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Poulos et al.

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(54) **PRODUCTION OF CANNABINOIDS IN YEAST**

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(22) Filed: **Jul. 9, 2015**

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(51) **Int. Cl.**

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C12N 15/00	(2006.01)
C12N 9/88	(2006.01)
C12N 9/10	(2006.01)
C12N 9/00	(2006.01)
C12N 15/81	(2006.01)

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

CPC ***C12P 7/42*** (2013.01); ***C12N 9/1029*** (2013.01); ***C12N 9/1085*** (2013.01); ***C12N 9/88*** (2013.01); ***C12N 9/93*** (2013.01); ***C12N 15/81*** (2013.01); ***C12Y 203/01206*** (2015.07); ***C12Y 205/01*** (2013.01); ***C12Y 504/99*** (2013.01); ***C12Y 602/01*** (2013.01)

(58) **Field of Classification Search**

CPC C12N 9/93; C12N 9/88; C12N 9/1029; C12N 9/1085; C12N 15/81; C12P 7/42

See application file for complete search history.

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(57) **ABSTRACT**

Exemplary embodiments provided herein include genetically engineering microorganisms, such as yeast or bacteria, to produce cannabinoids by inserting genes that produce the appropriate enzymes for the metabolic production of a desired compound.

3 Claims, 8 Drawing Sheets

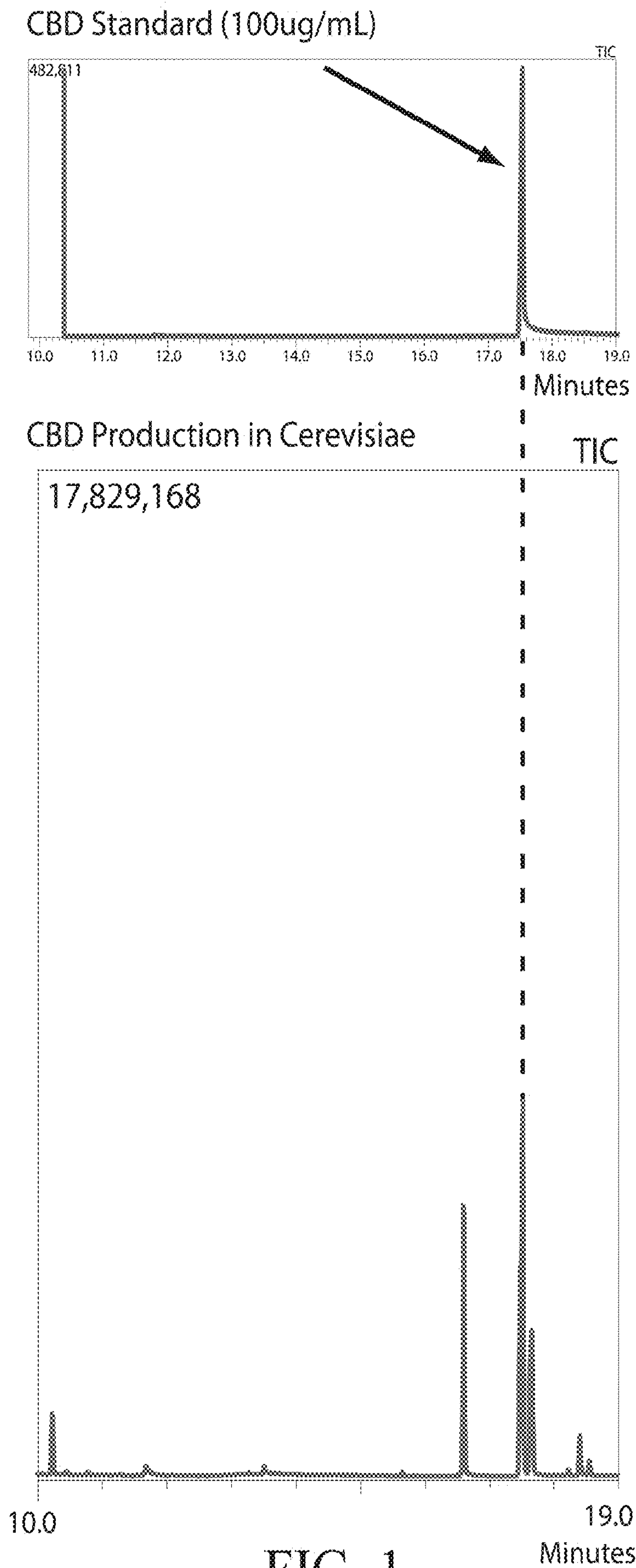


FIG. 1

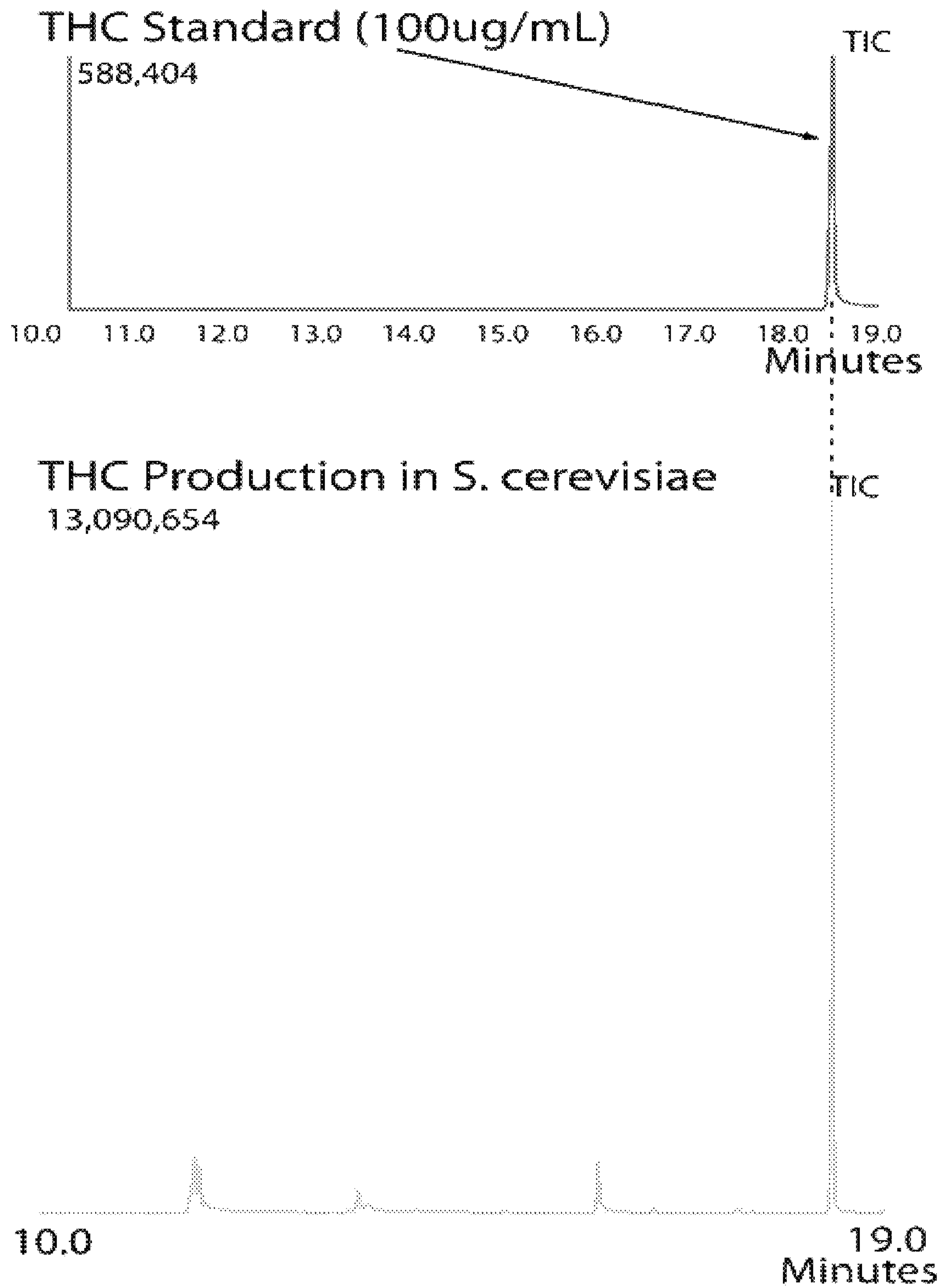


FIG. 2

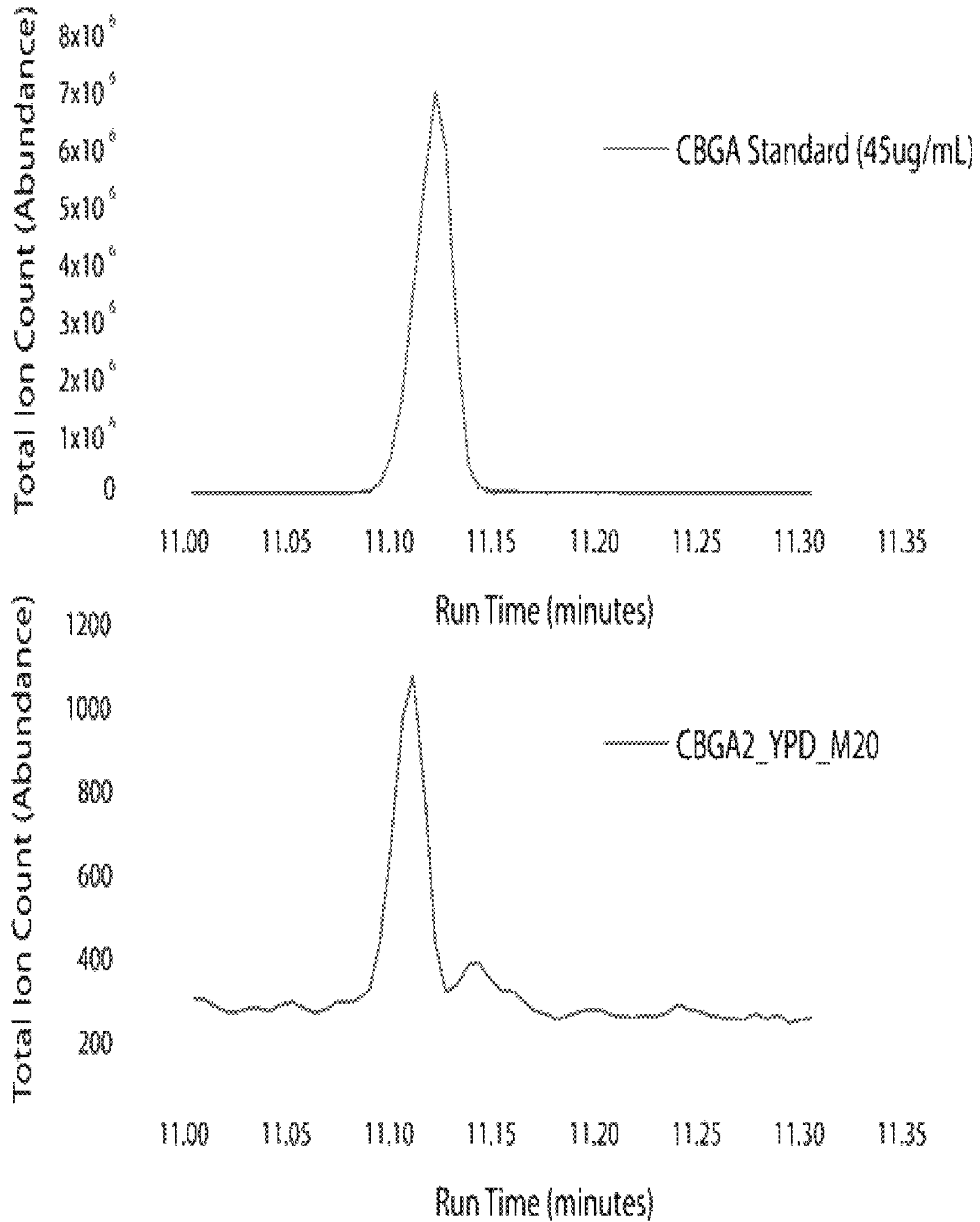


FIG. 3

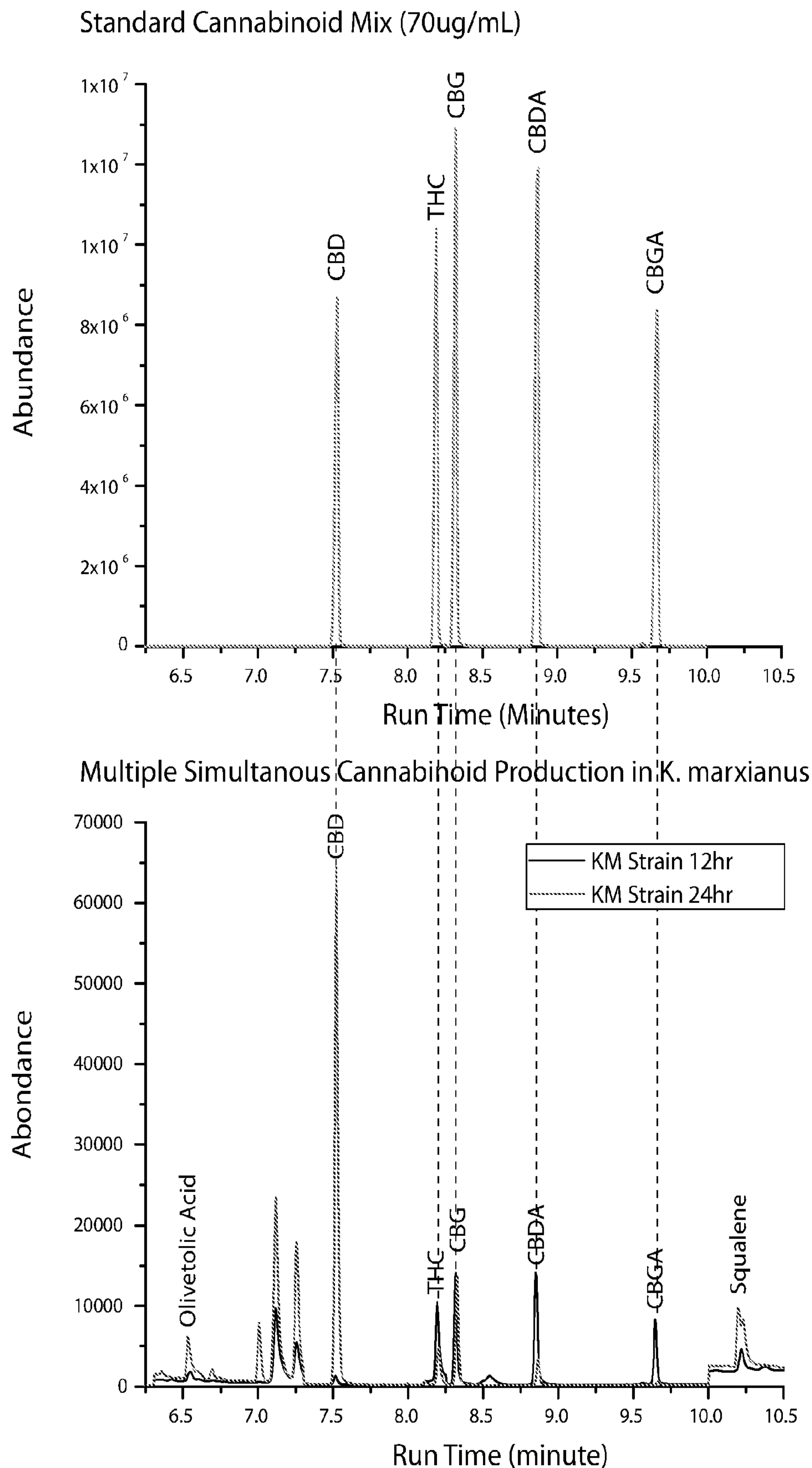


FIG. 4

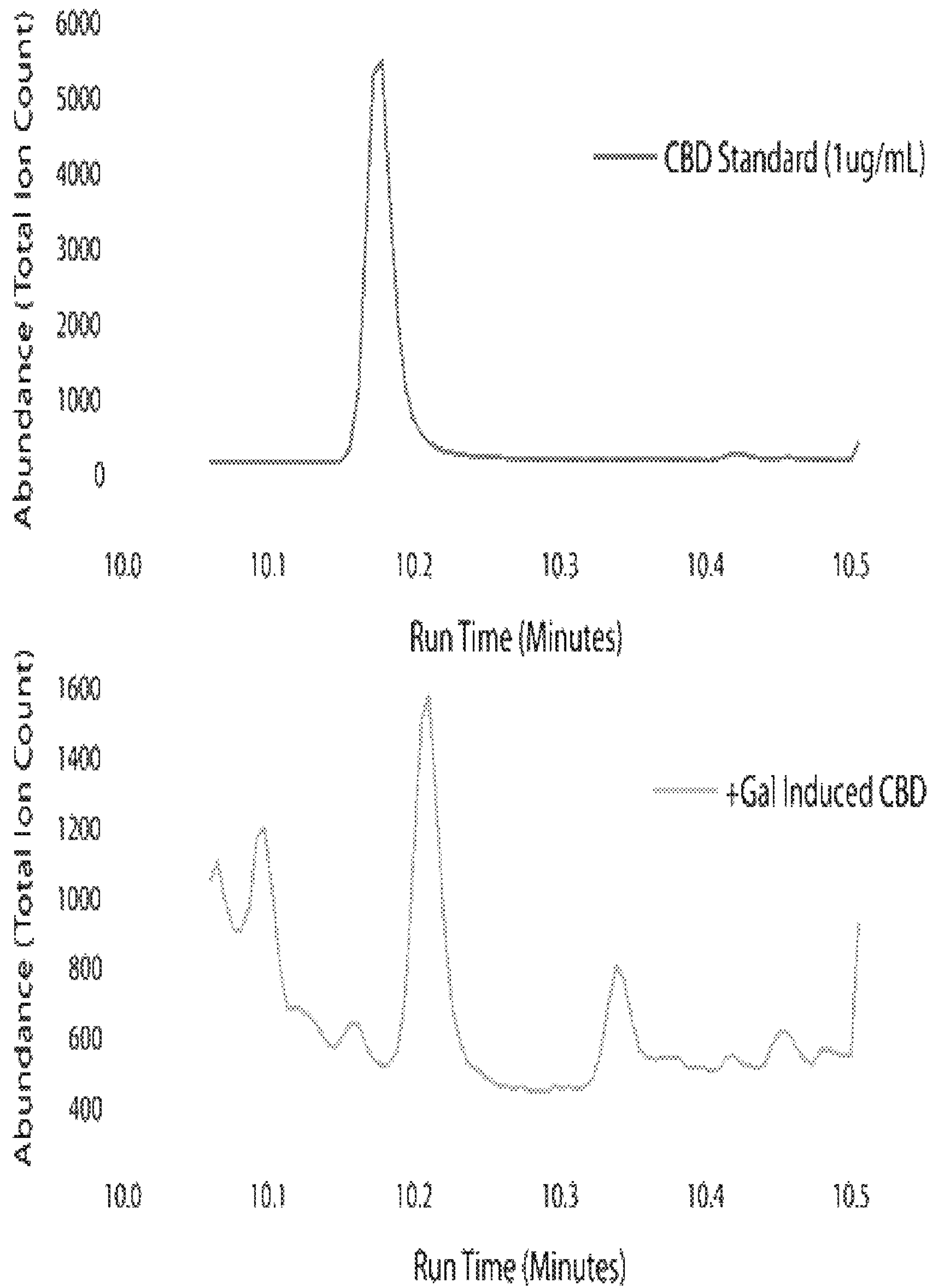
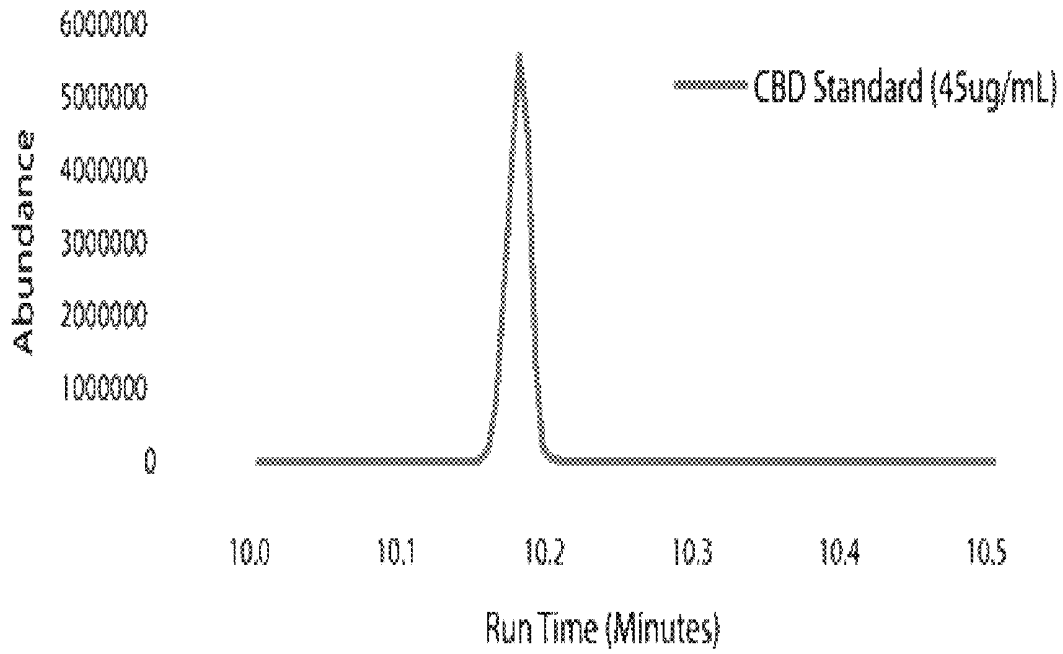


FIG. 5



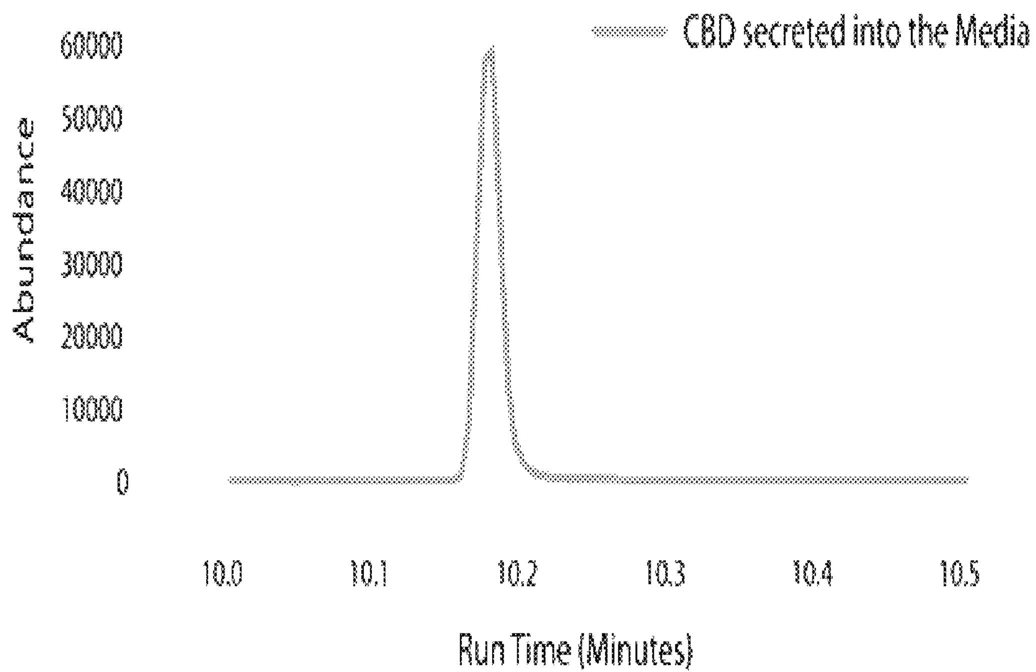


FIG. 6

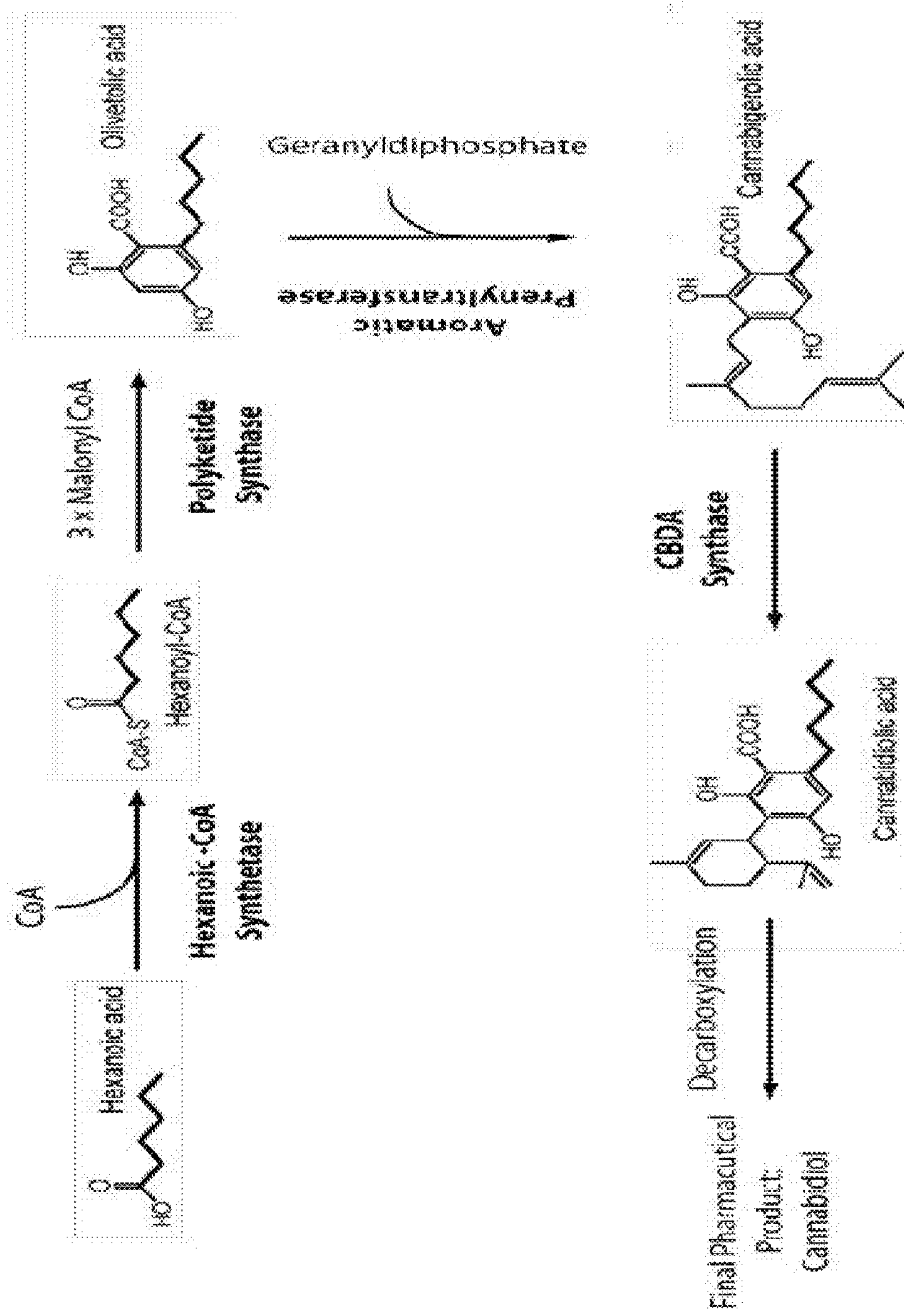


FIG. 7

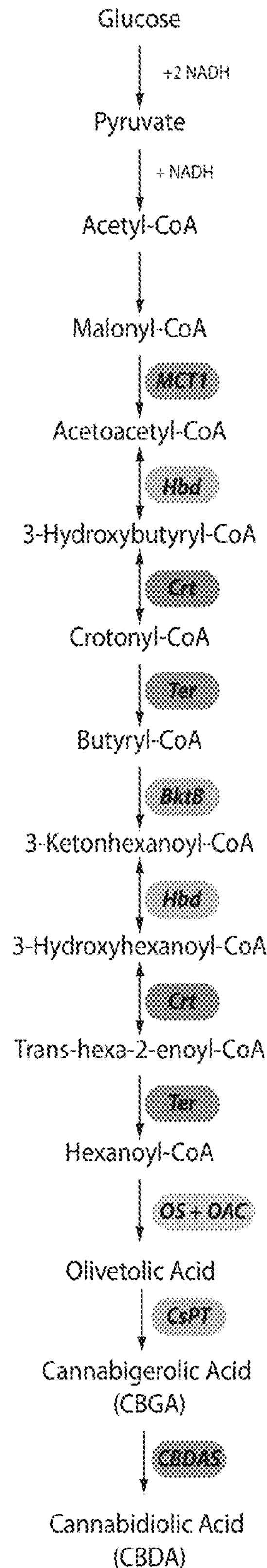


FIG. 8

PRODUCTION OF CANNABINOIDS IN YEAST

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit and priority of U.S. Provisional Patent Application Ser. No. 62/024,099 filed Jul. 14, 2014, titled "Terpenophenolic Production in Microorganisms," which is incorporated by reference herein.

