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(54) **SYNTHETIC FUSION GENE AND ITS USE THEREOF**

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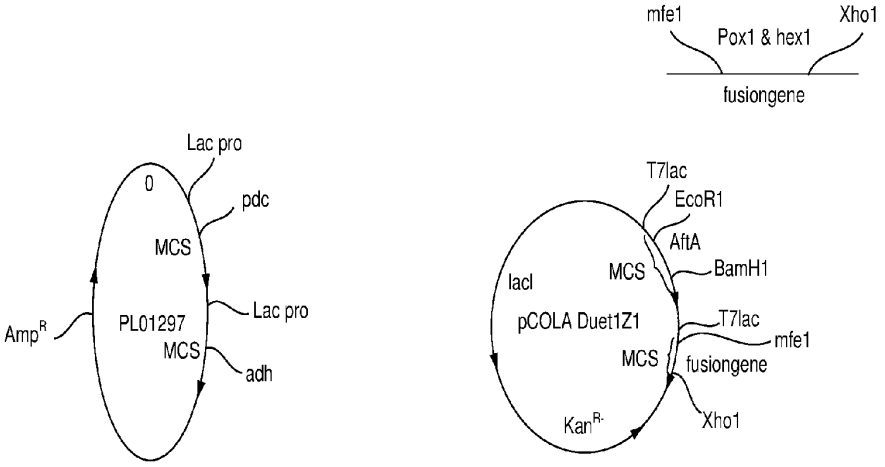
(57) **ABSTRACT**

The present invention discloses synthetic fusion gene comprising hex1 and pox1 genes, their process of preparation, polypeptide(s) encoded by the same and its use thereof for biological pre-treatment of biomass for the production of biodiesel.

**Specification includes a Sequence Listing.**

## Plasmid showing Synthetic fusion gene along with biodiesel producing gene

These two plasmid express together in a bacteria can produce biodiesel from lignocelluloses




- Bacteria-Zymomonas mobilis-(gene) pdc and adhB-help in conversion glucose to ethanol
- Bacteria-Acinetobacter baylyi-(gene) aftA-esterfication of ethanol to FAEE(Biodiesel)
- Fungus (hypocrite jecorina) tricoderma reesei (gene) hex1 degrade crystalline cellulosehemicelluloses into glucose
- Fungus (basidiomycetes) pleurotus ostreatus (gene) pox1 degrade crytalline lignin into glucose

Figure 1

Plasmid showing Synthetic fusion gene along with biodiesel producing gene

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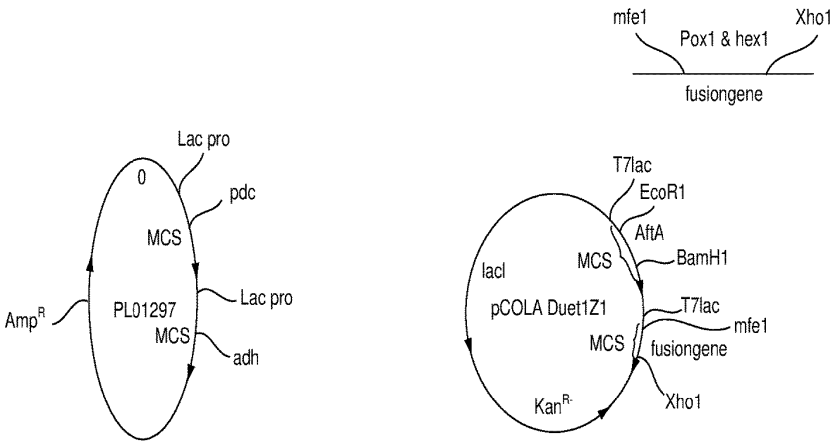


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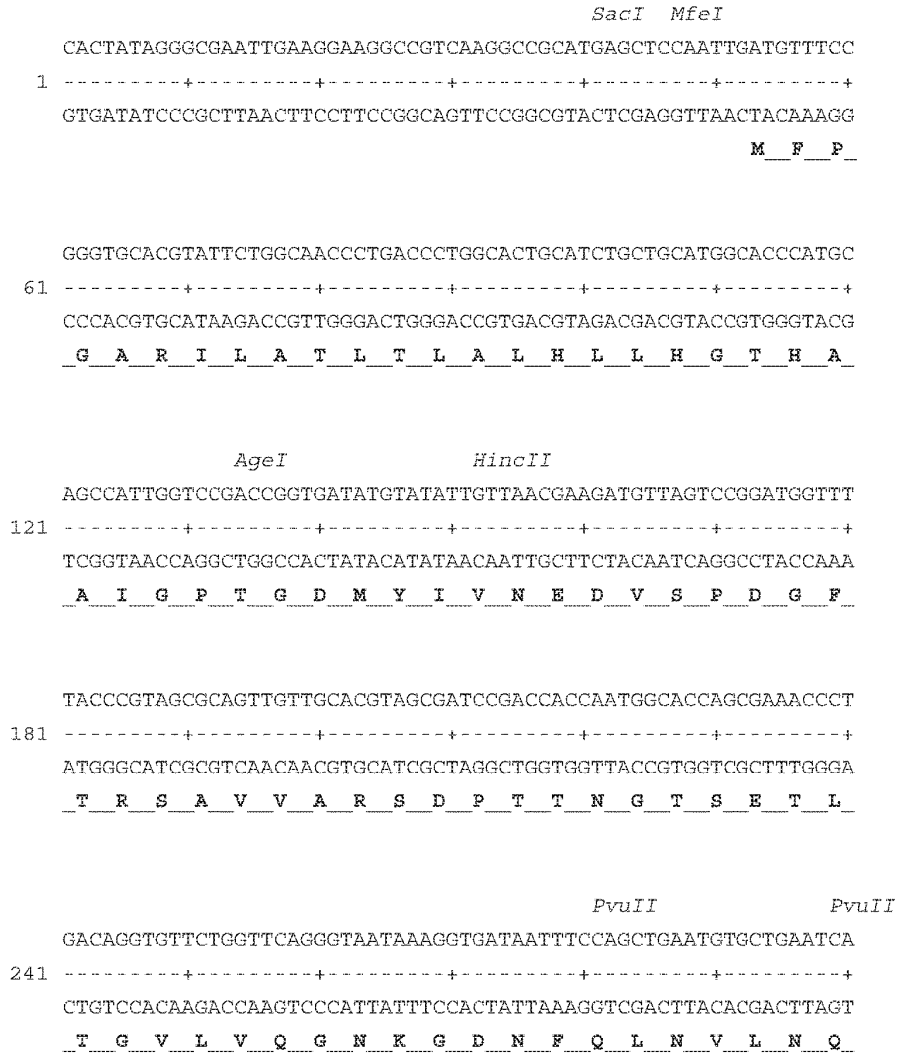
Figure 2

