



(12) 发明专利

(10) 授权公告号 CN 111139261 B

(45) 授权公告日 2022.05.31

(21) 申请号 202010119498.9

(22) 申请日 2020.02.26

(65) 同一申请的已公布的文献号
申请公布号 CN 111139261 A

(43) 申请公布日 2020.05.12

(66) 本国优先权数据
201910152467.0 2019.02.28 CN

(73) 专利权人 山东省农业科学院作物研究所
地址 250100 山东省济南市历城区工业北路202号

(72) 发明人 张淑娟 李根英 李玉莲 张荣志
高洁 宋国琦 李玮 陈明丽
刘敏 李吉虎

(74) 专利代理机构 济南诚智商标专利事务所有
限公司 37105
专利代理师 房一粟

(51) Int. Cl.
C12N 15/82 (2006.01)
C12N 15/113 (2010.01)
A01H 5/10 (2018.01)
A01H 6/46 (2018.01)

(56) 对比文件

CN 101508990 A, 2009.08.19
CN 107653256 A, 2018.02.02
CN 108866092 A, 2018.11.23
CN 1260836 A, 2000.07.19
CN 102154347 A, 2011.08.17
CN 104884626 A, 2015.09.02
US 2005229267 A1, 2005.10.13
DE 10225066 A1, 2003.12.18

黄婷等. 小麦多酚氧化酶(PPO)活性的基因型环境及其互作效应的分析.《安徽农业科学》.2007,第35卷(第32期),
陈锋等. 小麦籽粒多酚氧化酶及其控制基因研究进展.《麦类作物学报》.2011,第31卷(第4期),
Deepa Jaganathan等. CRISPR for Crop Improvement: An Update Review.《Frontiers in Plant Science》.2018,第9卷 (续)

审查员 张珍珍

权利要求书2页 说明书6页
序列表16页 附图7页

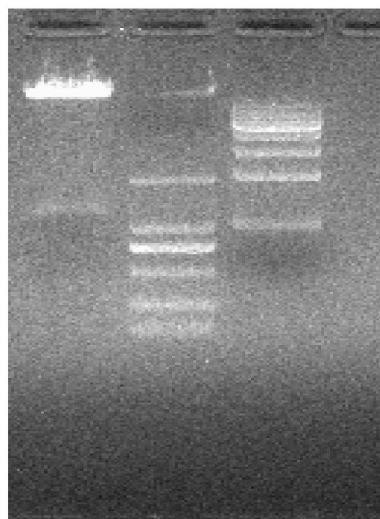
(54) 发明名称

一种利用基因编辑降低小麦籽粒多酚氧化酶含量的方法

(57) 摘要

本发明公开了一种利用基因编辑降低小麦籽粒多酚氧化酶含量的方法。本发明通过特异性引导单链gRNA的设计,构建了可以实现PPO基因体内编辑的表达载体,可以敲除小麦PPO基因。在实际应用中,通过一个基因转化过程,就可实现基因组水平胚乳特异表达PPO基因的沉默,从而降低小麦籽粒多酚氧化酶含量,减少面粉褐变,提高面粉白度。为利用基因工程技术改良小麦面粉的白度,提高小麦加工品质提供有力的工具。

1 2 3



CN 111139261 B

[接上页]

(56) 对比文件

Shujuan Zhang等. CRISPR/Cas9-mediated genome editing for wheat grain quality

improvement.《Plant Biotechnol J》.2021,第19卷(第9期),

1. 一种具有敲除PPO基因功能的pBUE411-PP0双元载体,其特征在于,包括表达盒A和表达盒B;

所述表达盒A具体自上游至下游依次包括如下元件:来自小麦的TaU3启动子A,所述PPO基因的sgRNA、终止子A;

所述表达盒B具体自上游至下游依次包括如下元件:启动子B、玉米Cas9编码序列、终止子B;

所述PPO基因的sgRNA的序列如SEQ ID NO.10所示;

所述玉米Cas9编码序列如SEQ ID NO.13所示;

所述启动子A的序列如SEQ ID NO.1所示;

所述启动子B序列如SEQ ID NO.2所示;

所述终止子A序列如SEQ ID NO.3所示;

所述终止子B序列如SEQ ID NO.4所示;

所述pBUE411-PP0双元载体含有抗性标记基因,所述标记基因为bar基因;

所述pBUE411-PP0双元载体的核苷酸序列如SEQ ID NO.7所示。

2. 根据权利要求1所述的pBUE411-PP0双元载体,其特征在于,所述表达盒A具体可如序列SEQ ID NO.5所示;

所述表达盒B具体可如序列SEQ ID NO.6所示;

所述表达盒A位于所述表达盒B的上游,表达盒A和表达盒B相互串联。

3. 如权利要求1或2所述的pBUE411-PP0双元载体的构建方法,其特征在于,包括如下步骤:

S11: 根据靶向PPO基因设计其sgRNA,sgRNA的寡核苷酸对应的是PP0-gR1-TaU3-F和PP0-gR1-TaU3-R,即PP0-gR1-TaU3-F序列如SEQ ID NO.8所示,PP0-gR1-TaU3-R序列如SEQ ID NO.9所示;

S12: 将步骤S11合成的sgRNA的2条寡核苷酸进行磷酸化和退火形成双链,获得带有粘性末端的双链sgRNA;

S13: 利用限制性内切酶BsaI酶切pBUE411载体,回收含有所述启动子A、所述终止子A、所述启动子B、所述终止子B的载体片段;

S14: 将步骤S13回收的pBUE411载体片段和步骤S12获得的双链sgRNA通过T4连接酶进行连接反应,获得最终的pBUE411-PP0双元载体。

4. 根据权利要求3所述的pBUE411-PP0双元载体的构建方法,其特征在于,所述PPO基因为GENBANK ACCESSION NUMBER:EF070147.1所示的DNA。

