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(54) Titre : MODULATION DU PROFIL DE CANNABINOÏDE DANS LE CANNABIS
(54) Title: MODULATION OF CANNABINOID PROFILE IN CANNABIS

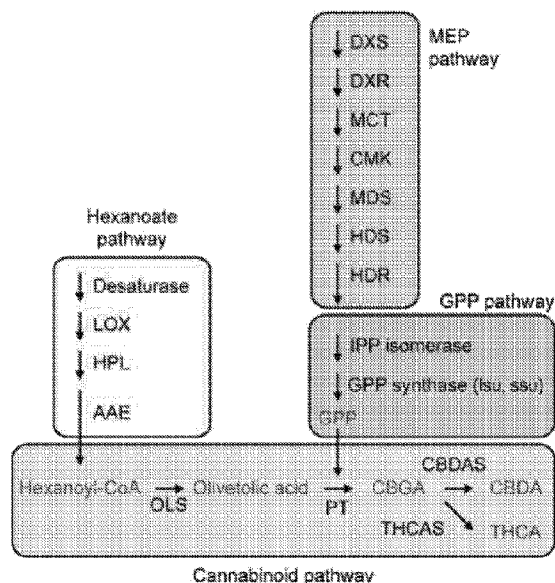


FIG. 2

(57) **Abrégé/Abstract:**

The present invention discloses a Cannabis plant with reduced delta-9 -tetrahydrocannabinol (THC) content, or reduced cannabidiol (CBD) content, or reduced THC and CBD content. According to a core aspect of the invention, the Cannabis plant comprises at least one targeted genome modification effective in decreasing expression of at least one Cannabis gene encoding a cannabinoid biosynthesis enzyme selected from the group consisting of Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme 1 (CsAAEI) and any combination thereof. The present invention further discloses methods for production of the Cannabis plants and use thereof,

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(54) Title: MODULATION OF CANNABINOID PROFILE IN CANNABIS

(57) Abstract: The present invention discloses a Cannabis plant with reduced delta-9 -tetrahydrocannabinol (THC) content, or reduced cannabidiol (CBD) content, or reduced THC and CBD content. According to a core aspect of the invention, the Cannabis plant comprises at least one targeted genome modification effective in decreasing expression of, at least one Cannabis gene encoding a cannabinoid biosynthesis enzyme selected from the group consisting of Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme 1 (CsAAEI) and any combination thereof. The present invention further discloses methods for production of the Cannabis plants and use thereof,

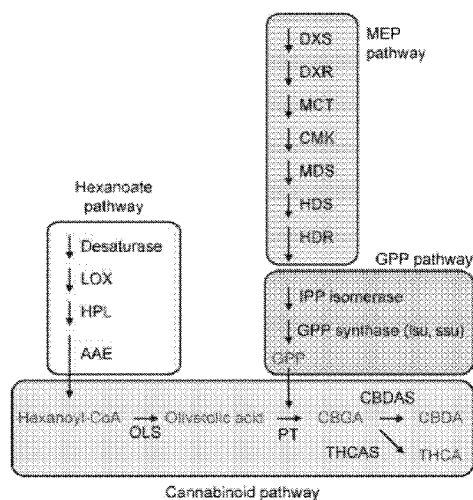


FIG. 2

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MODULATION OF CANNABINOID PROFILE IN CANNABIS

FIELD OF THE INVENTION

[0001] The present invention relates generally to Cannabis plants with altered expression of cannabinoids and/or altered expression of cannabinoid synthesizing enzymes. More specifically, the present disclosure relates to methods for controlling genes associated with cannabinoids synthesis in Cannabis plants.

BACKGROUND OF THE INVENTION

[0002] Cannabis has been bred by many different cultures for various uses such as food, fiber and medicine since the dawn of agricultural societies. In the last few decades, Cannabis breeding has stopped as it became illegal and non-economic to do so. With the recent legislation converting Cannabis back to legality, there is a growing need for the implementation of new and advanced breeding techniques in future Cannabis breeding programs. This will allow speeding up the long process of classical breeding and accelerate reaching new and genetically improved Cannabis varieties for fiber, food and medicine products. Developing and implementing molecular biology tools to support the breeders, will allow creating new traits and tracking the movement of such desired traits across breeders germplasm.

[0003] According to some publications, the Cannabis plant chemical profile is composed of at least 483 known chemical compounds, which include cannabinoids, terpenoids, flavonoids, nitrogenous compounds, amino acids, proteins, glycoproteins, enzymes, sugars and related compounds, hydrocarbons, alcohols, aldehydes, ketones, acids, fatty acids, esters, lactones, steroids, terpenes, non-cannabinoid phenols, vitamins, and pigments.

[0004] Cannabinoids are of particular interest for research and commercialization. There are at least 113 different cannabinoids isolated from Cannabis, exhibiting varied effects. The classical cannabinoids are concentrated in a viscous resin produced in structures known as glandular trichomes. The most notable cannabinoid is the phytocannabinoid delta 9 tetrahydrocannabinol (THC), the primary psychoactive compound in Cannabis. Cannabidiol (CBD) is another major constituent of the plant.

[0005] Other cannabinoids of interest include, Cannabigerol (CBG), Cannabigerolic Acid (CBGA), Cannabinol (CBN), Cannabichromene (CBC), Tetrahydrocannabivarin (THCV), Cannabigerovarin (CBGV), Cannabigerovarinic Acid (CBGVA), tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), cannabichromene (CBC), cannabicyclol (CBL), cannabivarin (CBV), cannabidivarin (CBDV), cannabichromevarin (CBCV), cannabigerol monomethyl ether (CBGM), cannabielsoin (CBE) and cannabicitran (CBT).

[0006] Cannabis plants can exhibit wide variation in the quantity and type of cannabinoids they produce. The mixture of cannabinoids produced by a plant is known as the plant's cannabinoid profile. Selective breeding has been used to control the genetics of plants and modify the cannabinoid profile. For example, strains that are used as fiber (commonly called hemp) are usually bred such that they are low in psychoactive chemicals like THC. Strains used in medicine are often bred for high CBD content, and strains used for recreational purposes are usually bred for high THC content or for a specific chemical balance.

[0007] Quantitative analysis of a plant's cannabinoid profile is often determined by analytical methods such as gas chromatography (GC), gas chromatography combined with mass spectrometry (GC/MS) and liquid chromatography (LC) techniques.

[0008] A variety of growing and cultivating techniques have been developed for increasing the production of secondary compounds within plants of genus Cannabis. These techniques include outdoor cultivation, indoor cultivation, hydroponics, fertilization, atmospheric manipulation, cloning, crossbreeding etc. There is very limited if any molecular tools supporting or leading the breeding process. Traditional Cannabis breeding is done by mixing breeding material with hope to find the desired traits and phenotypes by random crosses. These methods have allowed the construction of the leading Cannabis varieties on the market today.

[0009] As the cultivation of Cannabis intensifies, breeding and farming techniques fail to provide the level of control of cannabinoid production and yield needed. Cannabinoid research is still new and having plants producing modified levels of certain cannabinoids would be advantageous for research and medical purposes. Furthermore, separating and isolating specific molecules of the plant out of hundreds could be challenging and time consuming.

[0010] In view of the above there is an unmet and long felt need for non-GMO, advanced breeding of Cannabis plants producing particular amounts of predetermined cannabinoids. In particular,

there is a need for Cannabis plants selectively producing predetermined ratios and/or concentrations of cannabinoids for medical use.

SUMMARY OF THE INVENTION

[0011] It is one object of the present invention to disclose a Cannabis plant with reduced delta-9-tetrahydrocannabinol (THC) content, or reduced cannabidiol (CBD) content, or reduced THC and CBD content, wherein said plant comprises at least one targeted genome modification effective in decreasing expression of at least one Cannabis gene encoding a cannabinoid biosynthesis enzyme selected from the group consisting of Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme 1 (CsAAE1) and any combination thereof.

[0012] It is a further object of the present invention to disclose the Cannabis plant as defined above, wherein said plant comprises reduced THC content, or reduced CBD content, or reduced THC and CBD content relative to a Cannabis plant of a similar genotype or genetic background that does not comprise said targeted genome modification.

[0013] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said targeted genome modification is located in the cannabinoid biosynthesis enzyme gene locus.

[0014] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said targeted genome modification interrupts or interferes with or down regulate or silence transcription and/or translation of said Cannabis gene encoding said cannabinoid biosynthesis enzyme.

[0015] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said plant comprises an endonuclease enzyme targeting a nucleic acid sequence coding for said at least one cannabinoid biosynthesis enzyme.

[0016] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said plant does not comprise within its genome exogenous genetic material and said plant is a non-naturally occurring Cannabis plant or cell thereof.

[0017] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein the nucleotide sequence encoding said at least one cannabinoid biosynthesis enzyme is selected from the group consisting of: CsTHCAS having a genomic nucleotide sequence as set forth in SEQ ID NO:1 or a functional variant thereof, CsCBDAS having a genomic nucleotide sequence as set forth in SEQ ID NO:4 or a functional variant thereof, CsPT having a genomic nucleotide sequence as set forth in SEQ ID NO:7 or a functional variant thereof, CsOLS having a genomic nucleotide sequence as set forth in SEQ ID NO:10 or a functional variant thereof and CsAAE1 having a genomic nucleotide sequence as set forth in SEQ ID NO:13 or a functional variant thereof.

[0018] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said functional variant has at least 75% sequence identity to the nucleotide sequence of said cannabinoid biosynthesis enzyme or a codon degenerate nucleotide sequence thereof.

[0019] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said plant has decreased expression levels of at least one of CsTHCAS protein having an amino acid sequence with at least 75% identity to the amino acid sequence as set forth in SEQ ID NO:3 or a conservatively substituted amino acid sequence thereof, CsCBDAS protein having an amino acid sequence with at least 75% identity to the amino acid sequence as set forth in SEQ ID NO:6 or a conservatively substituted amino acid sequence thereof, CsPT protein having an amino acid sequence with at least 75% identity to the amino acid sequence as set forth in SEQ ID NO:9 or a conservatively substituted amino acid sequence thereof, CsOLS protein having an amino acid sequence with at least 75% identity to the amino acid sequence as set forth in SEQ ID NO:12 or a conservatively substituted amino acid sequence thereof, and CsAAE1 protein having an amino acid sequence with at least 75% identity to the amino acid sequence as set forth in SEQ ID NO:15 or a conservatively substituted amino acid sequence thereof.

[0020] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said Cannabis plant has modulated expression of one or more of the cannabinoids, optionally cannabigerolic acid, cannabigerol, DELTA.9-tetrahydrocannabinolic acid, cannabidiolic acid, cannabichromenic acid, DELTA.9-tetrahydrocannabinol, cannabidiol,

cannabichromene, THC, D9-THC, D8-THC, THCA, THCV, D8-THCV, D9-THCV, THCVA, CBD, CBDA, CBDV, CBDVA, CBC, CBCA, CBCV, CBCVA, CBG, CBGA, CBGV, CBGVA, CBN, CBNA, CBNV, CBNVA, CBND, CBNDA, CBNDV, CBNDVA, CBE, CBEA, CBEV, CBEVA, CBL, CBLA, CBLV, or CBLVA and cannabidiol.

[0021] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said Cannabis plant has reduced expression of one or more of the cannabinoids, optionally cannabigerolic acid, cannabigerol, DELTA.9-tetrahydrocannabinolic acid, cannabidiolic acid, cannabichromenic acid, DELTA.9-tetrahydrocannabinol, cannabidiol, cannabichromene, THC, D9-THC, D8-THC, THCA, THCV, D8-THCV, D9-THCV, THCVA, CBD, CBDA, CBDV, CBDVA, CBC, CBCA, CBCV, CBCVA, CBG, CBGA, CBGV, CBGVA, CBN, CBNA, CBNV, CBNVA, CBND, CBNDA, CBNDV, CBNDVA, CBE, CBEA, CBEV, CBEVA, CBL, CBLA, CBLV, or CBLVA and cannabidiol, as compared to a Cannabis plant of a similar genotype or genetic background that does not comprise said targeted genome modification.

[0022] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said targeted genome modification is introduced using CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas) genes (CRISPR/Cas) system, Transcription activator-like effector nuclease (TALEN), Zinc Finger Nuclease (ZFN), meganuclease or any combination thereof.

[0023] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said CRISPR/Cas genes or proteins are selected from the group consisting of Cas3, Cas4, Cas5, Cas5e (or CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9, Cas10, Cas10d, Cas12, Cas13, Cas14, CasX, CasF, CasG, CasH, Csy1, Csy2, Csy3, Cse1 (or CasA), Cse2 (or CasB), Cse3 (or CasE), Cse4 (or CasC), Csc1, Csc2, Csa5, Csn1, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Cpf1, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csz1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cu1966.

[0024] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said targeted genome modification is introduced via (i) at least one RNA-guided (gRNA) endonuclease or nucleic acid encoding at least one RNA-guided endonuclease, and (ii) at least one guide RNA (gRNA) or DNA encoding at least one guide RNA

(gRNA), further wherein each of said at least one gRNA directs said endonuclease to a targeted site located in the genomic sequence of said at least one Cannabis gene encoding a cannabinoid biosynthesis enzyme.

[0025] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein the gRNA nucleotide sequence targeted to CsTHCAS genomic sequence is as set forth in nucleotide sequence selected from the group consisting of SEQ. ID. NO.: 16-167 and any combination thereof.

[0026] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein the gRNA nucleotide sequence targeted to CsCBDAS genomic sequence is as set forth in nucleotide sequence selected from the group consisting of SEQ. ID. NO.: 168-304, SEQ. ID. NO.: 824-825, and any combination thereof.

[0027] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein the gRNA nucleotide sequence targeted to CsPT genomic sequence is as set forth in nucleotide sequence selected from the group consisting of SEQ. ID. NO.: 305-458 and any combination thereof.

[0028] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein the gRNA nucleotide sequence targeted to CsOLS genomic sequence is as set forth in nucleotide sequence selected from the group consisting of SEQ. ID. NO.: 459-509 and any combination thereof.

[0029] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein the gRNA nucleotide sequence targeted to CsAAE1 genomic sequence is as set forth in nucleotide sequence selected from the group consisting of SEQ. ID. NO.: 510-823, SEQ. ID. NO.: 826, and any combination thereof.

[0030] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said plant is mutated in a gene selected from the group consisting of CsTHCAS, CsSP, CsCBDAS, CsPT, CsOLS, CsAAE1 and any combination thereof.

[0031] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said mutated CsTHCAS, CsSP, CsCBDAS, CsPT, CsOLS and/or CsAAE1 gene is a CRISPR/Cas9- induced heritable mutated allele.

[0032] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said genome modification is a missense mutation, nonsense mutation, insertion, deletion, indel, substitution or duplication.

[0033] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein the insertion or the deletion produces a gene comprising a frameshift.

[0034] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said plant is homozygous for said at least one mutated gene.

[0035] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said mutation is in the coding region of said gene, a mutation in the regulatory region of said gene, a mutation in a gene downstream of said gene in the cannabinoid biosynthesis pathway or an epigenetic factor.

[0036] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said mutation is a silencing mutation, a knockdown mutation, a knockout mutation, a loss of function mutation or any combination thereof.

[0037] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said genome modification is generated *in planta*.

[0038] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above wherein said genome modification is generated *in planta* via introduction of a construct comprising (a) Cas DNA and gRNA sequence selected from the group consisting of SEQ ID NO:16-SEQ ID NO:826 and any combination thereof, or (b) a ribonucleoprotein (RNP) complex comprising Cas protein and gRNA sequence selected from the group consisting of SEQ ID NO:16-826 and any combination thereof.

[0039] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above wherein said gRNA sequence comprises a 3' NGG Protospacer Adjacent Motif (PAM).

[0040] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above wherein said gRNA is introduced into the plant cells via Agrobacterium infiltration, virus based plasmids for delivery of the genome editing molecules or mechanical

insertion such as polyethylene glycol (PEG) mediated DNA transformation, electroporation or gene gun biolistics.

[0041] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above wherein said Cannabis plant is selected from the group of species that includes, but is not limited to, *Cannabis sativa* (*C. sativa*), *C. indica*, *C. ruderalis* and any hybrid or cultivated variety of the genus *Cannabis*.

[0042] It is a further object of the present invention to disclose a Cannabis plant, plant part or plant cell as defined in any of the above wherein said plant does not comprise a transgene.

[0043] It is a further object of the present invention to disclose a plant part, plant cell or plant seed of a plant as defined in any of the above.

[0044] It is a further object of the present invention to disclose a tissue culture of regenerable cells, protoplasts or callus obtained from the Cannabis plant as defined in any of the above.

[0045] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above wherein said plant genotype is obtainable by deposit under accession number with NCIMB Aberdeen AB21 9YA, Scotland, UK, or wherein said plant genotype is obtainable by deposit under accession number with ATCC, LGC Standards, Queens Road Teddington Middlesex TW11 0LY UK.

[0046] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said Cannabis plant comprises a DNA encoding an antisense RNA or a siRNA effective to suppress expression of CsTHCAS, CsSP, CsCBDAS, CsPT, CsOLS, CsAAE1 and any combination thereof, the DNA operably linked to a heterologous promoter, wherein the Cannabis plant comprises reduced THC content, reduced CBD content, or decreased THC and CBD content relative to a Cannabis plant of a similar genotype that does not comprise the DNA.

[0047] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said Cannabis plant has a THC content of up to 30% by weight, particularly between about %0.1 to about 30% by weight, more particularly between about %0.3 to about 30%, even more particularly between about %0.3 to about 10% by weight.

[0048] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said Cannabis plant has a CBD content of up to 30% by weight,

particularly between about %0.1 to about 30% by weight, more particularly between about %0.3 to about 30%, even more particularly between about %0.3 to about 10% by weight.

[0049] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said plant has a THC and/or CBD content of not more than about 0.5% by weight.

[0050] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said plant is THC free.

[0051] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said plant has at least one targeted genome modification in at least one Cannabis gene encoding cannabinoid precursor synthesis enzyme selected from the group consisting of CsAAE, CsPT and CsOLS.

[0052] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said plant exhibits reduced expression of THC, CBD or both relative to a Cannabis plant of a similar genotype or genetic background lacking said targeted genome modification.

[0053] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said plant has a THC and/or CBD content of up to 30% by weight, particularly between about %0.1 to about 30% by weight, more particularly between about %0.3 to about 30%, even more particularly between about %0.3 to about 10% by weight.

[0054] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said plant has a THC and/or CBD content of not more than about 0.5% by weight.

[0055] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said plant has at least one targeted genome modification in at least one Cannabis gene encoding cannabinoid synthesis enzyme selected from the group consisting of CsTHCAS and CsCBDAS.

[0056] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said plant has at least one targeted genome modification in CsTHCAS and said plant exhibits reduced expression of THCA or THC, and elevated expression of CBD or

CBDA relative to a Cannabis plant of a similar genotype or genetic background lacking said targeted genome modification.

[0057] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said plant has a THC and/or THCA content of up to 30% by weight, particularly between about %0.1 to about 30% by weight, more particularly between about %0.3 to about 30%, even more particularly between about %0.3 to about 10% by weight.

[0058] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said plant has a THC and/or THCA content of not more than about 0.5% by weight.

[0059] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said plant has at least one targeted genome modification in CsCBDAS and said plant exhibits reduced expression of CBDA or CBD, and elevated expression of THC or THCA relative to a Cannabis plant of a similar genotype or genetic background lacking said targeted genome modification.

[0060] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said plant has a THC and/or CBD content of up to 30% by weight, particularly between about %0.1 to about 30% by weight, more particularly between about %0.3 to about 30%, even more particularly between about %0.3 to about 10% by weight.

[0061] It is a further object of the present invention to disclose the Cannabis plant as defined in any of the above, wherein said plant has a CBD and/or CBDA content of not more than about 0.5% by weight.

[0062] It is a further object of the present invention to disclose a Cannabis plant derived product from the plant as defined in any of the above.

[0063] It is a further object of the present invention to disclose the Cannabis plant derived product as defined above, comprising a combined cannabidiolic acid and cannabidiol concentration of about 0.3% to about 30% by weight .

[0064] It is a further object of the present invention to disclose the Cannabis plant derived product as defined in any of the above, comprising a combined delta-9-tetrahydrocannabinol and tetrahydrocannabinolic acid concentration of between about 0.3% to about 30% by weight .

[0065] It is a further object of the present invention to disclose the Cannabis plant derived product as defined in any of the above, comprising Cannabis oil, Cannabis tincture, dried Cannabis flowers, and/or dried Cannabis leaves.

[0066] It is a further object of the present invention to disclose the Cannabis plant derived product as defined in any of the above, for medical use.

[0067] It is a further object of the present invention to disclose the Cannabis plant derived product as defined in any of the above, formulated for inhalation, oral consumption, sublingual consumption, or topical consumption.

[0068] It is a further object of the present invention to disclose a medical composition derived from the plant as defined in any of the above.

[0069] It is a further object of the present invention to disclose a method for producing a Cannabis plant with reduced delta-9-tetrahydrocannabinol (THC) content, or reduced cannabidiol (CBD) content, or reduced THC and CBD content, wherein said method comprises steps of introducing into said Cannabis plant genome or a cell thereof at least one targeted genome modification effective in decreasing expression of a at least one Cannabis gene encoding a cannabinoid biosynthesis enzyme selected from the group consisting of tetrahydrocannabinolic acid synthase (THCAS), cannabidiolic acid synthase (CBDAS), aromatic prenyltransferase (PT), olivetol synthase (OLS), acyl-activating enzyme 1 (AAE1) and any combination thereof.

[0070] It is a further object of the present invention to disclose the method as defined above, further comprising steps of: (a) constructing an endonuclease enzyme targeting a nucleic acid sequence coding for a cannabinoid biosynthesis enzyme selected from the group consisting of CsTHCAS, CsCBDAS, CsPT, CsOLS, CsAAE1 and any combination thereof; (b) introducing the endonuclease enzyme into the genome of the Cannabis plant of; and (c) decreasing expression of the at least one cannabinoid biosynthesis enzyme within the genome.

[0071] It is a further object of the present invention to disclose the method as defined in any of the above, further comprising steps of: (a) introducing into the Cannabis plant or a cell thereof (i) at least one RNA-guided endonuclease or nucleic acid encoding at least one RNA-guided endonuclease, and (ii) at least one guide RNA (gRNA) or DNA encoding at least one gRNA; (b) assaying the Cannabis plant or a cell thereof for an endonuclease-mediated modification in the

DNA of said at least one Cannabis cannabinoid biosynthesis enzyme gene locus; and (c) identifying the Cannabis plant, or a cell thereof, or a progeny cell thereof as comprising a modification in said at least one gene locus.

[0072] It is a further object of the present invention to disclose the method as defined in any of the above, further comprising steps of culturing the Cannabis plant or cell thereof such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence of said at least one Cannabis cannabinoid biosynthesis enzyme, enabling the RNA-guided endonuclease introduce a double-stranded break in the targeted site, and repair of the double-stranded break by a DNA repair process such that the chromosomal sequence is modified, wherein the targeted site is located in the gene locus of the at least one Cannabis cannabinoid biosynthesis enzyme and the chromosomal modification interrupts or interferes with transcription and/or translation of said at least one gene encoding Cannabis cannabinoid biosynthesis enzyme.

[0073] It is a further object of the present invention to disclose the method as defined in any of the above, wherein the endonuclease enzyme is a CRISPR/Cas9 system.

[0074] It is a further object of the present invention to disclose the method as defined in any of the above, comprising steps of interfering with expression of a cannabinoid biosynthesis enzyme selected from the group consisting of CsTHCAS, CsCBDAS, CsPT, CsOLS, CsAAE1 and any combination thereof.

[0075] It is a further object of the present invention to disclose the method as defined in any of the above, wherein said plant comprises reduced THC content, or reduced CBD content, or reduced THC and CBD content relative to a Cannabis plant of a similar genotype or genetic background that does not comprise said targeted genome modification.

[0076] It is a further object of the present invention to disclose the method as defined in any of the above, wherein said targeted genome modification is located in the cannabinoid biosynthesis enzyme gene locus.

[0077] It is a further object of the present invention to disclose the method as defined in any of the above, wherein said targeted genome modification interrupts or interferes with or down regulate or silence transcription and/or translation of said Cannabis gene encoding said cannabinoid biosynthesis enzyme.

[0078] It is a further object of the present invention to disclose the method as defined in any of the above, wherein said plant comprises an endonuclease enzyme targeting a nucleic acid sequence coding for said at least one cannabinoid biosynthesis enzyme.

[0079] It is a further object of the present invention to disclose the method as defined in any of the above, wherein said plant does not comprise within its genome exogenous genetic material and said plant is a non-naturally occurring Cannabis plant or cell thereof.

[0080] It is a further object of the present invention to disclose the method as defined in any of the above, wherein the nucleotide sequence encoding said at least one cannabinoid biosynthesis enzyme is selected from the group consisting of: CsTHCAS having a genomic nucleotide sequence as set forth in SEQ ID NO:1 or a functional variant thereof, CsCBDAS having a genomic nucleotide sequence as set forth in SEQ ID NO:4 or a functional variant thereof, CsPT having a genomic nucleotide sequence as set forth in SEQ ID NO:7 or a functional variant thereof, CsOLS having a genomic nucleotide sequence as set forth in SEQ ID NO:10 or a functional variant thereof and CsAAE1 having a genomic nucleotide sequence as set forth in SEQ ID NO:13 or a functional variant thereof.

[0081] It is a further object of the present invention to disclose the method as defined in any of the above, wherein said functional variant has at least 75% sequence identity to the nucleotide sequence of said cannabinoid biosynthesis enzyme or a codon degenerate nucleotide sequence thereof.

[0082] It is a further object of the present invention to disclose the method as defined in any of the above, wherein said plant has decreased expression levels of at least one of CsTHCAS protein having an amino acid sequence with at least 75% identity to the amino acid sequence as set forth in SEQ ID NO:3 or a conservatively substituted amino acid sequence thereof, CsCBDAS protein having an amino acid sequence with at least 75% identity to the amino acid sequence as set forth in SEQ ID NO:6 or a conservatively substituted amino acid sequence thereof, CsPT protein having an amino acid sequence with at least 75% identity to the amino acid sequence as set forth in SEQ ID NO:9 or a conservatively substituted amino acid sequence thereof, CsOLS protein having an amino acid sequence with at least 75% identity to the amino acid sequence as set forth in SEQ ID NO:12 or a conservatively substituted amino acid sequence thereof, and CsAAE1 protein having

an amino acid sequence with at least 75% identity to the amino acid sequence as set forth in SEQ ID NO:15 or a conservatively substituted amino acid sequence thereof.

[0083] It is a further object of the present invention to disclose the method as defined in any of the above, wherein said Cannabis plant has modulated expression of one or more of the cannabinoids, optionally cannabigerolic acid, cannabigerol, DELTA.9-tetrahydrocannabinolic acid, cannabidiolic acid, cannabichromenic acid, DELTA.9-tetrahydrocannabinol, cannabidiol, cannabichromene, THC, D9-THC, D8-THC, THCA, THCV, D8-THCV, D9-THCV, THCVA, CBD, CBDA, CBDV, CBDVA, CBC, CBCA, CBCV, CBCVA, CBG, CBGA, CBGV, CBGVA, CBN, CBNA, CBNV, CBNVA, CBND, CBNDA, CBNDV, CBNDVA, CBE, CBEA, CBEV, CBEVA, CBL, CBLA, CBLV, or CBLVA and cannabidiol.

[0084] It is a further object of the present invention to disclose the method as defined in any of the above, wherein said Cannabis plant has reduced expression of one or more of the cannabinoids, optionally cannabigerolic acid, cannabigerol, DELTA.9-tetrahydrocannabinolic acid, cannabidiolic acid, cannabichromenic acid, DELTA.9-tetrahydrocannabinol, cannabidiol, cannabichromene, THC, D9-THC, D8-THC, THCA, THCV, D8-THCV, D9-THCV, THCVA, CBD, CBDA, CBDV, CBDVA, CBC, CBCA, CBCV, CBCVA, CBG, CBGA, CBGV, CBGVA, CBN, CBNA, CBNV, CBNVA, CBND, CBNDA, CBNDV, CBNDVA, CBE, CBEA, CBEV, CBEVA, CBL, CBLA, CBLV, or CBLVA and cannabidiol, as compared to a Cannabis plant of a similar genotype or genetic background that does not comprise said targeted genome modification.