stomer **Universiti Brunei Darussalam, Prabitha Kumar**

me of the gene **pox1_and_hex1**

imized for Escherichia coli



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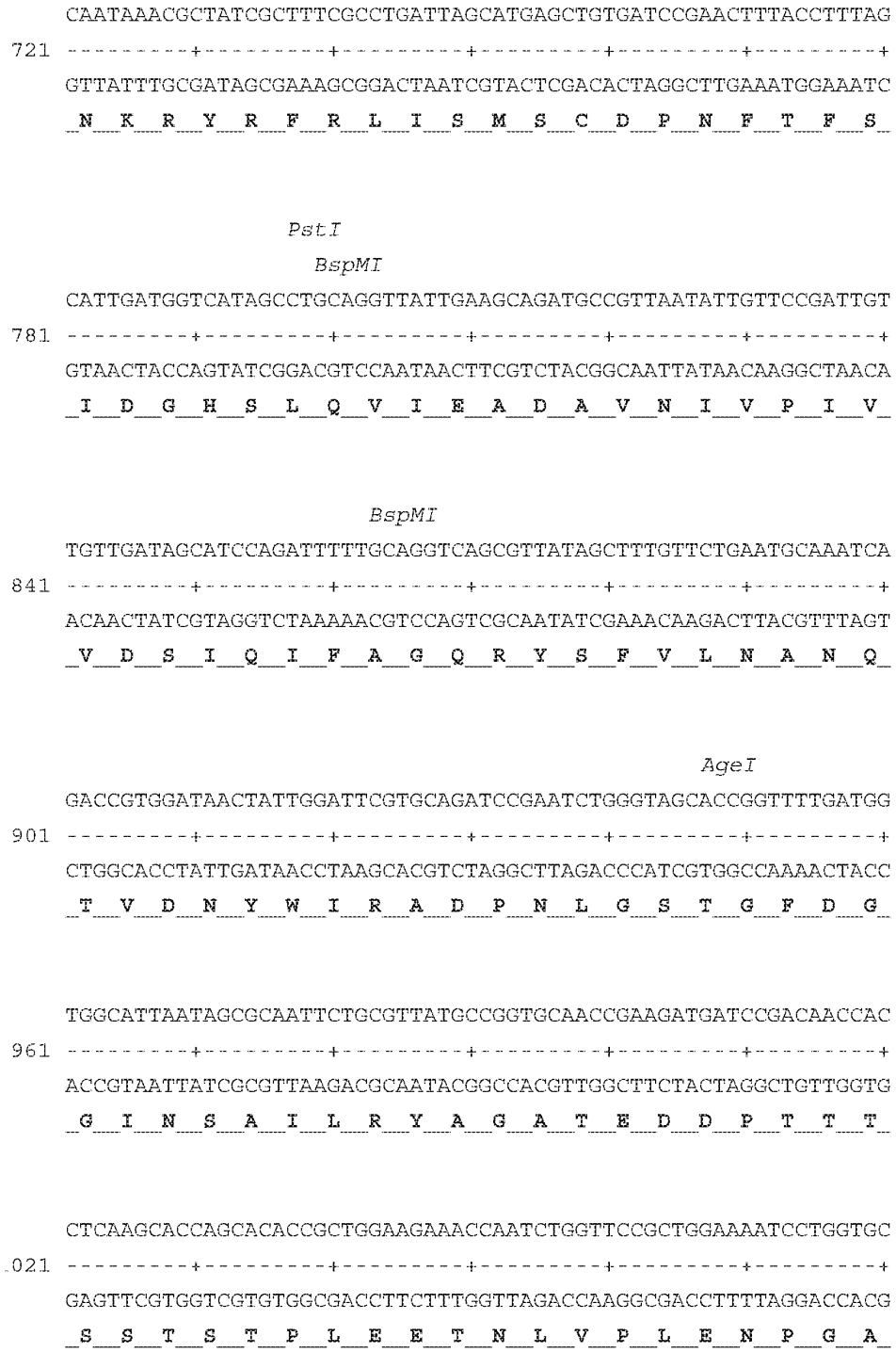