5. 如权利要求1-3任一项所述pBUE411-PP0双元载体在降低小麦胚乳中PPO基因表达水平中的应用。

6. 如权利要求1-3任一项所述pBUE411-PP0双元载体在小麦面粉色泽改良中的应用。

7. 如权利要求1-3任一项所述pBUE411-PP0双元载体在培育转基因小麦中的应用。

8. 一种利用基因编辑降低小麦籽粒多酚氧化酶含量的方法,其特征在于,包括如下步骤:

S1 构建如权利要求1-3任一项所述的pBUE411-PP0双元载体;

S2 将步骤S1获得的pBUE411-PP0双元载体通过农杆菌转化到普通小麦中,获得转基因

小麦。

一种利用基因编辑降低小麦籽粒多酚氧化酶含量的方法

技术领域

[0001] 本发明涉及一种利用基因编辑降低小麦籽粒多酚氧化酶含量的方法,属于基因工程领域。

背景技术

[0002] 小麦胚乳中多酚氧化酶(Polyphenol oxidase, PPO)容易引起面团褐变,从而影响面制品的白度。小麦面粉中的PPO活性仅占全籽粒的3%左右,但可以解释面粉及面制品加工和贮存过程中色泽褐变的50-70%。我国很多小麦品种的面粉色泽不能满足加工要求,面粉生产及食品加工企业采用向面粉中添加增白剂的方式,增加其产品的白度。过氧化苯甲酰(Benzoyl peroxide, BPO)是一种氧化剂,为当前使用最广泛的面粉增白剂。BPO的分解产物苯甲酸和苯酚均需肝脏中进行解毒,长期食用BPO超标的面粉及其制品会引起慢性中毒。因此,人们很久以来就期待着无增白剂面制品的问世,低PPO小麦品种的培育,将对面制品的加工工艺产生重要影响,无增白剂食品将具有巨大的市场空间和产业化前景。因此,培育具有低PPO活性的小麦品种是对面制品色泽性状进行改良的重要途径。

[0003] 小麦籽粒PPO活性主要受位于第二同源群上基因的调控,分别位于2AL和2DL,其中2AL上的QTL的效应较大,贡献率37.9-50.0%;位于2DL上的QTL贡献率为25.0-29.1%(D.J.Sun, Z.H.He, X.C.Xia, L.P.Zhang, C.F.Morris, R.Appels, W.J.Ma, H.Wang. A novel STS marker for polyphenol oxidase activity in bread wheat. Mol Breeding, 2005, 16:209-218)。利用电子克隆技术克隆2A和2D染色体上小麦籽粒PPO基因的全部编码序列,并利用模式植物对基因的功能进行了初步验证。Ppo-A1、Ppo-D1均包含一个1731bp的读码框(X.Y.He, Z.H.He, L.P.Zhang, D.J.Sun, C.F.Morris, E.P.Furerst, and X.C.Xia. Allelic variation of polyphenol oxidase (PPO) genes located on chromosomes 2A and 2D and development of functional markers for the PPO genes in common wheat, Theor Appl Genet, 2007, 115:47-58。),可编码一条含577个氨基酸的多肽(64kD),其基因编码区序列相似性达95%以上,这不仅显示了它们的同源进化关系,也为利用RNAi技术同步沉默2A和2D上的PPO基因提供了便利。

发明内容

[0004] 本发明克服了上述现有技术的不足,提供一种利用基因编辑降低小麦籽粒多酚氧化酶含量的方法。本发明的方法通过构建具有敲除PPO基因功能的表达载体pBUE411-PPO,通过基因转化过程降低胚乳中PPO基因的表的水平。

[0005] 一种具有敲除PPO基因功能的pBUE411-PPO双元载体,包括表达盒A和表达盒B;

[0006] 所述表达盒A具体自上游至下游依次包括如下元件:所述来自小麦的TaU3启动子A,所述PPO基因的sgRNA、终止子A;

