

(12) STANDARD PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. **AU 2017310339 B2**

(54) Title
High thebaine poppy and methods of producing the same

(51) International Patent Classification(s)
C12N 15/113 (2010.01) **C12P 17/18** (2006.01)
A01H 1/00 (2006.01) **A01H 5/00** (2006.01)
C12N 5/10 (2006.01) **A61K 31/485** (2006.01)
C12N 9/02 (2006.01) **A61P 25/04** (2006.01)
C12N 15/53 (2006.01) **C08L 7/02** (2006.01)
C12N 15/82 (2006.01)

(21) Application No: **2017310339** (22) Date of Filing: **2017.08.11**

(87) WIPO No: **WO18/027324**

(30) Priority Data

(31) Number	(32) Date	(33) Country
62/374,682	2016.08.12	US
2,941,315	2016.09.07	CA

(43) Publication Date: **2018.02.15**

(44) Accepted Journal Date: **2019.01.17**

(71) Applicant(s)
API Labs Inc.

(72) Inventor(s)
Kovalchuk, Igor

(74) Agent / Attorney
FB Rice Pty Ltd, Level 23 44 Market Street, Sydney, NSW, 2000, AU

(56) Related Art
WO 2009109012 A1
WO 2011058446 A2
WIJEKOON, C.P. et al., "Systematic knockdown of morphine pathway enzymes in opium poppy using virus-induced gene silencing", The Plant Journal, (2012), vol. 69, ISSN 1365-313X, pages 1052-1063.
WO 9802033 A
ALLEN R.S. et al., 'RNAi-mediated replacement of morphine with the nonnarcotic alkaloid reticuline in opium poppy', Nature Biotechnology. (2004), vol. 22, no. 12, pages 1559-1566.



(51) International Patent Classification:

C12N 15/113 (2010.01) **C12P 17/18** (2006.01)
A01H 1/00 (2006.01) **A61K 31/485** (2006.01)
C12N 15/53 (2006.01) **A61P 25/04** (2006.01)
C12N 15/82 (2006.01) **C08L 7/02** (2006.01)
C12N 5/10 (2006.01) **A01H 5/00** (2006.01)
C12N 9/02 (2006.01)

(21) International Application Number:

PCT/CA2017/050951

(22) International Filing Date:

11 August 2017 (11.08.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/374,682 12 August 2016 (12.08.2016) US
 2,941,315 07 September 2016 (07.09.2016) CA

(71) **Applicant:** API LABS INC. [CA/CA]; 289 Heritage Blvd. West, Lethbridge, Alberta T1K 7M5 (CA).

(72) **Inventor:** KOVALCHUK, Igor; 16 Sandstone Road South, Lethbridge, Alberta T1K 7X8 (CA).

(74) **Agent:** SMART & BIGGAR; 2300-1055 West Georgia Street, PO Box 11115, Vancouver, BC V6E 3P3 (CA).

(81) **Designated States** (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: HIGH THEBAINE POPPY AND METHODS OF PRODUCING THE SAME

(57) **Abstract:** This disclosure relates to the production of opium poppy plants having high levels of thebaine. More particularly, the disclosure relates to the production of opium poppies having high levels of thebaine by simultaneously reducing the expression of genes encoding thebaine 6-O-demethylase (T6ODM) and codeine 3-O-demethylase (CODM).

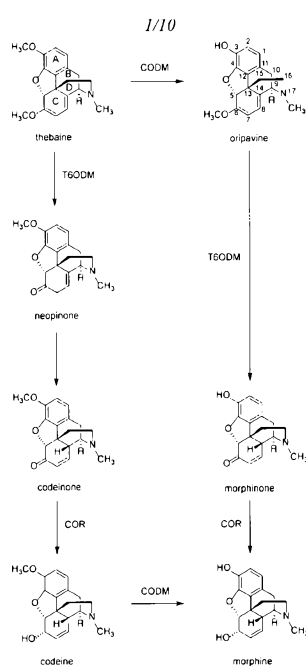


Figure 1

Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

- *with international search report (Art. 21(3))*
- *with sequence listing part of description (Rule 5.2(a))*

HIGH THEBAINE POPPY AND METHODS OF PRODUCING THE SAME

BACKGROUND OF THE DISCLOSURE

1. Field of Disclosure

5 This disclosure relates to the production of opium poppies having high levels of thebaine. More particularly, the disclosure relates to the production of opium poppies having high levels of thebaine by simultaneously reducing the expression/activity of thebaine 6-O-demethylase (T6ODM) and codeine 3-O-demethylase (CODM).

10 2. Description of Related Art

Opioids are psychoactive substances derived from the opium poppy (*Papaver somniferum*), or their synthetic analogues. Opioids have the potential to cause substance dependence that is characterized by a strong desire to take opioids, impaired control over opioid use, persistent opioid use despite harmful consequences, a higher priority given to opioid use than to other activities and obligations, increased tolerance, and a physical withdrawal reaction when opioids are discontinued. As of 2014, there were an estimated 17 million people who suffer from opioid dependence (i.e. an addiction to opioids). The majority of people dependent on opioids use illicitly cultivated and manufactured heroin. Due to their pharmacological effects, opioids in high doses can cause respiratory depression and death. As of 2014, an estimated 15 69 000 people die worldwide from opioid overdose each year.

In the World Drug Report 2016, the United Nations Office on Drugs and Crime indicated that recent declines in opium production would not lead to major shortages in the global heroin market given the high opium production levels of previous years. Thus, it may take a period of sustained decline in opium production for the repercussions to be felt in the heroin market. It would be desirable to have a method of disrupting opium production by turning off poppy plant genes necessary for the production of psychoactive alkaloids in the field.

Hydroxymorphinans, such as oxycodone, naloxone, naltrexone, nalbuphine and nalmefene are important opiate derivatives due to their utility as potent analgesics and/or narcotic antagonists. The most practical synthetic routes to the preparation of these pharmaceuticals use the alkaloid, thebaine, as a starting material. Other important opiate derivatives such as 30

the ring-C bridged compounds buprenorphine and etorphine are also most practically prepared from thebaine.

Unfortunately, thebaine is costly due to its limited availability. Total synthesis is difficult and, in poppy plants, thebaine typically accumulates to low levels of only 0.5 to 2% of the total alkaloids in opium poppy. Referring to Figure 1, thebaine exists at a branch point of morphine biosynthesis, being the substrate for two competing enzymes. Thebaine 6-O-demethylase (T6ODM) converts thebaine to oripavine, and codeine 3-O-demethylase (CODM) converts thebaine to neopinone.

Mutants of opium poppy accumulating thebaine and oripavine rather than morphine and codeine have been reported, including the TOP1 variety derived through chemical mutagenesis (Millgate et al. 2004). Although the metabolic block in TOP1 was suggested to result from a defect in the enzyme catalyzing the 6-O-demethylation of thebaine and oripavine, the biochemical basis for the phenotype was not determined. Moreover, a microarray was used to identify 10 genes underexpressed in TOP1, which list did not include any enzymes theoretically capable of O-demethylation. A plant line containing the TOP1 mutation was deposited under the Budapest Treaty with the American Type Culture Collection on March 20, 2008, under ATCC Patent Deposit Designation PTA-9110. WO2009/109012 discloses the mutagenesis of the line designated PTA-9110 to produce a further line accumulating high levels of thebaine, which was deposited under the Budapest Treaty with the American Type Culture Collection on March 20, 2008, under ATCC[®] Patent Deposit Designation PTA-9109. However, the biochemical basis for the phenotype was not explored.

Researchers have been interested in using molecular approaches to engineer opium poppy to produce opioids of choice for several years, however, the results have at times been unexpected and frustrating. For example, Allen et al. (Nature Biotechnology 22:1559-1556) used RNA interference (RNAi) to silence the genes encoding codeinone reductase (COR), the penultimate enzyme of morphine biosynthesis. COR converts codeinone to codeine. However, rather than resulting in the accumulation of codeinone, elimination of COR activity resulted in accumulation of reticuline, i.e. seven enzymatic steps before COR. The surprising accumulation of reticuline suggests a feedback mechanism preventing intermediates from general benzyloquinoline synthesis entering the morphine-specific branch.

SUMMARY

This disclosure relates to the production of opium poppies having high levels of thebaine. More particularly, the disclosure relates to the production of opium poppies having high levels of thebaine by simultaneously reducing the expression/activity of thebaine 6-O-demethylase (T6ODM) and codeine 3-O-demethylase (CODM).

Various aspects of the disclosure relate to a method of increasing accumulation of thebaine in an opium poppy plant, the method comprising genetically modifying the plant to simultaneously reduce the activity of thebaine 6-O-demethylase (T6ODM) and codeine 3-O-demethylase (CODM) in the poppy plant. The wild type T6ODM may have the amino acid sequence of SEQ ID NO: 1. The wild type CODM may have the amino acid sequence of SEQ ID NO: 3.

In some instances, genetically modifying the plant to simultaneously reduce the activity of T6ODM comprises introducing an expression construct to reduce the accumulation of transcripts from an endogenous gene encoding T6ODM. In some instances, the sequence of the expression construct to reduce the accumulation of transcripts from the endogenous gene encoding T6ODM comprises a portion of SEQ ID NO: 2 or SEQ ID NO: 4. In some instances, genetically modifying the plant to simultaneously reduce the activity of T6ODM comprises introducing a loss of function mutation in an endogenous gene encoding T6ODM.

In some instances, genetically modifying the plant to simultaneously reduce the activity of CODM comprises introducing an expression construct to reduce the accumulation of transcripts from an endogenous gene encoding CODM. In some instances, the sequence of the expression construct to reduce the accumulation of transcripts from the endogenous gene encoding CODM comprises a portion of SEQ ID NO: 2 or SEQ ID NO: 4. In some instances, genetically modifying the plant to simultaneously reduce the activity of CODM comprises introducing a loss of function mutation in an endogenous gene encoding CODM.

Various aspects of the disclosure relate to a method of producing an opium poppy plant with increased levels of thebaine relative to a wild type plant, the method comprising: crossing a first parent having at least one loss of function allele of the gene encoding thebaine 6-O-demethylase with a second parent having at least one loss of function allele of the gene

encoding codeine 3-O-demethylase; and allowing progeny that have both the loss of function mutation allele of the gene encoding thebaine 6-O-demethylase and the loss of function allele of the gene encoding codeine 3-O-demethylase to self pollinate to produce a plant that is homozygous for the loss of function mutation allele of the gene encoding thebaine 6-O-demethylase and homozygous for the loss of function allele of the gene encoding codeine 3-O-demethylase.

Various aspects of the disclosure relate to a method for producing an opium poppy plant with increased thebaine content, the method comprising: decreasing the expression an endogenous gene encoding an endogenous thebaine 6-O-demethylase (T6ODM) in the plant; and decreasing the expression of an endogenous gene encoding codeine 3-O-demethylase (CODM) in the plant. In some instances, decreasing the expression of the endogenous gene encoding T6ODM comprises introducing or producing a loss of function allele in the endogenous gene encoding T6ODM. In some instances, decreasing the expression of the endogenous gene encoding CODM comprises introducing or producing a loss of function allele in the endogenous gene encoding CODM.

In some instances, decreasing the expression of the endogenous gene encoding T6ODM comprises expressing a first heterologous nucleic acid molecule homologous to a portion of the endogenous gene encoding T6ODM, wherein the first heterologous nucleic acid molecule decreases expression of the endogenous gene encoding T6ODM. In some instances, decreasing the expression of the endogenous gene encoding CODM comprises introducing or producing a loss of function allele in the endogenous gene encoding CODM. In some instances, decreasing expression of the endogenous gene encoding CODM comprises expressing a second heterologous nucleic acid molecule homologous to a portion of the endogenous gene encoding CODM, wherein the second heterologous nucleic acid molecule decreases expression of the endogenous gene encoding CODM.

In some instances, decreasing expression of the endogenous gene encoding CODM comprises expressing a heterologous nucleic acid molecule homologous to a portion of the endogenous gene encoding CODM, wherein the heterologous nucleic acid molecule decreases expression of the endogenous gene encoding CODM. In some instances,

decreasing T6ODM activity comprises introducing or producing a loss of function allele in the endogenous gene encoding T6ODM.

5 In some instances, the loss of function allele comprises a disruption or point mutation in the gene. The disruption may be a deletion or an insertion. An insertion may be a T-DNA or a transposable element.

10 In some instances, the first heterologous nucleic acid molecule decreases expression of the endogenous gene encoding T6ODM by RNA interference.

In some instances, the second heterologous nucleic acid molecule decreases expression of the endogenous gene encoding CODM by RNA interference.

15 In some instances, the heterologous nucleic acid molecule decreases expression of the endogenous gene encoding CODM by RNA interference.

20 In some instances, the heterologous nucleic acid molecule comprises a portion of SEQ ID NO: 2 or SEQ ID NO: 4. In some instances, the heterologous nucleic acid molecule comprises a portion of SEQ ID NO: 7. In some instances, the heterologous nucleic acid molecule comprises a portion of SEQ ID NO: 8.

In some instances, the T6ODM has an amino acid sequence at least 95% identical to SEQ ID NO: 1 and the CODM has an amino acid sequence at least 95% identical to SEQ ID NO: 3.

25 Various aspects of the disclosure relate to a method of generating an opium poppy plant having increased thebaine content relative to a wild type opium poppy plant, the method comprising: i) using a molecular methodology to identify a first plant as comprising a loss of function allele in an endogenous gene encoding codeine 3-O-demethylase (CODM); ii) establishing a cross of said first plant to a second plant having a loss of function allele in an
30 endogenous gene encoding thebaine 6-O-demethylase (T6ODM); iii) allowing progeny from the cross to self-fertilize; and iv) screening progeny from self-fertilized plants for a plant that is homozygous for both the loss of function allele in the endogenous gene encoding CODM and the loss of function allele in the endogenous gene encoding T6ODM.

In some instances, the second plant having the loss of function allele in the endogenous gene encoding T6ODM is identified using a molecular methodology. In some instances, the loss of function allele in the endogenous gene encoding T6ODM is generated by genetic modification of the second plant or an ancestor thereof. In some instances, the second plant having the loss of function allele in the endogenous gene encoding T6ODM is a plant of the line deposited as ATCC PTA-9110.

Various aspects of the disclosure relate to a method of generating an opium poppy plant having an thebaine content relative to a wild type opium poppy plant, the method comprising: i) using a molecular methodology to identify a first plant as comprising a loss of function allele in an endogenous gene encoding thebaine 6-O-demethylase (T6ODM); ii) establishing a cross of said first plant to a second plant having a loss of function allele in an endogenous gene encoding codeine 3-O-demethylase (CODM); iii) allowing progeny from the cross to self-fertilize; and iv) screening progeny from self-fertilized plants for a plant that is homozygous for both the loss of function allele in the endogenous gene encoding CODM and the loss of function allele in the endogenous gene encoding T6ODM. In some instances, the second plant having the loss of function allele in the endogenous gene encoding CODM is identified using a molecular methodology. In some instances, the loss of function allele in the endogenous gene encoding CODM is generated by genetic modification of the second plant or an ancestor thereof. In some instances, the second plant having the loss of function allele in the endogenous gene encoding CODM is a plant of the line deposited as ATCC PTA-9109.

Various aspects of the disclosure relate to a method of generating an opium poppy plant having increased thebaine content relative to a wild type opium poppy plant, the method comprising: i) using a molecular methodology to identify a plant as comprising a loss of function allele in an endogenous gene encoding thebaine 6-O-demethylase (T6ODM); ii) genetically modifying the plant to introduce a loss of function allele in an endogenous gene encoding codeine 3-O-demethylase (CODM); iii) allowing the plant to self-fertilize; and iv) screening progeny from the self-fertilized plant for a plant that is homozygous for both the loss of function allele in the endogenous gene encoding CODM and the loss of function allele in the endogenous gene encoding T6ODM.

Various aspects of the disclosure relate to a method of generating an opium poppy plant having increased thebaine content relative to a wild type opium poppy plant, the method comprising: i) using a molecular methodology to identify a plant as comprising a loss of function allele in an endogenous gene encoding codeine 3-O-demethylase (CODM); ii) 5 genetically modifying the plant to introduce a loss of function allele in an endogenous gene encoding thebaine 6-O-demethylase (T6ODM) in the plant by genetic modification; iii) allowing the plant to self-fertilize; and iv) screening progeny from the self-fertilized plant for a plant that is homozygous for both the loss of function allele in the endogenous gene encoding CODM and the loss of function allele in the endogenous gene encoding T6ODM.

10

Various aspects of the disclosure relate to a method of generating an opium poppy plant having increased thebaine content relative to a wild type opium poppy plant, the method comprising: i) using a molecular methodology to identify a plant as comprising a loss of function allele in an endogenous gene encoding thebaine 6-O-demethylase (T6ODM); ii) 15 genetically modifying the plant to reduce expression of an endogenous gene encoding codeine 3-O-demethylase (CODM); iii) allowing the plant to self-fertilize; and iv) screening progeny from the self-fertilized plant for a plant that is homozygous for the loss of function allele in the endogenous gene encoding T6ODM and has reduced expression of the endogenous gene encoding CODM. In some instances, genetically modifying the plant to reduce expression of 20 the endogenous gene encoding CODM comprises introducing an expression construct to express a hairpin RNA targeting the endogenous gene encoding CODM.

25

Various aspects of the disclosure relate to a method of generating an opium poppy plant having increased thebaine content relative to a wild type opium poppy plant, the method comprising: i) using a molecular methodology to identify a plant as comprising a loss of function allele in an endogenous gene encoding codeine 3-O-demethylase (CODM); ii) 25 genetically modifying the plant to reduce expression of an endogenous gene encoding thebaine 6-O-demethylase (T6ODM); iii) allowing the plant to self-fertilize; and iv) screening progeny from the self-fertilized plant for a plant that is homozygous for both the loss of function 30 allele in the endogenous gene encoding CODM and has reduced expression of the endogenous gene encoding T6ODM. In some instances, genetically modifying the plant to reduce expression of the endogenous gene encoding T6ODM comprises introducing an

expression construct to express a hairpin RNA targeting the endogenous gene encoding T6ODM.

5 In some instances, the molecular methodology comprises targeting induced local lesions in genomes (TILLING) methodology.

Various aspects of the disclosure relate to an opium poppy plant produced by a method as described above.

10 Various aspects of the disclosure relate to a genetically modified opium poppy plant or plant cell having reduced activity of thebaine 6-O-demethylase (T6ODM) and codeine 3-O-demethylase (CODM) relative to a wild type plant, wherein the opium poppy plant is genetically modified to have reduced expression of T6ODM, and CODM, or both.

15 In some instances, the plant comprises a first expression construct for reducing the expression of T6ODM and a second expression construct for reducing expression of CODM. In some instances, the first expression construct comprises a first nucleic acid molecule encoding a first hairpin RNA for reducing expression of an endogenous gene encoding T6ODM. In some instances, the endogenous gene encoding T6ODM encodes an mRNA comprising having the
20 sequence of SEQ ID NO: 15. In some instances, the nucleic acid molecule encoding the first hairpin RNA comprises a portion of SEQ ID NO: 2.

In some instances, the second expression construct comprises a second nucleic acid molecule encoding a second hairpin RNA for reducing expression of an endogenous gene
25 encoding CODM. In some instances, the endogenous gene encoding CODM encodes an mRNA comprising having the sequence of SEQ ID NO: 16. In some instances, the nucleic acid molecule encoding the second hairpin RNA comprises a portion of SEQ ID NO: 4.

In some instances, the plant or plant cell comprises an expression construct comprising a
30 nucleic acid molecule for reducing the expression of T6ODM and CODM. In some instances, the nucleic acid molecule encodes a hairpin RNA for reducing expression of an endogenous gene encoding CODM. In some instances, the nucleic acid molecule encodes a hairpin RNA for reducing expression of an endogenous gene encoding T6ODM. In some instances, the

nucleic acid molecule encodes a single hairpin RNA sufficient to reduce expression of endogenous genes encoding T6ODM and CODM. In some instances, the nucleic acid molecule comprises a portion of SEQ ID NO:2, SEQ ID NO:4, or both.

- 5 In some instances, the expression construct comprises a first nucleic acid molecule encoding a first hairpin RNA for reducing expression of an endogenous gene encoding T6ODM and a second nucleic acid molecule encoding a second hairpin RNA for reducing expression of an endogenous gene encoding CODM. In some instances, the endogenous gene encoding T6ODM encodes an mRNA comprising having the sequence of SEQ ID NO: 15. In some
10 instances, the endogenous gene encoding T6ODM encodes a polypeptide having the sequence of SEQ ID NO 1. In some instances, the endogenous gene encoding CODM encodes an mRNA comprising having the sequence of SEQ ID NO: 16. In some instances, the endogenous gene encoding T6ODM encodes a polypeptide having the sequence of SEQ ID NO 3. In some instances, each of the first nucleic acid molecule and the second nucleic
15 acid molecule comprise a portion of SEQ ID NO: 2, SEQ ID NO: 4, or both.

In some instances, the nucleic acid molecule encoding the hairpin RNA(s) comprises a portion of SEQ ID NO: 8. In some instances, the nucleic acid molecule encoding the hairpin RNA(s) comprises a portion of SEQ ID NO: 7.

- 20 In some instances, the first nucleic acid molecule comprises a portion of SEQ ID NO: 8. In some instances, the first nucleic acid molecule comprises a portion of SEQ ID NO: 7.

- In some instances, the second nucleic acid molecule comprises a portion of SEQ ID NO: 8. In
25 some instances, the second nucleic acid molecule comprises a portion of SEQ ID NO: 7.

- In some instances, the plant or plant cell is genetically modified to have reduced activity of T6ODM, and the reduced activity of CODM is conferred by a mutation in the endogenous gene encoding CODM that was not introduced by genetic modification of the plant or plant cell. In
30 some instances, mutation in the endogenous gene encoding CODM that was not introduced by genetic modification of the plant or plant cell is the mutation present in seeds of the plant deposited under Patent Deposit Designation PTA-9109.

In some instances, the plant is genetically modified to have reduced activity of CODM, and wherein reduced activity of T6ODM is conferred by a mutation in the endogenous gene encoding T6ODM that was not introduced by genetic modification of the plant or plant cell. In some instances, the mutation in the endogenous gene encoding T6ODM that was not introduced by genetic modification of the plant or plant cell is the mutation present in seeds of the plant deposited under Patent Deposit Designation PTA-9110.

Various aspects of the disclosure relate to a genetically modified poppy plant or plant cell having reduced expression of endogenous genes encoding 6-O-demethylase (T6ODM) and codeine 3-O-demethylase (CODM), the genetically modified plant comprising: a transgenic expression construct decreasing the expression of an endogenous gene encoding T6ODM in the plant or plant cell; and a transgenic expression construct decreasing the expression of an endogenous gene encoding CODM in the plant or plant cell.

Various aspects of the disclosure relate to seed of an opium poppy plant as described above.

Various aspects of the disclosure relate to use of a plant as described above for the production of thebaine.

Various aspects of the disclosure relate to poppy straw from a plant as described above.

Various aspects of the disclosure relate to latex isolated from a plant as defined above.

Various aspects of the disclosure relate to a method of producing thebaine, said method comprising isolating thebaine from latex or poppy straw harvested from a plant as described above.

Various aspects of the disclosure relate to an isolated nucleic acid molecule, wherein the sequence of the nucleic acid molecule comprises a portion of SEQ ID NO:7.

Various aspects of the disclosure relate to an expression vector for simultaneously reducing the expression of endogenous genes encoding thebaine 6-O-demethylase (T6ODM) and

codeine 3-O-demethylase (CODM) in an opium poppy plant, the expression vector comprising a nucleic acid molecule that comprises a portion of SEQ ID NO:7.

Various aspects of the disclosure relate to use of a portion of a polynucleotide molecule having a sequence comprising a portion of SEQ ID NO: 2 or SEQ ID NO: 4 for simultaneously reducing the expression of endogenous genes encoding thebaine 6-O-demethylase (T6ODM) and codeine 3-O-demethylase (CODM) in an opium poppy plant.

Other aspects and features of the present invention will become apparent to those ordinarily skilled in the art upon review of the following description of specific embodiments of the invention in conjunction with the accompanying figures.

The methods disclosed herein may be useful for producing poppy plants having an increased ratio of thebaine:morphine.

Particular aspects of the disclosure relate to a method of increasing accumulation of thebaine in an opium poppy plant or plant cell, the method comprising genetically modifying the genome of the plant or plant cell to include one or more stable genetic modifications to simultaneously reduce the activity of thebaine 6-O-demethylase (T6ODM) and codeine 3-O-demethylase (CODM) in the poppy plant or plant cell.

Particular aspects of the disclosure relate to a genetically modified opium poppy plant or plant cell having reduced activity of thebaine 6-O-demethylase (T6ODM) and codeine 3-O-demethylase (CODM) relative to a wild type plant or plant cell, wherein the genetically modified opium poppy plant or plant cell comprises one or more stable genetic modifications to reduce expression of T6ODM, CODM, or both.

Particular aspects of the disclosure relate to a method for producing an opium poppy plant with increased thebaine content, the method comprising: (a) decreasing the expression of an endogenous gene encoding an endogenous thebaine 6-O-demethylase (T6ODM) in the plant; and (b) decreasing the expression of an endogenous gene encoding codeine 3-O-demethylase (CODM) in the plant, wherein decreasing the expression of the endogenous

gene encoding T6ODM comprises genetically modifying the plant to have a loss of function allele in the endogenous gene encoding T6ODM.

Particular aspects of the disclosure relate to a method for producing an opium poppy plant with increased thebaine content, the method comprising: (a) decreasing the expression an endogenous gene encoding an endogenous thebaine 6-O-demethylase (T6ODM) in the plant; and (b) decreasing the expression of an endogenous gene encoding codeine 3-O-demethylase (CODM) in the plant, wherein decreasing the expression of the endogenous gene encoding T6ODM comprises expressing a heterologous nucleic acid molecule homologous to a portion of the endogenous gene encoding T6ODM, wherein expression of the heterologous nucleic acid molecule decreases expression of the endogenous gene encoding T6ODM.

Particular aspects of the disclosure to a method for producing an opium poppy plant with increased thebaine content, the method comprising: (a) decreasing the expression an endogenous gene encoding an endogenous thebaine 6-O-demethylase (T6ODM) in the plant; and (b) decreasing the expression of an endogenous gene encoding codeine 3-O-demethylase (CODM) in the plant, wherein decreasing the expression of the endogenous gene encoding CODM comprises genetically modifying the plant to have a loss of function allele in the endogenous gene encoding CODM.

Particular aspects of the disclosure to a method for producing an opium poppy plant with increased thebaine content, the method comprising: (a) decreasing the expression of an endogenous gene encoding an endogenous thebaine 6-O-demethylase (T6ODM) in the plant; and (b) decreasing the expression of an endogenous gene encoding codeine 3-O-demethylase (CODM) in the plant, wherein decreasing the expression of the endogenous gene encoding CODM comprises expressing a heterologous nucleic acid molecule homologous to a portion of the endogenous gene encoding CODM, wherein expression of the heterologous nucleic acid molecule decreases expression of the endogenous gene encoding CODM.

Particular aspects of the disclosure relate to a genetically modified poppy plant or plant cell having reduced expression of endogenous genes encoding 6-O-demethylase (T6ODM) and

codeine 3-O-demethylase (CODM), the genetically modified plant comprising: a stably inherited transgenic expression construct for decreasing the expression of an endogenous gene encoding T6ODM in the plant or plant cell; and a stably inherited transgenic expression construct for decreasing the expression of an endogenous gene encoding CODM in the plant or plant cell.

Particular aspects of the disclosure relate to a method of generating an opium poppy plant having increased thebaine content relative to a wild type opium poppy plant, the method comprising: i) using a molecular methodology to identify a first plant as comprising a loss of function allele in an endogenous gene encoding codeine 3-O-demethylase (CODM); ii) establishing a cross of said first plant to a second plant having a loss of function allele in an endogenous gene encoding thebaine 6-O-demethylase (T6ODM); iii) allowing progeny from the cross to self-fertilize; and iv) screening progeny from self-fertilized plants for a plant that is homozygous for both the loss of function allele in the endogenous gene encoding CODM and the loss of function allele in the endogenous gene encoding T6ODM.

