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(54) Title: A METHOD FOR REGULATING BIOSYNTHESIS OF MONOLIGNOLS

(57) Abstract: A method for reducing the synthesis of monolignols in cytoplasm of the plant of *Hevea brasiliensis*, comprising the step of down-regulating expression of a nucleic acid template containing at least one nucleotide sequence set forth in SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 or SEQ ID NO. 12 in at least one cell. Also claimed are nucleotide sequences encoding phenylalanine ammonia-lyase 1 (PAL1), phenylalanine ammonia-lyase 2 (PAL2), cinnamate 4-hydroxylase (CH4), coumarate 3-hydroxylase 1 (C3H1), coumarate 3-hydroxylase 2 (C3H2), caffeic acid/5-hydroxyconiferaldehyde O-methyltransferase (COMT), Hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl transferase (HCT), ferulate 5-hydroxylase (F5H), 4-coumarate CoA ligase (4CL), 4-coumarate caffeoyl-CoA O-methyltransferase (CCoAOMT), 4-coumarate cinnamoyl coenzyme A reductase (CCR1), and 4-coumarate cinnamyl alcohol dehydrogenase (CAD) enzymes, all from *Hevea brasiliensis*.



A METHOD FOR REGULATING BIOSYNTHESIS OF MONOLIGNOLS

FIELD OF INVENTION

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The present invention relates to the specific genes encoding for a series of enzymes involved in the biosynthesis of lignin in the plant of *Hevea brasiliensis*. More particularly, the present invention provides the polynucleotide sequences of the genes encoding for the series of enzymes involved in the biosynthesis of the lignin precursors, monolignols, which allow specific genetic intervention to be conducted for regulating the biosynthesis of lignin in *H. brasiliensis*.

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BACKGROUND OF THE INVENTION

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Lignin limits various applications of plant material for fibre, chemical and energy production, therefore, strategies for its down-regulation are of considerable interest. There are three different levels of lignin biosynthesis pathway in plants, namely, the biosynthesis of monolignols, the transport and storage of the synthesized monolignols, as well as the polymerization of the transported monolignols at the targeted sites. In plants, the biosynthesis of lignin precursors, also known as monolignols (p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol) takes place in cytoplasm through phenylpropanoid pathway, these monomers are then transported into the cell wall and oxidized by peroxidases and laccases to phenoxy radicals that couple to form the lignin heteropolymer. Therefore, the biosynthesis process of monolignols is of significant importance as the presence of monolignols initiates the formation of lignin heteropolymer.

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Ten enzyme families are found to be associated with the biosynthesis of monolignols, in which phenylalanine is the first precursor molecule in the biosynthesis pathway.

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The first enzyme involved in the phenylpropanoid pathway is phenylalanine ammonia-lyase (PAL) that causes the deamination of phenylalanine producing *p*-cinnamic acid. The second enzyme involved in this pathway, cinnamate 4-hydroxylase (C4H) which belongs to a cytochrome P-450-linked monooxygenases family, catalyzes the hydroxylation of *p*-cinnamic acid to *p*-coumarate, which is followed by subsequent hydroxylation and methylation steps and the pathway becomes branched.

The hydroxylation steps are catalyzed by two cytochrome P450 enzymes, coumarate 3-hydroxylase (C3H) and ferulate 5-hydroxylase (F5H), which respectively perform the C3 and C5 hydroxylation processes. Another two enzymes, caffeoyl-CoA O-methyltransferase (CcoAOMT) and caffeic acid/5-hydroxyconiferaldehyde O-methyltransferase (COMT) carry out the methylation steps. Hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl transferase (HCT) catalyzes the conversion of *p*-coumaroyl CoA to form caffeoyl CoA. There are only two enzymes which are specific for monolignols biosynthesis pathway to form the lignin subunits, including cinnamoyl coenzyme A reductase (CCR) and cinnamyl alcohol dehydrogenase (CAD). CCR catalyzes the reduction of the hydroxycinnamoyl CoA esters to produce cinamaldehydes; whereas CAD genes catalyzes their reduction to cinnamyl alcohols.

Besides, 4-coumarate CoA ligase (4CL) catalyzes the formation of CoA thioester of cinnamic acids in the biosynthesis of a wide variety of phenolic derivatives, including benzoic acid, condensed tannins, flavonoids, and the cinnamyl alcohols. Coniferaldehyde 5-hydroxylase (Cald5H) is the enzyme responsible for the last hydroxylation of the syringyl-type lignin precursors, it catalyzes hydroxylation of coniferaldehyde to 5-hydroxyconiferaldehyde.

Some of the enzymes involved in the biosynthesis pathway of monolignols have been studied and characterized using various biological sources derived from different plant species. Significant progress has also been made in recent years towards an understanding of lignin biosynthesis through characterization of lignin biosynthetic

pathway enzymes and genes from both herbaceous and tree crops. However, the complete lignin biosynthesis pathway of rubber plant, *H. brasiliensis*, is thus far not provided by the prior art. Past efforts to reduce tree lignin content by down-regulating genes encoding caffeate *O*-methyltransferase or cinnamyl alcohol dehydrogenase did not succeed but instead modified the lignin structure. Therefore, a more innovative and effective method is required for regulating the lignin content in plant by genetic intervention of its biosynthesis pathway.

In view of the fact that the ten enzyme families associated with monolignol biosynthesis, namely, PAL, C4H, 4CL, HCT, C3H, CCoAOMT, F5H, COMT, CCR and CAD play significant roles in regulating the biosynthesis pathway of lignin in *H. brasiliensis*, it is therefore desirable for the industry to completely identify these biosynthetic genes and their encoded proteins, thus utilizing the molecular biology and genetic information to regulate the synthesis of lignin in this plant. Even though these enzymes are also present in other plants apart from *H. brasiliensis*, it is known in the art that genes encoding different enzymes from different species are potentially specific and exclusive. For example, genes encoding the PAL subunits were found to show tissue-specific patterns of expression in several angiosperms. A species-specific approach is hence preferred in order to yield a cost-effective result considering the rubber biosynthesis pathway and genetic makeup of each species of plant are potentially varied among one another.

SUMMARY OF INVENTION

The primary object of the present invention is to provide the polynucleotide sequences encoding for a series of enzymes, including PAL, C4H, 4CL, HCT, C3H, CCoAOMT, F5H, COMT, CCR and CAD which are involved in the biosynthesis of lignin, particularly monolignols, in the plant of *H. brasiliensis*.

Another object of the present invention is to provide the molecular biology and genetic information of the genes and enzymes set forth in the primary object to be exploited for the regulation of the biosynthesis of lignin or monolignols in the plant of *H. brasiliensis*.

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Still another object of the present invention is to provide the isolated polypeptides or the enzymes encoded by the polynucleotide sequences provided.

Yet another object of the present invention is to manipulate or regulate the biosynthesis of lignin in *H. brasiliensis* by genetically intervening the biosynthesis pathway of monolignols.

Further object of the present invention is to produce a transgenic plant of *H. brasiliensis* with reduced monolignols production, which, potentially, demonstrates increased yield of rubber.

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Another further object of the present invention is to provide a potential commercially feasible way to increase the yield of rubber in order to keep up with the increasing global demand on rubber-based products.

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At least one of the preceding objects is met, in whole or in part, by the present invention, in which one of the embodiments of the present invention describes a method for reducing the synthesis of monolignols in cytoplasm of the plant of *H. brasiliensis*, comprising the step of down-regulating expression of a nucleic acid template containing at least one nucleotide sequence set forth in SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 or SEQ ID NO. 12 in at least one cell.

30 In one of the preferred embodiments of the present invention, the down-regulating

step is conducted by introducing a dsRNA having a polynucleotide strand containing nucleotide sequence which is at least 60% complementary to the nucleic acid template in the cell.

- 5 Another preferred embodiment of the present invention discloses that the down-regulating step is conducted by transforming the cell with a recombinant gene construct encoding open reading frame containing nucleotide sequence which is at least 70% identical to SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ
10 ID NO. 10, SEQ ID NO. 11 or SEQ ID NO. 12 and expressing the nucleotide sequence of the gene construct under expression control of a lignification-associated promoter.

- Still another embodiment of the present invention is a transgenic plant of *H. brasiliensis* with reduced monolignol, or potentially lignin content, characterized in
15 that expression of a nucleic acid template containing at least one nucleotide sequence set forth in SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 or SEQ ID NO. 12 in at least one cell is down-regulated.

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To down regulate expression of the nucleic acid template containing the nucleotide sequences as set forth in the preceding embodiment, antisense inhibitory or RNA interference (RNAi) system can be applied.

- 25 Yet another embodiment of the present invention is an isolated polynucleotide encoding enzymes for catalyzing biosynthesis of monolignols in the plant of *H. brasiliensis* comprising nucleotide sequence set forth in SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 12 or
30 any of complementary sequences thereof.

Preferably, the polynucleotide is isolated from the plant of *H. brasiliensis* clone RRIM 600. Still another preferred embodiment of the present invention discloses a recombinant gene construct containing the isolated polynucleotide as set forth in the preceding description.

Further embodiment of the present invention is an isolated polypeptide for catalyzing biosynthesis of monolignols in the plant of *H. brasiliensis* comprising amino acid sequence set forth in SEQ ID NO. 13, SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NO. 16, SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23 or SEQ ID NO. 24. These polypeptides are a series of enzymes involved in the biosynthesis of monolignols, including PAL, C4H, 4CL, HCT, C3H, CCoAOMT, F5H, COMT, CCR and CAD.

With the full availability of the rubber genome sequence, an *in silico* bioinformatic approach can be taken for the complete identification of lignin biosynthesis of *H. brasiliensis*. The present invention is capable of providing the appropriate molecular biology and genetic information of the genes and enzymes involved in the biosynthesis of monolignols, a comprehensive step toward the biosynthetic pathway as well as a basis for further functional analysis. The functional characterization of the polynucleotides and polypeptides of the present invention allows for potential production of plant biomass with expected lignin content and composition for fibre, pulp and biofuel industry. Besides, the present invention also provides *in silico* methods for identification of all related genes and their encoded proteins or enzymes involved in the biosynthesis pathway of monolignols in *H. brasiliensis*.

One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The embodiments described herein are not intended as limitations on the scope of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

5 For the purpose of facilitating an understanding of the invention, there is illustrated in the accompanying drawing the preferred embodiments from an inspection of which when considered in connection with the following description, the invention, its construction and operation and many of its advantages would be readily understood and appreciated.

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Figure 1 shows the nucleotide sequence SEQ ID NO. 1 of the polynucleotide encoding enzyme PAL 1 of the plant *H. brasiliensis* and the amino acid sequence SEQ ID NO. 13 of the polypeptide encoded thereof as described in one of the preferred embodiments of the present invention.

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Figure 2 shows the nucleotide sequence SEQ ID NO. 2 of the polynucleotide encoding enzyme PAL 2 of the plant *H. brasiliensis* and the amino acid sequence SEQ ID NO. 14 of the polypeptide encoded thereof as described in one of the preferred embodiments of the present invention.

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Figure 3 shows the nucleotide sequence SEQ ID NO. 3 of the polynucleotide encoding enzyme C4H of the plant *H. brasiliensis* and the amino acid sequence SEQ ID NO. 15 of the polypeptide encoded thereof as described in one of the preferred embodiments of the present invention.

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Figure 4 shows the nucleotide sequence SEQ ID NO. 4 of the polynucleotide encoding enzyme C3H 1 of the plant *H. brasiliensis* and the amino acid sequence SEQ ID NO. 16 of the polypeptide encoded thereof as described in one of the preferred embodiments of the present invention.

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Figure 5 shows the nucleotide sequence SEQ ID NO. 5 of the polynucleotide encoding enzyme C3H 2 of the plant *H. brasiliensis* and the amino acid sequence SEQ ID NO. 17 of the polypeptide encoded thereof as described in one of the preferred embodiments of the present invention.

Figure 6 shows the nucleotide sequence SEQ ID NO. 6 of the polynucleotide encoding enzyme COMT of the plant *H. brasiliensis* and the amino acid sequence SEQ ID NO. 18 of the polypeptide encoded thereof as described in one of the preferred embodiments of the present invention.

Figure 7 shows the nucleotide sequence SEQ ID NO. 7 of the polynucleotide encoding enzyme HCT of the plant *H. brasiliensis* and the amino acid sequence SEQ ID NO. 19 of the polypeptide encoded thereof as described in one of the preferred embodiments of the present invention.

Figure 8 shows the nucleotide sequence SEQ ID NO. 8 of the polynucleotide encoding enzyme F5H of the plant *H. brasiliensis* and the amino acid sequence SEQ ID NO. 20 of the polypeptide encoded thereof as described in one of the preferred embodiments of the present invention.

Figure 9 shows the nucleotide sequence SEQ ID NO. 9 of the polynucleotide encoding enzyme 4CL of the plant *H. brasiliensis* and the amino acid sequence SEQ ID NO. 21 of the polypeptide encoded thereof as described in one of the preferred embodiments of the present invention.

Figure 10 shows the nucleotide sequence SEQ ID NO. 10 of the polynucleotide encoding enzyme CCoAOMT of the plant *H. brasiliensis* and the amino acid sequence SEQ ID NO. 22 of the polypeptide encoded thereof as described in one of the preferred embodiments of the present invention.