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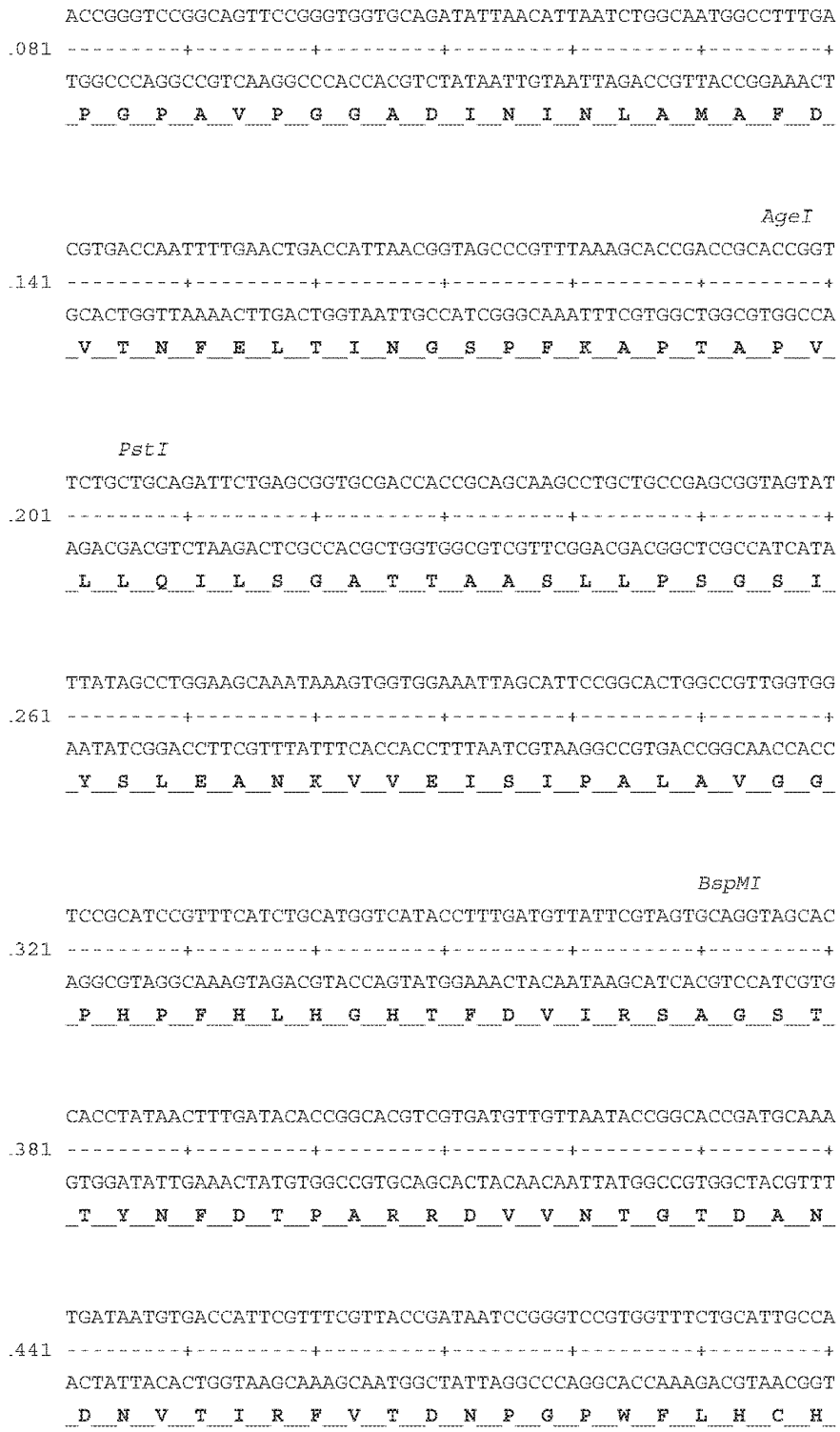
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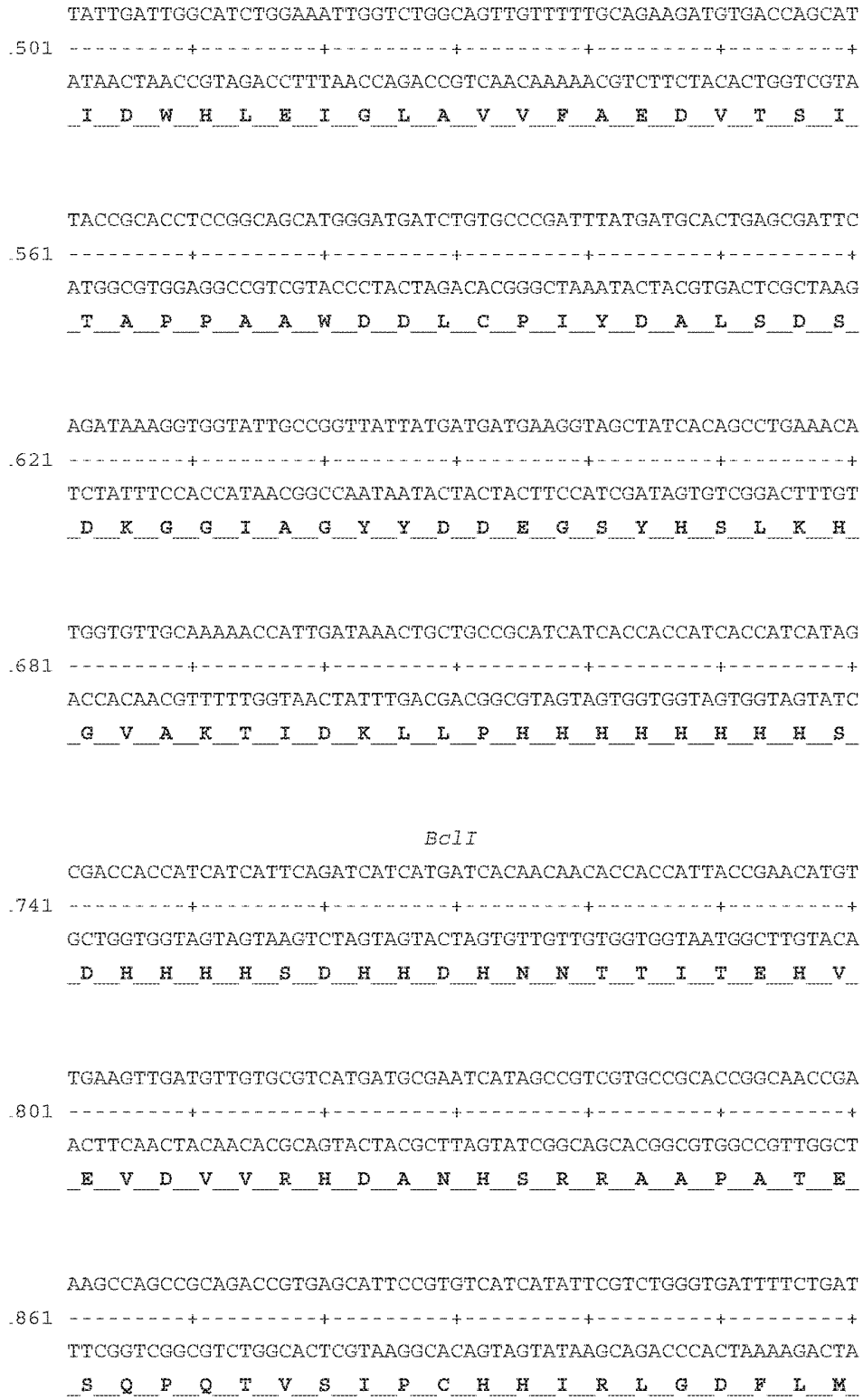