Particular aspects of the disclosure relate to a method of generating an opium poppy plant having increased thebaine content relative to a wild type opium poppy plant, the method comprising: i) using a molecular methodology to identify a first plant as comprising a loss of function allele in an endogenous gene encoding thebaine 6-O-demethylase (T6ODM); ii) establishing a cross of said first plant to a second plant having a loss of function allele in an endogenous gene encoding codeine 3-O-demethylase (CODM); iii) allowing progeny from the cross to self-fertilize; and iv) screening progeny from self-fertilized plants for a plant that is homozygous for both the loss of function allele in the endogenous gene encoding CODM and the loss of function allele in the endogenous gene encoding T6ODM.

Particular aspects of the disclosure relate to a method of generating an opium poppy plant having increased thebaine content relative to a wild type opium poppy plant, the method comprising: i) using a molecular methodology to identify a plant as comprising a loss of function allele in an endogenous gene encoding thebaine 6-O-demethylase (T6ODM); ii) genetically modifying the plant to introduce a loss of function allele in an endogenous gene encoding codeine 3-O-demethylase (CODM); iii) allowing the plant to self-fertilize; and iv) screening progeny from the self-fertilized plant for a plant that is homozygous for both the loss

of function allele in the endogenous gene encoding CODM and the loss of function allele in the endogenous gene encoding T6ODM.

Particular aspects of the disclosure relate to a method of generating an opium poppy plant
5 having increased thebaine content relative to a wild type opium poppy plant, the method comprising: i) using a molecular methodology to identify a plant as comprising a loss of function allele in an endogenous gene encoding codeine 3-O-demethylase (CODM); ii) genetically modifying the plant to introduce a loss of function allele in an endogenous gene encoding thebaine 6-O-demethylase (T6ODM) in the plant by genetic modification; iii) allowing
10 the plant to self-fertilize; and iv) screening progeny from the self-fertilized plant for a plant that is homozygous for both the loss of function allele in the endogenous gene encoding CODM and the loss of function allele in the endogenous gene encoding T6ODM.

Particular aspects of the disclosure relate to a method of generating an opium poppy plant
15 having increased thebaine content relative to a wild type opium poppy plant, the method comprising: i) using a molecular methodology to identify a plant as comprising a loss of function allele in an endogenous gene encoding thebaine 6-O-demethylase (T6ODM); ii) genetically modifying the plant to reduce expression of an endogenous gene encoding codeine 3-O-demethylase (CODM); iii) allowing the plant to self-fertilize; and iv) screening progeny
20 from the self-fertilized plant for a plant that is homozygous for the loss of function allele in the endogenous gene encoding T6ODM and has reduced expression of the endogenous gene encoding CODM.

Particular aspects of the disclosure relate to a method of generating an opium poppy plant
25 having increased thebaine content relative to a wild type opium poppy plant, the method comprising: i) using a molecular methodology to identify a plant as comprising a loss of function allele in an endogenous gene encoding codeine 3-O-demethylase (CODM); ii) genetically modifying the plant to reduce expression of an endogenous gene encoding thebaine 6-O-demethylase (T6ODM); iii) allowing the plant to self-fertilize; and iv) screening
30 progeny from the self-fertilized plant for a plant that is homozygous for both the loss of function allele in the endogenous gene encoding CODM and has reduced expression of the endogenous gene encoding T6ODM.

Particular aspects of the disclosure relate to an isolated nucleic acid molecule, wherein the sequence of the nucleic acid molecule comprises SEQ ID NO:7.

5 Particular aspects of the disclosure relate to a use of a polynucleotide molecule having a sequence comprising a portion of SEQ ID NO: 2 or SEQ ID NO: 4 for simultaneously reducing the expression of endogenous genes encoding thebaine 6-O-demethylase (T6ODM) and codeine 3-O-demethylase (CODM) in an opium poppy plant.

10 Particular aspects of the disclosure relate to poppy straw harvest from a plant or plant cell as claimed.

Particular aspects of the disclosure related to latex harvested from a plant or a plant cell as claimed.

15 BRIEF DESCRIPTION OF THE DRAWINGS

In drawings which illustrate embodiments of the invention,

Figure 1 is a schematic diagram of the morphine biosynthesis pathway in opium poppy, showing two routes from thebaine to morphine.

20 Figure 2 is a schematic diagram of a hairpin RNA expression cassette for expressing a hairpin RNA comprising T6ODM sequences for simultaneous reduction of expression of the genes encoding CODM and T6ODM.

Figure 3 is an alignment of cDNA sequences encoding CODM and T6ODM. The underlined portion identifies T6ODM sequences used in the creation of the hairpin RNA expression construct to reduce expression of both the endogenous gene encoding CODM and the endogenous gene encoding T6ODM. Differences between the T6ODM and CODM coding sequences within the underlined portion are highlighted in black.

Figure 4 are histograms showing the expression of the endogenous genes encoding CODM and T6ODM after transient expression of the expression construct.

30 Figure 5 is a histogram showing expression of the expression construct in transgenic lines AM1, AM2, and AM3.

Figure 6 are histograms showing reduced expression of the endogenous genes encoding (A) T6ODM and (B) CODM in lines AM1, AM2, and AM3.

Figure 7 is a chromatograph showing the accumulation of thebaine in lines (A) AM1, (B), AM2, and (C) AM3 relative to (D) line AM10 that does not have the expression construct.

5 DETAILED DESCRIPTION

This disclosure relates to a genetically modified opium poppy plants, seeds, cells, straw, progeny thereof, or produced latex thereof, which genetically modified plant produces a latex having increased levels of thebaine relative to wild type plants due to the combined reduction in the activity of the enzymes thebaine 6-O-demethylase (T6ODM) and codeine 3-O-demethylase (CODM) during opiate biosynthesis. The disclosure also relates to methods of obtaining such genetically modified opium poppy plants.

Definitions

“Opium poppy plant” or “poppy plant” as used herein refers to a plant of the species *Papaver Somniferum*.

A “field” of plants as used herein, refers to a plurality of opium plants cultivated together in close proximity.

“Activity” or as used herein refers to the level of a particularly enzymatic function in a plant cell. In the context of the present disclosure, reduced T6ODM activity refers to a reduction in O-demethylation activity at position 6, whereas reduced CODM activity refers to a reduction in O-demethylation activity at position 3. Reduction in activity can be the result of diminished functionality of the protein due to, for example, mutation, or the result of reduced expression of the protein, for example, due to reduced translation.

A “genetic modification” as used herein broadly refers to any a novel combination of genetic material obtained with techniques of modern biotechnology. Genetic modifications include, but are not limited to, “transgenes” in which the genetic material has been altered by the insertion of exogenous genetic material. However, genetic modifications also include alterations (e.g. insertions, deletions, or substitutions) in endogenous genes introduced in a targeted manner with techniques such as CRISPR/Cas9, TALENS, etc. as discussed below. However, for the

purposes of this disclosure “genetic modification” is not intended to include novel combinations of genetic material resulting from mutations generated by traditional means of random mutagenesis following by traditional means of breeding.

5 "Transgene" as used herein refers to a recombinant gene or genetic material that has been transferred by genetic engineering techniques into the plant cell. "Transgenic plants" or "transformed plants" as used herein refers to plants that have incorporated or integrated exogenous nucleic acid sequences or DNA fragments into the plant cell. A transgene may include a homologous or heterologous promoter operably linked to a DNA molecule encoding
10 the RNA or polypeptide of interest.

"Operably linked" refers to a functional linkage between a promoter and a second DNA sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second DNA sequence. Generally, operably linked means that
15 the nucleic acid sequences being linked are contiguous.

A “genetically modified” plant or plant cell as used herein broadly refers to any plant or plant cell that possesses a genetic modification as defined herein.

20 As used herein, the term “polypeptide” encompasses any chain of naturally or non-naturally occurring amino acids (either D- or L-amino acids), regardless of length (e.g., at least 5, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 40, 50, 100 or more amino acids) or post-translational modification (e.g., glycosylation or phosphorylation) or the presence of e.g. one or more non-amino acyl groups (for example, sugar, lipid, etc.) covalently linked to the peptide, and
25 includes, for example, natural proteins, synthetic or recombinant polypeptides and peptides, hybrid molecules, peptoids, peptidomimetics, etc. As used herein, the terms “polypeptide”, “peptide” and “protein” may be used interchangeably.

30 “Nucleotide sequence”, “polynucleotide sequence”, “nucleic acid” or “nucleic acid molecule” as used herein refers to a polymer of DNA or RNA which can be single or double stranded and optionally containing synthetic, non-natural or altered nucleotide bases capable of incorporation into DNA or RNA polymers. “Nucleic acid”, “nucleic acid sequence”, “polynucleotide sequence” or “nucleic acid molecule” encompasses genes, cDNA, DNA and

RNA encoded by a gene. Nucleic acids, nucleic acid sequences, polynucleotide sequence and nucleic acid molecule may comprise at least 3, at least 10, at least 100, at least 1000, at least 5000, or at least 10000 nucleotides or base pairs.

- 5 A “fragment”, a “fragment thereof”, “gene fragment” or a “gene fragment thereof” as used herein refers to a portion of a “nucleotide sequence”, “polynucleotide sequence”, “nucleic acid” or “nucleic acid molecule” that may still reduce expression of the gene(s) encoding CODM and/or T6ODM. In one embodiment, the fragment comprises at least 20, at least 40, at least 60, at least 80, at least 100, at least 150, at least 200, at least 150, at least 300, at least 350,
10 at least 400, at least 450 or at least 500 contiguous nucleotides.

A “non-natural variant” as used herein refers to nucleic acid sequences native to an organism but comprising modifications to one or more of its nucleotides introduced by mutagenesis.

- 15 An “allele” or “allelic variant” as used herein refers to an alternate form of the same gene at a specific location of the genome.

“Wildtype” as used herein refers to a plant or plant material that was not transformed with a nucleic acid molecule or construct, genetically modified, or otherwise mutated as described
20 herein. A “wildtype” may also refer to a plant or plant material in which T6ODM activity and CODM activity were not reduced.

The term “identity” as used herein refers to sequence similarity between two polypeptide or polynucleotide molecules. Identity can be determined by comparing each position in the
25 aligned sequences. A degree of identity between amino acid or nucleic acid sequences is a function of the number of identical or matching amino acids or nucleic acids at positions shared by the sequences, for example, over a specified region. Optimal alignment of sequences for comparisons of identity may be conducted using a variety of algorithms, as are known in the art, including the Clustal W™ program, available at <http://clustalw.genome.ad.jp>,
30 the local homology algorithm of Smith and Waterman, 1981, Adv. Appl. Math 2: 482, the homology alignment algorithm of Needleman and Wunsch, 1970, J. Mol. Biol. 48:443, the search for similarity method of Pearson and Lipman, 1988, Proc. Natl. Acad. Sci. USA 85:2444, and the computerised implementations of these algorithms (such as GAP, BESTFIT,

FASTA and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, Madison, Wis., U.S.A.). Sequence identity may also be determined using the BLAST algorithm (e.g. BLASTn and BLASTp), described in Altschul et al., 1990, J. Mol. Biol. 215:403-10 (using the published default settings). Software for performing BLAST analysis is available
5 through the National Center for Biotechnology Information (through the Internet at <http://www.ncbi.nlm.nih.gov/>). For instance, sequence identity between two nucleic acid sequences can be determined using the BLASTn algorithm at the following default settings: expect threshold 10; word size 11; match/mismatch scores 2, -3; gap costs existence 5, extension 2. Sequence identity between two amino acid sequences may be determined using
10 the BLASTp algorithm at the following default settings: expect threshold 10; word size 3; matrix BLOSUM 62; gap costs existence 11, extension 1. In another embodiment, the person skilled in the art can readily and properly align any given sequence and deduce sequence identity/homology by mere visual inspection.

15 As used herein, "heterologous", "foreign" and "exogenous" DNA and RNA are used interchangeably and refer to DNA or RNA that does not occur naturally as part of the plant genome in which it is present or which is found in a location or locations in the genome that differ from that in which it occurs in nature. Thus, heterologous or foreign DNA or RNA is nucleic acid that is not normally found in the host genome in an identical context (i.e. linked to
20 identical 5' and 3' sequences). In one aspect, heterologous DNA may be the same as the host DNA but introduced into a different place in the host genome and/or has been modified by methods known in the art, where the modifications include, but are not limited to, insertion in a vector, linked to a foreign promoter and/or other regulatory elements, or repeated at multiple copies. In another aspect, heterologous DNA may be from a different organism, a different
25 species, a different genus or a different kingdom, as the host DNA. Further, the heterologous DNA may be a transgene. As used herein, "transgene" refers to a segment of DNA containing a gene sequence that has been isolated from one organism and introduced into a different organism. In the context of the present disclosure, the nucleic acid molecules may comprise nucleic acid that is heterologous to the plant in which CODM and T6ODM activity is reduced.

30 "Expression" or "expressing", as used herein refers to the process by which information from a gene is used in the synthesis of a functional gene product, and may relate to production of any detectable level of a product, or activity of a product, encoded by a gene. Gene expression

may be modulated (i.e. initiated, increased, decreased, terminated, maintained or precluded) at many levels including transcription, RNA processing, translation, post-translational modification, protein degradation. Gene expression can also be modulated by the introduction of mutations that affect the activity of the gene product, e.g. the ability of a gene product to
5 convert substrate. In the context of the present disclosure, reduced expression of the endogenous gene(s) encoding CODM and/or T6ODM, or reduced expression of the CODM and/or T6ODM polypeptides, can be effected by reduced transcription of the endogenous gene(s) encoding CODM and/or T6ODM, by reduced translation of mRNA transcripts coding for CODM and/or T6ODM, or by the introduction of mutations that either prevent the
10 translation of functional polypeptides or result in the translation of polypeptides with reduced abilities to convert substrate. Such reduced expression of the endogenous genes may result from expression of transgenes comprising expression constructs designed to reduce expression of the endogenous genes.

15 "Poppy straw" as used herein refers to the straw material resulting from threshing of mature poppy capsules and the poppy capsule stems to remove the seeds.

"Latex" as used herein refer to the air-dried, milky exudation from lanced, unripe poppy capsules.

20 The term "increased thebaine content" or "increased level of thebaine" as used herein refers to a significantly increased levels of thebaine in one or more tissues as compared to the levels of thebaine in a corresponding wild type plant. The term "increased" also encompasses levels of thebaine that are significantly increased in one or more tissues compared to the same tissues
25 of a wild type plant, while wild type levels of thebaine persist elsewhere in the plant.

The term "reduced morphine content" as used herein refers to a significantly decreased levels of morphine in one or more tissues as compared to the levels of morphine in a corresponding wild type plant. The term "reduced" also encompasses levels of morphine that are significantly
30 reduced in one or more tissues compared to the same tissues of a wild type plant, while wild type levels of morphine persist elsewhere in the plant.

"Decreasing expression", "decreasing activity", "reducing expression", and "reducing activity" are intended to encompass well known equivalent terms regarding expression and activity such as "inhibiting", "down-regulating", "knocking out", "silencing", etc.

- 5 "substantially no" when referring to alkaloid content means that the particular alkaloid or combination of alkaloids constitutes less than 0.6% by weight, preferably, less than 0.5% by weight, more preferably, less than 0.4% by weight, or less than 0.2% by weight of the alkaloid combination of the poppy straw, concentrate of poppy straw or opium.
- 10 "Expression construct" as used herein refers to any type of genetic construct containing a nucleic acid coding for a gene product in which part or all of the nucleic acid encoding sequence is capable of being transcribed. The transcript may be translated into a protein, but it need not be. In certain embodiments, expression includes both transcription of a gene and translation of mRNA into a gene product. In other embodiments, expression only includes
- 15 transcription of the nucleic acid encoding a gene of interest into, for example, an siRNA.

An expression construct of the disclosure nucleic acid molecule may further comprise a promoter and other regulatory elements, for example, an enhancer, a silencer, a polyadenylation site, a transcription terminator, a selectable marker or a screenable marker.

20

As used herein, a "vector" or a "construct" may refer to any recombinant polynucleotide molecule such as a plasmid, cosmid, virus, vector, autonomously replicating polynucleotide molecule, phage, or linear or circular single-stranded or double-stranded DNA or RNA polynucleotide molecule, derived from any source. A "vector" or a "construct" may comprise a

25 promoter, a polyadenylation site, an enhancer or silencer and a transcription terminator, in addition to a nucleotide sequence encoding a gene or a gene fragment of interest. As used herein, a "transformation vector" may refer to a vector used in the transformation of, or in the introduction of DNA into, cells, plants or plant materials.

- 30 As used herein, a "promoter" refers to a nucleotide sequence that directs the initiation and rate of transcription of a coding sequence (reviewed in Roeder, Trends Biochem Sci, 16: 402, 1991). The promoter contains the site at which RNA polymerase binds and also contains sites for the binding of other regulatory elements (such as transcription factors). Promoters may be

naturally occurring or synthetic (see Datla et al. Biotech Ann. Rev 3:269, 1997 for review of plant promoters). Further, promoters may be species specific (for example, active only in *B. napus*); tissue specific (for example, the napin, phaseolin, zein, globulin, dlec2, γ -kafirin seed specific promoters); developmentally specific (for example, active only during embryogenesis);

5 constitutive (for example maize ubiquitin, rice ubiquitin, rice actin, Arabidopsis actin, sugarcane bacilliform virus, CsVMV and CaMV 35S, Arabidopsis polyubiquitin, *Solanum bulbocastanum* polyubiquitin, *Agrobacterium tumefaciens*-derived nopaline synthase, octopine synthase, and mannopine synthase gene promoters); or inducible (for example the stilbene synthase promoter and promoters induced by light, heat, cold, drought, wounding, hormones,

10 stress and chemicals). A promoter includes a minimal promoter that is a short DNA sequence comprised of a TATA box or an Inr element, and other sequences that serve to specify the site of transcription initiation, to which regulatory elements are added for control of expression. A promoter may also refer to a nucleotide sequence that includes a minimal promoter plus DNA

15 elements that regulates the expression of a coding sequence, such as enhancers and silencers. Thus in one aspect, the expression of the constructs of the present disclosure may be regulated by selecting a species specific, a tissue specific, a development specific or an inducible promoter.

"Constitutive promoter" as used herein refers to a promoter which drives the expression of the

20 downstream-located coding region in a plurality of or all tissues irrespective of environmental or developmental factors.

The skilled person will understand that it would be important to use a promoter that effectively directs the expression of the construct in the tissue in which thebaine is being synthesized. For

25 example, the endogenous T6ODM or CODM promoters could be used. Alternatively, constitutive, tissue-specific, or inducible promoters useful under the appropriate conditions to direct high level expression of the introduced expression construct during opioid biosynthesis can be employed.

30 Enhancers and silencers are DNA elements that affect transcription of a linked promoter positively or negatively, respectively (reviewed in Blackwood and Kadonaga, Science, 281: 61, 1998).

Polyadenylation site refers to a DNA sequence that signals the RNA transcription machinery to add a series of the nucleotide A at about 30 bp downstream from the polyadenylation site.

Transcription terminators are DNA sequences that signal the termination of transcription.

5 Transcription terminators are known in the art. The transcription terminator may be derived from *Agrobacterium tumefaciens*, such as those isolated from the nopaline synthase, mannopine synthase, octopine synthase genes and other open reading frame from Ti plasmids. Other terminators may include, without limitation, those isolated from CaMV and other DNA viruses, dlec2, zein, phaseolin, lipase, osmotin, peroxidase, PinII and ubiquitin
10 genes, for example, from *Solanum tuberosum*.

In the context of the disclosure the nucleic acid construct may further comprise a selectable marker. Selectable markers may be used to select for plants or plant cells that contain the exogenous genetic material. The exogenous genetic material may include, but is not limited to,
15 an enzyme that confers resistance to an agent such as a herbicide or an antibiotic, or a protein that reports the presence of the construct.

Numerous plant selectable marker systems are known in the art and are consistent with this invention. The following review article illustrates these well known systems: Miki and McHugh;
20 *Journal of Biotechnology* 107: 193-232; Selectable marker genes in transgenic plants: applications, alternatives and biosafety (2004).

Examples of a selectable marker include, but are not limited to, a neo gene, which codes for kanamycin resistance and can be selected for using kanamycin, NptII, G418, hpt etc.; an amp
25 resistance gene for selection with the antibiotic ampicillin; an hygromycinR gene for hygromycin resistance; a BAR gene (encoding phosphinothricin acetyl transferase) which codes for bialaphos resistance including those described in WO/2008/070845; a mutant EPSP synthase gene, aadA, which encodes glyphosate resistance; a nitrilase gene, which confers resistance to bromoxynil; a mutant acetolactate synthase gene (ALS), which confers
30 imidazolinone or sulphonylurea resistance, ALS, and a methotrexate resistant DHFR gene.

Further, screenable markers that may be used in the context of the invention include, but are not limited to, a β -glucuronidase or uidA gene (GUS), which encodes an enzyme for which

various chromogenic substrates are known, green fluorescent protein (GFP), and luciferase (LUX).

Alkaloid Production in *Papaver somniferum*

5

Figure 1 is a schematic diagram depicting two routes of morphine biosynthesis from thebaine. O-demethylation of thebaine at position 6 (ring C) is catalyzed by thebaine 6-O-demethylase (T6ODM) whereas O-demethylation at position 3 (ring A) is catalyzed by codeine O-demethylase (CODM). Thus, thebaine can undergo O-demethylation at position 6 or position 3 to yield
10 neopinone or oripavine, respectively. Neopinone converts spontaneously to codeinone, which is then reduced to codeine by codeinone reductase (COR). Codeine is demethylated at position 3 by CODM to produce morphine. Demethylation of oripavine at position 6 by T6ODM yields morphinone, which is then reduced to morphine by COR.

15 The present inventor hypothesized that it may be possible to produce plants containing elevated levels of thebaine, and reduced levels of codeine and morphine, compared to parental plants by simultaneously reducing the activity of the T6ODM and CODM enzymes.

Wild type amino acid sequences of the T6ODM and CODM enzymes are presented in SEQ ID
20 NOs: 1 and 3, respectively. The cDNA sequence corresponding to the endogenous gene coding for the T6ODM enzyme is presented as SEQ ID NO: 2, and the cDNA sequence corresponding to the endogenous gene coding for the CODM enzyme is presented as SEQ ID NO: 4. However, the skilled person will readily understand that naturally occurring variations in the T6ODM and CODM genes may exist between varieties, with slightly different nucleic acid
25 sequences that encode the same functional protein.

Reduction of CODM and T6ODM Activity or Expression

CODM and T6ODM expression and/or activity in genetically modified plants of the present
30 invention may be reduced by any method that results in reduced activity of these enzymes in the plant. This may be achieved by e.g. by altering CODM and T6ODM activity at the DNA, mRNA and/or protein levels.

As used herein, "activity" refers to the biochemical reaction of an enzyme with its cognate substrate. In the context of the invention, reduced T6ODM (or CODM) activity may result from reduced protein levels of T6ODM (or CODM) enzyme and/or the reduced rate at which a T6ODM (or CODM) enzyme catalyzes its reaction with thebaine.

5

Mutating Endogenous Genes encoding CODM and T6ODM

In one aspect, the present disclosure relates to genetic modifications targeting the endogenous genes encoding CODM and T6ODM to alter CODM and T6ODM expression
10 and/or activity. The endogenous CODM and T6ODM genes may be altered by, without limitation, knocking-out CODM and T6ODM genes; or knocking-in a heterologous DNA to disrupt CODM and T6ODM genes. The skilled person would understand that these approaches may be applied to the coding sequences, the promoter or other regulatory elements necessary for gene transcription. For example, technologies such as CRISPR/Cas9
15 and TALENS can be used to introduce loss of function mutations in both the endogenous genes encoding CODM and T6ODM. Plants having at least one allele of each gene comprising such loss of function mutations can then be self-fertilized to produce progeny homozygous for the loss of function alleles in the genes encoding CODM and T6ODM. In some embodiments, genetic modification of the endogenous gene encoding the CODM (or
20 T6ODM) enzyme results in a polypeptide that differs in sequence by one or more amino acid insertions, deletions, or substitutions, and has diminished or no CODM (or T6ODM) activity.

Deletions involve lack one or more residues of the endogenous protein. For the purposes of this disclosure, a deletion variant includes embodiments in which no amino acids of the
25 endogenous protein are translated, e.g. where the initial "start" methionine is substituted or deleted.

Insertional mutations typically involve the addition of material at a non-terminal point in the polypeptide, but may include fusion proteins comprising amino terminal and carboxy terminal
30 additions. Substitutional variants typically involve a substitution of one amino acid for another at one or more sites within the protein, and may be designed to modulate one or more properties of the polypeptide. Substitutions of this kind may, in some embodiments, be conservative, i.e. where one amino acid is replaced with one of similar shape, size, charge,

hydrophobicity, hydrophilicity, etc. Conservative substitutions are well known in the art and include, for example, the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; glycine to proline; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or leucine.

Accordingly, the CODM enzyme may have an amino acid sequence that possesses at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to SEQ ID NO: 1. Accordingly, the T6ODM enzyme may have an amino acid sequence that possesses at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to SEQ ID NO: 2.

Expression of transgenes targeting the endogenous genes

In another aspect, the present disclosure relates to reducing the expression and/or activity of CODM and T6ODM by targeting their respective mRNA transcripts. In this regard, levels of CODM and T6ODM mRNA transcripts may be reduced by methods known in the art including, but not limited to, co-suppression, antisense expression, small hair pin (shRNA) expression, interfering RNA (RNAi) expression, double stranded (dsRNA) expression, inverted repeat dsRNA expression, micro interfering RNA (miRNA), simultaneous expression of sense and antisense sequences, or a combination thereof.

In one embodiment, the present disclosure relates to the use of nucleic acid molecules that are complementary, or essentially complementary, to at least a portion of the molecules set forth in SEQ ID NO:2 or SEQ ID NO:4. Nucleic acid molecules that are "complementary" are those that are capable of base-pairing according to the standard Watson-Crick complementary rules. As used herein, the term "complementary sequences" means nucleic acid sequences that are substantially complementary, as may be assessed by the same nucleotide comparison set forth above, or as defined as being capable of hybridizing to the nucleic acid

segment of SEQ ID NO:2 or SEQ ID NO:4 under relatively stringent conditions such as those described herein. Nucleic acid molecules may be substantially complementary (or are homologues/have identity) if the two sequences hybridize to each other under moderately stringent, or preferably stringent, conditions. Hybridization to filter-bound sequences under moderately stringent conditions may, for example, be performed in 0.5 M NaHPO₄, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65° C., and washing in 0.2×SSC/0.1% SDS at 42° C. (see Ausubel, et al. (eds), 1989, Current Protocols in Molecular Biology, Vol. 1, Green Publishing Associates, Inc., and John Wiley & Sons, Inc., New York, at p. 2.10.3). Alternatively, hybridization to filter-bound sequences under stringent conditions may, for example, be performed in 0.5 M NaHPO₄, 7% SDS, 1 mM EDTA at 65° C., and washing in 0.1×SSC/0.1% SDS at 68° C. (see Ausubel, et al. (eds), 1989, supra). Hybridization conditions may be modified in accordance with known methods depending on the sequence of interest (see Tijssen, 1993, Laboratory Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Acid Probes, Part I, Chapter 2 “Overview of principles of hybridization and the strategy of nucleic acid probe assays”, Elsevier, N.Y.). Generally, stringent conditions are selected to be about 5° C. lower than the thermal melting point for the specific sequence at a defined ionic strength and pH.

The phenomenon of co-suppression in plants relates to the introduction of transgenic copies of a gene resulting in reduced expression of the transgene as well as the endogenous gene. The observed effect depends on sequence identity between the transgene and the endogenous gene.