[0007] 所述表达盒B具体自上游至下游依次包括如下元件:所述启动子B、所述玉米Cas9编码序列、所述终止子B。

- [0008] 进一步的,所述PPO基因的sgRNA的序列如SEQ10所示;
- [0009] 所述玉米Cas9编码序列如SEQ13所示;
- [0010] 所述启动子A的序列如SEQ1所示;
- [0011] 所述启动子B序列如SEQ2所示;
- [0012] 所述终止子A序列如SEQ3所示;
- [0013] 所述终止子B序列如SEQ4所示。
- [0014] 进一步的,所述表达盒A具体可如序列SEQ5所示;
- [0015] 所述表达盒B具体可如序列SEQ6所示;
- [0016] 所述表达盒A位于所述表达盒B的上游,表达盒A和表达盒B相互串联。
- [0017] 进一步的,所述pBUE411-PP0双元载体还含有抗性标记基因,如bar基因。
- [0018] 进一步的,所述pBUE411-PP0双元载体的核苷酸序列如SEQ7所示。
- [0019] 一种构建上述pBUE411-PP0双元载体的方法,包括如下步骤:
- [0020] S11根据靶向PP0基因设计其sgRNA,sgRNA的寡核苷酸对应的是PP0-gR1-TaU3-F和PP0-gR1-TaU3-R,即PP0-gR1-TaU3-F序列如SEQ8所示,PP0-gR1-TaU3-R序列如SEQ9所示;
- [0021] S12将步骤①合成的sgRNA的2条寡核苷酸进行磷酸化和退火形成双链,获得带有粘性末端的双链sgRNA;
- [0022] S13利用限制性内切酶BsaI酶切pBUE411载体,回收含有所述启动子A、所述终止子A、所述启动子B、所述终止子B的载体片段;
- [0023] S14将步骤S13回收的pBUE411载体片段和双链sgRNA通过T4连接酶进行连接反应,获得最终的pBUE411-PP0双元载体。
- [0024] 进一步的,所述PP0基因可为GENBANK ACCESSION NUMBER:EF070147.1 (VERSION ABK62801.1)所示的DNA。
- [0025] 含有所述pBUE411-PP0双元载体的转基因细胞系或重组菌也属于本发明的保护范围。
- [0026] 所述pBUE411-PP0双元载体可应用于降低小麦胚乳中PPO基因表达水平。
- [0027] 所述pBUE411-PP0双元载体在小麦面粉色泽改良中的应用。
- [0028] 所述pBUE411-PP0双元载体在培育转基因小麦中的应用;与转基因前小麦相比,所述转基因小麦的面粉白度增加。
- [0029] 本发明还保护一段DNA片段I,所述DNA片段I由PP0基因的引导sgRNA组成,其序列具体可如序列SEQ10所示。
- [0030] 一种利用基因编辑降低小麦籽粒多酚氧化酶含量的方法,包括如下步骤:
- [0031] S1构建一种pBUE411-PP0双元载体;
- [0032] S2将步骤S1获得的pBUE411-PP0双元载体通过农杆菌转化到普通小麦中,获得转基因小麦。
- [0033] 有益效果:
- [0034] 本发明通过特异性引导单链gRNA的设计,构建了可以实现PP0基因体内编辑的表达载体,可以敲除小麦PP0基因。在实际应用中,通过一个基因转化过程,就可实现基因组水平胚乳特异表达PP0基因的沉默,从而降低小麦籽粒多酚氧化酶含量,减少面粉褐变,提高面粉白度。本发明为利用基因工程技术改良小麦面粉的白度,提高小麦加工品质提供有力

的工具。

附图说明

- [0035] 图1PPO基因的编码区。
 [0036] 图2pBUE411载体BsaI酶切电泳图。
 [0037] 图3靶向PPO基因CRISPR/Cas9重组载体pBUE411-PPO测序结果。
 [0038] 图4Bar试纸条检测转基因小麦植株。
 [0039] 图5野生型小麦PPO基因的推导氨基酸序列。
 [0040] 图6突变型小麦PPO基因的推导氨基酸序列。
 [0041] 图7野生型和突变型的PPO基因核苷酸序列比对图。
 [0042] 图8野生型和突变型的PPO基因的推导氨基酸序列比对图。
 [0043] 图9Hi-tom检测转基因株系PPO基因编辑位点图。
 [0044] 图10基因编辑株系及对照植株籽粒PPO基因相对表达。

具体实施方式

[0045] 以下的实施例便于更好地理解本发明,但并不限定本发明。下述实施例中的实验方法,如无特殊说明,均为常规方法。下述实施例中所用的试验材料,如无特殊说明,均为自常规生化试剂商店购买得到的。以下实施例中的定量试验,均设置三次重复实验,结果取平均值。PCR扩增所需的高保真酶、DNA片段连接所需的T4连接酶、酶切片段回收所需的凝胶回收试剂盒以及质粒提取试剂盒均购自大连宝生物公司。质粒提取试剂盒购自天根生物,培养基配制所需的无机盐购自国药集团,维生素和抗生素以及激素购自Sigma公司。植物CRISPR/Cas9基因编辑载体为pBUE411,含有小麦U3启动子TaU3,用以启动sgRNA,Cas9p模拟了禾本科植物基因具有5'端GC含量较高的特征,是设计合成的植物优化密码子基因。本实验室所用的大肠杆菌菌株是E.coli DH5 α 。

[0046] T4连接酶、DNA Marker DL2000等试剂购自Takara公司;FastPfu、2*TaQ Mix、DH5 α 等购自北京全式金公司;限制性内切酶BsaI-HF购自NEB公司;所使用的引物由青岛擎科梓熙生物技术有限公司合成,序列如下:

[0047] 表1本研究中使用的引物

引物	序列(5'-3')
PPO-gR1-TaU3-F	GGCGGAAGAAGACGCTGCTGTTCC
PPO-gR1-TaU3-R	AAACGGAACAGCAGCGTCTTCTCT
[0048] pBUE411-F	TTGTAAAACGACGGCCAGTG
pBUE411-R	TGCACTGCAGGCATGCAA
Primer-F	ggagtgagtacggtgtgcATATTGTTAAGGTAACCACAGA
Primer-R	gagttgagatcggatggTGCCCATGTCTCCAAGTT

[0049] 小麦品种JW1是山东省农科院作物所自行选育的具有良好组织培养能力的新种质,公众可从山东省农业科学院作物研究所获得

[0050] 质粒pBUE411如序列表的序列7所示(17430bp),由中国农业大学孙其信课题组提供;公众可从中国农业大学获得。

[0051] 实验例1表达载体的构建

[0052] 1. 靶向PPO的sgRNA的设计

[0053] 在小麦品种JW1里扩增TaPPO基因的序列,扩增序列见图1所示,下划线标记序列为靶向PPO基因的gRNA序列,下划线部分后三位标记为PAM序列,与NCBI数据库中的小麦的该基因序列100%相似。该基因全长1734个碱基,通过网站CRISPRdirect (<http://crispr.dbcls.jp/>) 在PPO基因的编码区寻找1个适合的打靶位点,在PAM结构前找到20bp序列片段设定为靶序列,sgRNA序列选定为GAAGAAGACGCTGCTGTTCC。

