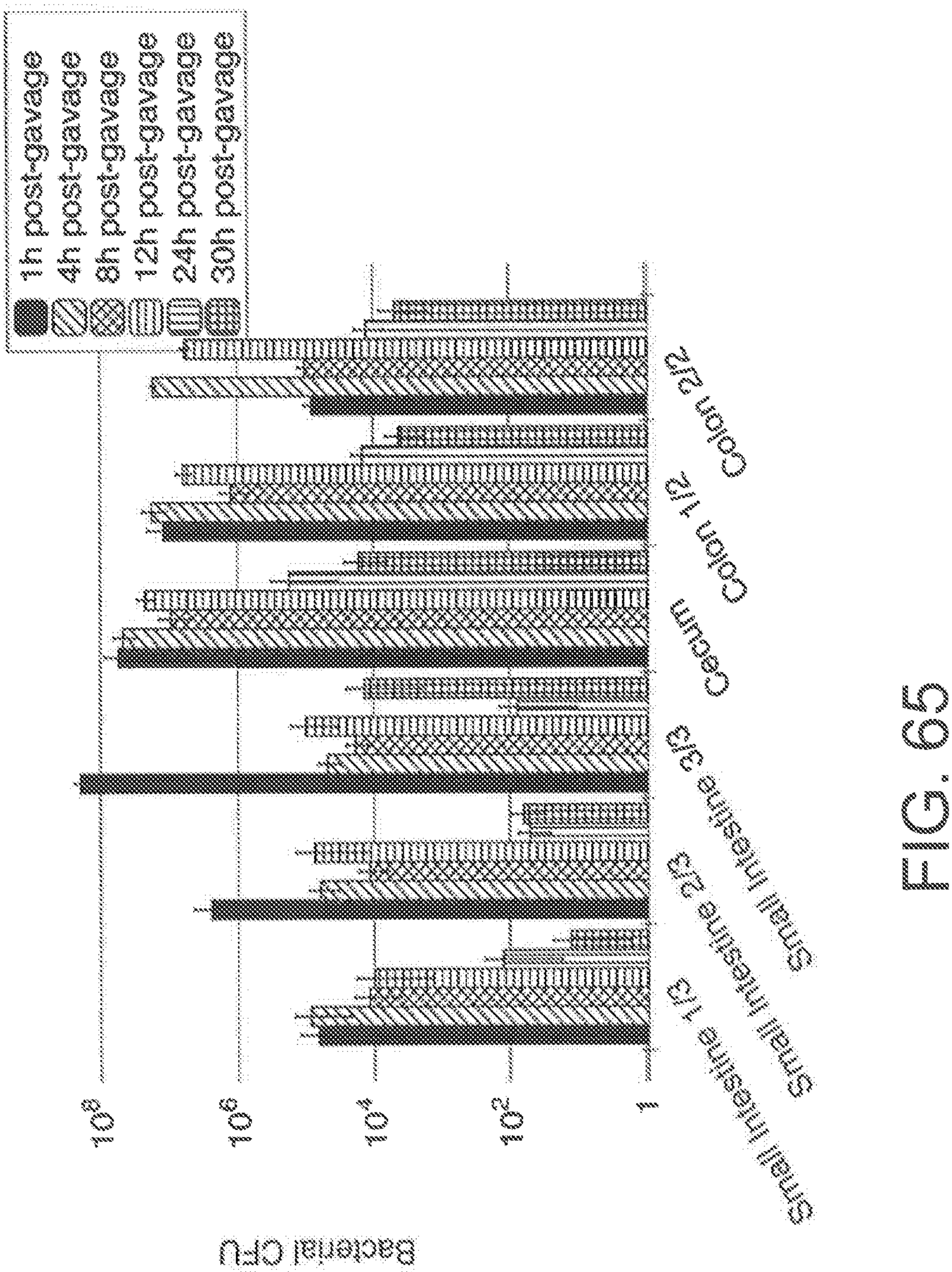

1h post-gavage
4h post-gavage
8h post-gavage
12h post-gavage
24h post-gavage
30h post-gavage
Colon 1/2
Colon 2/2
Cecum
s/c ausgasun neus
s/2 ausgasun neus
s/1 ausgasun neus
Bacterial CFU
10^8
10^6
10^4
10^2
1