[0085] It is a further object of the present invention to disclose the method as defined in any of the above, wherein said targeted genome modification is introduced using CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas) genes (CRISPR/Cas) system, Transcription activator-like effector nuclease (TALEN), Zinc Finger Nuclease (ZFN), meganuclease or any combination thereof.

[0086] It is a further object of the present invention to disclose the method as defined in any of the above, wherein said CRISPR/Cas genes or proteins are selected from the group consisting of Cas3, Cas4, Cas5, Cas5e (or CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9, Cas10, Cas10d, Cas12, Cas13, Cas14, CasX, CasF, CasG, CasH, Csy1, Csy2, Csy3, Cse1 (or CasA), Cse2 (or CasB), Cse3 (or CasE), Cse4 (or CasC), Csc1, Csc2, Csa5, Csn1, Csn2, Csm2,

Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Cpf1, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csz1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cu1966.

[0087] It is a further object of the present invention to disclose the method as defined in any of the above, wherein said targeted genome modification is introduced via (i) at least one RNA-guided (gRNA) endonuclease or nucleic acid encoding at least one RNA-guided endonuclease, and (ii) at least one guide RNA (gRNA) or DNA encoding at least one guide RNA (gRNA), further wherein each of said at least one gRNA directs said endonuclease to a targeted site located in the genomic sequence of said at least one Cannabis gene encoding a cannabinoid biosynthesis enzyme.

[0088] It is a further object of the present invention to disclose the method as defined in any of the above, wherein (a) the gRNA nucleotide sequence targeted to CsTHCAS genomic sequence is as set forth in nucleotide sequence selected from the group consisting of SEQ. ID. NO.: 16-167 and any combination thereof; (b) the gRNA nucleotide sequence targeted to CsCBDAS genomic sequence is as set forth in nucleotide sequence selected from the group consisting of SEQ. ID. NO.: 168-304, SEQ. ID. NO.: 824-825, and any combination thereof; (c) the gRNA nucleotide sequence targeted to CsPT genomic sequence is as set forth in nucleotide sequence selected from the group consisting of SEQ. ID. NO.: 305-458 and any combination thereof; (d) the gRNA nucleotide sequence targeted to CsOLS genomic sequence is as set forth in nucleotide sequence selected from the group consisting of SEQ. ID. NO.: 459-509 and any combination thereof; and (e) the gRNA nucleotide sequence targeted to CsAAE1 genomic sequence is as set forth in nucleotide sequence selected from the group consisting of SEQ. ID. NO.: 510-823, SEQ. ID. NO.: 826, and any combination thereof.

[0089] It is a further object of the present invention to disclose the method as defined in any of the above, wherein said plant is mutated in a gene selected from the group consisting of CsTHCAS, CsSP, CsCBDAS, CsPT, CsOLS, CsAAE1 and any combination thereof.

[0090] It is a further object of the present invention to disclose the method as defined in any of the above, wherein said at least one genome modification is generated *in planta*.

[0091] It is a further object of the present invention to disclose the method as defined in any of the above, wherein said genome modification is generated *in planta* via introduction of a construct comprising (a) Cas DNA and gRNA sequence selected from the group consisting of SEQ ID NO:16-SEQ ID NO:826 and any combination thereof, or (b) a ribonucleoprotein (RNP) complex

comprising Cas protein and gRNA sequence selected from the group consisting of SEQ ID NO:16-826 and any combination thereof.

[0092] It is a further object of the present invention to disclose the method as defined in any of the above, wherein said gRNA is introduced into the plant cells via Agrobacterium infiltration, virus based plasmids for delivery of the genome editing molecules or mechanical insertion such as polyethylene glycol (PEG) mediated DNA transformation, electroporation or gene gun biolistics.

[0093] It is a further object of the present invention to disclose a plant part, plant cell or plant seed produced by the method as defined in any of the above.

[0094] It is a further object of the present invention to disclose the method as defined in any of the above, wherein said Cannabis plant comprises a DNA encoding an antisense RNA or a siRNA effective to suppress expression of CsTHCAS, CsSP, CsCBDAS, CsPT, CsOLS, CsAAE1 and any combination thereof, the DNA operably linked to a heterologous promoter, wherein the Cannabis plant comprises reduced THC content, reduced CBD content, or decreased THC and CBD content relative to a Cannabis plant of a similar genotype that does not comprise the DNA.

[0095] It is a further object of the present invention to disclose a method for producing a medical Cannabis composition, the method comprising: (a) obtaining the Cannabis plant of claim 1; and (b) formulating a medical Cannabis composition from said plant.

[0096] It is a further object of the present invention to disclose a method for manipulating a content of one or more cannabinoids in a Cannabis plant, the method comprising down-regulating activity of at least one Cannabis gene encoding a cannabinoid biosynthesis enzyme selected from the group consisting of tetrahydrocannabinolic acid synthase (THCAS), cannabidiolic acid synthase (CBDAS), aromatic prenyltransferase (PT), olivetol synthase (OLS), acyl-activating enzyme 1 (AAE1) and any combination thereof.

[0097] It is a further object of the present invention to disclose the method as defined in any of the above, comprises steps of: (a) identifying at least one Cannabis gene locus encoding a cannabinoid biosynthesis enzyme selected from the group consisting of tetrahydrocannabinolic acid synthase (THCAS), cannabidiolic acid synthase (CBDAS), aromatic prenyltransferase (PT), olivetol synthase (OLS), acyl-activating enzyme 1 (AAE1) and any combination thereof; (b) identifying at least one endonuclease recognition sequence in or proximal to the at least one cannabinoid

biosynthesis enzyme gene locus; (c) providing at least one guide RNA (gRNA) comprising a nucleotide sequence at least partially complementary to said at least one identified gene locus; (d) introducing into Cannabis plant cells a construct comprising (i) an endonuclease nucleotide sequence operably linked to said gRNA, or (ii) a ribonucleoprotein (RNP) complex comprising an endonuclease protein and said gRNA; (e) assaying the Cannabis plant or a cell thereof for an endonuclease-mediated modification in the DNA of the at least one cannabinoid biosynthesis enzyme gene locus; and (f) identifying the Cannabis plant, a cell thereof, or a progeny cell thereof as comprising a modification in the at least one cannabinoid biosynthesis enzyme gene locus.

[0098] It is a further object of the present invention to disclose the method as defined in any of the above, further comprises steps of (a) screening the genome of the transformed Cannabis plant cells for induced targeted mutations in at least one of said cannabinoid biosynthesis enzyme gene locus comprising obtaining a nucleic acid sample from said transformed plant and carrying out nucleic acid amplification and optionally restriction enzyme digestion to detect a mutation in said at least one of said cannabinoid biosynthesis enzyme gene locus; (b) confirming the presence of said genetic mutation in the genome of said plant cells by sequencing said at least one cannabinoid biosynthesis enzyme gene locus; (c) regenerating plants carrying said genetic modification; and (d) screening said regenerated plants for a plant with modified cannabinoid content.

[0099] It is a further object of the present invention to disclose the method as defined in any of the above, wherein the endonuclease is expressed transiently or stably in the Cannabis plant.

[00100] It is a further object of the present invention to disclose an isolated nucleotide sequence having at least 75% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:10, and SEQ ID NO:13.

[00101] It is a further object of the present invention to disclose an isolated nucleotide sequence having at least 75% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11 and SEQ ID NO:14.

[00102] It is a further object of the present invention to disclose an isolated amino acid sequence having at least 75% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:12 and SEQ ID NO:15.

[00103] It is a further object of the present invention to disclose an isolated nucleotide sequence having at least 75% sequence identity to a nucleotide sequence as set forth in SEQ ID NO:16-826.

[00104] It is a further object of the present invention to disclose a vector, construct or expression system or cassette comprising the nucleic acid sequence as defined in any one of claims 90-93.

[00105] It is a further object of the present invention to disclose use of a nucleotide sequence as set forth in at least one of SEQ ID NO:16-826 and any combination thereof for down regulation of at least one Cannabis cannabinoid biosynthesis enzyme gene selected from the group consisting of Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme 1 (CsAAE1) and any combination thereof.

[00106] It is a further object of the present invention to disclose use of a nucleotide sequence as set forth in at least one of SEQ ID NO:16-167 and any combination thereof for targeted genome modification of Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS) gene.

[00107] It is a further object of the present invention to disclose use of a nucleotide sequence as set forth in at least one of SEQ ID NO:168-304, SEQ ID NO:824-825, and any combination thereof for targeted genome modification of Cannabis cannabidiolic acid synthase (CsCBDAS).

[00108] It is a further object of the present invention to disclose use of a nucleotide sequence as set forth in at least one of SEQ ID NO:305-458 and any combination thereof for targeted genome modification of Cannabis aromatic prenyltransferase (CsPT).

[00109] It is a further object of the present invention to disclose use of a nucleotide sequence as set forth in at least one of SEQ ID NO:459-509 and any combination thereof for targeted genome modification of Cannabis olivetol synthase (CsOLS).

[00110] It is a further object of the present invention to disclose use of a nucleotide sequence as set forth in at least one of SEQ ID NO:510-823, SEQ ID NO:826, and any combination thereof for targeted genome modification of Cannabis acyl-activating enzyme 1 (CsAAE1).

BRIEF DESCRIPTION OF THE DRAWINGS

[00111] Non-limiting examples of embodiments of the disclosure are described below with reference to figures attached hereto that are listed following this paragraph. Identical features that appear in more than one figure are generally labeled with a same label in all the figures in which they appear. A label labeling an icon representing a given feature of an embodiment of the disclosure in a figure may be used to reference the same given feature in other embodiments. Dimensions of features shown in the figures are chosen for convenience and clarity of presentation and are not necessarily shown to scale.

[00112] The invention, features and advantages thereof, may best be understood by reference to the following detailed description when read with the accompanied drawings. Embodiments of the invention are illustrated by way of example and not limitation in the figures of the accompanying drawings, in which like reference numerals indicate corresponding, analogous or similar elements, and in which:

[00113] **Fig. 1** is schematically presenting CRISPR/Cas9 mode of action as depicted by Xie, Kabin, and Yinong Yang. "RNA-guided genome editing in plants using a CRISPRtCas system." Molecular plant 6.6 (2013): 1975-1983;

[00114] **Fig. 2** is schematically illustrating the cannabinoid biosynthesis pathway as depicted by the *C. sativa* (Cannabis) Genome Browser internet site;

[00115] **Fig. 3** is photographically presenting staining of Cannabis plants after transient GUS transformation of (A) axillary buds (B) leaf (C) calli, and (D) cotyledons;

[00116] **Fig. 4** is presenting regenerated transformed Cannabis tissue;

[00117] **Fig. 5** is photographically presenting PCR detection of Cas9 DNA in shoots of Cannabis plants transformed using biolistics; and

[00118] **Fig. 6** is illustrating *in vitro* cleavage activity of CRISPR/Cas9; (A) a scheme of genomic area targeted for editing, and (B) a gel showing digestion of PCR amplicon containing RNP complex of Cas9 and gene specific gRNA.

[00119] It will be appreciated that, for simplicity and clarity of illustration, elements shown in the figures have not necessarily been drawn accurately or to scale. For example, the dimensions of some of the elements may be exaggerated relative to other elements for clarity, or several

physical components may be included in one functional block or element. Further, where considered appropriate, reference numerals may be repeated among the figures to indicate corresponding or analogous elements.

DETAILED DESCRIPTION OF THE INVENTION

[00120] In the following detailed description, numerous specific details are set forth in order to provide a thorough understanding of the invention. However, it will be understood by those skilled in the art that the present invention may be practiced without these specific details. In other instances, well-known methods, procedures, and components, modules, units and/or circuits have not been described in detail so as not to obscure the invention. Some features or elements described with respect to one embodiment may be combined with features or elements described with respect to other embodiments. For the sake of clarity, discussion of same or similar features or elements may not be repeated.

[00121] In the following detailed description of the preferred embodiments, reference is made to the accompanying drawings that form a part hereof, and in which are shown by way of illustration specific embodiments in which the invention may be practiced. It is understood that other embodiments may be utilized and structural changes may be made without departing from the scope of the present invention. The present invention may be practiced according to the claims without some or all of these specific details. For the purpose of clarity, technical material that is known in the technical fields related to the invention has not been described in detail so that the present invention is not unnecessarily obscured.

[00122] The present invention discloses manipulation of the biosynthesis pathways of a Cannabis plant of genus Cannabis. Accordingly, Cannabis plants of the present invention having a modified therapeutic component(s) profile may be useful in the production of medical Cannabis and/or may also be useful in the production of specific components or therapeutic formulations derived therefrom.

[00123] According to other main aspects of the present invention, THC free (e.g, hemp) plants for seeds, fiber and/or medical use are produced

[00124] According to a main embodiment, the present invention provides a Cannabis plant with reduced delta-9-tetrahydrocannabinol (THC) content, or reduced cannabidiol (CBD) content,

or reduced THC and CBD content, wherein said plant comprises at least one targeted genome modification effective in decreasing expression of at least one Cannabis gene encoding a cannabinoid biosynthesis enzyme selected from the group consisting of Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme 1 (CsAAE1) and any combination thereof.

[00125] According to one embodiment of the present invention, genes encoding cannabinoid precursor synthesis enzymes, namely AAE, PT and OLS are down regulated using targeted genome modification (e.g. gene editing techniques as *inter alia* presented). These enzymes are responsible for the production of a main cannabinoid precursor cannabigerolic acid (CBGA).

[00126] The final steps catalysing the synthesis of major active cannabinoids, namely, cannabichromenic acid (CBCA), cannabidiolic acid (CBDA) and Δ^9 -tetrahydrocannabinolic acid (THCA), are performed by oxidocyclases, namely, CBCA synthase (CBCAS), CBDA synthase (CBDAS) and THCA synthase (THCAS).

[00127] According to a further embodiment of the present invention, cannabinoid biosynthesis enzymes namely cannabidiolic acid (CBDA) synthase (CBDAS) and/or Δ^9 -tetrahydrocannabinolic acid (THCA) synthase (THCAS) are down regulated using targeted genome modification (e.g. gene editing techniques as *inter alia* presented). As a result, the production of the major cannabinoids CBDA (converted into CBD by decarboxylation) and/or THCA (converted into THC by decarboxylation) is significantly reduced and/or totally abolished.

[00128] Thus, according to one embodiment, targeted genome modification of one or more of the herein identified Cannabis genes encoding cannabinoid precursor synthesis enzymes, namely, CsAAE, CsPT and CsOLS, negatively affects the production of the cannabinoid precursor CBGA. As a result Cannabis plants with reduced content, or free of, THCA (and/or THC) and reduced content or free of CBDA (and/or CBD) are provided by the present invention.

[00129] According to a further embodiment of the present invention, targeted genome modification of the herein identified Cannabis gene encoding cannabinoid synthesis enzyme CsTHCAS results in reduced or no production of THCA and thus Cannabis plants with reduced content, or free of, THCA and/or THC are provided by the present invention.

[00130] According to a further embodiment of the present invention, targeted genome modification of the herein identified Cannabis gene encoding cannabinoid synthesis enzyme CsCBDAS results in reduced or no production of CBDA and thus Cannabis plants with reduced content, or free of, CBDA and/or CBD are provided by the present invention.

[00131] Breeding Cannabis plants is currently mostly done by small Cannabis growers. There is very limited if any molecular tools supporting or leading the breeding process. Traditional Cannabis breeding is done by mixing breeding material with hope to find the desired traits and phenotypes in random crosses. These methods have allowed the construction of the leading Cannabis varieties on the market today. During the last few decades, most of the breeding was focused on the psychoactive phytochemicals of the Cannabis plant. These phytochemicals, known as cannabinoids, are the compounds responsible for the medical attributes of the Cannabis plant.

[00132] As the medical Cannabis pharmaceutical industry is focusing on developing new cannabinoid based drugs, and these are mostly extracted from the Cannabis plant, there is a growing need for Cannabis plants bred for producing high levels of specific cannabinoids. In addition, there is a need for advanced breeding programs for food and fiber (Hemp) as well.

[00133] The present invention is aimed at enhancing cannabinoid breeding capabilities by using advanced molecular genome editing technologies in order to maximize the plants' phytochemical molecules production potential.

[00134] According to a further aspect of the present invention, a method or a tool is provided that enables the regulation *in planta* or the production of specific cannabinoid molecules.

[00135] It is further within the scope of the present invention to provide means and methods for *in planta* modification of specific genes that relate to and/or control the cannabinoid biosynthesis pathways (as indicated in Fig. 2). More specifically, but not limited to, the present invention achieves the use of the CRISPR/Cas technology (see Fig. 1), such as, but not limited to Cas9 or Cpf1, in order to generate knockout alleles of the genes depicted in Fig. 2, rendering the enzymes inactive thereby controlling *in planta* the production of the resulting cannabinoid products depicted in Fig. 2.

[00136] According to some embodiments of the present invention, the above *in planta* modification can be based on alternative gene silencing technologies such as Zinc Finger

Nucleases (ZFN's), Transcription activator-like effector nucleases (TALEN's), RNA silencing, amiRNA or any other gene silencing technique known in the art.

[00137] According to some other embodiments of the present invention, DNA introduction into the plant cells can be done by Agrobacterium infiltration, viral based plasmids for virus induced gene silencing (VIGS) and by mechanical insertion of DNA (PEG, gene gun etc).

[00138] According to further aspects of the present invention, it is possible to directly insert the Cas9 protein together with a gRNA in order to bypass the need for *in vivo* transcription and translation of the Cas9 + gRNA plasmid *in planta* in order to achieve the same desired outcome.

[00139] It is a core aspect of the present invention that the above CRISPR/Cas system allows the modification of specific DNA sequences. This is achieved by combining the Cas nuclease (Cas9, Cpf1 or the like) with a guide RNA molecule (gRNA). The gRNA is designed such that it should be complementary to a specific DNA sequence targeted for editing in the plant genome and which guides the Cas nuclease to a specific nucleotide sequence (see Fig. 1). Gene specific gRNA's are cloned into the same plasmid as the Cas gene and this plasmid is inserted into plant cells. Insertion of this plasmid DNA can be done, but not limited to, by different delivery systems biological and or mechanical.

[00140] Without wishing to be bound by theory, according to further specific aspects of the present invention, upon reaching the specific DNA sequence, the Cas9 nuclease cleaves both DNA strands to create double stranded breaks leaving blunt ends. This cleavage site is then repaired by the cellular non homologous end joining DNA repair mechanism resulting in insertions or deletions which eventually creates a mutation around the cleavage site. The deletion form of the mutation consists of at least 1 base pair deletion. As a result of this base pair deletion the gene coding sequence is disrupted and the translation of the encoded protein is compromised either by a premature stop codon or disruption of a functional or structural property of the protein.

[00141] It is further within the scope that by introducing a gRNA with homology to a specific site of a gene described in Fig. 2, and sub cloning this gRNA into a plasmid containing the Cas9 gene, and upon insertion of the described plasmid into the plant cells, site specific mutations are generated in the genes herein described (delta-9-tetrahydrocannabinol (THC) content, or reduced cannabidiol (CBD) content, or reduced THC and CBD content, wherein said plant comprises a targeted genome modification effective in decreasing expression of a at least

one Cannabis gene encoding a cannabinoid biosynthesis enzyme selected from the group consisting of Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme 1 (CsAAE1) and any combination thereof). Thus effectively creating non-active proteins in the cannabinoid biosynthesis pathway, resulting in inactivation of their enzymatic activity. As a result, the present disclosure enables altering cannabinoid content in the genome edited plant. This alteration of cannabinoid content can result in a plant with significantly reduced synthesis of the molecules depicted in Fig. 2 and/or of one or more cannabinoids produced by these enzymes.

[00142] It is herein acknowledged that as the pharma industry is interested in extracting the cannabinoids from the Cannabis plant, individual Cannabis plants or strains or varieties containing modulated levels of such cannabinoids can be developed, tailored to the specific needs of the pharma industry thereby increasing the cost effectiveness and attractiveness of this crop.

[00143] The solution proposed by the current invention is using genome editing such as the CRISPR/Cas system in order to create cultivated Cannabis plants with modulated levels or ratios of cannabinoids. More specifically alternation of specific cannabinoids, i.e. THC and CBD is achieved by using genome editing techniques to reduce the expression of enzymes in the cannabinoid biosynthesis pathway.

[00144] Breeding using genome editing allows a precise and significantly shorter breeding process in order to achieve these goals with a much higher success rate. Thus genome editing, has the potential to generate improved varieties faster and at a lower cost.

[00145] In order to generate a reproducible product, Cannabis growers are currently using vegetative propagation (cloning or tissue culture). However, in conventional agricultural, genetic stability of field crops and vegetables is maintained by using F1 hybrid seeds. These hybrids are generated by crossing homozygous parental lines.

[00146] The next step for the Cannabis industry is the adoption and use of hybrid seeds for propagation, which is common practice in the conventional seed industry (from field crops to vegetables). This will allow growing and supplying high quality and reproducible raw material for the pharmaceutical industry.

[00147] The current invention discloses the generation of non GMO Cannabis plants with manipulated and controlled cannabinoid content, using the genome editing technology, e.g., the CRISPR/Cas9 highly precise tool. The generated mutations can be introduced into elite or locally adapted Cannabis lines rapidly, with relatively minimal effort and investment.

[00148] Genome editing is an efficient and useful tool for increasing crop productivity traits, and there is particular interest in advancing manipulation of genes controlling cannabinoids biosynthesis in Cannabis species, to produce strains which are adapted to specific therapeutic or regulatory needs.

[00149] Genome-editing technologies, such as the clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein-9 nuclease (Cas9) (CRISPR–Cas9) provide opportunities to address these deficiencies, with the aims of increasing quality and yield.

[00150] A major obstacle for CRISPR–Cas9 plant genome editing is lack of efficient tissue culture and transformation methodologies. The present invention achieves these aims and surprisingly provides transformed and regenerated Cannabis plants with modified desirable cannabinoids content.

[00151] To that end, guide RNAs (gRNAs) were designed for each of the target genes herein identified in Cannabis to induce mutations in at least one cannabinoid biosynthesis enzyme including Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme 1 (CsAAE1) and any combination thereof.

[00152] The present invention shows that Cannabis plants which contain genome editing events with at least one of the CsAAE1, CsOLS, CsPT genes or any combination thereof, express not more than 0.5% THC (or THCA) and CBD (or THCA) by weight. In specific embodiments, such plants express less than 0.5%, preferably less than 0.4%, less than 0.3%, less than 0.2%, less than 0.1% or 0% THC and CBD by weight (e.g. by dry weight).

[00153] It is further within the scope that Cannabis plants which contain genome editing events with at least one of the CsAAE1, CsOLS, CsPT genes or any combination thereof, express not more than 0.5% THC (or THCA) and/or CBD (or THCA) by weight. In specific embodiments,

such plants express less than 0.5%, preferably less than 0.4%, less than 0.3%, less than 0.2%, less than 0.1% or 0% THC and/or CBD by weight (e.g. by dry weight).

[00154] The present invention further shows that Cannabis plants containing genome editing events within the CsTHCAS gene express higher levels of CBD (or CBDA) compared to non-edited plants. In a further embodiment, the CsTHCAS edited plants contain very low levels of THCA (or THC), preferably not more than 0.5% by weight.

[00155] The present invention further shows that plants containing genome editing events within the CsCBDAS gene express higher levels of THC (or THCA) as compared to non-edited plants. In a further embodiment, the CsCBDAS edited plants contain very low levels of CBDA (or CBD), preferably not more than 0.5% by weight.

[00156] It is a further aspect of the present invention to provide the Cannabis plant as defined in any of the above, wherein said plant has a THC and/or CBD content of up to 30% by weight, particularly between about %0.1 to about 30% by weight, more particularly between about %0.3 to about 30%, even more particularly between about %0.3 to about 10% by weight.

[00157] As used herein the term "**about**" denotes $\pm 25\%$ of the defined amount or measure or value.

[00158] As used herein the term "**similar**" denotes a correspondence or resemblance range of about $\pm 20\%$, particularly $\pm 15\%$, more particularly about $\pm 10\%$ and even more particularly about $\pm 5\%$.

[00159] As used herein the term "**corresponding**" generally means similar, analogous, like, alike, akin, parallel, identical, resembling or comparable. In further aspects it means having or participating in the same relationship (such as type or species, kind, degree, position, correspondence, or function). It further means related or accompanying. In some embodiments of the present invention it refers to plants of the same Cannabis species or strain or variety or to sibling plant, or one or more individuals having one or both parents in common.

[00160] A "**plant**" as used herein refers to any plant at any stage of development, particularly a seed plant. The term "plant" includes the whole plant or any parts or derivatives thereof, such as plant cells, seeds, plant protoplasts, plant cell tissue culture from which tomato plants can be regenerated, plant callus or calli, meristematic cells, microspores, embryos, immature

embryos, pollen, ovules, anthers, fruit, flowers, leaves, cotyledons, pistil, seeds, seed coat, roots, root tips and the like.

[00161] The term "**plant cell**" used herein refers to a structural and physiological unit of a plant, comprising a protoplast and a cell wall. The plant cell may be in a form of an isolated single cell or a cultured cell, or as a part of higher organized unit such as, for example, plant tissue, a plant organ, or a whole plant.

[00162] The term "**plant cell culture**" as used herein means cultures of plant units such as, for example, protoplasts, regenerable cells, cell culture, cells, cells in plant tissues, pollen, pollen tubes, ovules, embryo sacs, zygotes and embryos at various stages of development, leaves, roots, root tips, anthers, meristematic cells, microspores, flowers, cotyledons, pistil, fruit, seeds, seed coat or any combination thereof.

[00163] The term "**plant material**" or "**plant part**" used herein refers to leaves, stems, roots, root tips, flowers or flower parts, fruits, pollen, egg cells, zygotes, seeds, seed coat, cuttings, cell or tissue cultures, or any other part or product of a plant or a combination thereof.

[00164] A "**plant organ**" as used herein means a distinct and visibly structured and differentiated part of a plant such as a root, stem, leaf, flower, flower bud, or embryo.

[00165] The term "**Plant tissue**" as used herein means a group of plant cells organized into a structural and functional unit. Any tissue of a plant *in planta* or in culture is included. This term includes, but is not limited to, whole plants, plant organs, plant seeds, tissue culture, protoplasts, meristematic cells, calli and any group of plant cells organized into structural and/or functional units. The use of this term in conjunction with, or in the absence of, any specific type of plant tissue as listed above or otherwise embraced by this definition is not intended to be exclusive of any other type of plant tissue.

[00166] As used herein, the term "**progeny**" or "**progenies**" refers in a non limiting manner to offspring or descendant plants. According to certain embodiments, the term "progeny" or "progenies" refers to plants developed or grown or produced from the disclosed or deposited seeds as detailed *inter alia*. The grown plants preferably have the desired traits of the disclosed or deposited seeds, i.e. loss of function mutation in at least one CsSP gene or at least one CsSP5G gene.

[00167] The term “**Cannabis**” refers hereinafter to a genus of flowering plants in the family Cannabaceae. Cannabis is an annual, dioecious, flowering herb that includes, but is not limited to three different species, *Cannabis sativa*, *Cannabis indica* and *Cannabis ruderalis*. The term also refers to hemp. Cannabis plants produce a group of chemicals called cannabinoids. Cannabinoids, terpenoids, and other compounds are secreted by glandular trichomes that occur most abundantly on the floral calyxes and bracts of female Cannabis plants.

[00168] The term “**nonpsychoactive**” refers hereinafter to products or compositions or elements or components of Cannabis not significantly affecting the mind or mental processes.

[00169] The term “**cannabinoid**” refers hereinafter to a class of diverse chemical compounds that act on cannabinoid receptors on cells that repress neurotransmitter release in the brain. These receptor proteins include the endocannabinoids (produced naturally in the body by humans and animals), the phytocannabinoids (found in Cannabis and some other plants), and synthetic cannabinoids.