Figure 11 shows the nucleotide sequence SEQ ID NO. 11 of the polynucleotide encoding enzyme CCR 1 of the plant *H. brasiliensis* and the amino acid sequence SEQ ID NO. 23 of the polypeptide encoded thereof as described in one of the preferred embodiments of the present invention.

Figure 12 shows the nucleotide sequence SEQ ID NO. 12 of the polynucleotide encoding enzyme CAD of the plant *H. brasiliensis* and the amino acid sequence SEQ ID NO. 24 of the polypeptide encoded thereof as described in one of the preferred embodiments of the present invention.

Figure 13 is the electrophoresed agarose gel image showing the polymerase chain reaction (PCR) amplification result of PAL gene 1, in which lane L is 1kbp DNA ladder marker, lanes 1 to 3 are samples of PAL gene 1.

Figure 14 is the electrophoresed agarose gel image showing the PCR amplification result of PAL gene 2, in which lane L is 1kbp DNA ladder marker, and lanes 1 to 4 are samples of PAL gene 2.

Figure 15 is the electrophoresed agarose gel image showing the PCR amplification result of C4H gene, in which lane L is 1kbp DNA ladder marker, and lanes 1 and 2 are samples of C4H.

Figure 16 is the electrophoresed agarose gel image showing the PCR amplification result of C3H gene, in which lane L is 1kbp DNA ladder marker, and lanes 1 and 2 are samples of C3H gene 1 and 2, respectively.

Figure 17 is the electrophoresed agarose gel image showing the PCR amplification result of COMT gene, in which 1 to 3 are samples of

COMT gene, lane NTC is negative control, and lane L is 1kbp DNA ladder marker.

5 Figure 18 is the electrophoresed agarose gel image showing the PCR amplification result of HCT gene, in which lane L is 1kbp DNA ladder marker, and lanes 1 to 3 are samples of HCT gene.

10 Figure 19 is the electrophoresed agarose gel image showing the PCR amplification result of F5H gene, in which lane L is 1kbp DNA ladder marker, lanes 1 and 3 are blank, and lanes 2 and 4 are samples of F5H gene.

15 Figure 20 is the electrophoresed agarose gel image showing the PCR amplification result of 4CL gene, in which lane L is 1kbp DNA ladder marker, and lanes 1 and 2 are samples of 4CL gene.

20 Figure 21 is the electrophoresed agarose gel image showing the PCR amplification result of CCR gene, in which lane L is 1kbp DNA ladder marker, and lanes 1 and 2 are samples of CCR gene.

Figure 22 is the electrophoresed agarose gel image showing the PCR amplification result of CCoAOMT gene, in which lane L is 1kbp DNA ladder marker, and lanes 1 and 2 are samples of CCoAOMT gene.

25 Figure 23 is the electrophoresed agarose gel image showing the PCR amplification result of CAD gene, in which lane L is 1kbp DNA ladder marker, lane 1 is negative control and lane 2 is sample of CAD gene.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the specific genes encoding for a series of enzymes involved in the biosynthesis of lignin in the plant of *H. brasiliensis*. More particularly, the present invention provides the polynucleotide sequences of the genes encoding for the series of enzymes involved in the biosynthesis of the lignin precursors, monolignols, including PAL, C4H, 4CL, HCT, C3H, CCoAOMT, F5H, COMT, CCR and CAD, which allow specific genetic intervention to be conducted for regulating the biosynthesis of lignin in *H. brasiliensis*.

Hereinafter, the invention shall be described according to the preferred embodiments of the present invention and by referring to the accompanying description and drawings. However, it is to be understood that limiting the description to the preferred embodiments of the invention and to the drawings is merely to facilitate discussion of the present invention and it is envisioned that those skilled in the art may devise various modifications without departing from the scope of the appended claim.

The following terms used throughout the specification have the indicated meanings unless expressly indicated to have a different meaning.

The term “gene” is defined as the genomic sequence of the plant *H. brasilliensis* particularly polynucleotide sequences encoding polypeptide sequence of the series of enzymes involved in the biosynthesis pathway of monolignols.

The term “polynucleotide”, as used herein, is a nucleic acid chain containing a sequence greater than 100 nucleotides in length.

The term “polypeptide”, as used herein, is a single linear chain of amino acids bonded together by peptide bonds, and having a sequence greater than 100 amino acids in length.

The term "isolated polynucleotide" or "isolated nucleotide sequence" used herein refers to polymer of RNA or DNA acquired from biological sample or produced chemically via any known method in the art. The polymer can be single- or double-stranded.

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The term "primer", as used herein, is an oligonucleotide capable of binding to a target nucleic acid sequence and priming the nucleic acid synthesis. An amplification oligonucleotide as defined herein will preferably be 10 to 50, most preferably 15 to 25 nucleotides in length. While the amplification oligonucleotides of the present invention may be chemically synthesized and such oligonucleotides are not naturally-occurring nucleic acids.

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The abbreviation used throughout the specification to refer to nucleic acids comprising nucleotide sequences are the conventional one-letter abbreviations. Thus, when included in a nucleic acid, the naturally occurring encoding nucleotides are abbreviated as follows: adenine (A), guanine (G), cytosine (C), thymine (T) and uracil (U). Also, unless otherwise specified, the nucleic acid sequences presented herein in the 5'→3' direction.

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As used herein, the term "complementary" and derivatives thereof are used in reference to pairing of nucleic acids by the well-known rules that A pairs with T or U and C pairs with G. Complement can be "partial" or "complete". In partial complement, only some of the nucleic acid bases are matched according to the base pairing rules; while in complete or total complement, all the bases are matched according to the pairing rule. The degree of complement between the nucleic acid strands may have significant effects on the efficiency and strength of hybridization between nucleic acid strands as well known in the art. This may be of particular in detection method that depends upon binding between nucleic acids.

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The term “hybridization” or “hybridizing” used herein refers to the pairing action of two at least substantially complementary strands under appropriate conditions of temperature and ionic strength. The conditions of temperature and ionic strength also control stringency of the “hybridization” defining the extent of complementarity of the two strands needed for successful pairing.

The term “host cell” or “transformed cell” used herein refers to a cell that has received a foreign gene material or a recombinant gene construct and is capable of producing a product according to the genetic information presented in the foreign gene material.

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The term “*in silico*” used herein refers to experiments conducted via computer-based simulations or mathematical models relating to animal biochemistry.

The present invention discloses a method for reducing the synthesis of monolignols in the cytoplasm of the plant of *H. brasiliensis*, comprising the step of down-regulating expression of a nucleic acid template containing at least one nucleotide sequence set forth in SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 or SEQ ID NO. 12 in at least one cell. The present invention is highly lignin-specific with less interference towards the biosynthesis pathway. In more particular, the present method merely genetically intervenes the monolignol biosynthesis pathway in *H. brasiliensis*, which is responsible for lignin production.

The nucleotide sequence of SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 and SEQ ID NO. 12 are respectively shown in Figures 1 to 12. These nucleotide sequences are obtained from the plant of *H. brasiliensis* and respectively encode for the series of catalytic enzymes involved in the biosynthesis pathway of monolignols. SEQ ID NO. 1 and SEQ ID NO. 2 respectively encode for the isoforms PAL 1 and PAL 2 which can catalyze the deamination of

phenylalanine to form cinnamate. Cinnamate can be converted by C4H which is encoded by SEQ ID NO. 3 into *p*-coumarate; whereas *p*-coumarate can then be ligased into *p*-coumaroyl CoA, catalyzed by 4CL which is encoded by SEQ ID NO. 9.

5 The biosynthesis pathway of monolignols in *H. brasiliensis* also involves a series of hydroxylation and methylation steps. SEQ ID NO. 4 and SEQ ID NO. 5 respectively encode for the isoforms of C3H which perform the subsequent C3 hydroxylation steps. Besides, SEQ ID NO. 7 encodes for HCT which also helps in the C3 hydroxylation steps. As for the C5 hydroxylation steps, F5H encoded by SEQ ID NO.
10 8 is involved. The methylation step for C3 can be catalyzed by CCoAOMT encoded by SEQ ID NO. 10; whereas that of C5 can be catalyzed by COMT encoded by SEQ ID NO. 6. Further, SEQ ID NO. 11 and SEQ ID NO. 12 respectively encode for the reductases of CCR and CAD.

15 The present invention is provided to potentially reduce the lignification process of the rubber plant, *H. brasiliensis*, by applying a method for reducing the biosynthesis of monolignols, including the lignin precursors of *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol, which respectively forms the *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) lignin subunits at the end of the pathway. When the H, G and S
20 subunits are formed, they can be transported and then oxidized at a later stage to form lignin heteropolymer. Therefore, reduced availability of the monolignols synthesis-associated enzymes in the cytoplasm or the cells of *H. brasiliensis* is capable of substantially decreasing the biosynthesis process of monolignols, thus substantially prohibits subsequent lignin production therefrom.

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As set forth in the preceding description, the present method aims to disrupt the biosynthesis process of monolignols by genetically intervening the expression of gene encoding the ten families of enzymes involved and reducing the availability of these enzymes. Hence, the availability of monolignols or H, G and S subunits that primes
30 the lignin formation can be regulated accordingly.

The down-regulation process of the nucleic acid template expression can be conducted by two types of genetic intervention approaches. According to one of the preferred embodiments of the present invention, the down-regulation process can be performed by introducing a dsRNA having a polynucleotide strand containing nucleotide sequence which is at least 60% complementary to the nucleic acid template in the cell. This RNAi system is capable of silencing the expression of the related enzymes set forth in the foregoing description.

10 The complementary polynucleotide strand introduced, coupled with the RNA-induced silencing complexes in the transfected cells is able to initiate degradation of the mRNA of the related enzymes and thus prohibits subsequent creation of the corresponding enzymes in the affected cells. Preferably, the mRNA of the related enzymes can have a nucleotide sequence set forth in SEQ ID NO. 1, SEQ ID NO. 2, 15 SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 or SEQ ID NO. 12, respectively encode for the series of specific enzymes involved in the biosynthesis pathway of monolignols.

20 In accordance with another preferred embodiment of the present invention, another genetic intervention, which is the antisense inhibitory system, can also be applied. Accordingly, the down-regulation process is conducted by transforming the cell with a recombinant gene construct encoding open reading frame containing nucleotide sequence which is at least 70% identical to SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID 25 NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 or SEQ ID NO. 12 and expressing the nucleotide sequence of the gene construct under expression control of a lignification-associated promoter.

30 In this antisense inhibitory system, the open reading frame containing nucleotide

sequence which is at least 70% identical to SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 or SEQ ID NO. 12 allows generation of an antisense mRNA which hybridizes to the complementary mRNA having
5 nucleotide sequence set forth in SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 or SEQ ID NO. 12, and thus preventing the transcription process of a particular monolignols synthesis-associated enzyme through their natural pathway. The antisense mRNA may not need complete complementary
10 sequence to successfully hybridize onto the sense mRNA. It may only require at least 70% sequential nucleotides complimentary to the sense mRNA. Preferably, the dsRNA applied in this RNAi system may have a length of 20bp to a few hundreds bp.

Besides, it is also preferably for the expression of the antisense mRNA in the present
15 invention to be initiated by the lignification-associated promoter located at upper stream of the open reading frame. By using the lignification-associated promoter in the present invention, the expression of the antisense mRNA corresponding to the expression of the related target enzymes mRNA in the transfected cell can be activated upon receipt of the particular signal for generating lignin in the *H. brasiliensis*. In
20 other words, the lignification-associated promoter serves as a regulatory mechanism to control expression of the antisense RNA so that only in the specific cell types is signaled by the plant to produce lignin. The present method is capable of controlling the generation of antisense RNA specifically in only those lignin-producing cells, but not other transfected non-lignin-producing cell types. More preferably, tissue-specific
25 promoters can be used to drive the expression of the recombinant gene construct in the plant, so that the desired events can be limited to only the lignifying tissues or lignin-producing cells, thus preventing the disruption of other physiological pathways in the transgenic plant. The transformed genes could then be stably inherited over generations in the genetically modified plant of *Hevea brasiliensis*.

According to another embodiment of the present invention, a transgenic plant of *H. brasiliensis* with potentially reduced monolignol, or lignin content, is provided. The transgenic plant is characterized by its expression of a nucleic acid template containing at least one nucleotide sequence set forth in SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 or SEQ ID NO. 12 in at least one cell therein, which is down-regulated. Preferably, the transgenic plant of the present invention may possess secondary wall with reduced thickness compared to wild type plant of *H. brasiliensis*. Such feature may also be able to ease the rubber-tapping process as well as latex collection from the transgenic plant, thus effecting in the increase of the yield of rubber.

As set forth in the foregoing description, the expression of a nucleic acid template containing at least one nucleotide sequence set forth in SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 or SEQ ID NO. 12 in at least one cell in the transgenic plant of *H. brasiliensis* is down-regulated. The down-regulated expression process can be achieved by antisense inhibitors or RNA interference (RNAi) system as set forth in the preceding description. As set forth in the preceding description, tissue-specific promoters can be used to drive the expression of the recombinant gene construct in the plant so that the desired events can be limited to only the lignifying tissues or lignin-producing cells.