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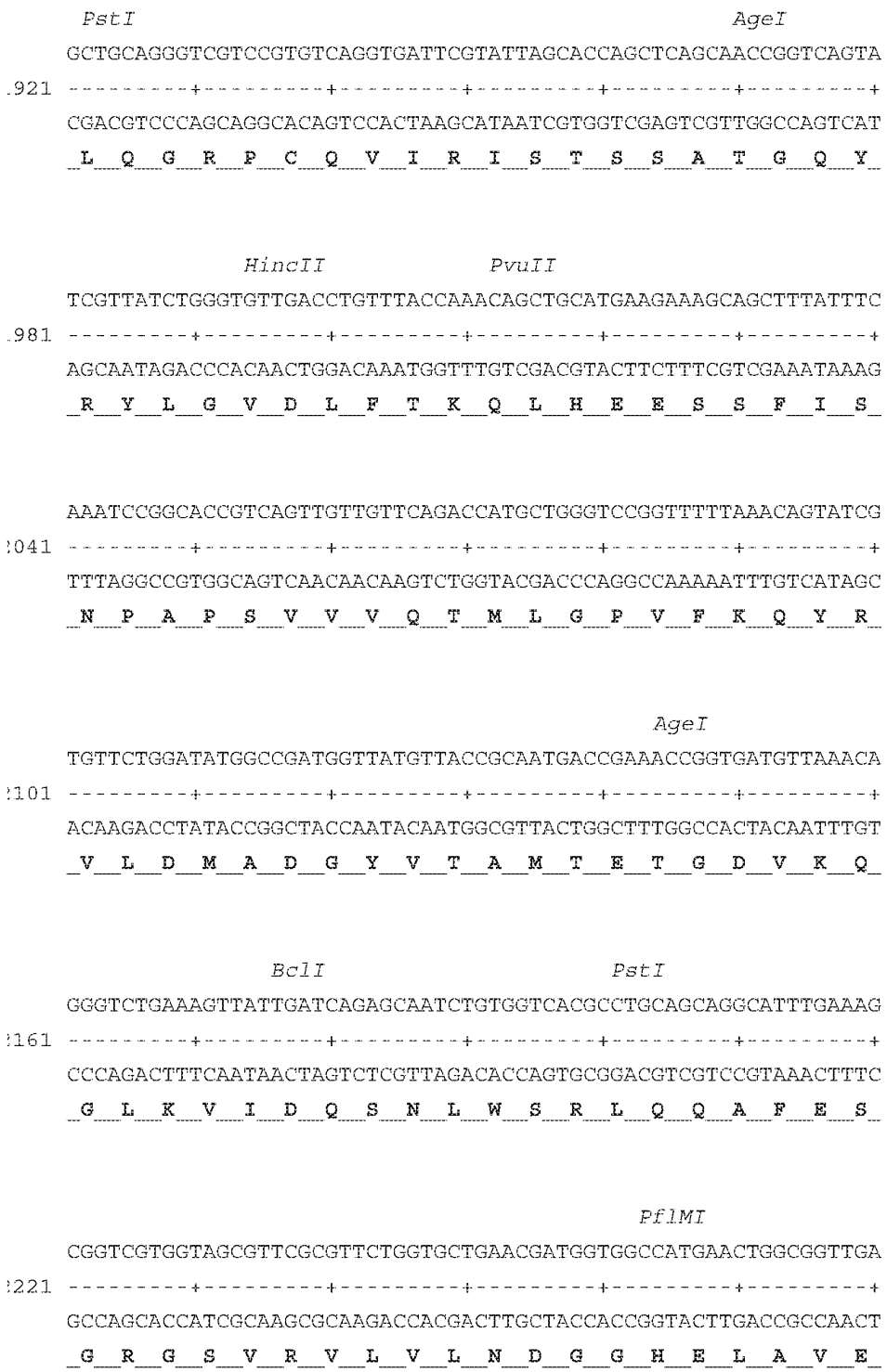
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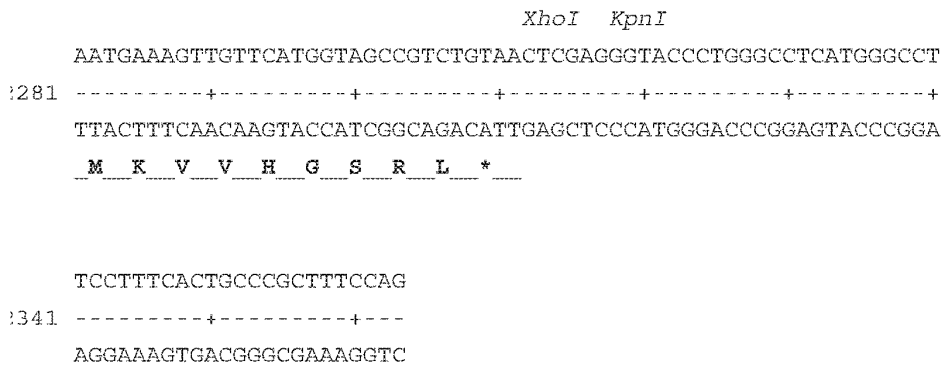
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Figure 3