REFERENCE TO SEQUENCE LISTINGS

The present application is filed with sequence listing(s) attached hereto and incorporated by reference, including Appendix 1B titled "Sequence IDs".

FIELD OF THE INVENTION

This invention relates to molecular biology, and more specifically to the transformation of yeast cells and the production of cannabinoids.

SUMMARY OF THE INVENTION

Exemplary embodiments provided herein include genetically engineering microorganisms, such as yeast or bacteria, to produce cannabinoids by inserting genes that produce the appropriate enzymes for the metabolic production of a desired compound.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows exemplary experimental data achieved in connection with Example 1 of Appendix 1A titled "Additional Examples" for cannabidiol ("CBD")/cannabidiolic acid ("CBDA") production in *S. cerevisiae.*

FIG. **2** shows exemplary experimental data achieved in connection with Example 2 of Appendix 1A titled "Additional Examples" for tetrahydrocannabinol ("THC")/tetrahydrocannabinolic acid ("THCA") production in *S. cerevisiae.*

FIG. **3** shows exemplary experimental data achieved in connection with Example 3 of Appendix 1A titled "Additional Examples" for cannabigerol ("CBG")/cannabigerolic acid ("CBGA") production in *S. cerevisiae.*

FIG. **4** shows exemplary experimental data achieved in connection with Example 4 of Appendix 1A titled "Additional Examples" for CBGA, CBDA, CBD, CBG and THC production in *K. marxianus.*

FIG. **5** show exemplary experimental data achieved in connection with Example 5 of Appendix 1A titled "Additional Examples" for galactose induced CBD production in *S. cerevisiae.*

FIG. **6** shows exemplary experimental data achieved in connection with Example 6 of Appendix 1A titled "Additional Examples" for secretion of CBD into media by *S. cerevisiae.*

FIG. **7** shows an exemplary metabolic pathway for the production of cannabinoids by *Cannabis sativa.*

FIG. **8** shows an exemplary biosynthetic route for the production of CBDA from glucose.

DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

The present application relates to the field of cannabinoid production in yeasts. Cannabinoids are a general class of chemicals that act on cannabinoid receptors and other target molecules to modulate a wide range of physiological behavior such as neurotransmitter release. Cannabinoids are produced naturally in humans (called endocannabinoids) and by several plant species (called phytocannabinoids) including *Cannabis sativa*. Cannabinoids have been shown to have several beneficial medical/therapeutic effects and therefore they are an active area of investigation by the pharmaceutical industry for use as pharmaceutical products for various diseases.

Currently the production of cannabinoids for pharmaceutical or other use is done by chemical synthesis or through the extraction of cannabinoids from plants that are producing these cannabinoids, for example *Cannabis sativa*. There are several drawbacks to the current methods of cannabinoid production. The chemical synthesis of various cannabinoids is a costly process when compared to the extraction of cannabinoids from naturally occurring plants. The chemical synthesis of cannabinoids also involves the use of chemicals that are not environmentally friendly, which can be considered as an additional cost to their production. Furthermore, the synthetic chemical production of various cannabinoids has been classified as less pharmacologically active as those extracted from plants such as *Cannabis sativa*. Although there are drawbacks to chemically synthesized cannabinoids, the benefit of this production method is that the end product is a highly pure single cannabinoid. This level of purity is preferred for pharmaceutical use. The level of purity required by the pharmaceutical industry is reflected by the fact that no plant extract based cannabinoid production has received FDA approval yet and only synthetic compounds have been approved.

In contrast to the synthetic chemical production of cannabinoids, the other method that is currently used to produce cannabinoids is production of cannabinoids in plants that naturally produce these chemicals; the most used plant for this is *Cannabis sativa*. In this method, the plant *Cannabis sativa* is cultivated and during the flowering cycle various cannabinoids are produced naturally by the plant. The plant can be harvested and the cannabinoids can be ingested for pharmaceutical purposes in various methods directly from the plant itself or the cannabinoids can be extracted from the plant. There are multiple methods to extract the cannabinoids from the plant *Cannabis sativa*. All of these methods typically involve placing the plant, *Cannabis sativa* that contains the cannabinoids, into a chemical solution that selectively solubilizes the cannabinoids into this solution. There are various chemical solutions used to do this such as hexane, cold water extraction methods, CO2 extraction methods, and others. This chemical solution, now containing all the different cannabinoids, can then be removed, leaving behind the excess plant material. The cannabinoid containing solution can then be further processed for use.

There are several drawbacks of the natural production and extraction of cannabinoids in plants such as *Cannabis sativa*. Since there are numerous cannabinoids produced by *Cannabis sativa* it is often difficult to reproduce identical cannabinoid profiles in plants using an extraction process. Furthermore, variations in plant growth will lead to different levels of cannabinoids in the plant itself making reproducible extraction difficult. Different cannabinoid profiles will have different pharmaceutical effects which are not desired for a pharmaceutical product. Furthermore, the extraction of cannabinoids from *Cannabis sativa* extracts produces a mixture of cannabinoids and not a highly pure single pharmaceutical compound. Since many cannabinoids are similar

in structure it is difficult to purify these mixtures to a high level resulting in cannabinoid contamination of the end product.

Disclosed herein are strategies for creating cannabinoids in microorganisms such as yeast and methods to produce various cannabinoids in yeast from a simple sugar source. The general methods involve genetically engineering yeast to produce various cannabinoids, where the main carbon source available to the yeast is a sugar (glucose, galactose, fructose, sucrose, honey, molasses, raw sugar, etc.). Genetic engineering of the microorganism involves inserting various genes that produce the appropriate enzymes and/or altering the natural metabolic pathway in the microorganism to achieve the production of a desired compound. Through genetic engineering of microorganisms these metabolic pathways can be introduced into these microorganisms and the same metabolic products that are produced in the plant *Cannabis sativa* can be produced by the microorganisms. The benefit of this method is that once the microorganism is produced, the production of the cannabinoid is low cost and reliable, only a specific cannabinoid is produced or a subset is produced, depending on the organism. The purification of the cannabinoid is straight forward since there is only a single cannabinoid or a selected few cannabinoids present in the microorganism. The process is a sustainable process which is more environmentally friendly than synthetic production.

FIG. **1** shows exemplary experimental data achieved in connection with Example 1 of Appendix 1A titled "Additional Examples" for cannabidiol ("CBD")/cannabidiolic acid ("CBDA") production in *S. cerevisiae.*

FIG. **1** shows gas chromatography-mass spectrometry of cannabidiol (CBD) produced in *S. cervisiae*. After processing the yeast cells, as described in Example 1 of Appendix 1A, the whole cell ethyl acetate extract is analyzed for the presences of CBD. The samples were prepared in a way similar to that shown in Appendix A1 except that no MSTFA derivatization was used in this sample (therefore CBDA turns into CBD upon heating), the oven protocol was also slightly different than that shown in Appendix 1A. Initially, a standard CBD solution is run (100 ug/mL; TOP). After running the standard the inventors determined the run time of 17.5 minutes. After running the standard the inventors repeated the GC-MS experiment with their whole cell extract (BOTTOM). At a run time of 17.5 minutes, the inventors saw the same peak as in the standard. Mass spectrometry analysis of the peaks showed identical mass for the two samples (standard and extract) indicating the presence of CBD in their whole cell extract.

FIG. **2** shows exemplary experimental data achieved in connection with Example 2 of Appendix 1A titled "Additional Examples" for tetrahydrocannabinol ("THC")/tetrahydrocannabinolic acid ("THCA") production in *S. cerevisiae.*

FIG. **2** shows gas chromatography-mass spectrometry of tetrahydrocannabinol (THC) produced in *S. cervisiae*. After processing the yeast cells, as described in Example 2 of Appendix 1A, the whole cell ethyl acetate extract was analyzed for the presences of THC. The samples were prepared in a way similar to that shown in Appendix 1A except that no MSTFA derivatization was used in this sample (therefore THCA turns into THC upon heating), the oven protocol was also slightly different than that shown in Appendix 1A. Initially, a standard CBD solution was run (100 ug/mL; TOP). After running the standard the inventors determined the run time of 18.5 minutes. After running the standard the inventors repeated the GC-MS experiment with their whole cell extract (BOTTOM). At a run time of 18.5 minutes, the inventors saw the same peak as in the standard. Mass spectrometry analysis of the peaks showed identical mass for the two samples (standard and extract) indicating the presence of THC in their whole cell extract.

FIG. **3** shows exemplary experimental data achieved in connection with Example 3 of Appendix 1A titled "Additional Examples" for cannabigerol ("CBG")/cannabigerolic acid ("CBGA") production in *S. cerevisiae.*

FIG. **3** shows gas chromatography-mass spectrometry of cannabigerolic acid (CBGA) produced in *S. cervisiae*. After processing the yeast cells, as described in Example 3 of Appendix 1A, the whole cell ethyl acetate extract was analyzed for the presences of CBGA. The samples were prepared in a way as described in Appendix 1A, but the oven protocol was also slightly different than that shown in Appendix 1A. Initially, a standard CBGA solution was run (45 ug/mL; TOP). After running the standard, the inventors determined the run time of 11.1 minutes. After running the standard, the inventors repeated the GC-MS experiment with their whole cell extract (BOTTOM). At a run time of 11.1 minutes, the inventors saw the same peak as in the standard. Mass spectrometry analysis of the peaks showed identical mass for the two samples (standard and extract) indicating the presence of CBGA in their whole cell extract.

FIG. **4** shows exemplary experimental data achieved in connection with Example 4 of Appendix 1A titled "Additional Examples" for CBGA, CBDA, CBD, CBG and THC production in *K. marxianus.*

FIG. **4** shows gas chromatography-mass spectrometry of cannabinoid production (CBGA, CBDA, CBD, CBG, THC) produced in *K. marxianus*. After processing the yeast cells, as described in Example 4 of Appendix 1A, the whole cell ethyl acetate extract was analyzed for the presence of cannabinoids. The samples were prepared in a way as described in Appendix 1A, but the oven protocol was also slightly different than that shown in Appendix 1A. Initially, a standard solution containing CBD, CBG, THC, CBDA, and CBGA was run (70 ug/mL each; TOP). After running the standard, the inventors determined the run time for each compounds. After running the standard, the inventors repeated the GC-MS experiment with their whole cell extract (BOTTOM). At each run time the inventors saw the same peaks as in the standard. Mass spectrometry analysis of the peaks showed identical mass for the two samples (standard and extract) indicating the presence of cannabinoids in their whole cell extract.

FIG. **5** show exemplary experimental data achieved in connection with Example 5 of Appendix 1A titled "Additional Examples" for galactose induced CBD production in *S. cerevisiae.*

FIG. **5** shows gas chromatography-mass spectrometry of induced cannabidiol (CBD) production in *S. cervisiae*. After processing yeast cells, as described in Example 5 of Appendix 1A, the whole cell ethyl acetate extract was analyzed for the presences of CBD. The samples were prepared in a way as described in Appendix 1A. Initially, a standard solution containing CBD was run (1 ug/mL; TOP). After running the standard the inventors determined the run time for CBD as 10.2 minutes. After running the standard, the inventors repeated the GC-MS experiment with their whole cell extract (BOTTOM). At 10.2 minutes, the inventors saw the same peak as in the standard. Mass spectrometry analysis of the peaks showed identical mass for the two samples (standard and extract) indicating the presence of CBD in their whole cell extract.

FIG. **6** shows exemplary experimental data achieved in connection with Example 6 of Appendix 1A titled "Additional Examples" for secretion of CBD into media by *S. cerevisiae*.

FIG. **6** shows gas chromatography-mass spectrometry of induced cannabidiol production (CBD) produced in *S. cervisiae* and secreted into the media. After processing the growth media, as described in Example 6 of Appendix 1A, the media ethyl acetate extract was analyzed for the presence of CBD. The samples were prepared in a way as described in Appendix 1A. Initially, a standard solution containing CBD was run (45 ug/mL; TOP). After running the standard the inventors determined the run time for CBD as 10.2 minutes. After running the standard, the inventors repeated the GC-MS experiment with their whole cell extract (BOTTOM). At 10.2 minutes, the inventors saw the same peak as in the standard. Mass spectrometry analysis of the peaks showed identical mass for the two samples (standard and extract) indicating the presence of CBD in their whole cell extract.

Biosynthetic Production of Cannabidiolic Acid (CBDA) in *S. cerevisiae*.

Through genetic engineering the inventors have reconstituted the cannabidiolic acid (CBDA) metabolic pathway found in *Cannabis sativa* into *S. cerevisiae* (a species of yeast).

Producing CBGA is an initial step in producing many cannabinoids from *Cannabis sativa* in *S. cerevisiae*. Once CBGA is produced a single additional enzymatic step is required to turn CBGA into many other cannabinoids (CBDA, THCA, CBCA, etc.). The acidic forms of the cannabinoids can be used as a pharmaceutical product or the acidic cannabinoids can be turned into their neutral form for use, for example Cannabidiol (CBD) is produced from CBDA through decarboxylation. The resulting cannabinoid products will be used in the pharmaceutical/nutraceutical industry to treat a wide range of health issues.

FIG. **7** shows an exemplary metabolic pathway for the production of cannabinoids by *Cannabis sativa*.

The biosynthetic route for the production of cannabidiolic acid in *Cannabis sativa* is shown in FIG. **7**. The pathway begins with the conversion of Hexanoic acid (a simple fatty acid) to Hexanoyl-CoA by Hexanoyl-CoA Synthetase. Hexanoyl-CoA is converted to Oleviolic acid (OA), a polyketide, by a Polyketide synthase. OA is then prenylated with the monoterpene geranyl diphosphate to cannabigerolic acid by an Aromatic prenyltransferase. Finally, cannabidiolic acid (CBDA) is produced by cyclizing cannabigerolic acid via CBDA synthase (CBDAs). In summary, it takes four enzymatic steps to produce CBDA from Hexanoic acid. The inventors have engineered this metabolic pathway into *S. cerevisiae* (a species of yeast) for the production of CBDA.

There are a few key differences between plant polyketide and terpene biosynthesis when compared to yeast. Yeast does not contain many of the enzymes and fatty acids required for the production of CBDA. Moreover, yeast do not express high levels of geranyl diphosphate (GPP), a chemical required for the production of cannabigerolic acid, the precursor to CBDA. Yet, through genetic engineering many of the required enzymes can be added and the production of GPP can be increased. In order to add the required enzymes for CBDA production in yeast the inventors created plasmids that contain the essential genes in the CBDA biosynthetic pathway. The inventors have transformed these genes into *S. cerevisiae*.

Synthesis of Fusion Genes Required for CBDA Production in *S. cerevisiae*.

The genome of *Cannabis sativa* has been investigated and the acyl-activating enzymes CsAAE1 was determined to convert hexanoic acid to hexanoyl-CoA (Step **1** in FIG. **7**). The inventors have overexpressed CsAAE1 in yeast while simultaneously supplementing the growth media with Hexanoic acid. By supplementing the media with hexanoic acid, the inventors ensured that the yeast have the required starting materials for the production of hexanoyl-CoA.

The next enzymatic step that was engineered into the yeast strain was for the production of Olivetolic acid (OA) from hexanoyl-CoA. This step requires the substrates hexanoyl-CoA and 3 malonyl-CoA molecules, with the malonyl-CoA molecule produced by yeast naturally. Olivetolic acid production requires two enzymes for the condensation and subsequent cyclization of malonyl-CoA with hexanoyl-CoA. This process requires the tetraketide synthase, olivetolic synthase (OS), and the polyketide cyclase, olivetolic acid cyclase (OAC). In some embodiments, stoichiometric amounts of both of these enzymes are preferred; as it has been experimentally determined that OAC binds a chemical intermediate made by OS. In various embodiments, in order to ensure the proper amounts of OS and OAC the inventors have created a single gene that is a fusion of OS, a self cleaving T2A peptide, and the OAC gene (OS-T2A-OAC) and in certain cases an HA tag was inserted at the C-terminus of OAC to verify protein expression. This entire fusion protein was produced in yeast and the self cleaving peptide is spliced in vivo to produce OS and OAC.

The next enzymatic step requires the production of geranyl pyrophosphate (GPP). In yeast the prenyltransferace Erg20 condenses isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAP) to geranyl pyrophosphate (GPP) and feranyl pyrophosphate (FPP) naturally. While only these two products are produced in yeast, a greater quantity of FPP when compared to GPP is produced. More GPP is required for the production of CBDA. In order to increase the production of GPP compared to FPP the inventors inserted a mutant prenyltransferase, Erg20(K179E) in the yeast strain. This mutant has been shown to shift the ratio of GPP:FPP to 70:30. This Erg20(K179E) mutant was placed on a fusion gene with CsAAE1, the enzyme for hexanoyl-CoA, and a self-cleaving peptide, T2A (CsAAE1-T2A-Erg20(K179E). We also added a FLAG tag to the C-terminus of the Erg20p (K197E) enzyme (CsAAE1-T2A-Erg20(K179E)-FLAG) to verify expression of this fusion protein in yeast in certain yeast strains. After production in yeast the self-cleaving peptide was cut producing CsAAE1 and Erg20(K179E).