In accordance with yet another embodiment of the present invention, an isolated polynucleotide encoding enzymes for catalyzing biosynthesis of monolignols in the plant of *Hevea brasiliensis* is disclosed. The polynucleotide comprises nucleotide sequence set forth in SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 12 or any of complementary sequences thereof. Preferably, the polynucleotide is isolated from the plant of *H. brasiliensis*

clone RRIM 600. This rubber tree clone is preferably used for the production of natural rubber as it gives higher yield, more adaptable to the environment and known to be less susceptible to climatic variations. Suitable primers or amplification oligonucleotides can be designed from the genomic database available to identify and
5 obtain the polynucleotide of the genes of interest, which encodes the series of monolignols synthesis-associated enzymes. An example of the oligonucleotides sequences used is tabulated in Example 1.

According to the preferred embodiment of the present invention, the partial or
10 complete sequence of these isolated polynucleotides can be utilized for synthesizing partial or complete polynucleotides of the enzymes PAL, C4H, 4CL, HCT, C3H, CCoAOMT, F5H, COMT, CCR or CAD. Still another preferred embodiment of the present invention discloses a recombinant gene construct containing the isolated polynucleotide as set forth in the preceding description. Preferably, the recombinant
15 gene construct further comprises a promoter region operably-linked to control the expression of the nucleic acid template. The recombinant gene construct can be used to facilitate the gene silencing process as set forth in the preceding description. From these polynucleotides or the recombinant gene constructs, polypeptides of the related enzymes can also be synthesized using biological agents such as bacteria or plant. An
20 example of the isolation of polynucleotides and the construction of recombinant gene constructs is further detailed in Example 1.

Further embodiment of the present invention is an isolated polypeptide for catalyzing biosynthesis of monolignols in the plant of *Hevea brasiliensis* comprising amino acid
25 sequence set forth in SEQ ID NO. 13, SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NO. 16, SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23 or SEQ ID NO. 24. These polypeptides are a series of enzymes involved in the biosynthesis of monolignols. They can either be synthesized chemically or biologically. A particular enzyme of interest synthesized can
30 be used in the production of the target lignin precursor or intermediate molecules *in*

vitro for various biological studies or industrial usage.

The present disclosure includes as contained in the appended claims, as well as that of the foregoing description. Although this invention has been described in its preferred
5 form with a degree of particularity, it is understood that the present disclosure of the preferred form has been made only by way of example and that numerous changes in the details of construction and the combination and arrangements of parts may be resorted to without departing from the scope of the invention.

10 EXAMPLE

Examples are provided below to illustrate different aspects and embodiments of the present invention. These examples are not intended in any way to limit the disclosed invention, which is limited only by the claims.

15

Example 1

Total RNA was isolated from the young or tender leaves of 8 months-old *H. brasiliensis* cultivar RRIM 600 using QIAGEN- RNeasy Mini Kit according to the manufacturer's instructions. The quality or the integrity of the RNA was checked by
20 agarose gel electrophoresis and was quantified using Thermo Scientific Nano Drop 2000 as per standard procedures taking. cDNA first strand was synthesized using SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen) following the manufacturer's instructions. The gene was amplified from the cDNA by PCR using the gene specific primers for PAL gene 1, PAL gene 2, C4H, C3H 1, C3H 2, COMT, HCT, F5H,
25 CCoAOMT, 4CL, CCR and CAD respectively, as tabulated in Table 1. The PCR reaction (50 µL) contained 1 µL of cDNA, 20 pmoles of each primers, 5 µL of 10X Pfu Buffer, 5 µL of 2.5 mM dNTP mix and 1.0 unit of PfuTurbo® DNA polymerase (Stratagene). PCR was carried out in Veriti™ Thermal Cycler (Applied Biosystems) using the following conditions. Initial denaturation for 5 min at 95°C followed by 35

- cycles of denaturation at 95°C for 30 sec, annealing at 54-55°C for 30 sec and extension at 72°C for 1 to 2.0 min depending on the length of the targeted gene, with a final extension at 72°C for 7 min. The PCR product was analysed by 1% agarose gel using 1X TAE buffer and the amplicon was eluted from the gel using GENE CLEAN®
- 5 TURBO Gel band elution kit (MP Biomedicals) following the manufacturer's instructions. The PCR products of the respective genes or isolated polynucleotides on the electrophoresed agarose gels are shown in Figures 13 to 23. The purified PCR product was ligated into pCR® 4 Blunt TOPO® Vector (Invitrogen) and transformed into One Shot® Mach1™-T1R Chemically Competent *E. coli* cells (Invitrogen).
 - 10 Plasmids were isolated from putative colonies using QIAprep Spin® Miniprep Kit (Qiagen) following the manufacturer's instructions. The presence of the insert was checked by digesting with EcoRI (NEB) and positive plasmids were subjected to sequencing.

15

Table 1

No.	Gene	Primer sequence	Annealing temperature
1.	PAL GENE 1	Forward ATGGCTACAAACGGAATTGCCTCTG	55°C
		Reverse AAATACAGGGGATCTCTCTTTCCAATC	
2.	PAL GENE 2	Forward ATGGAAGTTTCCGGCGGAG	54°C
		Reverse ACAAATTGGCAGAGGCGTGC	
3.	C4H	Forward ATGGATCTCCTCCTCCTAGAG	54°C
		Reverse AAATGATCTTGGCTTGGCAACAATG	
4.	C3H 1	Forward ATGGCTCTTCTTCTAATACCAATATC	55°C
		Reverse CATTTCACAGCCACACGTTTG	
5.	C3H 2	Forward ATGAGTTTTTCTCTCCTGTTCTTTTC	54°C
		Reverse TTGGGTATCTAAACCCTCATTTTTG	

6.	COMT	Forward ATGGATCAACAAGAGGAAGCATG	55°C
		Reverse CTTGTAGGATTCTATGACAGACC	
7.	HCT	Forward ATGGCACCAACACCACCCAC	55°C
		Reverse CAAGGCAGATACTATAAATTTGGA	
8.	F5H	Forward ATGGAAGCTCTTCTCCAAGCTC	55°C
		Reverse GAGCGGGCACAACAAACGC	
9.	4CL	Forward ATGGCACACGATTGCAGCTTC	54°C
		Reverse CACATATGCCATGGGAAATTGCC	
10.	CCoAOMT	Forward ATGGCATCCAACAATGAACAGC	54°C
		Reverse CTTGATCCGACGGCAGAG	
11.	CCR	Forward ATGTCAATCCAAAACAAAGAGCCAG	55 °C
		Reverse TGATGTTGACATTTTGTGCTTTAGG	
12.	CAD	Forward ATGGGTTAGTCTTGAAACTGAGAGAA	55 °C
		Reverse GGGATCCAGCTTGCTACCG	

Example 2

The nucleotide sequences and the amino acid sequences were analysed by BLASTN and BLASTP programmes respectively. The nucleotide sequences of the polynucleotides as well as the amino acid of the polypeptides isolated are shown in Figures 1 to 12.

CLAIMS

- 1.A method for reducing the synthesis of monolignols in cytoplasm of the plant of *Hevea brasiliensis*, comprising the step of down-regulating expression of a nucleic acid template containing at least one nucleotide sequence set forth in SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 or SEQ ID NO. 12 in at least one cell.
- 2.A method according to claim 1, wherein the down-regulating step is conducted by introducing a dsRNA having a polynucleotide strand containing nucleotide sequence which is at least 60% complementary to the nucleic acid template in the cell.
- 3.A method according to claim 1, wherein the down-regulating step is conducted by transforming the cell with a recombinant gene construct encoding open reading frame containing nucleotide sequence which is at least 70% identical to SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 or SEQ ID NO. 12 and expressing the nucleotide sequence of the gene construct under expression control of a lignification-associated promoter.
- 4.A transgenic plant of *Hevea brasiliensis* with reduced monolignol or lignin content, characterized in that expression of a nucleic acid template containing at least one nucleotide sequence set forth in SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 or SEQ ID NO. 12 in at least one cell is down-regulated.

5.A transgenic plant according to claim 4, wherein the down-regulation of the nucleic acid template containing at least one nucleotide sequence set forth in SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 or SEQ ID NO. 12 is conducted by introducing a dsRNA having a polynucleotide strand containing nucleotide sequence which is at least 60% complementary to the nucleic acid template in the cell.

6.A transgenic plant according to claim 4, wherein the down-regulation of the nucleic acid template containing at least one nucleotide sequence set forth in SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 or SEQ ID NO. 12 is conducted by transforming the cell with a recombinant gene construct encoding open reading frame containing nucleotide sequence which is at least 70% identical to SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 or SEQ ID NO. 12 and expressing the nucleotide sequence of the gene construct under expression control of a lignification-associated promoter.

7.An isolated polynucleotide encoding enzymes for catalyzing biosynthesis of monolignols in the plant of *Hevea brasiliensis* comprising nucleotide sequence set forth in SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 12 or any of complementary sequences thereof.

8.An isolated polynucleotide according to claim 7, wherein the plant of *Hevea brasiliensis* is clone RRIM 600.

9.A recombinant gene construct containing an isolated polynucleotide according to claim 7.

10.An isolated polypeptide for catalyzing biosynthesis of monolignols in the plant of
5 *Hevea brasiliensis* comprising amino acid sequence set forth in SEQ ID NO. 13, SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NO. 16, SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23 or SEQ ID NO. 24.

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SEQ ID NO. 1

ATGGCTACAAACGGAATTGCCTCTGTTTTTGATAGTGAAAATCACCCCTTTTGCAATCCT
TGTGTGCCTTGCCACCACATTGGAAGAAGGCTGCAGAGTCTCTCCAATGTGCCCATTTT
GATGAAGTCTCTCAAATGGTGTCCCAATTTGCCAATGCCAAAACAGTTGAAATCCAAGGT
ACCACCCTCACTGTTGCTCAGGTCACTGCTGTTGCTCGTCGGGTCGAAGTAAATGTTTGC
CTTGACGAGGATGCTGCTCGTGGCCGAGTGGCAAAGAGCGCCGATTGGGTTGCGGTGAAC
ATCTCTCGTGGGACTGACACTTATGGTGTACCACCGGCTTTGGTGCCACTTCTCATAGG
CGTACCAGTAAAACTGGTGACCTTCAGACAGAGCTCATTTCGGTTCCTCAATGCTGGGGTC
ATTGGCAAGGAGAATCTTCCGACCAGTTATTCCAAGGCAGCTATGCTCGTCCGTACAAAC
ACACTAATGCAAGGTTATTCTGGTATACGGTGGGAGATTCTTGAATCTATTGCCAAGCTT
ATGAATGAAAATTTGATCCCTAAGCTGCCTCTGAGAGGGACTATCACAGCTTCAGGCGAT
CTTGTGCCATTGTCTTATATTGCTGGTTTGTAACTGGCCGTCACAATTCCAAGGTAGAA
ACCCCTCAAGGGGAAGTGATTACAGCTATTGAAGCTTTGAAGAGAGCAGGAATTGAGGCT
CCATTCGAGCTACAAGCGAAGGAAGGTTTGGCTCTTGTGAATGGAACAGCGGTTGGCTCA
GCTGTAGCTGCAACTGTTTGTGTTTGATGCCAATATTTTGGCTCTGCTAGCAGAAATTCTC
TCTGCTTTGTTCTGTGAGGTCATGCATGGAAAGCCAGAGTACACAGACCCACTTACTCAT
GAGCTTAAGCATCATCCTGGCCAGATTGAAGCAGCTGCTATCATGAAACACCTGTTAGAT
GAAAGTGACTACATGAAAGAAGCAAAAATTCGCCATGAGAAAGACCCGCTTACAAAGCCT
AAACAGGACAGATATGCAATTAGAACCTCTCCACAGTGGCTGGGTCCACAAATTGAGGTT
ATTCGGGCAGCCACTCATTCAATTGAGAGGGAGATCAATTCTGTCAACGATAATCCATTG
ATTGATGTAGCTCGAGACCTTGCCCTTCATGGAGGAAATTTCCAAGGAACACCAATTGGT
GTTTCTATGGACAATCTCAGGATCGCCATTGCAGCAATTGGAAAGCTCATGTTTGCTCAG
TTCTCTGAACTCGTTTGCGACTACTACAACAATGGGTTGCCTTCCAATTTGAGCGGAGGA
CCTAATCCAAGCTTGGACTATGGATTAAAAGGTGCAGAAATAGCGATGGCCGCATATTGC
TCAGAGCTTCAGTACTTGGCAAATCCTGTAATACTCATGTTCAAAGTGCTGAACAGCAC
AATCAAGATGTGAACTCTCTAGGCTTGATTTCGGCAAGAAAAAGCGCTGAAGCCATTGAA
ATCCTGAAGCTTATGTCCGCGACTTTCATGGTGCCACTTTGCCAGGCAATTGATCTAAGG
CATTTGGAGGAGAACATGAGGGAAGTTGTGAAACAAGTGCTCCGCCAGGCGGTAAGGAAA
ATCCTATATATTTCTGAAGATGGAATGCTGCTCGAATCGCGTTTCTGCGAGAAAGAGCTC
TTGACAGTTTGTGAGCATCAACCTGTATTCTCCTATCTCGATGACCCAACAAATCCTTCT
TATGCACTTCTGCCTAAACTCCAAGAGGTAATCGTCAAGAAAGCACTTAAAGATCCTAAA
TCTGAACGTGCTGATGAAAATGGCTACACAATATTCAAGCGAATACCAGTTTTTATGGAG
GAATTGAAGGCAAGACTGGAAGAAGAAGTGCAAAAGCGAGAGGGAGATTTGATGGTGGA
GAATTTGCAATAGCAAACAGGATCAAGAAATGCAGGACTTATCCAATATATGAGTTTGTG
AGAAGTGAAAGTGGGGACTGAGCTTCTTAGTGGGGAAAAGAAAGTGAGCCAGGAGAGGAT
ATAGAGAAAGTCCACGAGGCTATCAATGAAGGGAAACTTGGGGAGGTTTTGATCAAGTGC
TTAGCAGATTGGAAAGAGAGATCCCTGTATTTAA