SEQUENCE LISTING (SEQ ID NO:2)

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# SYNTHETIC FUSION GENE AND ITS USE THEREOF

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** The present application claims priority to Brunei Patent Application No. BN/N/2014/0037, entitled "Synthetic Fusion Gene and its use thereof" and filed on Apr. 3, 2014, the contents of which are hereby incorporated by reference.

## FIELD OF THE INVENTION

**[0002]** The present invention relates to synthetic fusion gene comprising hex1 and pox1 genes, their process of preparation, polypeptide(s) encoded by the same and their use thereof.

## BACKGROUND OF THE INVENTION

**[0003]** There is a growing interest in using renewable feedstock for manufacturing biofuels, such as bioethanol, biochemical and biodiesel. As such, pre-treatment of the biomass is needed to increase the rate and/or yield of biofuel production.

**[0004]** Currently, the main methods used for pre-treatment are physical, such as milling, or chemical, such as acid pre-treatment. However, biological methods are a promising alternative since no harmful chemicals are used and less energy input is required.

**[0005]** However, pre-treatment attempts to date have fallen short of the desired economic and technical performance. Thereby, there exists a need for effective, economical pre-treatments to make these polysaccharides available at a sufficiently high yield and acceptable cost.

## BRIEF SUMMARY OF THE INVENTION

**[0006]** The present invention discloses synthetic fusion gene comprising hex1 and pox 1 genes, their process of preparation, polypeptide(s) encoded by the same and its use thereof for biological pre-treatment of biomass for the production of biodiesel.

**[0007]** In an embodiment, the present invention provides a polypeptide comprising an amino acid sequence of SEQ ID NO:1.

**[0008]** In another embodiment, it provides a polynucleotide comprising a nucleotide sequence of SEQ ID NO:2. It further describes the polynucleotide, capable of encoding a polypeptide, wherein the polypeptide comprising an amino acid sequence of SEQ ID NO. 1.

**[0009]** In another embodiment, the polynucleotide comprising SEQ ID NO 2 is obtained from hex 1 and pox1 gene.

**[0010]** In yet another embodiment, it discloses a vector comprising a polynucleotide, wherein the polynucleotide comprising a nucleotide sequence of SEQ ID NO:2.

**[0011]** The invention further provides a method of biological pretreatment for biofuel production comprising, introducing a vector in a host cell under conditions suitable for the expression of the vector, wherein the vector comprising a nucleotide sequence of SEQ ID NO:2 which is capable of encoding a polypeptide, the polypeptide comprising an amino acid sequence of SEQ ID NO:1.

**[0012]** It further discloses the method of biological pretreatment for biofuel production, wherein the polypeptide is capable of hydrolysing lignocellulo sic biomass.

**[0013]** The present invention solves the long standing need of pure biological treatment, removing the need of any additional physical or chemical pre-treatment step. Further, since polynucleotide comprising a nucleotide sequence of SEQ ID NO:2 is a pure biological agent there is no hazard to the environment.

**[0014]** The present invention, also provides quick and cheaper method of pre-treatment of biomass as nucleotide sequence of SEQ ID NO:2 can be replicated and can be used two or more times.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0015]** FIG. **1** shows the plasmid containing synthetic fusion gene along with biodiesel producing gene.

**[0016]** FIG. **2** shows the polypeptide comprising an amino acid sequence according to the present invention encoded by the synthetic fusion gene comprising hex1 and pox 1 genes. SEQ ID NO: 1 is referred by bold letter in sequence listing and SEQ ID NO: 2 is referred by light letters in sequence listing.

**[0017]** FIG. **3** shows the polynucleotide sequence of synthetic fusion gene comprising hex1 and pox1 genes. (SEQ ID NO: 2)

## DEFINITIONS

**[0018]** The term "polypeptide" corresponds to any chain of amino acids, regardless of length or post-translational modification (glycosylation or phosphorylation).

**[0019]** The term "polynucleotide" corresponds to any genetic material of any length and any sequence, comprising single-stranded and double-stranded DNA and RNA molecules, including regulatory elements, structural genes, groups of genes, plasmids, whole genomes and fragments thereof.

**[0020]** The term "nucleic acid molecule" is intended to indicate any single- or double stranded nucleic acid molecule of cDNA, genomic DNA, synthetic DNA or RNA, PNAS or LNA origin.

**[0021]** The term "plasmid", "vector system" or "expression vector" means a construct capable of in-vivo or in-vitro expression.

**[0022]** The term "host cell" in relation to the present invention includes any cell that comprises either the nucleic acid molecule or an expression vector as described above and which is used in the production of polypeptide having the specific properties as defined herein or in the methods of the present invention.

## DETAILED DESCRIPTION OF THE INVENTION

**[0023]** For the purpose of promoting and understanding of the principles of the invention, reference will now be made to embodiments and specific language will be used to describe the same. It will nevertheless be understood that no limitation of the scope of the invention is thereby intended, such alterations and further modifications in the disclosed process, and such further applications of the principles of the invention therein being contemplated as would normally occur to one skilled in the art to which the invention relates.

**[0024]** Reference throughout this specification to "one embodiment" "an embodiment" or similar language means that a particular feature, structure, or characteristic described in connection with the embodiment is included in at least

one embodiment of the present invention. Thus, appearances of the phrase "in one embodiment", "in an embodiment" and similar language throughout this specification may, but do not necessarily, all refer to the same embodiment.

**[0025]** The present invention discloses synthetic fusion gene comprising hex1 and pox1 genes, their process of preparation, polypeptide(s) encoded by the same and its use thereof for biological pre-treatment of biomass for the production of biodiesel.

**[0026]** The invention further discloses that the synthetic fusion gene was constructed using bioinformatics tools to help complete hydrolysis of lignocelluloses of Laila paddy into glucose for the production of biodiesel from (husk/straw) of Laila paddy.

**[0027]** The present disclosure relates to a polypeptides comprising an amino acid sequence of SEQ ID NO: 1 as referred by bold letter in sequence listing of FIG. **2**.