Once the inventors verified that they had enough GPP to prenylate Olivetolic acid to cannabigerolic acid the inventors inserted the aromatic prenyltransferase (CsPt1) gene into the yeast. In this final enzymatic step the inventors placed the cannabidiolic acid synthase (CBDAs) gene into yeast for the conversion of cannabigerolic acid to CBDA. Similar to the inventors' previous approach, they introduced a single gene containing CsPt1, a self-cleaving peptide T2A, CBDs, and in certain cases a MYC tag was inserted at the C-terminus of CBDs in order to verify production of each enzyme (CsPt1-T2A-CBDs-MYC).

Creation of a Stable Yeast Strain Producing the Metabolic Pathway for CBDA.

Three stable transformations of *S. cerevesaie* where created utilizing selection for leucine, uracil and tryptophan. The inventors first transformed an auxotrophic yeast strain (his3D1/leu2/trp1-289/ura3-52) with the CsAAE1-T2A-

Erg20(K197E)-FLAG gene in an integrating vector. 5 μg of CsAAE1-T2A-Erg20(K197E)-FLAG in a vector containing a gene for tryptophan depletion resistance was linearized with the restriction enzyme EcoRV, transformed into chemically competent InVSc1, and grown on Yeast Nitrogen Base without amino acids and 0.5% ammonium sulfate (YNBA) agar plates supplemented with histidine, leucine, tryptophan, 1% glucose and 2% lactic acid are grown at 30° C. until colonies are formed. Any yeast colonies that did not incorporate the plasmid, that contains the CsAAE1-T2A-Erg20 (K197E)-FLAG gene died since the starting yeast strain is a tryptophan auxotroph. All colonies, with successful plasmid incorporation, where picked and grown in YNBA supplemented with histidine, leucine and uracil, 1% glucose and 2% lactic acid. All colonies were screened for protein production by taking whole cell extracts of each induced clone and the total protein was subjected to SDS-PAGE followed by western blotting against the c-terminal tag of Erg20(K197E). Positive clones where stored at −80° C. in glycerol stocks. The highest expressing clone was taken for the second transformation and was be designated as VscGPHA.

Using the VscGPHA strains the inventors added 5 μg of OS-T2A-OAC-HA in the a vector containing a gene for leucine depletion resistance. This plasmid was linearized with the restriction enzyme AseI and transformed into chemically competent VscGPHA and grown on YNBA agar plates supplemented with histidine and uracil, 1% glucose and 2% lactic acid and grown at 30° C. until colonies were formed. Any yeast colonies that did not incorporate the plasmid that contains the OS-T2A-OAC-HA gene died since the VscGPHA is a leucine auxotroph. All colonies, with successful plasmid incorporation, were picked and grown in YNBA supplemented with histidine, and leucine. All colonies were screened for protein production by taking whole cell extracts of each induced clone and subjected the total protein to SDS-PAGE followed by western blotting against the c-terminal HA tag of OAC. Positive clones were stored at −80° C. in glycerol stocks. The highest expressing clone was taken for the second stable transformation and was designated VscGPHOA.

The final stable transformation was done in a similar way as the previous transformation. The CsPT-T2A-CBDAs-MYC gene was placed in the vector containing a gene for uracil depletion resistance 5μg of this plasmid was linearized with EcorV and transformed into chemically competent VscGPHOA. Transformed VscGPHOA was grown in YNBA supplemented with histidine, 1% glucose and 2% lactic acid. Any yeast colonies that did not incorporate the plasmid that contains the CsPT-T2A-CBDAs-MYC gene died since they lacked leucine. All colonies were picked and grown in YNBA supplemented with histidine, 1% glucose and 2% lactic acid. All colonies were screened for protein production by taking whole cell extracts of each induced clone and subjecting the total protein to SDS-PAGE followed by western blotting against the c-terminal Myc tag of CBDAs. Positive clones are stored at −80° C. in glycerol stocks. The highest expressing CBDAs was taken for the final strain and designated VscCBDA.

Production of CBDA in Yeast.

To initiate the reconstituted metabolic pathway of CBDA a colony of VscCBDA was freshly streaked on a plate of a frozen glycerol stock of VscCBDA. A small culture of VscCBDA was grown in YNBA supplemented with 0.05% histidine, 1% glucose, 2% lactic acid, and 0.03% hexanoic acid overnight at 30° C. The overnight culture was transferred to 1 L of YNBA supplemented with 0.05% histidine, 1% glucose, 2% lactic acid, and 0.03% hexanoic acid and was grown at 30° C. until mid log phase. Cells were pelleted by centrifugation then washed with 200 ml of phosphate buffered saline (PBS) and repelleted. Pelleted cells were resuspended with 1 L of YNBA supplemented with 0.05% histidine, 2% galactose, and 0.03% hexanoic acid and grown at 30° C. overnight.

Overnight 1 L cultures were pelleted by centrifugation, resuspended, washed one time in PBS and pelleted. Cell pellets were resuspended in 40% (wt/vol) KOH and 50% (vol/vol) ethanol solution and boiled for 10 minutes. Metabolite extraction was done by extracting from the boiled extracts 3 times with hexane, then 3 times with ethyl acetate. The spent supernatant broth was extracted in a similar fashion as described above. Organic phases of extracts of each sample were pooled then dried by a rotary evaporator and stored for liquid chromatography mass spectrometry (LC-MS) and gas chromatography mass spectrometry (GC-MS) analysis to confirm and quantitate how much CBDA is produced from strain VscCBDA.

Biosynthetic Production of Cannabidiolic Acid (CBDA) in *K. marxianus*

Through genetic engineering the inventors have reconstituted the cannabidiolic acid (CBDA) metabolic pathway found in *Cannabis sativa* into *K. marxianus* (a species of yeast). Producing CBGA is an initial step in producing many cannabinoids from *Cannabis sativa* in *K. marxianus*. Once CBGA is produced a single additional enzymatic step is required to turn CBGA into many other cannabinoids (CBDA, THCA, CBCA, etc.). The acidic forms of the cannabinoids can be used as a pharmaceutical product or the acidic cannabinoids can be turned into their neutral form for use, for example Cannabidiol (CBD) is produced from CBDA through decarboxylation. The resulting cannabinoid products will be used in the pharmaceutical/nutraceutical industry to treat a wide range of health issues.

Synthesis of Fusion Genes Required for CBDA Production in *k. Marxianus.*

FIG. **8** shows an exemplary biosynthetic route for the production of CBDA from glucose.

The biosynthetic route for the production of cannabidiolic acid in *Cannabis sativa*, from glucose to CBDA is shown in FIG. **8**. The pathway begins with the conversion of glucose to malonyl-CoA through a series of steps that are common to many strains of yeast. The conversion of malonyl-CoA to Acetoacetyl-CoA is conducted by the enzyme MCT1, an acyl-carrier-protein. Acetoacetyl-CoA is converted to 3-Hydroxybutyryl-CoA by the enzyme 3-hydroxybutyryl-CoA dehydrogenase (Hbd) from *Clostridium acetobutylicum*. Next, 3-Hydroxybutyryl-CoA is converted into Crotonyl-CoA by the enzyme crotonase (Crt) from *Clostridium acetobutylicum* and the conversion of Crotonyl-CoA to Butyryl-CoA is controlled by the enzyme trans-enoyl-CoA reducatase (Ter) from *Treponema denticola*. The Butyryl-CoA is converted to 3-Ketonhexanoyl-CoA by the enzyme β-ketothiolase (Bktb) from *Ralstonia Eutropha*. 3-Ketonhexanoyl-CoA is converted to 3-Hydroxyhexanoyl-CoA by the enzyme Hbd. Hydroxyhexanoyl-CoA is converted to Trans-hexa-2-enoyl-CoA by the enzyme Crt. Trans-hexa-2-enoyl-CoA is converted to Hexanoyl-CoA by the enzyme Ter. Hexanoyl-CoA, with 3 malonyl-CoAs, is converted to Oleviolic acid (OA) by a Polyketide synthase and cyclase, OA and OAC respectively. OA is then prenylated with the monoterpene geranyl diphosphate to cannabigerolic acid by an Aromatic prenyltransferase, CsPT. Finally, cannabidiolic acid (CBDA) is produced by cyclizing cannabigerolic acid via CBDA synthase (CBDAS). We have engineered this

metabolic pathway into *K. marxianus* (a species of yeast) for the production of CBDA (FIG. **8**).

There are a few key differences between plant polyketide and terpene biosynthesis when compared to yeast. Yeast does not contain many of the enzymes and fatty acids required for the production of CBDA. Moreover, yeast do not express high levels of geranyl diphosphate (GPP), a chemical required for the production of cannabigerolic acid, the precursor to CBDA. Yet, the inventors through genetic engineering created many of the required enzymes that can be added so the production of GPP was increased. In order to add the required enzymes for CBDA production in yeast the inventors created plasmids that contain the essential genes in the CBDA biosynthetic pathway. The inventors have transformed these genes into *K. marxianus.*

Creation of a Stable *K. marxianus* Strain Producing the Metabolic Pathways for Hexonyl-coA and CBDA.

Two stable transformations of *K. marxianus* were created utilizing selection for uracil and G418 (Geneticin). The inventors first transformed an auxotrophic *K. marxianus* strain (ATCC 17555 KM5) with 5 different genes needed to produce high levels of hexanoyl-CoA. After functional conformation of the genes required for hexanoyl-CoA the inventors did a second transformation with the genes responsible for CBDA production. The molecular biology methods required for biosynthetic production of CBDA in *K. marxianus* are outlined below.

Gene names Crt, Bktb, MCT1, TeR, Hbd, Erg20p(K179E) were codon optimized, synthesized and subclonned into puc57 and p426 ATCC with the restriction enzymes SpeI and SalI.

Genes Crt, Bktb, MCT1, TeR, Hbd, and Erg20p(K179E) were amplified via PCR using the primers GPD_F and URA_R and all 6 amplicons were electroporated into *K. Marxianus* ATCC 17555 KM5 at a concentration of 200 nM and selected onto yeast nitrogen base (YNB) supplemented with amino acid dropout mix (DO supplement-Ura Clonetech 630416) 2% glucose, and 2% Agar plates.

Gene integration and functional gene expression of Crt, Bktb, MCT1, TeR, Hbd, and Erg20p(K179E) was validated by genomic PCR and RT-PCR methods respectively. The final strain produced containing the functional expression of Crt, Bktb, MCT1, TeR, Hbd, and Erg20p(K179E) was labeled kMarxHex1.

Gene names CBDAs, CsPt, OS, and OAC were codon optimized and synthesized by Genscript. The codon optimized gene sequences of CBDAs and CsPt were cloned in frame with a nucleotide sequence containing a self cleaving T2A peptide and designated as CstTCbds. The codon optimized gene sequences of OS and OAC were cloned in frame with a nucleotide sequence containing a self cleaving T2A peptide and designated as OSTOc. CsTCbds and OSTOc were cloned in frame with an *S. cerevisiae* internal ribosomal entry site (IRES), Ure2, into a galactose inducible vector and the final gene sequence pcen/arsGal-OSTOc-IRES-CsTCbds plasmid can be seen below. The plasmid pcen/arsGal-OSTOc-IRES-CstTCbds was used to synthesize a functional gene fragment that expresses the enzymes CBDAs, CsPt, OS, and OAC by using the primers GalIRES_F, GalIRES_R.

The Gibson Assembly method was used to subclone the PCR fragment from [0057] into the plasmid HO-polyKanMx4-HO (ATCC 87804) using the primers KmXIRES_F and KmXIRES_R to create the plasmid pHOOSCstKnMxHO.

The plasmid pHOOSCstKnMxHO was digested with NotI and transformed into kMarxHex1 using standard electroporation methods. The selection of stable integrants was done with yeast nitrogen base (YNB) supplemented with amino acid dropout mix (DO supplement-Ura Clonetech 630416) 2% glucose, lmg/ml G418 (Gibco) and 2% Agar plates.

Gene integration and functional gene expression of pHOOSCstKnMxHO validated by genomic PCR and RT-PCR methods respectively. The final strain produced containing the functional expression of Crt, Bktb, MCT1, TeR, Hbd, Erg20p(K179E) CBDAs, CsPt, OS, and OAC was labeled k.MarxCBDA.

Production of CBDA in *K. marxianus.*

To initiate the reconstituted metabolic pathway of CBDA, a colony from *K. marx* CBDA was freshly streaked onto an agar plate from a frozen glycerol stock of k.MarxCBDA. A small culture of VscCBDA was grown in YNBA base (YNB) supplemented with amino acid dropout mix (DO supplement-Ura Clonetech 630416) 2% glucose, 1 mg/ml G418 (Gibco) and 2% Agar plates was grown overnight at 30° C. The overnight culture was transferred to 1 L of YNBA supplemented with base (YNB) supplemented with amino acid dropout mix (DO supplement-Ura Clonetech 630416) 2% glucose, and 1 mg/ml G418 (Gibco) and was grown at 30° C. until mid log phase. Cells were pelleted by centrifugation then washed with 200 ml of phosphate buffered saline (PBS) and repelleted. Pelleted cells were resuspended with 1 L of YNBA supplemented with base (YNB) supplemented with amino acid dropout mix (DO supplement-Ura Clonetech 630416) 2% galactose, and 1 mg/ml G418 (Gibco) and grown at 30° C. overnight.

Processing CBDA for Analysis of Cannabinoid Production.

Overnight 1 L cultures were pelleted by centrifugation, resuspended, washed one time in PBS and pelleted. The process for extracting cannabinoids from the yeast generally follows the following basic steps:

1. Remove the yeast cells from the media by centrifugation or filtration.
2. Lysis the cells using either chemical or mechanical methods or a combination of methods. Mechanical methods can include a French Press or glass bead milling or other standard methods. Chemical methods can include enzymatic cell lysis, solvent cell lysis, or detergent based cell lysis.
3. Perform a liquid-liquid extraction of the cannabinoids form the cell lysate using the appropriate chemical solvent. An appropriate solvent is any solvent where the cannabinoids are highly soluble in this solvent and the solvent is not miscible in water. Examples of this are hexane, ethyl acetate, and cyclohexane. Preferred solvents can be straight or branched alkane chains (C5-C8) work well; mixtures of these solvents can also be use.

Protocol Used for Cannabinoid Extraction from Yeast Cell Lysate

1. After lysising the cells using any mechanical technique, add 1 mL of 4M KCl, pH2.0 to each 1 mL of cell lysate.
2. Add 1-2 mLs of ethyl acetate for each 1 mL of cell lysate.
3. Rigorously mix for 1 min.
4. Centrifuge the mixture for 5 min at 1000×g.
5. Remove the top ethyl acetate layer. Cannabinoids are present in this layer.
   a. The ethyl acetate can be removed under vacuum if desired.
   b. Cannabinoids can be further purified through liquid chromatography methods if desired.