SEQ ID NO. 13

MATNGIASVFDSENHPPFCNPCVPCPPHWKKAAESLQCAHFDEVSQMV SQF
ANAKTVEIQGTTLTVAQVTAVARRVEVNVCLDEDAARGRVAKSADWVAVN
ISRGTDTYGVTTGFGATSHRRTSKTGD LQTELIRFLNAGVIGKENLPTSYK
AAMLVRTNTLMQGYSGIRWEILESIAKLMNENLIPKLPLRGTTITASGDLVPL
SYIAGLLTGRHNSKVETPQGEVITAIEALKRAGIEAPFELQAKEGLALVNGT
AVGSAVAATVCFDANILALLAEILSALFCEVMH GKPEYTDPLTHELKHHPG
QIEAAAIMKHLLEDSDYMKEAKIRHEKDPLTKPKQDRYAIRTSPQWLGPQI
EVIRAATHSIEREINSVNDNPLIDVARDLALHGGNFQGTPIGVSMNLR IAI
AAIGKLMFAQFSELVCDYYNNGLP SNLSGGPNPSLDYGLKGAEIAMAAYCS
ELQYLANPVTTHVQSAEQHNQDVNSLGLISARKSAEAI EILKLMSATFMVA
LCQAIDLRHLEENMREVVKQVLRQAVRKTLYISEDGM LLESRFCEKELLQ
VVEHQPVFSYLD DPTNPSYALLPKLQEV LVKKALKDPKSERADENGYTIFK
RIPVFMEELKARLEEEVSKARGRFDGGEFAIANRIKKCR TYPIYEFVRSEVG
TELLSGEKKVSPGEDIEKVHEAINEGKLGEVLIKCLADWKERSPVF Stop

Figure 1

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SEQ ID NO. 2

ATGGAAGTTTCCGGCGGAGAGATAAATGGAGATCATATATATAGCAATGGGTTATGCGTA
AAGGACCCATTGAAGTGGGGTCTAGCGGCGGAGGCATTGAAGGGTAGCCATGTAGAGGAG
GTGAAGAAAATGGTTAAGGAATATAGGAAGGCGGTGGTTCGCCTTGGGGGTGAGACGCTA
ACCGTAGGACAGGTAGCTGCGGTGGCTACTAGAGGTGGCAATGGAGTAACGGTGGAGTTA
TGTGAGGCGGCCAGGATTGGTGTGAAGGCAAGCAGTGATTGGGTAATGGAGAGTATGAAT
AAGGGGACAGATAGTTATGGGGTGTAGTACAGGGTTCGGGGCCACTTCCCATAGGAGAACC
AAGCAAGGAGCTGCCCTGCAGCAGGAGCTTATCAGGTTCTTGAATGCTGGAATCTTTGGC
AAAGGAGTAGAATCAGGCCACATATTGCCACATACAGCAACAAGGGCTGCTATGCTTGTG
AGGATCAATACTCTTCTCCATGGCTACTCAGGCATCAGGTTTGAGATCCTAGAGGCCGTT
ACAAAGCTAATTAATCACAACGTCACTCCATGCTTGCCTCTGCGCGGCTCAATAACTGCC
TCGGGTGATTTAGTACCCCTTTTCGTACATTGCCGGACTCCTAACAGGCAGACCGAATTCC
ACTGCGGTGGGGCCTAAAGGAGAATCACTTGATGCCGGAGAAGCCTTTCGCCTGGCTGGT
ATTGGTGGCGGATTTTTTCAGTTGCAGCCTAAGGAGGGTCTTGCAATTGTTAATGGCACT
GGAGTTGGTGTGCTGGCTTGGCATCTATAGTACTTTTTGATGCTAATATATTGACTCTTTTA
TCAGAAGTTTTGTCTGCAATTTTCGCTGAAGTTATGCAAGGAAAACCTGAGTTTACAGAT
CATTTGACACACAAATTAAGCATCATCCTGGACAGATTGAAGCTGCAGCTATTATGGAA
CACATTTTGGATGGAAGTTCATACGTTCAAGGAGGCAAAGAAATTACATGAAATGGATCCC
CTGCAGAAGCCTAAGCAGGATCGGTACGCTCTTCGCACTTCACCACAATGGTTAGGTCCA
CAAATTGAAGTGATTTCGATCATCAACCAAGTCAATTGAAAGGGAGATTAAATTCGGTGAAT
GATAATCCACTGATCGATGTGTCAAGGAACAAAGCCTTGCACGGAGGGAATTTCCAGGGC
ACTCCAATTGGTGTTCATGGATAACACTAGATTGGCATTAGCTTCCATAGGGAAACTC
ATGTTTGTCTCAATTCTCTGAGCTGGTTAATGATTTCTACAATAATGGTTTGCCTTCAAAT
CTCACAGGAGGAAGGAATCCTAGCTTGGATTATGGTTTTAAGGGTGCTGAGATAGCCATG
GCAGCCTATTGCTCTGAGCTCCAATACCTTGCGAACCTGTTACAAACCATGTCCAAAGT
GCTGAGCAACATAATCAAGATGTCAACTCATTAGGGCTGATTTCTTCAAGGAAAACAGCA
GAAGCAGTTGATATCTTGAAGCTCATGTCCTCAACTTTCTTGGTTGCACTTTGCCAGGCA
ATAGATTTAAGGCATTTAGAAGAGAATTTGAAGAGCACAGTTAAGAACACAGTGAGCCAG
GTGGCTAAGAAAGTTCTAACCATTGGAGTCAATGGTGAGCTTCATCCTTCAAGATTCTGC
GAGAAAGATCTGCTCAGGGTAGTCGATCGCGAGCAAGTGTTTGCATACATTGATGATCCT
TGTAGTTCAACATACCCACTAATGCAGAAGATGAGACAAGTCATTGTGGATCATGCATTA
ACCAACAATGATTATATAAACAACATAGATGCTTCAATCTTCCTCAAGATAGGTGCTTTC
GAAGACGAATTGAAGTCTGTCTTGCCAACAGAAGTAGAAGCTGCAAGAAGTGGTTTTGAG
AGTGGAATCCTGCAATTCCAAACAGAATCAAGGACTGCAGGTCTTATCCACTGTACAAG
TTTGTGAAAGAAGATTTGGGAAGTGAAGTCTTAACAGGAGAGAACTCAGGTCTCCTGGC
GAGGAATTTGATAAGTTGTTTACAGCAATTTGTGAAGGAAAGATTATTGATCCTCTCCTT
GAATGTCTTAAGGATTGGGATGGCACGCCTCTGCCAATTTGTTAG

SEQ ID NO. 14

MEVSGGEINGDHIYSNGLCVKDPLKWGLAAEALKGSHVEEVKKMVKEYR
KAVVRLGGETLTVGQVA AVATRGNGVTVELCEAARIGVKASSDWVMES
MNKGTDSYGVSTGFGATSHRRTKQGAALQQELIRFLNAGIFGKGVESGHIL
PHTATRAAMLVRINTLLHGYSGIRFEILEAVTKLINHNVTPLPLRGSITASG
DLVPFSYIAGLLTGRPNSTAVGPKGESLDAGEAFRLAGIGGGFFQLQPKEGL
AIVNGTGVGAGLASIVLFDANILTLSEVLSAIFAEVMQ GKPEFTDHLTHKL
KHHPGQIEAAAIMEHILDGSSYVQEA KKLHEMDPLQKPKQDRYALRTSPQ
WLGPPQIEVIRSSTKSIEREINSVNDNPLIDVSRNKALHGGNFQGTPIGVSM
NTRLALASIGKLMFAQFSELVNDFYNNGLPSNLTGGRNPSLDYGFKGAEIA
MAAYCSELQYLANPVTNHVQSAEQHNQDVNSLGLISSRKTA EAVDILKLMS
STFLVALCQAIDLRHLEENLKVSTVKNQTVS VAKKVLTIGVNGELHPSRFCE
KDLLRVVDREQVFAYIDDPSCSTYPLMQKMRQVIVDHALTNNDYINNIDAS
IFLKIGAFEDELKSVLPTEVEAARSGFESGNPAIPNRIKDCRSYPLYKFVKE
DLGTELLTGEKLRSPGEEFDKLF TAICEGKIIDPLLECLKDWDGTPLPIC Stop

Figure 2

SEQ ID NO. 3

ATGGATCTCCTCCTCTAGAGAAGACCCTTTTGGGTCTCTTCTTTGCTTTTATCGTGGCC
ATTGTCGTTTCTAAATTACGCGGCAAGCGTTTAAAGCTCCCGCCGGGTCCTTTACCTGTG
CCAGTTTTTGGCAACTGGCTTCAAGTCGGCGATGACTTGAACCACCGCAACCTCACAGAA
CTCGCTAAGAAATTCGGTGACATCTTGCTTCTCCGTATGGGGCAACGTAATCTCGCAGTT
GTCTCTTCACCGGAGTTAGCTAGGGAGGTTTTACATACTCAAGGTGTTGAGTTCGGGTCA
AGAGCCCCGAATGTTGTCTTTGATATTTTACCGGTAAGGGACAAGACATGGTGTTCACC
GTGTACGGTGAGCACTGGAGGAAGATGAGGCGGATTATGACAGTTCATTTTTACAAAC
AAGGTTGTTGAGCAGTATCGATATGGGTGGGAAGACGAGGCGACGAAAGTGGTGGAGGAT
GTGAAGAAGAACCCAGAGGCTGCCACAAGTGGTATTGTGTTGAGGCGGCGGTTGCAGCTG
ATGATGTATAATAATATGTACAGGATTATGTTTGACAGGAGATTTGAGAGCGTGGAGGAT
CCATTGTTTGTGAAGCTGAAGGCTTTGAATGGAGAGAGGAGTAGATTGGCTCAGAGCTTT
GAGTATAATTATGGTGATTTTATCCCATTTTGAAGACCTTTCTTGAGAGGTTACTTGAAG
ATTTGTAAGGAAGTGAAGGACATGAGATTGCAGCTCTTCAAGGACTACTTTGTTGAAGAG
AGGAAGAACTTTCAAGCACCAAGAGCATGAACAATGAAGGGTTGAAATGCGCGATAGAT
CATATTTTGGACTCACAGCAGAAGGGAGAGATCAATGAGGATAACGTTCTTTACATTGTT
GAGAATATCAACGTCGCGAGCAATTGAAACAACGCTATGGTCGATTGAGTGGGGAATTGCT
GAGCTTGTGAACCATCCCGAAATCCAAAAGAAGCTGCGAGATGAGCTTGACACTGTGCTT
GGACCTGGTCACCAAGTCACTGAGCCTGACACCCACAAGCTCCCCTATCTTCAGGCTGTT
ATCAAAGAGACTCTCCGTCTCAGAATGGCAATTCCTCTCCTTGTCCTCCACATGAATCTC
CACGACGCAACGCTTAAAGGTTTCGATATTCCTGCAGAAAGCAAAATTTTGGTGAATGCT
TGGTGGCTTGCCAACAATCCTGCTCACTGGAAGAATCCAGGAGAATTCAGGCCAGAGAGG
TTCTTGGAAGAGGAGTCCAACGTTGAGGCTAATGGAAATGATTTCAGGTACCTGCCATTC
GGTGTGGAAGAAGAAGCTGCCCTGGAATTATTCTTGCAATTGCCAATTCTTGGTATTACT
TTGGGACGTTTGGTACAGAATTTGAGCTCTTACCACCTCCAGGACAGTCAAATCTTGAT
ACCACAGAGAAAGGTGGACAATTCAGCTTGCATATATTGAAGCACTCCACCATTGTTGCC
AAGCCAAGATCATTTTAA

SEQ ID NO. 15

MDLLLLLEKTLGLFFAFIVAIVVSKLRGKRFLPPGPPLPVPVFGNWLQVGD
DLNHRNLTELAKKFGDILLRMGQRNLAVVSSPELAREVLHTQGVEFGSR
ARNVVFDIFTGKGQDMVFTVYGEHWRKMRRIMTVPFFTNKVVQQYRYGW
EDEATKVVEDVKKNPEAATSGIVLRRRLQLMMYNNMYRIMFDRRFESVED
PLFVKLKALNGERSRLAQSFYNYGDFIPILRPFLRGYLIKKEVKDMRLQ
LFKDYFVEERKKLSSTKSMNNEGLKCAIDHILDSQQKGEINEDNVLYIVEN
INVAAIETTLWSIEWGIAELVNHPEIQKKLRDELDTVLGPGHQVTEPDTHK
LPYLQAVIKETLRLRMAIPLLVPHMNLHDATLKGFDPAESKILVNAWWLA
NNPAHWKNPGEFRPERFLEESNVEANGNDFRYLPFGVGRRSCPGIILALPI
LGITLGRVLVQNFELLPPPGQSNLDTTEKGGQFSLHILKHSTIVAKPRSF Stop