**[0028]** In another aspect, the disclosure provides a polynucleotide comprising a nucleotide sequence of SEQ ID NO:2. It further describes the polynucleotide, capable of encoding a polypeptide, wherein the polypeptide comprising an amino acid sequence of SEQ ID NO: 1.

**[0029]** In further discloses that, the polynucleotide comprising SEQ ID NO: 2 is obtained from hex1 and pox1 gene.

**[0030]** In order to produce a polypeptide, it discloses a vector comprising a polynucleotide, wherein the polynucleotide comprising a nucleotide sequence of SEQ ID NO: 2.

**[0031]** The invention further provides a method of biological pre-treatment for biofuel production, wherein the polynucleotide comprising SEQ ID NO: 2 is included in an expression cassette and/or cloned into a suitable expression vector by standard molecular cloning techniques. The expression cassette or vector is introduced into a suitable expression host cell, which then expresses the corresponding polypeptide comprising SEQ ID NO: 1.

**[0032]** It further discloses the method of biological pre-treatment for biofuel production, wherein the polypeptide is capable of hydrolysing lignocellulosic biomass.

**[0033]** The present invention solves the long standing need of pure biological treatment, removing the need of any additional physical or chemical pre-treatment step. Further, since polynucleotide comprising a nucleotide sequence of SEQ ID NO:2 is a pure biological agent there is no hazard to the environment.

**[0034]** The present invention, also provides quick and cheaper method of pre-treatment of biomass as nucleotide sequence of SEQ ID NO: 2 can be replicated and can be used two or more times.

**[0035]** In an embodiment, the present invention arranges synthetic fusion gene to help complete hydrolysis of lignocelluloses of Laila paddy into glucose for production of biodiesel from hust/straw of laila paddy. The invention arranges Hex 1 and Pox1 . Hex1 is a fungi which is mainly used for hydrolysis of cellulose and hemicelluloses in pre-treatment. Pox1 is a mushroom which is used to break down lignin in lignocelluloses. The invention joins Hex1 and Pox1 together by arranging their cDNA nucleotides to construct new synthetic fusion gene.

**[0036]** In another embodiment, end codon of *Tricoderma recei* (hex1) is spliced and start codon of *pleurotus sojar caju* (pox1) is attached to it by synthetic arrangement of nucleotides. *Pleurotus sojar cajus* (pox1) stop codon act as end codons for both genes. Therefore, fusion gene work as a single gene having both properties of hex 1 and Pox 1.

**[0037]** For any Production of Biodiesel/micro diesel from ligno cellulose (Cellulose, hemicellulose & lignin) it is necessary to do a pre-treatment. However, no additional pretreatment process is required when novel fusion gene is used and fermentation step can be carried out without pretreatment. Synthetic fusion gene can be placed in same plasmid along with other gene used for production of biodiesel.

**[0038]** A Pre-treatment is a phase in which the lignocelluloses materials such as wood or straw is amenable to hydrolysis. Pre-treatment technique has been generally divided into physical, chemical and biological. Physical Treatment includes Milling and Grinding, Chemical Treatment includes Using Acids or alkali, and Biological Treatment includes mostly using Rot Fungi in combination with Physical treatment. However using the synthetic gene there is no need for a pre-treatment as the fusion gene is placed in the same plasmid along with biodiesel producing gene. Hence, hydrolysis of ligno cellulose takes place along with biodiesel production during fermentation.

**[0039]** Generally lignocelluloses has to undergo first a Combined pretreatment (Physical, Chemical, and an expensive Enzymatic treatment) prior to fermentation process. However the pre-treatment does not break the lignin or through some enzymes only partial breakdown of lignin is achieved. Thus a major part of lignin remain intact which is also a carbon source that is wasted. The present invention is a one step process in which invitro enzymatic pretreatment resulting from fusion gene hex1pox1 & fermentation takes place in the same medium. Also complete breakdown of lignin is achieved thereby increasing the production of biodiesel as all sources of carbon from lignocelluloses are utilized.

**[0040]** In another embodiment, Aerobic Fermentation was used. Fermentation flask was immersed in a temperature controlled water bath maintained at 37° C. and stirred at 250-280 rpm for 24 hours. pH was maintained within the range of 6.8 to 7. Lower temperatures upto 30 deg C. was tried, the growth of bacteria was not seen in the medium. Higher temperature yielded a turbid medium within 6 hours indicating full growth however resulted a very low yield. Thus 37 deg C. was the optimal temperature which resulted in the best yield.

**[0041]** The synthetic fusion gene comprises of hex1 and pox1 gene by synthetic arrangement of nucleotides, wherein hex1 and pox1 helps in hydrolysis of cellulose, hemicelluloses and lignin. The synthetic fusion gene helps in complete hydrolysis of lignocellulose into glucose without the use of any pre-treatment method for biodiesel production from paddy.

**[0042]** In another embodiment, the synthetic fusion gene can also be used as a pure biological pre-treatment tool for any production of micro diesel. The Synthetic gene is also used in p Cola duet 1 Z.

**[0043]** In another embodiment Genes hex1 and pox1 play the role of degradation of lignocellulose.

**[0044]** In an embodiment, the invention may be used on Biomass to produce biofuel. The biomass includes Laila paddy, husk and straw. *Oriza sativa* having same chemical component or any lignocellulose biomass having same component with different ratio can also be used as biomass.

**[0045]** In an embodiment, Fad E. del *E. coli* is used as host organism. Gene is optimized for *E. coli*. For other host

organism like mammalian, insect and yeast expression a KOZAK sequence is recommended to the upstream of the construct.

**[0046]** In another embodiment, other gene that are combined with pox1 hex1 are pdc, adh, and aft A. pdc & adh help in production of ethanol and aft A helps in the conversion of ethanol into biodiesel). Thus these gene (pdc, adh, aft A) complement fusion gene in producing biodiesel not in any hydrolytic process of lignocellulose.