Protocol Used for Cannabinoid Extraction from Growth Media (for Secreted Cannabinoid Samples)

1. Add 1 mL of ethyl acetate for every 1 mL of growth media.
2. Rigorously mix for 1 min.
3. Centrifuge the mixture for 5 min at 1000×g.
4. Remove the top ethyl acetate layer. Cannabinoids are present in this layer.
   a. The ethyl acetate can be removed under vacuum if desired.
   b. Cannabinoids can be further purified through liquid chromatography methods if desired.

Protocol Used for GC-MS Analysis of Cannabinoid Extracts for k.Marx CBDA

1. Remove solvent from samples under vacuum.
2. Re-suspend dry samples in either 100 uL of dry hexane or dry ethyl acetate
3. Add 20 uL of N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA)
4. Briefly mix
5. Heat solution to 60° C. for 10-15 minutes
6. GC-MS Method
   a. Instrument Agilent 6890-5975 GC-MS (Model Number: Agilent 19091S-433)
   b. Column HP-5MS 5% Phenyl Methyl Siloxane
   c. OVEN:
      i. Initial temp: 100 'C (On) Maximum temp: 300 'C
      ii. Initial time: 3.00 min Equilibration time: 0.50 min
      iii. Ramps:
         # Rate Final temp Final time
         1—30.00 280 1.00
         2—70.00 300 5.00
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tcccttgtag tggttctaac attggtgcag aattgagaga tggtgacata tcttgggatt   1680

actttttaga aagagccaag gagtttaaaa actgcgagtt tactgccaga gaacaacctg   1740

ttgatgctta tactaacatc ttattctcca gtggtactac aggtgaacca aaagcaattc   1800

cttggacaca agccacccca ttgaaggctg ctgctgatgg ttggtctcat ttggatatta   1860

gaaaaggtga cgttatagta tggccaacta atttgggttg gatgatgggt ccttggttgg   1920

tttatgctag tttgttaaat ggtgcatcta ttgccttgta caacggtagt cctttagtct   1980

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gcttttcttc atccggtgaa gcctctaatg tagacgaata tttgtggtta atgggtagag   2160

ctaactacaa gccagttata gaaatgtgtg gtggtacaga aatcggtggt gctttttctg   2220

ctggttcatt tttgcaagct caatctttaa gttctttttc atcccaatgt atgggttgca   2280

ccttgtacat attagataag aacggttacc caatgcctaa aaataagcca ggtatcggtg   2340

aattggcatt aggtcctgtt atgtttggtg cctcaaaaac attgttaaac ggtaatcatc   2400

acgatgtcta tttcaagggt atgccaacct tgaatggtga agtattgaga agacatggtg   2460

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aattggtcat atttttcgta ttgaaggatt caaacgacac aaccattgat ttgaaccaat   2700

tgagattatc ctttaacttg ggtttgcaaa agaaattgaa cccattattc aaagttacta   2760

gagttgtccc attgtcatcc ttacctagaa ctgcaacaaa caagatcatg agaagagttt   2820

tgagacaaca attcagtcat ttcgaagaag gtagaggttc tttgttaaca tgtggtgacg   2880

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acgtattccc aaagttagtt gaagaattga acgctagttt gttagcttat ggtatgccta   3000

aagaagcctg cgattggtat gctcactctt taaactacaa tactccaggt ggtaaattga   3060

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taggtcaaga agaatacgaa aaggtcgcta tcttgggttg gtgtattgaa ttgttgcaag   3180

catacttttt ggttgccgat gacatgatgg ataagtctat aacaagaaga ggtcaaccat   3240

gctggtacaa agttccagaa gttggtgaaa tagccataaa tgatgctttt atgttggaag   3300

ccgctatcta taaattgttg aagtcacatt tcagaaacga aaagtactac atcgatatta   3360

ccgaattatt ccacgaagtt actttccaaa cagaattggg tcaattgatg gatttgataa   3420

ctgcacctga agataaagtt gacttgtcaa agttttcctt gaagaaacat tcattcatcg   3480

tcacctttga aactgcttat tactccttct atttgccagt cgccttggct atgtacgtag   3540

ctggtattac tgatgaaaaa gacttgaagc aagcaagaga tgttttgata cctttgggtg   3600

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aaatcggtac agatattcaa gacaataagt gcagttgggt tattaacaag gctttggaat   3720

tagcatctgc cgaacaaaga aagactttgg atgaaaacta cggtaaaaag gactcagttg   3780
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ctgaagcaaa gtgtaagaaa atttttaatg atttgaagat tgaacaattg taccatgaat   3840

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gttttaaagc cgacgttttg acagctttct tgaataaggt ctacaagaga tcaaaggatt   3960

acaaggatca tgacggtgac tataaagacc acgatattga ctacaaagat gacgatgaca   4020

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cacatccgct ctaaccgaaa aggaaggagt tagacaacct gaagtctagg tccctattta   4140

tttttttata gttatgttag tattaagaac gttatttata tttcaaattt ttcttttttt   4200

tctgtacaga cgcgtgtacg catgtaacat tatactgaaa accttgcttg agaaggtttt   4260

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cc                                                                  4322
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<210> SEQ ID NO 7
<211> LENGTH: 2522
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 7

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ctgctgtaac ccgtacatgc caaaataggg ggcgggttac acagaatata taacactgat    180

ggtgcttggg tgaacaggtt tattcctggc atccactaaa tataatggag cccgcttttt    240

aagctggcat ccagaaaaaa aaagaatccc agcaccaaaa tattgttttc ttcaccaacc    300

atcagttcat aggtccattc tcttagcgca actacagaga acagggcaca aacaggcaaa    360

aaacgggcac aacctcaatg gagtgatgca acctgcctgg agtaaatgat gacacaaggc    420

aattgaccca cgcatgtatc tatctcattt tcttacacct tctattacct tctgctctct    480

ctgatttgga aaaagctgaa aaaaaaggtt taaaccagtt ccctgaaatt attcccctac    540

ttgactaata agtatataaa gacggtaggt attgattgta attctgtaaa tctatttctt    600

aaacttctta aattctactt ttatagttag tctttttttt agttttaaaa caccaagaac    660

ttagtttcga cggatctaga actagtatga accatttgag agccgaaggt cctgcctccg    720

tattagccat aggtacagcc aacccagaaa acatattgat ccaagatgaa tttcctgatt    780

attacttcag agttaccaag agtgaacaca tgactcaatt gaaggaaaag tttagaaaaa    840

tatgtgataa gtctatgatc agaaagagaa actgcttctt gaacgaagaa catttgaagc    900

aaaatccaag attggtagaa cacgaaatgc aaacattgga tgccagacaa gacatgttag    960

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aaccaaagtc aaagatcact catttgattt ttacaagtgc atctactaca gatatgcctg   1080

gtgcagacta ccactgtgcc aaattgttag gtttgtcacc atccgttaag agagtcatga   1140

tgtatcaatt aggttgctac ggtggtggta ctgttttgag aatcgctaag gatattgcag   1200

aaaacaacaa gggtgccaga gtattagctg tttgttgcga cattatggct tgcttgttta   1260

gaggtccaag tgattctgac ttggaattgt tagttggtca agctatcttc ggtgacggtg   1320

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aattagtcag tacaggtcaa accatcttgc ctaattctga aggtacaatt ggtggtcata   1440

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acatagaaaa gtgtttgata gaagcattca ctcctatagg tatctcagat tggaactcta   1560
ttttctggat aacacatcca ggtggtaaag ccattttgga taaggttgaa gaaaaattgg   1620
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aatctactac tggtgacggt tttgaatggg gtgtcttatt tggtttcggt cctggtttga   1800
ccgtcgaaag agtagttgtc agatcagtac caattaaata tgaaggtaga ggttccttgt   1860
taacttgtgg tgacgttgaa gaaaacccag gtcctatggc cgtcaagcat ttgatagtat   1920
tgaagtttaa agatgaaatc acagaagctc aaaaggaaga atttttcaag acctacgtta   1980
atttggtcaa cattatacct gctatgaaag atgtatactg gggtaaagac gttacacaaa   2040
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cataagtcga cctcgagtca tgtaattagt tatgtcacgc ttacattcac gccctccccc   2280
cacatccgct ctaaccgaaa aggaaggagt tagacaacct gaagtctagg tccctattta   2340
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tctgtacaga cgcgtgtacg catgtaacat tatactgaaa accttgcttg agaaggtttt   2460
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cc                                                                  2522
```

<210> SEQ ID NO 8
<211> LENGTH: 3887
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 8

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ctgctgtaac ccgtacatgc caaaataggg ggcgggttac acagaatata taacactgat    180
ggtgcttggg tgaacaggtt tattcctggc atccactaaa tataatggag cccgcttttt    240
aagctggcat ccagaaaaaa aaagaatccc agcaccaaaa tattgttttc ttcaccaacc    300
atcagttcat aggtccattc tcttagcgca actacagaga acagggcaca aacaggcaaa    360
aaacgggcac aacctcaatg gagtgatgca acctgcctgg agtaaatgat gacacaaggc    420
aattgaccca cgcatgtatc tatctcattt tcttacacct tctattacct tctgctctct    480
ctgatttgga aaaagctgaa aaaaaaggtt taaaccagtt ccctgaaatt attcccctac    540
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aaacttctta aattctactt ttatagttag tctttttttt agttttaaaa caccaagaac    660
ttagtttcga cggatctaga actagtatgg gtttatcatc cgtctgtact ttctccttcc    720
aaactaacta tcatacctta ttgaatcctc acaacaacaa tccaaaaaca tcattgttgt    780
gttacagaca tccaaagaca cctattaagt actcttacaa caactttcca tcaaaacatt    840
gttcaaccaa gtccttccac ttacaaaata agtgctccga aagtttgtct atagctaaga    900
actctatcag agctgcaact acaaatcaaa ctgaaccacc tgaaagtgat aatcactctg    960
```

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ttgccacaaa aattttgaac ttcggtaaag catgttggaa gttgcaaaga ccatacacca   1020
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acttaatttc atggtccttg atgttcaagg catttttctt tttagttgcc atcttgtgca   1140
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aaccagattt gcctttggct tcaggtgaaa tatccgtcaa tactgcatgg atcatgtcta   1260
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cctctgacgt tgaaggtgac acaaagttcg gtattagtac cttggcttct aagtacggtt   1620
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tcaaaaagat aatggaaatc catgaattag ttaaattggt caataagtgg caaaacatcg   2760
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ataaccaagg taaaaataag actgctatcc acacatactt ttcttcagtt ttcttgggtg   2880
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catatttgaa ctacagagat ttggacatcg gtattaacga tccaaagaat cctaacaact   3420

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agtcatgtaa ttagttatgt cacgcttaca ttcacgccct ccccccacat ccgctctaac   3660

cgaaaaggaa ggagttagac aacctgaagt ctaggtccct atttattttt ttatagttat   3720

gttagtatta agaacgttat ttatatttca aatttttctt ttttttctgt acagacgcgt   3780

gtacgcatgt aacattatac tgaaaacctt gcttgagaag gttttgggac gctcgaaggc   3840

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<210> SEQ ID NO 9
<211> LENGTH: 8963
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 9

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tgagcacgtg agtatacgtg attaagcaca caaaggcagc ttggagtatg tctgttatta    480

atttcacagg tagttctggt ccattggtga aagtttgcgg cttgcagagc acagaggccg    540

cagaatgtgc tctagattcc gatgctgact tgctgggtat tatatgtgtg cccaatagaa    600

agagaacaat tgacccggtt attgcaagga aaatttcaag tcttgtaaaa gcatataaaa    660

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gagtttaaaa actgcgagtt tactgccaga gaacaacctg ttgatgctta tactaacatc   1080

ttattctcca gtggtactac aggtgaacca aaagcaattc cttggacaca agccacccca   1140

ttgaaggctg ctgctgatgg ttggtctcat ttggatatta gaaaaggtga cgttatagta   1200

tggccaacta atttgggttg gatgatgggt ccttggttgg tttatgctag tttgttaaat   1260

ggtgcatcta ttgccttgta caacggtagt cctttagtct ctggtttcgc taaatttgtt   1320

caagatgcaa aggtcacaat gttgggtgtc gtaccatcta ttgtaagatc atggaaatcc   1380

acaaattgtg tttcaggtta cgattggtcc accataagat gcttttcttc atccggtgaa   1440

gcctctaatg tagacgaata tttgtggtta atgggtagag ctaactacaa gccagttata   1500

gaaatgtgtg gtggtacaga aatcggtggt gctttttctg ctggttcatt tttgcaagct   1560

caatctttaa gttctttttc atcccaatgt atgggttgca ccttgtacat attagataag   1620

aacggttacc caatgcctaa aaataagcca ggtatcggtg aattggcatt aggtcctgtt   1680

atgtttggtg cctcaaaaac attgttaaac ggtaatcatc acgatgtcta tttcaagggt   1740

atgccaacct tgaatggtga agtattgaga agacatggtg acattttcga attgacctct   1800

aacggttact accatgcaca cggtagagcc gatgacacta tgaacatcgg tggtatcaaa   1860

attagttcta tcgaaatcga aagagtctgt aatgaagtag atgacagagt tttgaaaacc   1920

actgctattg gtgttccacc tttgggtggt ggtccagaac aattggtcat atttttcgta   1980

ttgaaggatt caaacgacac aaccattgat ttgaaccaat tgagattatc ctttaacttg   2040

ggtttgcaaa agaaattgaa cccattattc aaagttacta gagttgtccc attgtcatcc   2100

ttacctagaa ctgcaacaaa caagatcatg agaagagttt tgagacaaca attcagtcat   2160

ttcgaagaag gtagaggttc tttgttaaca tgtggtgacg ttgaagaaaa tccaggtcct   2220

atggcttcag aaaaggaaat aagaagagaa agattcttga acgtattccc aaagttagtt   2280

gaagaattga acgctagttt gttagcttat ggtatgccta aagaagcctg cgattggtat   2340

gctcactctt taaactacaa tactccaggt ggtaaattga atagaggttt gagtgtagtt   2400

gatacttatg ctatcttgtc taacaaaacc gttgaacaat taggtcaaga agaatacgaa   2460

aaggtcgcta tcttgggttg gtgtattgaa ttgttgcaag catacttttt ggttgccgat   2520
```

```
gacatgatgg ataagtctat aacaagaaga ggtcaaccat gctggtacaa agttccagaa   2580

gttggtgaaa tagccataaa tgatgctttt atgttggaag ccgctatcta taaattgttg   2640

aagtcacatt tcagaaacga aaagtactac atcgatatta ccgaattatt ccacgaagtt   2700

actttccaaa cagaattggg tcaattgatg gatttgataa ctgcacctga agataaagtt   2760

gacttgtcaa agttttcctt gaagaaacat tcattcatcg tcacctttga aactgcttat   2820

tactccttct atttgccagt cgccttggct atgtacgtag ctggtattac tgatgaaaaa   2880

gacttgaagc aagcaagaga tgttttgata cctttgggtg aatacttcca aatccaagat   2940

gactacttag actgtttcgg tactccagaa caaataggta aaatcggtac agatattcaa   3000

gacaataagt gcagttgggt tattaacaag gctttggaat tagcatctgc cgaacaaaga   3060

aagactttgg atgaaaacta cggtaaaaag gactcagttg ctgaagcaaa gtgtaagaaa   3120

atttttaatg atttgaagat tgaacaattg taccatgaat acgaagaatc catcgctaaa   3180

gacttaaagg caaagattag tcaagttgat gaatcaagag gttttaaagc cgacgttttg   3240

acagctttct tgaataaggt ctacaagaga tcaaaggatt acaaggatca tgacggtgac   3300

tataaagacc acgatattga ctacaaagat gacgatgaca agtaagcggc cgc          3353
```

```
<210> SEQ ID NO 15
<211> LENGTH: 1553
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 15