Figure 3

SEQ ID NO. 4

ATGGGCTCTTCTTCTAATACCAATATCAATCCTCCTCCTCCTCCTTAGCATACAAACTCTACTTCCGC
ATCCGCTTCAAACCTCGCCCCCGGCCACGCCCTTGGCCGATCGTCGGCAACCTCTACGACGTCAAG
CCAGTTAGGTTCCGATGCTACGCCGAGTGGGCTAAGTCTTATGGTCCGATCATTTTCAGTTTGGCTTGG
TTCAACTTTAAATGTGATCGTTTCGAATACAGAATTGGCGAAAGAAGTGCTGAAGGAACATGATCAA
CAGTTAGCTGATCGGCATCGGAGTAGGTCAGCTGCAAAGTTTAGCAAAGATGGGCAGGATCTTATAT
GGGCTGACTATGGACCTCACTATGTGAAGGCTAGGAAAGTGTGTACACTTGAGCTTTTTACACCTAA
GAGACTCGAGGCTTTGAAACCCATTAGGGAAGATGAGGTCAGTCCATGGTTGAATCCATTTTCAAC
GACTGCACCAATCCTGAAACCAATGGCAAAAGTTTGCTAGTGAAGAAGTATTTGGGAGCAGTTGCA
TTCAACAACATAACAAGGCTTGCATTTGGTAAGCGATTTGTGAAGTCCGGAGGGCATAATTGACGAGC
AAGGCCAAGAATTTAAGGCAATTGTGGCCAACGGTCTTAAGCTAGGTGCATCACTTGCCATGGCAG
AGCACATTCCATGGCTTCGCTGGATGTTCCCACTAGAGGAGGAGGCTTTCCGCAAGCATGGTGCTCG
AAGGGACCGTCTCACTCGAGCTATTATGGAAGAACATACACTTGCTCGCCAGAAGAGTGAGGAGTGC
CAAGCAACATTTTGTGATGCATTGCTTACATTACAGGAGAAATATGACCTTAGTGAGGACACAATTA
TTGGACTCCTTTGGGATATGATTACTGCTGGCATGGACACAACCTGCAATCACTGTAGAATGGGCAAT
GGCTGAGCTAATTAAGAACCCAAGGGTGCAACAGAAGGCTCAAGAGGAGCTAGACCGTGTCATTG
GGTTCGAACGAGTGTTGACTGAGGCTGATTTCTCAAGTCTCCCTTACCTACAATGTGTGGCTAAAGA
GGGACTAAGGTTGCATCCTCCAACACCGCTAATGCTTCCTCACCGTGCCAATTCTAATGTCAAGATT
GGTGGCTATGATATACCTAAGGGATCAAATGTTCTTGTAATGTATGGGCTGTAGCTCGTGATCCAGC
AGTCTGGAAGAACCCAGAAGAGTTTCGACCGGAGAGGTTCTCGAGGAGGATGTTGACATGAAGG
GTCATGATTTCCGGCTACTTCCATTTGGTGCTGGGAGGAGGGTGTCCTGGTACACAACACTGGTAT
CAATTTGGTCACGTCTATGTTAGGTCACCTATTGACCATTTTTCGTTGGACACCACTGATGGTGTGA
AGCCAGGGGAAATTGATATGTCAGAAAATCCTGGATTGGTCACTTCATGAAAACCCCATTAACAAGC
AGTGGCAACCCCTCGGCTGCCTTCAGAAATTGTACAAACGTGTGGCTGTGGAAATGTAA

SEQ ID NO. 16

MALLLIPISILLLLLLLAYKLYFRIRFKLAPGPRPWPIVGNLYDVKPVFRFRCYA
EWAKSYGPIISVWLGSTLNVIIVSNTELAKEVLKEHDQQLADRHRSRSAKF
SKDGQDLIWADYGPHYVKARKVCTLELFTPKRLEALKPIREDEV TAMVESI
FNDCTNPETNGKSLLVKKYLGAFAFNITRLAFGKR FVNSEGIIDEQQQEF
KAIVANGLKLGASLAMA EHIPWLRWMFPLEEEAF AKHGARRDRLTRAIMEE
HTLARQKSGGAKQH FVDALLTLQEKYDLSEDTIIGLLWDMITAGMDTTAIT
VEWAMAELIKNPRVQQKAQEELDRVIGFERVLTEADFSSLPYLQCVAKEGL
RLHPPTPLMLPHRANSNVKIGGYDIPKGSNVLNVWAVARDPAVWKNPEE
FRPERFLEEDVDMKGHDFRLLPFGAGRRVCPGTQLGINLVTSMLGHLH
FRWTPPDGVKPG EIDMSEN PGLVTFMKTPLQAVATPRLPSELYKRVAVEM
Stop

Figure 4

5/16

SEQ ID NO. 5

ATGAGTTTTTCTCTCCTGTTCTTTTCCATCATATTCATCTTCCTTGCTTACGAATATTCG
TACCGCAGGCTAAAATACAAGCTCCCTTCAGGACCCTTTCCATGGCCCATACTGGGAAAC
CTTCCCTATCTTGAACCAGACATTTTCCAGTGTCTTGACAAGTGGTCACAAATTTATGGT
CAAATTATATCGCTCTGGCTTGGTTCCACCTTAAACGTTGTGGTCTCATCCTCCGAGTTG
GCCAAGGAAGTGCTCAAAGACAAGGATCAGCTATTGGCTCATAGGCCAAGAAATAAGCCT
GTGAGTATAATGTCAAAGATGGCAAAGGCATTCTGTGGGCAGATTATGGTCCTCATTAC
GTTAAACTGAGAAAAATTTGTACTCTCGAGCTCTTCTCCCCAAAGGGTATTGAAGCTCTC
AGACCTATCAGAGAAGGTGAAGTCAGAGCTATGGTTGAGTCAATTTTTAAAGAGACTATT
AATCCTGATAATTATAGCAAGATTTTGTACTTAGAAAGTATTTGCAGCCCGTAGTCTCA
AACAGCGTTACAATGTTAGTATTAGGGAAGCGATTCTTGTCTAGAGGTGGTGTGACAAGC
GAACTGGGACAGGAGTTTAAGGCAACATTGGGTAATGAAATGAAGCTTGCAGGCTCATT
TTCCCTGCCGAGCACTTCTGGTGGCTAAATTGGATCTTTTGGCTCCGGAATAACACGTTT
TCCAGGCAGCTGGCACGTAGAGACAAGCTCATTCGAGCTGTAATAGAGGCTTACAGGGCT
GAAAAAAGGAATGGTGCTAATGCCAAGCCAAATCTTTTAGATTCATTCTTGGTTTCCAA
GAACTTATGGCCTTAGCGAGGAAAGCATTAAAGAACTACTCTGGGACGTAACTCAAGTGA
GGCCTGGAGACAAGTAGTATAGTTGTTGAATGCGCCATGGCTCAATTGGTCAAGAATCCA
AGGGTGCAATTCAGGGCTCAAGAAGAGCTTGACAAAGTAATTGGGCTCAACCGTGTAATG
GCCGAATCCGATATCTCTAACTCCCTTACCTGAAATGCATTGTCAAGGAGGCACTAAGG
TTGCACCCTCCAGCCCCATTGCTGCTACCTCACAAGGCAAATTCCAATATCAAGATTGGT
GGCTACGACGTTCTTAAGGGAACCTACGTTGATGTGAATGTTAGGGCCATAGGACGTGAC
CCAGCAGTGTGGCAAGACCCAGTAGAATTTAGACCTGAAAGGTTCTTGGAGAAAGATGTA
GAAATTAAGGGTCATGATTTTCGATTGCTGCCATTTGGTGCAGGAAGGCGAATGTGCCCT
GCTGCCCAATTGGGCATGAATTTGGTTACGTCGATGTTGGGTGAGCTGTTGCATAATTT
AGCTGGGCTCTTCTGAAGGGGTACTGCCTGAAGAGGTTGACTTGTGTGGGATCTCAGGG
ATGGCTAGTTTCATGCAGAAACCATTACAAGTTGTGCCTAGTGTGCGATTGCCAGCCAC
TTGTACAAAAATGAGGGTTTAGATACCCAATAG

SEQ ID NO. 17

MSFSLFFSIIIFIFLAYEYSYRRLKYKLPSGPPFPWPILGNLPYLEPDIFQCLD
KWSQIYGQIISLWLGSTLNVVSSSELAKEVLKDKDQLLAHRPRNKPVSIM
SKDGKGILWADYGPHYVKLRKICTLELFSPKGIEALRPIREGEVRAMVESIF
KETINPDNYSKILLRKYLPVVSNSVTMLVLGKRFLSRGGVTSELGQEFK
ATLGNEMLAGSFFPAEHFWLNLWIFWLRNNTFSRQLARRDKLIRAVIEA
YRAEKRNGANAKPNLLDSLLGFQETYGLSEESI KELLWDVTTAGLETSSIV
VECAMAQLVKNPRVQFRAQEELDKVIGLNRVMAESDISKLPLYLKCIVKEAL
RLHPPAPLLLPHKANSNIKIGGYDVPKGTHVHVNVRAIGRDPVWQDPVEF
RPERFLEKDVEIKGHDFRLLPFGAGRMRMCPAAQLGMNLVTSMLGQLLHNF
SWALPEGVLPEEVDLCGISGMASFMQKPLQVPSVRLPAHLYKNEGLDTQ
Stop

Figure 5

SEQ ID NO. 6

ATGGATCAACAAGAGGAAGCATGTCTAGATGCCTTGATATTTCTGGCTCTCATGTATTT
CCTCTGGTTCTTCACACAGCTATCGACCTAAATCTTTTGATATCATAGCAAAAGCAGGC
CCTGGAGCTTATGTTTCTGCTTCTGAAATTGCCTCTCAGCTTCCCACAACAAATCCTGAT
GCCCCCTTCTATGCTTGATCGTATCCTGCGTCTCTTCGCCGCTCACTCTCTCCTCTCCTAC
ACCTCACGAACCTCTCGAAGATGGCAGGATTGAAAACTTTATGGCCTAACACCTGCCTGT
AAATTCTTTATTGGGGCTGTAGAAGAAGGCAATATCGCTCCCATATCTGCTCTTGCCTAC
CACAGAGCCACTTTGGAGGTTTGGTTACATATGAAGGACCTAATTCTTGAAGGTGGAAAT
CTGTTCAAGAAAGTTCATGGGATGTCTATATTCGAGTACATGAACAAAGATCCAGAATTC
AACACCATCTTCAACCAGGCAATAATGGCTGGTGGTCTCTCTACAGTAGTCATTATGAAT
GGAATTCTAGCAATTTACAAAGGATTCGAGGGACTCACTTCACTTGTTGATGTTGGTGGT
GGCACTGGCAGGACTCTCAATATGATCATTTCCTCAAGTACCCTTCCATCAAAGGCATCAAC
TATGATCTCCCTCATGTGATCCAAACTGCACCACCTTATCCTGGAATCCAGCATGTGGGA
GGAAATATGCTCACAAGCATTCCACAAGCAGAAGCAATCATGATAAAGGATATATGCCAC
AACTGGAGTGATGAAAATGTGGTGAAAGTATTGAAGAACATTTATGAGGTGCTGCCAAAT
AATGGGAAACTTATAGTGATGAATGTATTATTACCAGAAGAACCAGAGACAAGCAAGGCT
TCTCAATATGTTTCAAGACTTGACAATACCATGTTATCCCAACCTTCAGGCAAAGAAAGG
ACTGCAAGAGAATTTGAAGTCTTGGCTAGAGCAGCTGGGTTCACAACTTCAAAGTTGCT
TGTGTTGCTCATGGTATTTGGTCTGTCATAGAATCCTACAAGTAA

SEQ ID NO. 18

MDQQEEACLDALIFSGSHVFPVLVHTAIDLNLFDIIAKAGPGAYVSASEIAS
QLPTTNPDPAPSMLDRILRLFAAHSLLSYTSRTLEDGRIEKLYGLTPACKFFI
GAVEEGNIAPISALAYHRATLEVWLHMKDLILEGGNLFKKVHGMSIFEYM
NKDPEFNTIFNQAIMAGGLSTVIMNGILAIYKGFEGLTSLVDVGGGTGRT
LNMIISKYPSIKGINYDLPHVIQTAPPYPGIQHVGGNMLTSIPQAEAIMIKDI
CHNWSDENVVVKVLKNIYEVLPNNGKLIVMNVLLPEEPETSKASQYVSRLD
NTMLSQPSGKERTAREFEVLARAAGFTNFKVACVAHGIWSVIESYK Stop