[00170] The main cannabinoids are concentrated in a viscous resin produced in structures known as glandular trichomes. Up until now, at least 113 different cannabinoids have been isolated from the Cannabis plant. The main classes of cannabinoids from Cannabis are THC (tetrahydrocannabinol), THCA (tetrahydrocannabinolic acid), CBD (cannabidiol), CBDA (cannabidiolic acid), CBN (cannabinol), CBG (cannabigerol), CBC (cannabichromene), CBL (cannabicyclol), CBV (cannabivarin), THCV (tetrahydrocannabivarin), CBDV (cannabidivarin), CBCV (cannabichromevarin), CBGV (cannabigerovarin), CBGM (cannabigerol monomethyl ether), CBE (cannabielsoin), CBT (cannabicitran) and any combination thereof.

[00171] The best studied cannabinoids include tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN).

[00172] Reference is now made to Tetrahydrocannabinol (THC), the primary psychoactive component of the Cannabis plant. Delta-9-tetrahydrocannabinol (Δ^9 -THC, THC) and delta-8-tetrahydrocannabinol (Δ^8 -THC), through intracellular CB1 activation, induce anandamide and 2-arachidonoylglycerol synthesis produced naturally in the body and brain. These cannabinoids produce the effects associated with Cannabis by binding to the CB1 cannabinoid receptors in the brain.

[00173] Tetrahydrocannabinolic acid (THCA, 2-COOH-THC; conjugate base tetrahydrocannabinolate) is a precursor of tetrahydrocannabinol (THC), the active component of cannabis. THCA is found in variable quantities in fresh, undried cannabis, but is progressively decarboxylated to THC with drying, and especially under intense heating such as when cannabis is smoked or cooked into cannabis edibles. In the context of the present invention, the term THC also refers to THCA and *vice versa*.

[00174] Reference is now made to Cannabidiol (CBD) which is considered as non-psychoactive. Cannabidiol has little affinity for CB1 and CB2 receptors but acts as an indirect antagonist of cannabinoid agonists. It is further acknowledged herein that it is an antagonist at the putative cannabinoid receptor, GPR55, a GPCR expressed in the caudate nucleus and putamen. Cannabidiol has also been shown to act as a 5-HT1A receptor agonist. Cannabis produces CBD-carboxylic acid through the same metabolic pathway as THC, until the next to last step, where CBDA synthase performs catalysis instead of THCA synthase. CBDA is converted into CBD by decarboxylation. In the context of the present invention, the term CBD also refers to CBDA and *vice versa*.

[00175] CBD shares a precursor with THC and is the main cannabinoid in CBD-dominant Cannabis strains.

[00176] In the context of the current invention, enzymes within the biosynthetic pathway of THC and CBD, especially AAE, OLS, PT, CBDAS and THCAS (depicted in Fig. 2), are down regulated to control the content of CBD and/or THC in the Cannabis plant.

[00177] Reference is now made to Fig. 2 schematically illustrating the proposed pathway leading to the major cannabinoids Δ^9 -tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), which decarboxylate to yield Δ^9 -tetrahydrocannabinol (THC) and cannabidiol (CBD), respectively. The biosynthesis of THC and CBD in Cannabis follows a similar pathway.

[00178] Cannabigerolic acid (CBGA), the precursor to all natural cannabinoids, is cyclized into tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA) by THCA and CBDA synthase (THCAS and CBDAS in Fig. 2), respectively. The final products of THC and CBD are formed via decarboxylation of these acidic forms. Structurally, there is an important difference between these major cannabinoids. Where THC contains a cyclic ring, CBD contains a hydroxyl

group. This seemingly small difference in molecular structure may give the two compounds their different pharmacological properties.

[00179] A more specific and detailed description of the biosynthetic pathway of cannabinoids in the Cannabis plant follows below:

[00180] The isoprenoid and prenyl precursors for cannabigerolic acid (CBGA), are provided by the hexanoate and 2-C-methyl-D-erythritol 4-phosphate (MEP) pathways, respectively. Geranyl diphosphate (GPP), is a key intermediate metabolite and building block for both cannabinoid and terpenoid biosynthesis. The seven-step mevalonate (MVA) pathway converts pyruvate and glyceraldehyde-3-phosphate (G-3-P) into isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Key catalytic enzymes controlling flux through this pathway include the first two steps, 1-deoxy-D-xylulose 5-phosphate synthase (DXS) and 1-deoxy-D-xylulose 5-phosphate reductase (DXR). In the six-step MEP pathway, three units of acetyl coenzyme A (CoA) are converted to IPP, which is isomerized with DMAPP by IPP isomerase. The enzyme catalysing the synthesis of MEV, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), is considered to control flux through this pathway. The number of consecutive condensations of the five-carbon monomer isopentenyl diphosphate (IPP) to its isomer, dimethylallyl diphosphate (DMAPP) is indicated by 1x, 2x, 3x. Longer-chain isoprenoids, GPP, farnesyl diphosphate (FPP) and geranyl geranyl diphosphate (GGPP), are the products of IPP and DMAPP condensation catalysed by GPP synthase, FPP synthase and GGPP synthase, respectively. GPP, FPP and geranyl-geranyl diphosphate (GGPP) are the precursors for mono-, sequi-, and di-terpines, respectively. The final steps catalysing the synthesis of major active cannabinoids, cannabichromenic acid (CBCA), cannabidiolic acid (CBDA) and Δ^9 -tetrahydrocannabinolic acid (THCA), are oxidocyclases, CBCA synthase (CBCAS), CBDA synthase (CBDAS) and THCA synthase (THCAS).

[00181] In the current invention, AAE, refers to acyl-activating enzyme; CBD: cannabidiol; CYP76F39, α/β -santalene monooxygenase; GPP synthase small subunit; OLS, olivetol synthase; P450: haemoprotein cytochrome P450; PT, prenyltransferase; STS, santalene synthase; TS, gamma-terpinene synthase; and TXS, taxadiene synthase.

[00182] As used herein the term "**genetic modification**" or "**genome modification**" refers hereinafter to genetic manipulation or modulation, which is the direct manipulation of an

organism's genes using biotechnology. It also refers to a set of technologies used to change the genetic makeup of cells, including the transfer of genes within and across species, targeted mutagenesis and genome editing technologies to produce improved organisms. According to main embodiments of the present invention, modified Cannabis plants with altered cannabinoid content traits are generated using genome editing mechanism. This technique enables to achieve *in planta* modification of specific genes that control the biosynthesis of main cannabinoids, namely, THC and/or CBD in the Cannabis plant.

[00183] The term "**genome editing**", or "**genome/genetic modification**" or "**genome engineering**" generally refers hereinafter to a type of genetic engineering in which DNA is inserted, deleted, modified or replaced in the genome of a living organism. Unlike previous genetic engineering techniques that randomly insert genetic material into a host genome, genome editing targets the insertions to site specific locations.

[00184] It is within the scope of the present invention that the common methods for such editing use engineered nucleases, or "molecular scissors". These nucleases create site-specific double-strand breaks (DSBs) at desired locations in the genome. The induced double-strand breaks are repaired through nonhomologous end-joining (NHEJ) or homologous recombination (HR), resulting in targeted mutations ('edits'). Families of engineered nucleases used by the current invention include, but are not limited to: meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector-based nucleases (TALEN), and the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) system.

[00185] Reference is now made to exemplary genome editing terms used by the current disclosure:

Genome Editing Glossary

Cas = CRISPR-associated genes

Cas9, Csn1 = a CRISPR-associated protein containing two nuclease domains, that is programmed by small RNAs to cleave DNA

crRNA = CRISPR RNA

dCAS9 = nuclease-deficient Cas9

DSB = Double-Stranded Break

gRNA = guide RNA

HDR = Homology-Directed Repair

HNH = an endonuclease domain named for characteristic histidine and asparagine residues

Indel = insertion and/or deletion

NHEJ = Non-Homologous End Joining

PAM = Protospacer-Adjacent Motif

RuvC = an endonuclease domain named for an *E. coli* protein involved in DNA repair

sgRNA = single guide RNA

tracrRNA, trRNA = trans-activating crRNA

TALEN = Transcription-Activator Like Effector Nuclease

ZFN = Zinc-Finger Nuclease

[00186] According to specific aspects of the present invention, the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas) genes are used for the first time for generating genome modification in targeted genes in the Cannabis plant. It is herein acknowledged that the functions of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas) genes are essential in adaptive immunity in select bacteria and archaea, enabling the organisms to respond to and eliminate invading genetic material. These repeats were initially discovered in the 1980s in *E. coli*. Without wishing to be bound by theory, reference is now made to a type of CRISPR mechanism, in which invading DNA from viruses or plasmids is cut into small fragments and incorporated into a CRISPR locus comprising a series of short repeats (around 20 bps). The loci are transcribed, and transcripts are then processed to generate small RNAs (crRNA, namely CRISPR RNA), which are used to guide effector endonucleases that target invading DNA based on sequence complementarity.

[00187] According to further aspects of the invention, Cas protein, such as Cas9 (also known as Csn1) is required for gene silencing. Cas9 participates in the processing of crRNAs, and is responsible for the destruction of the target DNA. Cas9's function in both of these steps relies on the presence of two nuclease domains, a RuvC-like nuclease domain located at the amino

terminus and a HNH-like nuclease domain that resides in the mid-region of the protein. To achieve site-specific DNA recognition and cleavage, Cas9 is complexed with both a crRNA and a separate trans-activating crRNA (tracrRNA or trRNA), that is partially complementary to the crRNA. The tracrRNA is required for crRNA maturation from a primary transcript encoding multiple pre-crRNAs. This occurs in the presence of RNase III and Cas9.

[00188] Without wishing to be bound by theory, it is herein acknowledged that during the destruction of target DNA, the HNH and RuvC-like nuclease domains cut both DNA strands, generating double-stranded breaks (DSBs) at sites defined by a 20-nucleotide target sequence within an associated crRNA transcript. The HNH domain cleaves the complementary strand, while the RuvC domain cleaves the noncomplementary strand.

[00189] It is further noted that the double-stranded endonuclease activity of Cas9 also requires that a short conserved sequence, (2–5 nts) known as protospacer-associated motif (PAM), follows immediately 3'- of the crRNA complementary sequence.

[00190] According to further aspects of the invention, a two-component system may be used by the current invention, combining trRNA and crRNA into a single synthetic single guide RNA (sgRNA) for guiding targeted gene alterations.

[00191] It is further within the scope that Cas9 nuclease variants include wild-type Cas9, Cas9D10A and nuclease-deficient Cas9 (dCas9).

[00192] Reference is now made to **Fig. 1** schematically presenting an example of CRISPR/Cas9 mechanism of action as depicted by Xie, Kabin, and Yinong Yang. "*RNA-guided genome editing in plants using a CRISPR–Cas system.*" *Molecular plant* 6.6 (2013): 1975-1983. As shown in this figure, the Cas9 endonuclease forms a complex with a chimeric RNA (called guide RNA or gRNA), replacing the crRNA–transcrRNA heteroduplex, and the gRNA could be programmed to target specific sites. The gRNA–Cas9 should comprise at least 15-base-pairing (gRNA seed region) without mismatch between the 5'-end of engineered gRNA and targeted genomic site, and an NGG motif (called protospacer-adjacent motif or PAM) that follows the base-pairing region in the complementary strand of the targeted DNA.

[00193] The term "**meganucleases**" as used herein refers hereinafter to endodeoxyribonucleases characterized by a large recognition site (double-stranded DNA

sequences of 12 to 40 base pairs); as a result this site generally occurs only once in any given genome. Meganucleases are therefore considered to be the most specific naturally occurring restriction enzymes.

[00194] The term "**protospacer adjacent motif**" or "**PAM**" as used herein refers hereinafter to a 2-6 base pair DNA sequence immediately following the DNA sequence targeted by the Cas9 nuclease in the CRISPR bacterial adaptive immune system. PAM is a component of the invading virus or plasmid, but is not a component of the bacterial CRISPR locus. PAM is an essential targeting component which distinguishes bacterial self from non-self DNA, thereby preventing the CRISPR locus from being targeted and destroyed by nuclease.

[00195] The term "**Next-generation sequencing**" or "**NGS**" as used herein refers hereinafter to massively, parallel, high- throughput or deep sequencing technology platforms that perform sequencing of millions of small fragments of DNA in parallel. Bioinformatics analyses are used to piece together these fragments by mapping the individual reads to the reference genome.

[00196] The term "**gene knockdown**" as used herein refers hereinafter to an experimental technique by which the expression of one or more of an organism's genes is reduced. The reduction can occur through genetic modification, i.e. targeted genome editing or by treatment with a reagent such as a short DNA or RNA oligonucleotide that has a sequence complementary to either gene or an mRNA transcript. The reduced expression can be at the level of RNA or at the level of protein. It is within the scope of the present invention that the term gene knockdown also refers to a loss of function mutation and /or gene knockout mutation in which an organism's genes is made inoperative or nonfunctional.

[00197] The term "**gene silencing**" as used herein refers hereinafter to the regulation of gene expression in a cell to prevent the expression of a certain gene. Gene silencing can occur during either transcription or translation. In certain aspects of the invention, gene silencing is considered to have a similar meaning as gene knockdown. When genes are silenced, their expression is reduced. In contrast, when genes are knocked out, they are completely not expressed. Gene silencing may be considered a gene knockdown mechanism since the methods used to silence genes, such as RNAi, CRISPR, or siRNA, generally reduce the expression of a gene by at least 70% but do not completely eliminate it.

[00198] The term "**loss of function mutation**" as used herein refers to a type of mutation in which the altered gene product lacks the function of the wild-type gene. A synonyms of the term included within the scope of the present invention is null mutation.

[00199] The term "**microRNAs**" or "**miRNAs**" refers hereinafter to small non-coding RNAs that have been found in most of the eukaryotic organisms. They are involved in the regulation of gene expression at the post-transcriptional level in a sequence specific manner. MiRNAs are produced from their precursors by Dicer-dependent small RNA biogenesis pathway. MiRNAs are candidates for studying gene function using different RNA-based gene silencing techniques. For example, artificial miRNAs (amiRNAs) targeting one or several genes of interest is a potential tool in functional genomics.

[00200] The term "***in planta***" means in the context of the present invention within the plant or plant cells. More specifically, it means introducing CRISPR/Cas complex into plant material comprising a tissue culture of several cells, a whole plant, or into a single plant cell, without introducing a foreign gene or a mutated gene. It also used to describe conditions present in a non-laboratory environment (e.g. *in vivo*).

[00201] The term "**genotype**" or "**genetic background**" refers hereinafter to the genetic constitution of a cell or organism. An individual's genotype includes the specific alleles, for one or more genetic marker loci, present in the individual's haplotype. As is known in the art, a genotype can relate to a single locus or to multiple loci, whether the loci are related or unrelated and/or are linked or unlinked. In some embodiments, an individual's genotype relates to one or more genes that are related in that the one or more of the genes are involved in the expression of a phenotype of interest. Thus, in some embodiments a genotype comprises a summary of one or more alleles present within an individual at one or more genetic loci. In some embodiments, a genotype is expressed in terms of a haplotype. It further refers to any inbreeding group, including taxonomic subgroups such as subspecies, taxonomically subordinate to species and superordinate to a race or subrace and marked by a pre-determined profile of latent factors of hereditary traits.

[00202] The term "**orthologue**" as used herein refers hereinafter to one of two or more homologous gene sequences found in different species.

[00203] The term "**functional variant**" or "**functional variant of a nucleic acid or amino acid sequence**" as used herein, for example with reference to SEQ ID NOs: 1, 4 or 7 refers to a

variant of a sequence or part of a sequence which retains the biological function of the full non-variant allele and hence has the activity of the expressed gene or protein. A functional variant also comprises a variant of the gene of interest encoding a polypeptide which has sequence alterations that do not affect function of the resulting protein, for example, in non-conserved residues. Also encompassed is a variant that is substantially identical, i.e. has only some sequence variations, for example, in non-conserved residues, to the wild type nucleic acid or amino acid sequences of the alleles as shown herein, and is biologically active.

[00204] The term "**variety**" or "**cultivar**" used herein means a group of similar plants that by structural features and performance can be identified from other varieties within the same species.

[00205] The term "**allele**" used herein means any of one or more alternative or variant forms of a gene or a genetic unit at a particular locus, all of which alleles relate to one trait or characteristic at a specific locus. In a diploid cell of an organism, alleles of a given gene are located at a specific location, or locus (loci plural) on a chromosome. Alternative or variant forms of alleles may be the result of single nucleotide polymorphisms, insertions, inversions, translocations or deletions, or the consequence of gene regulation caused by, for example, by chemical or structural modification, transcription regulation or post-translational modification/regulation. An allele associated with a qualitative trait may comprise alternative or variant forms of various genetic units including those that are identical or associated with a single gene or multiple genes or their products or even a gene disrupting or controlled by a genetic factor contributing to the phenotype represented by the locus. According to further embodiments, the term "allele" designates any of one or more alternative forms of a gene at a particular locus. Heterozygous alleles are two different alleles at the same locus. Homozygous alleles are two identical alleles at a particular locus. A wild type allele is a naturally occurring allele.

[00206] As used herein, the term "**locus**" (loci plural) means a specific place or places or region or a site on a chromosome where for example a gene or genetic marker element or factor is found. In specific embodiments, such a genetic element is contributing to a trait.

[00207] As used herein, the term "**homozygous**" refers to a genetic condition or configuration existing when two identical or like alleles reside at a specific locus, but are

positioned individually on corresponding pairs of homologous chromosomes in the cell of a diploid organism.

[00208] Conversely, as used herein, the term "**heterozygous**" means a genetic condition or configuration existing when two different or unlike alleles reside at a specific locus, but are positioned individually on corresponding pairs of homologous chromosomes in the cell of a diploid organism.

[00209] As used herein, the phrase "**genetic marker**" or "**molecular marker**" or "biomarker" refers to a feature in an individual's genome e.g., a nucleotide or a polynucleotide sequence that is associated with one or more loci or trait of interest. In some embodiments, a genetic marker is polymorphic in a population of interest, or the locus occupied by the polymorphism, depending on context. Genetic markers or molecular markers include, for example, single nucleotide polymorphisms (SNPs), indels (i.e. insertions deletions), simple sequence repeats (SSRs), restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), cleaved amplified polymorphic sequence (CAPS) markers, Diversity Arrays Technology (DArT) markers, and amplified fragment length polymorphisms (AFLPs) or combinations thereof, among many other examples such as the DNA sequence per se. Genetic markers can, for example, be used to locate genetic loci containing alleles on a chromosome that contribute to variability of phenotypic traits. The phrase "genetic marker" or "molecular marker" or "biomarker" can also refer to a polynucleotide sequence complementary or corresponding to a genomic sequence, such as a sequence of a nucleic acid used as a probe or primer.

[00210] As used herein, the term "**germplasm**" refers to the totality of the genotypes of a population or other group of individuals (e.g., a species). The term "germplasm" can also refer to plant material; e.g., a group of plants that act as a repository for various alleles. Such germplasm genotypes or populations include plant materials of proven genetic superiority; e.g., for a given environment or geographical area, and plant materials of unknown or unproven genetic value; that are not part of an established breeding population and that do not have a known relationship to a member of the established breeding population.

[00211] The terms "**hybrid**", "hybrid plant" and "hybrid progeny" used herein refers to an individual produced from genetically different parents (e.g., a genetically heterozygous or mostly heterozygous individual).

[00212] As used herein, **"sequence identity"** or **"identity"** in the context of two nucleic acid or polypeptide sequences makes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins, it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. The term further refers hereinafter to the amount of characters which match exactly between two different sequences. Hereby, gaps are not counted and the measurement is relational to the shorter of the two sequences.

[00213] It is further within the scope that the terms **"similarity"** and **"identity"** additionally refer to local homology, identifying domains that are homologous or similar (in nucleotide and/or amino acid sequence). It is acknowledged that bioinformatics tools such as BLAST, SSEARCH, FASTA, and HMMER calculate local sequence alignments which identify the most similar region between two sequences. For domains that are found in different sequence contexts in different proteins, the alignment should be limited to the homologous domain, since the domain homology is providing the sequence similarity captured in the score. According to some aspects the term similarity or identity further includes a sequence motif, which is a nucleotide or amino-acid sequence pattern that is widespread and has, or is conjectured to have, a biological significance. Proteins may have a sequence motif and/or a structural motif, a motif formed by the three-dimensional arrangement of amino acids which may not be adjacent.

[00214] As used herein, the terms **"nucleic acid"**, **"nucleic acid sequence"**, **"nucleotide"**, **"nucleic acid molecule"** or **"polynucleotide"** are intended to include DNA molecules (e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA), natural occurring, mutated, synthetic DNA or RNA molecules, and analogs of the DNA or RNA generated using nucleotide analogs. It can be single-stranded or double-stranded. Such nucleic acids or polynucleotides include, but are not limited to, coding sequences of structural genes, anti-sense sequences, and non-coding regulatory sequences that do not encode mRNAs or protein products. These terms also encompass a gene. The term **"gene"**, **"allele"** or **"gene sequence"** is used broadly to refer to a DNA nucleic acid associated with a biological function. Thus, genes may include introns and exons as in the genomic sequence, or may comprise only a coding sequence as in cDNAs, and/or may include cDNAs in

combination with regulatory sequences. Thus, according to the various aspects of the invention, genomic DNA, cDNA or coding DNA may be used. In one embodiment, the nucleic acid is cDNA or coding DNA.

[00215] The terms "**peptide**", "polypeptide" and "protein" are used interchangeably herein and refer to amino acids in a polymeric form of any length, linked together by peptide bonds.

[00216] According to other aspects of the invention, a "modified" or a "mutant" plant is a plant that has been altered compared to the naturally occurring wild type (WT) plant. Specifically, the endogenous nucleic acid sequences of one or more of the Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme 1 (CsAAE1), and any combination thereof, homologs in Cannabis have been altered compared to wild type sequences using mutagenesis and/or genome editing methods as described herein. This causes inactivation of at least one of these endogenous genes and thus disables their function in production of THC and/or CBD, depending on the gene or combination of genes down regulated.

[00217] Such plants have an altered cannabinoid profile which may be suitable for treatment of different medical conditions or diseases. Therefore, the cannabinoid profile is affected by the presence of at least one mutated endogenous cannabinoid biosynthesis enzyme gene in the Cannabis plant genome which has been specifically targeted using genome editing technique.

[00218] As used herein, the term "cannabinoid biosynthesis enzyme" refers to a protein acting as a catalyst for producing one or more cannabinoids in a plant of genus Cannabis.

[00219] Examples of cannabinoid biosynthesis enzymes within the context of this disclosure include, but are not limited to: tetrahydrocannabinolic acid synthase (THCAS), cannabidiolic acid synthase (CBDAS), aromatic prenyltransferase (PT), olivetol synthase (OLS), acyl-activating enzyme 1 (AAE1), polyketide synthase (PKS), olivetolic acid cyclase (OAC), tetraketide synthase (TKS), type III PKS, chalcone synthase (CHS), prenyltransferase, CBCA synthase, GPP synthase, FPP synthase, Limonene synthase, aromatic prenyltransferase, and geranylphosphate: olivetolate geranyltransferase.

[00220] Disclosed herein, is a method of controlling cannabinoid synthesis in a plant of genus Cannabis. In some embodiments, the method comprising: Manipulating expression of a gene

coding for a cannabinoid biosynthesis enzyme selected from the group consisting of Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme 1 (CsAAE1) and any combination thereof.

[00221] As used herein, the term "controlling" refers to directing, governing, steering, and/or manipulating, specifically reducing, decreasing or down regulating or silencing the amount of a cannabinoid or cannabinoids produced in a plant of genus Cannabis. In one embodiment, controlling comprises modifying a plant of genus Cannabis to produce an unnaturally occurring concentration of a first cannabinoid. In one embodiment, controlling comprises modifying a plant of genus Cannabis to produce an unnaturally occurring ratio of a first cannabinoid. In one embodiment, controlling comprises modifying a plant of genus Cannabis to produce an unnaturally occurring concentration of a second cannabinoid. In one embodiment, controlling comprises modifying a plant of genus Cannabis to produce an unnaturally occurring ratio of a second cannabinoid.

[00222] As used herein, the term "expression of a gene" refers to a plant's ability to utilize information from genetic material for producing functional gene products. Within the context of this disclosure, expression is meant to encompass the plant's ability to produce proteins, such as enzymes, and various other molecules from the plant's genetic material. In one embodiment, the plant expresses mutated or modified cannabinoid biosynthesis enzymes for cannabinoid biosynthesis. In one embodiment it refers to transcription (RNA) or translation (protein) levels of gene expression.

[00223] As used herein, the term "manipulating expression of a gene" refers to intentionally changing the genome of a plant of genus Cannabis to control the expression of certain features.

[00224] In one embodiment, the plant's genome is manipulated to express less CBDA synthase.

[00225] In one embodiment, the plant's genome is manipulated to express less THCA synthase.

[00226] In one embodiment, the plant's genome is manipulated to express less aromatic prenyltransferase (PT).

[00227] In one embodiment, the plant's genome is manipulated to express less olivetol synthase (OLS).

[00228] In one embodiment, the plant's genome is manipulated to express less acyl-activating enzyme 1 (AAE1).

[00229] According to a further embodiment, the plant's genome is manipulated to express less of any combination of the above mentioned cannabinoid biosynthesis enzymes.

[00230] As used herein, the term "coding" refers to storing genetic information and accessing the genetic information for producing functional gene products.

[00231] According to further aspects of the present invention, the altered THC and/or CBD content trait is not conferred by the presence of transgenes expressed in Cannabis.

[00232] Cannabis plants of the invention are modified plants compared to wild type plants which comprise and express at least one mutant Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme 1 (CsAAE1) and any combination thereof allele.

[00233] Main aspects of the invention involve targeted mutagenesis methods, specifically genome editing, and exclude embodiments that are solely based on generating plants by traditional breeding methods.

[00234] Described are polynucleotides as well as methods for modifying metabolite biosynthesis pathways in Cannabis plants and/or Cannabis plant cells, Cannabis plants and/or plant cells exhibiting modified metabolite biosynthesis pathways. In particular, described are methods for modifying production of THC and/or CBD in Cannabis plants by modulating the expression and/or activity of at least one of Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme 1 (CsAAE1) and any combination thereof and Cannabis plants having modified expression and/or activity of at least one of these genes/proteins.

[00235] Accordingly, in certain embodiments, the present invention provides methods of downregulating production of THC and/or CBD. In particular embodiments, there is provided methods of downregulating expression and/or activity THCA synthase and/or CBDA synthase.

[00236] Also provided are plants and/or plant cells having modified production of THC and/or CBD. In certain embodiments, there are provided Cannabis plants and/or cells having down-regulated expression of and/or activity of at least one of Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme 1 (CsAAE1) and any combination thereof.

[00237] Down regulation of key steps in metabolic pathway re-directs intermediates and energy to alternative metabolic pathways and results in increased production and accumulation or reduced production and elimination of other end products. THC and other Cannabis metabolites share a biosynthetic pathway; that cannabigerolic acid is a precursor of THC, CBD and Cannabichromene. In particular, THCA synthase catalyzes the production of delta-9-tetrahydrocannabinolic acid from cannabigerolic acid; delta-9-tetrahydrocannabinolic undergoes thermal conversion to form THC. CBDA synthase catalyzes the production of cannabidiolic acid from cannabigerolic acid; cannabidiolic acid undergoes thermal conversion to CBD. CBCA synthase catalyzes the production of cannabichromenic acid from cannabigerolic acid; cannabichromenic acid undergoes thermal conversion to cannabichromene.

[00238] A reduction in the production of THC, CBD, or Cannabichromene will enhance production of the remaining metabolites in this shared pathway. For example, production of CBD and/or Cannabichromene is enhanced by inhibiting production of THC. THC production may be inhibited by inhibiting expression and/or activity of tetrahydrocannabinolic acid (THCA) synthase enzyme.

[00239] Described are certain embodiments of enhancing production of one or more secondary metabolites by downregulation of the production of one or more metabolites having a shared biosynthetic pathway. Certain embodiments provide methods of enhancing production of one or more secondary metabolites that share steps and intermediates in the THC and/or CBD biosynthetic pathway by downregulation of THC and/or CBD production. In specific embodiments, there are provided methods of enhancing production of CBD and/or

Cannabichromene by inhibiting production of THC. In other specific embodiments, there are provided methods of enhancing production of THC by inhibiting production of CBD.