[0054] 2. sgRNA的退火及双链的形成

[0055] PPO基因的sgRNA的2条oligo是由青岛擎科梓熙生物技术有限公司合成,其中sgRNA的寡核苷酸对应的是PPO-gR1-TaU3-F和PPO-gR1-TaU3-R(表1),引物中的下划线碱基部分是与pBUE411载体中的序列匹配。分别将sgRNA的2条oligo进行磷酸化,直接退火形成双链,体系为:PPO-gR1-TaU3-F (10 μ M) : 4 μ L, PPO-gR1-TaU3-R (10 μ M) : 4 μ L, 10 \times T4 PNK buffer: 1.5 μ L, PNK: 1 μ L, ATP: 1 μ L, ddH₂O补足15 μ L。程序在PCR仪中按下列条件运行: 37 $^{\circ}$ C, 30min; 95 $^{\circ}$ C, 5min; ramp to 25 $^{\circ}$ C at 5 $^{\circ}$ C/min (0.08 $^{\circ}$ C/s)。或者是沸水中煮5min,之后自然冷却至室温。

[0056] 3. pBUE411载体的线性化

[0057] 用BsaI的内切酶酶切pBUE411载体3h,电泳后回收线性载体片段,回收含有所述启动子A、所述终止子A、所述启动子B、所述终止子B的载体片段酶切电泳图如图2所示,1为pBUE411用BsaI酶切;2为DL2000 Marker;3为1kb Marker。

[0058] 4. pBUE411载体与sgRNA的连接反应

[0059] 连接反应体系为: 5 \times ligation Buffer 2 μ L, annealed oligo duplex 4 μ L, pBUE411/BsaI 2 μ L (100ng), T4 ligase 1 μ L, ddH₂O补足10 μ L, 25 $^{\circ}$ C 30min进行连接反应。

[0060] 5. 转化

[0061] 将连接产物转化大肠杆菌,重组质粒经转化到DH5 α 感受态细胞,之后于LB(kan)平板上培养至克隆长出后,挑单克隆进行菌液PCR鉴定及测序,引物用的是pBUE411-F和pBUE411-R。测序结果(如图3)检测到了sgRNA的靶序列,同时在靶序列上游都检测到了TaU3启动子序列,测序结果说明sgRNA表达盒构建成功,并成功的组装进入pBUE411双元表达载体,证明了PPO的CRISPR/Cas9基因编辑载体构建成功。

[0062] 实验例2基因编辑载体的转化普通小麦

[0063] 1. 培养基配制: 见文献Kan Wang (ed.), *Agrobacterium Protocols: Volume 1, Methods in Molecular Biology*, vol.1223D0I10.007/978-1-4939-1695-5_15, Springer Science+Business Media New York 2015

[0064] 2. 农杆菌转化: 取授粉15天左右的小麦穗子,取粒剥胚。在试验前一天农杆菌悬浮液摇菌,160r, 28 $^{\circ}$ C培养24小时,穗子准备好后,开始准备农杆菌悬浮液取1ml菌液于1.5ml离心管中,加入1.4 μ l乙酰丁香酮(0.1M)混匀。加入准备好的菌液侵染5分钟后放到共培培养基上,23 $^{\circ}$ C暗培养3天。共培后放到休息培养基上暗培养5天,25 $^{\circ}$ C。将愈伤转移至筛选培养基1上。用封口膜封好培养皿,在25.5 $^{\circ}$ C培养箱中暗培养2周。将愈伤切割后转移到筛选培养基2上。

[0065] 用封口膜封好培养皿,在25.5 $^{\circ}$ C培养箱中暗培养2周。愈伤切割筛选2周后,表现有

抗性的愈伤转移到再生培养基上。抗性愈伤一般有绿芽或者绿点,或者有美丽的米黄色球状结构。

[0066] 糊状的和褐色的愈伤不要转移。有增生的愈伤可以再切成更小的愈伤。来自同一个愈伤的小块应该再摆到同一条线上。注意愈伤的方向,例如,绿芽和绿点朝上。

[0067] 封好培养皿,放到25度培养箱中光照(16h)培养2周。再生2周后,将健康成长的小苗转移到新的抗性再生小盒里。待苗长到一定大小可以取样检测。

[0068] 3.转基因苗的突变体检测

[0069] 3.1T0代转基因株系阳性鉴定及突变体筛选

[0070] 由于此次转化所用载体上T-DNA区域有cas9、bar和gRNA三个表达框,其中bar基因的表达检测已经非常成熟,已经有商业化的检测试剂条,样品经过粗磨后用试剂条在10分钟内就可以判断是否带有bar基因,比PCR扩增快速高效。因此,我们以未转基因植株作为阴性对照,利用bar基因检测试剂盒quickstix™对转基因小麦T0植株进行检测,筛选阳性转基因苗子具体操作如下:

[0071] 取转基因苗3-5cm,放入1.5m离心管中,用研磨棒研磨后加入Buffer 500μL,插入Bar试纸条,静置5min,根据试条显示带数纪录数据,2条带表示阳性,1条带代表阴性。上述试剂盒购自麦蒂森科技有限公司。

[0072] 结果如图4所示,样品1-10为T0代再生苗,11为阳性对照,12为阴性对照,其中样品1,3,4,5,6,7,8,10出现两条带,与阳性对照11结果一致,为阳性苗;2,9出现一条带,与阴性对照一致数字1-10,分别代表ePPO-01,-02,-03,-04,-05,06,-07,-08,-09,-10。