FIG. 65




FIG. 66A

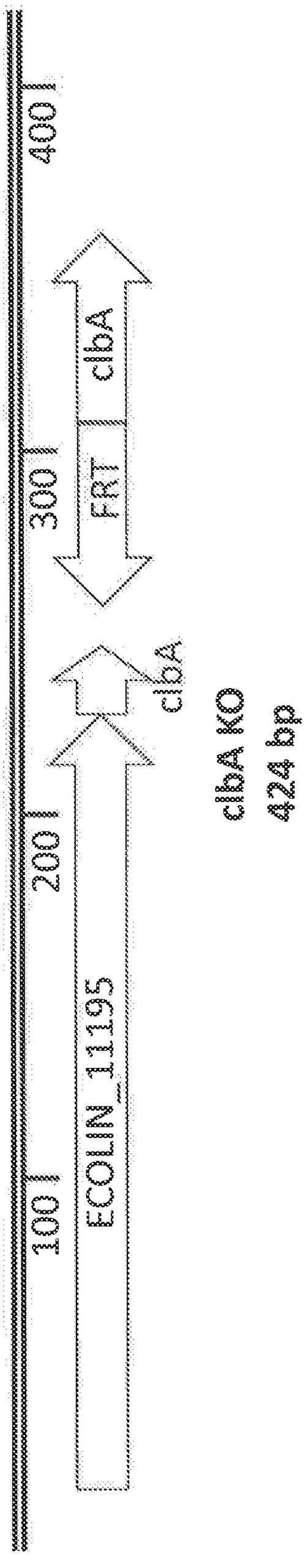



FIG. 66B

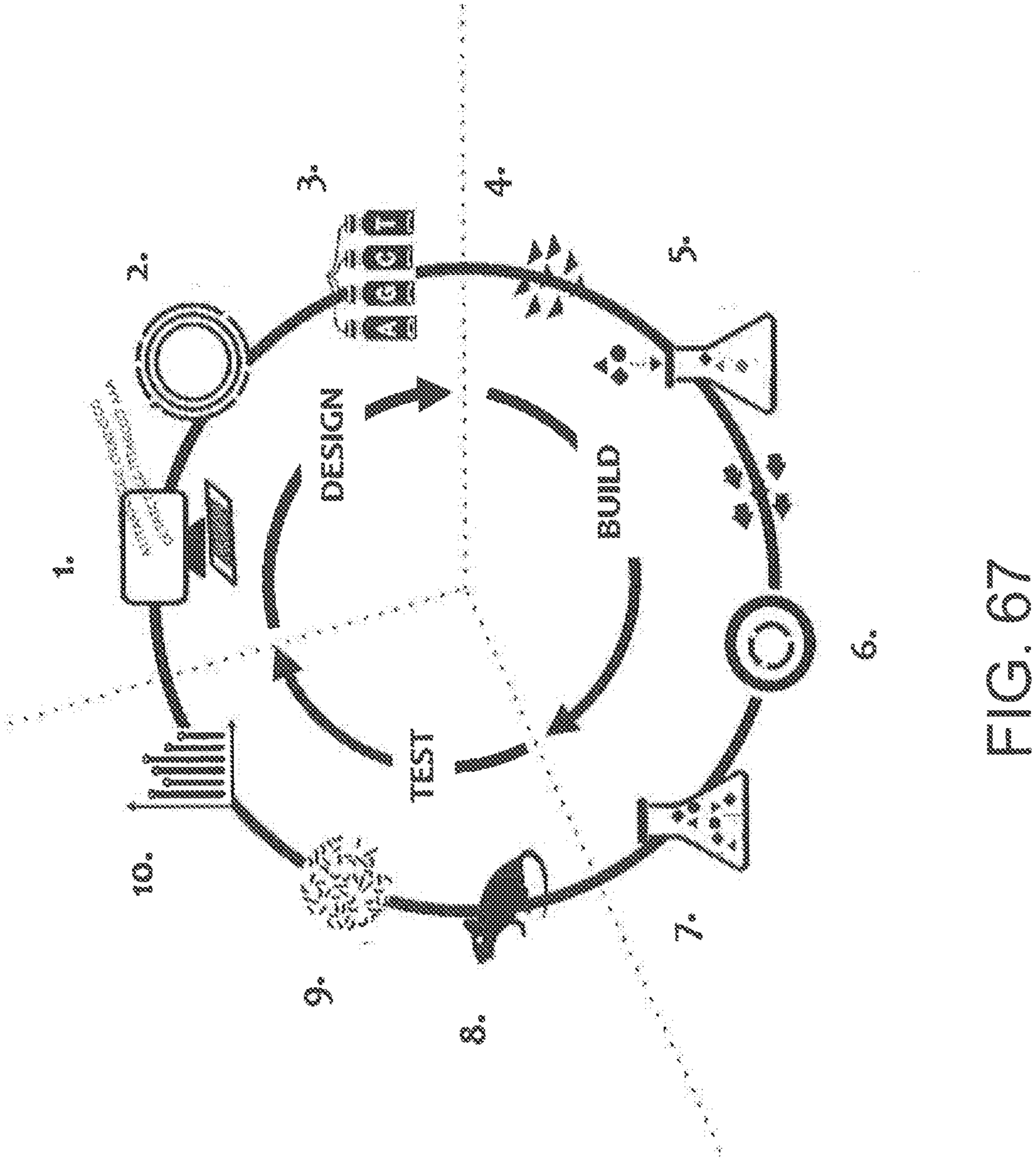



FIG. 67