[00240] In other specific embodiments, both the production of CBD and THC is inhibited by targeting at least one of the herein identified genes CsAAE1 (SEQ ID NO:13), or CsOLS (SEQ ID NO:10), or CsPT (SEQ ID NO:7) or any combination thereof.

[00241] In other specific embodiments, the production of CBD is inhibited (and THC is induced or not affected) by targeting the herein identified gene CsCBDAS (SEQ ID NO:4).

[00242] In other specific embodiments, the production of THC is inhibited (and CBD is induced or not affected) by targeting the herein identified gene CsTHCAS (SEQ ID NO:1).

[00243] Certain embodiments provide methods of enhancing production of one or more secondary metabolites which share steps and intermediates in the THC biosynthetic pathway by downregulation of expression and/or activity of CsTHCA synthase (SEQ ID NO:1).

[00244] In specific embodiments, there are provided methods of enhancing production of CBD and/or Cannabichromene by downregulation of expression and/or activity of THCA synthase.

[00245] Also provided are plants and plant cells having modified production of one or more metabolites having a shared biosynthetic pathway. In certain embodiments, there are provided Cannabis plants and cells enhanced production of one or more secondary metabolites and downregulation of one or more other metabolites having a shared biosynthetic pathway. In certain embodiments, there are provided Cannabis plants and cells having enhanced production of one or more secondary metabolites and downregulation of one or more other metabolites in the THC and or CBD biosynthetic pathway. In certain embodiments, there are provided Cannabis plants and cells having enhanced production of one or more secondary metabolites in the THC biosynthetic pathway and downregulated THC production. In specific embodiments, there are provided Cannabis plants and cells having enhanced production of CBD and/or Cannabichromene and downregulated THC production.

[00246] In specific embodiments, there are provided Cannabis plants and/or cells having enhanced production of CBD and/or Cannabichromene and downregulated expression and/or activity of THCA synthase.

[00247] The loss of function mutation may be a deletion or insertion ("indels") with reference the wild type allele sequence. The deletion may comprise 1-20 or more nucleotides, for example 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or 20 nucleotides or more in one or more strand. The insertion may comprise 1-20 or more nucleotides, for example 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or 20 or more nucleotides in one or more strand.

[00248] The plant of the invention includes plants wherein the plant is heterozygous for the each of the mutations. In other embodiment however, the plant is homozygous for the mutations. Progeny that is also homozygous can be generated from these plants according to methods known in the art.

[00249] It is further within the scope that variants of a particular nucleotide or amino acid sequence according to the various aspects of the invention will have at least about 50%-99%, for example at least 75%, for example at least 85%, 86%, 87%, 88%, 89%, 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% or more sequence identity to that particular non-variant nucleotide sequence of the Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme 1 (CsAAE1) allele as shown in SEQ ID NO 1, 4, 7, 10 or 13; and/or SEQ ID NO 2, 5, 8, 11 or 14, respectively. Sequence alignment programs to determine sequence identity are well known in the art.

[00250] Also, the various aspects of the invention encompass not only a Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme 1 (CsAAE1) nucleic acid sequence or amino acid sequence, but also fragments thereof. By "fragment" is intended a portion of the nucleotide sequence or a portion of the amino acid sequence and hence of the protein encoded thereby. Fragments of a nucleotide sequence may encode protein fragments that retain the biological activity of the native protein, in this case cannabinoid biosynthesis enzymes.

[00251] According to further embodiments of the present invention, DNA introduction into the plant cells can be done by Agrobacterium infiltration, virus based plasmids for delivery of the genome editing molecules and mechanical insertion of DNA (PEG mediated DNA transformation, biolistics, etc.).

[00252] In addition, it is within the scope of the present invention that the Cas9 protein is directly inserted together with a gRNA (ribonucleoprotein- RNP's) in order to bypass the need for *in vivo* transcription and translation of the Cas9+gRNA plasmid *in planta* to achieve gene editing.

[00253] It is within the scope of the present invention that the usage of CRISPR/Cas system for the generation of Cannabis plants with at least one improved domestication trait, allows the modification of predetermined specific DNA sequences without introducing foreign DNA into the genome by GMO techniques. According to one embodiment of the present invention, this is achieved by combining the Cas nuclease (e.g. Cas9, Cpf1 and the like) with a predefined guide RNA molecule (gRNA). The gRNA is complementary to a specific DNA sequence targeted for editing in the plant genome and which guides the Cas nuclease to a specific nucleotide sequence (for example see Fig. 1). The predefined gene specific gRNA's are cloned into the same plasmid as the Cas gene and this plasmid is inserted into plant cells. Insertion of the aforementioned plasmid DNA can be done, but not limited to, using different delivery systems, biological and/or mechanical, e.g. Agrobacterium infiltration, virus based plasmids for delivery of the genome editing molecules and mechanical insertion of DNA (PEG mediated DNA transformation, biolistics, etc.).

[00254] It is further within the scope of the present invention that upon reaching the specific predetermined DNA sequence, the Cas9 nuclease cleaves both DNA strands to create double stranded breaks leaving blunt ends. This cleavage site is then repaired by the cellular non homologous end joining DNA repair mechanism resulting in insertions or deletions which eventually create a mutation at the cleavage site. For example, it is acknowledged that a deletion form of the mutation consists of at least 1 base pair deletion. As a result of this base pair deletion the gene coding sequence is disrupted and the translation of the encoded protein is compromised either by a premature stop codon or disruption of a functional or structural property of the protein. Thus DNA is cut by the Cas9 protein and re-assembled by the cell's DNA repair mechanism.

[00255] It is further within the scope that manipulation of cannabinoid biosynthesis enzymes in Cannabis plants is herein achieved by generating gRNA with homology to a specific site of predetermined genes in the Cannabis genome i.e. Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme

1 (CsAAE1) genes, sub cloning this gRNA into a plasmid containing the Cas9 gene, and insertion of the plasmid into the Cannabis plant cells. In this way site specific mutations in the aforementioned genes are generated thus effectively creating non-active molecules, resulting in loss of function of at least one of the enzymes, reduced content of THC, CBD or both of the cannabinoids in the genome edited plant.

[00256] In order to understand the invention and to see how it may be implemented in practice, a plurality of preferred embodiments will now be described, by way of non-limiting example only, with reference to the following examples.

EXAMPLE 1

Production of Cannabis plants with modulated cannabinoid expression

[00257] Production of Cannabis lines with mutated Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS) or Cannabis cannabidiolic acid synthase (CsCBDAS) or Cannabis aromatic prenyltransferase (CsPT) or Cannabis olivetol synthase (CsOLS) or Cannabis acyl-activating enzyme 1 (CsAAE1) gene or any combination thereof may be achieved by at least one of the following breeding/cultivation schemes:

[00258] Scheme 1:

- line stabilization by self pollination
- Generation of F6 parental lines
- Genome editing of parental lines
- Crossing edited parental lines to generate an F1 hybrid plant

[00259] Scheme 2:

- Identifying genes/alleles of interest
- Designing gRNA
- Transformation of plants with Cas9+gRNA constructs
- Screening and identifying editing events
- Genome editing of parental lines

[00260] It is noted that line stabilization may be performed by the following:

- Induction of male flowering on female (XX) plants
- Self pollination

According to some embodiments of the present invention, line stabilization requires about 6 self-crossing (6 generations) and done through a single seed descent (SSD) approach.

[00261] F1 hybrid seed production: Novel hybrids are produced by crosses between different Cannabis strains.

[00262] According to a further aspect of the current invention, shortening line stabilization is performed by Doubled Haploids (DH). More specifically, the CRISPR-Cas9 system is transformed into microspores to achieve DH homozygous parental lines. A doubled haploid (DH) is a genotype formed when haploid cells undergo chromosome doubling. Artificial production of doubled haploids is important in plant breeding. It is herein acknowledged that conventional inbreeding procedures take about six generations to achieve approximately complete homozygosity, whereas doubled haploidy achieves it in one generation.

[00263] It is within the scope of the current invention that genetic markers specific for Cannabis are developed and provided by the current invention:

- Sex markers- molecular markers are used for identification and selection of female vs male plants in the herein disclosed breeding program
- Genotyping markers- germplasm used in the current invention is genotyped using molecular markers, in order to allow a more efficient breeding process and identification of the Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS) or Cannabis acyl-activating enzyme 1 (CsAAE1) editing event.

[00264] It is further within the scope of the current invention that allele and genetic variation is analysed for the Cannabis strains used.

[00265] Reference is now made to optional stages that have been used for the production of mutated Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme 1 (CsAAE1) Cannabis plants by genome editing:

[00266] **Stage 1:** Identifying *Cannabis sativa* (*C. sativa*), *C. indica* and *C. ruderalis* tetrahydrocannabinolic acid synthase (THCAS), cannabidiolic acid synthase (CBDAS), aromatic prenyltransferase (PT), olivetol synthase (OLS), acyl-activating enzyme 1 (AAE1) orthologues/homologs.

[00267] The following homologs have herein been identified in *Cannabis sativa* (*C. sativa*), *C. indica* and *C. ruderalis*, namely Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS) and Cannabis acyl-activating enzyme 1 (CsAAE1). These homologous genes have been sequenced and mapped.

[00268] CsTHCAS has been mapped to CM011610.1:22243181-22246809 and has a genomic sequence as set forth in SEQ ID NO:1. The CsTHCAS gene has a coding sequence as set forth in SEQ ID NO:2 and it encodes an amino acid sequence as set forth in SEQ ID NO:3.

[00269] CsCBDAS has been mapped to CM011610.1:21836038-21839672 and has a genomic sequence as set forth in SEQ ID NO:4. The CsCBDAS gene has a coding sequence as set forth in SEQ ID NO:5 and it encodes an amino acid sequence as set forth in SEQ ID NO:6.

[00270] CsPT has been mapped to CM011614.1:1184501-1186728 and has a genomic sequence as set forth in SEQ ID NO:7. The CsPT gene has a coding sequence as set forth in SEQ ID NO:8 and it encodes an amino acid sequence as set forth in SEQ ID NO:9.

[00271] CsOLS has been mapped to CM011613.1:2335391-2338392 and has a genomic sequence as set forth in SEQ ID NO:10. The CsOLS gene has a coding sequence as set forth in SEQ ID NO:11 and it encodes an amino acid sequence as set forth in SEQ ID NO:12.

[00272] CsAAE1 has been mapped to CM011611.1:1210973-1228229 and has a genomic sequence as set forth in SEQ ID NO:13. The CsAAE1 gene has a coding sequence as set forth in SEQ ID NO:14 and it encodes an amino acid sequence as set forth in SEQ ID NO:15.

[00273] **Stage 2:** Designing and synthesizing gRNA molecules corresponding to the sequence targeted for editing, i.e. sequences of each of the genes Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme

1 (CsAAE1). It is noted that the editing event is preferably targeted to a unique restriction site sequence to allow easier screening for plants carrying an editing event within their genome.

[00274] According to some aspects of the invention, the nucleotide sequence of the gRNAs should be completely compatible with the genomic sequence of the target gene. Therefore, for example, suitable gRNA molecules should be constructed for different Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme 1 (CsAAE1) homologues of different Cannabis strains.

[00275] Reference is now made to Tables 1-5 presenting gRNA molecules targeted for silencing Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme 1 (CsAAE1), respectively. The term 'PAM' refers hereinafter to Protospacer Adjacent Motif, which is a 2-6 base pair DNA sequence immediately following the DNA sequence targeted by the Cas9 nuclease in the CRISPR bacterial adaptive immune system.

[00276] **Table 1: gRNA sequences targeted for CsTHCAS**

Position on SEQ. ID. NO.: 1	Strand	Sequence	PAM	Specificity Score	Efficiency Score	SEQ. ID NO
919	-1	TATAACTTTATATTGGAGCG	GGG	91.68505	59.10928	16
920	-1	CTATAACTTTATATTGGAGC	GGG	81.97792	39.46084	17
921	-1	TCTATAACTTTATATTGGAG	CGG	59.73169	58.95429	18
926	-1	TCCTTTCTATAACTTTATAT	TGG	56.2663	28.95317	19
936	1	TCCAATATAAAGTTATAGAA	AGG	52.27911	43.64749	20
1013	1	ACTACTCAACATTCTCCTTT	AGG	78.87146	29.78268	21
1017	-1	AATTTTGTAACAAACCTAA	AGG	47.82242	50.8107	22
1085	-1	CAATTTAGGAAATTTCTTG	AGG	53.52986	57.27972	23
1099	-1	TATATTGGGAGAAGCAATTT	AGG	72.42583	23.48898	24
1113	-1	TGGATTGTTATGAATATATT	GGG	39.7547	29.09505	25
1114	-1	CTGGATTGTTATGAATATAT	TGG	62.7209	37.8461	26
1133	-1	TATACGAGTTTTAGATTTGC	TGG	72.07547	31.53966	27
1168	-1	TCAGGACAGACATATACAAT	TGG	85.35755	47.27478	28
1186	-1	GATTTTGTATTGTCAAATTC	AGG	60.33738	31.33693	29

1218	-1	TGGTTTTGGGGTTGTATCAG	AGG	87.42544	54.0448	30
1230	-1	GACAATAACGAGTGGTTTTG	GGG	52.23624	53.9614	31
1231	-1	TGACAATAACGAGTGGTTTT	GGG	75.404	22.91377	32
1232	-1	GTGACAATAACGAGTGGTTT	TGG	84.75157	30.78067	33
1238	-1	GAAGGAGTGACAATAACGAG	TGG	81.66494	66.45396	34
1256	-1	TGGATATGGGAGACATTTGA	AGG	79.3898	47.59851	35
1269	-1	TAGAATAGTGGCTTGGATAT	GGG	90.41741	42.20222	36
1270	-1	ATAGAATAGTGGCTTGGATA	TGG	86.00061	40.03718	37
1276	-1	TGGAGCATAGAATAGTGGCT	TGG	91.91503	51.2944	38
1281	-1	TTTCTTGGAGCATAGAATAG	TGG	71.98696	51.24085	39
1296	-1	AATCTGCAAGCCAACCTTCT	TGG	83.889	23.52241	40
1297	1	ATTCTATGCTCCAAGAAAGT	TGG	76.79961	44.57193	41
1321	1	TTGCAGATTCGAACTCGAAG	CGG	91.27714	68.55563	42
1324	1	CAGATTCGAACTCGAAGCGG	TGG	95.1427	59.66476	43
1336	-1	AGGACAAACCCTCAGCATCA	TGG	91.96485	64.63997	44
1338	1	AAGCGGTGGCCATGATGCTG	AGG	89.20633	53.30037	45
1339	1	AGCGGTGGCCATGATGCTGA	GGG	92.84794	63.02093	46
1356	-1	AAATGGGACTTGAGATGTGT	AGG	82.02117	58.19267	47
1372	-1	TCAAGTCTACTATAACAAAT	GGG	66.95566	44.7251	48
1373	-1	CTCAAGTCTACTATAACAAA	TGG	79.05133	34.85902	49
1398	1	AGACTTGAGAAACATGCATT	CGG	78.16459	54.20818	50
1429	-1	CGGCTTCAACCCACGCAGTT	TGG	99.57277	40.06864	51
1430	1	ATATTCGTAGCCAACTGCG	TGG	92.73046	65.82128	52
1431	1	TATTCGTAGCCAACTGCGT	GGG	97.53917	62.7453	53
1441	1	CAAACCTGCGTGGGTGAAGC	CGG	95.87721	52.5429	54
1449	-1	AACTTCTCCAAGGGTAGCTC	CGG	72.36938	46.01821	55
1453	1	GTTGAAGCCGGAGCTACCCT	TGG	97.29983	54.79341	56
1458	-1	CCAATAATAAACTTCTCCAA	GGG	74.16963	63.94759	57
1459	-1	TCCAATAATAAACTTCTCCA	AGG	73.52665	52.58996	58
1469	1	CCCTTGAGAGAAGTTTATTAT	TGG	83.24938	16.35292	59
1504	1	AATGAGAATCTTAGTTTTCC	TGG	66.81185	30.01853	60
1507	1	GAGAATCTTAGTTTTCTGG	TGG	85.44233	66.58972	61
1508	1	AGAATCTTAGTTTTCTGGT	GGG	81.70394	50.34528	62
1511	-1	ACAGTAGGGCAATACCCACC	AGG	96.88637	64.82422	63
1525	1	GGTGGGTATTGCCCTACTGT	TGG	78.50407	53.56142	64
1525	-1	GTCCACCTACGCCAACAGTA	GGG	89.72665	55.22571	65
1526	-1	TGTCCACCTACGCCAACAGT	AGG	89.39852	57.68998	66

1531	1	TATTGCCCTACTGTTGGCGT	AGG	94.7426	55.96514	67
1534	1	TGCCCTACTGTTGGCGTAGG	TGG	95.81918	46.27569	68
1546	1	GGCGTAGGTGGACACTTTAG	TGG	66.85409	46.38521	69
1549	1	GTAGGTGGACACTTTAGTGG	AGG	13.8355	69.31429	70
1552	1	GGTGGACACTTTAGTGGAGG	AGG	16.66937	53.37905	71
1558	1	CACTTTAGTGGAGGAGGCTA	TGG	45.06996	40.83788	72
1579	1	GGAGCATTAAATGCGAAATTA	TGG	83.83888	31.63254	73
1591	-1	CAATGATATTATCAGCTGCG	AGG	87.7846	69.05545	74
1627	1	GCACACTTAGTCAATGTTGA	TGG	81.81927	49.79998	75
1653	1	AGTTCTAGATCGAAAATCCA	TGG	82.25517	53.08044	76
1654	1	GTTCTAGATCGAAAATCCAT	GGG	87.53577	49.36309	77
1655	1	TTCTAGATCGAAAATCCATG	GGG	80.87126	69.96673	78
1656	1	TCTAGATCGAAAATCCATGG	GGG	87.02608	75.88737	79
1659	-1	CCAAAATAGATCTTCCCCCA	TGG	52.2372	64.79896	80
1670	1	CCATGGGGGAAGATCTATTT	TGG	40.87315	10.50835	81
1671	1	CATGGGGGAAGATCTATTTT	GGG	45.38645	26.29582	82
1681	1	GATCTATTTTGGGCTATACG	TGG	92.9509	65.38681	83
1684	1	CTATTTTGGGCTATACGTGG	TGG	93.89012	56.74158	84
1687	1	TTTTGGGCTATACGTGGTGG	TGG	89.67677	47.46932	85
1690	1	TGGGCTATACGTGGTGGTGG	AGG	89.86004	51.37554	86
1702	1	GGTGGTGGAGGTGAAAACCTT	TGG	82.68444	59.30357	87
1718	1	ACTTTGGAATCATTGCAGCG	TGG	94.22981	66.30813	88
1731	1	TGCAGCGTGGAATTAGAC	TGG	88.9567	50.21323	89
1748	1	GACTGGTTGCTGTCCCATCA	AGG	98.25535	54.41897	90
1749	1	ACTGGTTGCTGTCCCATCAA	GGG	90.45162	53.09475	91
1750	-1	TGAATATAGTAGCCCTTGAT	GGG	89.11324	51.54889	92
1751	-1	CTGAATATAGTAGCCCTTGA	TGG	94.41378	50.50505	93
1772	1	CTACTATATTCAGTGTTAAA	AGG	79.2234	36.92709	94
1779	1	ATTCAGTGTTAAAAGGAATA	TGG	65.11384	40.20542	95
1789	1	AAAAGGAATATGGAGATACA	TGG	63.91743	56.49626	96
1790	1	AAAGGAATATGGAGATACAT	GGG	68.15381	55.96289	97
1814	1	TTGTCAAGTTATTTAACAAA	TGG	47.11745	34.12702	98
1874	1	TCATGACTCACTTCATAACC	AGG	35.67077	53.19672	99
1881	-1	TTGATTATCTATAATATTCC	TGG	75.11494	33.49257	100
1894	1	AGGAATATTATAGATAATCA	AGG	61.99209	56.41876	101
1918	1	AAGAATAAGACTACAGTACA	CGG	22.03371	69.66059	102
1942	1	TACTTCTCTTGCAATTTCCA	TGG	70.76593	46.22116	103

1945	1	TTCTCTTGCATTTTCCATGG	TGG	64.23993	47.59533	104
1948	-1	CTAGACTATCCACTCCACCA	TGG	94.11237	66.93721	105
1950	1	TTGCATTTTCCATGGTGGAG	TGG	86.29578	54.03153	106
1992	1	GAACAAGAGCTTTCCTGAGT	TGG	94.74542	48.70955	107
1993	1	AACAAGAGCTTTCCTGAGTT	GGG	91.66248	41.21334	108
1994	-1	GTTTTTTTAATACCCAACCTC	AGG	81.54763	48.23303	109
2027	1	CTGATTGCAAAGAATTGAGC	TGG	91.63062	45.41598	110
2049	-1	TACAACACCACTGTAGAAGA	TGG	87.1194	55.28908	111
2053	1	GATACTACCATCTTCTACAG	TGG	85.64352	61.29416	112
2088	1	TAACACTACTAATTTTCAA	AGG	52.892	37.63415	113
2113	1	ATTTTGCTTGATAGATCAGC	TGG	89.54269	45.49052	114
2114	1	TTTTGCTTGATAGATCAGCT	GGG	83.06148	69.66798	115
2168	-1	ACAATTGCAGTTTCTGGAAT	TGG	83.65549	27.85148	116
2174	-1	ATTTTGACAATTGCAGTTTC	TGG	71.89285	13.8671	117
2190	1	AACTGCAATTGTCAAAATTT	TGG	51.26105	21.65345	118
2215	1	AAATTGTATGAAGAAGATGT	AGG	50.42741	60.89104	119
2221	1	TATGAAGAAGATGTAGGAGT	TGG	74.0566	40.67797	120
2245	1	GTGTATGTATTGTACCCTTA	CGG	87.47868	52.95626	121
2248	1	TATGTATTGTACCCTTACGG	TGG	88.53147	70.67558	122
2248	-1	TGTCCATTATACCACCGTAA	GGG	98.58031	65.38253	123
2249	-1	TTGTCCATTATACCACCGTA	AGG	96.99197	52.80478	124
2256	1	GTACCCTTACGGTGGTATAA	TGG	86.96972	36.4762	125
2291	-1	ATTCCAGCTCGATGAGGGAA	AGG	85.04932	58.09939	126
2296	-1	ACATGATTCCAGCTCGATGA	GGG	95.76248	60.07786	127
2297	-1	TACATGATTCCAGCTCGATG	AGG	96.20958	66.08229	128
2299	1	ATTCCTTTCCCTCATCGAGC	TGG	72.65637	45.46834	129
2318	1	CTGGAATCATGTACGAAGTT	TGG	91.44916	38.23198	130
2333	1	AAGTTTGGTACGCAGCTACC	TGG	97.19351	52.48753	131
2334	1	AGTTTGGTACGCAGCTACCT	GGG	99.34096	54.73117	132
2340	-1	ATTATCTTCTTGCTTCTCCC	AGG	65.57768	47.7251	133
2369	1	ATAATGAAAAGCATATAAAC	TGG	54.90991	38.48962	134
2370	1	TAATGAAAAGCATATAAACT	GGG	52.19307	58.36084	135
2408	-1	CTTGGATTTTGGGACACATA	AGG	86.23392	45.11168	136
2418	-1	ATACGCCATTCTTGGATTTT	GGG	83.33913	19.22763	137
2419	-1	GATACGCCATTCTTGGATTTT	TGG	84.63939	21.34065	138
2424	1	TGTGTCCCAAAATCCAAGAA	TGG	72.13536	54.39396	139
2426	-1	TAATTGAGATACGCCATTCT	TGG	94.47859	25.9349	140

2441	1	GAATGGCGTATCTCAATTAT	AGG	87.03451	33.66134	141
2442	1	AATGGCGTATCTCAATTATA	GGG	83.63841	42.44805	142
2455	1	AATTATAGGGACCTTGATTT	AGG	51.02913	28.76282	143
2455	-1	GATCAGTTTTTCCTAAATCA	AGG	81.50991	49.62644	144
2477	-1	GTGTAATTATTAGGACTCTT	GGG	63.03567	37.12308	145
2478	-1	GGTGAATTATTAGGACTCT	TGG	87.51322	36.60593	146
2486	-1	CGTGCTTGGGTGTAATTATT	AGG	89.37979	20.5205	147
2499	-1	TTCACCCCAGATACGTGCTT	GGG	96.64962	52.4944	148
2500	-1	TTTCACCCCAGATACGTGCT	TGG	98.66657	52.02383	149
2504	1	ATTACACCCAAGCACGTATC	TGG	74.79545	34.14523	150
2505	1	TTACACCCAAGCACGTATCT	GGG	95.37111	41.9996	151
2506	1	TACACCCAAGCACGTATCTG	GGG	98.75694	62.87462	152
2521	1	ATCTGGGGTGAAAAGTACTT	TGG	87.2143	49.68151	153
2547	1	AACTTTGACAAGTTAGTTA	AGG	62.31057	39.88018	154
2565	-1	AAAATTATTGGGATCAACTT	TGG	58.80076	33.4693	155
2576	-1	TCGTTTCTAAAAAATTATT	GGG	48.06619	22.76069	156
2577	-1	CTCGTTTCTAAAAAATTAT	TGG	55.23725	14.78939	157
2608	-1	GACGTCGTGGCGGAAGAGGT	GGG	98.09602	65.65899	158
2609	-1	TGACGTCGTGGCGGAAGAGG	TGG	91.81254	50.45154	159
2612	-1	TAATGACGTCGTGGCGGAAG	AGG	98.07448	47.29841	160
2618	-1	AATAATTAATGACGTCGTGG	CGG	89.39021	64.62174	161
2621	-1	AAAAATAATTAATGACGTCG	TGG	78.57945	59.53964	162
2684	-1	ATATATAACAGATACATGTA	TGG	62.7441	56.45533	163
2719	-1	CTTAGGAGCATACATAGTAC	AGG	83.60953	55.16134	164
2736	-1	TACTGTAGATGTTTCATACTT	AGG	72.89098	47.9896	165
2776	1	TGTAGACATCATAAGATATA	TGG	61.18482	42.96127	166
2807	1	AAATTATCTTTCTATTATAA	TGG	40.09022	10.00087	167

[00277] **Table 2: gRNA sequences targeted for CsCBDAS**

Position on SEQ. ID. NO.: 4	Strand	Sequence	PAM	Efficiency Score	SEQ. ID NO.
927	-1	AGCTTTATATATTGGAGCAG	GGG	54.68597	168
928	-1	TAGCTTTATATATTGGAGCA	GGG	56.13836	169
929	-1	ATAGCTTTATATATTGGAGC	AGG	39.33975	170
935	-1	CTATTTATAGCTTTATATAT	TGG	24.05235	171
947	1	CAATATATAAAGCTATAAAT	AGG	27.93541	172

971	-1	TAATGAATTTTGAATTACTA	TGG	34.40115	173
1022	1	AGTACTCAACATTCTCCTTT	TGG	25.84144	174
1026	-1	TATCTTGCAAACAAACAAA	AGG	54.35998	175
1075	-1	GAGGATTAGCAATGGAAGTT	TGG	35.85374	176
1083	-1	GTTTTCTCGAGGATTAGCAA	TGG	61.84745	177
1094	-1	CATTTAAGGAAGTTTTCTCG	AGG	62.59449	178
1108	-1	TATATTGCGAGAAGCATTTA	AGG	22.98833	179
1133	-1	TTTAGATTTGTTGCATTATT	GGG	18.30136	180
1134	-1	TTTTAGATTTGTTGCATTAT	TGG	22.12177	181
1177	-1	TTAGGACAGACATATACAAT	GGG	50.41844	182
1178	-1	TTTAGGACAGACATATACAA	TGG	52.3325	183
1195	-1	GATTGTGTATTGTCAATTT	AGG	29.00652	184
1239	-1	GACGATAACAAGTGGTTTTG	GGG	53.7312	185
1240	-1	TGACGATAACAAGTGGTTTT	GGG	22.17034	186
1241	-1	GTGACGATAACAAGTGGTTT	TGG	32.43789	187
1247	-1	GAAGGAGTGACGATAACAAG	TGG	66.33896	188
1265	-1	TGGATATGAGAGACATGTGA	AGG	69.66263	189
1279	1	TCACATGTCTCTCATATCCA	AGG	60.60093	190
1285	-1	TGGAGCATAGAATAGTGCCT	TGG	51.2944	191
1305	-1	AATCTGCAAGCCAACCTTCT	TGG	23.52241	192
1306	1	ATTCTATGCTCCAAGAAAGT	TGG	44.57193	193
1330	1	TTGCAGATTGGAAGTGAAG	TGG	63.85956	194
1333	1	CAGATTGGAAGTGAAGTGG	TGG	60.21029	195
1347	1	AAGTGGTGGTCATGATTCTG	AGG	61.14171	196
1348	1	AGTGGTGGTCATGATTCTGA	GGG	66.1321	197
1365	-1	AAATGGGACTTGAGATATGT	AGG	48.94352	198
1381	-1	TCAAGTCTACTATAACAAAT	GGG	44.7251	199
1382	-1	CTCAAGTCTACTATAACAAA	TGG	34.85902	200
1438	-1	CGGCTTCAACCCATGCAGTT	TGG	41.98925	201
1439	1	ATGTTTCATAGCCAAACTGCA	TGG	53.04758	202
1440	1	TGTTTCATAGCCAAACTGCAT	GGG	62.17149	203
1450	1	CAAAGTGCATGGGTTGAAGC	CGG	57.17967	204
1458	-1	AACTTCTCCAAGGGTAGCTC	CGG	46.01821	205
1462	1	GTTGAAGCCGGAGCTACCCT	TGG	56.02393	206
1467	-1	CCAATAATAAACTTCTCCAA	GGG	65.85225	207
1468	-1	CCCAATAATAAACTTCTCCA	AGG	53.53392	208
1478	1	CCCTTGAGAGAAGTTTATTAT	TGG	20.92657	209