The term "RNA interference" (RNAi) refers to well-known methods for down-regulating or silencing expression of a naturally occurring gene in a host plant. RNAi employs a double-stranded RNA molecule or a short hairpin RNA to change the expression of a nucleic acid sequence with which they share substantial or total homology. For a review, see e.g. Agrawal, N. et al (2003) Microbiol Mol Biol Rev. 67(4): 657-685. RNA is both an initiator and target in the process. This mechanism targets RNA from viruses and transposons and also plays a role in regulating development and genome maintenance. Briefly, double stranded RNA is cleaved by the enzyme dicer resulting in short fragments of 21-23 bp (siRNA). One of the two strands of each fragment is incorporated into the RNA-induced silencing complex (RISC). The RISC associated RNA strand pairs with mRNA and induces cleavage of the mRNA. Alternatively,

RISC associated RNA strand pairs with genomic DNA resulting in epigenetic changes that affect gene transcription. Micro RNA (miRNA) is a type of RNA transcribed from the genome itself and works in a similar way. Similarly, shRNA may be cleaved by dicer and associate with RISC resulting in mRNA cleavage.

5

Specific examples of gene silencing in poppy have been reported using RNAi approaches. In 2008, Allen et al. reported suppression of the gene encoding the morphinan pathway enzyme salutaridinol 7-O-acetyltransferase (SalAT) in opium poppy. Hairpin RNA-mediated suppression of SalAT resulted in the accumulation of salutaridine to 23% of total alkaloids. As discussed above in the Description of Related Art, Allen et al. (2004) silenced codeinone reductase (COR) in opium poppy using a chimeric hairpin RNA construct designed to silence all members of the multigene COR family through RNAi.

10

Antisense suppression of gene expression does not involve the catalysis of mRNA degradation, but instead involves single-stranded RNA fragments binding to mRNA and blocking protein translation.

15

Both antisense and sense suppression are mediated by silencing RNAs (sRNAs) produced from either a sense-antisense hybrid or double stranded RNA (dsRNA) generated by an RNA-dependant RNA polymerase. Major classes of sRNAs include short-interfering RNAs (siRNAs) and microRNAs (miRNAs) which differ in their biosynthesis.

20

Processing of dsRNA precursors by Dicer-Like complexes yields 21-nucleotide siRNAs and miRNAs guide cleavage of target transcripts from within RNA-induced silencing complexes (RISC).

25

T6ODM and CODM expression may be suppressed using a synthetic gene(s) or an unrelated gene(s) that contain about 21 bp regions or longer of high homology (preferably 100% homology) to the endogenous coding sequences for T6ODM and CODM.

30

See, for example, Jorgensen R A, Doetsch N, Muller A, Que Q, Gendler, K and Napoli C A (2006) A paragenetic perspective on integration of RNA silencing into the epigenome and in the biology of higher plants. Cold Spring Harb. Symp. Quant. Biol. 71:481-485. For a further

review, see for example, Ossowski S, Schwab R and Weigel D (2008) Gene silencing in plants using artificial microRNAs and other small RNAs. *The Plant Journal* 53:674-690.

Nucleic acid molecules that are substantially identical to portions of the endogenous coding sequences for CODM and T6ODM may also be used in the context of the disclosure. As used herein, one nucleic acid molecule may be "substantially identical" to another if the two molecules have at least 60%, at least 70%, at least 80%, at least 82.5%, at least 85%, at least 87.5%, at least 90%, at least 92.5%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity. Thus, a nucleic acid sequence comprising a nucleic acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92.5%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 2 or SEQ ID NO: 4 may be suitable for use in the context of this disclosure. In one embodiment, the two nucleic acid molecules each comprise at least 20 identical contiguous nucleotides.

Fragments of nucleic acid sequences encoding CODM or T6ODM may be used. Such fragments may have lengths of at least 20, at least 50, at least 100, at least 150, at least 200, at least 300 or at least 400 contiguous nucleotides of a nucleic acid sequence encoding a CODM or T6ODM as the case may be. Alternatively such fragments may have a minimum length of at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, or at least 50 contiguous nucleotides and a maximum length less than 3000, less than 2000, less than 1750, less than 1500, less than 1250, less than 1000, less than 750 or less than 500 contiguous nucleotides or any combination of such minimum and maximum lengths of a nucleic acid sequence encoding CODM or T6ODM as the case may be.

In one embodiment, a genetically modified opium poppy plant of the disclosure comprises, stably integrated into its genome a first nucleic acid molecule heterologous to the plant. The first nucleic acid molecule encodes an RNA, e.g. a hairpin RNA, for reducing expression of the CODM enzyme. The genetically modified opium poppy plant further comprises and a second nucleic acid molecule heterologous to the plant. The second nucleic acid molecule encodes an RNA, e.g. a hairpin RNA, for reducing expression of the T6ODM enzyme.

The first and second nucleic acid molecules may be present in a single genetic construct or in multiple constructs. In one embodiment, the first and/or second nucleic acid molecules may be arranged in the sense orientation relative to a promoter. In another embodiment, the first and/or second nucleic acid molecules may be arranged in the anti-sense orientation relative to a promoter. In a further embodiment, a genetic construct may comprise at least two nucleic acid molecules in both the sense and anti-sense orientations, relative to a promoter. A genetic construct comprising nucleic acids in both the sense and anti-sense orientations may result in mRNA transcripts capable of forming stem-loop (hairpin) structures.

One or both of the nucleic acid molecules may be under transcriptional control of the same promoter.

In various instances, the first and second heterologous nucleic acid molecules respectively comprise:

at least 20, at least 50, at least 100, at least 150, at least 200, at least 300 or at least 400 contiguous nucleotides of a nucleic acid sequence possessing at least 80%, at least 90% or 100% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 4;

at least 20, at least 50, at least 100, at least 150, at least 200, at least 300 or at least 400 contiguous nucleotides of a nucleic acid sequence possessing at least 80%, at least 90% or 100% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 2.

In various instances, the first and second nucleic acid molecules respectively comprise:

a nucleic acid molecule with a minimum length of at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, or at least 50 contiguous nucleotides and a maximum length less than 1750, less than 1500, less than 1250, less than 1000, less than 750 or less than 500 contiguous nucleotides or any combination of such minimum and maximum lengths of a nucleic acid sequence possessing at least 80%, at least 90% or 100% sequence identity to the nucleic acid sequence set forth in SEQ ID NO:4; and

a nucleic acid molecule with a minimum length of at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, or at least 50 contiguous nucleotides and a maximum length less than 1750, less than 1500, less than 1250, less than 1000, less than 750 or less than 500 contiguous nucleotides or any combination of such minimum and maximum lengths of a nucleic acid sequence possessing at least 80%, at least 90% or 100% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 2.

The skilled person will also appreciate that the reduction in activity of CODM and T6ODM may not be limited by the number of different nucleic acid molecules introduced into a plant or plant cell. In one embodiment, one nucleic acid molecule may target one or both endogenous genes encoding the enzymes. Accordingly, in another embodiment, a genetically modified opium poppy plant of the disclosure comprises, stably integrated into its genome a nucleic acid molecule heterologous to the plant. The nucleic acid molecule encodes a single transcript comprising an RNA (e.g., a hairpin RNA) for reducing expression of the CODM enzyme and an RNA (e.g. a hairpin RNA) for reducing expression of the T6ODM enzyme. In the working embodiment specifically exemplified in this disclosure, the nucleic acid molecule encodes a single transcript comprising a single hairpin RNA for reducing expression of both the CODM enzyme and the T6ODM enzyme.

In one aspect, a nucleic acid molecule may comprise a portion(s) of the coding sequence for CODM (SEQ ID NO: 4); T6ODM (SEQ ID NO: 2); an allelic variant thereof; a non-natural variant thereof; a fragment thereof; or any combination thereof.

In one embodiment, a fragment of the coding sequence for T6ODM (SEQ ID NO: 2) is suitable for the production of an expression construct coding for an RNAi hairpin that targets expression of the endogenous coding sequences of both CODM and T6ODM. In the working embodiment specifically exemplified in this disclosure, such fragment comprises SEQ ID NO: 7. In the working embodiment specifically exemplified in this disclosure, such expression construct comprises SEQ ID NO: 5. In the working embodiment specifically exemplified in this disclosure, the RNAi hairpin is encoded by a nucleic acid comprising SEQ ID NO: 6.

An expression construct comprising nucleic acids in both orientations relative to a promoter may further comprise a spacer to separate the nucleic acid molecules in sense orientation and those in the anti-sense orientation. As used herein, a “spacer” may comprise at least 2, at least 5, at least 10, at least 20, at least 30, at least 40, at least 50, at least 75, at least 100, at least 150, or at least 200 nucleotides.

The skilled person will also readily understand that although in the foregoing illustrative examples partial CODM and T6ODM coding sequences were suggested for constructing the CODM and T6ODM constructs, complete CODM and T6ODM coding sequences, alternative CODM and/or T6ODM coding sequences, 5'UTR and/or 3'UTR, or mutated derivatives of these sequences can also be used. The maximum number of nucleic acid molecules that may be used in the context of the invention may be limited only by the maximum size of the construct that may be delivered to a target plant or plant cell using a given transformation method.

In various embodiments, genetically modified plants of the present disclosure may further comprise a third nucleic acid molecule heterologous to the plant. The third nucleic acid molecule is for increasing expression of Cyp80B3 to increase the total level of morphinans.

Expression of transgenes targeting the endogenous CODM and T6ODM polypeptides

In a further aspect, the disclosure relates to reducing CODM and/or T6ODM activity by targeting CODM and T6ODM at the protein level. For example, CODM (or T6ODM) activity may be reduced by affecting the post-translational modification of the enzyme; or by the introduction of a heterologous protein (e.g. a mutated form of CODM or (T6ODM) may be expressed such that it associates with the wildtype enzyme and alters its activity or outcompetes the wildtype enzyme for substrate without being able to convert the substrate; or an antibody that binds specifically to the CODM (or T6ODM) enzyme.

The skilled person would also appreciate that a nucleic acid molecule comprising the sequence of a CODM or T6ODM gene promoter and/or other regulatory elements may be used in the context of the invention. In an embodiment, a heterologous nucleic acid molecule comprising sequences of a CODM (or T6ODM as the case may be) gene promoter and/or

regulatory element may be used to bias the cellular machinery away from an endogenous CODM (or T6ODM as the case may be) gene promoter thus resulting in reduced CODM (or T6ODM) expression.

- 5 The size or length of the nucleic acid construct or elements thereof, are not limited to the specific embodiments described herein. For example, the skilled person would appreciate that the size of a transgene element may be defined instead by transgene element function; and that the promoter element may be determined instead as one that was capable of driving transcription at a sufficient level and in the desired tissues. Similarly, the stem loop structure
- 10 formed by the mRNA transcribed by a nucleic acid construct of the invention, may comprise a number of gene segments which may vary in length. For example, the stem loop may comprise 3 gene segments of about 21-30 basepairs each, in addition to a spacer, such as an intron (126 bp plus intron).
- 15 The skilled person would appreciate that the size of the gene segments may be established by the sum of the element sizes combined and may depend on the transformation method used to deliver the transgene into the target organism. For example, each transformation method (Agrobacterium, biolistics, VIGS-based delivery systems) may be limited to theoretical maximum transgene sizes.

20

Plant Transformation

- The introduction of DNA into plant cells by Agrobacterium mediated transfer is well known to those skilled in the art. If, for example, the Ti or Ri plasmids are used for the transformation of the plant cell, at least the right border, although more often both the right and the left border of the T-DNA contained in the Ti or Ri plasmid must be linked to the genes to be inserted as flanking region. If agrobacteria are used for the transformation, the DNA to be integrated must be cloned into special plasmids and specifically either into an intermediate or a binary vector. The intermediate vectors may be integrated into the Ti or Ri plasmid of the agrobacteria by
- 25 homologous recombination due to sequences, which are homologous to sequences in the T-DNA. This also contains the vir-region, which is required for T-DNA transfer. Intermediate vectors cannot replicate in agrobacteria. The intermediate vector can be transferred to Agrobacterium tumefaciens by means of a helper plasmid (conjugation). Binary vectors are
- 30

able to replicate in *E. coli* as well as in *agrobacteria*. They contain a selection marker gene and a linker or polylinker framed by the right and left T-DNA border region. They can be transformed directly into *agrobacteria*. The *agrobacterium* acting as host cell should contain a plasmid carrying a *vir*-region. The *vir*-region is required for the transfer of the T-DNA into the plant cell. Additional T-DNA may be present. Such a transformed *agrobacterium* is used for the transformation of plant cells. The use of T-DNA for the transformation of plant cells has been intensively studied and has been adequately described in standard review articles and manuals on plant transformation. Plant explants cultivated for this purpose with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* can be used for the transfer of DNA into the plant cell.

Agrobacterium transformation can be used to transform opium poppy plants (Chitty et al. (Meth. Molec. Biol, 344:383-391; Chitty et al. (Functional Plant Biol, 30: 1045-1058); Facchini et al. (Plant Cell Rep., 27(4) :719-727)). Facchini et al. (2008) disclosed *A. tumefaciens*-mediated genetic transformation protocol via somatic embryogenesis for the production of fertile, herbicide-resistant opium poppy plants. Transformation was mediated using pCAMBIA3301, a transformation vector that harbors the phosphinothricin acetyltransferase (*pat*) gene driven by the cauliflower mosaic virus (CaMV) 35S promoter and the β -glucuronidase (*GUS*) gene also driven by the CaMV 35S promoter. Explants were co-cultivated with *A. tumefaciens* in the presence of 50 1M ATP and 50 1M MgCl₂. Root explants pre-cultured on callus induction medium were then used for transformation. Herbicide-resistant, proliferating callus was obtained from explants on a medium containing both 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzyladenine (BA). Globular embryo genic callus was induced by removal of the BA from the medium, and placed on a hormone-free medium to form somatic embryos. The somatic embryos were converted to plantlets under specific culture conditions and transferred to soil. Plants were allowed to mature and set seed. PAT and GUS transcripts and enzyme activities were detected in the transgenic lines tested.

Nevertheless, the present invention is not limited to any particular method for transforming plant cells, and the skilled person will readily understand that any other suitable method of DNA transfer into plant may be used.. Methods for introducing nucleic acids into cells (also referred to herein as "transformation") are known in the art and include, but are not limited to: Viral methods (Clapp. Clin Perinatol, 20: 155-168, 1993; Lu et al. J Exp Med, 178: 2089-2096,

1993; Eglitis and Anderson. *Biotechniques*, 6: 608-614, 1988; Eglitis et al, *Avd Exp Med Biol*, 241: 19-27, 1988); physical methods such as microinjection (Capecchi. *Cell*, 22: 479-488, 1980), electroporation (Wong and Neumann. *Biochim Biophys Res Commun*, 107: 584-587, 1982; Fromm et al, *Proc Natl Acad Sci USA*, 82: 5824-5828, 1985; U.S. Pat. No. 5,384,253) and the gene gun (Johnston and Tang. *Methods Cell Biol*, 43: 353-365, 1994; Fynan et al. *Proc Natl Acad Sci USA*, 90: 11478-11482, 1993); chemical methods (Graham and van der Eb. *Virology*, 54: 536-539, 1973; Zatloukal et al. *Ann NY Acad Sci*, 660: 136-153, 1992); and receptor mediated methods (Curiel et al. *Proc Natl Acad Sci USA*, 88: 8850-8854, 1991; Curiel et al. *Hum Gen Ther*, 3: 147-154, 1992; Wagner et al. *Proc Natl Acad Sci USA*, 89: 6099-6103, 1992).

Another method for introducing DNA into plant cells is by biolistics. This method involves the bombardment of plant cells with microscopic particles (such as gold or tungsten particles) coated with DNA. The particles are rapidly accelerated, typically by gas or electrical discharge, through the cell wall and membranes, whereby the DNA is released into the cell and incorporated into the genome of the cell. This method is used for transformation of many crops, including corn, wheat, barley, rice, woody tree species and others. Biolistic bombardment has been proven effective in transfecting a wide variety of animal tissues as well as in both eukaryotic and prokaryotic microbes, mitochondria, and microbial and plant chloroplasts (Johnston. *Nature*, 346: 776-777, 1990; Klein et al. *Bio/Technol*, 10: 286-291, 1992; Pecorino and Lo. *Curr Biol*, 2: 30-32, 1992; Jiao et al, *Bio/Technol*, 11: 497-502, 1993).

Another method for introducing DNA into plant cells is by electroporation. This method involves a pulse of high voltage applied to protoplasts/cells/tissues resulting in transient pores in the plasma membrane which facilitates the uptake of foreign DNA. The foreign DNA enter through the holes into the cytoplasm and then to the nucleus.

Plant cells may be transformed by liposome mediated gene transfer. This method refers to the use of liposomes, circular lipid molecules with an aqueous interior, to deliver nucleic acids into cells. Liposomes encapsulate DNA fragments and then adhere to the cell membranes and fuse with them to transfer DNA fragments. Thus, the DNA enters the cell and then to the nucleus.

Other well-known methods for transforming plant cells which are consistent with the present invention include, but are not limited to, pollen transformation (See University of Toledo 1993 U.S. Pat. No. 5,177,010); Whiskers technology (See U.S. Pat. Nos. 5,464,765 and 5,302,523).

- 5 The nucleic acid constructs of the present invention may be introduced into plant protoplasts. Plant protoplasts are cells in which its cell wall is completely or partially removed using either mechanical or enzymatic means, and may be transformed with known methods including, calcium phosphate based precipitation, polyethylene glycol treatment and electroporation (see for example Potrykus et al., *Mol. Gen. Genet.*, 199: 183, 1985; Marcotte et al., *Nature*, 335: 10 454, 1988). Polyethylene glycol (PEG) is a polymer of ethylene oxide. It is widely used as a polymeric gene carrier to induce DNA uptake into plant protoplasts. PEG may be used in combination with divalent cations to precipitate DNA and effect cellular uptake. Alternatively, PEG may be complexed with other polymers, such as poly(ethylene imine) and poly L lysine.
- 15 A nucleic acid molecule of the present invention may also be targeted into the genome of a plant cell by a number of methods including, but not limited to, targeting recombination, homologous recombination and site-specific recombination (see review Baszcynski et al. *Transgenic Plants*, 157: 157-178, 2003 for review of site-specific recombination systems in plants). Homologous recombination and gene targeting in plants (reviewed in Reiss. 20 *International Review of Cytology*, 228: 85-139, 2003) and mammalian cells (reviewed in Sorrell and Kolb. *Biotechnology Advances*, 23: 431-469, 2005) are known in the art.

As used herein, "targeted recombination" refers to integration of a nucleic acid construct into a site on the genome, where the integration is facilitated by a construct comprising sequences 25 corresponding to the site of integration.

Homologous recombination relies on sequence identity between a piece of DNA that is introduced into a cell and the cell's genome. Homologous recombination is an extremely rare event in higher eukaryotes. However, the frequency of homologous recombination may be 30 increased with strategies involving the introduction of DNA double-strand breaks, triplex forming oligonucleotides or adeno-associated virus.

As used herein, "site-specific recombination" refers to the enzymatic recombination that occurs when at least two discrete DNA sequences interact to combine into a single nucleic acid sequence in the presence of the enzyme. Site-specific recombination relies on enzymes such as recombinases, transposases and integrases, which catalyse DNA strand exchange between DNA molecules that have only limited sequence homology. Mechanisms of site specific recombination are known in the art (reviewed in Grindley et al. Annu Rev Biochem, 75: 567-605, 2006). The recognition sites of site-specific recombinases (for example Cre and att sites) are usually 30-50 bp. The pairs of sites between which the recombination occurs are usually identical, but there are exceptions e.g. attP and attB of λ integrase (Landy. Ann Rev Biochem, 58: 913-949, 1989).

Additional methods might be selected from the recent years of development of methods and compositions to target and cleave genomic DNA by site specific nucleases e.g. Zinc Finger Nucleases, ZFNs, Meganucleases, Transcription Activator-Like Effector Nucleases, TALENS and Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated nuclease (CRISPR/Cas) with an engineered crRNA/tracr RNA), to induce targeted mutagenesis, induce targeted deletions of cellular DNA sequences, and facilitate targeted recombination of an exogenous donor DNA polynucleotide within a predetermined genomic locus. Current methods for targeted insertion of exogenous DNA typically involve co-transformation of plant tissue with a donor DNA polynucleotide containing at least one transgene and a site specific nuclease, e.g., ZFN, which is designed to bind and cleave a specific genomic locus of an actively transcribed coding sequence. This causes the donor DNA polynucleotide to stably insert within the cleaved genomic locus resulting in targeted gene addition at a specified genomic locus comprising an actively transcribed coding sequence.

As used herein the term "zinc fingers," defines regions of amino acid sequence within a DNA binding protein binding domain whose structure is stabilized through coordination of a zinc ion.

A "zinc finger DNA binding protein" (or binding domain) is a protein, or a domain within a larger protein, that binds DNA in a sequence-specific manner through one or more zinc fingers, which are regions of amino acid sequence within the binding domain whose structure is stabilized through coordination of a zinc ion. The term zinc finger DNA binding protein is often abbreviated as zinc finger protein or ZFP. Zinc finger binding domains can be "engineered" to

bind to a predetermined nucleotide sequence. Non-limiting examples of methods for engineering zinc finger proteins are design and selection. A designed zinc finger protein is a protein not occurring in nature whose design/composition results principally from rational criteria. Rational criteria for design include application of substitution rules and computerized algorithms for processing information in a database storing information of existing ZFP designs and binding data. (U.S. Pat. No. 6,453,242; see also WO 98/53058).

A "TALE DNA binding domain" or "TALE" is a polypeptide comprising one or more TALE repeat domains/units. The repeat domains are involved in binding of the TALE to its cognate target DNA sequence. A single "repeat unit", also referred to as a "repeat", is typically 33-35 amino acids in length and exhibits at least some sequence homology with other TALE repeat sequences within a naturally occurring TALE protein. (U.S. Patent Publication No. 2011/0301073).

The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas (CRISPR Associated) nuclease system. Briefly, a "CRISPR DNA binding domain" is a short stranded RNA molecule that acting in concert with the CAS enzyme can selectively recognize, bind, and cleave genomic DNA. The CRISPR/Cas system can be engineered to create a double-stranded break (DSB) at a desired target in a genome, and repair of the DSB can be influenced by the use of repair inhibitors to cause an increase in error prone repair. (Jinek et al (2012) Science 337, p. 816-821).

Zinc finger, CRISPR and TALE binding domains can be "engineered" to bind to a predetermined nucleotide sequence, for example via engineering (altering one or more amino acids) of the recognition helix region of a naturally occurring zinc finger. Similarly, TALEs can be "engineered" to bind to a predetermined nucleotide sequence, for example by engineering of the amino acids involved in DNA binding (the repeat variable diresidue or RVD region). Therefore, engineered DNA binding proteins (zinc fingers or TALEs) are proteins that are non-naturally occurring. Non-limiting examples of methods for engineering DNA-binding proteins are design and selection. A designed DNA binding protein is a protein not occurring in nature whose design/composition results principally from rational criteria. Rational criteria for design include application of substitution rules and computerized algorithms for processing information in a database storing information of existing ZFP and/or TALE designs and binding

data. (U.S. Pat. No. 6,453,242; see also WO 98/53058; and U.S. Publication Nos. 2011/0301073).

5 A "selected" zinc finger protein, CRISPR or TALE is a protein not found in nature whose production results primarily from an empirical process such as phage display, interaction trap or hybrid selection.

10 In one embodiment, the polynucleotide encodes a zinc finger protein that binds to a gene encoding a T6ODM or a CODM polypeptide, resulting in reduced expression of the gene. In particular embodiments, the zinc finger protein binds to a regulatory region of a gene encoding T6ODM or CODM. In other embodiments, the zinc finger protein binds to a messenger RNA encoding a T6ODM or a CODM polypeptide and prevents its translation. Methods of selecting sites for targeting by zinc finger proteins have been described, for example, in US 6,453,242, and methods for using zinc finger proteins to inhibit the expression of genes in plants are
15 described, for example, in US2003/0037355, each of which is herein incorporated by reference. Methods of selecting sites for targeting by TALE proteins have been described in e.g. Moscou MJ, Bogdanove AJ, 2009, A simple cipher governs DNA recognition by TAL effectors. Science 326:1501.

20 The nucleic acid molecule becomes stably integrated into the plant genome such that it is heritable to daughter cells in order that successive generations of plant cells have reduced CODM and T6ODM expression. This may involve the nucleic acid molecules of the present invention integrating, for instance integrating randomly, into the plant cell genome. Alternatively, the nucleic acid molecules of the present invention may remain as exogenous,
25 self-replicating DNA that is heritable to daughter cells. As used herein, exogenous, self-replicating DNA that is heritable to daughter cells is also considered to be "stably integrated into the plant genome".

Testing for Reduction of CODM and T6ODM Activity or Expression

30 Disruption of endogenous genes encoding CODM and T6ODM, their expression, or CODM and T6ODM enzymatic activity may be confirmed by methods known in the art of molecular biology. For example, disruption of endogenous genes may be assessed by PCR followed by

Southern blot analysis. CODM and T6ODM mRNA levels may, for example, be measured by real time PCR, RT-PCR, Northern blot analysis, micro-array gene analysis, and RNase protection. CODM and T6ODM protein levels may, without limitation, be measured by enzyme activity assays, ELISA and Western blot analysis. CODM and T6ODM expression, or lack thereof, may be used as a predictor of increased thebaine accumulation. CODM and/or T6ODM enzymatic activity may be assessed biochemically or functionally.

For example, CODM (and/or T6ODM) activity may be measured biochemically by methods known in the art including, but not limited to, the detection of products formed by the enzyme in the presence of any number of heterologous substrates, for example, thebaine. CODM (and/or T6ODM) activity may also be measured functionally, for example, by assessing thebaine levels in the poppy tissues.

A genetically modified opium poppy plant of the present disclosure may result in the reduction of CODM and/or T6ODM activity in said plant or its seed, seedling, straw, capsules, or progeny thereof, by at least 57%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% relative to a wild type seed, seedling, straw, capsules, or progeny thereof.

Targeted Screening for Loss of Function Mutations in CODM and/or T6ODM.

This disclosure further relates to methods of generating opium poppy plants with high levels of thebaine that involve targeted screening for loss of function mutations in the endogenous genes encoding CODM and/or T6ODM and subsequent breeding of plants to combine the mutations to obtain plants homozygous for the loss of function mutations at both loci. Opium poppy breeders have used a variety of selection techniques in the development of improved cultivars. However, the most successful breeding method involving the hybridization of parents with a variety of different desired characteristics. Such approach has been used successfully to increase capsule numbers, seed and opium yield, morphine content, and lodging resistance.

The term "T-DNA insertion" refers to methods utilizing transfer-DNA (T-DNA) for disrupting genes via insertional mutagenesis. Down-regulating or silencing expression of the

endogenous gene(s) encoding CODM and/or T6ODM in an opium poppy plant can thus be achieved by T-DNA mutagenesis, wherein the T-DNA is used to randomly inserting in the plant genome to introduce mutations . Subsequently, plants can be screen for T-DNA insertions in the genes encoding CODM and/or T6ODM by PCR, using a primer pair comprising one primer
5 specific for the T-DNA and one primer specific for the gene encoding CODM (or T6ODM as the case may be), or other high-throughput technologies. For a review of T-DNA as an insertional mutagen, see e.g. Krysan, P.J. et al. (1999) Plant Cell, 11: 2283-2290. Insertional mutagenesis using transposons could also be employed.

10 Mutations (including deletions, insertions, and point mutations) can also be introduced randomly into the genome of a plant cell by various forms of mutagenesis to produce non-natural variants. Methods for mutagenesis of plant materials, including seeds, and subsequent screening or selection for desired phenotypes are well known, as described in WO2009109012. Mutagenized plants and plant cells can also be specifically screened for
15 mutations in the genes encoding CODM and/or T6ODM, for example, by TILLING (Targeting Induced Local Lesions IN Genomes). Loss of function mutations present in natural plant populations can be identified by EcoTILLING.