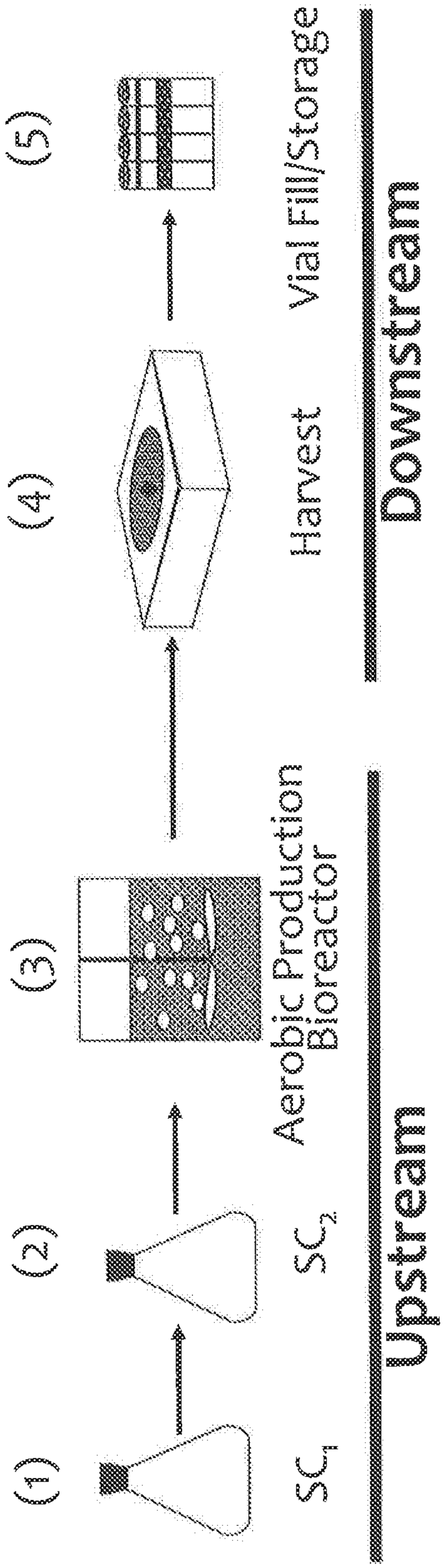

Downstream
Vial Filling/Storage
(5)
Harvest
(4)
Aerobic Production Bioreactor
(3)
$SC_2$
(2)
Upstream
(1)

FIG. 68