1479	1	CCTTGGAGAAGTTTATTATT	GGG	22.0283	210
1509	1	GAAAAATGAGAGTCTTAGTT	TGG	32.53304	211
1516	1	GAGAGTCTTAGTTTGGCTGC	TGG	40.11193	212
1517	1	AGAGTCTTAGTTTGGCTGCT	GGG	53.68777	213
1534	-1	GTCCACCTGCGCAAAACAGTA	GGG	51.52452	214
1540	1	TATTGCCCTACTGTTTGC GC	AGG	51.06289	215
1543	1	TGCCCTACTGTTTGC GCAGG	TGG	42.48452	216
1552	1	GTTTGC GCAGGTGGACACTT	TGG	56.37796	217
1558	1	GCAGGTGGACACTTTGGTGG	AGG	55.26903	218
1561	1	GGTGGACACTTTGGTGGAGG	AGG	53.00302	219
1567	1	CACTTTGGTGGAGGAGGCTA	TGG	43.62549	220
1580	-1	AGGCCATAGCTTCTCATCAA	TGG	51.67311	221
1588	1	GGACCATTGATGAGAAGCTA	TGG	51.46816	222
1596	1	GATGAGAAGCTATGGCCTCG	CGG	72.19046	223
1600	-1	CAATGATATTATCAGCCGCG	AGG	70.84796	224
1636	1	GCACACTTAGTCAACGTTCA	TGG	51.20136	225
1662	1	AGTGCTAGATCGAAAATCTA	TGG	38.12486	226
1663	1	GTGCTAGATCGAAAATCTAT	GGG	38.40821	227
1664	1	TGCTAGATCGAAAATCTATG	GGG	57.41791	228
1665	1	GCTAGATCGAAAATCTATGG	GGG	68.96928	229
1679	1	CTATGGGGGAAGATCTCTTT	TGG	21.51399	230
1680	1	TATGGGGGAAGATCTCTTTT	GGG	27.66662	231
1690	1	GATCTCTTTTGGGCTTTACG	TGG	59.22284	232
1693	1	CTCTTTTGGGCTTTACGTGG	TGG	46.97883	233
1696	1	TTTTGGGCTTTACGTGGTGG	TGG	42.65142	234
1711	1	GGTGGTGGAGCAGAAAGCTT	CGG	56.63543	235
1727	1	GCTTCGGAATCATTGTAGCA	TGG	58.92273	236
1740	1	TGTAGCATGGAAAATTAGAC	TGG	47.49685	237
1759	-1	CACTAAACATAGTAGACTTT	GGG	33.44838	238
1760	-1	ACACTAAACATAGTAGACTT	TGG	43.66107	239
1785	1	GTTTAGTGTTAAAAAGATCA	TGG	50.38465	240
1820	1	TTGTCAAGTTAGTTAACAAA	TGG	43.40152	241
1880	1	TCATGACTCACTTCATAACT	AGG	50.92123	242
1900	1	AGGAACATTACAGATAATCA	AGG	55.24406	243
1901	1	GGAACATTACAGATAATCAA	GGG	59.25054	244
1948	1	TACTTCTCTTCAGTTTTCT	TGG	33.58233	245
1951	1	TTCTCTTCAGTTTTCTTGG	TGG	33.88507	246

1954	-1	CTAGACTATCCACTCCACCA	AGG	70.9998	247
1956	1	TTCAGTTTTCTTGGTGGAG	TGG	49.36462	248
1998	1	GAACAAGAGTTTTCTGAGT	TGG	44.24263	249
1999	1	AACAAGAGTTTTCTGAGTT	GGG	38.37804	250
2000	-1	GTTTTTTTAATACCCAACTC	AGG	47.70664	251
2013	1	TGAGTTGGGTATTAACAAAAA	CGG	35.28865	252
2033	1	CGGATTGCAGACAATTGAGC	TGG	51.29451	253
2059	1	GATACTATCATCTTCTATAG	TGG	50.42687	254
2094	1	CGACACTGATAATTTTAACA	AGG	47.46003	255
2119	1	ATTTTGCTTGATAGATCCGC	TGG	45.52498	256
2120	1	TTTTGCTTGATAGATCCGCT	GGG	66.15602	257
2124	-1	GAAAGCACCGTTCTGCCAG	CGG	74.32675	258
2128	1	GATAGATCCGCTGGGCAGAA	CGG	60.09095	259
2174	-1	ACAAATACAGATTCTGGAAT	TGG	29.64316	260
2180	-1	ATTTGGACAAATACAGATTC	TGG	32.93366	261
2196	1	ATCTGTATTTGTCCAAATTT	TGG	19.19094	262
2197	-1	CATATAATTTTCCAAAATT	TGG	22.7704	263
2221	1	AAATTATATGAAGAAGATAT	AGG	48.31475	264
2227	1	TATGAAGAAGATATAGGAGC	TGG	37.66845	265
2228	1	ATGAAGAAGATATAGGAGCT	GGG	55.50564	266
2251	1	ATGTATGCGTTGTACCCTTA	CGG	51.83563	267
2254	1	TATGCGTTGTACCCTTACGG	TGG	70.89895	268
2254	-1	CATCCATTATACCACCGTAA	GGG	65.50071	269
2255	-1	TCATCCATTATACCACCGTA	AGG	52.60015	270
2262	1	GTACCCTTACGGTGGTATAA	TGG	37.81553	271
2297	-1	ATTCCAGCTCGATGAGGGAA	TGG	52.19017	272
2302	-1	ACAAGATTCCAGCTCGATGA	GGG	58.64874	273
2303	-1	TACAAGATTCCAGCTCGATG	AGG	64.02127	274
2305	1	ATTCCATTCCCTCATCGAGC	TGG	52.09796	275
2324	1	CTGGAATCTTGATGAGTTA	TGG	42.0067	276
2339	1	AGTTATGGTACATATGTAGC	TGG	42.23778	277
2340	1	GTTATGGTACATATGTAGCT	GGG	57.33278	278
2375	1	ATAACGAAAAGCATCTAAAC	TGG	36.44159	279
2414	-1	CTTGGATTTTGGGACACATA	AGG	45.61377	280
2424	-1	ATATGCCAATCTTGATTTT	GGG	20.28758	281
2425	-1	GATATGCCAATCTTGATTTT	TGG	27.04467	282
2430	1	TGTGTCCCAAAATCCAAGAT	TGG	48.79389	283

2432	-1	TAATTGAGATATGCCAATCT	TGG	37.73585	284
2461	1	AATTATAGAGACCTTGATAT	AGG	48.98627	285
2461	-1	GATCATTTATTCCTATATCA	AGG	43.92608	286
2483	-1	GTGTAATTATTTGGATTCTT	GGG	35.25388	287
2484	-1	TGTGTAATTATTTGGATTCT	TGG	32.83863	288
2492	-1	CGTGCTTGTGTGAATTATT	TGG	14.08447	289
2510	1	ATTACACACAAGCACGTATT	TGG	23.81028	290
2511	1	TTACACACAAGCACGTATTT	GGG	22.17912	291
2512	1	TACACACAAGCACGTATTTG	GGG	52.47749	292
2527	1	ATTTGGGGTGAGAAGTATTT	TGG	23.04503	293
2543	1	ATTTTGGTAAAAATTTTGAC	AGG	51.19317	294
2565	1	GCTAGTAAAAGTGAAAACCC	TGG	70.67066	295
2571	-1	AAAATTATTGGGATCAACCA	GGG	60.21659	296
2572	-1	AAAAATTATTGGGATCAACC	AGG	51.83397	297
2582	-1	TCGTTTCTAAAAAAATTATT	GGG	23.03526	298
2583	-1	TTCGTTTCTAAAAAAATTAT	TGG	16.97799	299
2614	-1	GATGATGCCGTGGAAGAGGT	GGG	70.41151	300
2615	-1	TGATGATGCCGTGGAAGAGG	TGG	55.81165	301
2618	1	AAAGCATCCACCTCTTCCA	CGG	62.29392	302
2618	-1	TAATGATGATGCCGTGGAAG	AGG	53.28713	303
2624	-1	GATCATTAATGATGATGCCG	TGG	59.64588	304
1535	-1	TGTCCACCTGCGCAACAGT	AGG	50.51317	824
1555	-1	TGCGCAGGTGGACACTTTGG	TGG	53.04254	825

[00278] **Table 3: gRNA sequences targeted for CsPT**

Position on SEQ. ID. NO.: 7	Strand	Sequence	PAM	Specificity Score	Efficiency Score	SEQ. ID NO.
2533	1	CTATAAATAATATAATGTGT	TGG	50.15504	52.66784	305
2548	-1	TCATATAATTAAGACACATT	AGG	58.08987	39.51504	306
2605	-1	GAAGATTTTACGAGTTCATG	TGG	78.97558	61.36868	307
2630	-1	GGTTGTACAATGCAGGAAGC	AGG	94.76609	53.10248	308
2637	-1	TATATTGGGTTGTACAATGC	AGG	89.85941	51.19467	309

2651	-1	ATCTGATTATATTTTATATT	GGG	39.36258	19.27719	310
2652	-1	CATCTGATTATATTTTATAT	TGG	30.46803	22.76394	311
2717	1	TTTTGTATGTACCAATAAAG	AGG	62.64158	47.70007	312
2717	-1	TGTGTTATTATCCTCTTTAT	TGG	59.87869	13.1344	313
2736	1	GAGGATAATAACACAATTAA	TGG	53.00849	32.95598	314
2758	1	GTAGTCATTTTGAGTATTAC	CGG	65.22014	38.84988	315
2759	1	TAGTCATTTTGAGTATTACC	GGG	71.968	55.98069	316
2766	-1	TGCGTTAACAAATTACGACC	CGG	95.86365	58.36396	317
2886	1	ATAGATAATTTAATTCAAAA	TGG	40.48669	36.67217	318
2895	1	TTAATTCAAAATGGCAAACC	TGG	77.80096	45.1845	319
2898	1	ATTCAAAATGGCAAACCTGG	AGG	80.97824	62.29508	320
2902	-1	AGCATTTTGGTTAATCCTCC	AGG	91.75956	45.33793	321
2915	-1	TTCATGCAATCTTAGCATTT	TGG	58.15822	22.94305	322
2962	-1	ATCATTTCTTATTTAATTAC	AGG	45.18918	19.61169	323
2988	1	GAAATGATGAGAAGAAATGA	TGG	34.5444	57.85824	324
2997	1	AGAAGAAATGATGGCAAACC	TGG	78.33764	53.71781	325
3004	-1	TAAGAATTTATTAATAAAACC	AGG	51.75369	55.62077	326
3817	1	TATAGATGAGATGAGATACC	TGG	85.29248	42.90625	327
3818	1	ATAGATGAGATGAGATACCT	GGG	81.64461	53.0755	328
3819	1	TAGATGAGATGAGATACCTG	GGG	90.29784	74.15808	329
3824	-1	TGCTGGGATTATCTGGCCCC	AGG	99.76501	49.69658	330
3831	-1	CTATACTTGCTGGGATTATC	TGG	91.47874	32.32128	331
3840	-1	ATGTGGCTGCTATACTTGCT	GGG	78.11018	54.0404	332
3841	-1	TATGTGGCTGCTATACTTGC	TGG	89.74351	29.36968	333
3854	1	AGCAAGTATAGCAGCCACAT	AGG	88.70643	65.60028	334
3857	-1	AATTGTTCTCCTATCCTATG	TGG	83.08341	61.11113	335
3859	1	GTATAGCAGCCACATAGGAT	AGG	88.86311	52.55338	336
3880	-1	AACTTGACATTATTTGTTC	TGG	60.92679	30.32567	337
3896	1	ACAAAATAATGTCAAGTTTC	TGG	60.06363	20.93761	338
3910	-1	TCAACGTTGGCAAGTAAATA	TGG	58.30165	30.24285	339
3923	-1	AAGATTTGGCATATCAACGT	TGG	83.63482	54.17247	340
3937	-1	TATATATTTCTTTCAAGATT	TGG	43.05887	26.00648	341
3994	1	AATTATTTAAACTAATTATA	AGG	27.59395	24.43668	342
4038	-1	AAAGATGCTTCAGACGTTGA	AGG	87.29059	53.27521	343
4069	-1	TAAATCAATGGGTGCAGCTT	TGG	87.80614	30.65775	344
4080	-1	CTAGCATTTATTAATCAAT	GGG	56.10289	36.01011	345
4081	-1	GCTAGCATTTATTAATCAA	TGG	77.76418	42.71614	346

4095	1	TTGATTTAATAAATGCTAGC	AGG	80.47522	49.30411	347
4109	1	GCTAGCAGGAAAGTAAAAGA	AGG	82.20668	59.95433	348
4121	-1	ATTTCAATTTGATTATTTTC	AGG	42.72452	19.67918	349
4166	1	ATACAATCAAATTTAAATAC	AGG	41.34514	46.2972	350
4167	1	TACAATCAAATTTAAATACA	GGG	38.73103	56.21882	351
4186	1	AGGGAAATCGTTTATGTTAT	TGG	77.90025	22.30105	352
4224	-1	GAAACACGTATTTTAGAGAT	TGG	86.63062	41.49631	353
4473	-1	ACCACTTAAGAATTTCTTT	TGG	55.0929	24.80139	354
4483	1	GCCAAAAGAAAATTCTTAAG	TGG	64.12831	50.01367	355
4515	-1	CAAGATATACTATATAATAT	AGG	44.93458	38.54408	356
4527	1	CTATATTATATAGTATATCT	TGG	56.9374	31.81188	357
4528	1	TATATTATATAGTATATCTT	GGG	49.01302	47.17769	358
4573	-1	CCTACCATTGAGTTGAGGT	GGG	84.17055	57.97852	359
4574	-1	TCCTACCATTGAGTTGAGG	TGG	72.81109	48.59222	360
4577	-1	TTGTCCTACCATTGAGTTG	AGG	79.45986	49.37752	361
4580	1	ATTACCCACCTCAACTCAAA	TGG	78.76399	43.71813	362
4584	1	CCCACCTCAACTCAATGGT	AGG	88.70205	65.11965	363
4603	1	TAGGACAAGAGCTGCTCTGC	TGG	96.15743	52.64779	364
4635	1	ATGTGAAATTTGTAATAATA	TGG	46.50782	21.55043	365
4636	1	TGTGAAATTTGTAATAATAT	GGG	44.42897	39.03579	366
4648	-1	TTTTTTAATCTGCTTGCAC	AGG	81.28024	42.68662	367
4763	1	TACTCTTATAGTAACCAGAG	AGG	91.51612	71.60058	368
4766	-1	ATAGTAATTAGTTACCTCTC	TGG	88.7712	40.73368	369
4898	-1	TTATATAAAAAATATATTCGT	TGG	52.69209	44.66746	370
4915	1	AATATATTTTATATAAATA	TGG	25.28327	23.82603	371
4916	1	ATATATTTTATATAAATAT	GGG	20.87203	33.8773	372
4989	1	AATTATCTCATCTAACTAAA	TGG	61.01435	39.06579	373
5099	1	TGTTAGTAAAGTAAAATACC	AGG	75.01818	60.50068	374
5106	-1	TGCATTTCTTCTCAATTTCC	TGG	55.3698	27.57883	375
5121	1	GAAATTGAGAAGAAATGCAG	TGG	66.10407	61.792	376
5125	1	TTGAGAAGAAATGCAGTGG	AGG	82.89142	54.57658	377
5146	1	GGATTTTGCTTCCATCTAAA	TGG	80.36516	33.72734	378
5146	-1	ATTCTGTTCCACCATTTAGA	TGG	81.59639	41.29933	379
5149	1	TTTTGCTTCCATCTAAATGG	TGG	73.22228	58.83392	380
5177	-1	TACTGTTTGGTATTTTGG	TGG	52.22343	46.50211	381
5180	-1	GGCTACTGTTTGGTATTTT	TGG	57.1959	14.32438	382
5189	-1	TATATATTTGGCTACTGTTT	TGG	70.57808	21.40652	383

5201	-1	GGTGGACCACTCTATATATT	TGG	88.25064	28.7384	384
5206	1	CAGTAGCCAAATATATAGAG	TGG	80.8101	56.01321	385
5219	-1	ATAACTATAAAAAATGAAGGG	TGG	58.67371	66.9197	386
5222	-1	ATAATAACTATAAAAAATGAA	GGG	32.04873	55.75115	387
5223	-1	GATAATAACTATAAAAAATGA	AGG	44.11535	43.57819	388
5249	-1	AGCATAATTGTGGCACTGTT	TGG	91.98088	35.4872	389
5259	-1	TTGGATTATGAGCATAATTG	TGG	56.2773	59.04583	390
5278	-1	AAATATCAGTAAACACAGCT	TGG	83.11907	51.55396	391
5301	-1	TGATCTACCACTAGCTTCAG	GGG	89.84025	61.37871	392
5302	-1	CTGATCTACCACTAGCTTCA	GGG	92.57816	49.17639	393
5303	-1	CCTGATCTACCACTAGCTTC	AGG	91.25108	35.18826	394
5305	1	GATATTTCCCTGAAGCTAG	TGG	85.99172	60.18662	395
5314	1	CCTGAAGCTAGTGGTAGATC	AGG	92.20769	47.61895	396
5403	1	TACAAAAAATTGTTGTAGCT	AGG	60.78952	43.73403	397
5404	1	ACAAAAAATTGTTGTAGCTA	GGG	62.02877	48.6504	398
5484	-1	TGTGGGTCATAAATAAAGTT	GGG	63.20534	39.71502	399
5485	-1	TTGTGGGTCATAAATAAAGT	TGG	76.86574	49.65417	400
5501	-1	ATAACAATTATAATTTTTGT	GGG	35.20761	38.75031	401
5502	-1	GATAACAATTATAATTTTTG	TGG	40.09979	38.6558	402
5524	-1	TATGCTATTGAACCTCTAGT	AGG	73.91656	50.40142	403
5565	-1	TAATTTGAGTTAATAATTTT	AGG	35.14236	12.79093	404
5737	-1	TTTACGATCTTCACATTGAC	AGG	86.84326	43.60669	405
5760	1	AAGATCGTAAATCTGATTGA	TGG	79.39694	53.97011	406
5802	-1	CAAGGCATTCTTTTTTTTGG	TGG	78.28594	36.74021	407
5805	-1	GTTCAAGGCATTCTTTTTTT	TGG	77.61678	15.8281	408
5820	-1	AAGTTGGTCTCTGATGTTCA	AGG	78.39446	48.10956	409
5836	-1	ATAACACAAATTAATAAGT	TGG	49.51096	36.82689	410
5872	-1	GGGAGAGCAGTGGATTGTTT	GGG	90.26781	39.34907	411
5873	-1	TGGGAGAGCAGTGGATTGTT	TGG	88.36253	32.36556	412
5882	-1	ATTTTGGTATGGGAGAGCAG	TGG	94.63991	58.35153	413
5892	-1	ATTTGTTTTAATTTTGGTAT	GGG	41.95799	36.82488	414
5893	-1	TATTTGTTTTAATTTTGGTA	TGG	44.60475	38.88369	415
5898	-1	ATATATATTTGTTTTAATTT	TGG	27.84114	26.40511	416
5979	-1	TTAGATTTGAGAGTTAAATG	TGG	55.46527	69.10913	417
5998	1	AACTCTCAAATCTAAATTTT	TGG	45.16858	7.464133	418
5999	1	ACTCTCAAATCTAAATTTTT	GGG	45.01203	12.96086	419
6000	1	CTCTCAAATCTAAATTTTTG	GGG	47.56245	39.64619	420

6033	1	TTAATCTTTTTGTTATTTA	AGG	39.86712	15.52071	421
6036	1	ATCTTTTTGTTATTTAAGG	TGG	61.57787	43.97947	422
9091	-1	ATACTTTCTGTGCAAAAATA	TGG	63.71265	24.27214	423
9190	-1	TAGCATTTACTTCATGCGCT	TGG	88.85683	40.57145	424
9214	1	GTAAATGCTATGATTGTATA	TGG	67.17232	42.70364	425
9238	-1	TAAACTTTGGGAAGGCATGT	TGG	82.83401	50.01002	426
9246	-1	TAAAATTTTAACTTTGGGA	AGG	42.81657	55.00889	427
9250	-1	CAACTAAAATTTTAACTTT	GGG	34.37113	28.08549	428
9251	-1	GCAACTAAAATTTTAACTTT	TGG	48.74808	29.25676	429
9286	1	ACTGAATGATTATCAGATTC	TGG	81.6358	34.3321	430
9289	1	GAATGATTATCAGATTCTGG	AGG	77.96775	65.74172	431
9324	-1	AATAATAATAATGTGTTAAC	AGG	53.34883	37.14458	432
9379	1	GTTGTGTATTTTTTCTTTT	AGG	44.5762	18.62322	433
9420	-1	TAATATTATATTTAATTAGA	GGG	39.10845	36.29759	434
9421	-1	TTAATATTATATTTAATTAG	AGG	31.68565	39.80062	435
9510	1	GAGACACACACATACCCTAA	TGG	86.59004	50.40612	436
9513	-1	AATCGCAAAAAATCCATTA	GGG	57.75175	49.45903	437
9514	-1	CAATCGCAAAAAATCCATT	AGG	59.61224	37.96305	438
9567	1	GTTTTGTAGATGAAAACCTCT	TGG	57.02108	52.35675	439
9570	1	TTGTAGATGAAAACCTCTGG	TGG	76.57239	57.2048	440
9585	1	CTTGGTGGAGCAATGTTTAG	AGG	81.85424	43.12889	441
9586	1	TTGGTGGAGCAATGTTTAGA	GGG	81.38342	49.63563	442
9613	1	TTATTGTAAGAGTATTTAAT	TGG	42.08458	25.1192	443
9621	1	AGAGTATTTAATTGGTGTTT	TGG	28.15366	29.48476	444
9622	1	GAGTATTTAATTGGTGTTTT	GGG	59.62235	18.36114	445
9623	1	AGTATTTAATTGGTGTTTTG	GGG	51.98403	49.93003	446
9642	1	GGGGTGTGCGATATAATAATG	AGG	74.06144	54.09539	447
9648	1	TCGATATAATAATGAGGTTT	TGG	66.02342	29.35407	448
9649	1	CGATATAATAATGAGGTTTT	GGG	54.71808	18.42158	449
9664	1	GTTTTGGGATTATTATTGTG	AGG	54.73719	60.27909	450
9680	1	TGTGAGGATTTAATAAAGTA	TGG	62.9832	45.48923	451
9702	1	GTAATTAGTTTGAAATGAAA	AGG	42.78812	38.59876	452
9731	-1	CAATCAATAAATCTTCAT	GGG	51.22321	46.33554	453
9732	-1	TCAATCAATAAATCTTCA	TGG	52.54212	42.7126	454
9765	1	AATATTAAGAAAAAATG	AGG	32.91192	50.40054	455
9766	1	ATATTAAGAAAAAATGA	GGG	31.58425	59.29349	456
9775	1	AGAAAAAATGAGGGAAAAGAA	AGG	37.46114	45.55358	457

9776	1	GAAAAAATGAGGGAAAGAAA	GGG	19.94773	54.89242	458
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[00279] **Table 4: gRNA sequences targeted for CsOLS**

Position on SEQ. ID. NO.: 10	Strand	Sequence	PAM	Specificity Score	Efficiency Score	SEQ. ID NO.
606	1	CATACATAATATATATATAT	AGG	38.80015	37.5899	459
694	1	TATGAATCATCTTCGTGCTG	AGG	92.51688	55.65201	460
695	1	ATGAATCATCTTCGTGCTGA	GGG	92.26269	55.16668	461
700	1	TCATCTTCGTGCTGAGGGTC	CGG	97.38454	45.93456	462
708	-1	CCGATGGCGAGAACGGAGGC	CGG	99.23283	53.34917	463
712	-1	GGTGCCGATGGCGAGAACGG	AGG	99.09248	64.53414	464
715	-1	GGCGGTGCCGATGGCGAGAA	CGG	98.67263	50.70203	465
719	1	CCGGCCTCCGTCTCGCCAT	CGG	99.67834	47.54908	466
724	-1	CTCCGGATTGGCGGTGCCGA	TGG	99.54478	39.05761	467
733	1	CGCCATCGGCACCGCCAATC	CGG	99.93027	36.80185	468
733	-1	TAAAAATGTTCTCCGGATTGG	CGG	90.17454	51.19588	469
736	-1	TATTAATGTTCTCCGGAT	TGG	88.80068	45.44233	470
741	-1	TCTTGATTAATGTTCTC	CGG	73.04651	45.0436	471
771	-1	GTGACCCGAAAGTAGTAGTC	AGG	96.00665	45.73853	472
777	1	AGTTTCCTGACTACTACTTT	CGG	89.23006	34.52546	473
778	1	GTTTCCTGACTACTACTTTC	GGG	92.15777	29.58151	474
793	-1	TTGAGTCATGTGTTCACTTT	TGG	73.05001	32.95869	475
876	-1	CATGGAAAAGTATTATTAGT	TGG	56.37872	42.87624	476
894	-1	AAATAGATAATGCTTATACA	TGG	65.92159	54.09676	477
917	1	ATTATCTATTTATATAATAA	AGG	38.73228	37.78037	478
1000	1	CTTACTTTATATGTATATGT	AGG	48.96629	45.21245	479
1019	1	TAGGTGACAAAAGTATGATA	AGG	63.42832	45.85195	480
1071	1	TCTAAAGCAAAACCCAAGAT	TGG	65.43717	53.59795	481
1072	-1	TCTCGTGCTCCGCCAATCTT	GGG	95.9415	42.77202	482
1073	-1	ATCTCGTGCTCCGCCAATCT	TGG	97.96263	28.38723	483
1074	1	AAAGCAAAACCCAAGATTGG	CGG	51.53179	58.86478	484
1095	1	GGAGCACGAGATGCAAACTC	TGG	94.77341	54.21811	485