Once the loss of function mutations in the endogenous genes encoding CODM and T6ODM
20 have been identified, they can be combined through traditional breeding processes to produce plants homozygous for the loss of function mutations at both loci. Alternatively, a loss of function mutation identified in the endogenous gene encoding CODM can be combined with mutations in the endogenous gene encoding T6ODM that are introduced by genetic modification, and vice versa. Alternatively, a loss of function mutation identified in the
25 endogenous gene encoding CODM can be combined with genetic modification comprising an expression construct designed to reduce expression of T6ODM as described above, and vice versa.

Alkaloid collection and analysis

30 Opium poppy cultivation and opium harvesting traditionally involved the processes of manually lancing the seed capsule and collecting the latex. However, methods to extract morphine and related compounds from opium poppy straw circumvented the traditional technique and makes

it possible to obtain high quality seeds and pharmaceutically valuable raw materials simultaneously. Recovering thebaine from poppy straw or latex of an opium poppy plant is well known in the art as discussed in WO2009109012. IN addition to the particular methods described below, methods of analyzing alkaloid extracts from opium poppy straw or latex are also discussed in WO2009109012.

Examples

Referring to Figure 2, the inventor used a single hairpin construct targeting genes coding for CODM and T6ODM enzymes to test the hypothesis that plants containing elevated levels of thebaine (and reduced levels of codeine and morphine) compared to parental plants can be produced by simultaneously reducing the activity of the T6ODM and CODM enzymes.

Referring to Figure 3, the coding sequences for CODM and T6ODM genes have very high level of identity. Accordingly, the inventor created a single expression construct to target both the endogenous gene encoding CODM and the endogenous gene encoding T6ODM from a portion of the T6ODM coding sequence by RNAi. The portion of the T6ODM coding sequence used for the sense and antisense portions of the RNAi gene construct depicted in Figure 2 is underlined in Figure 3. A 342 base pair sense fragment was amplified from cDNA isolated from opium poppy plant material using primers AAAGGCGCGCCCCTTGTCTCAACCAAAT and AAAATTTAAATTCCACTTTTAAACAAAGC). A 342 base pair antisense fragment was amplified from cDNA isolated from opium poppy plant material using primers AAAACTAGTCCTTGTCTCAACCAAAT), OPP026 (AAAGGATCCTCCACTTTTAAACAAAGC). These two fragments were used to create a nucleotide molecule with these two fragments interposed by sequences from β -glucuronidase.

The sequence of the nucleic acid molecule to be transcribed to produce a hairpin RNA is provided as SEQ ID NO: 6. The T6ODM sequences are underlined, whereas the intervening "hairpin" sequence between the T6ODM sequences is derived from coding sequences for β -glucuronidase.

The complete expression construct comprising SEQ ID NO: 6 along with the Cauliflower Mosaic Virus 35S promoter and transcription and translation termination sequences from octopine synthase was cloned into a TDNA transfer vector. The sequences between the left and right borders of the vector are provided as provided as SEQ ID NO: 5. The sequence 5' to the first underlined region (i.e. the "sense" T6ODM-specific sequence) comprises the 35S promoter sequence, whereas the sequences 3' to the second underlined region (i.e. the "antisense" T6ODM-specific sequence) comprises the transcription and translation termination sequences from octopine synthase.

While this expression construct was generated using a combination of traditional polymerase chain reaction (PCR) and cloning techniques (e.g. with restriction enzymes and ligations), the skilled person will understand that various conventional techniques could be used to produce the construct, including overlap extension PCR cloning or direct synthesis.

The sequence of the entire T-DNA comprising SEQ ID NO:6, from Right Border to Left Border, is provided as SEQ ID NO: 4.

Virus-induced gene silencing (VIGS) was used to transiently test the ability of the gene cassette to silence the endogenous genes encoding T6ODM and CODM. VIGS is a plant RNA-silencing technique that uses viral vectors carrying a fragment of a gene of interest to generate double-stranded RNA, which initiates the silencing of the target gene. pTRV1 (helper plasmid) and pTRV2 (binary vector) TRV-based VIGS vectors to express the expression construct. Tissues were taken in 48 hrs, 72 hrs, 5 day, 7 days, and 2 weeks after infiltration. As indicated in Figures 4a and 4b, transient expression of the expression construct resulted in substantial downregulation of transcripts from the endogenous genes encoding CODM and T6ODM at 48 h post transformation (first bar from the right in both Figure 3a and 3b above).

Plants stably transformed with the expression construct were then generated according to the following protocol.

TRANSFORMATION PROTOCOL
Transformation of Poppy hypocotyls/roots

Media required:

5 ***LB***

Agrobacterium suspension medium

B5 salts and vitamins containing 20 g/l sucrose, pH – 5.6 – 5.8 ± 0.2.

Shoot germination medium

10 Half strength Murashige and Skoog basal medium supplemented with 20 g/l sucrose, 8 g/l agar. pH – 5.8 ± 0.2.

Primary callus induction medium

B5 medium containing 30 g/l sucrose, 2.0 mg/l NAA, and 0.1 mg/l BAP, 8 g/l agar.

15

Somatic embryo induction medium

B5 medium containing 1.0 mg/l NAA, 0.5 mg/l BAP, 50 mg/l paromomycin, 300 mg/l timentin and 8 g/l agar.

20 ***Embryo induction Medium***

Murashige and Skoog basal medium supplemented with 30 g/l sucrose, 0.25 g/l MES, 0.2 g/l myo-inositol, 1 mg/l 2,4-D, 2.5 mg/l AgNO₃, 8 g/l agar. pH – 5.6 ± 0.2. (Plus antibiotic - Timentin 300 mg/l)

25 ***Embryo Maturation and germination***

Murashige and Skoog basal medium supplemented with 30 g/l sucrose, 0.25 g/l MES, 0.2 g/l myo-inositol, 1 mg/l Benzyl adenine, 1 mg/l Zeatin, 2.5 mg/l AgNO₃, 8 g/l agar. pH – 5.6 ± 0.2. (Plus antibiotic - Timentin 300 mg/l)

30 ***Phytohormone-free plant regeneration medium***

B5 medium containing 50 mg/L paromomycin, 300 mg/L timentin, and 8 g/L agar

Shoot elongation medium

Murashige and Skoog basal medium supplemented with 30 g/l sucrose, 0.25 g/l MES, 0.2 g/l myo-inositol, 0.5 mg/l Benzyl adenine, 2.5 mg/l AgNO₃, 8 g/l agar. pH – 5.6 ± 0.2. (Plus antibiotic - Timentin 300 mg/l)

5

Rooting Medium

Murashige and Skoog basal medium supplemented with 30 g/l sucrose, 0.25 g/l MES, 0.2 g/l myo-inositol, 2.5 mg/l AgNO₃, 8 g/l agar. pH – 5.6 ± 0.2. (Plus antibiotic – Timentin 300 mg/l)

10 **Seed sterilization and germination**

The seeds were surface-sterilized with 70% (vv⁻¹) ethanol for 30 s and 1% (vv⁻¹) sodium hypochlorite solution for 2 min each three times, then rinsed five times in sterile water. Approximately 50 seeds were placed on 25 ml of agar-solidified culture medium in jars. The basal medium consisted of ½ Murashige and Skoog basal medium supplemented with 30 g/l sucrose (Gamborg et al. 1968) and solidified with 0.8% (vv⁻¹) agar. The medium was adjusted to pH 5.6-5.8 before adding the agar, and then sterilized by autoclaving. The seeds were germinated in a growth chamber at 25 °C under standard cool white fluorescent tubes and a 16-h photoperiod.

20 **Preparation of *Agrobacterium tumefaciens***

The binary vector pORE::ALM-MIRMAC was mobilized by electroporation in *Agrobacterium tumefaciens* strain EHA105. *A. tumefaciens* cultures were grown at 28 °C on a gyratory shaker at 180 rpm in liquid Luria-Bertani medium [1% tryptone, 0.5% yeast extract, and 1% NaCl, pH 7.0] containing 50 mg/l kanamycin and rifampicin 100 mg/l, to A₆₀₀=0.8. The bacterial cells were collected by centrifugation for 10 min at 4000 rpm and resuspended at a cell density of A₆₀₀=0.5 in liquid inoculation medium (B5 salts and vitamins containing 20 g/l sucrose).

25

Production of transgenic plants

Excised cotyledons from 12-day-old seedlings, line 118, were isolated by longitudinal bisection of the hypocotyl. The hypocotyls were dipped into the *A. tumefaciens* culture in liquid inoculation medium for 15 min, blotted dry on sterile filter paper, and incubated in the dark at 25 °C on primary callus induction medium. After 2 days of co-cultivation with *A. tumefaciens*, the hypocotyls were transferred to fresh primary callus induction medium containing 50 mg/L

30

paromomycin and 300 mg/L timentin. After 4-5 weeks of incubation, primary calli were subcultured on somatic embryo induction medium. After 3 weeks of cultivation on induction medium, somatic embryos were transferred to phytohormone-free plant regeneration medium. Mature embryos were placed on phytohormone-free medium and immature embryos were transferred to embryo maturation and germination medium. Then, when first cotyledons appeared they were transplanted onto shoot elongation medium. Finally, when shoots were about 0.5-1 cm, they were placed on rooting medium. Regenerated putative transgenic plantlets were grown in a growth chamber at 25°C under standard cool-white conditions and a 16-h photoperiod. Rooted plantlets were then transferred to pots containing autoclaved soil, covered with polyethylene bags for 1 week to sustain high humidity, and maintained in the growth chamber at 25°C for 1-2 weeks before the plants were transferred to the greenhouse.

Method:

1. Pick a single colony of the desired construct in *Agrobacterium* and inoculate it in 2 ml LB liquid medium containing appropriate antibiotics; culture overnight at 28°C to prepare a starter culture.
2. Inoculate 100 ml LB liquid medium containing appropriate antibiotics with the starter culture and incubate overnight at 28°C. Incubate cells until a desired OD₆₀₀ = 0.5-0.8 is attained.
3. Pellet the cells by centrifugation at 4000 rpm for 10 mins.
4. Resuspend the cells in *Agrobacterium* suspension medium to a final OD₆₀₀ = 0.5.
5. Roots and hypocotyl segments from 12 day old seedlings were cut into ~ 5 mm segments while dipping in the *Agrobacterium* suspension.
6. Incubate the roots and hypocotyl segments on Petri dish for 15 mins in the *Agrobacterium* suspension with occasional swirling.
7. Blot dry the roots and hypocotyl segments on a sterile filter paper and transfer to plates containing primary callus induction medium. Incubate the plates for 2 days at 22 ± 2°C in a growth cabinet under dark (covered with aluminium foil).

8. After 2 days of co-cultivation, wash the root and hypocotyl segments with sterile distilled water, blot dry on a sterile filter paper and transfer to plates containing primary callus induction medium (antibiotic paromomycin was added).
9. Incubate the plates in growth chamber at standard conditions (16/8 h photoperiod) in dark (covered with aluminium foil) for approximately 4-5 weeks.
10. After 4-5 weeks of incubation, primary calli were subcultured on somatic embryo induction medium (antibiotic paromomycin was added).
11. After 3 weeks of cultivation on induction medium, mature somatic embryos were transferred to phytohormone free medium (no antibiotic). Immature embryos were placed to another round of selection on embryo maturation and germination medium (no antibiotic) and incubated at $22 \pm 2^\circ\text{C}$ in a growth cabinet under 16/8 h photoperiod until embryos matured and started germinating.
12. Transfer the germinating embryos to plates containing shoot elongation medium until shoots appear and subsequently transfer to rooting medium.
13. Transfer ~ 0.5-1.0 cm shoots to rooting medium.
14. Rooted plantlets are washed under tap water to remove the entire adhering agar and then are transferred to sterile soil in small pots, covered with saran wrap and incubated in a growth cabinet for acclimatization.
15. Obtained putative transformants are analysed for transgene presence and expression.

Notes: - no antibiotic in rooting media.

Chemicals

1. 50 mg/ml Paromomycin sulfate stock: Prepare by dissolving powder in water and sterilize by filtration, aliquot, and store at -20°C .
2. 50 mg/ml Kanamycin stock: Prepare by dissolving powder in water and sterilize by filtration, aliquot, and store at -20°C .
3. 100 mg/ml Rifampicin stock: Prepare by dissolving powder in DMSO, aliquot, and store at -20°C .
4. 300 mg/ml Timentin stock: Prepare by dissolving powder in water and sterilize by filtration, aliquot, and store at -20°C .

5. 2.0 mg/ml NAA stock: Prepare by dissolving powder in 1N NaOH, adjust volume with water and sterilize by filtration, aliquot, and store at -20C.
6. 2.0 mg/ml BAP stock: Prepare by dissolving powder in 1N NaOH, adjust volume with water and sterilize by filtration, aliquot, and store at -20C.
- 5 7. 5 mg/ml AgNO₃ stock: Prepare by dissolving powder in water and sterilize by filtration, and store at +4C.
8. 1.0 mg/ml Zeatin stock: Prepare by dissolving powder in 1N NaOH, adjust volume with water and sterilize by filtration, aliquot, and store at -20C.
9. All the chemicals used for media are added to autoclaved medium once it has cooled
10 to about 50C. Swirl to mix thoroughly the medium before pouring into 90 x 25-mm Petri dishes.

CHARACTERIZATION OF REGENERATED TRANSGENIC PLANTS

- 15 The T-DNA comprising the expression construct included the nptII gene, which confers resistance to paromycin. Six plantlets (AM1 to AM6) were identified as resistant to paromomycin, suggesting that these plants were transformed with the expression construct. Polymerase chain reaction on genomic DNA isolated from these plantlets using primers specific for the hairpin expression construct (SEQ ID NO: 9, TAACCGACTTGCTGCCCCGA;
20 SEQ ID NO: 10, AAATAGAGATGCTTGCAGAAGATCCCG) showed that plants AM1, AM2 and AM3 contain amplicon from genomic DNA. Primers for actin (SEQ ID NO: 11, CGTTTGAATCTTGCTGGCCGTGAT; SEQ ID NO: 12, TAGACGAGCTGCCTTTGGAAGTGT) were used as a positive control to confirm that the samples contained genomic DNA from *Papaver somniferum*.

- 25 Referring to Figure 4 reverse transcription polymerase chain reaction (RT-PCR) was performed on RNA extracts from plants AM1 to AM6 was performed using primers specific for the hairpin (SEQ ID NO: 9, TAACCGACTTGCTGCCCCGA; SEQ ID NO: 10, AAATAGAGATGCTTGCAGAAGATCCCG), and demonstrated that the expression construct
30 was expressed in plants AM1, AM2, and AM3.

RT-PCR was performed on the RNA extracts from plants AM1 to AM6 using primers specific for endogenous transcripts encoding CODM and T6ODM. Referring to Figure 5, expression of

the expression construct appeared sufficient to downregulate the expression of the endogenous gene encoding T6ODM relative to plants that did not express the expression construct. Referring to Figure 6, expression of the expression construct appeared sufficient to downregulate the expression of the endogenous gene encoding CODM relative to plants that did not express the expression construct.

Alkaloid Analysis

Acidic extraction: 0.100g of ground capsule or stems (from poppy) was mixed with 5 ml of a solution of 10% acetic acid, 10% water and 80% methanol followed by agitation for 30 minutes, and then filtered. The filtrate was directly injected into the HPLC.

All samples were run on an HPLC Gradient System having an Kinetex 2.6 μ m C18, 50x2.1 mm column, with a 2 microliter injection volume, operating at 280 nm at a temperature of 45 °C and a flow rate of 0.8 ml/min. Eluent A was 10 mM Ammonium acetate Buffer, pH 5.5, whereas Eluent B was Acetonitrile (100%). The gradient profile is as follows

Step No.	Time (min)	Pct A	Pct B
1	0	95	5
2	0.25	85	15
3	2.00	60	40
4	2.01	95	5
5	3.00	20	80
6	4.00	10	90
7	5.00	0	100

The reported figures are percent by weight of the dry starting material.

Referring to Figures 7A to 7D, analysis of alkaloids in the capsule of AM 1 and the leaves of AM2 and AM3 indicated increased accumulation of thebaine and decreased accumulation of morphine compared to a plant in which the expression construct was not present and had a wild type level of T6ODM and CODM expression. Subsequent analysis of alkaloids in the capsules of the AM2 and AM3 plants showed that thebaine accumulated to 4.82% of alkaloids

in the AM2 capsules and to 3.13% of total alkaloids in the AM3 capsules. Substantially no morphine was detected in AM1, AM2, and AM3 plants.

Table 1. Concentration of alkaloids in transgenic plants per 100 mg dry capsule.

	<u>Codeine (Conc.wt%)</u>	<u>Thebaine (Conc.wt%)</u>
AM1	0.23	3.87
AM2	0.11	4.82
AM3	0.12	3.13

The progeny of the self-fertilized AM1, AM2, and AM3 transformants appear to segregate 3:1, indicating that the transgenes are stably integrated and inherited.

Six additional individual transformants that carry the expression construct, and in which the endogenous CODM and T6ODM transcripts could not be detected, were isolated (AM12, AM13, AM16, AM17, and AM19). Analysis in the capsules of these additional plants showed that thebaine accumulated to as much as 8.28% of alkaloids in the capsules (see Table 2).

Table 2. Concentration of thebaine in transgenic plants per 100 mg dry capsule.

	<u>Thebaine (Conc.wt%)</u>
AM12	5.87
AM13	6.20
AM16	8.28
AM17	7.18
AM19	4.95

Plants were grown from progeny seed of AM1 that contained the expression construct. The concentration of morphine, oripavine, codeine, and morphine in poppy straw from six AM1 progeny carrying the expression construct are provided in Table 3.

Table 3. Concentration of thebaine, codeine, oripavine, and morphine in poppy straw of progeny of AM1.

straw from six AM1 progeny carrying the expression construct are provided in Table 3.

Table 3. Concentration of thebaine, codeine, oripavine, and morphine in poppy straw of progeny of AM1.

Individual	Amount of Each Component (% w/w)			
	Morphine	Oripavine	Codeine	Thebaine
AM1-1	0.07	0.01	0.09	4.94
AM1-13	0.05	0.20	0.10	4.46
AM1-28	0.10	0.01	0.08	3.69
AM1-30	0.07	0.02	0.14	6.68
AM1-38	0.05	0.01	0.07	3.20
AM1-82	0.06	0.03	0.13	5.31

Operation

While specific embodiments of the invention have been described and illustrated, such embodiments should be considered illustrative of the invention only and not as limiting the invention as construed in accordance with the accompanying claims.

This description contains a sequence listing in electronic form in ASCII text format. The sequences in the sequence listing are reproduced in the following Table.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated step or element or integer or group of steps or elements or integers but not the exclusion of any other step or element or integer or group of elements or integers.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended claims.

Sequence Table

SEQ ID NO: 1 (T6ODM)

5	Met	Glu	Lys	Ala	Lys	Leu	Met	Lys	Leu	Gly	Asn	Gly	Met	Glu	Ile	Pro
	1				5					10					15	
	Ser	Val	Gln	Glu	Leu	Ala	Lys	Leu	Thr	Leu	Ala	Glu	Ile	Pro	Ser	Arg
			20						25					30		
10	Tyr	Val	Cys	Ala	Asn	Glu	Asn	Leu	Leu	Leu	Pro	Met	Gly	Ala	Ser	Val
			35					40					45			
	Ile	Asn	Asp	His	Glu	Thr	Ile	Pro	Val	Ile	Asp	Ile	Glu	Asn	Leu	Leu
		50					55					60				
15	Ser	Pro	Glu	Pro	Ile	Ile	Gly	Lys	Leu	Glu	Leu	Asp	Arg	Leu	His	Phe
	65					70					75					80
	Ala	Cys	Lys	Glu	Trp	Gly	Phe	Phe	Gln	Val	Val	Asn	His	Gly	Val	Asp
20					85					90						95
	Ala	Ser	Leu	Val	Asp	Ser	Val	Lys	Ser	Glu	Ile	Gln	Gly	Phe	Phe	Asn
				100					105					110		
25	Leu	Ser	Met	Asp	Glu	Lys	Thr	Lys	Tyr	Glu	Gln	Glu	Asp	Gly	Asp	Val
			115					120					125			
	Glu	Gly	Phe	Gly	Gln	Gly	Phe	Ile	Glu	Ser	Glu	Asp	Gln	Thr	Leu	Asp
		130					135					140				
30	Trp	Ala	Asp	Ile	Phe	Met	Met	Phe	Thr	Leu	Pro	Leu	His	Leu	Arg	Lys
	145					150					155					160
	Pro	His	Leu	Phe	Ser	Lys	Leu	Pro	Val	Pro	Leu	Arg	Glu	Thr	Ile	Glu
35					165					170					175	
	Ser	Tyr	Ser	Ser	Glu	Met	Lys	Lys	Leu	Ser	Met	Val	Leu	Phe	Asn	Lys
				180					185					190		
40	Met	Glu	Lys	Ala	Leu	Gln	Val	Gln	Ala	Ala	Glu	Ile	Lys	Gly	Met	Ser
			195					200					205			
	Glu	Val	Phe	Ile	Asp	Gly	Thr	Gln	Ala	Met	Arg	Met	Asn	Tyr	Tyr	Pro
		210					215					220				
45	Pro	Cys	Pro	Gln	Pro	Asn	Leu	Ala	Ile	Gly	Leu	Thr	Ser	His	Ser	Asp
	225					230					235					240
	Phe	Gly	Gly	Leu	Thr	Ile	Leu	Leu	Gln	Ile	Asn	Glu	Val	Glu	Gly	Leu
50					245					250					255	
	Gln	Ile	Lys	Arg	Glu	Gly	Thr	Trp	Ile	Ser	Val	Lys	Pro	Leu	Pro	Asn
				260					265					270		

53

	Ala	Phe	Val	Val	Asn	Val	Gly	Asp	Ile	Leu	Glu	Ile	Met	Thr	Asn	Gly	
			275					280					285				
5	Ile	Tyr	His	Ser	Val	Asp	His	Arg	Ala	Val	Val	Asn	Ser	Thr	Asn	Glu	
		290				295						300					
	Arg	Leu	Ser	Ile	Ala	Thr	Phe	His	Asp	Pro	Ser	Leu	Glu	Ser	Val	Ile	
10	305					310					315					320	
	Gly	Pro	Ile	Ser	Ser	Leu	Ile	Thr	Pro	Glu	Thr	Pro	Ala	Leu	Phe	Lys	
					325					330					335		
	Ser	Gly	Ser	Thr	Tyr	Gly	Asp	Leu	Val	Glu	Glu	Cys	Lys	Thr	Arg	Lys	
15				340					345					350			
	Leu	Asp	Gly	Lys	Ser	Phe	Leu	Asp	Ser	Met	Arg	Ile					
		355						360									
20	SEQ ID NO: 2 (T6ODM)																
	gttccttaatt	cattaatttaa	tttagaaaaa	tcattggagaa	agcaaaaactt	atgaagctag											60
	gtaattggtat	ggaaataacca	agtgttcaag	aattggctaa	actcacgctt	gccgaaattc											120
	catctcgata	cgtatgcgcc	aatgaaaacc	ttttgttgcc	tatgggtgca	tctgtcataa											180
25	atgatcatga	aaccattcct	gtcatcgata	tagaaaattt	attatctcca	gaaccaataa											240
	tcggaaaagt	agaattagat	aggcttcatt	ttgcttgcaa	agaatggggg	ttttttcagg											300
	tagtgaacca	tggagtcgac	gcttcattgg	tggatagtgt	aaaatcagaa	attcaagggt											360
	tctttaacct	ttctatggat	gagaaaacta	aatatgaaca	ggaagatgga	gatgtggaag											420
	gatttggaca	aggctttatt	gaatcagagg	accaaact	tgattgggca	gatataattt											480
30	tgatgttcac	tcttccactc	catttaagga	agcctcactt	atcttcaaaa	ctcccagtg											540
	ctctcaggga	gacaatcgaa	tctactcat	cagaaatgaa	aaagttatcc	atggttctct											600
	ttaataagat	ggaaaaagct	ctacaagtac	aagcagccga	gattaagggt	atgtcagagg											660
	tgtttataga	tgggacacaa	gcaatgagga	tgaactatta	tcccccttgt	cctcaaccaa											720
	atctcgccat	cggctcttac	tgcactcgg	atcttgccgg	tttgacaatc	ctccttcaaa											780
35	tcaacgaagt	ggaaggatta	cagataaaaa	gagaggggac	atggatttca	gtcaaacctc											840
	tacctaattg	gttcgtagtg	aatgttggag	atattttgga	gataatgact	aatggaattt											900
	accatagtgt	cgatcacccg	gcagtagtaa	actcaacaaa	tgagaggctc	tcaatcgcaa											960
	catttcatga	ccctagtcta	gagtcggtaa	taggcccatt	atcaagcttg	attactccag											1020
	agacacctgc	tttgttttaa	agtggatcta	catatgggga	tcttgtggag	gaatgtaaaa											1080
40	caaggaagct	cgatggaaaa	tcatttcttg	actccatgag	gatttgaaaa	ctcaagaaaa											1140
	aataatacga	cgtgattgca	tgtcagattc	aactatcctt	ttgtcgtttt	ttggtgctcg											1200
	agtccttaat	tgttttgatc	attgcttttg	attctaatta	ataagacttt	tctcaagaac											1260
	cacatgtaat	gtacctttac	tttcagaaaa	taaaaagtat	tgaggcacia	atgagaaaat											1320
	tgagagagt	cttgagaagt	gtaattttct	gaaagtgcgt	tgtgtttgaa	aaaaaaaaaa											1380
45	aaaaaa																1386
	SEQ ID NO: 3 (CODM)																
	Met	Glu	Thr	Pro	Ile	Leu	Ile	Lys	Leu	Gly	Asn	Gly	Leu	Ser	Ile	Pro	
50	1				5					10					15		
	Ser	Val	Gln	Glu	Leu	Ala	Lys	Leu	Thr	Leu	Ala	Glu	Ile	Pro	Ser	Arg	
				20					25					30			
55	Tyr	Thr	Cys	Thr	Gly	Glu	Ser	Pro	Leu	Asn	Asn	Ile	Gly	Ala	Ser	Val	