Figure 6

7/16

SEQ ID NO. 7

ATGGCACCAACACCACCCACCTCCCTAGAGTTCAAGGTTTCATAGGCGTGAACCTGAACTA
ATCGCCCCTGCTAAGCCTACCCCTCATGAGTTCAAACCATTATCGGACATTGATGACCAA
GAGGGTCTTCGATTTACATTCCGGTGATACAGTTTTATCGGTATGATCCCTCCATGCAA
GGGAGAGATCCTGTTATGGTGATAAGAGAGGCACTCGCTAGAACACTAGTGTTTTACTAT
CCATTTGCTGGTAGGCTCAGGGAAGGGCCTAAACGCAAGCTTATAGTGGAATGTACCAGT
GAAGGTGTGTTGTTTATTGAGGCTGAAGCTGATGTTGCTCTTGAGCAATTCCGTGATGCA
CTTCAACCTCCATTCCCTTGCTTGGAAGAGCTTCTTTTTGATGTCCCTGGCTCCAGTGGG
GTGCTTAACTGCCCTTGCTGCTTATTCAGGTGACGCGCCTAAAATGCGGTGGTTTTATA
TTTGCTCTCCGTCTCAACCACACCATGAGCGATGCTCCTGGCCTAGTCCAATTCATGTCC
ACCGTAGGAGAGATGGCACGTGGCGCACAAGCGCCGTCTGTTCTTCCGGTATGGGAAAGG
CATTTCTTAACGCTCGGGATCCACCACGTGTGACACGCACCCATCGTGAATACGATGAG
GTTGCCGACACCAAAGGCACAATAATCCCGCTCGATGACATGGCTCACCAATCATTCTTC
TTTGGAACCAATGAAGTCGCTGCTCTCCGAAGATTTGTGCCGAAGCACCTCCGCCAATGC
TCTAACTTTGAATTATTGACGGCATGTCTGTGGAAGTGCCGTACCATTGCACTTCAACCA
GATCCCACTGAAGAGATGCGTATTATTGTATTGTCAATGGACGCAACAAATTCAATCCT
CCGTTACCGACTGGATATTACGGCAACGGATTTGTGTTTCCGGTGGCGGTGGCAAGAGCA
AAAGAACTAATTGAGAAACCGCTAGGCTTCGCATTAGAATTGGTAAAAGAGGTTAAAGAT
AGTGTGACGGAAGAGTACATGCGATCCGTGGCGGATCTAATGGTGATTAAAGGTAGGCCC
CATTTTACAGTGGTGAGGAGTTACCTAGTGTGCGATGTGACGCGTGCAGGATTTGGAGAG
GTGGATTTTGGATGGGGTAAGGCTGCATATGGTGGGCCAGCGAAAGGTGGGGTAGGGGCC
ATTCCTGGAGTGGCAAGCTTTATATACCGTTTCAGAAACAAGAAAGGAGAAAATGGGATT
GTGTTGCCAATTTGCTTGCCAGCACAGGCAATGGAACGATTTGTAAAGGAGCTAAAGGGT
ATGTTAGAGGAGCAGCCAATTAATGGTGCCAAAAAATCCAAATTATAGTATCTGCCTTG
TAA

SEQ ID NO. 19

MAPTPPTSLEFKVHRREPELIAPAKPTPHEFKPLSDIDDQEGRLRFHIPVFIQFY
RYDPSMQGRDPVMVIREALARTLVFYYPFAGRLREGPKRKLIVECTSEGVL
FIEAEADVALEQFGDALQPPFPCLEELLFDVPGSSGVLNCPLLLIQVTRLKC
GGFIFALRLNHTMSDAPGLVQFMSTVGEMARGAQAPSVLPVWERHFLNAR
DPPRVTRTHREYDEVADTKGTIIPLDDMAHQSF FGPNEVAALRRFVPKHL
RQCSNFELLTACLWKCR TIALQPDPT EEMRIICIVNGRNKFNPLPTGY YGN
GFVFPVAVARAKELIEKPLGFALELVKEVKDSVTEEYMRSVADLMVIKGRP
HFTVVR SYLVSDVTRAGFGEVDFGWGKAAYGGPAKGGVGAIPGVASFYIPF
RNKKGENGIVLPICLPAQAMERFVKELKGMLEEQPINGAKKSKFIVSAL Stop

Figure 7

SEQ ID NO. 8

ATGGAAGCTCTTCTCCAAGCTCTGCAACCTTTACCCTTCACTCTTTTCCTTATTGTTCCCTTTACTATTC
CTACTAGGTCTAATCTCTCTCCTTCGTAGACGATTGCCATATCCTCCAGGGCCTAAAGGCTTGCCAAT
TATAGGTAACCTGCTCATGATGGACCAGTTAACTACCGGGGTTTAGCTAAACTAGCTAAAGAATATG
GTGGCCTCTTCCATCTTCGCATGGGAAACATTACATGTTTGCTGTTTCTTCTCCTGAGATAGCTCGC
CAAGTACTTCAAGTCCAAGATAATATTTTCTCAAATAGACCTGCCACCATAGCCATCAGTTATCTCAC
ATATGACCGTGCAGATATGGCCTTCGCCCCACTACGGCCCTTTCTGGCGCCAGATGCGCAAGCTCTGC
GTCATGAAACTTTTCAGCCGTAAACGGGCTGAGTCATGGGAATCTGTGAGGGATGAGGTTGATTATA
TGGTTAAAACCGTTGTGGCCAACAAAGGGAAGCCAGTGAATGTTGGAGAGTTGATTTTTACTCTAA
CCATGAATATTATTTATAGGGCTGCTTTCGGTTCGAAGAACGAGGGACAGGACGAGTTTATTTCAGATT
TTGCAGGAGTTCTCCAAGCTTTTGGTGCTTTCAACATTGCTGATTTTCATTCTTGGCTTGGATGGAT
AGACCCACAAGGCCTCAAGCTCAACTCAAGACTTGCCAAGGCTCGTAAATCACTTGACAGATTTCAT
CGACTTGATCATCGACGAACACATGCATAAGAGTAAGCAGGGTAATGTCTCTGATGATAACTCTGATA
TGGTGGATGATTTGCTAGCTTTCTACAGCGATGAGCCTAAAGTAAACGAATCAGACGATCTTCAGAA
TTCCTTCAAACCTCACTCGAGACAACATCAAAGCAATTATCATGGACGTAATGTTTGGTGGGACAGAG
ACAGTGGCATCGGCGATTGAGTGGGCCCTGACGGAGTTGATGAGGGCTCCTGAAGAGTTGAAAAA
GGTCCAGCAAGAGCTTGCCGATGTGGTGGGTCTAGAGCGGCGCGTGAGGAGAGTGATTTTCGACA
AGCTGACATACCTAAAATGCACAGTCAAAGAACTCTAAGACTTCACCCACCAATCCCACTTCTCTT
ACATGAGACAGCCGAGGACGCAGAGGTCGCCGGGTATTACATTCCAGCGAAGTCTAGGGTCATGAT
CAATGCATGGGCTATTGGAAGGGACAATAACTCATGGGAGGACCCTGAACTTTTCAGGCCATCCAG
GTTTTTAAAAGAGGGAGTGCCTGATTTTAAAGGAAACAATTTTCGAGTTTCATTCCGATCGGGC
CGGAGATCCTGCCCCGGCATGCAACTCGGACTCTACTCTTGTGATTTGGCTGTGGCCCATCTTCTTCA
CTGTTTTACATGGCAGTTGCCTGATGGGATGAAACCAAGTGAAATTGACACGAGCGATGTGTTTGA
CTACCGCTCCTCGGGCAACCCGACTGGTAGCTGTTCCAAACGCGCGTTTGTGTGCCCGCTCTAA

SEQ ID NO. 20

MEALLQALQPLPFTLFLIVPLLFLGLISLLRRRLPYPPGPKGLPIIGNLLMM
DQLTHRGLAKLAKEYGGLFHLRMGNIHMFVSSPEJARQVLQVQDNIFSN
RPATIAISYLTYDRADMAFAHYGPFWRQMRKLCVMKLF SRKRAESWESVR
DEVDMVKTVVANKGKPVNVGELIFTLTMNIIYRAAFGSKNEGQDEFIQIL
QEF SKLFGAFNIADFI PWLGWIDPQGLKLN SRLAKARKSLDRFIDLIIDEHM
HKSKQGNVSDDNSDMVDDLLAFYSDEPKVNESDDLQNSFKLTRDN IKAI
MDVMFGGTETVASAIEWALTELMRAPEELKKVQQELADV VGLERRVEESD
FDKLT YLKCTVKETLR LHPP IPLLHETAEDA EVAGYYIPAKSRV MINAWAI
GRDNNSWEDPETFRPSRFLKEGVPDFKGN NFEFIPFGSGRRSCPGMQLGL
YSLDLAVAHLLHCFTWQLPDGMKPSEIDTSDFGLTAPRATRLVAVPNARL
LCPL Stop

Figure 8

9/16

SEQ ID NO. 9

ATGGCACACGATTGCAGCTTCGAGATCGACCCGAGAAGCGGCTTTTGCAGATCTAATTCTATTTTCT
GCAGTAAACGCAAGCCTATTCTCTCCACACAATTACTGCATCGACATCACCACCTTTATTTTCGTCC
CAGGCTCACCGTGGCAAGACAGCCTTCATTGACGCCACCACTGGCCTCCACCTCACCTTCATCGAC
CTATGGAAATCCGTCGATTCCGTCGCCACCTCTCTATTTGAGATAGGAGTCCGCAAGGGCCACGTTAT
CCTTCTCCTTTCCCCAAACTCCATTTTCTTTCTGTTGTTTGCCTTTCTGTTATGTCCCTTGGTGCCAT
AATTACCACCACCAACCCTCTCAACACTCCCCGTGAAATCGCCAAGCAAATCGCCGACTCCAAACC
TTTATTCGCCTTTACCACCCCCCAACTAGTCCCCAAACTCACCGAGTCGAACTCGAATCTCCCCATCA
TCCTCATAGACGACCATGATTCCATCAAAGCCCAATCCATAATCGTGACAACTTTAAGCGAGATGATG
AAGGAGCCTAGTGGGAGTCGTGTCAGGGAGCGAGTCAACATGGACGACACAGCTACTCTGCTCTAC
TCTTCAGGTACAACCGGAGCAAGCAAGGGCGTAGTTTCCTCGCATCGCAACCTAATCGCGATGGTTC
AAACCATCATTGGACGCTTTAACGAAGACAGAGAGCATAAATTTATCTGCACCGTCCCAATGTTTCA
CATTTACGGTCTAGCATTGTTTCGCGACGGGATTACTCGCGTCAGGATCAACAATCATTGTGCTCTCAA
AGTTCGAGATTCATGAGATGCTATCCACGATCGAGAGGTATCGTGCCACGGATCTCCATCTGGTACC
ACCTATACTAGTAGCACTGATCAACGGCGCTGATCAGATAAGGTCCAAATATGATTTGAGTTCGCTAC
AAACAGTTGTGTGTGGAGGAGCCCCACTGAGCAAGGAGGTGATAGAGGGGTTTCATGGAGAAATAT
CAAACGTTGAGAATCTTCAGGGTTACGGGTTGACTGAATCGACGGGGGTGGGTGCATCAACAGAT
ACTTTAGAGGAAAGTAGGAGATACGGCACCGCGGGGCTGCTGACGTCGAGCATGGAGGCAAAGAT
TGTGGACCCAGAAAGCGGAAAAGCTCTGCCGGTGAACCAGACCGGTGAGCTTTGGCTTAGAGGTC
CTTCTATTATGAAAGGTTACTTTTTAAATACTGAAGCTACATCAACAACCTCTTGACTCCGAGGGATGG
TTAAGAACTGGGGATCTATGCTACATTGATGATGACGGCTTTATTTTGTGTTGATCGACTGAAGGA
GTTGATCAAATACAAGGGATATCAGGTCCCTCCTGCGGAAGTAGAGGCCTTGTTGCTTACTCATGAG
GAAATTTCCGATGCTGCTGTAATCCCGTTTCCGGACAAGGAGGCTGGGCAATTTCCCATGGCATATG
TGTA

SEQ ID NO. 21

MAHDCSFEIDPRSGFRCRSNSIFCSKRKPIPLPHNYCIDITTFISSQAHRGKTA
FIDATTGLHLTFIDLWKSVDVSLFEIGVRKGHVILLSPNSIFFPVVCLS
VMSLGAIITTTNPLNTPREIAKQIADSKPLFAFTTPQLVPKLTESNSNLPIILI
DDHDSIKAQSIIVTTLSEMMKEPSGSRVRERNMDDTATLLYSSGTTGASK
GVVSSHRNLIAMVQTIIGRFNEDREHKFICTVPMFHIYGLALFATGLLASGS
TIIVLSKFIEHMLSTIERYRATDLHLVPPILVALINGADQIRSKYDLSSLQT
VVCGGAPLSKEVIEGFMEKYQTLRILQGYGLTESTGVGASTDTLEESRRYG
TAGLLTSSMEAKIVDPESGKALPVNQTGELWLRGPSIMKGYFLNTEATSTT
LDSEGWLRTGDLCYIDDDGFIFVVDRLKELIKYKGYQVPPAELEALLTHE
EISDAAVIPFPDKEAGQFPMAYVS