1116	1	GGATGCACGTCAAGACATGT	TGG	94.27103	64.06157	486
1125	1	TCAAGACATGTTGGTAGTTG	AGG	77.53627	54.5857	487
1138	1	GTAGTTGAGGTTCCAAAACCT	TGG	70.93462	44.35067	488
1139	1	TAGTTGAGGTTCCAAAACCTT	GGG	68.9903	55.5133	489
1139	-1	CAAGCATCCTTCCCAAGTTT	TGG	89.31912	22.9458	490
1143	1	TGAGGTTCCAAAACCTGGGA	AGG	81.7532	56.98226	491
1158	1	TGGGAAGGATGCTTGTGCAA	AGG	86.76519	63.49574	492
1170	-1	GGGTTGACCCATTCTTTGA	TGG	82.91197	29.12733	493
1172	1	GTGCAAAGGCCATCAAAGAA	TGG	72.44945	46.30634	494
1173	1	TGCAAAGGCCATCAAAGAAT	GGG	70.64931	38.21922	495
1174	1	GCAAAGGCCATCAAAGAATG	GGG	78.23762	71.1964	496
1190	-1	AAATGAGTGATTTTAGACTT	GGG	54.17939	50.10922	497
1191	-1	TAAATGAGTGATTTTAGACT	TGG	64.67096	46.53421	498
1233	-1	GTCTGCACCGGGCATGTCAG	TGG	97.80392	53.96746	499
1237	1	GCATCAACCACTGACATGCC	CGG	92.69975	59.55302	500
1244	-1	GCGCAATGGTAGTCTGCACC	GGG	99.19901	57.07458	501
1245	-1	AGCGCAATGGTAGTCTGCAC	CGG	98.52043	48.84276	502
1258	-1	GTCCGAGAAGCTTAGCGCAA	TGG	94.35214	55.9059	503
1267	1	TACCATTGCGCTAAGCTTCT	CGG	91.29961	37.63117	504
1286	-1	ATCATCACACGCTTCACTGA	GGG	89.91737	65.13509	505
1287	-1	CATCATCACACGCTTCACTG	AGG	96.68067	65.997	506
1309	1	CGTGTGATGATGTATCAACT	AGG	84.90515	64.72637	507
1318	1	ATGTATCAACTAGGCTGTTA	TGG	89.65638	40.36473	508
1321	1	TATCAACTAGGCTGTTATGG	TGG	89.40848	68.01826	509

[00280] **Table 5: gRNA sequences targeted for CsAAE1**

Position on SEQ. ID. NO.: 13	Strand	Sequence	PAM	Specificity Score	Efficiency Score	SEQ. ID No.
1017	1	ATTCAAAGTGAGAAAATTGT	TGG	46.82883	42.08966	510
1036	-1	AACCAACAAGATCATGAGAA	GGG	65.50328	65.57937	511
1037	-1	CAACCAACAAGATCATGAGA	AGG	69.62583	61.96231	512
1045	1	AACCTTCTCATGATCTTGT	TGG	26.15799	41.67638	513

1058	1	ATCTTGTTGGTTGCTGTTCT	CGG	83.86054	29.09087	514
1073	1	GTTCTCGGAAGTGATGAAAG	AGG	79.35978	74.00854	515
1099	-1	TGTTTGGTTAATTATTAAAT	AGG	33.15963	19.05195	516
1115	-1	ACTAATTCTAATTTGGTGTT	TGG	59.84418	32.05943	517
1122	-1	ATTGTATACTAATTCTAATT	TGG	54.12193	21.41009	518
1157	1	ATAAGATCATCATCATAGTG	TGG	66.94162	60.62696	519
1168	1	ATCATAGTGTGGAAGTGTAT	AGG	73.55537	49.07951	520
1213	-1	TTTTTAGTCTTGACTCTCAA	GGG	77.37359	51.59533	521
1214	-1	ATTTTGTCTTGACTCTCA	AGG	74.23792	43.89766	522
1259	1	AAAGTTAATAATGATGAAAT	TGG	35.52392	37.97787	523
1274	1	GAAATTGGTACCTTGAACAG	AGG	81.08644	61.31573	524
1305	-1	TTGAGGTTATCTTTCAACTT	GGG	64.77667	37.47441	525
1306	-1	ATTGAGGTTATCTTTCAACT	TGG	77.67685	49.4408	526
1322	-1	CAATTGACTTAAATCAATTG	AGG	58.54721	51.56257	527
1382	1	AAGAAAATTACTAATTGCTC	AGG	70.79317	49.81138	528
1395	-1	GGGGTGCCACCTTTGGGCGG	TGG	98.94197	45.7826	529
1397	1	TGCTCAGGTCCACCGCCCAA	AGG	99.42523	59.45534	530
1398	-1	ATTGGGGTGCCACCTTTGGG	CGG	95.01333	60.80505	531
1400	1	TCAGGTCCACCGCCCAAAGG	TGG	97.56412	60.82488	532
1401	-1	GCTATTGGGGTGCCACCTTT	GGG	95.35281	30.579	533
1402	-1	TGCTATTGGGGTGCCACCTT	TGG	97.59112	22.5998	534
1414	-1	TTTCGAGACAACTGCTATTG	GGG	95.02736	53.10779	535
1415	-1	TTTTCGAGACAACTGCTATT	GGG	91.01659	36.67599	536
1416	-1	GTTTTCGAGACAACTGCTAT	TGG	93.27798	46.45164	537
1555	-1	GTTACTTTTGATAATTTGAT	AGG	45.16312	42.37079	538
7139	-1	AATGAATATTGGAGGCATCA	AGG	69.38955	59.92139	539
7147	-1	GATGATACAATGAATATTGG	AGG	65.15451	59.16492	540
7150	-1	GCAGATGATACAATGAATAT	TGG	70.32188	47.43563	541
7177	-1	AATGGTTATTATCATGCACA	TGG	69.87898	60.70248	542
7195	-1	ATTTTGTAGCTTACATCTAA	TGG	68.8764	35.50075	543
7220	-1	AGAGGTTTTAAGGAGGCATG	GGG	90.60318	66.45911	544
7221	-1	GAGAGGTTTTAAGGAGGCAT	GGG	82.79088	50.05129	545
7222	-1	GGAGAGGTTTTAAGGAGGCA	TGG	80.93082	48.72577	546
7227	-1	TGAATGGAGAGGTTTTAAGG	AGG	74.09145	69.25707	547
7230	-1	CATTGAATGGAGAGGTTTTA	AGG	69.65457	16.77595	548
7238	-1	AATGCCTACATTGAATGGAG	AGG	85.66528	65.63677	549
7243	-1	AAGGGAATGCCTACATTGAA	TGG	89.92094	39.76854	550
7245	1	AAAACCTCTCCATTCAATGT	AGG	79.28148	56.68625	551
7261	-1	CACCATGATGTTTATTTTAA	GGG	58.97317	9.801739	552

7262	-1	TCACCATGATGTTTATTTTA	AGG	58.8467	15.41337	553
7270	1	TTCCCTTAAATAAACATCA	TGG	61.84936	57.29224	554
7288	-1	GCATCGAAGACTCTGTTGAA	TGG	91.56656	52.95186	555
7312	-1	GCGCTCGGTCCAGTCATGTT	TGG	94.43272	39.68016	556
7314	1	TTCGATGCTCCAAACATGAC	TGG	86.8283	47.62248	557
7327	-1	GGAATTGGTGAATTAGCGCT	CGG	96.74799	68.37405	558
7341	1	AGCGCTAATTCACCAATTCC	TGG	93.7153	36.16436	559
7342	-1	CCTAAAAACAAACCAGGAAT	TGG	86.34378	41.08083	560
7348	-1	ATGCAGCCTAAAAACAAACC	AGG	90.91529	63.74234	561
7353	1	CCAATTCCTGGTTTGTITTT	AGG	66.41673	7.967407	562
7417	1	AAATGTAACATAATTTTATA	TGG	30.24509	21.5658	563
7418	1	AATGTAACATAATTTTATAT	GGG	36.43879	34.67268	564
7461	-1	ACTTTTCAAATAAGATTTGA	TGG	49.31759	24.4411	565
7489	-1	AAAATATAATTTAAAAATAT	TGG	23.50641	25.15222	566
7523	1	ATAAGTTTATTAATTACCAT	TGG	43.96007	52.6669	567
7528	-1	TGACAACAATGGTTATCCAA	TGG	81.46885	63.06849	568
7539	-1	TTATACATACTTGACAACAA	TGG	72.70705	49.88993	569
7569	-1	TCATTTAGTTCACAATGTAT	GGG	69.87154	43.07873	570
7570	-1	TTCATTTAGTTCACAATGTA	TGG	64.11188	34.27846	571
7620	-1	ATTGGTGGTGCATTTTCTGC	TGG	75.70066	41.54012	572
7635	-1	TGTGGTGGCACAGAAATTGG	TGG	86.97385	62.37008	573
7638	-1	ATGTGTGGTGGCACAGAAAT	TGG	91.18051	35.67967	574
7650	-1	CCTGTATCGAAATGTGTGG	TGG	86.4401	69.73002	575
7653	-1	AAGCCTGTTATCGAAATGTG	TGG	86.07817	69.79364	576
7661	1	CCACCACACATTCGATAAC	AGG	91.98911	34.09153	577
7688	-1	ATGAATACCTATGGTTGATG	GGG	79.66939	61.20548	578
7689	-1	GATGAATACCTATGGTTGAT	GGG	77.02024	47.22441	579
7690	-1	AGATGAATACCTATGGTTGA	TGG	76.43674	51.54312	580
7692	1	TTGCTCTCCCCATCAACCAT	AGG	89.17718	57.68331	581
7697	-1	CTAATGTAGATGAATACCTA	TGG	79.03258	52.66036	582
7726	1	ATTAGATGCTTCACCAGAAG	AGG	76.7559	48.05163	583
7728	-1	TGTAGTTGCTTTTCTCTTC	TGG	70.77754	18.10598	584
7745	1	GAGGAAAAGCAACTACAAGA	AGG	77.08372	61.08762	585
7746	1	AGGAAAAGCAACTACAAGAA	GGG	59.40687	56.12144	586
7780	-1	ATTATATTAAACGTATTATA	TGG	58.83333	26.89437	587
7828	1	TGTGCATAGATAAGAAATAT	TGG	49.90268	36.74205	588
7853	1	AATATATTATAATTCTTTAC	CGG	49.5763	28.18052	589
7857	1	TATTATAATTCTTTACCGGA	TGG	78.22362	52.20352	590
7860	1	TATAATTCCTTACCGGATGG	TGG	93.78137	54.29303	591

7861	-1	GCTATGATTGGTCCACCATC	CGG	82.3879	55.49635	592
7873	-1	ATTGTGTTAGTGGCTATGAT	TGG	85.22929	56.76141	593
7883	-1	AAAAGTAGCAATTGTGTTAG	TGG	71.54968	45.76116	594
7906	-1	TCCCTAGCATTGTTGATCA	TGG	95.58084	52.55436	595
7915	1	TTCCATGATCGAACAATGCT	AGG	89.83435	51.35939	596
7916	1	TCCATGATCGAACAATGCTA	GGG	83.34292	59.40933	597
7929	-1	TAAAGTAACAATGCTAGGTG	TGG	86.0951	63.10284	598
7934	-1	GATGCTAAAGTAACAATGCT	AGG	68.92833	59.04437	599
7956	-1	ATGTTTTCTAATATGTGTGT	AGG	35.74139	53.28168	600
8037	-1	AATAAAAAACATGATAAGTT	CGG	49.74918	45.14228	601
8086	1	AATAAAAAATGATGTGATTAT	TGG	45.03911	43.8586	602
8114	-1	ATGTCTAATTCATTATAGAA	GGG	64.65734	51.67938	603
8115	-1	AATGTCTAATTCATTATAGA	AGG	55.7369	45.68541	604
8199	1	CTAAAAAGTTTATTAGTTAA	TGG	50.73953	27.333	605
8223	-1	TAGCTTCGCCAAATTTGTGC	AGG	88.30821	49.16688	606
8226	1	TTAGTTTACCTGCACAAATT	TGG	74.90104	37.25004	607
8245	1	TTGGCGAAGCTAGAAACAAG	TGG	82.9657	68.53364	608
8261	-1	GCTTCTCTTGCTTGTATAA	TGG	81.45785	36.91329	609
8262	1	AAGTGGTGATCCATTATACA	AGG	80.45179	62.09832	610
8282	1	AGGCAAGAGAAGCCCCATTA	AGG	93.10259	42.00278	611
8283	-1	CTATGCTTCACTCCTTAATG	GGG	92.90881	51.33474	612
8284	-1	TCTATGCTTCACTCCTTAAT	GGG	80.41893	33.5345	613
8285	-1	GTCTATGCTTCACTCCTTAA	TGG	92.13485	27.46679	614
8305	1	AGTGAAGCATAGACCAGCCA	AGG	93.2408	55.91368	615
8307	-1	TTGGATGATGGGTCCTTGGC	TGG	89.87415	37.38458	616
8311	-1	TTGGTTGGATGATGGGTCCT	TGG	90.64716	46.50603	617
8318	-1	ACTAATCTTGGTTGGATGAT	GGG	55.88848	43.22222	618
8319	-1	CACTAATCTTGGTTGGATGA	TGG	64.35474	40.68855	619
8326	-1	TTTGGCCCACTAATCTTGGT	TGG	63.95374	49.8337	620
8330	-1	ATTGTTTGGCCCACTAATCT	TGG	83.91578	42.92642	621
8331	1	CATCATCCAACCAAGATTAG	TGG	45.46742	53.20986	622
8332	1	ATCATCCAACCAAGATTAGT	GGG	44.6141	53.44581	623
8344	-1	GGAAAGGTGATGTCATTGTT	TGG	78.65278	38.65702	624
8360	-1	AGCCATTGGACATTAGGAA	AGG	84.2141	58.69325	625
8365	-1	GGTGGAGCCATTTGGACATT	AGG	86.64479	40.00615	626
8369	1	CACCTTTCCTAATGTCCAAA	TGG	75.0476	45.46945	627
8373	-1	TGCAGATGGGTGGAGCCATT	TGG	93.51908	44.95053	628
8383	-1	TAAAAGCAGCTGCAGATGGG	TGG	93.4923	62.38047	629
8386	-1	CTTTAAAAGCAGCTGCAGAT	GGG	88.26499	50.81653	630

8387	-1	CCTTTAAAAGCAGCTGCAGA	TGG	86.2835	54.0118	631
8398	1	CCATCTGCAGCTGCTTTTAA	AGG	87.21073	15.05639	632
8408	1	CTGCTTTTAAAGGAGTTGCT	TGG	88.7393	43.96243	633
8409	1	TGCTTTTAAAGGAGTTGCTT	GGG	70.87059	39.59579	634
8416	1	AAAGGAGTTGCTTGGGTCCA	TGG	92.13848	46.59847	635
8422	-1	GGGAGCCAAAGGCAATTCCA	TGG	82.72992	60.08041	636
8428	1	TGGGTCCATGGAATTCCTT	TGG	83.84223	36.75466	637
8433	-1	TTTTGGTATAGGGGAGCCAA	AGG	62.72308	58.65204	638
8442	-1	ACAATATTGTTTTGGTATAG	GGG	70.04203	44.96968	639
8443	-1	GACAATATTGTTTTGGTATA	GGG	65.06063	24.34171	640
8444	-1	AGACAATATTGTTTTGGTAT	AGG	60.62704	36.77509	641
8450	-1	GATGGAAGACAATATTGTTT	TGG	49.88722	31.0149	642
8468	-1	ATCAATCTAAACACTTTTGA	TGG	65.25066	22.9316	643
8497	1	TTGATGAACAAAATAATTA	AGG	60.26021	31.42456	644
8506	1	AAAATAATTAAGGATATTA	AGG	28.77328	23.99012	645
8507	1	AAATAATTAAGGATATTAA	GGG	47.82816	35.89387	646
8508	1	AAATAATTAAGGATATTAAG	GGG	56.45894	52.91201	647
8509	1	ACTAATTAAGGATATTAAGG	GGG	66.78309	63.26044	648
8529	1	GGGCTCATGAGATTTTTTAA	CGG	72.44404	35.38053	649
8573	-1	ATTCAGTAAATAATGATGCT	TGG	74.0009	56.25971	650
8598	-1	TTTTAAGAGCGCAAACTAAA	AGG	69.75899	34.44909	651
8648	1	GTTTTGTTTAAAGATAATAG	CGG	63.36248	63.28955	652
8649	1	TTTTGTTTAAAGATAATAGC	GGG	66.03681	52.89056	653
8681	1	TCAAAATTTTATATTGTAAG	CGG	40.99317	51.22546	654
8758	-1	AGACAATAAGAGAGTTTATA	CGG	63.10426	33.70903	655
8777	1	ACTCTCTTATTGTCTTAAAC	AGG	82.59379	27.0699	656
8778	1	CTCTCTTATTGTCTTAAACA	GGG	76.21161	46.02143	657
8779	1	TCTCTTATTGTCTTAAACAG	GGG	81.69869	60.34407	658
8801	-1	GATTTAATGATCAAGAATTT	AGG	52.45697	36.47312	659
8853	-1	GAATTTATTAATAATAGCATT	TGG	58.39351	33.17128	660
8873	1	ATTTTAATAAATTCAAAACA	TGG	42.10593	49.13089	661
8876	1	TTAATAAATTCAAAACATGG	CGG	51.71372	68.38405	662
8879	1	ATAAATTCAAAACATGGCGG	TGG	87.21197	46.77449	663
8900	-1	CTCTTCTCATCTGGAACAAC	AGG	83.95127	38.49164	664
8909	-1	ACAAACATCCTCTTCTCATC	TGG	82.47663	39.35798	665
8912	1	CTGTTGTTCCAGATGAGAAG	AGG	82.81433	53.17806	666
8925	1	TGAGAAGAGGATGTTTGTAT	AGG	75.94583	50.65718	667
8934	1	GATGTTTGTATAGGCATCAA	CGG	83.23553	48.99018	668
8935	1	ATGTTTGTATAGGCATCAAC	GGG	71.89291	50.59686	669

8970	1	AGTAAATTCACAATTTCTGC	AGG	72.90705	51.93162	670
8984	-1	GGGACTAATATCTTCTTGAG	TGG	81.64895	66.64607	671
9004	-1	AAAATAATAAGCATATATGT	GGG	46.06348	54.81391	672
9005	-1	CAAAATAATAAGCATATATG	TGG	50.75457	48.01509	673
9017	1	CACATATATGCTTATTATTT	TGG	49.76422	8.576781	674
9121	-1	TTTTTAATTAAGTAACAC	AGG	60.1073	53.94147	675
9187	-1	TTAATTTAGGAGTTATTTTG	GGG	44.33331	53.49833	676
9188	-1	ATTAATTTAGGAGTTATTTT	GGG	41.75312	20.1865	677
9189	-1	TATTAATTTAGGAGTTATTT	TGG	46.05579	9.863159	678
9200	-1	AAGTTGGGCATTATTAATTT	AGG	64.26833	29.09929	679
9215	-1	ATCAGGGGTTTTATCAAGTT	GGG	73.93607	33.45243	680
9216	-1	TATCAGGGGTTTTATCAAGT	TGG	84.29187	44.06854	681
9230	-1	ATCACGTTTCTTATTATCAG	GGG	80.66179	58.62752	682
9231	-1	TATCACGTTTCTTATTATCA	GGG	82.42224	47.4101	683
9232	-1	GTATCACGTTTCTTATTATC	AGG	58.04329	29.70134	684
9261	-1	AAAATACATGTGAATGTTAG	TGG	69.1364	51.00635	685
9289	1	TATTTTAGTTCAAACCTCAA	TGG	60.62746	52.04271	686
9294	-1	ATATGAACATTTGGATCCAT	TGG	46.78738	41.8429	687
9303	-1	TATCTATATATATGAACATT	TGG	54.10978	35.78905	688
9338	-1	GTGGAATATATAAACAGTAG	AGG	80.1669	56.2241	689
9357	-1	AATTCGTATAGAGATTAATG	TGG	73.95175	62.50764	690
9459	-1	GCGTGATGGCGATATTTCTT	GGG	86.54713	37.70124	691
9460	-1	TGCGTGATGGCGATATTTCT	TGG	91.24965	32.52202	692
9473	-1	ATTGGTGCGAATTGCGTGA	TGG	89.64882	66.39713	693
9491	-1	CCTTGAGTGGCTCTAATAT	TGG	83.67391	26.74643	694
9502	1	CCAATATTAGAGCCACTACA	AGG	88.94997	62.54443	695
9503	-1	GCCATTGTTATTCCTTAG	TGG	86.75924	41.11451	696
9513	1	GCCACTACAAGGAATAACAA	TGG	78.65214	62.36806	697
9519	1	ACAAGGAATAACAATGGCCA	TGG	84.70821	54.69464	698
9520	1	CAAGGAATAACAATGGCCAT	GGG	88.76204	62.99032	699
9525	-1	TGTGGAAGCCAAGTCTCCCA	TGG	96.83523	60.94207	700
9528	1	AACAATGGCCATGGGAGACT	TGG	91.04348	37.2658	701
9543	-1	TAAATTGTGCAGTAGAGTTG	TGG	74.79858	59.21973	702
9574	1	TTAACTCTTTTTTTGTGAGA	TGG	67.00048	39.91071	703
9617	1	ATAATTTATTATTATTTGTC	AGG	34.22417	35.90263	704
13019	1	TGCTAGTACATGTCTATATA	AGG	51.98406	29.87248	705
13060	-1	AGCAAAAGCCATTTTACAC	AGG	81.1356	53.00551	706
13063	1	TATATATACCTGTGTAAAAA	TGG	60.80392	35.0674	707
13103	1	CGAAGTCTTGTTGATATTC	AGG	57.63384	20.64668	708

13152	-1	TATCTAGCTATTGTTCTTGC	GGG	77.9103	33.48401	709
13153	-1	CTATCTAGCTATTGTTCTTG	CGG	74.55985	43.94593	710
13180	-1	GCCAATGCATGTGGATGCTG	TGG	92.72347	60.88652	711
13189	-1	AATTGATATGCCAATGCATG	TGG	77.53646	64.53936	712
13190	1	ACCACAGCATCCACATGCAT	TGG	81.43998	59.13625	713
13224	-1	GAAGAAATGGGTTTGGAGAA	GGG	49.22523	49.88879	714
13225	-1	TGAAGAAATGGGTTTGGAGA	AGG	69.09616	48.87133	715
13231	-1	TGCACTTGAAGAAATGGGTT	TGG	71.57783	32.7508	716
13236	-1	GGTTATGCACTTGAAGAAAT	GGG	58.12192	38.87319	717
13237	-1	TGGTTATGCACTTGAAGAAA	TGG	67.04056	33.35969	718
13257	-1	TAATTAATTTGATGGTTAGT	TGG	56.6831	44.59873	719
13265	-1	ATGTATGGTAATTAATTTGA	TGG	46.32665	36.88262	720
13280	-1	TTGGATATTGAACTATGTA	TGG	64.43349	55.56421	721
13299	-1	TTAATATGATATGGTTTATT	TGG	51.05981	16.59851	722
13308	-1	TTCATAAAGTTAATATGATA	TGG	45.86946	38.61687	723
13386	-1	ACCAATTGCGTAAACGTGTT	GGG	92.64873	38.15694	724
13387	-1	GACCAATTGCGTAAACGTGT	TGG	94.58325	51.95048	725
13396	1	ACCCAACACGTTTACGCAAT	TGG	94.35879	35.38154	726
13419	1	TCAAGTGTCATTTGTTTAG	AGG	60.27782	38.1724	727
13444	-1	ATGATTGTATGGCGTGATGA	AGG	93.06641	56.01275	728
13455	-1	TGAATGATACAATGATTGTA	TGG	65.25489	43.79748	729
13524	1	GCTGAGTTAAGATAACCTCC	TGG	95.01444	53.02965	730
13528	-1	GGTAGTGAATGGCTTCCAGG	AGG	97.82923	69.29733	731
13531	-1	GGGGGTAGTGAATGGCTTCC	AGG	97.10675	35.56817	732
13539	-1	ATAATCCAGGGGGTAGTGAA	TGG	91.39645	59.2292	733
13545	1	GGAAGCCATTCACTACCCCC	TGG	99.45732	52.97305	734
13549	-1	GATGATATTAATAATCCAGG	GGG	87.72204	72.36028	735
13550	-1	TGATGATATTAATAATCCAG	GGG	70.74045	64.58516	736
13551	-1	ATGATGATATTAATAATCCA	GGG	56.34847	64.43657	737
13552	-1	GATGATGATATTAATAATCC	AGG	64.68399	47.30268	738
13584	1	TCTCTACGCAATATACATTC	TGG	85.48205	37.55777	739
13598	-1	GATGAAGATAAGTTTTTCAA	AGG	34.9946	46.22336	740
13625	-1	GTATTGGAGAACATTACTAA	TGG	81.61714	54.192	741
13641	-1	TTATATAATGTTAGGTGTAT	TGG	50.0441	37.07111	742
13649	-1	GGTTAATATTATATAATGTT	AGG	46.14634	41.29752	743
13670	-1	TGTTAATTATTTGTAATTAA	TGG	30.06382	19.30784	744
13699	-1	TTTATTATTTTATTTGAGGG	TGG	56.87973	54.11921	745
13702	-1	CTATTTATTATTTTATTTGA	GGG	22.86306	26.95378	746
13703	-1	ACTATTTATTATTTTATTTG	AGG	24.37755	35.02855	747

13732	1	ATAGTTATTATTATTACCTC	AGG	69.1903	42.38884	748
13733	1	TAGTTATTATTATTACCTCA	GGG	66.22878	58.43041	749
13737	-1	ATTTTCTGTAAGAAACCCTG	AGG	86.29961	70.70242	750
13754	1	GGTTTCTTACAGAAAATTCT	TGG	66.82242	29.73772	751
13776	1	GAAATGAGAAAAGCTTGAAA	TGG	53.70676	37.62262	752
13777	1	AAATGAGAAAAGCTTGAAAT	GGG	61.18077	43.36214	753
13791	-1	GTTTTTGGGAGTCAAGTATA	AGG	79.38507	47.93209	754
13805	-1	AAGCGAGGAAAAGAGTTTTT	GGG	66.05009	27.99152	755
13806	-1	GAAGCGAGGAAAAGAGTTTT	TGG	65.56542	30.89956	756
13820	-1	GGCGCACTTTTGAGAAGCG	AGG	98.3174	63.90309	757
13830	-1	CACCAATATGGGCGCACTTT	TGG	96.32144	22.47601	758
13839	1	CTCCAAAAGTGCGCCCATAT	TGG	94.31583	41.35959	759
13841	-1	AAAGTAAAGTCCACCAATAT	GGG	68.75283	36.52054	760
13842	1	CAAAAGTGCGCCCATATTGG	TGG	97.30856	48.92306	761
13842	-1	GAAAGTAAAGTCCACCAATA	TGG	84.33286	38.41171	762
13866	-1	TTTTCTTTTATTATGATTCA	GGG	39.04863	43.80485	763
13867	-1	TTTTCTTTTATTATGATTC	AGG	29.8602	27.80478	764
15978	1	GAAAAAATATATATAGTA	AGG	36.13766	41.8761	765
15995	1	GTAAGGAAAGAGAGAGATTA	CGG	54.88255	36.8303	766
15996	1	TAAGGAAAGAGAGAGATTAC	GGG	57.53794	51.49059	767
16000	1	GAAAGAGAGAGATTACGGGT	CGG	86.61929	64.70693	768
16001	1	AAAGAGAGAGATTACGGGTC	GGG	93.1411	48.83463	769
16013	1	TACGGGTTCGGGTATCCATGC	AGG	98.86045	52.97598	770
16016	1	GGGTCGGGTATCCATGCAGG	AGG	97.58735	53.65399	771
16016	-1	TTGGACCCGCCCTCCTGCA	TGG	96.38923	49.42741	772
16017	1	GGTCGGGTATCCATGCAGGA	GGG	99.45455	58.97398	773
16018	1	GTCGGGTATCCATGCAGGAG	GGG	98.23654	50.30444	774
16021	1	GGGTATCCATGCAGGAGGGG	CGG	94.19216	43.31877	775
16022	1	GGTATCCATGCAGGAGGGGC	GGG	98.70541	48.06551	776
16035	-1	TATGGTTGCTATAAAGACTT	TGG	72.61024	46.52942	777
16053	-1	CTGCACCAGATGCTCTACTA	TGG	93.10997	49.44754	778
16059	1	AGCAACCATAGTAGAGCATC	TGG	93.28253	48.85983	779
16065	1	CATAGTAGAGCATCTGGTGC	AGG	93.11563	41.82045	780
16066	1	ATAGTAGAGCATCTGGTGCA	GGG	83.82199	65.30317	781
16072	1	GAGCATCTGGTGCAGGGAGA	AGG	95.16992	42.4774	782
16073	1	AGCATCTGGTGCAGGGAGAA	GGG	92.19717	45.18153	783
16074	1	GCATCTGGTGCAGGGAGAAG	GGG	81.91369	46.14286	784
16077	1	TCTGGTGCAGGGAGAAGGGG	AGG	92.48414	60.72469	785
16082	1	TGCAGGGAGAAGGGGAGGTC	AGG	93.10184	46.08437	786

16095	1	GGAGGTCAGGCGAGAGAATA	TGG	93.54221	39.68623	787
16099	1	GTCAGGCGAGAGAATATGGT	TGG	92.48836	56.48815	788
16119	1	TGGCAATATTGATCCATGTT	TGG	82.02049	32.56259	789
16120	1	GGCAATATTGATCCATGTTT	GGG	77.55255	27.19259	790
16121	1	GCAATATTGATCCATGTTTG	GGG	74.7566	63.12063	791
16121	-1	GCGCTGCCACTCCCCAAACA	TGG	96.37777	52.90008	792
16126	1	ATTGATCCATGTTTGGGGAG	TGG	87.10662	48.37676	793
16143	-1	GCCGAGATCGTGTGTAATTA	TGG	97.83461	28.59523	794
16153	1	GCCATAATTACACACGATCT	CGG	96.59886	55.77195	795
16165	-1	TGAGACACTCCATGGTAGAC	TGG	91.79216	52.52207	796
16167	1	CGATCTCGGCCAGTCTACCA	TGG	100	52.39825	797
16173	-1	GAAGTTGCTGAGACACTCCA	TGG	95.9579	67.21232	798
16187	1	TGGAGTGTCTCAGCAACTTC	AGG	90.0358	32.39625	799
16188	1	GGAGTGTCTCAGCAACTTCA	GGG	93.36081	51.14577	800
16189	1	GAGTGTCTCAGCAACTTCAG	GGG	89.51798	72.6193	801
16200	1	CAACTTCAGGGGTGATACCT	AGG	97.10935	43.25481	802
16201	1	AACTTCAGGGGTGATACCTA	GGG	90.00002	51.96256	803
16206	-1	GCCTCTGACTTCATAGCCCT	AGG	93.23889	55.05182	804
16216	1	ACCTAGGGCTATGAAGTCAG	AGG	96.23264	65.86427	805
16228	-1	CAAGTCCCTGGACTCTGTTG	TGG	96.47011	52.94814	806
16233	1	CAGAGGCCACAACAGAGTCC	AGG	98.55521	53.21894	807
16234	1	AGAGGCCACAACAGAGTCCA	GGG	94.01286	58.98263	808
16240	-1	GGGTAAGAATTACAAGTCCC	TGG	90.97415	50.38745	809
16260	-1	AGTTTAATAGAAGTAATAAT	GGG	46.88021	37.37628	810
16261	-1	CAGTTTAATAGAAGTAATAA	TGG	62.668	33.52716	811
16332	1	AATATTATATATATTTATAT	TGG	27.51617	28.97861	812
16339	1	TATATATTTATATTGGATTT	TGG	45.60089	18.21184	813
16340	1	ATATATTTATATTGGATTTT	GGG	41.67556	15.57417	814
16344	1	ATTTATATTGGATTTTGGGA	TGG	54.07712	37.86541	815
16345	1	TTTATATTGGATTTTGGGAT	GGG	52.6776	44.14789	816
16398	1	ATCAAAGACTTGCTCAATAA	TGG	65.21274	25.05297	817
16404	1	GACTTGCTCAATAATGGATT	AGG	79.79469	45.48502	818
16408	1	TGCTCAATAATGGATTAGGC	TGG	82.83171	48.09015	819
16415	1	TAATGGATTAGGCTGGAATT	TGG	87.54181	34.74462	820
16420	1	GATTAGGCTGGAATTTGGTT	TGG	73.47545	37.68495	821
16421	1	ATTAGGCTGGAATTTGGTTT	GGG	61.27861	39.60131	822
16447	-1	TCAAAGGCAACACAAGTGAT	AGG	89.10229	58.40638	823
1273	-1	GAAACTAAATCCTCTGTTCA	AGG	90.64196	43.40519	826

[00281] Reference is made to Table 6 presenting a summary of the sequences within the scope of the current invention.