54

			35					40					45					
5	Thr	Asp	Asp	Glu	Thr	Val	Pro	Val	Ile	Asp	Leu	Gln	Asn	Leu	Leu	Ser		
	50						55					60						
	Pro	Glu	Pro	Val	Val	Gly	Lys	Leu	Glu	Leu	Asp	Lys	Leu	His	Ser	Ala		
	65					70					75					80		
10	Cys	Lys	Glu	Trp	Gly	Phe	Phe	Gln	Leu	Val	Asn	His	Gly	Val	Asp	Ala		
					85					90					95			
	Leu	Leu	Met	Asp	Asn	Ile	Lys	Ser	Glu	Ile	Lys	Gly	Phe	Phe	Asn	Leu		
				100					105					110				
15	Pro	Met	Asn	Glu	Lys	Thr	Lys	Tyr	Gly	Gln	Gln	Asp	Gly	Asp	Phe	Glu		
			115					120					125					
	Gly	Phe	Gly	Gln	Pro	Tyr	Ile	Glu	Ser	Glu	Asp	Gln	Arg	Leu	Asp	Trp		
20		130					135					140						
	Thr	Glu	Val	Phe	Ser	Met	Leu	Ser	Leu	Pro	Leu	His	Leu	Arg	Lys	Pro		
	145					150					155					160		
25	His	Leu	Phe	Pro	Glu	Leu	Pro	Leu	Pro	Phe	Arg	Glu	Thr	Leu	Glu	Ser		
					165					170					175			
	Tyr	Leu	Ser	Lys	Met	Lys	Lys	Leu	Ser	Thr	Val	Val	Phe	Glu	Met	Leu		
				180					185					190				
30	Glu	Lys	Ser	Leu	Gln	Leu	Val	Glu	Ile	Lys	Gly	Met	Thr	Asp	Leu	Phe		
			195					200					205					
	Glu	Asp	Gly	Leu	Gln	Thr	Met	Arg	Met	Asn	Tyr	Tyr	Pro	Pro	Cys	Pro		
35		210					215					220						
	Arg	Pro	Glu	Leu	Val	Leu	Gly	Leu	Thr	Ser	His	Ser	Asp	Phe	Ser	Gly		
	225					230					235					240		
40	Leu	Thr	Ile	Leu	Leu	Gln	Leu	Asn	Glu	Val	Glu	Gly	Leu	Gln	Ile	Arg		
					245					250					255			
	Lys	Glu	Glu	Arg	Trp	Ile	Ser	Ile	Lys	Pro	Leu	Pro	Asp	Ala	Phe	Ile		
				260					265					270				
45	Val	Asn	Val	Gly	Asp	Ile	Leu	Glu	Ile	Met	Thr	Asn	Gly	Ile	Tyr	Arg		
			275					280					285					
	Ser	Val	Glu	His	Arg	Ala	Val	Val	Asn	Ser	Thr	Lys	Glu	Arg	Leu	Ser		
50		290					295					300						
	Ile	Ala	Thr	Phe	His	Asp	Ser	Lys	Leu	Glu	Ser	Glu	Ile	Gly	Pro	Ile		
						310					315					320		
	Ser	Ser	Leu	Val	Thr	Pro	Glu	Thr	Pro	Ala	Leu	Phe	Lys	Arg	Gly	Arg		
55						325					330					335		

Tyr Glu Asp Ile Leu Lys Glu Asn Leu Ser Arg Lys Leu Asp Gly Lys
 340 345 350

5 Ser Phe Leu Asp Tyr Met Arg Met
 355 360

SEQ ID NO: 4 (CODM)

10 gtaaagattg atatatgato tgaagatctg acaagaaagt tcatcaaata tagagttcat 60
 ggagacacca ataacttatca agctaggcaa tggtttgtca ataccaagtg ttcaggaatt 120
 ggctaaactc acgcttgcag aaattccatc tcgatacaca tgcaccggtg aaagcccgtt 180
 gaataatatt ggtgcgtctg taacagatga tgaacagtt cctgtcatcg atttgcaaaa 240
 15 ttactatct ccagaaccg tagttggaaa gttagaattg gataagcttc attctgcttg 300
 caaagaatgg ggtttctttc agctgggtta ccattggagtc gacgctttac tgatggacaa 360
 tataaaatca gaaattaaag gtttctttta ccttccaatg aatgagaaaa ctaatacgg 420
 acagcaagat ggagattttg aaggatttg acaaccctat attgaatcgg aggaccaaag 480
 acttgattgg actgaagtgt ttagcatgtt aagtcttctt cctcatttaa ggaagcctca 540
 20 ttgttttcca gaactccctc tgcctttcag ggagacactg gaatcctacc tatcaaaaat 600
 gaaaaaacta tcaacgggtg tctttgagat gttggaaaaa tctctacaat tagttgagat 660
 taaaggatg acagacttat ttgaagatgg gttgcaaaca atgaggatga actattatcc 720
 tccttgctct cgaccagagc ttgtacttgg tcttacgtca cactcggatt ttagcggttt 780
 gacaattctc ctccaactta atgaagttga aggattacaa ataagaaaag aagagaggtg 840
 25 gatttcaatc aaacctctac ctgatgcgtt catagtgaat gttggagaca ttttggagat 900
 aatgactaat gggattttacc gtagegtcga gcaccgggca gtagtaaaact caacaaagga 960
 gaggtctctc atcgcgacat ttcatgactc taaactagag tcagaaatag gcccaatttc 1020
 gagcttggtc acaccagaga cacctgcttt gttcaaaaaga ggtaggtatg aggatatttt 1080
 gaaggaaaat ctttcaagga agcttgatgg aaaatcattt ctcgactaca tgaggatgtg 1140
 30 agaaagtgtg aacatatatt ataactccaca ttgtgtttta tatatgatga aataagttgc 1200
 ttttgaagta tgatgaaata agttgggttt gaagaattca tattgtgctt aaatttcgtg 1260
 gatgactgag agatttatta tgtaataata atgtattggg ttgaagattc tcgtctcact 1320
 atatgtaaga ctctgttttg gtcaagtgat gtaatcacgg ttgaaataag ttgcttttga 1380
 agaattcata tgggtgctta tattatgtaa taaataatgt attggattga aaaaaaaaaa 1440
 35 aaaaaaaaaa aa 1452

SEQ ID NO: 5

tcctgtggttggcatgcacatacaaatggacgaacggataaaccttttcacgccccttttaaatatccgattattct
 40 aataaacgctcttttctcttaggtttaccgcgaatatatcctgtcaaactgatagtttaaaactgaaggcggga
 aacgacaatctgctagtggtatctccagtcacgacgttgtaaaacgggccccgcggaaagcttgctagccaatt
 ggggcccacgttctcgagaacgtggataacttggcagtggttacttggcttttcttttattttcttttggacggaa
 gcggtggttactttgtcacacatttaaaaaaacacgtgtttctcacttttttctattcccgtcacaaaacaatttta
 agaaagatccatctatcgtgatctttctatcaacaaaaagaaaaaggtcttcatagtaacgctacaacatcaaat
 atgtggttgctctgacatcagtcgggaaaaataaggatatggcggcattggccacatctattgggggtcccaacttcc
 45 ttccacaaaaaaattaaattgggtgtcccaacttttatctttgatatagtgacatgagtatcgggagcattggaca
 atggataaaatgagaactaaaaaaattctgggttaatttttgatcattgttatttaaaaggttattttatctataat
 ctaccatattgatcagttttattttaatttggtttagctaccgctccacgagagagatcctcatcttaaaaatgga
 atattggaattacacacgacccccaaaagtataatttttctctggagaatgctatttagagctttgactatatggtc
 tgaattagaagacggggaataaaatctgctaagtgtataagctctaagtaggcgatgtgtgatggagaacacct
 50 ttcttttaacagctcttcatgtttttacagattcgcgaacttcgaatatccctatacgggtctgtctaaccctcgtgtg
 tcttttgagtcgaagataaaaggccattatttgagtaacatagacatgctggaatccaaccattgaagtcacaactgt
 ccatgtagattctttggagaatctgaaaagtttaataaaaggtgggtgttcaaagaaaaacaaaacaaatgagttaa
 gaaaaaaaaatatcatgtagtggtcgagtattatgttattttattgtgtagctaccaatctttattcttttaaatctg

acataaaatgctacaaaactttttacctcgtctatagccccaaaaaacctaaccacggttctaaaaaccacacacagt
 gatttttggttgacgacaatgcctctccttcccaaacgattttattttacatttttttaaatcaaatgttacattttta
 taccataaattaagtctttttacagaatacttagatggaagagatgtataaaaaaggaggaaattgtaaaaacata
 5 tttcgatcaattaaaccaggattcataaaaaatataagtatatatataaatgatgtttcggttagcgatgaacttca
 ctcatatgataataacttaacaatataagtacataaaaaataaaaataaaatttattgtttacgaaaagtctacaaat
 actgcatgtataaattaatgttctctttattttattttattttaccttaccaagatatatctataaaccgcatagaaat
 agaaggcgaagagataatttccaaaaacaagaaaaacctctaagctcaaaagtctagaaggccttggtccacca
 10 tggaggttggtcacagtatcacttgtagcagttggtgatcactactttcttatacttaactcttcagagattcaagtcc
 taaagggttgccaccagggtccaaaacctggccaatagttggaaaccttcttcaacttggtgagaaacctcattct
 cagtttgctcagcttgctgaaacctatggtgatctcttttactgaaactaggaagtgaacggttggtgtagctt
 caactccattagcagctagcgagattctaaagacgcgatgatcggtgtctctctggtcgatacgtgtttcaagttt
 cggggtaaaggaacatgtggagaactctattgtgtggtctgaatgtaatgaaacatggaagaaactgcggaaagt
 tgtagaacggaactttttacgcagaagatgattgaaagtcaagctgaagttagagaaagttaaggctatggaaatgg
 15 tggagtatttgaagaaaaatgtaggaaatgaagtgaatgtgctgaagttgtatttgggacggttggtgaatatatt
 cggttaacttgatattttcacaaaatattttcaagttgggtgatgaaagtgtggaagtgtagaaatgaaagaacat
 ctatggagaatgctggaattggggaactcgacaaatccagctgattattttccatttttgggtaaatctgatttgt
 ttggacaaagaaaagatgttgctgattgtctgcaagggtttatagtgtttggggtgctatgctcaaagaaagaaa
 aatagccaagcagcataacaacagcaagaagaatgatttgggtgagattttgctcgattccggactcgatgaccag
 20 cagattaatgccttgctcatggaaatatttgggtgcgggaacagagacaaagtgcactacaatagaatgggcggttgt
 ctgagctcacaaaaaacctcaagtaacagccaatatgcggttggaaattgttatctgtggtagggaagaggccggt
 taaggaatccgacataccaaaacatgccttatcttcaagcttttgtaaagaaactctacggttcatccagcaact
 cctctgctgcttccacgtcgagcacttgagacctgcaaagttttgaaactatacgatcccgaaagagtgtcagatta
 tgggtgaacgcctggggcattgggtcgggatccaaaaaggtggactgatccattgaagttttcaccagagaggttctt
 25 gaattcgagcatttgatttcaaaggggaacgacttcgagttgataccatttgggtgcaggggagaaggatgtcctggt
 gtgcccttggcactcaatttatttagtcttatttgctgactagtttgggtacagaattttgattggggattaccgaagg
 gaattggtccttagccaactgatcatggaagagaatttgggtgacactgcaaaaaggaacctctgtatatattgtt
 cctaaaaactcgggattaaggggagaattcgtcgactttgcccgcgcacatcgatactgcaggagctcggtaccttttac
 tagtgatatccctgtgtgaaattgttatccgctacgcgtgatcggttcaaacatttggcaataaagtttcttaagat
 30 tgaatcctgttgccggtcttgogatgattatcatataatttctgttgaaattacgttaagcatgtaataattaacat
 gtaatgcatgacgttattttatgagatgggtttttatgattagagtcccgcattatacatttaatacgcgatagaa
 aacaaaatatagcgcgcaaaactaggataaattatcgcgcgcggtgtcatctatgttactagatcccattgggaagt
 cctattccgaagttcctattctctgaaaagtataggaacttcagcgatcgctccaatcccacaaaaatctgagctt
 aacagcacagttgctcctctcagagcagaatcgggtattcaacacctcatatcaactactacgttgtgtataacg
 35 gtccacatgccggtatatacgatgactgggggtgtacaaaggcggaacaaacggcggttcccggagttgcacacaa
 gaaatttggcactattacagaggcaagagcagcagctgacgcgtacacaacaagtgcagcaaacagacaggttgaac
 ttcatccccaaaggagaagctcaactcaagcccaagagctttgctaaggccctaacaagcccaccaaagcaaaaag
 cccactggctcacgctaggaaccaaaggccagcagtgatccagccccaaaagagactcctttgccccggagatt
 acaatggacgatttctctatctttacgatctaggaagggaagttcgaagggtgaagggtgacgcacactatgttcacca
 40 ctgataatgagaagggttagcctcttcaattttcagaaagaatgctgacccacagatgggttagagaggcctacgcagc
 aggtctcatcaagacgatctaccgagtaacaatctccaggagatcaaataccttcccagaaggttaaatgca
 gtcaaaagattcaggactaattgcatcaagaacacagagaaagacatatcttcaagatcagaagttactattccag
 tatggacgattcaggcttgcttcataaacaaggcaagtaataagagattggagttcttaaaaaggtagttcctac
 tgaatctaaggccatgcatggagtctaagattcaaatcgaggatctaacagaactcgccgtgaagactggcgaaca
 45 gttcatacagagtttttacgactcaatgacaagaagaaaatcttcgtcaacatgggtggagcacgacactctggtc
 tactccaaaaatgtcaagatacagttctcagaagaccaaagggtctattgagacttttcaacaaaggataatttcgg
 gaaacctcctcggtattccattgccagctatctgtcacttcatcgaaaggacagtagaaaaagggaaggtggctccta
 caaatgccatcattgogataaaggaaagggtatcattcaagatctctctgccgacagtggtcccaaagatggaccc
 ccacccacgaggagcatcgtggaaaaagaagacgttccaaccacgtcttcaaagcaagtggttgatgtgacatct
 50 ccactgacgtaagggtgacgcacaatcccactatccttcgaagacccttctctatataagggaagttcattttca
 tttggagaggacacgctcgagtataagagctctatttttacaacaattaccaacaacaacaacaacaacaacat
 tacaattacattttacaattaccatggggcgcccccttgtcctcaaccaaactctcgccatcggtcttacgtcgac
 tcggatttttggcggtttgacaatcctccttcaaatcaacgaagtgggaaggattacagataaaaagagaggggacat
 ggatttcagtcaaacctctacctaattgcgttcgtagtgaatgttggagatattttggagataatgactaatggaat
 55 ttaccatagtgtcgatcaccgggcagtagtaaaactcaacaaatgagaggctctcaatcgcaacatttcatgacct
 agtctagagtcggtaatataggcccaatatcaagcttgattactccagagacacctgctttgtttaaaagtggaaattt

5
10
15
20
25
30
35
40
45

aaatccccagatgaacatggcatcgtgggtgattgatgaaactgctgctgctcggttttaacctctcttttaggcattg
gtttcgaagcgggcaacaagccgaaagaactgtacagcgaagaggcagtcacacggggaaactcagcaagcgcactt
acaggcgattaaagagctgatagcgcgtgacaaaaaccaccaagcgtgggtgatgtggagtattgccaacgaaccg
gatacccggtccgcaaggtgcacgggaatatttcgcgccactggcggaagcaacgcgtaaactcgacccgacgcgtc
cgatcacctgcgtcaatgtaatgttctgcgacgctcacaccgataccatcagcgatctctttgatggggatcctcc
acttttaaacaaagcaggtgtctctggagtaatcaagcttgatattgggcctattaccgactctagactagggtca
tgaaatgttgcgattgagagcctctcatttgttgagtttactactgcccgggtgatcgacactatggtaaattccat
tagtcattatctccaaaatatctccaacattcactacgaacgcattaggtagaggtttgactgaaatccatgtccc
ctctctttttatctgtaatccttccacttcgttgatttgaaggaggattgtcaaacgccaaaatccgagtgcgac
gtaagaccgatggcgagatttgggttgaggacaaggactagtccttagagtcctgctttaatgagatatgcgagacg
cctatgatcgcatgatatttgccttcaattctgttggtgcacgttgtaaaaaacctgagcatgtgtagctcagatcc
ttaccgcgggtttcggttcattctaatgaatgaatatatcacccgttactatcgatttttatgaataatattctc
cgttcaatttactgattgtaccctactacttatatgtacaatattaaaatgaaaacaatatattgtgctgaatagg
tttatagcgacatctatgatagagcgccacaataacaaacaattgcgtttttattattacaaaatccaattttaaaaa
aagcgggcagaaccgggtcaaacctaaaagactgattacataaatcttattcaaatttcaaaagtgcgccaggggcta
gtatctacgacacaccgagcggcggaactaataacgctcactgaagggaactccggttccccgcggcgcgcatggg
tgagattccttgaagttgagttattggcgcgtccgctctaccgaaagttacgggcaccattcaaccgggtccagcacg
gcgccggggtaacgcacttgcgtgccccgagaattatgcagcatttttttggtgatgtgggccccaaatgaagtgc
aggtcaaaccttgacagtgacgacaaatcggttggggcggtccaggggcgaattttgcgacaacatgtcgaggctcag
caggggcgatcgcgagcgtcgggatctcttgcgaagcatctctatttccctgaaggtctaacctcgaagatttaagatt
taattacgtttataattacaaaattgattctagtatctttaatttaattgcttatacattattaattaatttagtac
tttcaatttgttttcagaaattattttactattttttataaaaataaaaggagaaaaatggctattttaataactagc
ctattttattttcaatttttagcttaaaatcagcccccaatttagcccccaatttcaaattcaaattgggtccagccccattc
ctaaaataacccacccctaacccgcgggtttcccttttgatccatgcagtcacacgcccagaatttccctatataa
tttttaattccccaaacccctaacctatccccattctcaccaaccgccacatagatctatctcttattctctc
aaactctctcgaaaccttccccctaaccttagcagcctctcatcctcactcactcaaaacccacggggcgccgatg
attgaacaagatggattgcacgcaggttctccggcgcgttgggtggagaggctattcggtatgactgggcacac
agacaatcggtgctctgatgcgcgcgtgttccggctgtcagcgcaggggaggccgggttctttttgtcaagaccga
cctgtccgggtgcccgaatgaacttcaagacgaggcagcgcggctatcggtggctggccacgacgggcttccctgc
gcagctgtgctcgacgttgtcactgaagcgggaagggaactggctgctattgggcgaagtgcgggggagggatctcc
tgtcatctcaccttgcctcctgcgagaaagtatccatcatggctgatgcaatgcggcggtgcatacgttgatcc
ggctacctgcccattcgaccaccaagcgaaacatcgcatcgagcagcagcgtactcggtggaagccgggtcttgctc
gatcaggatgatctggacgaagagcatcaggggctcgcgccagccgaactgttcgccaaggctcaaggcgcgcgatgc
ccgacggcgaggatctcgtcgtgactcatggcgatgcttgcgaatatcatgggtggaaaatggccgcttttc
tggattcatcgactgtggcgggtgggtgtggcggaaccgctatcaggacatagcgttgggtacccgtgatattgct
gaagagcttggcgggcgaatgggtgacgcgttccctcgtgctttacgggtatcgccgctcccgatctcgacgcgatcg
ccttctatcgcttcttgacgagttcttctgagggcgcgcgatcggttcaaactttggcaataaagtttcttaaga
ttgaatcctgttgccgggtcttgcatgattatcatataatttctgttgattacgttaagcatgtaataattaaca
tgtaatgcatgacgttatttatgagatgggtttttatgatttagagtcgccgaattatacatttaatacgcgataga
aaacaaaatatagcgcgcaaaactaggataaattatcgcgcgcggtgtcatctatgttactagatccctagggaagt
tccatttccgaagttcctattctctgaaaagtataggaaacttcttgcgtattgggcgctcttggcctttttggcc
accggctcgtagcgtttaaaccacccagctacattaaaacgctccgcaatgtgttattaagttgtctaagcgtcaat
ttgtttacaccacaatatatcctgccaccagccagccaacagctccccgaccggcagctcggcacaaaatcaccac
tcgatacaggcagcccatcagtc

SEQ ID NO: 6

50

gcgatcgctccaatcccacaaaaatctgagcttaacagcacagttgctcctctcagagcagaatcgggtattcaac
acctcatatcaactactacgttgtgtataacgggtccacatgccggtatatacgatgactggggtgtacaaaggc
ggcaacaaacggcggttcccggagttgcacacaagaaatttgccactattacagaggcaagagcagcagctgacgcg
tacacaacaagtacgcaaacagacaggttgaacttcatccccaaaggagaagctcaactcaagcccaagagcttgc
ctaaggccctaacaagcccaccaaagcaaaaagcccactggctcacgctaggaaccaaaggccagcagtgatcc
agccccaaaagagactcctttgccccggagattacaatggacgatttccctctatctttacgatctaggaaggaagt
tcgaaggtgaaggtgacgacactatgttcaccactgataatgagaaggttagcctcttcaatttcagaaagaatgc

5 tgacccacagatgggttagagaggcctacgcagcaggtctcatcaagacgatctacccgagtaacaatctccaggag
 atcaaataccttcccaagaagggttaaagatgcagtcacaaagattcaggactaattgcatcaagaacacagagaaag
 acatattttctcaagatcagaagtaactattccagtatggacgattcaaggcttgcttcataaaccaggcaagtaat
 10 agagattggagttctctaaaaaggtagttcctactgaatctaaggccatgcatggagttcaagattcaaatcgagga
 tctaacagaactcgccgtgaagactggcgaacagttcatacagagttctttacgactcaatgacaagaagaaaatc
 ttctgtaacatgggtggagcacgacactctgggtctactccaaaaatgtcaaagatacagttctcagaagaccaaagg
 ctattgagacttttcaacaaaggataaatttcgggaaacctcctcggattccattgcccagctatctgtcacttcat
 15 cgaaaggacagtagaaaagggaagggtgggtcctacaaatgccatcattgcgataaaggaaaggctatcattcaagat
 ctctctgccgacagtggtcccaagatggacccccacccacgaggagcatcgtggaaaaagaagacgttccaacca
 cgtcttcaaagcaagtggattgatgtgacatctccactgacgtaagggatgacgcacaatccactatccttcgca
 agacccttctctatataaggaagttcatttcatttggagaggacacgctcgagtataagagctctatttttaca
 caattaccaacaacaacaacaacaacaacattacaattacatttacaattaccatggggcgcgcccttgtcct
 caaccaaattctcgccatcggtcttacgtcgcaactcggtatttggcggttgacaatcctccttcaaatacaacgaag
 20 tgggaaggattacagataaaaaagagaggggacatggatttcagtcacaaacctctacctaattgcgttcgtagtgaatgt
 tggagataatttggagataatgactaatggaatttaccatagtgatcgatcaccgggcagtagtaaaactcaacaaat
 gagaggctctcaatcgcaacatttcatgacctagtcagagtcggtaaataggcccaatatcaagcttgattactc
 cagagacacctgctttgtttaaaagtggaaatttaaatccccagatgaacatggcatcgtgggtgattgatgaaactg
 ctgctgtcggttttaacctctcttttaggcattgggttccgaagcgggcaacaagccgaaagaactgtacagcgaaga
 25 ggcagtcacacgggaaactcagcaagcgcacttacaggcgattaaagagctgatagcgcgtgacaaaaaccacca
 agcgtgggtgatgtggagatttgccaaacgaaccggataaccgctccgcaagggtgcacgggaatatctcgccactgg
 cggaaagcaacgcgtaaactcgacccgacgcgtccgatcacctgcgtcaatgtaatgttctgcgacgctcacaccga
 taccatcagcgatctctttgatggggatcctccacttttaacaaagcagggtgtctctggagtaatacaagcttgat
 attgggcttattaccgactctagactagggtcatgaaatgttgcgattgagagcctctcatttgttgagttacta
 30 ctgcccgggtgatcgacactatggtaaatccattagtcattatctccaaaatatctccaacattcactacgaacgc
 attaggtagaggtttgactgaaatccatgtccccctctcttttatctgtaatccttccacttcgttgattgaagg
 aggtattgtcaaacgcgcaaaatccgagtcgcagcgtgaagccgatggcgagatttggttgaggacaaggactagtc
 cttagagtcctgcttttaattgagatatgagagacgcctatgatcgcatgatatttgctttcaattctgttgacgt
 tgtaaaaaacctgagcatgtgtagctcagatccttacgcgcggtttcggttcatttcaatgaatgaatatatcacc
 35 cgttactatcgtatttttatgaataatattctcgttcaatttactgattgtaccctactacttatatgtacaata
 ttaaaatgaaaacaatatattgtgtgtaatagggttatagcgacatctatgatagagcgccacaataacaaacaat
 tgcgtttttattattacaaatccaatttttaaaaaagcggcagaaccggtcaaacctaaaagactgattacataaat
 cttatttcaattttcaaaagtgcgccaggggctagtatctacgacacaccgagcggcgaactaataacgctcactga
 agggaactccggttccccgcggcgcgcatgggtgagattccttgaagttgagttattggccgctccgctctaccgaa
 40 agttacgggcaccattcaaccgggtccagcacggcgggcgggtgaaccgacttgctgccccgagaattatgcagcat
 ttttttggtgtatgtgggccccaaatgaagtgcaggtcaaaccttgacagtgacgacaaatcgttgggcgggtcca
 gggcgaattttgcgacaacatgtcgaggtcagcagggcgatcgca

SEQ ID NO: 7

40 ccccttgctcctcaaccaaattctcgccatcggtcttacgtcgcaactcggtatttggcggttgacaatcctccttca
 aatcaacgaagtgggaaggattacagataaaaaagagaggggacatggatttcagtcacaaacctctacctaattgcgttc
 gtatgtaattgtggagataatttggagataatgactaatggaatttaccatagtgatcgatcaccgggcagtagtaa
 actcaacaaatgagaggtctcattcgcaacatttcatgacctagtcagagtcggtaataaggcccaatatcaag
 45 cttgattactccagagacacctgctttgtttaaaagtggaaatttaaatccccagatgaacatggcatcgtgggtgat
 tgatgaaactgctgctgctcggttttaacctctcttttaggcattgggttccgaagcgggcaacaagccgaaagaactg
 tacagcgaagaggcagtcacacgggaaactcagcaagcgcacttacaggcgattaaagagctgatagcgcgtgaca
 aaaaccacccaagcgtgggtgatgtggagatttgccaaacgaaccggataaccgctccgcaagggtgcacgggaatat
 50 cgcgccactggcggaagcaacgcgtaaactcgacccgacgcgtccgatcacctgcgtcaatgtaatgttctgcgac
 gctcacaccgataccatcagcgatctctttgatggggatcctccacttttaacaaagcagggtgtctctggagtaa
 tcaagcttgatattgggcttattaccgactctagactagggtcatgaaatgttgcgattgagagcctctcatttgt
 tgagtttactactgcccgggtgatcgacactatggtaaatccattagtcattatctccaaaatatctccaacattc
 actacgaacgcattaggtagaggtttgactgaaatccatgtccccctctcttttatctgtaatccttccacttcgt
 tgatttgaaggaggattgtcaaacgcgcaaaatccgagtcgcagcgtgaagaccgatggcgagatttggttgaggaca
 agg

SEQ ID NO: 8

5 ccttgctcctcaaccaaattctcgccatcggtcttacgtcgcaactcggatTTTTggcggtttgacaatcctccttcaaa
tcaacgaagtggaaggattacagataaaaaagagaggggacatggatttcagtcaaacctctacctaatacggttcgt
agtgaatggttgagatattttggagataatgactaatggaatttaccatagtgtcgatcaccgggcagtagtaaac
tcaacaaatgagaggctctcaatcgcaacatttcatgaccctagtctagagtcggtaataggcccaatatcaagct
tgattactccagagacacctgctttgtttaaaagtgga

SEQ ID NO: 9

10 TAACCGACTTGCTGCCCCGA

SEQ ID NO: 10

AAATAGAGATGCTTGCAGAAGATCCCG

15 SEQ ID NO: 11

CGTTTGAATCTTGCTGGCCGTGAT

SEQ ID NO: 12

TAGACGAGCTGCCTTTGGAAGTGT

20

SEQ ID NO: 13:

CGTCTTGCGCACTGATTTGAA

SEQ ID NO 14:

25 CGTTTGAATCTTGCTGGCCGTGAT

SEQ ID NO: 15 (T6ODM)