[0073] 3.2T0代转基因植株PSY基因的特异性扩增和突变体鉴定

[0074] 目标基因编辑是否成功需要测序进行鉴定,本实验采用天津诺禾致源公司生产的Hi-TOM基因编辑位点检测试剂盒。该试剂盒通过PCR法自动完成高通量建库过程,并用Hi-TOM在线软件直接解析出多样品多位点的变异信息。利用靶向序列两侧的特异性引物(表1中的Primer-F和Primer-R)对PPO进行扩增,扩增产物建库后送到诺禾致源测序。目的基因小麦PPO基因的野生型核苷酸序列如SEQ11所示,其推导的氨基酸序列如图5所示,突变体的目的基因核苷酸序列如SEQ12所示,其推导的氨基酸序列如图6所示,如经比对,ePPO-2发生在+887处的核苷酸减少2个碱基GT,在突变序列的+1113处出现TGA,变成了终止密码子,造成蛋白质翻译的提前终止,核苷酸序列的比对结果如图7所示,标记为加粗下划线的部分为野生型和突变体有差异的部分,氨基酸序列比对结果如图8所示,标记为浅灰色的部分为野生型和突变体有差异的部分。

[0075] 第一轮PCR反应体系:以转pBUE411-PPO基因的小麦植株叶片DNA为模板1μL,试剂盒中的2×Taq Master Mix 10μL,Primer-F和Primer-R(表1)(10μM)各0.5μL,Nuclease-free Water补足体积至20μL。PCR反应条件为:94℃变性2min;94℃变性30s,65℃复性30s,72℃延伸20s,共33个循环;最后72℃延伸5min。PCR结束后取5μL琼脂糖凝胶电泳检测PCR产物,确保目标产物存在且特异性良好。

[0076] 之后进行第二轮PCR反应,试剂盒中的Hi-TOM Mix 12μL,以第一轮PCR产物为模板加入1μL,Nuclease-free Water补足体积至20μL。PCR反应程序:94℃变性2min;94℃变性30s,57℃复性30s,72℃延伸25s,共33个循环;最后72℃延伸5min。

[0077] 扩增产物建库后送到诺禾致源测序。如图9所示(样品1-12为不同的转基因株系,M

为DL2000 marker,——表示的是突变位点碱基变化),在此处确实2个碱基GT,经比对,ePPO-2发生在+887处的核苷酸减少2个碱基GT,在突变序列的+1113处出现TGA,变成了终止密码子,造成蛋白质翻译的提前终止。

[0078] 3.3基因编辑株系中PPO基因的表达量分析

[0079] 应用RNAprep Pure植物总RNA提取试剂盒(天根,北京,中国)提取转基因植株和对照植株不同发育时期(7、14、21和28DPA)籽粒总RNA,检测各转基因株系在籽粒不同发育时期PPO基因的相对表达量。基于小麦籽粒PPO同源基因cDNA序列间的保守性和差异性,设计能同时扩增A、B、D基因组同源基因的保守性引物,用于qRT-PCR检测。普通小麦 β -actin基因作为内参基因。qRT-PCR反应体系:2 \times SYBR Green Mix 5 μ L,Primer-F和Primer-R(表1)(10 μ M)各0.5 μ L,50ng cDNA,Nuclease-free Water补足体积至10 μ L。反应程序:95 $^{\circ}$ C变性5min;95 $^{\circ}$ C变性15s,60 $^{\circ}$ C复性20s,72 $^{\circ}$ C延伸20s,共40个循环。每个转基因株系选取3个生物学重复,每个样本3次技术重复。结果表明如图10所示(*表示P<0.05,WT表示野生型),基因编辑株系中PPO基因的表达量显著降低。

[0080] 表2 引物序列(二)

	PPO	PPO-F	CGCAACCTTCTCCGTGGCCTT
		PPO-R	ATCGCGCCTCGACCTTTGTCC
[0081]	β -actin	Actin-F	CTGATCGCATGAGCAAAGAG
		Actin-R	CCACCGATCCAGACACTGTA

[0082] 3.4PPO活性测定

[0083] 称取0.25g面粉样品,以邻苯二酚为底物,加入3ml pH6.5的50mmol/L MOPS缓冲液,振荡浸提30min,然后加入1mL120mmol/L邻苯二酚,振荡反应20min,于10000g/min离心10min,以缓冲液加邻苯二酚作对照,取上清液在405nm测定吸光值。每个转基因系测定3个生物学重复,每个样品重复检测三次。结果表明,与未转基因对照小麦相比(13.16min \cdot 1g \cdot 1),基因编辑株系籽粒中多酚氧化酶(PPO活性)显著降低(8.72min \cdot 1g \cdot 1)。