actagtatga accatttgag agccgaaggt cctgcctccg tattagccat aggtacagcc     60

aacccagaaa acatattgat ccaagatgaa tttcctgatt attacttcag agttaccaag    120

agtgaacaca tgactcaatt gaaggaaaag tttagaaaaa tatgtgataa gtctatgatc    180

agaaagagaa actgcttctt gaacgaagaa catttgaagc aaaatccaag attggtagaa    240

cacgaaatgc aaacattgga tgccagacaa gacatgttag ttgtcgaagt tcctaaattg    300

ggtaaagatg cttgtgcaaa agccattaag gaatggggtc aaccaaagtc aaagatcact    360

catttgattt ttacaagtgc atctactaca gatatgcctg gtgcagacta ccactgtgcc    420

aaattgttag gtttgtcacc atccgttaag agagtcatga tgtatcaatt aggttgctac    480

ggtggtggta ctgttttgag aatcgctaag gatattgcag aaaacaacaa gggtgccaga    540

gtattagctg tttgttgcga cattatggct tgcttgttta gaggtccaag tgattctgac    600

ttggaattgt tagttggtca agctatcttc ggtgacggtg ctgctgctgt tattgttggt    660

gcagaacctg acgaatctgt tggtgaaaga ccaatatttg aattagtcag tacaggtcaa    720

accatcttgc ctaattctga aggtacaatt ggtggtcata taagagaagc aggtttgatc    780

ttcgatttgc acaaagacgt tccaatgtta atctctaaca acatagaaaa gtgtttgata    840

gaagcattca ctcctatagg tatctcagat tggaactcta ttttctggat aacacatcca    900

ggtggtaaag ccattttgga taaggttgaa gaaaaattgg atttgaagaa agaaaagttt    960

gtagatagta gacatgtttt atctgaacac ggtaacatgt cttcatccac tgtcttgttc   1020

gtaatggatg aattgagaaa gagatcatta gaagagggta aatctactac tggtgacggt   1080

tttgaatggg gtgtcttatt tggtttcggt cctggtttga ccgtcgaaag agtagttgtc   1140

agatcagtac caattaaata tgaaggtaga ggttccttgt taacttgtgg tgacgttgaa   1200
```

```
gaaaacccag gtcctatggc cgtcaagcat ttgatagtat tgaagtttaa agatgaaatc   1260

acagaagctc aaaaggaaga atttttcaag acctacgtta atttggtcaa cattatacct   1320

gctatgaaag atgtatactg ggtaaagac gttacacaaa agaaagaaga aggttataca    1380

cacattgtcg aagtaacctt cgaatcagtt gaaactatcc aagattacat cattcatcca   1440

gctcacgttg gttttggtga cgtttacaga tccttctggg aaaaattgtt gatcttcgat   1500

tacaccccaa gaaagtaccc ttacgatgtt ccagactatg cataagcggc cgc          1553
```

<210> SEQ ID NO 16
<211> LENGTH: 2918
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 16

```
actagtatgg gtttatcatc cgtctgtact ttctccttcc aaactaacta tcatacctta     60

ttgaatcctc acaacaacaa tccaaaaaca tcattgttgt gttacagaca tccaaagaca    120

cctattaagt actcttacaa caactttcca tcaaaacatt gttcaaccaa gtccttccac    180

ttacaaaata agtgctccga aagtttgtct atagctaaga actctatcag agctgcaact    240

acaaatcaaa ctgaaccacc tgaaagtgat aatcactctg ttgccacaaa aattttgaac    300

ttcggtaaag catgttggaa gttgcaaaga ccatacacca taatcgcttt tacttcttgt    360

gcatgcggtt tattcggtaa agaattgttg cataacacta acttaatttc atggtccttg    420

atgttcaagg cattttcctt tttagttgcc atcttgtgca tcgcttcatt caccactaca    480

attaatcaaa tatacgattt gcacatcgac agaattaaca aaccagattt gcctttggct    540

tcaggtgaaa tatccgtcaa tactgcatgg atcatgtcta tcatagtagc cttgttcggt    600

ttgatcatca caattaaaat gaagggtggt ccattgtaca tcttcggtta ctgtttcggt    660

atcttcggtg gtattgtcta ttccgtacca ccttttagat ggaaacaaaa ccctagtact    720

gcctttttgt tgaatttctt agctcatatc atcacaaact tcaccttcta ctacgcttca    780

agagctgctt taggtttgcc attcgaattg agaccttcat tcacattttt gttggcattc    840

atgaaaagta tgggttctgc attagccttg atcaaggatg cctctgacgt tgaaggtgac    900

acaaagttcg gtattagtac cttggcttct aagtacggtt caagaaattt gactttgttc    960

tgctccggta tcgttttgtt aagttacgtc gcagccattt tggcaggtat catttggcca   1020

caagccttta attctaacgt tatgttgttg tcacatgcca tcttggcttt ctggttgatc   1080

ttgcaaacta gagatttcgc tttgacaaat tatgaccctg aagcaggtag aagattctac   1140

gagtttatgt ggaaattgta ctacgctgaa tatttggtat acgtttttat tgaaggtaga   1200

ggttctttgt tgacctgtgg tgacgttgaa gaaaatccag gtcctatgaa atgttcaact   1260

ttctcctttt ggttcgtatg caagatcatc ttcttttcct ttttccttta catccaaaca   1320

agtatcgcaa acccaagaga aaacttttg aagtgcttct cacaatacat acctaataac    1380

gccaccaatt tgaagttggt ttacactcaa aacaacccat tgtacatgtc cgtcttgaac   1440

agtacaatcc ataatttgag attcacttct gataccactc caaaaccttt ggtcattgta   1500

acccctagtc atgtatctca catccaaggt actatcttat gttctaaaaa ggttggtttg   1560

caaattagaa ctagatccgg tggtcatgat agtgaaggca tgtcatacat ctcccaagtt   1620

ccattcgtta tcgttgattt gagaaacatg agatcaatta aaatagacgt acactcacaa   1680

actgcttggg ttgaagctgg tgcaacattg ggtgaagtat actactgggt taacgaaaag   1740
```

```
aatgaaaact tatcattggc tgctggttac tgtccaacag tttgcgcagg tggtcatttt   1800

ggtggtggtg gttatggtcc tttaatgaga aactacggtt tggccgctga taacataatc   1860

gacgctcatt tggtaaatgt tcacggtaaa gttttggata gaaagtctat gggtgaagac   1920

ttattttggg ctttgagagg tggtggtgca gaatcattcg gtatcatagt tgcttggaag   1980

ataagattag tcgcagtacc aaagtctact atgttctcag tcaaaaagat aatggaaatc   2040

catgaattag ttaaattggt caataagtgg caaaacatcg catacaagta cgataaggac   2100

ttgttgttga tgactcattt catcacaaga aacatcaccg ataaccaagg taaaaataag   2160

actgctatcc acacatactt ttcttcagtt ttcttgggtg gtgtcgattc cttagtagac   2220

ttgatgaata agtcttttcc agaattaggt attaagaaaa ctgattgtag acaattgtct   2280

tggatcgaca ccatcatctt ttattcaggt gttgtcaact acgatacaga caacttcaac   2340

aaagaaatat tattggatag atccgcaggt caaaacggtg cctttaaaat taagttagac   2400

tacgttaaaa agccaatacc tgaatcagtt ttcgtccaaa tcttagaaaa attgtacgaa   2460

gaagatattg gtgcaggcat gtacgccttg tatccatacg gtggtataat ggacgaaatc   2520

agtgaatctg ccattccatt tcctcataga gctggtatct tatacgaatt gtggtacatt   2580

tgttcatggg aaaagcaaga agataacgaa aagcacttaa actggattag aaacatctat   2640

aacttcatga ctccatacgt ttctaaaaac cctagattgg catatttgaa ctacagagat   2700

ttggacatcg gtattaacga tccaaagaat cctaacaact atacccaagc tagaatttgg   2760

ggtgaaaaat acttcggtaa aaatttcgat agattagtaa aggttaagac attggttgac   2820

ccaaacaact tctttagaaa cgaacaatcc attccacctt tacctagaca tagacacgaa   2880

caaaaattaa taagtgaaga agatttgtaa gcggccgc                           2918
```

<210> SEQ ID NO 17
<211> LENGTH: 167
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 17

```
agccaaaata atgataacga gaataatatc aagaatacct tagaacaaca tcgacaacaa     60

caacaggcat tttcggatat gagtcacgtg gagtattcca gaattacaaa attttttcaa    120

gaacaaccac tggagggata tacccttttc tctcacaggt ctgcgcc                  167
```

<210> SEQ ID NO 18
<211> LENGTH: 1518
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 18

```
atggtttcca atcacttgtt tgacgcaatg agagccgctg cccctggtaa cgcccctttc     60

ataagaatag ataatactag aacttggaca tacgatgacg cctttgcttt atctggtaga    120

atagcatcag ctatggatgc tttgggtatc agaccaggtg acagagtcgc agttcaagta    180

gaaaaatccg ctgaagcatt gatcttgtat ttggcttgtt tgagaagtgg tgcagtttat    240

ttgccattga atactgccta cacattagct gaattggatt acttcatagg tgacgcagaa    300

cctagattgg ttgtagtcgc ctcttcagcc agagctggtg tagaaacaat tgctaaacca    360
```

```
agaggtgcaa tagtcgaaac cttagatgct gctggttctg gtagtttgtt agatttggcc    420

agagacgaac ctgctgattt tgttgacgct tcaagatcag ccgatgactt agccgctatt    480

ttgtacacct ctggtactac aggtagatca aagggtgcta tgttgactca tggtaatttg    540

ttgtcaaacg cattaacctt gagagatttc tggagagtta ctgccggtga cagattaatc    600

cacgctttgc caatttttca tactcacggt ttattcgttg ctaccaacgt aactttgtta    660

gcaggtgcct ccatgttctt gttgagtaag ttcgatccag aagaaatatt atctttgatg    720

cctcaagcta ctatgttgat gggtgtccca acattctacg ttagattgtt acaatcacct    780

agattagata agcaagctgt tgcaaacatc agattgttta tatccggtag tgctccattg    840

ttagcagaaa cccatactga atttcaagca agaacaggtc acgccatttt agaaagatac    900

ggtatgacag aaaccaatat gaacacttct aacccttatg aaggtaaaag aatagctggt    960

acagttggtt ttccattgcc tgatgtcaca gttagagtaa ccgacccagc cactggttta   1020

gctttgccac ctgaacaaac tggtatgatc gaaattaaag gtccaaacgt ttttaagggt   1080

tactggagaa tgcctgaaaa gactgctgct gagtttactg ctgatggttt ctttatctct   1140

ggtgacttag gtaaaattga tagagacggt tatgtccata ttgttggtcg tggtaaagat   1200

ttggttatat ccggtggtta taacatctac cctaaggaag tagaaggtga aatagatcaa   1260

atcgaaggtg ttgtagaatc agctgtaata ggtgtcccac atcctgattt tggtgaaggt   1320

gttacagcag tcgttgtaag aaaaccaggt gctgcattag atgaaaaggc aattgtttct   1380

gccttacaag acagattggc tagatacaag caaccaaaga gaataatctt cgcagaagat   1440

ttgcctagaa atactatggg taaagtacaa aagaacatct tgagacaaca atacgccgac   1500

ttatacacca gaacctga                                                 1518


<210> SEQ ID NO 19
<211> LENGTH: 2092
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 19

actagtatgg gtttatcatc cgtctgtact ttctccttcc aaactaacta tcatacctta     60

ttgaatcctc acaacaacaa tccaaaaaca tcattgttgt gttacagaca tccaaagaca    120

cctattaagt actcttacaa caactttcca tcaaaacatt gttcaaccaa gtccttccac    180

ttacaaaata agtgctccga aagtttgtct atagctaaga actctatcag agctgcaact    240

acaaatcaaa ctgaaccacc tgaaagtgat aatcactctg ttgccacaaa aattttgaac    300

ttcggtaaag catgttggaa gttgcaaaga ccatacacca taatcgcttt tacttcttgt    360

gcatgcggtt tattcggtaa agaattgttg cataacacta acttaatttc atggtcctat    420

gaactgctcc gcattctctt tctggttcgt ctgtaaaata atcttcttct tcttgtcctt    480

caacatccaa atctccatcg caaatccaca agaaaacttt ttgaagtgtt tctccgaata    540

catcccaaac aaccctgcta acccaaagtt tatatatact caacatgatc aattgtacat    600

gtccgttttg aacagtacca tccaaaattt gagattcact tctgacacta caccaaaacc    660

tttagtcatt gttacacctt ccaatgttag tcacattcaa gcttctatat tgtgctctaa    720

gaaagtaggt ttgcaaatca gaactagatc aggtggtcat gatgcagaag gcatgtctta    780

catctcacaa gttccattcg ttgtagtcga tttgagaaat atgcattcca taaagatcga    840

cgttcacagt caaacagcat gggtagaagc aggtgccacc ttgggtgaag tttactactg    900
```

```
gatcaacgaa aagaatgaaa acttttcttt ccctggtggt tactgtccaa cagtaggtgt    960

cggtggtcac ttttctggtg gtggttatgg tgcattgatg agaaactacg gtttagctgc   1020

agataatatt atagacgccc atttggttaa cgtagatggt aaagttttgg acagaaagtc   1080

tatgggtgaa gatttgtttt gggccataag aggtggtggt ggtgaaaatt tcggtatcat   1140

tgccgcttgg aaaattaagt tagtcgctgt tccttccaaa agtactattt tctctgtcaa   1200

aaagaacatg gaaatccacg gtttggttaa gttgtttaat aagtggcaaa acatcgctta   1260

caagtacgat aaggacttgg ttttgatgac ccatttcatc actaaaaata ttacagataa   1320

ccatggtaaa aataagacca ctgttcacgg ttatttttct tcaattttcc atggtggtgt   1380

agattctttg gttgatttga tgaataagtc attcccagaa ttgggtatta aaaagacaga   1440

ttgcaaggaa ttttcttgga tagacacaac catcttctat tcaggtgttg taaacttcaa   1500

caccgctaac ttcaaaaagg aaatcttgtt ggatagatcc gctggtaaaa agaccgcttt   1560

ttctattaaa ttggactacg ttaagaaacc aatccctgaa actgcaatgg tcaagatatt   1620

ggaaaagttg tacgaagaag atgtaggtgt cggcatgtac gttttgtatc catacggtgg   1680

tattatggaa gaaatatctg aatcagccat accatttcct cacagagctg gtatcatgta   1740

tgaattatgg tacacagcct catgggaaaa gcaagaagat aacgaaaagc atatcaactg   1800

ggtcagatcc gtttacaact tcactacacc ttacgttagt caaaacccaa gattggcata   1860

tttgaactac agagatttgg acttaggtaa aactaaccct gaatctccaa ataactatac   1920

acaagcaaga atttggggtg aaaagtactt tggtaaaaat ttcaacagat tagttaaagt   1980

aaagactaaa gccgacccta acaacttttt cagaaacgaa caatccatcc cacctttgcc   2040

acctcaccac cacgaacaaa aattaataag tgaagaagat ttgtaagtcg ac           2092
```

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<210> SEQ ID NO 20
<211> LENGTH: 7904
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 20
```

```
tcgcgcgttt cggtgatgac ggtgaaaacc tctgacacat gcagctcccg gagacggtca     60

cagcttgtct gtaagcggat gccgggagca gacaagcccg tcagggcgcg tcagcgggtg    120

ttggcgggtg tcggggctgg cttaactatg cggcatcaga gcagattgta ctgagagtgc    180

accatatcga ctacgtcgta aggccgtttc tgacagagta aaattcttga gggaactttc    240

accattatgg gaaatggttc aagaaggtat tgacttaaac tccatcaaat ggtcaggtca    300

ttgagtgttt tttatttgtt gtattttttt ttttttagag aaaatcctcc aatatcaaat    360

taggaatcgt agtttcatga ttttctgtta cacctaactt tttgtgtggt gccctcctcc    420

ttgtcaatat taatgttaaa gtgcaattct ttttccttat cacgttgagc cattagtatc    480

aatttgctta cctgtattcc tttactatcc tcctttttct ccttcttgat aaatgtatgt    540

agattgcgta tatagtttcg tctaccctat gaacatattc cattttgtaa tttcgtgtcg    600

tttctattat gaatttcatt tataaagttt atgtacaaat atcataaaaa aagagaatct    660

ttttaagcaa ggattttctt aacttcttcg gcgacagcat caccgacttc ggtggtactg    720

ttggaaccac ctaaatcacc agttctgata cctgcatcca aaaccttttt aactgcatct    780

tcaatggcct taccttcttc aggcaagttc aatgacaatt tcaacatcat tgcagcagac    840
```