30	guucuaauu	cauuauuuua	uuuagaaaaa	ucauggagaa	agcaaaacuu	augaagcuag	60
	guaaugguau	ggaaaauacca	aguguucaag	aaauuggcuua	acucacgcuu	gccgaaaauuc	120
	caucucgaua	cguaugcgcc	aaugaaaacc	uuuuguugcc	uaugggugca	ucugucauaa	180
	augaucauga	aaccuuuccu	gucaucgaua	uagaaaauuu	auuaucucca	gaaccaauaa	240
	ucggaaaguu	agaauuagau	aggcuucauu	uugcuugcaa	agaauggggu	uuuuuucagg	300
	uagugaacca	uggagucgac	gcuucauugg	uggauagugu	aaaaucagaa	auucaagguu	360
	ucuuuaaccu	uucuauggau	gagaaaacua	aaauaugaaca	ggaagaugga	gauguggaag	420
35	gauuuggaca	aggcuuuauu	gaaucaagagg	accaaacacu	ugauugggca	gauauauuuu	480
	ugauguucac	ucuuccacuc	cauuuaagga	agccucacuu	auuuucaaaa	cucccagugc	540
	cucucagggg	gacaaucgaa	uccuacucau	cagaaaugaa	aaaguuaucc	augguucucu	600
	uuauuaagau	ggaaaaagcu	cuacaaguac	aagcagccga	gauuaagggu	augucagagg	660
	uguuuauaga	ugggacacaa	gcaaugagga	ugaacuauua	ucccccuugu	ccucaaccaa	720
40	aucucgccau	cggucuuacg	ucgcacucgg	auuuuggcgg	uuugacaauc	cuccuucaaa	780
	ucaacgaagu	ggaaggauua	cagauaaaaa	gagaggggac	auggauuua	gucaaaccuc	840
	uaccuaaugc	guucguagug	aauguuggag	auauuuugga	gauaaugacu	aauggaauuu	900
	accuauagugu	cgauaccggg	gcaguaguua	acucaacaaa	ugagaggcuc	ucaaucgcaa	960
	cauuucauga	cccuagucua	gagucgguaa	uaggcccaau	aucaagcuug	auuacuccag	1020

60

	agacaccugc	uuuguuuuaa	aguggaucua	cauauagggga	ucuuguggag	gaauguaaaa	1080
	caaggaagcu	cgauaggaaa	ucauuucuu	acuccaugag	gauuugaaaa	cucaagaaaa	1140
	aaauaauacga	cgugauugca	ugucagauuc	aacuaucuu	uugucguuuu	uuggugcucg	1200
5	aguccuuauu	uguuuugauc	auugcuuuug	auucuaauua	auaagacuuu	ucucaagaac	1260
	cacauguaau	guaccuuuac	uuucagaaaa	uaaaaaguau	ugaggcacaa	augagaaaau	1320
	ugagagagug	cuugagaagu	guaauuucuc	gaaagugcgu	uguguuugaa	aaaaaaaaaa	1380
	aaaaaa						1386
10	SEQ ID NO: 16 (CODM)						
	guaaagauug	auauaugauc	ugaagaucug	acaagaaagu	ucaucaaaua	uagaguucan	60
	ggagacacca	auacuuauca	agcuaggcaa	ugguuuguca	auaccaagug	uucaggaaau	120
	ggcuaaaacuc	acgcuugcag	aaauuccauc	ucgauacaca	ugcaccggug	aaagcccguu	180
	gaauaauauu	ggugcgucug	uaacagauga	ugaaacaguu	ccugucaucg	auuugcaaaa	240
15	uuuacuauc	ccagaacccg	uaguuggaaa	guuagaauug	gauaagcuuc	auucugcuug	300
	caaagaauug	gguuucuuuc	agcugguuaa	ccauggaguc	gacgcuuuac	ugauggacaa	360
	uauaaaauca	gaaauuaaag	guuucuuuaa	ccuuccaaug	aaugagaaaa	cuaaaauacgg	420
	acagcaagau	ggagauuuug	aaggauuuug	acaaccuuau	auugaaucgg	aggaccaaaag	480
20	acuugauug	acugaagugu	uuagcauguu	aagucuuccu	cuccauuuua	ggaagccuca	540
	uuuguuucca	gaacucccuc	ugccuuucag	ggagacacug	gaauccuacc	uaucaaaaau	600
	gaaaaaacua	ucaacggguug	ucuuugagau	guuggaaaaa	ucucuacaau	uaguugagau	660
	uaaagguaug	acagacuauu	uugaagaugg	guugcaaaca	augaggaua	acuaauuaucc	720
	uccuuguccu	cgaccagagc	uuguacuugg	ucuuacguca	cacucggauu	uuagcgguuu	780
25	gacaaucuc	cuucaacuua	augaaguuga	aggauuacaa	auaagaaaag	aagagaggug	840
	gauuucaau	aaaccucuc	cugaugcggu	cauagugaau	guuggagaca	uuuuggagau	900
	aaugacuauu	gggaauuacc	guagcgucga	gcaccgggca	guaguaaacu	caacaaagga	960
	gaggcucuca	aucgcgacau	uucaugacuc	uaaacuagag	ucagaaauag	gccccauuuc	1020
	gagcuugguc	acaccagaga	caccugcuuu	guucaaaaaga	gguagguaug	aggauuuuuu	1080
	gaaggaaaau	cuuucagga	agcuugaugg	aaaaucauuu	cucgacuaca	ugaggauug	1140
30	agaaagugug	aacauauauu	auacuccaca	uuguguuuua	uauaugauga	aaauaaguugc	1200
	uuuugaagua	ugaugaaaua	aguugguuuu	gaagaauuca	uauugugcuu	aaauuucgug	1260
	gaugacugag	agaauuaaua	uguaauaaua	auguaauuggu	uugaagauuc	ucgucucacu	1320
	auauguaaga	cucuguuugg	gucaagugau	guaaucacgg	uugaaauaag	uugcuuuuga	1380
35	agaaaucaua	uggugcuuaa	uauuauguaa	uaauuaaugu	auuggauuga	aaaaaaaaaa	1440
	aaaaaaaaaa	aa					1452

What is claimed is:

1. A method of increasing accumulation of thebaine in an opium poppy plant, the method comprising genetically modifying the genome of the plant to include a nucleic acid molecule encoding a hairpin RNA for simultaneously reducing the activity of thebaine 6-O-demethylase (T6ODM) and codeine 3-O-demethylase (CODM) in the poppy plant, wherein the nucleic acid molecule comprises SEQ ID NO: 7.
2. The method of claim 1, wherein T6ODM has the amino acid sequence of SEQ ID NO: 1.
3. The method of claim 1 or 2, wherein CODM has the amino acid sequence of SEQ ID NO: 3.
4. The method of claim 1, 2, or 3, wherein simultaneously reducing the activity of T6ODM and CODM comprises reducing the accumulation of transcripts from an endogenous gene encoding T6ODM and an endogenous gene encoding CODM.
5. A method for producing an opium poppy plant with increased thebaine content, the method comprising:
 - (a) decreasing the expression of an endogenous gene encoding an endogenous thebaine 6-O-demethylase (T6ODM) in the plant; and
 - (b) decreasing the expression of an endogenous gene encoding codeine 3-O-demethylase (CODM) in the plant,

wherein decreasing the expression of the endogenous gene encoding T6ODM and CODM comprises genetically modifying the plant to include a nucleic acid molecule

encoding a hairpin RNA for simultaneously reducing the activity of T6ODM and CODM in the poppy plant, wherein the nucleic acid molecule comprises SEQ ID NO: 7

6. The method of claim 5, wherein the T6ODM has an amino acid sequence at least 95% identical to SEQ ID NO: 1 and the CODM has an amino acid sequence at least 95% identical to SEQ ID NO: 3.

7. A genetically modified opium poppy plant cell produced by a method as defined in claim 5 or 6.

8. A genetically modified opium poppy plant cell having reduced activity of thebaine 6-O-demethylase (T6ODM) and codeine 3-O-demethylase (CODM) relative to a wild type plant cell, wherein the genetically modified opium poppy plant cell comprises a nucleic acid molecule encoding a hairpin RNA for reducing the expression of an endogenous gene encoding T6ODM and an endogenous gene encoding CODM, wherein the nucleic acid molecule comprises SEQ ID NO: 7.

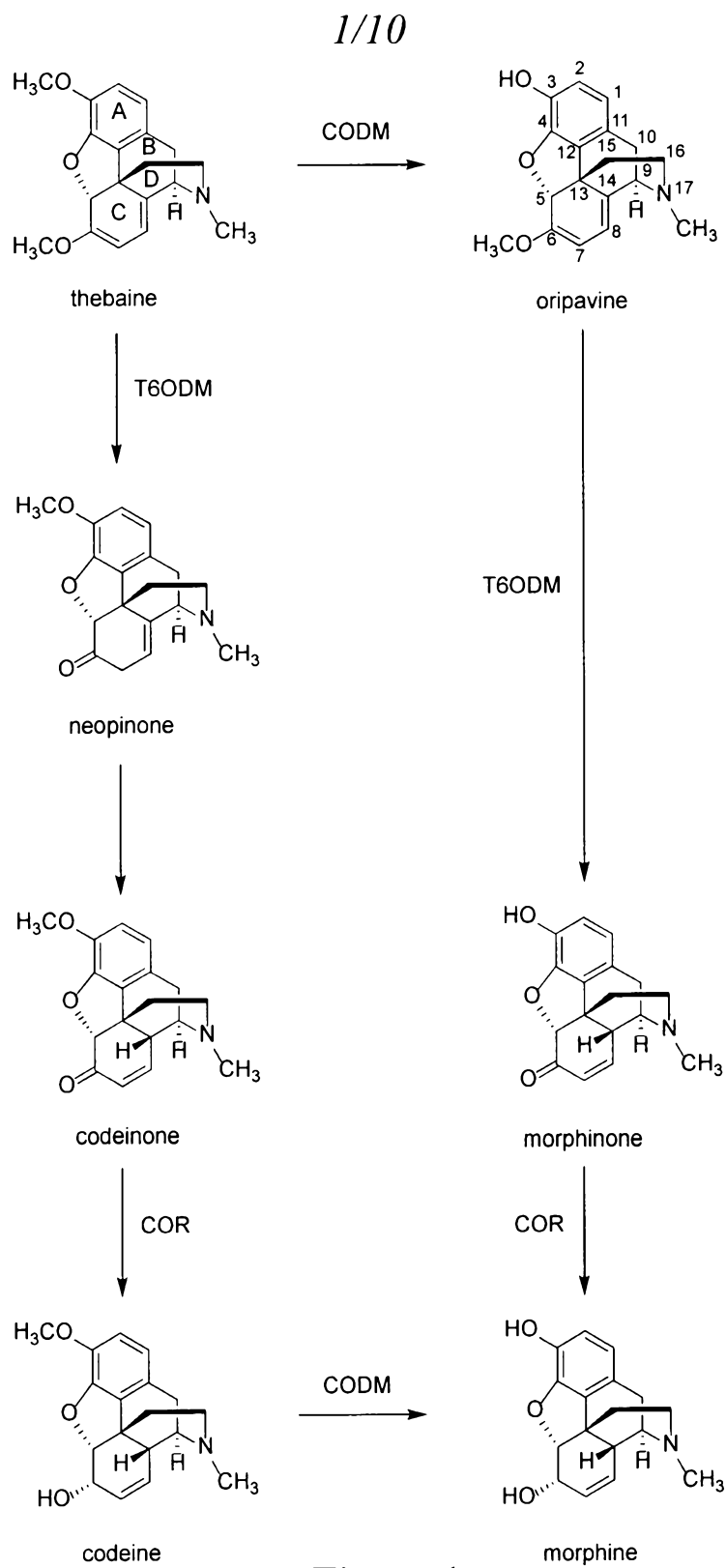
9. The genetically modified opium poppy plant cell of claim 8, wherein T6ODM has the amino acid sequence of SEQ ID NO: 1.

10. The genetically modified opium poppy plant cell of claim 8 or 9, wherein CODM has the amino acid sequence of SEQ ID NO: 3.

11. A method of producing thebaine, said method comprising isolating thebaine from latex or poppy straw harvested from the plant cell as defined in any one of claims 7 to 10.

12. An isolated nucleic acid molecule comprising a heterologous promoter operably linked to polynucleotide comprising the sequence of SEQ ID NO:7.

13. An expression vector for simultaneously reducing the expression of endogenous genes encoding thebaine 6-O-demethylase (T6ODM) and codeine 3-O-demethylase (CODM) in an opium poppy plant, the expression vector comprising an isolated nucleic acid as defined in claim 12.
14. Use of a polynucleotide molecule having a sequence comprising SEQ ID NO: 7 for generating a stable genetic modification in an opium poppy plant to simultaneously reduce the expression of endogenous genes encoding thebaine 6-O-demethylase (T6ODM) and codeine 3-O-demethylase (CODM) in the opium poppy plant.
15. Use of a plant comprising a genetically modified opium poppy plant cell as defined in any one of claims 7 to 10 for the production of thebaine.
16. Poppy straw from a plant comprising a plant cell as defined in any one of claims 7 to 10.
17. Latex from a plant cell as defined in any one of claims 7 to 10.

**Figure 1**

		1	3/10	50
CODM GQ500141	(1)	ATGGAGACACCAATACTTATCAAGCTAGGCAATGGTTTGTCAATACCAAG		
T6ODM GQ500139	(1)	ATGGAGAAAGCAAACTTATGAAGCTAGGTAATGGTATGGAAATACCAAG		
		51		100
CODM GQ500141	(51)	TGTTCAAGGAATTGGCTAAACTCACGCTTGCAGAAATCCATCTCGATACA		
T6ODM GQ500139	(51)	TGTTCAAGAATTGGCTAAACTCACGCTTGCCGAAATCCATCTCGATACG		
		101		150
CODM GQ500141	(101)	CATGCACCGGTGAAAGCCCGTTGAATAATATTGGTGCGTCTGTAACAGAT		
T6ODM GQ500139	(101)	TATGCGCCAATGAAAACCTTTTGTTCCTATGGGTGCATCTGTCATAAAT		
		151		200
CODM GQ500141	(151)	GAT---GAAACAGTTCCTGTCATCGATTGCAAATTTACTATCTCCAGA		
T6ODM GQ500139	(151)	GATCATGAAACCATTCTCTGTCATCGATATAGAAAATTTATTATCTCCAGA		
		201		250
CODM GQ500141	(198)	ACCCGTAGTTGGAAAGTTAGAATTGGATAAGCTTCATTCTGCTTGCAAAG		
T6ODM GQ500139	(201)	ACCAATAATCGGAAAGTTAGAATTAGATAGGCTTCATTTGCTTGCAAAG		
		251		300
CODM GQ500141	(248)	AATGGGGTTTCTTTTCAGCTGGTTAACCATGGAGTCGACGCTTTACTGATG		
T6ODM GQ500139	(251)	AATGGGGTTTTTTTTTCAGGTAGTGAACCATGGAGTCGACGCTTCATTGGTG		
		301		350
CODM GQ500141	(298)	GACAATATAAAATCAGAAATTAAAGGTTTCTTTAACCTTCCAATGAATGA		
T6ODM GQ500139	(301)	GATAGTGTAAAATCAGAAATTCAAGGTTTCTTTAACCTTCTATGGATGA		
		351		400
CODM GQ500141	(348)	GAAAACTAAATACGGACAGCAAGATGGAGATTTTGAAGGATTTGGACAAC		
T6ODM GQ500139	(351)	GAAAACTAAATATGAACAGGAAGATGGAGATGTGGAAGGATTTGGACAAG		
		401		450
CODM GQ500141	(398)	CCTATATTGAATCGGAGGACCAAAGACTTGATTGGACTGAAGTGTTTAGC		
T6ODM GQ500139	(401)	GCTTTATTGAATCAGAGGACCAAACACTTGATTGGGCAGATATATTTATG		
		451		500
CODM GQ500141	(448)	ATGTTAAGTCTTCCTCTCCATTTAAGGAAGCCTCATTTGTTTCCAGAACT		
T6ODM GQ500139	(451)	ATGTTCACTCTTCCACTCCATTTAAGGAAGCCTCACTTATTTTCAAACT		
		501		550
CODM GQ500141	(498)	CCCTCTGCCTTTCAGGGAGACACTGGAATCCTACCTATCAAAAATGAAAA		
T6ODM GQ500139	(501)	CCCAGTGCCTCTCAGGGAGACAATCGAATCCTACTCATCAGAAATGAAAA		
		551		600

Figure 3

4/10

CODM GQ500141	(548)	AACTATCAACGGTTGTCTTTGAGATGTTGGAAAAATCTCTACAATTA---	
T6ODM GQ500139	(551)	AGTTATCCATGGTTCTCTTTAATAAGATGGAAAAAGCTCTACAAGTACAA	
		601	650
CODM GQ500141	(595)	---GTTGAGATTAAAGGTATGACAGACTTATTTGAAGATGGGTTGCAAAC	
T6ODM GQ500139	(601)	GCAGCCGAGATTAAGGGTATGTCAGAGGTGTTTATAGATGGGACACAAGC	
		651	700
CODM GQ500141	(642)	AATGAGGATGAAC TATTATCC CCTTGTCCTC ACCA AACTTGTACTTG	
T6ODM GQ500139	(651)	AATGAGGATGAAC TATTATCC CCTTGTCCTC ACCA AACTTGTACTTG	
		701	750
CODM GQ500141	(692)	GTCTTACGTC CACTCGGATTTT GCGGTTTGACAAT CTCCTTCAAATT	
T6ODM GQ500139	(701)	GTCTTACGTC CACTCGGATTTT GCGGTTTGACAAT CTCCTTCAAATT	
		751	800
CODM GQ500141	(742)	AA GAAGT GAAGGATTACA AATAA AA AGA AGAGA GTGGATTTCAT	
T6ODM GQ500139	(751)	AA GAAGT GAAGGATTACA AATAA AA AGA AGAGA GTGGATTTCAT	
		801	850
CODM GQ500141	(792)	CAAACCTCTACCT ATGCGTTCT TAGTGAATGTTGGAGA ATTTTGGAGA	
T6ODM GQ500139	(801)	CAAACCTCTACCT ATGCGTTCT TAGTGAATGTTGGAGA ATTTTGGAGA	
		851	900
CODM GQ500141	(842)	TAATGACTAATGG AATTACCTAG GTCGA CACCGGGCAGTAGTAAAC	
T6ODM GQ500139	(851)	TAATGACTAATGG AATTACCTAG GTCGA CACCGGGCAGTAGTAAAC	
		901	950
CODM GQ500141	(892)	TCAACAAA GAGAGGCTCTCAATCGC ACATTTTCATGAC CTA ACTAGA	
T6ODM GQ500139	(901)	TCAACAAA GAGAGGCTCTCAATCGC ACATTTTCATGAC CTA ACTAGA	
		951	1000
CODM GQ500141	(942)	GTC GAATAGGCCCAAT TCGAGCTTG ATACCAGAGACACCTGCTT	
T6ODM GQ500139	(951)	GTC GAATAGGCCCAAT TCGAGCTTG ATACCAGAGACACCTGCTT	
		1001	1050
CODM GQ500141	(992)	TGTT AAAAG GGT --TAGGTATGAGGATATTTTGAAGGAAAATCTTTCA	
T6ODM GQ500139	(1001)	TGTT AAAAG GGT TCTACATATGGGGATCTTGTGGAGGAATGTAAACA	
		1051	1095
CODM GQ500141	(1039)	AGGAAGCTTGATGGAAAATCATTTCTCGACTACATGAGGATGTGA	
T6ODM GQ500139	(1051)	AGGAAGCTCGATGGAAAATCATTTCTTGACTCCATGAGGATTTGA	

Figure 3 (Continued)

5/10

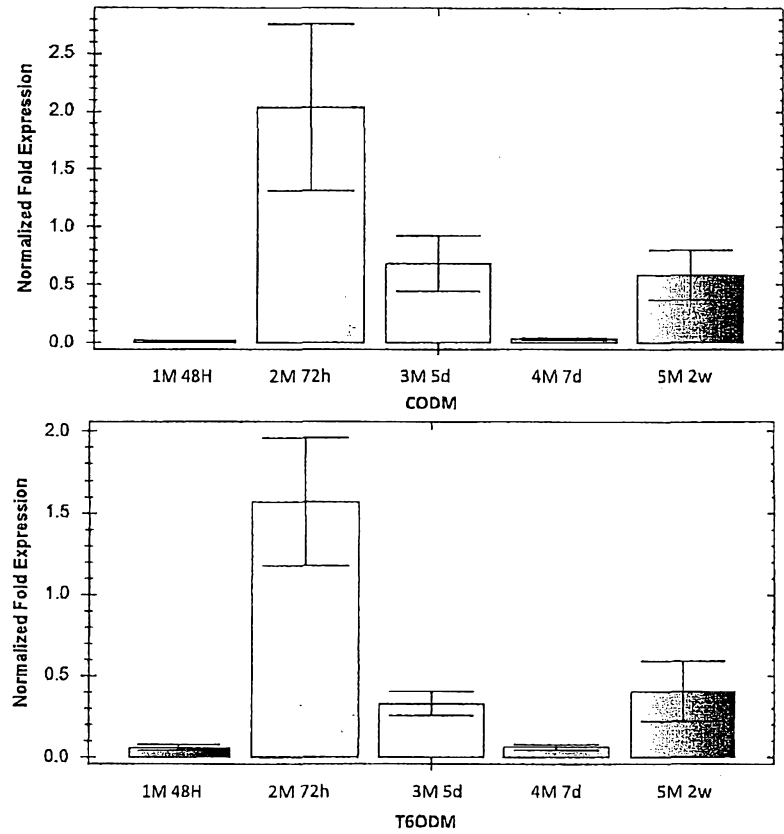


Figure 4

6/10

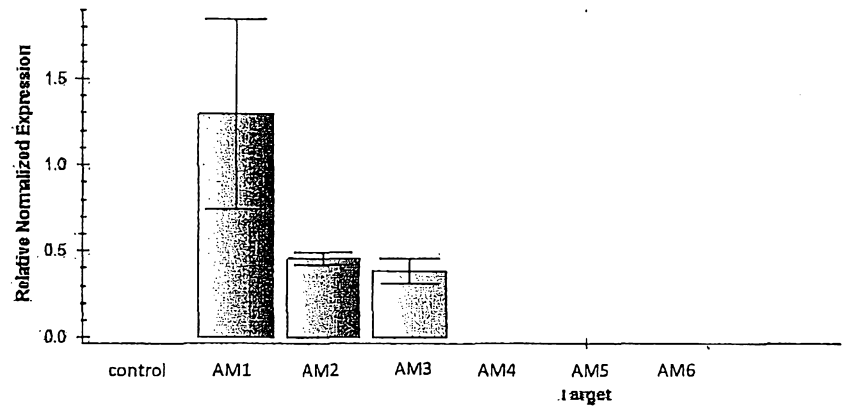


Figure 5

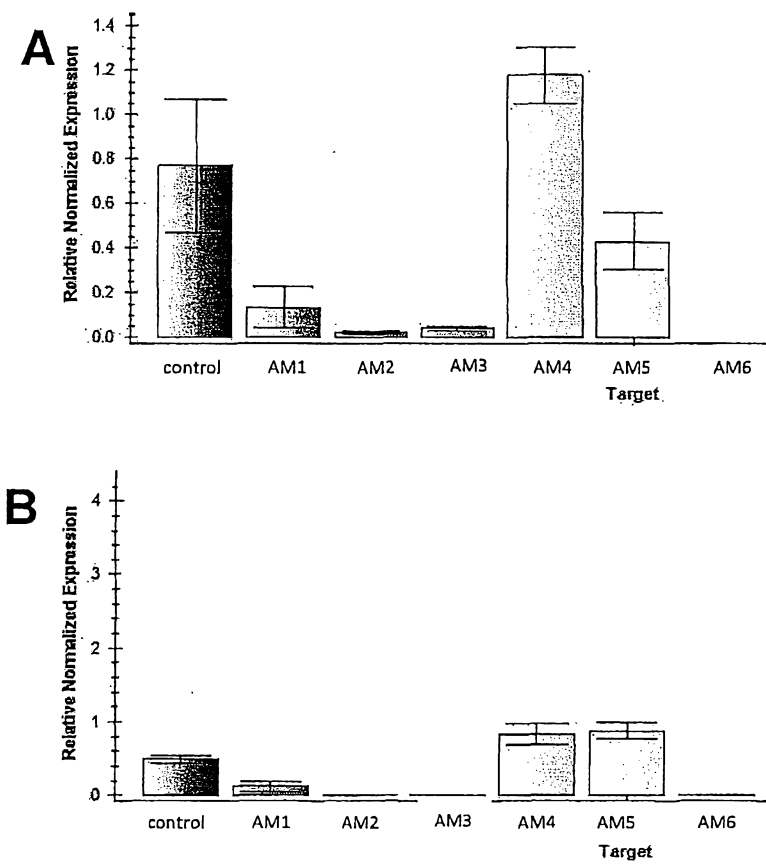
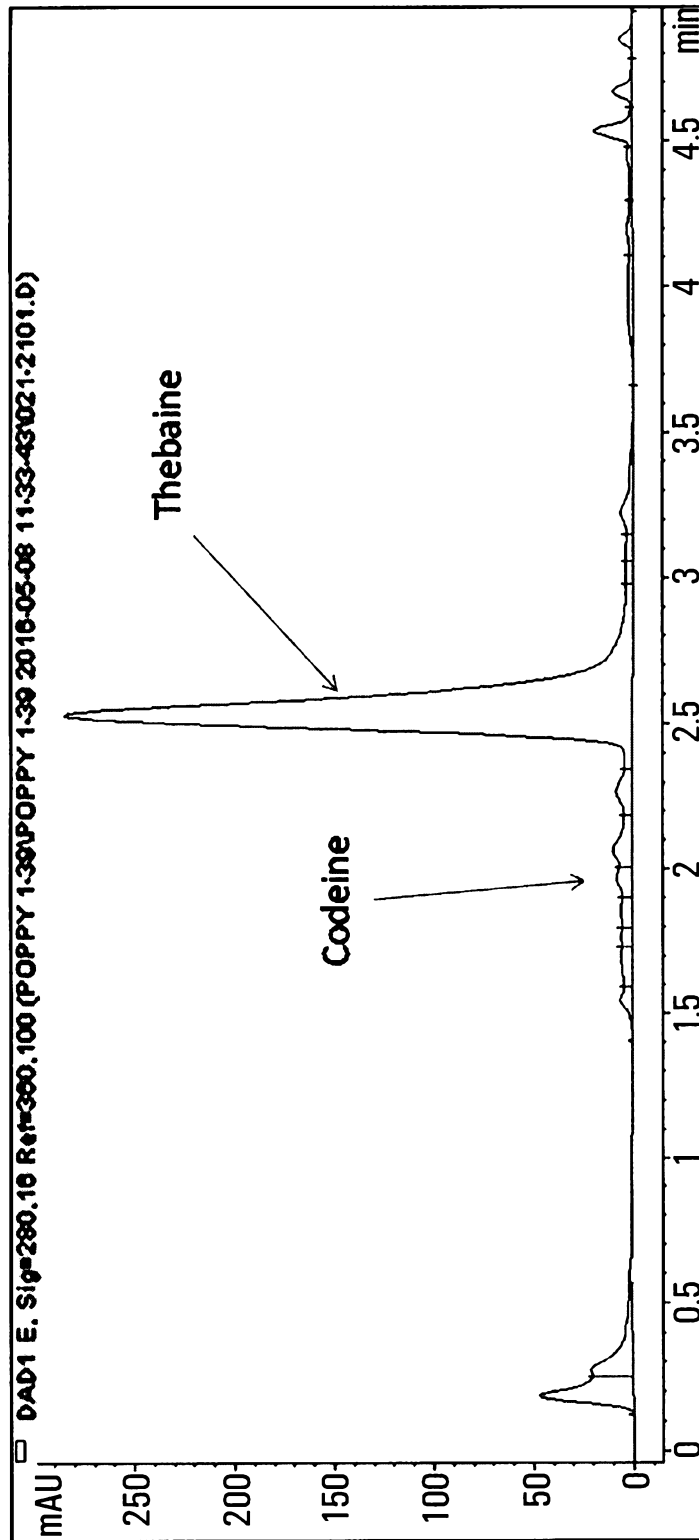