[0001] SEQUENCE LISTING

[0002] <110> 山东省农业科学院蔬菜所

[0003] <120> 一种利用基因编辑降低小麦籽粒多酚氧化酶含量的方法

[0004] <130> 0

[0005] <160> 13

[0006] <170> PatentIn version 3.3

[0007] <210> 1

[0008] <211> 527

[0009] <212> DNA

[0010] <213> 人工序列

[0011] <400> 1

[0012] catgaatcca aaccacacgg agttcaaatt cccacagatt aaggctcgtc cgtcgcacaa 60

[0013] ggtaatgtgt gaatattata tctgtcgtgc aaaattgcct ggctgcaca attgctgtta 120

[0014] tagttggcgg caggagagt tttacattg actagcgtgc tgataattg tgagaaataa 180

[0015] taattgacaa gtagactg acatttgaga agagcttctg aactgttatt agtaacaaaa 240

[0016] atggaaagct gatgcacgga aaaaggaaag aaaaagccat acttttttt aggtaggaaa 300

[0017] agaaaaagcc atacgagact gatgtctctc agatgggccc ggatctgtct atctagcagg 360

[0018] cagcagccca ccaacctcac gggccagcaa ttacgagtc ttctaaaage tcccgcag 420

[0019] gggcgtggc gctgctgtgc agcagcacgt ctaacattag tcccacctcg ccagtttaca 480

[0020] gggagcagaa ccagcttata agcggaggcg cggaccaag aagcggc 527

[0021] <210> 2

[0022] <211> 1992

[0023] <212> DNA

[0024] <213> 人工序列

[0025] <400> 2

[0026] ctgcagtgea gcgtgaccg gtcgtgcccc tctctagaga taatgagcat tgcattgteta 60

[0027] agttataaaa aattaccaca ttttttttt gtcacactg tttgaagtgc agtttatcta 120

[0028] tctttataca tatatttaa ctttactcta cgaataatat aatctatagt actacaataa 180

[0029] tatcagtgtt ttagagaatc atataaatga acagttagac atgggtctaaa ggacaattga 240

[0030] gtattttgac aacaggactc tacagtttta tcttttttagt gtgcatgtgt tctccttttt 300

[0031] ttttgcaaat agcttcacct atataatact tcatccattt tattagtaca tccatttagg 360

[0032] gtttagggtt aatggtttt atagactaat ttttttagta catctatttt attctatttt 420

[0033] agcctctaaa ttaagaaaac taaaactcta ttttagtttt tttatttaat aatttagata 480

[0034] taaaatagaa taaaataaag tgactaaaaa ttaacaaat accctttaag aaattaaaa 540

[0035] aactaaggaa acatttttct tgtttcgagt agataatgcc agcctgttaa acgccgtcga 600

[0036] cgagtctaac ggacaccaac cagcgaacca gcagcgtcgc gtcgggcaa gcgaagcaga 660

[0037] cggcacggca tctctgtcgc tgctctgga cccctctcga gagttccgct ccaccgttg 720

[0038] acttgcctcg ctgtcggcat ccagaaattg cgtggcggag cggcagacgt gagccggcac 780

[0039] ggcaggcggc ctctcctcc tctcagggca cggcagctac gggggattcc tttcccaccg 840

[0040] ctctctcgt ttccttctc cgcctcctc aataaataga caccctctc acaccctctt 900

[0041] tcccacact cgtgtgttc ggagcgcaca cacacacaac cagatctccc ccaatccac 960

[0042]	ccgtcggcac ctccgcttca aggtacgccg ctctcctcc ccccccccc ctctctacct	1020
[0043]	tctctagatc ggcgttccgg tccatggta gggcccgta gttctacttc ttttcatggt	1080
[0044]	tgtgttagat ccgtgtttgt gttagatccg tgctgctagc gttcgtacac ggatgcgacc	1140
[0045]	tgtacgtcag acacgttctg attgctaact tgccagtgt tctctttggg gaatcctggg	1200
[0046]	atggctctag ccgttccgca gacgggatcg atttcatgat tttttttggt tcgttgcata	1260
[0047]	gggtttggtt tgcccttttc ctttatttca atatatgccg tgcacttgtt tgcgggtca	1320
[0048]	tcttttcatg cttttttttg tcttggttgt gatgatgtgg tctggttggg cggtcgttct	1380
[0049]	agatcggagt agaattctgt ttcaaactac ctgggtgatt tattaatttt ggatctgtat	1440
[0050]	gtgtgtgcca tacatattca tagttacgaa ttgaagatga tggatggaaa tatcgatcta	1500
[0051]	ggataggtat acatgttgat gcgggtttta ctgatgcata tacagagatg ctttttggtc	1560
[0052]	gcttggttgt gatgatgtgg tgtggttggg cggtcgttca ttcgttctag atcggagtag	1620
[0053]	aatactgttt caaactacct ggtgtattta ttaattttgg aactgtatgt gtgtgtcata	1680
[0054]	catcttcata gttacgagtt taagatggat ggaaatatcg atctaggata ggtatacatg	1740
[0055]	ttgatgtggg ttttactgat gcatatacat gatggcatat gcagcatcta ttcatatgct	1800
[0056]	ctaaccttga gtacctatct attataataa acaagtatgt tttataatta tttgatctt	1860
[0057]	gatatacttg gatgatggca tatgcagcag ctatatgtgg attttttttag ccctgccttc	1920
[0058]	atacgtatt tatttgcttg gtactgtttc ttttgtcgat gctcacctg ttgtttggtg	1980
[0059]	ttacttctgc ag	1992
[0060]	<210>	3
[0061]	<211>	291
[0062]	<212>	DNA
[0063]	<213>	人工序列
[0064]	<400>	3
[0065]	tttttttttt cgttttgcat tgagttttct ccgtcgcgatg tttgcagttt tattttccgt	60
[0066]	tttgcattga aatttctccg tctcatgttt gcagcgtgtt caaaaagtac gcagctgtat	120
[0067]	ttcacttatt tacggcgcca cattttcatg ccgtttgtgc caactatccc gagctagtga	180
[0068]	atacagcttg gttcacaca acactggtga cccgctgacc tgctcgtacc tcgtaccgtc	240
[0069]	gtacggcaca gcatttgga ttaaagggtg tgatcgatac tgcttgctgc	t 291
[0070]	<210>	4
[0071]	<211>	571
[0072]	<212>	DNA
[0073]	<213>	人工序列
[0074]	<400>	4
[0075]	aagcggccag cggcgacgaa gaaggcgggg caggcgaaga agaagaagtg agctcagagc	60
[0076]	tttcttctgt atcatcgggt tcgacaacgt tcgtcaagtt caatgcatca gtttcattgc	120
[0077]	gcacacacca gaatctact gagtttgagt attatggcat tgggaaaact gtttttcttg	180
[0078]	taccatttgt tgtgcttcta atttactgtg ttttttattc gtttttctgt atcgaactgt	240
[0079]	gaaatggaaa tggatggaga agagttaatg aatgatatgg tccttttgtt cattctcaaa	300
[0080]	ttaatattat ttgttttttc tcttatttgt tgtgtgttga atttgaaatt ataagagata	360
[0081]	tgcaaacatt ttgttttgag taaaaatgtg tcaaatcgtg gcctctaag accgaagtta	420
[0082]	atatgaggag taaaacactt gtagttgtac cattatgctt attcactagg caacaaatat	480
[0083]	attttcagac ctagaaaagc tgcaaatgtt actgaataca agtatgtcct cttgtgtttt	540