Construction of Synthetic Fusion Gene:

**[0047]** *Tricoderma reesi* (hex1) is a fungi which is mainly used for hydrolysis of cellulose and hemicelluloses in pre-treatment and *Pleurotous sojar caju* (pox1) is a mushroom which is used to breakdown lignin in lignocelluloses. These two particular genes hex1 and pox1 play the role of degradation in these fungi. In the present invention, hex1 and pox1 were joined together by arranging their cDNA nucleotides, to construct new synthetic fusion gene. Here end codon of *Tricoderma reesi* (hex1) was spilced and *Pleurotous sojar caju* (pox1) start codon was attached to it by synthetic arrangement of nucleotides. The stop codon of *Pleurotous sojar caju* (pox1) acts as end codons for both the genes. Thus, the fusion gene works as single gene having both properties.

## SPECIFIC EMBODIMENTS ARE DESCRIBED BELOW

**[0048]** A polypeptide comprising an amino acid sequence of SEQ ID NO: 1 as referred by bold letter in sequence listing of FIG. **2**.

## FURTHER SPECIFIC EMBODIMENTS ARE DESCRIBED BELOW

**[0049]** A polynucleotide comprising a nucleotide sequence of SEQ ID NO: 2 as referred by light letter in sequence listing of FIG. **2**.

**[0050]** Such polynucleotide(s), capable of encoding a polypeptide, the polypeptide comprising an amino acid sequence of SEQ ID NO. 1.

**[0051]** Such polynucleotide(s), wherein the SEQ ID NO 2 is obtained from hex1 and pox1 gene.

## FURTHER SPECIFIC EMBODIMENTS ARE DESCRIBED BELOW

**[0052]** A vector comprising a polynucleotide, the polynucleotide comprising a nucleotide sequence of SEQ ID NO: 2.

## FURTHER SPECIFIC EMBODIMENTS ARE DESCRIBED BELOW

**[0053]** A method of biological pre-treatment for biofuel production comprising: introducing a vector in a host cell under conditions suitable for the expression of the vector, wherein the vector comprising a nucleotide sequence of SEQ ID NO:2 which is capable of encoding a polypeptide, the polypeptide comprising an amino acid sequence of SEQ ID NO:1.

**[0054]** Such method(s), wherein the polypeptide is capable of hydrolysing lignocellulosic biomass.

## INDUSTRIAL APPLICABILITY

**[0055]** The present invention discloses the method of biological pre-treatment for biofuel production, wherein the polypeptide is capable of hydrolysing lignocellulosic biomass.

**[0056]** Further, the present invention solves the long standing need of pure biological treatment, removing the need of any additional physical or chemical pre-treatment step. Further, since polynucleotide comprising a nucleotide sequence of SEQ ID NO: 2 is a pure biological agent there is no hazard to the environment.

**[0057]** Furthermore, the present invention, also provides quick and cheaper method of pre-treatment of biomass as nucleotide sequence of SEQ ID NO: 2 can be replicated and can be used two or more times

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SEQUENCE LISTING

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Fusion

<400> SEQUENCE: 1

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Leu Leu His Gly Thr His Ala Ala Ile Gly Pro Thr Gly Asp Met Tyr
            20                  25                  30

Ile Val Asn Glu Asp Val Ser Pro Asp Gly Phe Thr Arg Ser Ala Val
        35                  40                  45

Val Ala Arg Ser Asp Pro Thr Thr Asn Gly Thr Ser Glu Thr Leu Thr
    50                  55                  60

Gly Val Leu Val Gln Gly Asn Lys Gly Asp Asn Phe Gln Leu Asn Val
65                  70                  75                  80
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```
Leu Asn Gln Leu Ser Asp Thr Thr Met Leu Lys Thr Thr Ser Ile His
                85                  90                  95

Trp His Gly Phe Phe Gln Ser Gly Ser Thr Trp Ala Asp Gly Pro Ala
            100                 105                 110

Phe Val Asn Gln Cys Pro Ile Ala Ser Gly Asn Ser Phe Leu Tyr Asp
        115                 120                 125

Phe Asn Val Pro Asp Gln Ala Gly Thr Phe Trp Tyr His Ser His Leu
    130                 135                 140

Ser Thr Gln Tyr Cys Asp Gly Leu Arg Gly Pro Phe Ile Val Tyr Asp
145                 150                 155                 160

Pro Ser Asp Pro His Leu Ser Leu Tyr Asp Val Asp Asn Ala Asp Thr
                165                 170                 175

Ile Ile Thr Leu Glu Asp Trp Tyr His Val Val Ala Pro Gln Asn Ala
            180                 185                 190

Val Leu Pro Thr Ala Asp Ser Thr Leu Ile Asn Gly Lys Gly Arg Phe
        195                 200                 205

Ala Gly Gly Pro Thr Ser Ala Leu Ala Val Ile Asn Val Glu Ser Asn
    210                 215                 220

Lys Arg Tyr Arg Phe Arg Leu Ile Ser Met Ser Cys Asp Pro Asn Phe
225                 230                 235                 240

Thr Phe Ser Ile Asp Gly His Ser Leu Gln Val Ile Glu Ala Asp Ala
                245                 250                 255

Val Asn Ile Val Pro Ile Val Val Asp Ser Ile Gln Ile Phe Ala Gly
            260                 265                 270

Gln Arg Tyr Ser Phe Val Leu Asn Ala Asn Gln Thr Val Asp Asn Tyr
        275                 280                 285

Trp Ile Arg Ala Asp Pro Asn Leu Gly Ser Thr Gly Phe Asp Gly Gly
    290                 295                 300

Ile Asn Ser Ala Ile Leu Arg Tyr Ala Gly Ala Thr Glu Asp Asp Pro
305                 310                 315                 320

Thr Thr Thr Ser Ser Thr Ser Thr Pro Leu Glu Glu Thr Asn Leu Val
                325                 330                 335

Pro Leu Glu Asn Pro Gly Ala Pro Gly Pro Ala Val Pro Gly Gly Ala
            340                 345                 350