Figure 6

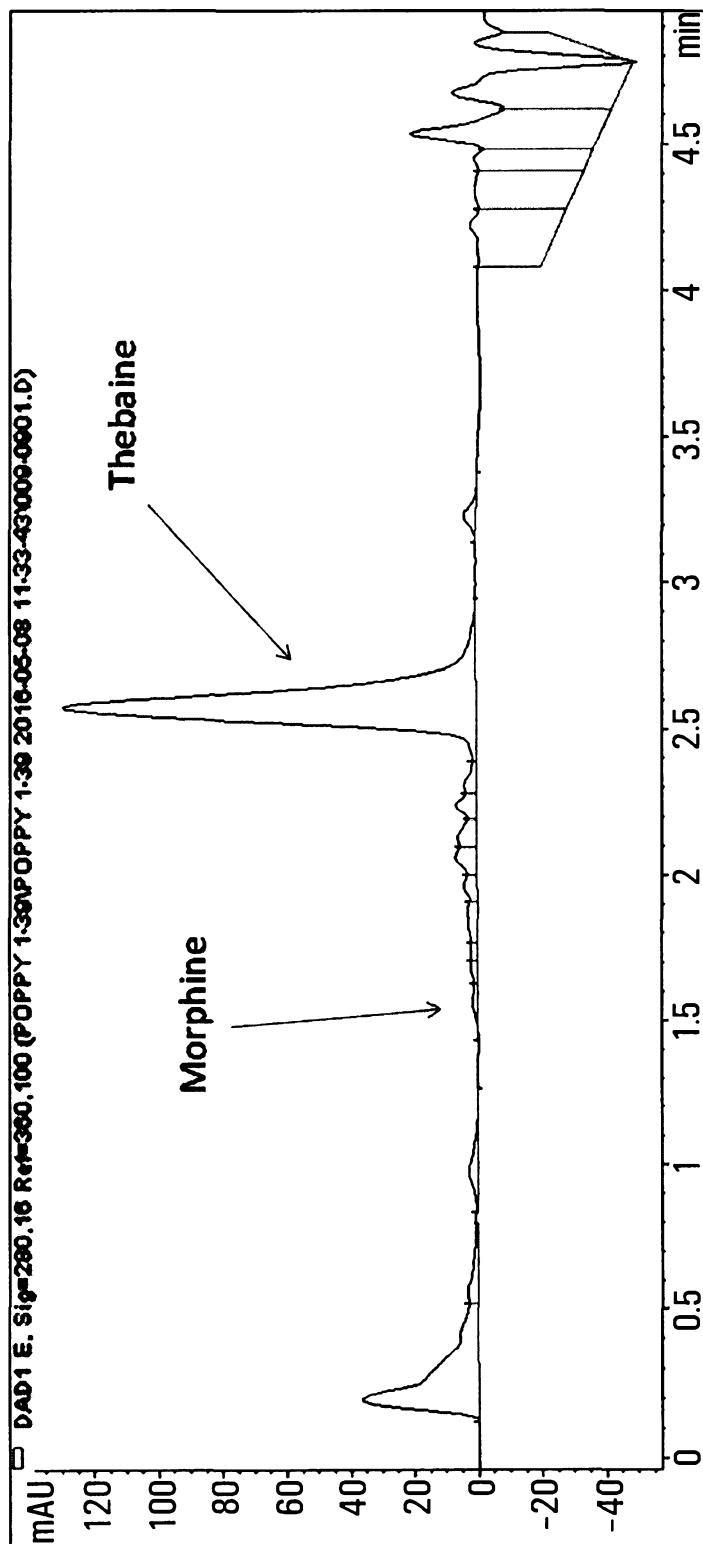
7/10



AM 1, capsules – 3.87% thebaine

Figure 7A

8/10



AM 2, dry leaves – 1.63% thebaine

Figure 7B

9/10

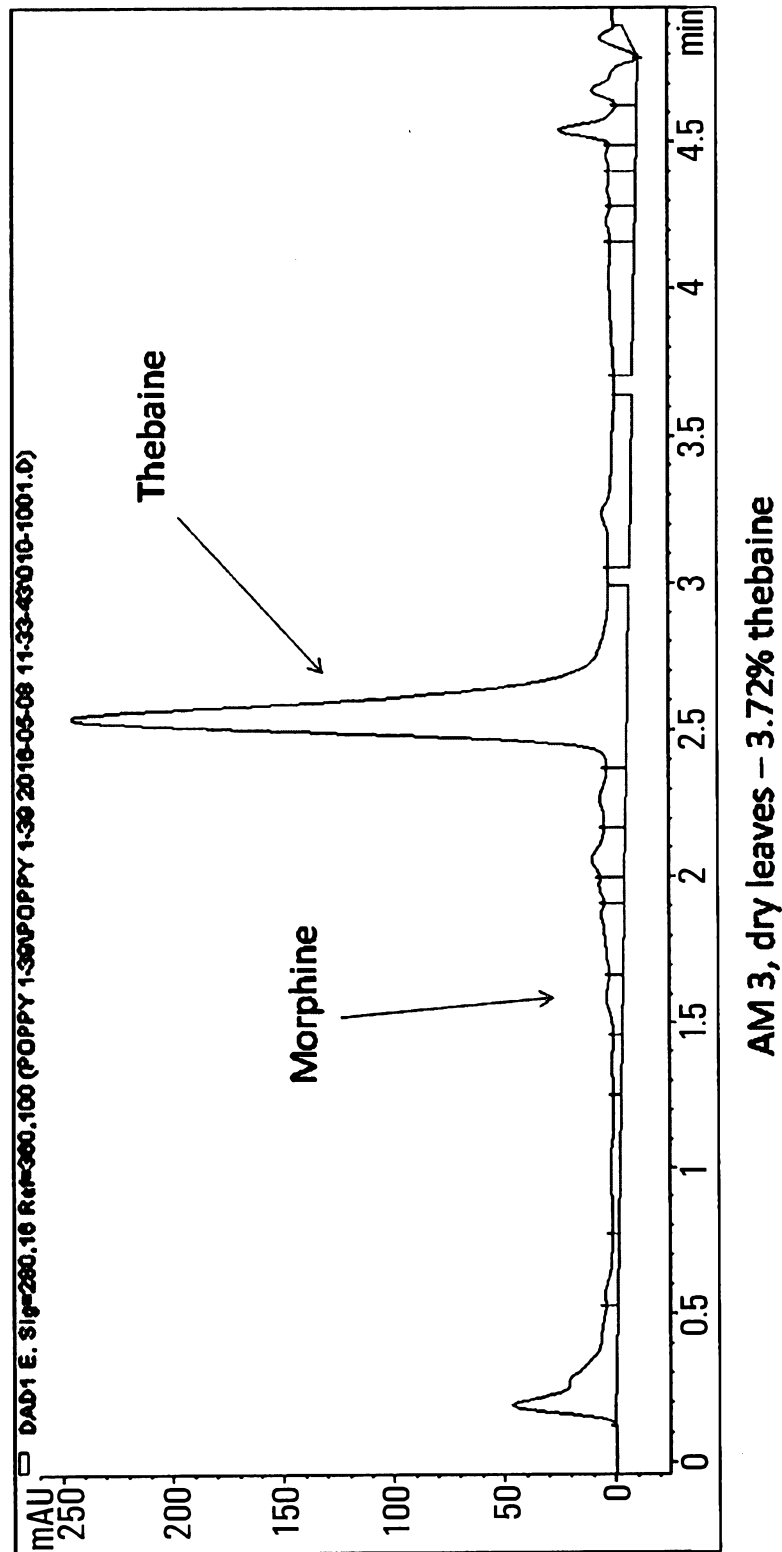


Figure 7C

10/10

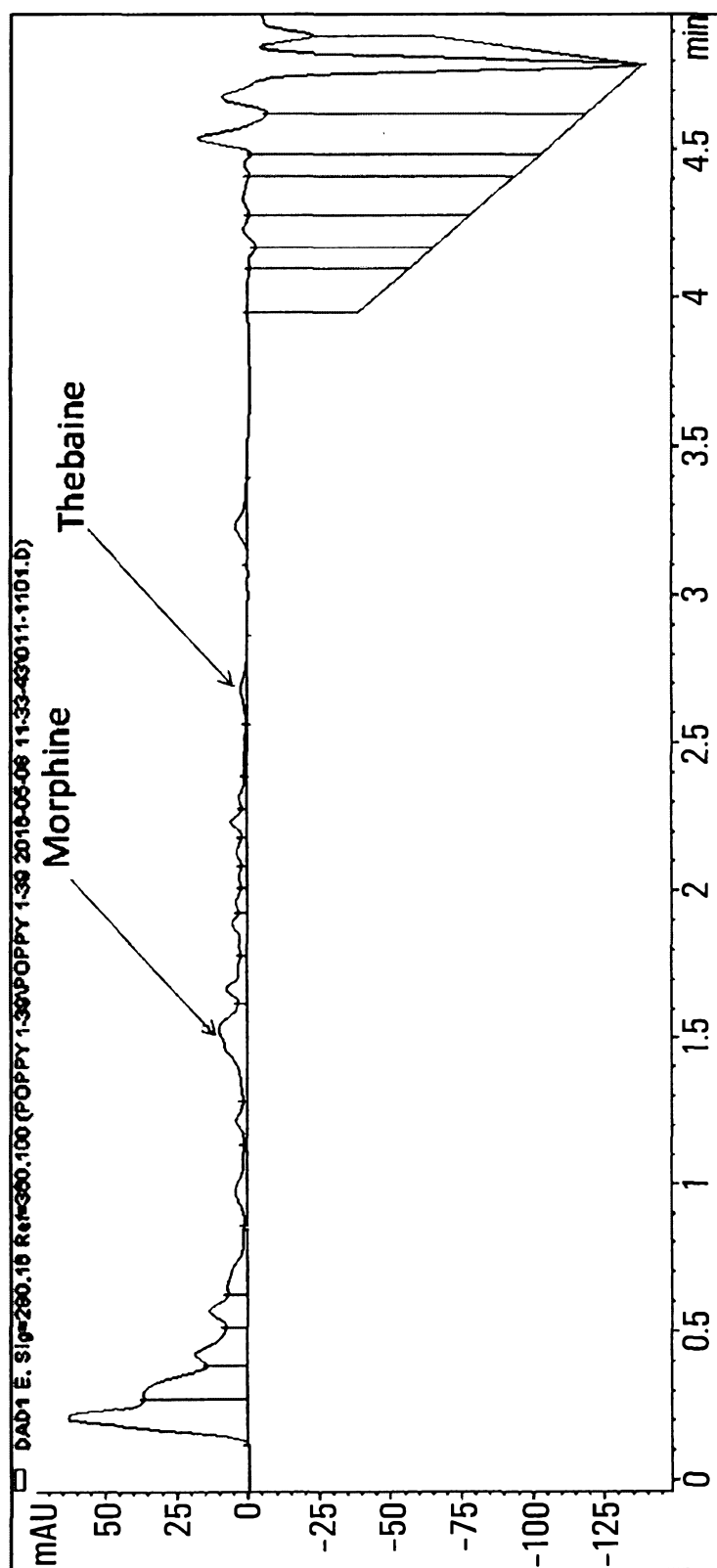


Figure 7D

pctca2017050951-seq1.txt
SEQUENCE LISTING

<110> API LABS INC.

<120> HIGH THEBAINE POPPY AND METHODS OF PRODUCING THE SAME

<130> 84058461 (16272-11)

<150> US 62/374,682
<151> 2016-08-12

<150> CA 2941315
<151> 2016-09-07

<160> 16

<170> PatentIn version 3.5

<210> 1
<211> 364
<212> PRT
<213> Papaver somniferum T6ODM

<400> 1
Met Glu Lys Ala Lys Leu Met Lys Leu Gly Asn Gly Met Glu Ile Pro
1 5 10 15
Ser Val Gln Glu Leu Ala Lys Leu Thr Leu Ala Glu Ile Pro Ser Arg
20 25 30
Tyr Val Cys Ala Asn Glu Asn Leu Leu Leu Pro Met Gly Ala Ser Val
35 40 45
Ile Asn Asp His Glu Thr Ile Pro Val Ile Asp Ile Glu Asn Leu Leu
50 55 60
Ser Pro Glu Pro Ile Ile Gly Lys Leu Glu Leu Asp Arg Leu His Phe
65 70 75 80
Ala Cys Lys Glu Trp Gly Phe Phe Gln Val Val Asn His Gly Val Asp
85 90 95
Ala Ser Leu Val Asp Ser Val Lys Ser Glu Ile Gln Gly Phe Phe Asn
100 105 110
Leu Ser Met Asp Glu Lys Thr Lys Tyr Glu Gln Glu Asp Gly Asp Val
115 120 125
Glu Gly Phe Gly Gln Gly Phe Ile Glu Ser Glu Asp Gln Thr Leu Asp
130 135 140
Trp Ala Asp Ile Phe Met Met Phe Thr Leu Pro Leu His Leu Arg Lys
145 150 155 160
Pro His Leu Phe Ser Lys Leu Pro Val Pro Leu Arg Glu Thr Ile Glu
165 170 175
Ser Tyr Ser Ser Glu Met Lys Lys Leu Ser Met Val Leu Phe Asn Lys
180 185 190
Met Glu Lys Ala Leu Gln Val Gln Ala Ala Glu Ile Lys Gly Met Ser
195 200 205
Glu Val Phe Ile Asp Gly Thr Gln Ala Met Arg Met Asn Tyr Tyr Pro
210 215 220
Pro Cys Pro Gln Pro Asn Leu Ala Ile Gly Leu Thr Ser His Ser Asp
225 230 235 240

pctca2017050951-seq1.txt

Phe Gly Gly Leu Thr Ile Leu Leu Gln Ile Asn Glu Val Glu Gly Leu
245 250 255
Gln Ile Lys Arg Glu Gly Thr Trp Ile Ser Val Lys Pro Leu Pro Asn
260 265 270
Ala Phe Val Val Asn Val Gly Asp Ile Leu Glu Ile Met Thr Asn Gly
275 280 285
Ile Tyr His Ser Val Asp His Arg Ala Val Val Asn Ser Thr Asn Glu
290 295 300
Arg Leu Ser Ile Ala Thr Phe His Asp Pro Ser Leu Glu Ser Val Ile
305 310 315 320
Gly Pro Ile Ser Ser Leu Ile Thr Pro Glu Thr Pro Ala Leu Phe Lys
325 330 335
Ser Gly Ser Thr Tyr Gly Asp Leu Val Glu Glu Cys Lys Thr Arg Lys
340 345 350
Leu Asp Gly Lys Ser Phe Leu Asp Ser Met Arg Ile
355 360

<210> 2
<211> 1386
<212> DNA
<213> Papaver somniferum T60DM

<400> 2
gttcttaatt cattaattaa tttagaaaaa tcatggagaa agcaaaactt atgaagctag 60
gtaatgggat ggaaataacca agtgttcaag aattggctaa actcacgctt gccgaaattc 120
catctcgata cgtatgcgcc aatgaaaacc ttttgttgcc tatgggtgca tctgtcataa 180
atgatcatga aaccattcct gtcatcgata tagaaaattt attatctcca gaaccaataa 240
tcggaaagt agaatagat aggccttcatt ttgcttgcaa agaatggggt ttttttcagg 300
tagtgaacca tggagtcgac gcttcattgg tggatagtgt aaaatcagaa attcaagggt 360
tcittaacct ttctatggat gagaaaaacta aatatgaaca ggaagatgga gatgtggaag 420
gatttggaca aggcctttatt gaatcagagg accaaacact tgattgggca gatataattt 480
tgatgttcac tcttccactc catttaagga agcctcactt attttcaaaa ctcccagtcg 540
ctctcagggg gacaatcgaa tcctactcat cagaaatgaa aaagttatcc atggttctct 600
ttaataagat ggaaaaagct ctacaagtac aagcagccga gattaagggt atgtcagagg 660
tgtttataga tgggacacaa gcaatgagga tgaactatta tcccccttgt cctcaaccaa 720
atctcgccat cggctcttac tgcgactcgg attttggcgg tttgacaatc ctccttcaaa 780
tcaacgaagt ggaaggatta cagataaaaa gagaggggac atggatttca gtcaaacctc 840
tacctaagtc gttcgtagt aatgttggag atattttgga gataatgact aatggaattt 900
accatagtgt cgatcacgg gcagtagtaa actcaacaaa tgagaggctc tcaatcgcaa 960
catttcatga ccctagtcta gagtcggtaa taggcccaat atcaagcttg attactccag 1020
agacacctgc tttgtttaaa agtggatcta catatgggga tcttgtggag gaatgtaaaa 1080
caaggaagct cgatggaaaa tcatttcttg actccatgag gatttgaaaa ctcaagaaaa 1140
aataatacga cgtgattgca tgtcagattc aactatcctt ttgtcgtttt ttggtgctcg 1200
agtccttaat tgttttgatc attgcttttg attctaatta ataagacttt tctcaagaac 1260
cacatgtaat gtacctttac tttcagaaaa taaaaagtat tgaggcacia atgagaaaaat 1320
tgagagagt cttgagaagt gtaatttctc gaaagtgcgt tgtgtttgaa aaaaaaaaaa 1380
aaaaaa 1386

<210> 3
<211> 360
<212> PRT
<213> Papaver somniferum CODM

<400> 3
Met Glu Thr Pro Ile Leu Ile Lys Leu Gly Asn Gly Leu Ser Ile Pro
1 5 10 15
Ser Val Gln Glu Leu Ala Lys Leu Thr Leu Ala Glu Ile Pro Ser Arg
20 25 30

pctca2017050951-seql.txt

Tyr Thr Cys Thr Gly Glu Ser Pro Leu Asn Asn Ile Gly Ala Ser Val
35 40 45

Thr Asp Asp Glu Thr Val Pro Val Ile Asp Leu Gln Asn Leu Leu Ser
50 55 60

Pro Glu Pro Val Val Gly Lys Leu Glu Leu Asp Lys Leu His Ser Ala
65 70 75 80

Cys Lys Glu Trp Gly Phe Phe Gln Leu Val Asn His Gly Val Asp Ala
85 90 95

Leu Leu Met Asp Asn Ile Lys Ser Glu Ile Lys Gly Phe Phe Asn Leu
100 105 110

Pro Met Asn Glu Lys Thr Lys Tyr Gly Gln Gln Asp Gly Asp Phe Glu
115 120 125

Gly Phe Gly Gln Pro Tyr Ile Glu Ser Glu Asp Gln Arg Leu Asp Trp
130 135 140

Thr Glu Val Phe Ser Met Leu Ser Leu Pro Leu His Leu Arg Lys Pro
145 150 155 160

His Leu Phe Pro Glu Leu Pro Leu Pro Phe Arg Glu Thr Leu Glu Ser
165 170 175

Tyr Leu Ser Lys Met Lys Lys Leu Ser Thr Val Val Phe Glu Met Leu
180 185 190

Glu Lys Ser Leu Gln Leu Val Glu Ile Lys Gly Met Thr Asp Leu Phe
195 200 205

Glu Asp Gly Leu Gln Thr Met Arg Met Asn Tyr Tyr Pro Pro Cys Pro
210 215 220

Arg Pro Glu Leu Val Leu Gly Leu Thr Ser His Ser Asp Phe Ser Gly
225 230 235 240

Leu Thr Ile Leu Leu Gln Leu Asn Glu Val Glu Gly Leu Gln Ile Arg
245 250 255

Lys Glu Glu Arg Trp Ile Ser Ile Lys Pro Leu Pro Asp Ala Phe Ile
260 265 270

Val Asn Val Gly Asp Ile Leu Glu Ile Met Thr Asn Gly Ile Tyr Arg
275 280 285

Ser Val Glu His Arg Ala Val Val Asn Ser Thr Lys Glu Arg Leu Ser
290 295 300

Ile Ala Thr Phe His Asp Ser Lys Leu Glu Ser Glu Ile Gly Pro Ile
305 310 315 320

Ser Ser Leu Val Thr Pro Glu Thr Pro Ala Leu Phe Lys Arg Gly Arg
325 330 335

Tyr Glu Asp Ile Leu Lys Glu Asn Leu Ser Arg Lys Leu Asp Gly Lys
340 345 350

Ser Phe Leu Asp Tyr Met Arg Met
355 360

<210> 4
<211> 1452
<212> DNA
<213> Papaver somniferum CODM

pctca2017050951-seq1.txt

```

<400> 4
gtaaagattg atatatgatc tgaagatctg acaagaaagt tcatcaaata tagagttcat 60
ggagacacca atacttatca agctaggcaa tggtttgtca ataccaagtg ttcaggaatt 120
ggctaaactc acgcttgcag aaattccatc tcgatacaca tgcaccggtg aaagcccgtt 180
gaataatatt ggtgcgtctg taacagatga tgaacaggtt cctgtcatcg atttgcaaaa 240
tttactatct ccagaacccg tagttggaaa gttagaattg gataagcttc attctgcttg 300
caaagaatgg ggtttctttc agctgggtta ccatggagtc gacgctttac tgatggacaa 360
tataaaatca gaaattaaag gtttctttta ccttccaatg aatgagaaaa ctaaatacgg 420
acagcaagat ggagattttg aaggatttgg acaaccctat attgaatcgg aggaccaaa 480
acttgattgg actgaagtgt ttagcatgtt aagtcttcct ctccatttta ggaagcctca 540
tttgtttcca gaactccctc tgcctttcag ggagacactg gaatcctacc tatcaaaaat 600
gaaaaaacta tcaacggttg tctttgagat gttggaaaaa tctctacaat tagttgagat 660
taaaggtatg acagacttat ttgaagatgg gttgcaaaca atgaggatga actattatcc 720
tccttgtcct cgaccagagc ttgtacttgg tcttacgtca cactcggatt tttagcggttt 780
gacaattctc cttcaactta atgaagtga aggattacaa ataagaaaag aagagaggtg 840
gatttcaatc aaacctctac ctgatgcgtt catagtgaat gttggagaca ttttggagat 900
aatgactaat gggattttacc gtagcgtcga gcaccgggca gtagtaaaact caacaaagga 960
gaggctctca atcgcgacat ttcatgactc taaactagag tcagaaatag gcccaatttc 1020
gagcttggtc acaccagaga cacctgcttt gttcaaaaga ggtaggtatg aggatatttt 1080
gaaggaaaaa ctttcaagga agcttggatgg aaaatcattt ctcgactaca tgaggatgtg 1140
agaaaagtgtg aacatatatt atactccaca ttgtgtttta tatatgatga aataagttgc 1200
ttttgaagta tgatgaaata agttggtttt gaagaattca tattgtgctt aaatttcgtg 1260
gatgactgag agatttatta tgtaataata atgtattggt ttgaagattc tcgtctcact 1320
atatgtaaga ctctgttttg gtcaagtgat gtaatcacgg ttgaaataag ttgcttttga 1380
agaattcata tgggtgctta tattatgtaa taaataatgt attggattga aaaaaaaaaa 1440
aaaaaaaaaa aa 1452

```

```

<210> 5
<211> 8688
<212> DNA
<213> Artificial Sequence

```

```

<220>
<223> TDNA sequence

```

```

<400> 5
tcctgtgggt ggcattgcaca tacaaatgga cgaacggata aaccttttca cgccctttta 60
aatatccgat tattctaata aacgctcttt tctcttaggt ttaccgcca atatatcctg 120
tcaaacactg atagtttaaa ctgaaggcgg gaaacgacaa tctgctagtg gatctcccag 180
tcacgacgtt gtaaaacggg cgccccgcgg aaagcttgct agccaattgg ggcccaacgt 240
tctcgagaac gtggatactt ggcagtggtt acttggcttt tcctttatct tcttttggac 300
ggaagcgggt gttactttgt cacacattta aaaaaacacg tgtttctcac ttttttctat 360
tcccgtcaca aacaatttta agaaagatcc atctatcgtg atctttctat caaacaanaag 420
aaaaaagggtc ttcatagtaa cgctacaaca tcaaatatgt ggttgctctg acatcagtcg 480
ggaaaataag gatatggcgg cattggccac atctattggg gtcccaactt cttttcacia 540
aaaaattaaa ttgggtgtcc caacttttat ctttgatata gtgacatgag tatcgggagc 600
attggacaat ggataaaatg agaactaaaa aaattctggt taatttttga tcattgttat 660
ttaaaggtt attttatcta taatctaccc atattgatca gttttattta aatttgttta 720
gctaccgtc cagagagag atcctcatc taaaaatgga atatggaat tacacagcag 780
cccaaaagta tatttttct ctggagaatg ctatttagag ctttgactat atggtctgaa 840
ttagaaagac gggaaataaa atctgctaag tgatataagc tctaagtagg cgatgtgtga 900
tggaagacac cttttcttta acagtcttca tgttttacag attcgcgaa ttcgaatata 960
cctatacggg ctgtctaacc ctctgtgtgc ttttgagtcc aagataaagg ccattattga 1020
gtaacataga catgctggaa tccaaccatt gaagtcacaa ctgtccatgt agattctttg 1080
gagaatctga aaagtcttaa taaagggtgt gtttcaaaga aaacaaaaca aatgagttaa 1140
gaaaaaaaaa tatcatgtag tggctgagta ttatgttatt tattgtgtag ctaccaatct 1200
ttattcttta aatctgacat aaaatgctac aaacttttta cctcgtctat agcccaaaaa 1260
aacctaacca cggttctaaa accacacaca gtgattttgg ttgacgacaa tgcctctcct 1320
tctcaaaac gatttattta ctttttttaa atcaaattgt acattttata ccataattaa 1380
gtctttttac agaatactta gatggaagag atgtataaaa aaggaggaaa ttgtaaaaaa 1440
catatttctg tcaattaaac caggattcat aaaaatataa gtatatatat aaatgattgt 1500
tcgtttagcg atgaacttca ctcatatgat aatacttaac aatataagta cataaaaaat 1560
aaaataaaat taattgttta cgaaaagtct acaaaactg catgtataat taatgttctc 1620
tttatttatt tatttatacc ttaccaagat atatctataa ccgcatagaa atagaaggcg 1680
aagagataat ttccaaaaac aagaaaaacc tctaagctca aaagtctaga aggccttggg 1740
tccacccatg gaggtgttca cagtatcact tgtagcagtt gtgataccta ctttcttata 1800
cttaattctc agagattcaa gtccataaagg ttggccacca ggtccaaaac cctggccaat 1860
agttggaaac cttcttcaac ttggtgagaa acctattctc cagtttgctc agcttgctga 1920