-continued

```
aagatagtgg cgatagggtc aaccttattc tttggcaaat ctggagcaga accgtggcat    900
ggttcgtaca aaccaaatgc ggtgttcttg tctggcaaag aggccaagga cgcagatggc    960
aacaaaccca aggaacctgg gataacggag gcttcatcgg agatgatatc accaaacatg   1020
ttgctggtga ttataatacc atttaggtgg gttgggttct taactaggat catggcggca   1080
gaatcaatca attgatgttg aaccttcaat gtagggaatt cgttcttgat ggtttcctcc   1140
acagtttttc tccataatct tgaagaggcc aaaacattag ctttatccaa ggaccaaata   1200
ggcaatggtg gctcatgttg tagggccatg aaagcggcca ttcttgtgat tctttgcact   1260
tctggaacgg tgtattgttc actatcccaa gcgacaccat caccatcgtc ttcctttctc   1320
ttaccaaagt aaatacctcc cactaattct ctgacaacaa cgaagtcagt acctttagca   1380
aattgtggct tgattggaga taagtctaaa agagagtcgg atgcaaagtt acatggtctt   1440
aagttggcgt acaattgaag ttctttacgg atttttagta aaccttgttc aggtctaaca   1500
ctaccggtac cccatttagg accacccaca gcacctaaca aaacggcatc aaccttcttg   1560
gaggcttcca gcgcctcatc tggaagtggg acacctgtag catcgatagc agcaccacca   1620
attaaatgat tttcgaaatc gaacttgaca ttggaacgaa catcagaaat agctttaaga   1680
accttaatgg cttcggctgt gatttcttga ccaacgtggt cacctggcaa aacgacgatc   1740
ttcttagggg cagacatagg ggcagacatt agaatggtat atccttgaaa tatatatata   1800
tattgctgaa atgtaaaagg taagaaaagt tagaaagtaa gacgattgct aaccacctat   1860
tggaaaaaac aataggtcct taaataatat tgtcaacttc aagtattgtg atgcaagcat   1920
ttagtcatga acgcttctct attctatatg aaaagccggt tccggcctct cacctttcct   1980
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<210> SEQ ID NO 21
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 21

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<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

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aactgcacag aacaaaaacc tgcaggaaac gaagataaat catgtcgaaa gctacatata 480

aggaacgtgc tgctactcat cctagtcctg ttgctgccaa gctatttaat atcatgcacg 540

aaaagcaaac aaacttgtgt gcttcattgg atgttcgtac caccaaggaa ttactggagt 600

tagttgaagc attaggtccc aaaatttgtt tactaaaaac acatgtggat atcttgactg 660

atttttccat ggagggcaca gttaagccgc taaaggcatt atccgccaag tacaattttt 720

tactcttcga agacagaaaa tttgctgaca ttggtaatac agtcaaattg cagtactctg 780

cgggtgtata cagaatagca gaatgggcag acattacgaa tgcacacggt gtggtgggcc 840

caggtattgt tagcggtttg aagcaggcgg cagaagaagt aacaaaggaa cctagaggcc 900

ttttgatgtt agcagaattg tcatgcaagg gctccctatc tactggagaa tatactaagg 960

gtactgttga cattgcgaag agcgacaaag attttgttat cggctttatt gctcaaagag 1020

acatgggtgg aagagatgaa ggttacgatt ggttgattat gacacccggt gtgggtttag 1080

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gatctgacat tattattgtt ggaagaggac tatttgcaaa gggaagggat gctaaggtag 1200

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cgcgtaacca ccacacccgc cgcgcttaat gcgccgctac agggcgcgtc gcgccattcg 1860

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acgtaacttt gttagcaggt gcctccatgt tcttgttgag taagttcgat ccagaagaaa   3240
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tgaatagagg tttgagtgta gttgatactt atgctatctt gtctaacaaa accgttgaac   4320
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aagcatactt tttggttgcc gatgacatga tggataagtc tataacaaga agaggtcaac   4440
catgctggta caaagttcca gaagttggtg aaatagccat aaatgatgct tttatgttgg   4500
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aagccgctat ctataaattg ttgaagtcac atttcagaaa cgaaaagtac tacatcgata   4560

ttaccgaatt attccacgaa gttactttcc aaacagaatt gggtcaattg atggatttga   4620

taactgcacc tgaagataaa gttgacttgt caaagttttc cttgaagaaa cattcattca   4680

tcgtcacctt tgaaactgct tattactcct tctatttgcc agtcgccttg gctatgtacg   4740

tagctggtat tactgatgaa aaagacttga agcaagcaag agatgttttg atacctttgg   4800

gtgaatactt ccaaatccaa gatgactact tagactgttt cggtactcca gaacaaatag   4860

gtaaaatcgg tacagatatt caagacaata agtgcagttg ggttattaac aaggctttgg   4920

aattagcatc tgccgaacaa agaaagactt tggatgaaaa ctacggtaaa aaggactcag   4980

ttgctgaagc aaagtgtaag aaaattttta atgatttgaa gattgaacaa ttgtaccatg   5040

aatacgaaga atccatcgct aaagacttaa aggcaaagat tagtcaagtt gatgaatcaa   5100

gaggttttaa agccgacgtt ttgacagctt tcttgaataa ggtctacaag agatcaaagt   5160

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gtcatagctg tttcctgtgt gaaattgtta tccgctcaca attccacaca acataggagc   5580

cggaagcata aagtgtaaag cctggggtgc ctaatgagtg aggtaactca cattaattgc   5640

gttgcgctca ctgcccgctt tccagtcggg aaacctgtcg tgccagctgc attaatgaat   5700

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aatacggtta tccacagaat caggggataa cgcaggaaag aacatgtgag caaaaggcca   5880

gcaaaaggcc aggaaccgta aaaaggccgc gttgctggcg tttttccata ggctcggccc   5940

ccctgacgag catcacaaaa atcgacgctc aagtcagagg tggcgaaacc cgacaggact   6000

ataaagatac caggcgttcc ccccctggaag ctccctcgtg cgctctcctg ttccgaccct   6060

gccgcttacc ggatacctgt ccgcctttct cccttcggga agcgtggcgc tttctcaatg   6120

ctcacgctgt aggtatctca gttcggtgta ggtcgttcgc tccaagctgg gctgtgtgca   6180

cgaacccccc gttcagcccg accgctgcgc cttatccggt aactatcgtc ttgagtccaa   6240

cccggtaaga cacgacttat cgccactggc agcagccact ggtaacagga ttagcagagc   6300

gaggtatgta ggcggtgcta cagagttctt gaagtggtgg cctaactacg gctacactag   6360

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tagctcttga tccggcaaac aaaccaccgc tggtagcggt ggtttttttg tttgcaagca   6480

gcagattacg cgcagaaaaa aaggatctca agaagatcct ttgatctttt ctacggggtc   6540

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gatcttcacc tagatccttt taaattaaaa atgaagtttt aaatcaatct aaagtatata   6660

tgagtaaact tggtctgaca gttaccaatg cttaatcagt gaggcaccta tctcagcgat   6720

ctgtctattt cgttcatcca tagttgcctg actgcccgtc gtgtagataa ctacgatacg   6780

ggagggctta ccatctggcc ccagtgctgc aatgataccg cgagacccac gctcaccggc   6840

tccagattta tcagcaataa accagccagc cggaagggcc gagcgcagaa gtggtcctgc   6900
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aactttatcc gcctccatcc agtctattaa ttgttgccgg gaagctagag taagtagttc   6960

gccagttaat agtttgcgca acgttgttgc cattgctaca ggcatcgtgg tgtcacgctc   7020

gtcgtttggt atggcttcat tcagctccgg ttcccaacga tcaaggcgag ttacatgatc   7080

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gtgtatgcgg cgaccgagtt gctcttgccc ggcgtcaata cgggataata ccgcgccaca   7320

tagcagaact ttaaaagtgc tcatcattgg aaaacgttct tcggggcgaa aactctcaag   7380

gatcttaccg ctgttgagat ccagttcgat gtaacccact cgtgcaccca actgatcttc   7440

agcatctttt actttcacca gcgtttctgg gtgagcaaaa acaggaaggc aaaatgccgc   7500

aaaaaaggga ataagggcga cacggaaatg ttgaatactc atactcttcc tttttcaata   7560

ttattgaagc atttatcagg gttattgtct catgagcgga tacatatttg aatgtattta   7620

gaaaaataaa caaatagggg ttccgcgcac atttccccga aaagtgccac ctgacgtcta   7680

agaaaccatt                                                          7690

<210> SEQ ID NO 23
<211> LENGTH: 2163
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 23

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atcacctcag aagtagcaga aacattacat ggtagattgg cagaaatcgt ttgtaattat    120

ggtgctgcaa cccctcaaac ttggatcaac atcgctaacc atatcttgtc accagatttg    180

cctttctcct tacaccaaat gttgttttat ggttgctaca aggatttcgg tccagcccca    240

cctgcttgga ttccagaccc tgaaaaagtc aagtcaacta atttgggtgc tttgttggaa    300

aagagaggta aagaattttt gggtgtaaag tacaaagatc caatttcttc tttttctcac    360

ttccaagaat ttctgttag aaaccctgaa gtctattgga gaacagtatt gatggatgaa    420

atgaaaatta gtttctctaa ggacccagaa tgtatcttga gaagagatga catcaacaac    480

ccaggtggtt ctgaatggtt acctggtggt tacttgaact cagctaaaaa ttgcttgaac    540

gtaaactcca ataagaaatt gaacgatact atgatcgttt ggagagacga gggtaacgat    600

gacttgcctt tgaataagtt gacattagat caattgagaa agagagtttg gttggttggt    660

tatgcattgg aagaaatggg tttagaaaaa ggttgtgcaa tagccatcga tatgccaatg    720

catgttgatg ctgttgttat atatttggcc atagtattgg ctggttacgt agttgtctct    780

atagcagatt cattttccgc ccctgaaatc tcaactagat tgagattatc caaagctaag    840

gcaattttca cacaagatca catcatcaga ggtaaaaaga gaataccatt gtattcaaga    900

gtagttgaag ctaaatcccc aatggcaata gttatccctt gtagtggttc taacattggt    960

gcagaattga gagatggtga catatcttgg gattactttt tagaaagagc caaggagttt   1020

aaaaactgcg agtttactgc cagagaacaa cctgttgatg cttatactaa catcttattc   1080

tccagtggta ctacaggtga accaaaagca attccttgga cacaagccac cccattgaag   1140

gctgctgctg atggttggtc tcatttggat attagaaaag gtgacgttat agtatggcca   1200
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actaatttgg gttggatgat gggtccttgg ttggtttatg ctagtttgtt aaatggtgca 1260

tctattgcct tgtacaacgg tagtccttta gtctctggtt tcgctaaatt tgttcaagat 1320

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tgtgtttcag gttacgattg gtccaccata agatgctttt cttcatccgg tgaagcctct 1440

aatgtagacg aatatttgtg gttaatgggt agagctaact acaagccagt tatagaaatg 1500

tgtggtggta cagaaatcgg tggtgctttt tctgctggtt catttttgca agctcaatct 1560

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tacccaatgc ctaaaaataa gccaggtatc ggtgaattgg cattaggtcc tgttatgttt 1680

ggtgcctcaa aacattgtt aaacggtaat catcacgatg tctatttcaa gggtatgcca 1740

accttgaatg gtgaagtatt gagaagacat ggtgacattt tcgaattgac ctctaacggt 1800

tactaccatg cacacggtag agccgatgac actatgaaca tcggtggtat caaaattagt 1860

tctatcgaaa tcgaaagagt ctgtaatgaa gtagatgaca gagtttttga aaccactgct 1920

attggtgttc cacctttggg tggtggtcca gaacaattgg tcatattttt cgtattgaag 1980

gattcaaacg acacaaccat tgatttgaac caattgagat tatcctttaa cttgggtttg 2040

caaaagaaat tgaacccatt attcaaagtt actagagttg tcccattgtc atccttacct 2100

agaactgcaa caaacaagat catgagaaga gttttgagac aacaattcag tcatttcgaa 2160

tga 2163

<210> SEQ ID NO 24
<211> LENGTH: 1059
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 24

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gaagaattga acgctagttt gttagcttat ggtatgccta aagaagcctg cgattggtat 120

gctcactctt taaactacaa tactccaggt ggtaaattga atagaggttt gagtgtagtt 180

gatacttatg ctatcttgtc taacaaaacc gttgaacaat taggtcaaga agaatacgaa 240

aaggtcgcta tcttgggttg gtgtattgaa ttgttgcaag catacttttt ggttgccgat 300

gacatgatgg ataagtctat aacaagaaga ggtcaaccat gctggtacaa agttccagaa 360

gttggtgaaa tagccataaa tgatgctttt atgttggaag ccgctatcta taaattgttg 420

aagtcacatt tcagaaacga aaagtactac atcgatatta ccgaattatt ccacgaagtt 480

actttccaaa cagaattggg tcaattgatg gatttgataa ctgcacctga agataaagtt 540

gacttgtcaa agttttcctt gaagaaacat tcattcatcg tcacctttga aactgcttat 600

tactccttct atttgccagt cgccttggct atgtacgtag ctggtattac tgatgaaaaa 660

gacttgaagc aagcaagaga tgttttgata cctttgggtg aatacttcca aatccaagat 720

gactacttag actgtttcgg tactccagaa caaataggta aaatcggtac agatattcaa 780

gacaataagt gcagttgggt tattaacaag gctttggaat tagcatctgc cgaacaaaga 840

aagactttgg atgaaaacta cggtaaaaag gactcagttg ctgaagcaaa gtgtaagaaa 900

atttttaatg atttgaagat tgaacaattg taccatgaat acgaagaatc catcgctaaa 960

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acagctttct tgaataaggt ctacaagaga tcaaagtag 1059

<210> SEQ ID NO 25
<211> LENGTH: 1158
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 25

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gaaaacatat tgatccaaga tgaatttcct gattattact tcagagttac caagagtgaa    120

cacatgactc aattgaagga aaagtttaga aaaatatgtg ataagtctat gatcagaaag    180

agaaactgct tcttgaacga agaacatttg aagcaaaatc caagattggt agaacacgaa    240

atgcaaacat tggatgccag acaagacatg ttagttgtcg aagttcctaa attgggtaaa    300

gatgcttgtg caaaagccat taaggaatgg ggtcaaccaa agtcaaagat cactcatttg    360

atttttacaa gtgcatctac tacagatatg cctggtgcag actaccactg tgccaaattg    420

ttaggtttgt caccatccgt taagagagtc atgatgtatc aattaggttg ctacggtggt    480

ggtactgttt tgagaatcgc taaggatatt gcagaaaaca acaagggtgc cagagtatta    540

gctgtttgtt gcgacattat ggcttgcttg tttagaggtc caagtgattc tgacttggaa    600

ttgttagttg gtcaagctat cttcggtgac ggtgctgctg ctgttattgt tggtgcagaa    660

cctgacgaat ctgttggtga aagaccaata tttgaattag tcagtacagg tcaaaccatc    720

ttgcctaatt ctgaaggtac aattggtggt catataagag aagcaggttt gatcttcgat    780

ttgcacaaag acgttccaat gttaatctct aacaacatag aaaagtgttt gatagaagca    840

ttcactccta taggtatctc agattggaac tctattttct ggataacaca tccaggtggt    900

aaagccattt tggataaggt tgaagaaaaa ttggatttga agaaagaaaa gtttgtagat    960

agtagacatg ttttatctga acacggtaac atgtcttcat ccactgtctt gttcgtaatg   1020

gatgaattga gaaagagatc attagaagag ggtaaatcta ctactggtga cggttttgaa   1080

tggggtgtct tatttggttt cggtcctggt ttgaccgtcg aaagagtagt tgtcagatca   1140

gtaccaatta aatattag                                                1158
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<210> SEQ ID NO 26
<211> LENGTH: 315
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 26

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atggccgtca agcatttgat agtattgaag tttaaagatg aaatcacaga agctcaaaag     60

gaagaatttt tcaagaccta cgttaatttg gtcaacatta tacctgctat gaaagatgta    120

tactggggta aagacgttac acaaaagaaa gaagaaggtt atacacacat tgtcgaagta    180

accttcgaat cagttgaaac tatccaagat tacatcattc atccagctca cgttggtttt    240

ggtgacgttt acagatcctt ctgggaaaaa ttgttgatct tcgattacac cccaagaaag    300

ttaaagccaa aataa                                                     315
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<210> SEQ ID NO 27
<211> LENGTH: 1188
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 27

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cctcacaaca acaatccaaa aacatcattg ttgtgttaca gacatccaaa gacacctatt    120
aagtactctt acaacaactt tccatcaaaa cattgttcaa ccaagtcctt ccacttacaa    180
aataagtgct ccgaaagttt gtctatagct aagaactcta tcagagctgc aactacaaat    240
caaactgaac cacctgaaag tgataatcac tctgttgcca caaaaatttt gaacttcggt    300
aaagcatgtt ggaagttgca aagaccatac accataatcg cttttacttc ttgtgcatgc    360
ggtttattcg gtaaagaatt gttgcataac actaacttaa tttcatggtc cttgatgttc    420
aaggcatttt tctttttagt tgccatcttg tgcatcgctt cattcaccac tacaattaat    480
caaatatacg atttgcacat cgacagaatt aacaaaccag atttgccttt ggcttcaggt    540
gaaatatccg tcaatactgc atggatcatg tctatcatag tagccttgtt cggtttgatc    600
atcacaatta aaatgaaggg tggtccattg tacatcttcg gttactgttt cggtatcttc    660
ggtggtattg tctattccgt accacctttt agatggaaac aaaaccctag tactgccttt    720
ttgttgaatt tcttagctca tatcatcaca aacttcacct tctactacgc ttcaagagct    780
gctttaggtt tgccattcga attgagacct tcattcacat tttgttggc attcatgaaa    840
agtatgggtt ctgcattagc cttgatcaag gatgcctctg acgttgaagg tgacacaaag    900
ttcggtatta gtaccttggc ttctaagtac ggttcaagaa atttgacttt gttctgctcc    960
ggtatcgttt tgttaagtta cgtcgcagcc attttggcag gtatcatttg gccacaagcc   1020
tttaattcta acgttatgtt gttgtcacat gccatcttgg ctttctggtt gatcttgcaa   1080
actagagatt tcgctttgac aaattatgac cctgaagcag gtagaagatt ctacgagttt   1140
atgtggaaat tgtactacgc tgaatatttg gtatacgttt ttatttag                1188
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<210> SEQ ID NO 28
<211> LENGTH: 1635
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 28

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atgaaatgtt caactttctc cttttggttc gtatgcaaga tcatcttctt tttcttttcc     60
tttaacatcc aaacaagtat cgcaaaccca agagaaaact tttgaagtg cttctcacaa    120
tacataccta ataacgccac caatttgaag ttggtttaca ctcaaaacaa cccattgtac    180
atgtccgtct tgaacagtac aatccataat ttgagattca cttctgatac cactccaaaa    240
cctttggtca ttgtaacccc tagtcatgta tctcacatcc aaggtactat cttatgttct    300
aaaaaggttg gtttgcaaat tagaactaga tccggtggtc atgatagtga aggcatgtca    360
tacatctccc aagttccatt cgttatcgtt gatttgagaa acatgagatc aattaaaata    420
gacgtacact cacaaactgc ttgggttgaa gctggtgcaa cattgggtga agtatactac    480
tgggttaacg aaaagaatga aaacttatca ttggctgctg gttactgtcc aacagtttgc    540
gcaggtggtc attttggtgg tggtggttat ggtcctttaa tgagaaacta cggtttggcc    600
gctgataaca taatcgacgc tcatttggta aatgttcacg gtaaagtttt ggatagaaag    660
tctatgggtg aagacttatt ttgggctttg agaggtggtg gtgcagaatc attcggtatc    720
atagttgctt ggaagataag attagtcgca gtaccaaagt ctactatgtt ctcagtcaaa    780
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aagataatgg aaatccatga attagttaaa ttggtcaata agtggcaaaa catcgcatac    840
aagtacgata aggacttgtt gttgatgact catttcatca caagaaacat caccgataac    900
caaggtaaaa ataagactgc tatccacaca tacttttctt cagttttctt gggtggtgtc    960
gattccttag tagacttgat gaataagtct tttccagaat taggtattaa gaaaactgat   1020
tgtagacaat tgtcttggat cgacaccatc atcttttatt caggtgttgt caactacgat   1080
acagacaact tcaacaaaga aatattattg gatagatccg caggtcaaaa cggtgccttt   1140
aaaattaagt tagactacgt taaaaagcca atacctgaat cagttttcgt ccaaatctta   1200
gaaaaattgt acgaagaaga tattggtgca ggcatgtacg ccttgtatcc atacggtggt   1260
ataatggacg aaatcagtga atctgccatt ccatttcctc atagagctgg tatcttatac   1320
gaattgtggt acatttgttc atgggaaaag caagaagata acgaaaagca cttaaactgg   1380
attagaaaca tctataactt catgactcca tacgtttcta aaaaccctag attggcatat   1440
ttgaactaca gagatttgga catcggtatt aacgatccaa agaatcctaa caactatacc   1500
caagctagaa tttggggtga aaaatacttc ggtaaaaatt tcgatagatt agtaaaggtt   1560
aagacattgg ttgacccaaa caacttcttt agaaacgaac aatccattcc acctttacct   1620
agacatagac actga                                                    1635
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<210> SEQ ID NO 29
<211> LENGTH: 1638
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon optimized

<400> SEQUENCE: 29

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ttcaacatcc aaatctccat cgcaaatcca caagaaaact tttgaagtg tttctccgaa    120
tacatcccaa acaaccctgc taacccaaag tttatatata ctcaacatga tcaattgtac    180
atgtccgttt tgaacagtac catccaaaat ttgagattca cttctgacac tacaccaaaa    240
cctttagtca ttgttacacc ttccaatgtt agtcacattc aagcttctat attgtgctct    300
aagaaagtag gtttgcaaat cagaactaga tcaggtggtc atgatgcaga aggcatgtct    360
tacatctcac aagttccatt cgttgtagtc gatttgagaa atatgcattc cataaagatc    420
gacgttcaca gtcaaacagc atgggtagaa gcaggtgcca ccttgggtga agtttactac    480
tggatcaacg aaaagaatga aaacttttct ttccctggtg gttactgtcc aacagtaggt    540
gtcggtggtc acttttctgg tggtggttat ggtgcattga tgagaaacta cggtttagct    600
gcagataata ttatagacgc ccatttggtt aacgtagatg gtaaagtttt ggacagaaag    660
tctatgggtg aagatttgtt ttgggccata agaggtggtg gtggtgaaaa tttcggtatc    720
attgccgctt ggaaaattaa gttagtcgct gttccttcca aagtactat tttctctgtc    780
aaaaagaaca tggaaatcca cggtttggtt aagttgttta ataagtggca aaacatcgct    840
tacaagtacg ataaggactt ggttttgatg acccatttca tcactaaaaa tattacagat    900
aaccatggta aaaataagac cactgttcac ggttattttt cttcaatttt ccatggtggt    960
gtagattctt tggttgattt gatgaataag tcattcccag aattgggtat taaaaagaca   1020
gattgcaagg aattttcttg gatagacaca accatcttct attcaggtgt tgtaaacttc   1080
aacaccgcta acttcaaaaa ggaaatcttg ttggatagat ccgctggtaa aaagaccgct   1140
```