Asp Ile Asn Ile Asn Leu Ala Met Ala Phe Asp Val Thr Asn Phe Glu
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Leu Thr Ile Asn Gly Ser Pro Phe Lys Ala Pro Thr Ala Pro Val Leu
    370                 375                 380

Leu Gln Ile Leu Ser Gly Ala Thr Thr Ala Ala Ser Leu Leu Pro Ser
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Gly Ser Ile Tyr Ser Leu Glu Ala Asn Lys Val Val Glu Ile Ser Ile
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Pro Ala Leu Ala Val Gly Gly Pro His Pro Phe His Leu His Gly His
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Thr Phe Asp Val Ile Arg Ser Ala Gly Ser Thr Thr Tyr Asn Phe Asp
        435                 440                 445

Thr Pro Ala Arg Arg Asp Val Val Asn Thr Gly Thr Asp Ala Asn Asp
    450                 455                 460

Asn Val Thr Ile Arg Phe Val Thr Asp Asn Pro Gly Pro Trp Phe Leu
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His Cys His Ile Asp Trp His Leu Glu Ile Gly Leu Ala Val Val Phe
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Leu Cys Pro Ile Tyr Asp Ala Leu Ser Asp Ser Asp Lys Gly Gly Ile
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Ala Gly Tyr Tyr Asp Asp Glu Gly Ser Tyr His Ser Leu Lys His Gly
    530                 535                 540

Val Ala Lys Thr Ile Asp Lys Leu Leu Pro His His His His His His
545                 550                 555                 560

His His Ser Asp His His His His Ser Asp His His Asp His Asn Asn
                565                 570                 575

Thr Thr Ile Thr Glu His Val Glu Val Asp Val Val Arg His Asp Ala
            580                 585                 590

Asn His Ser Arg Arg Ala Ala Pro Ala Thr Glu Ser Gln Pro Gln Thr
        595                 600                 605

Val Ser Ile Pro Cys His His Ile Arg Leu Gly Asp Phe Leu Met Leu
    610                 615                 620

Gln Gly Arg Pro Cys Gln Val Ile Arg Ile Ser Thr Ser Ser Ala Thr
625                 630                 635                 640

Gly Gln Tyr Arg Tyr Leu Gly Val Asp Leu Phe Thr Lys Gln Leu His
                645                 650                 655

Glu Glu Ser Ser Phe Ile Ser Asn Pro Ala Pro Ser Val Val Val Gln
            660                 665                 670

Thr Met Leu Gly Pro Val Phe Lys Gln Tyr Arg Val Leu Asp Met Ala
        675                 680                 685

Asp Gly Tyr Val Thr Ala Met Thr Glu Thr Gly Asp Val Lys Gln Gly
    690                 695                 700

Leu Lys Val Ile Asp Gln Ser Asn Leu Trp Ser Arg Leu Gln Gln Ala
705                 710                 715                 720

Phe Glu Ser Gly Arg Gly Ser Val Arg Val Leu Val Leu Asn Asp Gly
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<210> SEQ ID NO 2
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Fusion

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gacggcttca ctcgttcggc tgtcgtcgct cgctctgacc ccaccacaaa tgggacgtcg    180

gagacgctta ccggtgtcct cgtgcaagga aacaagggcg acaacttcca gctgaacgtt    240

ctcaatcaac tgtcggacac gactatgttg aagaccacta gtatccattg gcatggcttc    300

tttcaatccg gttctacgtg ggcagatgga cccgcgttcg tgaatcaatg ccccatcgcc    360

tcggggaaca gcttcctata tgacttcaac gtccccgacc aagctggcac gttctggtac    420

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ctcatcaatg gcaaaggtcg cttcgctggg gggcctactt ccgctttggc cgtcatcaac 660

gtcgaaagca acaagcgata tcgtttcaga cttatctcga tgtcttgcga ccccaatttc 720

acgttctcga tcgacggtca ctctttgcag gtcatcgagg cagacgctgt caatattgtg 780

cccattgtcg tggatagtat tcaaatcttc gcaggccaac gctattcctt cgtcttgaat 840

gccaatcaga ctgtcgacaa ttactggatt cgcgcagatc ccaacttggg atcgactggc 900

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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agccattggt ccgaccggtg atatgtatat tgttaacgaa gatgttagtc cggatggttt 180

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cagctttctg tatgatttta atgttccgga tcaggcaggc acctttttggt atcatagcca    480

tctgagcacc cagtattgtg atggtctgcg tggtccgttt attgtttatg atccgagcga    540

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caataaacgc tatcgctttc gcctgattag catgagctgt gatccgaact ttacctttag    780

cattgatggt catagcctgc aggttattga agcagatgcc gttaatattg ttccgattgt    840

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ctcaagcacc agcacaccgc tggaagaaac caatctggtt ccgctggaaa atcctggtgc   1080

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aatgaaagtt gttcatggta gccgtctgta actcgagggt accctgggcc tcatgggcct   2340

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```

What is claimed is:

**1**. A polypeptide comprising an amino acid sequence of SEQ ID NO:1.

**2**. A polynucleotide comprising a nucleotide sequence of SEQ ID NO:2.

**3**. The polynucleotide as claimed in claim **2**, capable of encoding a polypeptide, the polypeptide comprising an amino acid sequence of SEQ ID NO. 1.

**4**. The polynucleotide as claimed in claim **2**, wherein the SEQ ID NO 2 is obtained from hex1 and pox1 gene.

**5**. (canceled)

**6**. (canceled)

**7**. (canceled)

**8**. A synthetic fusion gene comprising hex1 and pox1 wherein hex1 and pox1 are joined together by arranging their eDNA nucleotides.

**9**. The method of claim **8** further comprising:

splicing end codon of hex1 and start codon of pox1 and attaching together by synthetic arrangement of nucleotides.

**10**. The method of claim **8** further comprising:

splicing codon of Hex1 and attaching codon of Pox1 by synthetic arrangement of nucleotides.

* * * * *