[00282] **Table 6: Summary of sequences within the scope of the present invention**

Sequence type	CsTHCAS	CsCBDAS	CsPT	CsOLS	CsAAE1
Genomic sequence	SEQ ID NO:1	SEQ ID NO:4	SEQ ID NO:7	SEQ ID NO:10	SEQ ID NO:13
Coding sequence (CDS)	SEQ ID NO:2	SEQ ID NO:5	SEQ ID NO:8	SEQ ID NO:11	SEQ ID NO:14
Amino acid sequence	SEQ ID NO:3	SEQ ID NO:6	SEQ ID NO:9	SEQ ID NO:12	SEQ ID NO:15
gRNA sequence	SEQ ID NO:16- SEQ ID NO:167 (Table 1)	SEQ ID NO:168- 304 & SEQ ID NO: 824-825 (Table 2)	SEQ ID NO:305- SEQ ID NO:458 (Table 3)	SEQ ID NO:459- SEQ ID NO:509 (Table 4)	SEQ ID NO:510- 823 & SEQ ID NO:826 (Table 5)

[00283] The above gRNA molecules have been cloned into suitable vectors and their sequence has been verified. In addition different Cas9 versions have been analyzed for optimal compatibility between the Cas9 protein activity and the gRNA molecule in the Cannabis plant.

[00284] The efficiency of the designed gRNA molecules have been validated by transiently transforming Cannabis tissue culture. A plasmid carrying a gRNA sequence together with the Cas9 gene has been transformed into Cannabis protoplasts. The protoplast cells have been grown for a short period of time and then were analyzed for existence of genome editing events. The positive constructs have been subjected to the herein established stable transformation protocol into Cannabis plant tissue for producing genome edited Cannabis plants in Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme 1 (CsAAE1) genes.

[00285] **Stage 3:** Transforming Cannabis plants using Agrobacterium or biolistics (gene gun) methods. For Agrobacterium and biolistics, a DNA plasmid carrying (Cas9 + gene specific gRNA) can be used. A vector containing a selection marker, Cas9 gene and relevant gene specific gRNA's is constructed. For biolistics, Ribonucleoprotein (RNP) complexes carrying (Cas9 protein + gene specific gRNA) are used. RNP complexes are created by mixing the Cas9 protein with relevant gene specific gRNA's.

[00286] According to some embodiments of the present invention, transformation of various Cannabis tissues was performed using particle bombardment of:

- DNA vectors
- Ribonucleoprotein complex (RNP's)

[00287] According to further embodiments of the present invention, transformation of various Cannabis tissues was performed using Agrobacterium (*Agrobacterium tumefaciens*) by:

- Regeneration-based transformation
- Floral-dip transformation
- Seedling transformation

[00288] Transformation efficiency by *A. tumefaciens* has been compared to the bombardment method by transient GUS transformation experiment. After transformation, GUS staining of the transformants has been performed.

[00289] Reference is now made to **Fig. 3** photographically presenting GUS staining after transient transformation of the following Cannabis tissues (A) axillary buds (B) leaf (C) calli, and (D) cotyledons. **Fig. 3** demonstrates that various Cannabis tissues have been successfully transiently transformed using biolistics system. Transformation has been performed into calli, leaves, axillary buds and cotyledons of Cannabis.

[00290] According to further embodiments of the present invention, additional transformation tools were used in Cannabis, including, but not limited to:

- Protoplast PEG transformation
- Extend RNP use
- Directed editing screening using fluorescent tags

- Electroporation

[00291] **Stage 4:** Regeneration in tissue-culture. When transforming DNA constructs into the plant, antibiotics is used for selection of positive transformed plants. An improved regeneration protocol was herein established for the Cannabis plant.

[00292] Reference is now made to **Fig. 4A-C** presenting regeneration of Cannabis tissue. In this figure, arrows indicate new meristem emergence.

[00293] **Stage 5:** Selection of positive transformants. Once regenerated plants appear in tissue culture, DNA is extracted from leaf sample of the transformed plant and PCR is performed using primers flanking the edited region. PCR products are then digested with enzymes recognizing the restriction site near the original gRNA sequence. If editing event occurred, the restriction site will be disrupted and the PCR product will not be cleaved. No editing event will result in a cleaved PCR product.

[00294] Reference is now made to **Fig. 5** showing PCR detection of Cas9 DNA in shoots of transformed Cannabis plants. DNA extracted from shoots of plants transformed with Cas9 using biolistics. This figure shows that three weeks post transformation, Cas9 DNA was detected in shoots of transformed plants.

[00295] Screening for CRISPR/Cas9 gene editing events has been performed by at least one of the following analysis methods:

- Restriction Fragment Length Polymorphism (RFLP)
- Next Generation Sequencing (NGS)
- PCR fragment analysis
- Fluorescent-tag based screening
- High resolution melting curve analysis (HRMA)

[00296] Reference is now made to **Fig. 6** presenting results of *in vitro* analysis of CRISPR/Cas9 cleavage activity. **Fig. 6A** schematically shows the genomic area targeted for editing (PAM is marked in red) and amplified by the reverse and forward designed primers **Fig. 6B** photographically presents a gel showing successful digestion of the resulted PCR amplicon containing the gene specific gRNA sequence, by RNP complex containing Cas9. The analysis included the following steps:

1) Amplicon was isolated from two exemplified Cannabis strains by primers flanking the sequence of the gene of interest targeted by the predesigned sgRNA.

2) RNP complex was incubated with the isolated amplicon.

- 3) The reaction mix was then loaded on agarose gel to evaluate Cas9 cleavage activity at the target site.

[00297] **Stage 6:** Selection of transformed Cannabis plants presenting reduced expression of at least one of Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme 1 (CsAAE1) as described above. It is within the scope that different gRNA promoters were tested in order to maximize editing efficiency.

[00298] Various embodiments have been presented. Each of these embodiments may of course include features from other embodiments presented, and embodiments not specifically described may include various features described herein.

CLAIMS

1. A Cannabis plant with reduced delta-9-tetrahydrocannabinol (THC) content, or reduced cannabidiol (CBD) content, or reduced THC and CBD content, wherein said plant comprises at least one targeted genome modification effective in decreasing expression of a at least one Cannabis gene encoding a cannabinoid biosynthesis enzyme selected from the group consisting of Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme 1 (CsAAE1) and any combination thereof.
2. The Cannabis plant according to claim 1, wherein said plant comprises reduced THC content, or reduced CBD content, or reduced THC and CBD content relative to a Cannabis plant of a similar genotype or genetic background that does not comprise said targeted genome modification.
3. The Cannabis plant according to claim 1, wherein said targeted genome modification is located in the cannabinoid biosynthesis enzyme gene locus.
4. The Cannabis plant according to claim 1, wherein said targeted genome modification interrupts or interferes with or down regulate or silence transcription and/or translation of said Cannabis gene encoding said cannabinoid biosynthesis enzyme.
5. The Cannabis plant according to claim 1, wherein said plant comprises an endonuclease enzyme targeting a nucleic acid sequence coding for said at least one cannabinoid biosynthesis enzyme.
6. The Cannabis plant according to claim 1, wherein said plant does not comprise within its genome exogenous genetic material and said plant is a non-naturally occurring Cannabis plant or cell thereof.
7. The Cannabis plant according to claim 1, wherein the nucleotide sequence encoding said at least one cannabinoid biosynthesis enzyme is selected from the group consisting of: CsTHCAS having a genomic nucleotide sequence as set forth in SEQ ID NO:1 or a

functional variant thereof, CsCBDAS having a genomic nucleotide sequence as set forth in SEQ ID NO:4 or a functional variant thereof, CsPT having a genomic nucleotide sequence as set forth in SEQ ID NO:7 or a functional variant thereof, CsOLS having a genomic nucleotide sequence as set forth in SEQ ID NO:10 or a functional variant thereof and CsAAE1 having a genomic nucleotide sequence as set forth in SEQ ID NO:13 or a functional variant thereof.

8. The Cannabis plant according to claim 7, wherein said functional variant has at least 75% sequence identity to the nucleotide sequence of said cannabinoid biosynthesis enzyme or a codon degenerate nucleotide sequence thereof.
9. The Cannabis plant according to claim 1, wherein said plant has decreased expression levels of at least one of CsTHCAS protein having an amino acid sequence with at least 75% identity to the amino acid sequence as set forth in SEQ ID NO:3 or a conservatively substituted amino acid sequence thereof, CsCBDAS protein having an amino acid sequence with at least 75% identity to the amino acid sequence as set forth in SEQ ID NO:6 or a conservatively substituted amino acid sequence thereof, CsPT protein having an amino acid sequence with at least 75% identity to the amino acid sequence as set forth in SEQ ID NO:9 or a conservatively substituted amino acid sequence thereof, CsOLS protein having an amino acid sequence with at least 75% identity to the amino acid sequence as set forth in SEQ ID NO:12 or a conservatively substituted amino acid sequence thereof, and CsAAE1 protein having an amino acid sequence with at least 75% identity to the amino acid sequence as set forth in SEQ ID NO:15 or a conservatively substituted amino acid sequence thereof.
10. The Cannabis plant according to claim 1, wherein said Cannabis plant has modulated expression of one or more of the cannabinoids, optionally cannabigerolic acid, cannabigerol, DELTA.9-tetrahydrocannabinolic acid, cannabidiolic acid, cannabichromenic acid, DELTA.9-tetrahydrocannabinol, cannabidiol, cannabichromene, THC, D9-THC, D8-THC, THCA, THCV, D8-THCV, D9-THCV, THCVA, CBD, CBDA, CBDV, CBDVA, CBC, CBCA, CBCV, CBCVA, CBG, CBGA, CBGV, CBGVA, CBN, CBNA, CBNV, CBNVA, CBND, CBNDA, CBNDV, CBNDVA, CBE, CBEA, CBEV, CBEVA, CBL, CBLA, CBLV, or CBLVA and cannabidiol.

11. The Cannabis plant according to claim 1, wherein said Cannabis plant has reduced expression of one or more of the cannabinoids, optionally cannabigerolic acid, cannabigerol, DELTA.9-tetrahydrocannabinolic acid, cannabidiolic acid, cannabichromenic acid, DELTA.9-tetrahydrocannabinol, cannabidiol, cannabichromene, THC, D9-THC, D8-THC, THCA, THCV, D8-THCV, D9-THCV, THCVA, CBD, CBDA, CBDV, CBDVA, CBC, CBCA, CBCV, CBCVA, CBG, CBGA, CBGV, CBGVA, CBN, CBNA, CBNV, CBNVA, CBND, CBNDA, CBNDV, CBNDVA, CBE, CBEA, CBEV, CBEVA, CBL, CBLA, CBLV, or CBLVA and cannabidiol, as compared to a Cannabis plant of a similar genotype or genetic background that does not comprise said targeted genome modification.
12. The Cannabis plant according to claim 1, wherein said targeted genome modification is introduced using CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas) genes (CRISPR/Cas) system, Transcription activator-like effector nuclease (TALEN), Zinc Finger Nuclease (ZFN), meganuclease or any combination thereof.
13. The Cannabis plant according to claim 12, wherein said CRISPR/Cas genes or proteins are selected from the group consisting of Cas3, Cas4, Cas5, Cas5e (or CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9, Cas10, Cas10d, Cas12, Cas13, Cas14, CasX, CasF, CasG, CasH, Csy1, Csy2, Csy3, Cse1 (or CasA), Cse2 (or CasB), Cse3 (or CasE), Cse4 (or CasC), Csc1, Csc2, Csa5, Csn1, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Cpf1, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csz1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cu1966.
14. The Cannabis plant according to claim 1, wherein said targeted genome modification is introduced via (i) at least one RNA-guided (gRNA) endonuclease or nucleic acid encoding at least one RNA-guided endonuclease, and (ii) at least one guide RNA (gRNA) or DNA encoding at least one guide RNA (gRNA), further wherein each of said at least one gRNA directs said endonuclease to a targeted site located in the genomic sequence of said at least one Cannabis gene encoding a cannabinoid biosynthesis enzyme.

15. The Cannabis plant according to claim 14, wherein the gRNA nucleotide sequence targeted to CsTHCAS genomic sequence is as set forth in nucleotide sequence selected from the group consisting of SEQ. ID. NO.: 16-167 and any combination thereof.
16. The Cannabis plant according to claim 14, wherein the gRNA nucleotide sequence targeted to CsCBDAS genomic sequence is as set forth in nucleotide sequence selected from the group consisting of SEQ. ID. NO.: 168-304, SEQ. ID. NO.: 824-825, and any combination thereof.
17. The Cannabis plant according to claim 14, wherein the gRNA nucleotide sequence targeted to CsPT genomic sequence is as set forth in nucleotide sequence selected from the group consisting of SEQ. ID. NO.: 305-458 and any combination thereof.
18. The Cannabis plant according to claim 14, wherein the gRNA nucleotide sequence targeted to CsOLS genomic sequence is as set forth in nucleotide sequence selected from the group consisting of SEQ. ID. NO.: 459-509 and any combination thereof.
19. The Cannabis plant according to claim 14, wherein the gRNA nucleotide sequence targeted to CsAAE1 genomic sequence is as set forth in nucleotide sequence selected from the group consisting of SEQ. ID. NO.: 510-823, SEQ. ID. NO.: 826, and any combination thereof.
20. The Cannabis plant according to claim 1, wherein said plant is mutated in a gene selected from the group consisting of CsTHCAS, CsSP, CsCBDAS, CsPT, CsOLS, CsAAE1 and any combination thereof.
21. The Cannabis plant according to claim 20, wherein said mutated CsTHCAS, CsSP, CsCBDAS, CsPT, CsOLS and/or CsAAE1 gene is a CRISPR/Cas9- induced heritable mutated allele.
22. The Cannabis plant according to claim 1, wherein said genome modification is a missense mutation, nonsense mutation, insertion, deletion, indel, substitution or duplication.
23. The Cannabis plant of claim 22, wherein the insertion or the deletion produces a gene comprising a frameshift.

24. The Cannabis plant of claim 20, wherein said plant is homozygous for said at least one mutated gene.
25. The Cannabis plant according to claim 20, wherein said mutation is in the coding region of said gene, a mutation in the regulatory region of said gene, a mutation in a gene downstream of said gene in the cannabinoid biosynthesis pathway or an epigenetic factor.
26. The Cannabis plant according to claim 20, wherein said mutation is a silencing mutation, a knockdown mutation, a knockout mutation, a loss of function mutation or any combination thereof.
27. The Cannabis plant according to claim 1 wherein said genome modification is generated *in planta*.
28. The Cannabis plant according to claim 1 wherein said genome modification is generated *in planta* via introduction of a construct comprising (a) Cas DNA and gRNA sequence selected from the group consisting of SEQ ID NO:16-SEQ ID NO:826 and any combination thereof, or (b) a ribonucleoprotein (RNP) complex comprising Cas protein and gRNA sequence selected from the group consisting of SEQ ID NO:16-826 and any combination thereof.
29. The Cannabis plant according to claim 14 wherein said gRNA sequence comprises a 3' NGG Protospacer Adjacent Motif (PAM).
30. The Cannabis plant according to claim 14 wherein said gRNA is introduced into the plant cells via Agrobacterium infiltration, virus based plasmids for delivery of the genome editing molecules or mechanical insertion such as polyethylene glycol (PEG) mediated DNA transformation, electroporation or gene gun biolistics.
31. The Cannabis plant according to claim 1 wherein said Cannabis plant is selected from the group of species that includes, but is not limited to, *Cannabis sativa* (*C. sativa*), *C. indica*, *C. ruderalis* and any hybrid or cultivated variety of the genus *Cannabis*.
32. A Cannabis plant, plant part or plant cell according to claim 1 wherein said plant does not comprise a transgene.

33. A plant part, plant cell or plant seed of a plant according to claim 1.
34. A tissue culture of regenerable cells, protoplasts or callus obtained from the Cannabis plant according to claim 1.
35. The Cannabis plant according to claim 1 wherein said plant genotype is obtainable by deposit under accession number with NCIMB Aberdeen AB21 9YA, Scotland, UK.
36. The Cannabis plant according to claim 1, wherein said Cannabis plant comprises a DNA encoding an antisense RNA or a siRNA effective to suppress expression of CsTHCAS, CsSP, CsCBDAS, CsPT, CsOLS, CsAAE1 and any combination thereof, the DNA operably linked to a heterologous promoter, wherein the Cannabis plant comprises reduced THC content, reduced CBD content, or decreased THC and CBD content relative to a Cannabis plant of a similar genotype that does not comprise the DNA.
37. The Cannabis plant according to claim 1, wherein said Cannabis plant has a THC content of up to 30% by weight, particularly between about 0.1% to about 30% by weight, more particularly between about 0.3% to about 30%, even more particularly between about 0.3% to about 10% by weight.
38. The Cannabis plant according to claim 1, wherein said Cannabis plant has a CBD content of up to 30% by weight, particularly between about 0.1% to about 30% by weight, more particularly between about 0.3% to about 30%, even more particularly between about 0.3% to about 10% by weight.
39. The Cannabis plant according to claim 1, wherein said plant has a THC and/or CBD content of not more than about 0.5% by weight.
40. The Cannabis plant according to claim 1, wherein said plant is THC free.
41. The Cannabis plant according to claim 1, wherein said plant has at least one targeted genome modification in at least one Cannabis gene encoding cannabinoid precursor synthesis enzyme selected from the group consisting of CsAAE, CsPT and CsOLS.

42. The Cannabis plant according to claim 41, wherein said plant exhibits reduced expression of THC, CBD or both relative to a Cannabis plant of a similar genotype or genetic background lacking said targeted genome modification.
43. The Cannabis plant according to claim 41, wherein said plant has a THC and/or CBD content of up to 30% by weight, particularly between about 0.1% to about 30% by weight, more particularly between about 0.3% to about 30%, even more particularly between about 0.3% to about 10% by weight.
44. The Cannabis plant according to claim 41, wherein said plant has a THC and/or CBD content of not more than about 0.5% by weight.
45. The Cannabis plant according to claim 1, wherein said plant has at least one targeted genome modification in at least one Cannabis gene encoding cannabinoid synthesis enzyme selected from the group consisting of CsTHCAS and CsCBDAS.
46. The Cannabis plant according to claim 45, wherein said plant has at least one targeted genome modification in CsTHCAS and said plant exhibits reduced expression of THCA or THC, and elevated expression of CBD or CBDA relative to a Cannabis plant of a similar genotype or genetic background lacking said targeted genome modification.
47. The Cannabis plant according to claim 46, wherein said plant has a THC and/or THCA content of up to 30% by weight, particularly between about 0.1% to about 30% by weight, more particularly between about 0.3% to about 30%, even more particularly between about 0.3% to about 10% by weight.
48. The Cannabis plant according to claim 46, wherein said plant has a THC and/or THCA content of not more than about 0.5% by weight.
49. The Cannabis plant according to claim 45, wherein said plant has at least one targeted genome modification in CsCBDAS and said plant exhibits reduced expression of CBDA or CBD, and elevated expression of THC or THCA relative to a Cannabis plant of a similar genotype or genetic background lacking said targeted genome modification.

50. The Cannabis plant according to claim 49, wherein said plant has a THC and/or CBD content of up to 30% by weight, particularly between about 0.1% to about 30% by weight, more particularly between about 0.3% to about 30%, even more particularly between about 0.3% to about 10% by weight.
51. The Cannabis plant according to claim 49, wherein said plant has a CBD and/or CBDA content of not more than about 0.5% by weight.
52. A Cannabis plant derived product from the plant of claim 1.
53. The Cannabis plant derived product of claim 52, comprising a combined cannabidiolic acid and cannabidiol concentration of about 0.3% to about 30% by weight .
54. The Cannabis plant derived product of claim 52, comprising a combined delta-9-tetrahydrocannabinol and tetrahydrocannabinolic acid concentration of between about 0.3% to about 30% by weight .
55. The Cannabis plant derived product of claim 52, comprising Cannabis oil, Cannabis tincture, dried Cannabis flowers, and/or dried Cannabis leaves .
56. The Cannabis plant derived product of claim 52, for medical use.
57. The Cannabis plant derived product of claim 52, formulated for inhalation, oral consumption, sublingual consumption, or topical consumption.
58. A medical composition derived from the plant according to claim 1.
59. A method for producing a Cannabis plant with reduced delta-9-tetrahydrocannabinol (THC) content, or reduced cannabidiol (CBD) content, or reduced THC and CBD content, wherein said method comprises steps of introducing into said Cannabis plant genome or a cell thereof at least one targeted genome modification effective in decreasing expression of a at least one Cannabis gene encoding a cannabinoid biosynthesis enzyme selected from the group consisting of tetrahydrocannabinolic acid synthase (THCAS), cannabidiolic acid synthase (CBDAS), aromatic prenyltransferase (PT), olivetol synthase (OLS), acyl-activating enzyme 1 (AAE1) and any combination thereof.

60. The method according to claim 59, further comprising steps of:

- a. constructing an endonuclease enzyme targeting a nucleic acid sequence coding for a cannabinoid biosynthesis enzyme selected from the group consisting of CsTHCAS, CsCBDAS, CsPT, CsOLS, CsAAE1 and any combination thereof;
- b. introducing the endonuclease enzyme into the genome of the Cannabis plant of; and
- c. decreasing expression of the at least one cannabinoid biosynthesis enzyme within the genome.

61. The method according to claim 59, further comprising steps of:

- a. introducing into the Cannabis plant or a cell thereof (i) at least one RNA-guided endonuclease or nucleic acid encoding at least one RNA-guided endonuclease, and (ii) at least one guide RNA (gRNA) or DNA encoding at least one gRNA;
- b. assaying the Cannabis plant or a cell thereof for an endonuclease-mediated modification in the DNA of said at least one Cannabis cannabinoid biosynthesis enzyme gene locus; and
- c. identifying the Cannabis plant, or a cell thereof, or a progeny cell thereof as comprising a modification in said at least one gene locus.

62. The method according to claim 59, further comprising steps of culturing the Cannabis plant or cell thereof such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence of said at least one Cannabis cannabinoid biosynthesis enzyme, enabling the RNA-guided endonuclease introduce a double-stranded break in the targeted site, and repair of the double-stranded break by a DNA repair process such that the chromosomal sequence is modified, wherein the targeted site is located in the gene locus of the at least one Cannabis cannabinoid biosynthesis enzyme and the chromosomal modification interrupts or interferes with transcription and/or translation of said at least one gene encoding Cannabis cannabinoid biosynthesis enzyme.

63. The method according to claim 59, wherein the endonuclease enzyme is a CRISPR/Cas9 system.

64. The method according to claim 59, comprising steps of interfering with expression of a cannabinoid biosynthesis enzyme selected from the group consisting of CsTHCAS, CsCBDAS, CsPT, CsOLS, CsAAE1 and any combination thereof.
65. The method according to claim 59, wherein said plant comprises reduced THC content, or reduced CBD content, or reduced THC and CBD content relative to a Cannabis plant of a similar genotype or genetic background that does not comprise said targeted genome modification.
66. The method according to claim 59, wherein said targeted genome modification is located in the cannabinoid biosynthesis enzyme gene locus.
67. The method according to claim 59, wherein said targeted genome modification interrupts or interferes with or down regulate or silence transcription and/or translation of said Cannabis gene encoding said cannabinoid biosynthesis enzyme.
68. The method according to claim 59, wherein said plant comprises an endonuclease enzyme targeting a nucleic acid sequence coding for said at least one cannabinoid biosynthesis enzyme.
69. The method according to claim 59, wherein said plant does not comprise within its genome exogenous genetic material and said plant is a non-naturally occurring Cannabis plant or cell thereof.
70. The method according to claim 59, wherein the nucleotide sequence encoding said at least one cannabinoid biosynthesis enzyme is selected from the group consisting of: CsTHCAS having a genomic nucleotide sequence as set forth in SEQ ID NO:1 or a functional variant thereof, CsCBDAS having a genomic nucleotide sequence as set forth in SEQ ID NO:4 or a functional variant thereof, CsPT having a genomic nucleotide sequence as set forth in SEQ ID NO:7 or a functional variant thereof, CsOLS having a genomic nucleotide sequence as set forth in SEQ ID NO:10 or a functional variant thereof and CsAAE1 having a genomic nucleotide sequence as set forth in SEQ ID NO:13 or a functional variant thereof.