ttttctatta aattggacta cgttaagaaa ccaatccctg aaactgcaat ggtcaagata 1200

ttggaaaagt tgtacgaaga agatgtaggt gtcggcatgt acgttttgta tccatacggt 1260

ggtattatgg aagaaatatc tgaatcagcc ataccatttc ctcacagagc tggtatcatg 1320

tatgaattat ggtacacagc ctcatgggaa aagcaagaag ataacgaaaa gcatatcaac 1380

tgggtcagat ccgtttacaa cttcactaca ccttacgtta gtcaaaaccc aagattggca 1440

tatttgaact acagagattt ggacttaggt aaaactaacc ctgaatctcc aaataactat 1500

acacaagcaa gaatttgggg tgaaaagtac tttggtaaaa atttcaacag attagttaaa 1560

gtaaagacta aagccgaccc taacaacttt ttcagaaacg aacaatccat cccacctttg 1620

ccacctcacc accactaa 1638

<210> SEQ ID NO 30
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 30

aggaaacgaa gataaatctc gagtttatca ttatcaatac tg 42

<210> SEQ ID NO 31
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 31

ggaaaaatca gtcaaggcaa attaaagcct tcgagcg 37

<210> SEQ ID NO 32
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 32

gatgggggat ccactagttc tagaatc 27

<210> SEQ ID NO 33
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 33

tgatgggctg caggaattcg atatc 25

<210> SEQ ID NO 34
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 34

gaactagtgg atcccccatc atgaaccatt tgagagcc 38

<210> SEQ ID NO 35
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 35

tattttggct ttaactttct tggggtgtaa tc 32

<210> SEQ ID NO 36
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 36

agaaagttaa agccaaaata atgataacga gaataatatc aag 43

<210> SEQ ID NO 37
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 37

ataaacccat ggcgcagacc tgtgagag 28

<210> SEQ ID NO 38
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 38

ggtctgcgcc atgggtttat catccgtc 28

<210> SEQ ID NO 39
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 39

cgaattcctg cagcccatca gtgtctatgt ctaggtaaag g 41

<210> SEQ ID NO 40
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 40

tgatgggctg caggaattcg atatc 25

<210> SEQ ID NO 41
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

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<400> SEQUENCE: 41

gatgggggat ccactagttc tagaatc                                          27


<210> SEQ ID NO 42
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 42

caccagaacc gaaggtagag gttctttgtt aac                                   33


<210> SEQ ID NO 43
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 43

cgaattcctg cagcccatca ctttgatctc ttgtagacct tattc                      45


<210> SEQ ID NO 44
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 44

gaactagtgg atcccccatc atggtttcca atcacttgtt tg                         42


<210> SEQ ID NO 45
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 45

ctctaccttc ggttctggtg tataagtcg                                        29


<210> SEQ ID NO 46
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 46

gatccactag ttctagaatc cg                                               22


<210> SEQ ID NO 47
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 47

tctagaacta gtggatcatg aaccatttga gagcc                                 35


<210> SEQ ID NO 48
<211> LENGTH: 28
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 48

tcgttatcac tttcttgggg tgtaatcg 28

<210> SEQ ID NO 49
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 49

ccaagaaagt gataacgaga ataatatcaa gaatac 36

<210> SEQ ID NO 50
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 50

aggtcgacgg tatcgttaaa taaaaacgta taccaaatat tcag 44

<210> SEQ ID NO 51
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 51

cgataccgtc gacctcga 18

<210> SEQ ID NO 52
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 52

ggttaaacta gtatgggtaa aaactataag tc 32

<210> SEQ ID NO 53
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 53

gtgcccgtcg actcattcga aatgactgaa ttg 33

What is claimed is:

1. A method for making cannabigerolic acid, the method comprising:

transforming *S. cervisiae* with a first nucleotide sequence comprising the nucleotide sequence of SEQ. ID. NO.2 expressing an acyl-activating enzyme and expressing a mutant prenyltransferase; and

transforming the *S. cervisiae* with a second nucleotide sequence comprising the nucleotide sequence of SEQ. ID. NO.13 expressing olivetolic synthase, expressing olivetolic acid cyclase and expressing aromatic prenyltransferase.

2. A method for making cannabigerolic acid, the method comprising:

transforming a *S. cervisiae* with a first nucleotide sequence comprising the nucleotide sequence of SEQ. ID. NO.1 expressing an acyl-activating enzyme;

transforming the *S. cervisiae* with a second nucleotide sequence comprising the nucleotide sequence of SEQ. ID. NO.22 expressing a mutant prenyltransferase;

transforming the *S. cervisiae* with a third nucleotide sequence comprising the nucleotide sequence of SEQ. ID. NO.10 expressing olivetolic synthase;

transforming the *S. cervisiae* with a fourth nucleotide sequence comprising the nucleotide sequence of SEQ. ID. NO.5 expressing olivetolic acid cyclase; and

transforming the *S. cervisiae* with a fifth nucleotide sequence comprising the nucleotide sequence of SEQ. ID. NO.13 expressing aromatic prenyltransferase.

3. A method for making cannabigerolic acid, the method comprising:

transforming *S. cervisiae* with a first nucleotide sequence comprising the nucleotide sequence of SEQ. ID. NO.23 expressing an acyl-activating enzyme;

transforming the *S. cervisiae* with a second nucleotide sequence comprising the nucleotide sequence of SEQ. ID. NO.24 expressing a mutant prenyltransferase;

transforming the *S. cervisiae* with a third nucleotide sequence comprising the nucleotide sequence of SEQ. ID. NO.25 expressing olivetolic synthase;

transforming the *S. cervisiae* with a fourth nucleotide sequence comprising the nucleotide sequence of SEQ. ID. NO.26 expressing olivetolic acid cyclase; and

transforming the *S. cervisiae* with a fifth nucleotide sequence comprising the nucleotide sequence of SEQ. ID. NO.27 expressing aromatic prenyltransferase.

* * * * *