[0084] agacatttat gaactttcct ttatgtaatt t 571
 [0085] <210> 5
 [0086] <211> 915
 [0087] <212> DNA
 [0088] <213> 人工序列
 [0089] <400> 5
 [0090] catgaatcca aaccacacgg agttcaaatt cccacagatt aaggctcgtc cgtcgcacaa 60
 [0091] ggtaatgtgt gaatattata tctgtcgtgc aaaattgcct ggctgcaca attgctgtta 120
 [0092] tagttggcgg caggagagt tttacattg actagcgtgc tgataattg tgagaaataa 180
 [0093] taattgacaa gtagatactg acatttgaga agagcttctg aactgttatt agtaacaaaa 240
 [0094] atggaaagct gatgcacgga aaaaggaaag aaaaagccat acttttttt aggtaggaaa 300
 [0095] agaaaaagcc atacgagact gatgtctctc agatgggccc ggatctgtct atctagcagg 360
 [0096] cagcagccca ccaacctcac gggccagcaa ttacgagtc ttctaaaagc tcccgccgag 420
 [0097] gggcgtggc gctgctgtgc agcagcacgt ctaacattag tcccacctc ccagtttaca 480
 [0098] gggagcagaa ccagcttata agcggaggcg cggcaccaag aagcggcggga agaagacgct 540
 [0099] gctgttccgt tttagagcta gaaatagcaa gttaaaataa ggctagtccg ttatcaactt 600
 [0100] gaaaaagtgg caccgagtcg gtgcttttt ttttcgttt gcattgagtt ttctccgtcg 660
 [0101] catgtttgca gttttatfff ccgttttgca ttgaaattc tccgtctcat gtttgacgcg 720
 [0102] tgttcaaaaa gtacgcagct gtatttact tatttacggc gccacattt catgccgttt 780
 [0103] gtgccaacta tcccagcta gtgaatacag cttggcttca cacaacactg gtgaccgct 840
 [0104] gacctgctc tacctctac cgtcgtacgg cacagcattt ggaattaaag ggtgtgatcg 900
 [0105] atactgcttg ctgct 915
 [0106] <210> 6
 [0107] <211> 6799
 [0108] <212> DNA
 [0109] <213> 人工序列
 [0110] <400> 6
 [0111] ctgcagtgca gcgtgaccg gtcgtgccc tctctagaga taatgagcat tgcattgtcta 60
 [0112] agttataaaa aattaccaca tattttttt gtcacactg tttgaagtgc agtttatcta 120
 [0113] tctttataca tatatttaa ctttactcta cgaataatat aatctatagt actacaataa 180
 [0114] tatcagtgtt ttagagaatc atataaatga acagtttagc atggctctaa ggacaattga 240
 [0115] gtattttgac aacaggactc tacagtttta tctttttagt gtgcatgtgt tctcctttt 300
 [0116] ttttgcaaat agcttcacct atataatact tcatccattt tattagtaca tccatttagg 360
 [0117] gtttagggtt aatggtttt atagactaat ttttttagta catctattt attctattt 420
 [0118] agcctctaaa ttaagaaaac taaaactcta ttttagttt tttatttaat aatttagata 480
 [0119] taaaatagaa taaaataaag tgactaaaaa ttaacaaat accctttaag aaattaaaa 540
 [0120] aactaaggaa acatttttct tgtttcgagt agataatgcc agcctgttaa acgccgtcga 600
 [0121] cgagtctaac ggacaccaac cagcgaacca gcagcgtcgc gtcgggccc gcaagcaga 660
 [0122] cggcacggca tctctgtcgc tgctctgga cccctctcga gagttccgct ccaccgttg 720
 [0123] acttgctccg ctgtcggcat ccagaaattg cgtggcggag cggcagacgt gagccggcac 780
 [0124] ggcagcggc ctctctctc tctcacggca cggcagctac gggggattc tttcccaccg 840
 [0125] ctctctcgt tctctctc cgtccgccc aataaataga caccctctc acaccctct 900