71. The method according to claim 70, wherein said functional variant has at least 75% sequence identity to the nucleotide sequence of said cannabinoid biosynthesis enzyme or a codon degenerate nucleotide sequence thereof.
72. The method according to claim 59, wherein said plant has decreased expression levels of at least one of CsTHCAS protein having an amino acid sequence with at least 75% identity to the amino acid sequence as set forth in SEQ ID NO:3 or a conservatively substituted amino acid sequence thereof, CsCBDAS protein having an amino acid sequence with at least 75% identity to the amino acid sequence as set forth in SEQ ID NO:6 or a conservatively substituted amino acid sequence thereof, CsPT protein having an amino acid sequence with at least 75% identity to the amino acid sequence as set forth in SEQ ID NO:9 or a conservatively substituted amino acid sequence thereof, CsOLS protein having an amino acid sequence with at least 75% identity to the amino acid sequence as set forth in SEQ ID NO:12 or a conservatively substituted amino acid sequence thereof, and CsAAE1 protein having an amino acid sequence with at least 75% identity to the amino acid sequence as set forth in SEQ ID NO:15 or a conservatively substituted amino acid sequence thereof.
73. The method according to claim 59, wherein said Cannabis plant has modulated expression of one or more of the cannabinoids, optionally cannabigerolic acid, cannabigerol, DELTA.9-tetrahydrocannabinolic acid, cannabidiolic acid, cannabichromenic acid, DELTA.9-tetrahydrocannabinol, cannabidiol, cannabichromene, THC, D9-THC, D8-THC, THCA, THCV, D8-THCV, D9-THCV, THCVA, CBD, CBDA, CBDV, CBDVA, CBC, CBCA, CBCV, CBCVA, CBG, CBGA, CBGV, CBGVA, CBN, CBNA, CBNV, CBNVA, CBND, CBNDA, CBNDV, CBNDVA, CBE, CBEA, CBEV, CBEVA, CBL, CBLA, CBLV, or CBLVA and cannabidiol.
74. The method according to claim 59, wherein said Cannabis plant has reduced expression of one or more of the cannabinoids, optionally cannabigerolic acid, cannabigerol, DELTA.9-tetrahydrocannabinolic acid, cannabidiolic acid, cannabichromenic acid, DELTA.9-tetrahydrocannabinol, cannabidiol, cannabichromene, THC, D9-THC, D8-THC, THCA, THCV, D8-THCV, D9-THCV, THCVA, CBD, CBDA, CBDV, CBDVA, CBC, CBCA, CBCV, CBCVA, CBG, CBGA, CBGV, CBGVA, CBN, CBNA, CBNV, CBNVA, CBND, CBNDV, CBNDVA, CBE, CBEA, CBEV, CBEVA, CBL, CBLA, CBLV, or CBLVA and cannabidiol.

CBNDA, CBNDV, CBNDVA, CBE, CBEA, CBEV, CBEVA, CBL, CBLA, CBLV, or CBLVA and cannabidiol, as compared to a Cannabis plant of a similar genotype or genetic background that does not comprise said targeted genome modification.

75. The method according to claim 59, wherein said targeted genome modification is introduced using CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas) genes (CRISPR/Cas) system, Transcription activator-like effector nuclease (TALEN), Zinc Finger Nuclease (ZFN), meganuclease or any combination thereof.
76. The method according to claim 59, wherein said CRISPR/Cas genes or proteins are selected from the group consisting of Cas3, Cas4, Cas5, Cas5e (or CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9, Cas10, Cas10d, Cas12, Cas13, Cas14, CasX, CasF, CasG, CasH, Csy1, Csy2, Csy3, Cse1 (or CasA), Cse2 (or CasB), Cse3 (or CasE), Cse4 (or CasC), Csc1, Csc2, Csa5, Csn1, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Cpf1, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csz1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cu1966.
77. The method according to claim 59, wherein said targeted genome modification is introduced via (i) at least one RNA-guided (gRNA) endonuclease or nucleic acid encoding at least one RNA-guided endonuclease, and (ii) at least one guide RNA (gRNA) or DNA encoding at least one guide RNA (gRNA), further wherein each of said at least one gRNA directs said endonuclease to a targeted site located in the genomic sequence of said at least one Cannabis gene encoding a cannabinoid biosynthesis enzyme.
78. The method according to claim 77, wherein
 - a. the gRNA nucleotide sequence targeted to CsTHCAS genomic sequence is as set forth in nucleotide sequence selected from the group consisting of SEQ. ID. NO.: 16-167 and any combination thereof;
 - b. the gRNA nucleotide sequence targeted to CsCBDAS genomic sequence is as set forth in nucleotide sequence selected from the group consisting of SEQ. ID. NO.: 168-304, SEQ. ID. NO.: 824-825, and any combination thereof;

- c. the gRNA nucleotide sequence targeted to CsPT genomic sequence is as set forth in nucleotide sequence selected from the group consisting of SEQ. ID. NO.: 305-458 and any combination thereof;
 - d. the gRNA nucleotide sequence targeted to CsOLS genomic sequence is as set forth in nucleotide sequence selected from the group consisting of SEQ. ID. NO.: 459-509 and any combination thereof; and
 - e. the gRNA nucleotide sequence targeted to CsAAE1 genomic sequence is as set forth in nucleotide sequence selected from the group consisting of SEQ. ID. NO.: 510-823, SEQ. ID. NO.: 826, and any combination thereof.
79. The method according to claim 59, wherein said plant is mutated in a gene selected from the group consisting of CsTHCAS, CsSP, CsCBDAS, CsPT, CsOLS, CsAAE1 and any combination thereof.
80. The method according to claim 59 wherein said at least one genome modification is generated *in planta*.
81. The method according to claim 59 wherein said genome modification is generated *in planta* via introduction of a construct comprising (a) Cas DNA and gRNA sequence selected from the group consisting of SEQ ID NO:16-SEQ ID NO:826 and any combination thereof, or (b) a ribonucleoprotein (RNP) complex comprising Cas protein and gRNA sequence selected from the group consisting of SEQ ID NO:16-826 and any combination thereof.
82. The method according to claim 77 wherein said gRNA is introduced into the plant cells via Agrobacterium infiltration, virus based plasmids for delivery of the genome editing molecules or mechanical insertion such as polyethylene glycol (PEG) mediated DNA transformation, electroporation or gene gun biolistics.
83. A plant part, plant cell or plant seed produced by the method according to claim 59.
84. The method according to claim 59, wherein said Cannabis plant comprises a DNA encoding an antisense RNA or a siRNA effective to suppress expression of CsTHCAS, CsSP, CsCBDAS, CsPT, CsOLS, CsAAE1 and any combination thereof, the DNA operably linked

to a heterologous promoter, wherein the Cannabis plant comprises reduced THC content, reduced CBD content, or decreased THC and CBD content relative to a Cannabis plant of a similar genotype that does not comprise the DNA.

85. A method for producing a medical Cannabis composition, the method comprising:

- a. obtaining the Cannabis plant of claim 1; and
- b. formulating a medical Cannabis composition from said plant.

86. A method for manipulating a content of one or more cannabinoids in a Cannabis plant, the method comprising down-regulating activity of at least one Cannabis gene encoding a cannabinoid biosynthesis enzyme selected from the group consisting of tetrahydrocannabinolic acid synthase (THCAS), cannabidiolic acid synthase (CBDAS), aromatic prenyltransferase (PT), olivetol synthase (OLS), acyl-activating enzyme 1 (AAE1) and any combination thereof.

87. The method according to claim 59, comprises steps of:

- a. identifying at least one Cannabis gene locus encoding a cannabinoid biosynthesis enzyme selected from the group consisting of tetrahydrocannabinolic acid synthase (THCAS), cannabidiolic acid synthase (CBDAS), aromatic prenyltransferase (PT), olivetol synthase (OLS), acyl-activating enzyme 1 (AAE1) and any combination thereof;
- b. identifying at least one endonuclease recognition sequence in or proximal to the at least one cannabinoid biosynthesis enzyme gene locus;
- c. providing at least one guide RNA (gRNA) comprising a nucleotide sequence at least partially complementary to said at least one identified gene locus;
- d. introducing into Cannabis plant cells a construct comprising (a) an endonuclease nucleotide sequence operably linked to said gRNA, or (b) a ribonucleoprotein (RNP) complex comprising an endonuclease protein and said gRNA;

- e. assaying the Cannabis plant or a cell thereof for an endonuclease-mediated modification in the DNA of the at least one cannabinoid biosynthesis enzyme gene locus; and
 - f. identifying the Cannabis plant, a cell thereof, or a progeny cell thereof as comprising a modification in the at least one cannabinoid biosynthesis enzyme gene locus.
88. The method according to claim 87, further comprises steps of
- a. screening the genome of the transformed Cannabis plant cells for induced targeted mutations in at least one of said cannabinoid biosynthesis enzyme gene locus comprising obtaining a nucleic acid sample from said transformed plant and carrying out nucleic acid amplification and optionally restriction enzyme digestion to detect a mutation in said at least one of said cannabinoid biosynthesis enzyme gene locus;
 - g. confirming the presence of said genetic mutation in the genome of said plant cells by sequencing said at least one cannabinoid biosynthesis enzyme gene locus;
 - h. regenerating plants carrying said genetic modification; and
 - i. screening said regenerated plants for a plant with modified cannabinoid content.
89. The method according to claim 88, wherein the endonuclease is expressed transiently or stably in the Cannabis plant.
90. An isolated nucleotide sequence having at least 75% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:10, and SEQ ID NO:13.
91. An isolated nucleotide sequence having at least 75% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11 and SEQ ID NO:14.
92. An isolated amino acid sequence having at least 75% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:12 and SEQ ID NO:15.

93. An isolated nucleotide sequence having at least 75% sequence identity to a nucleotide sequence as set forth in SEQ ID NO:16-826.
94. A vector, construct or expression system or cassette comprising the nucleic acid sequence as defined in any one of claims 90-93.
95. Use of a nucleotide sequence as set forth in at least one of SEQ ID NO:16-826 and any combination thereof for down regulation of at least one Cannabis cannabinoid biosynthesis enzyme gene selected from the group consisting of Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS), Cannabis cannabidiolic acid synthase (CsCBDAS), Cannabis aromatic prenyltransferase (CsPT), Cannabis olivetol synthase (CsOLS), Cannabis acyl-activating enzyme 1 (CsAAE1) and any combination thereof.
96. Use of a nucleotide sequence as set forth in at least one of SEQ ID NO:16-167 and any combination thereof for targeted genome modification of Cannabis tetrahydrocannabinolic acid synthase (CsTHCAS) gene.
97. Use of a nucleotide sequence as set forth in at least one of SEQ ID NO:168-304, SEQ ID NO:824-825 and any combination thereof for targeted genome modification of Cannabis cannabidiolic acid synthase (CsCBDAS).
98. Use of a nucleotide sequence as set forth in at least one of SEQ ID NO:305-458 and any combination thereof for targeted genome modification of Cannabis aromatic prenyltransferase (CsPT).
99. Use of a nucleotide sequence as set forth in at least one of SEQ ID NO:459-509 and any combination thereof for targeted genome modification of Cannabis olivetol synthase (CsOLS).
100. Use of a nucleotide sequence as set forth in at least one of SEQ ID NO:510-823, SEQ ID NO:826 and any combination thereof for targeted genome modification of Cannabis acyl-activating enzyme 1 (CsAAE1).

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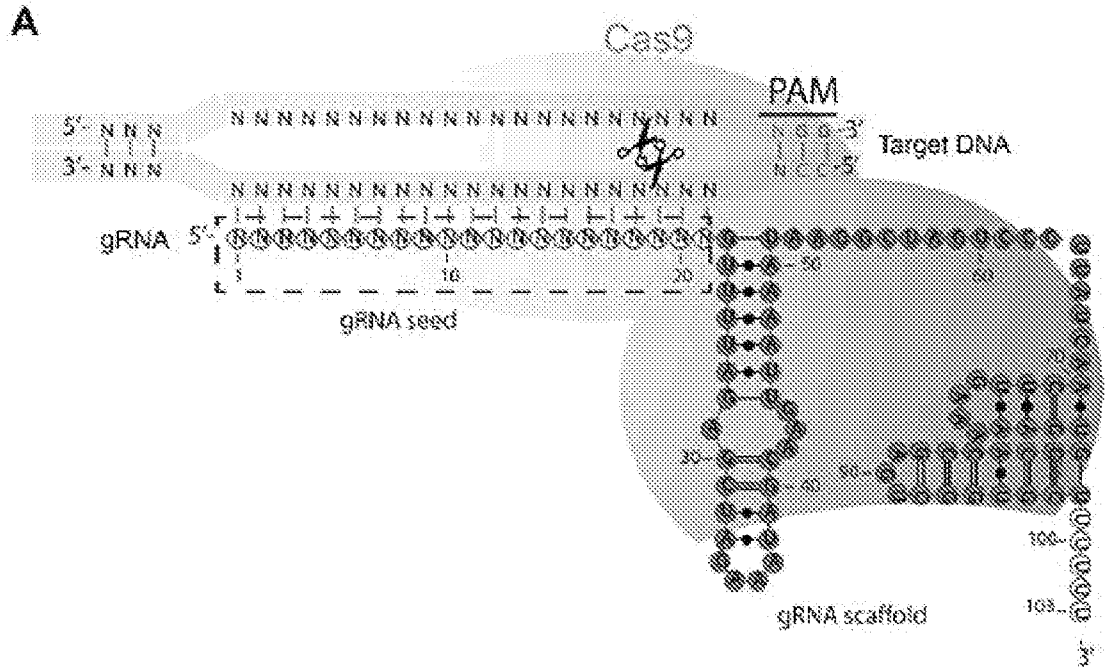


FIG. 1

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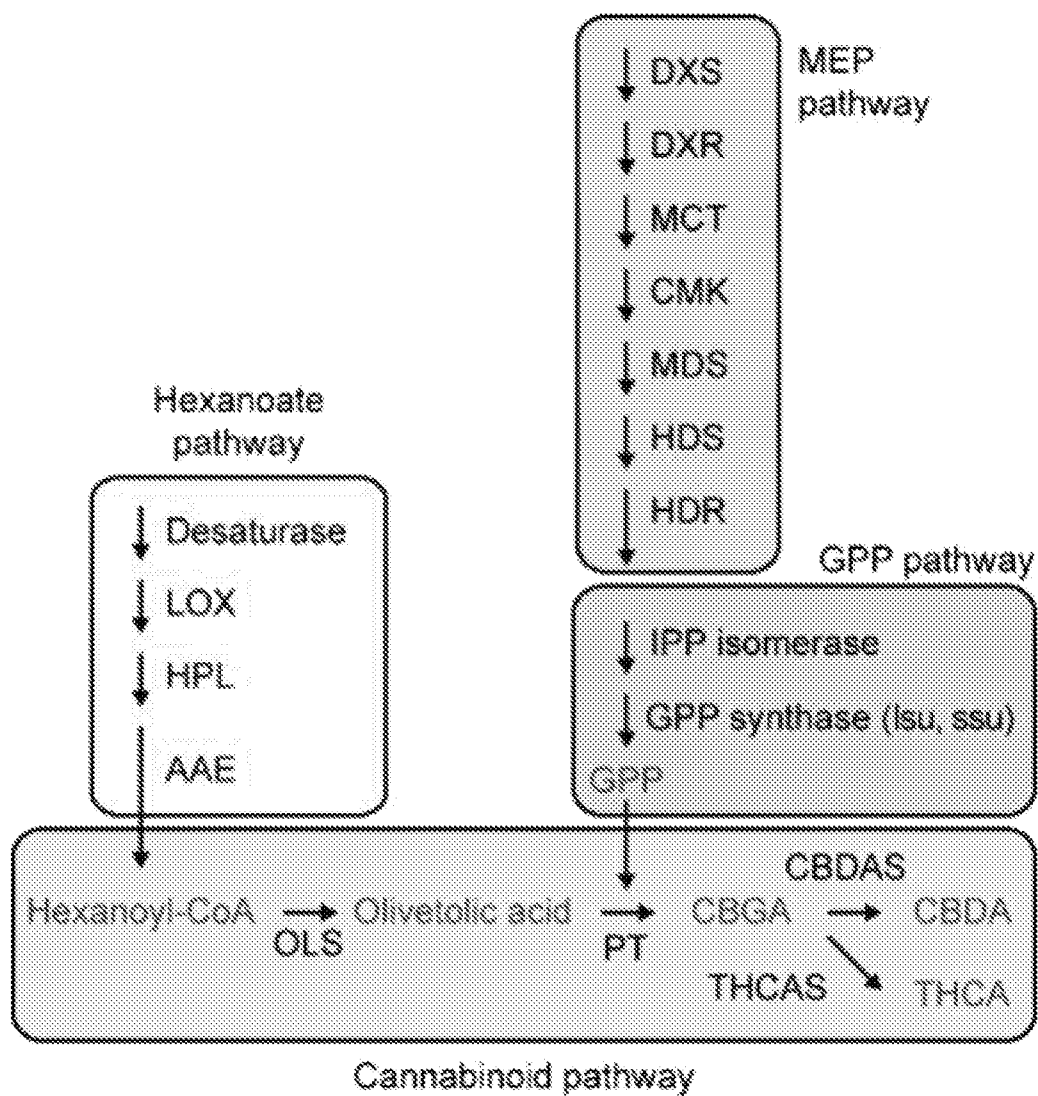


FIG. 2

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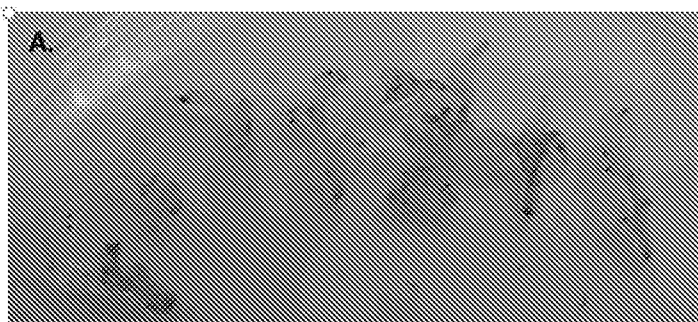


FIG. 3A

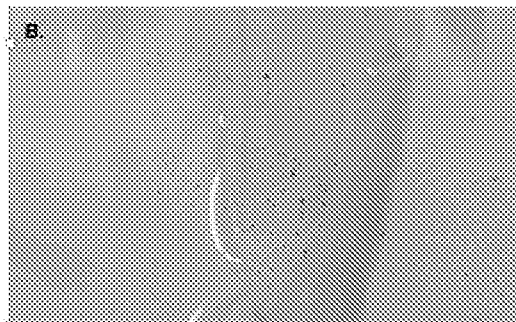


FIG. 3B

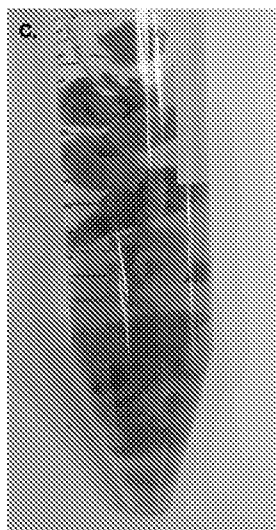


FIG. 3C

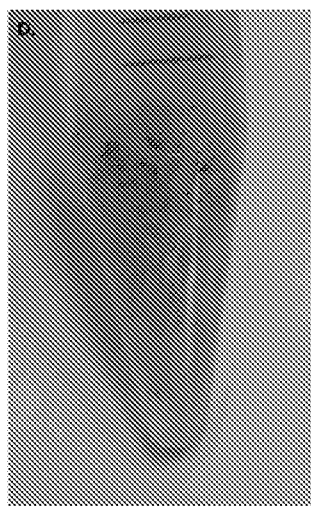


FIG. 3D

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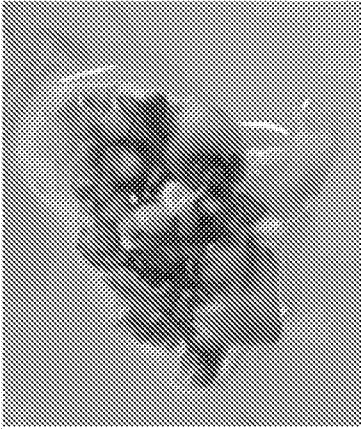


FIG. 4A

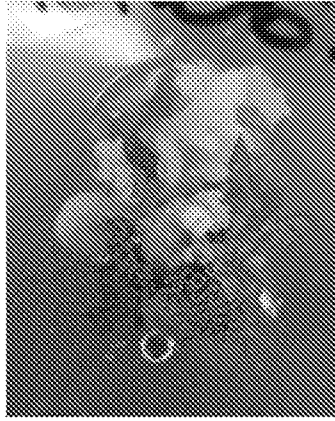


FIG. 4B



FIG. 4C

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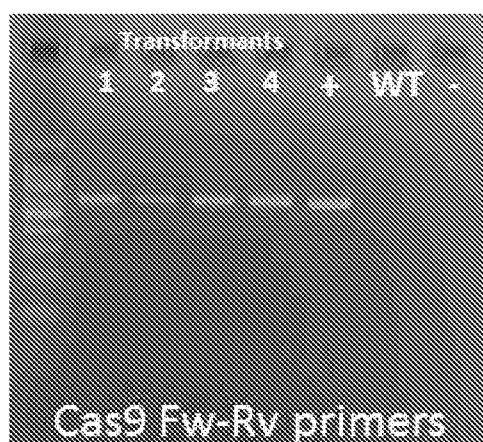


FIG. 5

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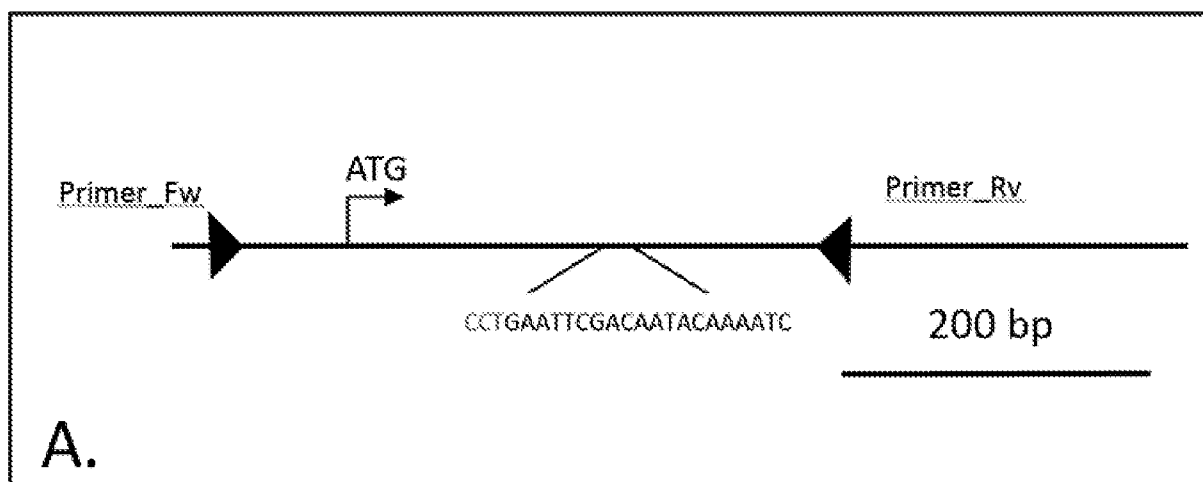


FIG. 6A

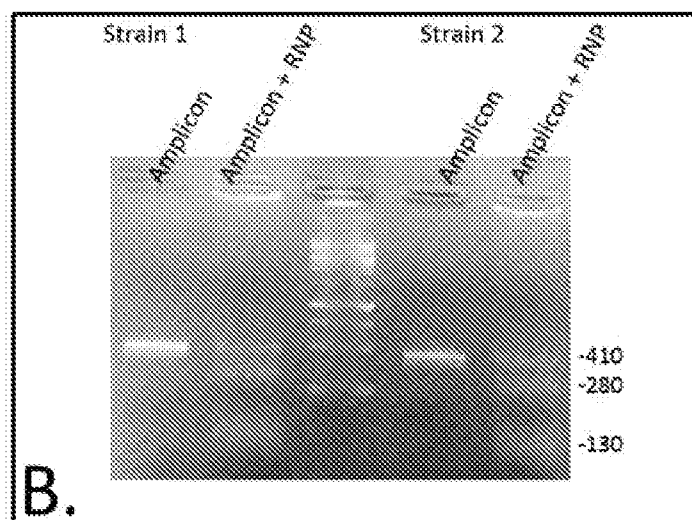


FIG. 6B

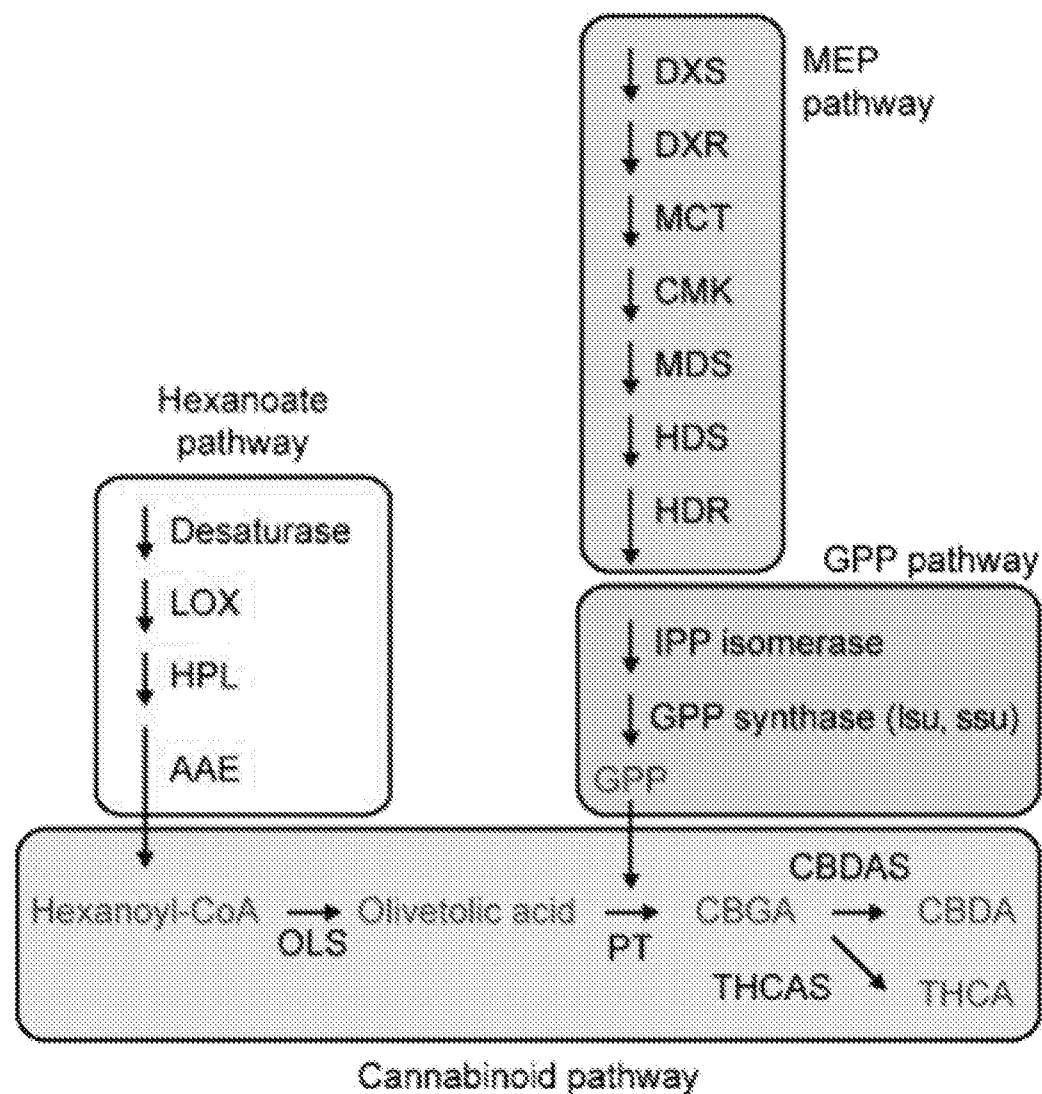


FIG. 2