Figure 9

10/16

SEQ ID NO. 10

ATGTCAATCCAAAACAAAGAGCCAGTCTGCGTCACTGGAGCCAATGGCTTCATTGGATCCTACCTGG
TCCGGACCCTCATAACCCATGGCTACACCACCATCCACGCCTCCATCTACCCCTACTCCGATGCTTCC
CACCTCTTCCACCTCCTTCCCTCCGCTGCCTACTCTAACGTCCAACCTTCGTATCTTTGAGGCCGACGT
TCTTGACTACGACGCCGTTGCCAAGGCCGTTGAGGGCTGTGCGGGGGGTTTTCCACGTGGCCTCTCC
CTGTACGCTAGAGGACCCTAAGGACCCGCAGAAGGAGCTTGTATTGCCTGCGGTGCAGGGCACGAT
TAATGTGCTGCAGGCTGCCAAGAAGCTTCGGTGTGAGGCGCGTGGTGGTCACGTCTCTATTTCGGC
GTTGGTTCCTAATCCTAATTGGCCGGCCGGTAAAGTGTTTGATGAGTCGTCTGGACTGACATCGATT
ACTGCAAGTCTCGTCAGAAGTGGTATCCAGTGTCAAAGACACTGGCAGAGAAAGAAGCGTGGGAC
TTTGCAAAGAAGCATGGAATGAATCTGGTGACAATCCATCCTGCTACATGTCTAGGCCCACTGTTGC
AGCCTAGTTTGAATGCTAGCTGTGCTGTGTTGAAGCAATTATTGGAGGGATCAGAAGACACTCAGG
AATATCACTGGTTGGGAGCTGTGCATGTCAAAGATGTGGCTGAGTCACAAATTTTGCTGTTTGAGAC
TCCTACTGCTTCTGGTAGATATCTCTGCACAAATGGTATTTATCAATTTGGTGATTTTGACAGAGAGG
TTTCCAAGCTCTTCCCTGAGTTTCTGTTTACAGGTTTGTGGGGAAACTCAACCGGGCCTGACAAC
TTGCAAAGATGCTGCCAAGAGATTAATTGAGATGGGGCTAGTTTTCACTCCAGTTGAAGATGCCATC
CAGGACACGGTGGAAGTCTAAAGGCTAAAGGATTCTAAAGCACAAATGTCAACATCATAG

SEQ ID NO. 22

MSIQNKEPVCVTGANGFIGSYLVRTLITHGYTTIHASIYPYSDASHLFLHLLPS
AAYSNVQLRIFEADVLDYDAVAKAVEGCRGVFHVASPTLEDPKDPQKEL
VLPAVQGTINVLQAAKNFGVRRVVVTSSISALVPNPWPAGKVFEDESSWTD
IDYCKSRQKWYPVSKTLAEKEAWDFAKKHGMNLVTIHPATCLGPLLQPSL
NASCAVLKQLLEGSEDTQEYHWLGAVHVKDVAESQILLFETPTASGRYLCT
NGIYQFGDFAERVSKLPPEFPVHRFVGETQPGLTTCKDAAKRLIEMGLVFT
PVEDAIQDTVESLKAKGFLKHKMST S Stop

Figure 10

11/16

SEQ ID NO. 11

ATGGCATCCAACAATGAACAGCAAAATCAGGCAGGCAGGCACCAAGAAGTTGGCCACAAG
AGCCTTCTGCAGAGTGATGCACTTTACCAGTACATACTTGAGACAAGTGTGTACCCAACA
GAGCCTGAACCCATGAAGGAGCTGAGGGAGCTAACTGCCAAGCACCCCTGGAACATAATG
ACTACATCAGCAGATGAAGGGCAGTTCTTGAACATGCTTTTGAAGCTTATGAATGCAAAG
AACACCATGGAAATTGGTGTATACACTGGATATTCTCTCTTGGCAACTGCTCTTGCTCTT
CCTGATGATGGAAAGATCCTGGCGATGGACATAAACAGAGAGAACTATGAGTTGGGTCTG
CCTGTTATCCAGAAAGCAGGTGTTGCTCACAAGATTGAATTCAGGGAAGGGCCTGCTTTG
CCTGTTCTTGACCAAATGATTGCTGAAGGGAAGTACCATGGAACCTTTTGACTTCATCTTT
GTGGACGCTGATAAAGACAATTATCTGAATTACCACAAGAGATTGATTGAGCTGGTCAAA
GTTGGTGGAGTGATCGGGTACGACAATACCCTATGGAATGGGTCTGTGGTGGCACCACCA
GATGCACCCCTCAGGAAGTATGTTAAGTACTACAGAGACTTTGTGATGGAGCTCAACAAG
GCCTTACCGGCGGACCCCAGAATTGAGATCTGCATGCTCCCTGTTGGTGTATGGGATCACT
CTCTGCCGTCGGATCAAGTGA

SEQ ID NO. 23

MASNNEQQNQAGRHQEVGHKSLLQSDALYQYILETSVYPTPEPEPMKELRE
LTAKHPWNIMTTSADEGQFLNMLLKL MN AKNTMEIGVYTGYSLLATALAL
PDDGKILAMDINRENYELGLPVIQKAGVAHKIEFREGPALPVLDQMIAEGK
YHGTFDFIFVDADKDNLYLNHKLIELVKVGGVIGYDNTLWNGSVVAPPD
APLRKYVKYYRDFVMELNKALPADPRIEICMLPVGDGITLCRRIK Stop

Figure 11

12/16

SEQ ID NO. 12

ATGGGTTAGTCTTGAAACTGAGAGAACTGTGGTAGGATGGGCAGCAAAAGATCCATCTGGG
GTCCTTTCTCCTTATTCTTACACTATCAGAAACACAGGCCAGAGGATATTTGTTGAAA
GTTCTCAGCTGTGGAATCTGCCACACTGATATTCATCAAGTGAAGAATGATCTTGGCATG
TCACGTTATCCCATGGTTCTTGGGCATGAGGTGGTTGGTGAGGTGGTGGAGGTGGGATCA
GAGGTGACTAAGTTCGGAGTGGGAGATGTAGTTGGAGTTGGATGTATTGTTGGAAGTTGC
AGGAGTTGCAACCCATGTAAATCAGATATTGAGCAGTACTGCAACAAGAAAATCTGGTCT
TATAATGATGTCTACACCGATGGAAAACCCACCCAGGGCGGCTTTGCCCAATCCATGATC
GTAGATCAAAAGTTTGTGGTAAAAATACCAGAGGGGATGTCACCAGAACAAGCAGCACCA
CTATTGTGTGCAGGATTGACAGTGTATAGCCCACTTAGCCACTTTGGGTTGAAGAAGAGT
GGACTAAGAGGTGGCATATTAGGACTTGGAGGAGTAGGGCACATGGGCGTGAAGATAGCG
AAGGCAATGGGACACCATGTTACTGTGATAAGTTCTTCAGACAAAAGAGGGAGGAGGCC
TTGGAGCATCTTGGTGTCTGATGAGTACTTGGTTAGTTCTGATGCAACTCGGATGCAAGAA
TTTGTCTGATACCCTTGATTATCTCATTGACACTGTACCTGTATTTACCCCTCTTGAGCCT
TATCTTTCTCTGTTGAAACTTGATGGGAAGCTGATTTTGATGGGTGTTATTAATACCCCA
TTGCAATTTCTCACTCCTTTGGTCATGCTTGGGAGAAAATCAATCACAGGGAGCTTCATA
GGAAGCATGAAAGAAACAGAGGAGATGCTTGAATTCTGCAAGGAAAAGGGATTGACCTCC
ACGATTGAAGTGGTAAAAATGGATTACATCAACACAGCAATGGAAAGGCTGGAGAAAAAC
GATGTCAGATATAGGTTTGTGTGGATGTTGCCGGTAGCAAGCTGGATCCCTGA

SEQ ID NO. 24

MGSLETERTVVVGWAAKDPSGVLSPYSYTIRNTGPEDILLKVLSCGICHTDIH
QVKNDLGMSTRYPMPVPGHEVVGEVVEVGSEVTKFGVGDVVVGVCIVGSCR
SCNPCKSDIEQYCNKKIWSYNDVYTDGKPTQGGFAQSMIVDQKFVVKIPE
GMSPEQAAPLLCAGLTVYSPLSHFGLKKSGLRGGILGLGGVGHMGMVKIAK
AMGHHVTVISSSDKKREEALEHLGADEYLVSSDATRMQEFADTLDYLIDTV
PVFHPLEPYLSLLKLDGKLILMGVINTPLQFLTPLVMLGRKSITGSFIGSMK
ETEEMLEFCKEKGLTSTIEVVKMDYINTAMERLEKNDVRYRFVVDVAGSK
LDP Stop

Figure 12

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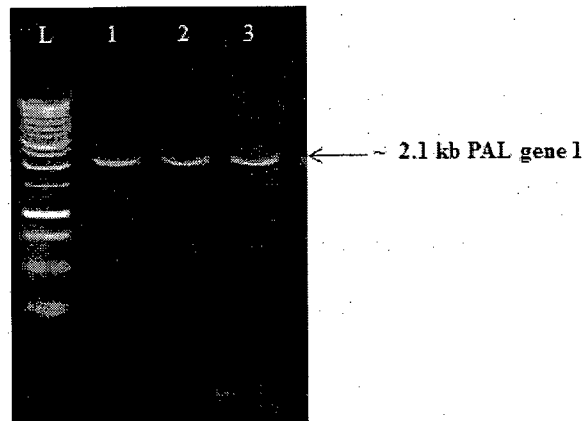


Figure 13

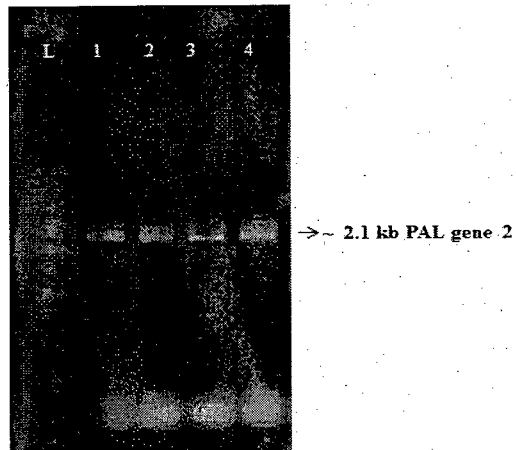


Figure 14

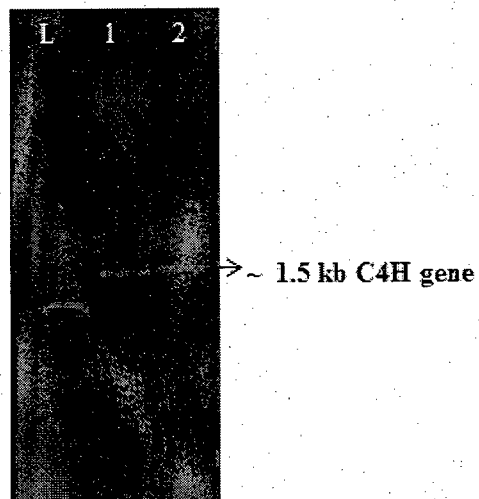


Figure 15

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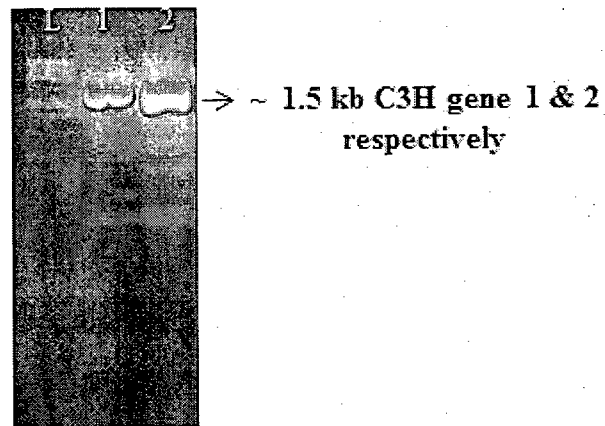


Figure 16

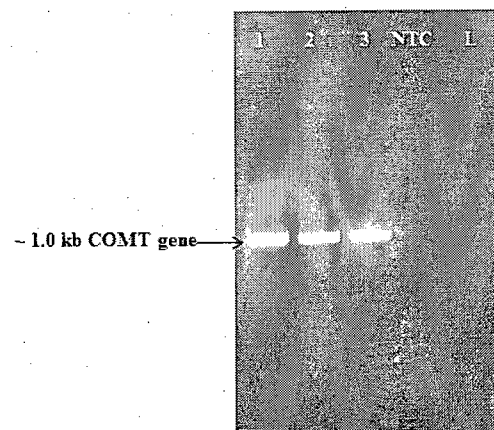


Figure 17

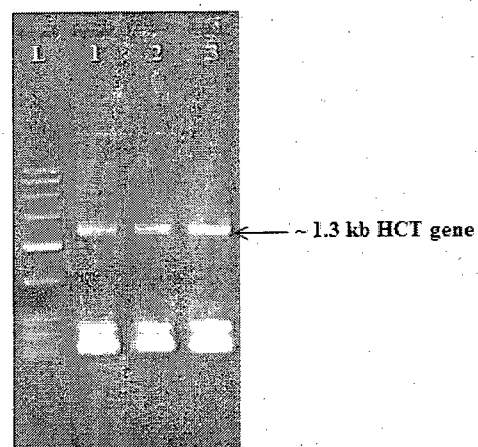


Figure 18

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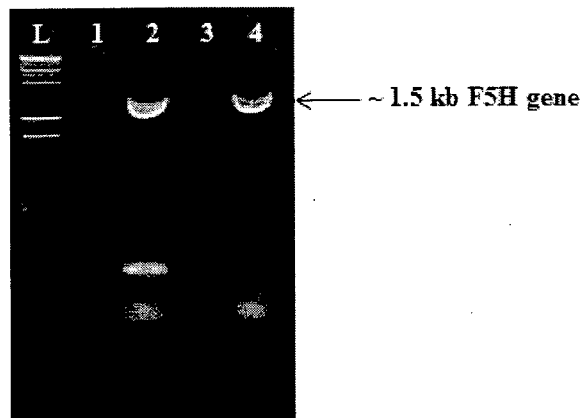