```

pctca2017050951-seq1.txt

aacctatggt	gatctctttt	cactgaaact	aggaagttaa	acgggtgttg	tagcttcaac	1980
tccattagca	gctagcgaga	ttctaaagac	gcgatgcgt	gttctctctg	gtcgatacgt	2040
gtttcaaagt	ttccgggtaa	aggaacatgt	ggagaactct	attgtgtggt	ctgaatgtaa	2100
tgaaacatgg	aagaaactgc	ggaaagtttg	tagaacggaa	ctttttacgc	agaagatgat	2160
tgaaagtcaa	gctgaagtta	gagaaagtaa	ggctatggaa	atggtggagt	atttgaagaa	2220
aaatgtagga	aatgaagtga	aaattgctga	agttgtattt	gggacgttgg	tgaatatatt	2280
cggtaacttg	atattttcac	aaaatatttt	caagttgggt	gatgaaagta	gtggaagtgt	2340
agaaaatgaaa	gaacatctat	ggagaatgct	ggaattgggg	aactcgacaa	atccagctga	2400
ttattttcca	tttttgggta	aattcgattt	gtttggacaa	agaaaagatg	ttgctgattg	2460
tctgcaaggg	atttatagtg	tttgggggtgc	tatgctcaaa	gaaagaaaaa	tagccaagca	2520
gcataacaac	agcaagaaga	atgattttgt	tgagattttg	ctcgattccg	gactcgatga	2580
ccagcagatt	aatgccttgc	tcattggaaat	atttgggtgcg	ggaacagaga	caagtgcatt	2640
tacaatagaa	tgggcgttgt	ctgagctcac	aaaaaacctt	caagtaacag	ccaatatgct	2700
gttggaattg	ttatctgttg	tagggaagag	gccggttaag	gaatccgaca	taccaaacat	2760
gccctatctt	agacgttttg	ttaaagaaac	tctacggctt	catccagcaa	ctcctctgct	2820
gcttccacgt	cgagcacttg	agacctgcaa	agttttgaac	tatacgatcc	cgaaagagtg	2880
tcagattatg	gtgaacgcct	ggggcattgg	tcgggatcca	aaaaggtgga	ctgatccatt	2940
gaagttttca	ccagagaggt	tcttgaattc	gagcattgat	ttcaaaggga	acgacttcga	3000
gttgatacca	tttgggtgcg	ggagaaggat	atgtcctggt	gtgcccttgg	caactcaatt	3060
tatttctctt	atttgttcta	gtttggtaca	gaattttgat	tggggattac	cgaagggaat	3120
ggatccctagc	caactgatca	tggaaagaaa	atttgggtga	cactgcaaaa	ggaaccacct	3180
ctgtatatgg	ttcctaaaac	tcgggattaa	gggagaattc	gtcgactttg	cggccgcatt	3240
gatactgcag	gagctcggta	ccttttacta	gtgatatccc	tgtgtgaaat	tggtatccgc	3300
tacgcgtgat	cgttcaaaca	tttggcaata	aagtttctta	agattgaatc	ctgttgccgg	3360
tcttgcgatg	attatcatat	aatttctggt	gaattacggt	aagcatgtaa	taattaacat	3420
gtaatgcatt	acgtttattt	tgagatgggt	ttttatgatt	agagtccgcg	aattatacat	3480
ttaatacgcg	atagaaaaca	aaatatagcg	cgcaaacatg	gataaattat	cgcgcgcggt	3540
gtcatctatg	ttactagatc	ccatgggaag	ttcctatttc	gaagttccta	ttctctgaaa	3600
agtataggaa	cttcagcgat	cgctccaatc	ccacaaaaat	ctgagcttaa	cagcacagtt	3660
gctcctctca	gagcagaatc	gggtattcaa	caccctcata	tcaactacta	cgttgtgtat	3720
aacgggtccac	atgccggtat	atacgatgac	tgggggttga	caaaggcggc	aacaaacggc	3780
gttcccggag	ttgcacacaa	gaaatttgcc	actattacag	aggcaagagc	agcagctgac	3840
gcgtacacaa	caagtcagca	aacagacagg	ttgaacttca	tccccaaagg	agaagctcaa	3900
ctcaagccca	agagctttgc	taaggcccta	acaagcccac	caaagcaaaa	agcccactgg	3960
ctcacgctag	gaacccaaaag	gcccagcagt	gatccagccc	caaaagagac	tcctttgccc	4020
cggagattac	aatggacgat	ttcctctatc	tttacgatct	aggaagggaag	ttcgaagggtg	4080
aaggtgacga	cactatgttc	accactgata	atgagaaggt	tagcctcttc	aatttccagaa	4140
agaatgctga	cccacagatg	gttagagagg	cctacgcagc	aggtctcatc	aagacgatct	4200
acccgagtaa	caatctccag	gagatcaaat	accttcccaa	gaagggttaa	gatgcagtca	4260
aaagattcag	gactaattgc	atcaagaaca	cagagaaaga	catatttctc	aagatcagaa	4320
gtactattcc	agtatggacg	attcaaggct	tgcttcataa	accaaggcaa	gtaatagaga	4380
ttggagtctc	taaaaaggta	gttcctactg	aatctaaggc	catgcatgga	gtctaagatt	4440
caaatcgagg	atctaacaga	actcgccgtg	aagactggcg	aacagttcat	acagagtcct	4500
ttacgactca	atgacaagaa	gaaaatcttc	gtcaacatgg	tggagcacga	cactctgggtc	4560
tactccaaaa	atgtcaaaga	tacagtctca	gaagaccaa	gggctattga	gacttttcaa	4620
caaaggataa	tttcgggaaa	cctcctcgga	ttccattgcc	cagctatctg	tcacttcatc	4680
gaaaggacag	tagaaaagga	aggtggctcc	tacaaatgcc	atcattgcga	taaaggaaaag	4740
gctatcattc	aagatctctc	tgccgacagt	gggtcccaaag	atggaccccc	acccacgagg	4800
agcatcgtgg	aaaaagaaga	cgttccaact	agcaagtgga	agcaagtgga	ttgatgtgac	4860
atctccactg	acgtaaggga	tgacgcacaa	tcccactatc	cttcgcaaga	cccttcctct	4920
atataaggaa	gttcatttca	tttggagagg	acacgctcga	gtataagagc	tctattttta	4980
caacaattac	caacaacaac	aaacaacaaa	caacattaca	attacattta	caattaccat	5040
ggggcgcgcc	ccttgtcctc	aaccaaattc	cgccatcggt	cttacgtcgc	actcggattt	5100
tggcggtttg	acaatcctcc	ttcaaattca	cgaagtggaa	ggattacaga	taaaaagaga	5160
ggggacatgg	atttcagtca	aacctctacc	taatgcgttc	gtagtgaatg	ttggagatat	5220
tttggagata	atgactaatg	gaattttacca	tagtgtcgat	caccgggcag	tagtaaaactc	5280
aacaaatgag	aggctctcaa	tcgcaacatt	tcattgacct	agtctagagt	cggtaatagg	5340
cccaatatca	agcttgatta	ctccagagac	acctgctttg	tttaaaagtg	gaattttaaat	5400
ccccagatga	acatggcatc	gtggtgattg	atgaaactgc	tgctgtcggc	tttaacctct	5460
ctttaggcat	tggtttcgaa	gcgggcaaca	agccgaaaga	actgtacagc	gaagaggcag	5520
tcaacgggga	aactcagcaa	gcgcacttac	aggcgattaa	agagctgata	gcgctgaca	5580
aaaaccaccc	aagcgtgggtg	atgtggagta	ttgccaacga	accggatacc	cgtccgcaag	5640
gtgcacggga	atatttcgcg	ccactggcgg	aagcaacgcg	taaactcgac	ccgacgcgtc	5700
cgatcacctg	cgtcaatgta	atgttctgcg	acgctcacac	cgataccatc	agcgatctct	5760
ttgatgggga	tcctccactt	ttaaacaaag	caggtgtctc	tggagtaatc	aagcttgata	5820
ttgggcctat	taccgactct	agactagggt	catgaaatgt	tgcgattgag	agcctctcat	5880
ttgttgatgt	tactactgcc	cggatgacga	cactatggtg	aattccatta	gtcattattct	5940
ccaaaatatc	tccaacattc	actacgaacg	cattaggtag	aggtttgact	gaaatccatg	6000

pctca2017050951-seq1.txt

tccccctctct	ttttatctgt	aatccttcca	cttcgttgat	ttgaaggagg	attgtcaaac	6060
cgccaaaatc	cgagtgcgac	gtaagaccga	tggttgagga	caaggactag		6120
tccctagagt	cctgctttta	tgagatatgc	gagacgccta	tgatcgcatg	atatttgctt	6180
tcaattctgt	tgtgcacgtt	gtaaaaaacc	tgagcatgtg	tagctcagat	ccttaccgcc	6240
ggtttcgggt	cattctaata	aatgaatata	tcacccgtta	ctatcgtatt	tttatgaata	6300
atattctccg	ttcaattttac	tgattgtacc	ctactactta	tatgtacaat	attaaaaatga	6360
aaacaatata	ttgtgctgaa	taggtttata	gcgacatcta	tgatagagcg	ccacaataac	6420
aaacaattgc	gttttattat	tacaaaatcca	atttttaaaaa	aagcggcaga	accgggtcaaa	6480
cctaaaagac	tgattacata	aatctttattc	aaattttcaaa	agtgtcccccag	gggctagtat	6540
ctacgacaca	ccgagcgggcg	aactaataac	gctcactgaa	gggaactccg	gttccccgcc	6600
ggcgcgcatg	ggtagatttc	cttgaagttg	agtattggcc	gtccgctcta	ccgaaagtta	6660
cgggcaccat	tcaacccggg	ccagcacggc	ggccgggtta	ccgacttgct	gccccgagaa	6720
ttatgcagca	tttttttggg	gtatgtgggc	cccaaataaa	gtgcagggtca	aaccttgaca	6780
gtgacgacaa	atcggtgggc	gggtccaggg	cgaattttgc	gacaacatgt	cagggtcag	6840
cagggcgatc	gcagacgtcg	ggatcttctg	caagcatctc	tatttcctga	aggtctaacc	6900
tcgaagattt	aagatttaaa	tacgtttata	attacaaaat	tgattctagt	atctttaatt	6960
taatgcttat	acattattaa	ttaatttagt	actttcaatt	tgttttcaga	aattattttta	7020
ctatttttta	taaaataaaa	gggagaaaat	ggctatttta	atactagcct	atttttatttc	7080
aatttttagt	taaaatcagc	cccaatttagc	cccaattttca	aattcaaata	gtccagccca	7140
attcctaagt	aacccacccc	taacccgccc	ggtttcccc	tttgatccat	gcagtgcaacg	7200
cccagaattt	ccctatataa	tttttttaatt	cccaaacc	cctaactcta	tcccattttct	7260
caccaaccgc	cacatagatc	tatcctctta	tctctcaaac	tctctcgaac	cttccccctaa	7320
ccctagcagc	ctctcatcat	cctcacctca	aaacccaccg	gggcccggcca	tgattgaaca	7380
agatggattg	cacgcaggtt	ctccggccgc	ttgggtggag	aggctatttcg	gctatgactg	7440
ggcacaacag	acaatcggt	gctctgatgc	cgccgtgttc	cggctgtcag	cgcaggggag	7500
gctcggttct	tttgtcaaga	ccgacctgtc	cgggtgccctg	aatgaacttc	aagacgaggg	7560
agcgcggcta	tcgtggctgg	ccacgacggg	cgttccttgc	gcagctgtgc	tcgacgttgt	7620
cactgaagcg	ggaagggact	ggctgctatt	gggcgaagtg	ccggggcagg	atctcctgtc	7680
atctcacctt	gctcctgccg	agaaagtatc	catcatggct	gatgcaatgc	ggcggctgca	7740
tacgcttgat	ccggctacct	gcccattcga	ccaccaagcg	aaacatcgca	tcgagcgagc	7800
acgtactcgg	atggaagccg	gtcttgctga	tcaggatgat	ctggacgaag	agcatcaggg	7860
gctcgcgcca	gccgaactgt	tcgccaggct	caaggcgcg	atgcccgacg	gcgaggatct	7920
cgctcgtgact	catggcgatg	cctgcttgcc	gaatatcatg	gtggaaaatg	gccgcttttc	7980
tggtatcatc	gactgtggcc	ggctgggtgt	ggcggaccgc	tatcaggaca	tagcgttggc	8040
tacccgtgat	attgctgaag	agcttgccgg	cgaatgggct	gaccgcttcc	tcgtgcttta	8100
cggtatcgcc	gctcccgaat	cgcagcgc	cgccttctat	cgccttcttg	acgagttcct	8160
ctgaggcgcg	ccgatcggtc	aaacatttgg	caataaagtt	tcttaagatt	gaatcctgtt	8220
gctcggtcttg	cgatgattat	catataattt	ctgttgtaatt	acgttaagca	tgtaataatt	8280
aacatgtaat	gcatgacgtt	atttatgaga	tgggttttta	tgattagagt	cccgaatta	8340
tacatttaaa	acgcgataga	aaacaaaata	tagcgcgcaa	actaggataa	attatcgcg	8400
gcggtgtcat	ctatgttact	agatccctag	ggaagttcct	attccgaagt	tcctattctc	8460
tgaagagtat	aggaacttct	ttgcgtattg	ggcgctcttg	gccttttttg	ccaccggctg	8520
tacggttaaa	accacccag	tacattaaaa	acgtccgcaa	tgtgttatta	agttgtctaa	8580
gcgtcaattt	gtttacacca	caatatatcc	tgccaccagc	cagccaacag	ctccccgacc	8640
ggcagctcgg	cacaaaatca	ccactcgata	caggcagccc	atcagttcc		8688

<210> 6

<211> 3238

<212> DNA

<213> Artificial Sequence

<220>

<223> expression cassette including promoter

<400> 6

gcgatcgctc	caatcccaca	aaaatctgag	cttaacagca	cagttgctcc	tctcagagca	60
gaatcgggta	ttcaacaccc	tcatatcaac	tactacgttg	tgtataacgg	tccacatgcc	120
ggtatatacg	atgactgggg	ttgtacaaag	gcggcaacaa	acggcggtcc	cggagttgca	180
cacaagaaat	ttgccactat	tacagaggca	agagcagcag	ctgacgcgta	cacaacaagt	240
cagcaaacag	acaggttgaa	cttcaccccc	aaaggagaag	ctcaactcaa	gccccagagc	300
ttgtctaagg	ccctaacaag	cccaaaaagg	caaaaagccc	actggctcac	gctaggaacc	360
aaaaggccca	gcagtgatcc	agcccaaaaa	gagactcctt	tgccccggag	attacaattg	420
acgatttcct	ctatctttac	gatctaggaa	ggaagttcga	aggtgaaggt	gacgacacta	480
tgttcaccac	tgataatgag	aagggttagcc	tcttcaattt	cagaaagaat	gctgaccac	540
agatggttag	agaggcctac	gcagcaggtc	tcataagac	gatctaccgc	agtaacaatc	600
tccaggagat	caaatacctt	cccaagaagg	ttaaagatgc	agtcaaaaaga	ttcaggacta	660
attgcatcaa	gaacacagag	aaagacatat	ttctcaagat	cagaagtact	attccagtat	720
ggacgattca	aggcttgctt	cataaaccaa	ggcaagtaat	agagattgga	gtctctaaaa	780

pctca2017050951-seq1.txt

aggtagttcc	tactgaatct	aaggccatgc	atggagtc	agattcaaat	cgaggatcta	840
acagaactcg	ccgtgaagac	tggcgaacag	ttcatacaga	gtcttttacg	actcaatgac	900
aagaagaaaa	tcttcgtcaa	catgggtggag	cacgacactc	tgggtctactc	caaaaatg	960
aaagatacag	tctcagaaga	ccaaagggct	attgagactt	ttcaacaaag	gataatttcg	1020
ggaaacctcc	tcggattcca	ttgcccagct	atctgtcact	tcacgaaag	gacagtagaa	1080
aagggaaggtg	gctcctacaa	atgccatcat	tgcgataaag	gaaaggctat	cattcaagat	1140
ctctctgccg	acagtgggtc	caaagatgga	ccccaccca	cgaggagcat	cgtggaaaaa	1200
gaagacgttc	caaccacgtc	ttcaaagcaa	gtggattgat	gtgacatctc	cactgacgta	1260
agggatgacg	cacaatccca	ctatccttcg	caagaccctt	cctctatata	aggaagttca	1320
tttcatttgg	agaggacacg	ctcgagtata	agagctctat	ttttacaaca	attaccaaca	1380
acaacaaaca	acaaacaaca	ttacaattac	atttacaatt	accatggggc	gcgccccttg	1440
tcctcaacca	aatctcgcca	tcggtcttac	gtcgcactcg	gattttggcg	gtttgacaat	1500
cctccttcaa	atcaacgaag	tgggaaggatt	acagataaaa	agagagggga	catggatttc	1560
agtcaaacct	ctaccataatg	cgttcgtatg	gaatgttggg	gatatttttg	agataatgac	1620
taatggcaatt	taccatagt	tcgatcacgg	ggcagtagta	aactcaacaa	atgagaggct	1680
ctcaatcgca	acatttcatg	accctagtct	agagtcggta	ataggcccaa	tatcaagctt	1740
gattactcca	gagacacctg	ctttgtttaa	aagtgggaatt	taaatcccca	gatgaacatg	1800
gcacgtgggt	gattgatgaa	actgctgctg	tcggctttta	cctctcttta	ggcatttggtt	1860
tcgaagcggg	caacaagccg	aaagaactgt	acagcgaaga	ggcagtcaac	ggggaaactc	1920
agcaagcgca	cttacaggcg	attaaagagc	tgatagcgcg	tgacaaaaac	caccacaagcg	1980
tgggtgatgtg	gagtattgcc	aacgaaccgg	ataccgcgtc	gcaaggtgca	cgggaatatt	2040
tcgcgccact	ggcgggaagca	acgcgtaaac	tcgaccgcac	gcgtccgatc	acctgcgtca	2100
atgtaatgtt	ctgcgacgct	cacaccgata	ccatcagcga	tctctttgat	ggggatcctc	2160
cactttttaa	caaagcaggt	gtctctggag	taatcaagct	tgatattggg	cctattaccg	2220
actctagact	agggtcata	aatgttgcca	ttgagagcct	ctcatttgtt	gagtttacta	2280
ctgcccgggtg	atcgacacta	tggtaaattc	cattagtcac	tatctccaaa	atatctccaa	2340
cattcactac	gaacgcatta	ggtagaggtt	tgactgaaat	ccatgtcccc	tctcttttta	2400
tctgtaatcc	ttccacttcg	ttgatttgaa	ggaggattgt	caaaccgcca	aaatccgagt	2460
gcgacgtaag	accgatggcg	agatttggtt	gaggacaagg	actagtcctt	agagtcctgc	2520
tttaatgaga	tatgcgagac	gcctatgatc	gcacgatatt	tgctttcaat	tctgtttgtc	2580
acgttgtaaa	aaacctgagc	atgtgtagct	cagatcctta	ccgccgggtt	cggttcattc	2640
gattccttga	atatatcacc	cgttactatc	gtatttttat	gaataatatt	ctccgttcaa	2700
tttactgatt	gtaccctact	acttatatgt	acaatattaa	aatgaaaaca	atatattgtg	2760
ctgaataggt	ttatagcgac	atctatgata	gagcgccaca	ataacaaaca	attgcgtttt	2820
attattacaa	atccaatttt	aaaaaaaagc	gcagaaccgg	tcaaacctaa	aagactgatt	2880
acataaatct	tattcaaatt	tcaaaagtgc	cccaggggct	agtatctacg	acacaccgag	2940
ggcggaacta	ataacgctca	ctgaaggga	ctccgggttc	ccgccggcgc	gcatgggtga	3000
gattccttga	agttgagtat	tggccgtccg	ctctaccgaa	agttacgggc	accattcaac	3060
ccgggtccagc	acggcgggcg	ggtaaccgac	ttgctgcccc	gagaattatg	cagcattttt	3120
ttggtgtatg	tgggccccaa	atgaagtgca	ggtcaaacct	tgacagtgac	gacaaatcgt	3180
tgggcgggtc	cagggcgaat	tttgcgacaa	catgtcgagg	ctcagcaggg	cgatcgca	3238

<210> 7
 <211> 1067
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> hairpin construct

<400> 7						
ccccttgctc	tcaaccaa	ctcgccatcg	gtcttacg	gcactcggat	tttggcgggt	60
tgacaatcct	ccttcaa	aacgaagtgg	aaggattaca	gataaaaaga	gaggggacat	120
ggatttcagt	caaacctcta	cctaattgctg	tcgtagttaa	tgttggagat	atttttgaga	180
taatgactaa	tggaatttac	catagtgtcg	atcaccgggc	agtagtaaac	tcaacaaatg	240
agaggctctc	aatcgcaaca	tttcatgacc	ctagtctaga	gtcggtaata	ggcccaatat	300
caagcttgat	tactccagag	acacctgctt	tgtttaaaag	tggaaattta	atccccagat	360
gaacatggca	tcgtgggtgat	tgatgaaact	gctgctgtcg	gctttaacct	ctcttttaggc	420
attggtttcg	aagcgggcaa	caagccgaaa	gaactgtaca	gcgaagaggc	agtcaacggg	480
gaaactcagc	aagcgcactt	acaggcgatt	aaagagctga	tagcgcgtga	caaaaaccac	540
ccaagcgtgg	tgatgtggag	tattgccaac	gaaccggata	cccgtccgca	aggtgcacgg	600
gaatatttgc	cgccactggc	ggaagcaacg	cgtaaaactc	acccgacgcg	tccgatcacc	660
tgcgtcaatg	taatgttctg	cgacgctcac	accgatacca	tcagcgatct	ctttgatggg	720
gatcctccac	ttttaacaa	agcaggtgtc	tctggagtaa	tcaagcttga	tattgggcct	780
attaccgact	ctagactagg	gtcatgaaat	gttgcgattg	agagcctctc	atttgtttgag	840
tttactactg	cccgggtgat	gacactatgg	taaattccat	tagtcattat	ctccaaaata	900
tctccaact	tcactacgaa	cgcatttagg	agaggtttga	ctgaaatcca	tgtcccctct	960
ctttttatct	gtaatccttc	cacttcggtt	atttgaagga	ggattgtcaa	accgccaaaa	1020

tccgagtgcg acgtaagacc gatggcgaga tttggttgag gacaagg 1067

<210> 8
 <211> 342
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> sense strand of hairpin construct

<400> 8
 ccttgtcctc aaccaaattc cgccatcggc cttacgtcgc actcggattt tggcggtttg 60
 acaatcctcc ttcaaataca cgaagtggaa ggattacaga taaaaagaga ggggacatgg 120
 atttcagtca aacctctacc taatgcgttc gtagtgaatg ttggagatat tttggagata 180
 atgactaatg gaatttacca tagtgctgat caccgggcag tagtaaaactc aacaaatgag 240
 aggctctcaa tcgcaacatt tcatgaccct agtctagagt cggtaatagg cccaatatca 300
 agcttgatta ctccagagac acctgctttg tttaaaagtg ga 342

<210> 9
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic primer

<400> 9
 taaccgactt gctgccccga 20

<210> 10
 <211> 27
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic primer

<400> 10
 aaatagagat gcttgcagaa gatcccg 27

<210> 11
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic primer

<400> 11
 cgtttgaatc ttgctggccg tgat 24

<210> 12
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic primer

<400> 12
 tagacgagct gcctttggaa gtgt 24

<210> 13
 <211> 21
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Synthetic primer

<400> 13

cgtcttgccg actgatttga a

21

<210> 14

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic primer

<400> 14

cgtttgaatc ttgctggccg tgat

24

<210> 15

<211> 1386

<212> DNA

<213> Artificial Sequence

<220>

<223> T60DM RNA transcript

<400> 15

guucuuaauu	cauuuaauuaa	uuuagaaaaa	ucauggagaa	agcaaaacuu	augaagcuag	60
guaaugguau	ggaaauacca	aguguucaag	aaugggcuaa	acucacgcuu	gccgaaauuc	120
caucucgaua	cguaugcgcc	aaugaaaacc	uuuuguugcc	uaugggugca	ucugucauaa	180
augaucauga	aaccauuccu	gucaucgaua	uagaaaauuu	auuaucucca	gaaccaauaa	240
ucggaaaguu	agaauuagau	aggcuucauu	uugcuugcaa	agaauggggu	uuuuuucagg	300
uagugaacca	uggagucgac	gcuucauugg	uggauagugu	aaaaucagaa	auucaagguu	360
ucuuuaaccu	uucuauggau	gagaaaacua	aaauagaaca	ggaagaugga	gauguggaag	420
gauuuggaca	aggcuuuauu	gaaucaagag	accaaacacu	ugauugggca	gauauauuaa	480
ugauguucac	ucuuccacuc	cauuuaagga	agccucacuu	auuuucaaaa	cucccagugc	540
cucucaggga	gacaaucgaa	uccuacucuu	cagaaaugaa	aaaguuaucc	augguucucu	600
uuauuaagau	ggaaaaagcu	cuacaaguac	aagcagccga	gauuaagggg	augucagagg	660
uguuuauaga	ugggacacaa	gcaaugagga	ugaacuauua	ucccccuugu	ccucaaccaa	720
aucucgccau	cggucuuacg	ucgcacucgg	auuuuggcgg	uuugacaauc	cuccuucaaa	780
ucaacgaagu	ggaaggauua	cagauaaaaa	gagaggggac	auggauuua	gucaaaccuc	840
uaccuaaugc	guucguagug	aauguuggag	auauuuugga	gauaaugacu	aauggaauuu	900
accuauagugu	cgauaccggg	gcaguaguua	acucaacaaa	ugagaggcuc	ucaaucgcaa	960
cauuucauga	cccuagucua	gagucgguaa	uaggcccaau	aucaagcuug	auuacuccag	1020
agacaccugc	uuuguuuuaa	aguggaucua	cauaugggga	ucuuguggag	gauguaaaaa	1080
caaggaagcu	cgauggaaaa	ucauuucuuu	acuccaugag	gauuugaaaa	cucaagaaaa	1140
aaauaauacga	cgugauugca	ugucagauuc	aacuaucuuu	uugucguuuu	uuggugcucg	1200
aguccuuauu	uguuuuugau	auugcuuuug	auucuaauua	auaagacuuu	ucucaagaac	1260
cacauguaau	guaccuuuac	uuucagaaaa	uaaaaaguau	ugaggcacaa	augagaaaau	1320
ugagagagug	cuugagaagu	guaauuucuc	gaaagugcgu	uguguuugaa	aaaaaaaaaa	1380
aaaaaa						1386

<210> 16

<211> 1452

<212> RNA

<213> Artificial Sequence

<220>

<223> CODM RNA transcript

<400> 16

guaaagauug	auauaugauc	ugaagaucug	acaagaaagu	ucaucaaaau	uagaguucuu	60
ggagacacca	auacuuauca	agcuaggcaa	ugguuuguca	auaccaagug	uucaggaauu	120
ggcuaaaacuc	acgcuugcag	aaauuccauc	ucgauacaca	ugcaccggug	aaagcccguu	180
gaauaaauuu	ggugcgucug	uaacagauga	ugaaacaguu	ccugucaucg	auuugcaaaa	240
uuuacuaucu	ccagaacccg	uaguuggaaa	guuagaauug	gauaagcuuc	auucugcuug	300
caaagaauug	gguuucuuuc	agcugguuaa	ccauggaguc	gacgcuuuac	ugauggacaa	360
uauaaaauca	gaaauuaaag	guuucuuuaa	ccuuccaau	aaugagaaaa	cuaaaucagg	420
acagcaagau	ggagauuuug	aaggauuuug	acaaccuuau	auugaaucgg	aggaccaaag	480
acuugauugg	acugaagugu	uuagcauguu	aagucuuccu	cuccauuuua	ggaagccuca	540

pctca2017050951-seq1.txt

uuuguuuucca	gaacucccuc	ugccuuucag	ggagacacug	gaauccuacc	uaucaaaaau	600
gaaaaaacua	ucaacgguug	ucuuugagau	guuggaaaaa	ucucuacaau	uaguugagau	660
uaaagguaug	acagacuuau	uugaagaugg	guugcaaaca	augaggauga	acuaauaucc	720
uccuuguccu	cgaccagagc	uuguacuugg	ucuuacguca	cacucggauu	uuagcgguuu	780
gacaaucuc	cuucaacuua	augaaguuga	aggauuacaa	auaagaaaag	aagagaggug	840
gauuucaauc	aaaccucuac	cugaugcguu	cauagugaau	guuggagaca	uuuuggagau	900
aaugacuaau	gggauuuacc	guagcgucga	gcaccgggca	guaguaaacu	caacaaagga	960
gaggcucuca	aucgcgacau	uucaugacuc	uaaacuagag	ucagaaaauag	gcccacuuuc	1020
gagcuugguc	acaccagaga	caccugcuuu	guucaaaga	gguagguaug	aggauauuuu	1080
gaaggaaaau	cuuucagga	agcuugaugg	aaaaucauuu	cucgacuaca	ugaggauugug	1140
agaaagugug	aacauauauu	auacuccaca	uuguguuuua	uauaugauga	aaauaguugc	1200
uuuugaagua	ugaugaaaua	aguugguuuu	gaagaauuca	uauugugcuu	aaauuucgug	1260
gaugacugag	agauuuauua	uguaauaaua	auguauuggu	uugaagauuc	ucgucucacu	1320
auauguaaga	cucuguuugg	gucaagugau	gaaucacgg	uugaaaauag	uugcuuuuga	1380
agaauucaua	uggugcuuaa	uauuauguaa	uaauaangu	auuggauuga	aaaaaaaaaa	1440
aaaaaaaaaa	aa					1452