Figure 19

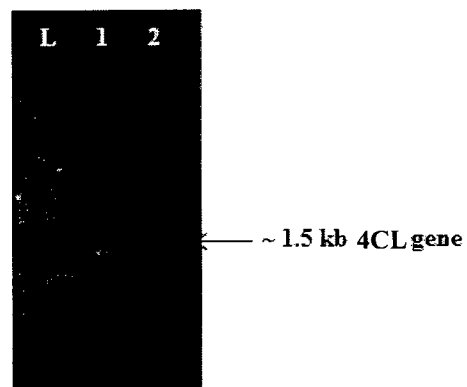


Figure 20

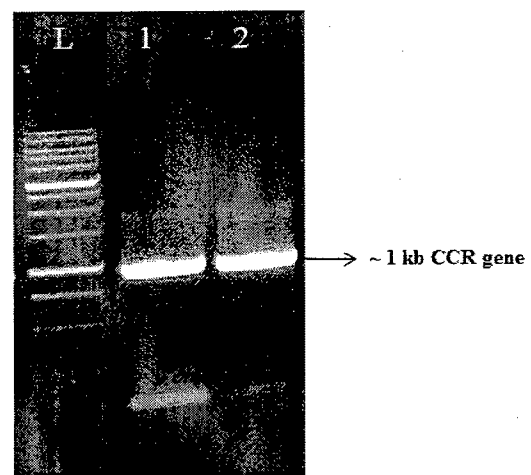


Figure 21

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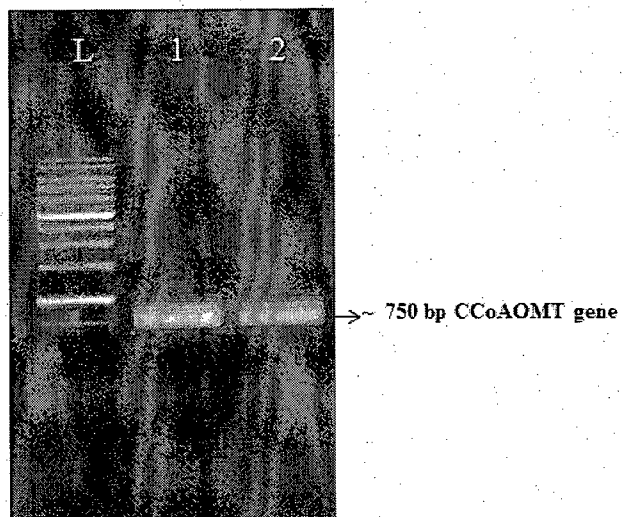


Figure 22

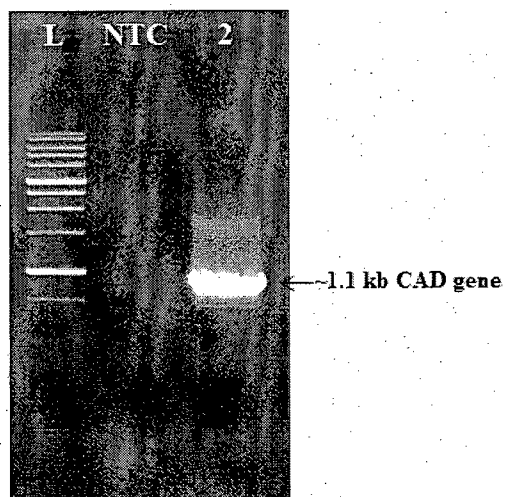


Figure 23

INTERNATIONAL SEARCH REPORT

International application No.

PCT/MY2012/000014

A. CLASSIFICATION OF SUBJECT MATTER

C12N 9/88 (OCT 2005)

C12N 15/60 (OCT 2005)

C07K 14/415 (OCT 2005)

C12N 15/29 (OCT 2005)

C12N 15/82 (OCT 2005)

A01H 1/00 (OCT 2005)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 MEDLINE, AGRICOLA, BIOSIS, CAPLUS: monolignol, lignin, hevea, rubber tree, phenylalanine ammonia-lyase, PAL, tree, wood, pulp and similar terms. GENOMEQUEST: SEQ IDs 1, 2, 13, 14.
 EPOQUE: monolignol, lignin, phenylalanine ammonia-lyase, PAL and similar terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

☒ Further documents are listed in the continuation of Box C☒ See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 28 June 2012	Date of mailing of the international search report 09 July 2012
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au Facsimile No.: +61 2 6283 7999	Authorized officer Jason MacKenzie AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262837934

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation).	DOCUMENTS CONSIDERED TO BE RELEVANT	PCT/MY2012/000014
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/012594 A2 (THE SAMUEL ROBERTS NOBLE FOUNDATION, INC) 02 February 2006 See the whole document, particularly the summary of the invention on pages 4-8, Figures 1-2, page 11 lines 19-26, and Example 1B.	1-10
X	WO 2006/036741 A2 (ARBORGEN LLC) 06 April 2006 See the whole document, particularly paragraph [0014], summary of the invention in paragraphs [0028]-[0057], Figures 93 and 223, and paragraph [0318].	1-10
X	WO 1998/011205 A2 (GENESIS RESEARCH & DEVELOPMENT CORPORATION LIMITED et al) 19 March 1998 See the whole document, particularly the summary of the invention on pages 2-4, Figure 1, page 6 lines 11-28, Examples 6 and 7 on pages 22-24	1-10
X	Xu F et al. Molecular cloning, characterization and expression of phenylalanine ammonia-lyase gene from Ginkgo biloba. Afr. J. Biotechnol. 7 (6), 721-729 (2008) & GENBANK Accession No. ABU49842 submitted on 28 July 2007 (shares 66.48% identity with SEQ ID 13). See the whole document, particularly the abstract, and cloning of the GbPAL DNA and cDNA on pages 722-723.	7-10
A	See the whole document.	1-6
X	Shi R et al. Towards a systems approach for lignin biosynthesis in Populus trichocarpa: transcript abundance and specificity of the monolignol biosynthetic genes. Plant Cell Physiol. 2010 Jan;51(1):144-63. Epub 2009 Dec 8. & GENBANK Accession No. ACC63889 submitted on 31 March 2008 (shares 82.02% identity with SEQ ID 14). GENBANK Accession No. EU603319 submitted on 31 March 2008 (shares 65.73% identity with SEQ ID 1). GENBANK Accession No. EU603320 submitted on 31 March 2008 (shares 66.41% identity with SEQ ID 1). GENBANK Accession No. EU603321 submitted on 31 March 2008 (shares 75.7% identity with SEQ ID 2). See the whole document, particularly the abstract, Figure 1, Table 1, and Supplementary Table S3 (available online from http://pcp.oxfordjournals.org/).	1-10

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:
 - a. (means)
☐ on paper
☐ in electronic form
 - b. (time)
☐ in the international application as filed
☐ together with the international application in electronic form
☐ subsequently to this Authority for the purposes of search
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

No sequence listing was provided in the standardised format as set out in Annex C of the Administrative Instructions under the Patent Cooperation Treaty. The sequences contained in the drawings provided to this ISA were OCR'd and used for the purposes of the search.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/MY2012/000014**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-10 (all in part), insofar as they relate to PAL1 (SEQ IDs 1 and 13), OR PAL2 (SEQ IDs 2 AND 14).

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Supplemental Box**Continuation of: Box III**

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- Claims 1-10 (all in part). The feature of phenylalanine ammonia-lyase 1 per se (PAL1, SEQ IDs 1 and 13), and the uses thereof is specific to this group of claims.
- Claims 1-10 (all in part). The feature of phenylalanine ammonia-lyase 2 per se (PAL2, SEQ IDs 2 and 14), and the uses thereof is specific to this group of claims.
- Claims 1-10 (all in part). The feature of cinnamate 4-hydroxylase per se (CH4, SEQ IDs 3 and 15), and the uses thereof is specific to this group of claims.
- Claims 1-10 (all in part). The feature of coumarate 3-hydroxylase 1 per se (C3H1, SEQ IDs 4 and 16), and the uses thereof is specific to this group of claims.
- Claims 1-10 (all in part). The feature of coumarate 3-hydroxylase 2 per se (C3H2, SEQ IDs 5 and 17), and the uses thereof is specific to this group of claims.
- Claims 1-10 (all in part). The feature of caffeic acid/5-hydroxyconiferaldehyde O-methyltransferase per se (COMT, SEQ IDs 6 and 18), and the uses thereof is specific to this group of claims.
- Claims 1-10 (all in part). The feature of Hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl transferase per se (HCT, SEQ IDs 7 and 19), and the uses thereof is specific to this group of claims.
- Claims 1-10 (all in part). The feature of ferulate 5-hydroxylase per se (F5H, SEQ IDs 8 and 20), and the uses thereof is specific to this group of claims.
- Claims 1-10 (all in part). The feature of 4-coumarate CoA ligase per se (4CL, SEQ IDs 9 and 21), and the uses thereof is specific to this group of claims.
- Claims 1-10 (all in part). The feature of 4-coumarate caffeoyl-CoA O-methyltransferase per se (CCoAOMT, SEQ IDs 10 and 22), and the uses thereof is specific to this group of claims.

Supplemental Box

- Claims 1-10 (all in part). The feature of 4-coumarate cinnamoyl coenzyme A reductase per se (CCR1, SEQ IDs 11 and 23), and the uses thereof is specific to this group of claims.
- Claims 1-10 (all in part). The feature of 4-coumarate cinnamyl alcohol dehydrogenase per se (CAD, SEQ IDs 12 and 24), and the uses thereof is specific to this group of claims.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a priori*.

There appears to be no significant homology amongst the defined SEQ IDs, nor is there any disclosure of a common biochemical property shared by the 12 enzyme activities defined in claims 1-10. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a priori*.

For the fee already paid, this ISA has decided to search inventions 1 and 2 insofar as they both relate to phenylalanine ammonia-lyase.

INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/MY2012/000014	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
WO 2006/012594 A2	02 Feb 2006	AU 2005266927 A1	02 Feb 2006
		AU 2005266927 B2	14 Jul 2011
		EP 1771568 A2	11 Apr 2007
		NZ 552941 A	25 Feb 2011
		US 2007079398 A1	05 Apr 2007
		US 7663023 B2	16 Feb 2010
		WO 2006012594 A2	02 Feb 2006
WO 2006/036741 A2	06 Apr 2006	AR 050887 A1	29 Nov 2006
		BR PI0506008 A	23 May 2006
		CN 101410515 A	15 Apr 2009
		CN 101410521 A	15 Apr 2009
		EP 1804569 A2	11 Jul 2007
		EP 1809094 A2	25 Jul 2007
		EP 2267123 A2	29 Dec 2010
		JP 2008513025 A	01 May 2008
		JP 2008513030 A	01 May 2008
		JP 2011055839 A	24 Mar 2011
		US 2006101535 A1	11 May 2006
		US 7402428 B2	22 Jul 2008
		US 2006130183 A1	15 Jun 2006
		US 7456338 B2	25 Nov 2008
		US 7799906 B1	21 Sep 2010
		US 2009077686 A1	19 Mar 2009
		US 7807880 B2	05 Oct 2010
		US 2009119796 A1	07 May 2009
		US 8030545 B2	04 Oct 2011
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		WO 2006036698 A2	06 Apr 2006
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WO 1998/011205 A2	19 Mar 1998	AR 020742 A1	29 May 2002
		AR 067725 A2	21 Oct 2009
		AU 733388 B2	10 May 2001
		AU 1083600 A	01 May 2000
		AU 777237 B2	07 Oct 2004

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

Form PCT/ISA/210 (Family Annex)(July 2009)

INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/MY2012/000014	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
		AU 4403697 A	02 Apr 1998
		BR 9711745 A	18 Jan 2000
		BR 9915021 A	14 Aug 2001
		CA 2344990 A1	20 Apr 2000
		CN 1329663 A	02 Jan 2002
		EP 0929682 A2	21 Jul 1999
		EP 1121421 A1	08 Aug 2001
		EP 1121421 B1	04 Apr 2007
		EP 1826265 A2	29 Aug 2007
		EP 1826265 B1	12 Jan 2011
		ID 30318 A	22 Nov 2001
		JP 4793963 B2	12 Oct 2011
		JP 2001500378 A	16 Jan 2001
		JP 2002527058 A	27 Aug 2002
		NZ 334565 A	25 Aug 2000
		NZ 510940 A	30 Jan 2004
		NZ 529839 A	27 Aug 2004
		NZ 533254 A	28 Apr 2006
		NZ 544044 A	21 Dec 2007
		NZ 553139 A	30 Jun 2008
		US 5850020 A	15 Dec 1998
		US 5952486 A	14 Sep 1999
		US 6204434 B1	20 Mar 2001
		US 6330922 B1	18 Dec 2001
		US 6410718 B1	25 Jun 2002
		US 6653528 B1	25 Nov 2003
		US 2003131373 A1	10 Jul 2003
		US 7087426 B2	08 Aug 2006
		US 2006183895 A1	17 Aug 2006
		US 7910326 B2	22 Mar 2011
		US 2012035354 A1	09 Feb 2012
		WO 0022099 A1	20 Apr 2000
		WO 9811205 A2	19 Mar 1998
		ZA 9710451 A	28 Jul 1999

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

Form PCT/ISA/210 (Family Annex)(July 2009)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/MY2012/000014

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report**Patent Family Member/s****Publication Number****Publication Date****Publication Number****Publication Date**

ZA 9810574 A

25 May 1999

ZA 200102534 A

16 Jul 2001

End of Annex