[0126]	tccccaacct	cgtgttgttc	ggagcgcaca	cacacacaac	cagatctccc	ccaaatccac	960
[0127]	ccgtcggcac	ctccgcttca	aggtacgccg	ctcgtcctcc	ccccccccc	ctctctacct	1020
[0128]	tctctagatc	ggcgttccgg	tccatggta	gggcccggta	gttctacttc	gtttcatgtt	1080
[0129]	tgtgttagat	ccgtgtttgt	gttagatccg	tgctgctagc	gttcgtacac	ggatgcgacc	1140
[0130]	tgtacgtcag	acacgttctg	attgctaact	tgccagtgtt	tctctttggg	gaatcctggg	1200
[0131]	atggctctag	ccgttccgca	gacgggatcg	atttcatgat	ttttttgtt	tcgttgcata	1260
[0132]	gggtttggtt	tgcccttttc	ctttatttca	atatatgccg	tgacttgtt	tgctcgggtca	1320
[0133]	tcttttcatg	cttttttttg	tcttggttgt	gatgatgtgg	tctggttggg	cggtcgttct	1380
[0134]	agatcggagt	agaattctgt	ttcaaactac	ctggtggatt	tattaatttt	ggatctgtat	1440
[0135]	gtgtgtgcca	tacatattca	tagttacgaa	ttgaagatga	tggatggaaa	tatcgatcta	1500
[0136]	ggataggtat	acatgttgat	gcgggtttta	ctgatgcata	tacagagatg	ctttttgttc	1560
[0137]	gcttggttgt	gatgatgtgg	tgtggttggg	cggtcgttca	ttcgttctag	atcggagtag	1620
[0138]	aatactgttt	caaacctact	ggtgtattta	ttaatttttg	aactgtatgt	gtgtgtcata	1680
[0139]	catcttcata	gttacgagtt	taagatggat	ggaaatatcg	atctaggata	ggtatacatg	1740
[0140]	ttgatgtggg	ttttactgat	gcatatacat	gatggcatat	gcagcatcta	ttcatatgct	1800
[0141]	ctaaccttga	gtacctatct	attataataa	acaagtatgt	tttataatta	ttttgatctt	1860
[0142]	gatatacttg	gatgatggca	tatgcagcag	ctatatgtgg	atTTTTTTtag	ccctgccttc	1920
[0143]	atacgtatt	tatttgcttg	gtactgtttc	ttttgtcgat	gctcacctg	ttgtttggtg	1980
[0144]	ttacttctgc	agccctaggc	ctactagatg	gattacaagg	accacgacgg	ggattacaag	2040
[0145]	gaccacgaca	ttgattacaa	ggatgatgat	gacaagatgg	ctccgaagaa	gaagaggaag	2100
[0146]	gttgcatcc	acggggtgcc	agctgctgac	aagaagtact	cgatcggcct	cgatattggg	2160
[0147]	actaactctg	ttggctgggc	cgtgatcacc	gacgagtaca	aggtgccctc	aaagaagttc	2220
[0148]	aagtcctgg	gcaacaccga	tcggcattcc	atcaagaaga	atctcattgg	cgctctcctg	2280
[0149]	ttcgacagcg	gcgagacggc	tgaggctacg	cggtcaagc	gcaccgcccg	caggcggtag	2340
[0150]	acgcgcagga	agaatcgcat	ctgctacctg	caggagattt	tctccaacga	gatggcgaag	2400
[0151]	gttgacgatt	ctttcttcca	caggctggag	gagtcattcc	tcgtggagga	ggataagaag	2460
[0152]	cacgagcggc	atccaatctt	cggcaacatt	gtcgacgagg	ttgcctacca	cgagaagtac	2520
[0153]	cctacgatct	accatctcgc	gaagaagctc	gtggactcca	cagataaggc	ggacctccgc	2580
[0154]	ctgatctacc	tcgctctggc	ccacatgatt	aagttcaggg	gccatttctt	gatcgagggg	2640
[0155]	gatctcaacc	cggacaatag	cgatgttgac	aagctgttca	tccagctcgt	gcagacgtac	2700
[0156]	aaccagctct	tcgaggagaa	ccccattaat	gcgtcaggcg	tcgacgcgaa	ggctatcctg	2760
[0157]	tccgctaggc	tctcgaagtc	tcggcgcctc	gagaacctga	tcgcccagct	gccgggcgag	2820
[0158]	agaagaacg	gcctgttcgg	gaatctcatt	gcgctcagcc	tggggctcac	gccaacttc	2880
[0159]	aagtcaatt	tcgatctcgc	tgaggacgcc	aagctgcagc	tctccaagga	cacatacgac	2940
[0160]	gatgacctgg	ataacctcct	ggcccagatc	ggcgatcagt	acgcggacct	gttctctcgt	3000
[0161]	gccaagaatc	tgctcgacgc	catcctctcg	tctgatattc	tcagggtgaa	caccgagatt	3060
[0162]	acgaaggctc	cgctctcagc	ctccatgatc	aagcgtacg	acgagacca	tcaggatctg	3120
[0163]	acctctctga	aggcgttgtt	caggcagcag	ctccccgaga	agtacaagga	gatcttcttc	3180
[0164]	gatcagtcga	agaacggcta	cgctgggtac	attgacggcg	ggcctctca	ggaggagttc	3240
[0165]	tacaagttca	tcaagccgat	tctggagaag	atggacggca	cggaggagct	gctggtgaag	3300
[0166]	ctcaatcgcg	aggacctcct	gaggaagcag	cggacattcg	ataacggcag	catccacac	3360
[0167]	cagattcadc	tcggggagct	gcacgctatc	ctgaggaggc	aggaggactt	ctaccctttc	3420

[0168] ctcaaggata accgcgagaa gatcgagaag attctgactt tcaggatccc gtactacgtc 3480
[0169] ggcccactcg ctaggggcaa ctcccgttc gcttggatga cccgcaagtc agaggagacg 3540
[0170] atcacgccgt ggaacttca ggaggtggtc gacaagggcg ctagecgtca gtcttcatc 3600
[0171] gagaggatga cgaatttca caagaacctg ccaaatgaga aggtgctccc taagcactcg 3660
[0172] ctctgtacg agtacttca agtctacaac gagctgacta aggtgaagta tgtgaccgag 3720
[0173] ggcatgagga agccggcttt cctgtctggg gagcagaaga aggccatcgt ggacctcctg 3780
[0174] ttcaagacca accggaaggt cacggttaag cagctcaagg aggactactt caagaagatt 3840
[0175] gagtgttcg attcggtcga gatctctggc gttgaggacc gcttcaacgc ctccctgggg 3900
[0176] acctaccag atctctgaa gatcattaag gataaggact tcctggacaa cgaggagaat 3960
[0177] gaggatatcc tcgaggacat tgtgtctgaca ctactctgt tcgaggaccg ggagatgatc 4020
[0178] gaggagcgc tgaagactta cgccatctc ttgatgaca aggtcatgaa gcagctcaag 4080
[0179] aggaggaggt acaccggctg ggggagctg agcaggaagc tcatcaacgg cattcgggac 4140
[0180] aagcagtcg ggaagacgat cctcgactt ctgaagagcg atggcttcgc gaaccgcaat 4200
[0181] ttcatgcagc tgattcacga tgacagcctc acattcaagg aggatatcca gaaggctcag 4260
[0182] gtgagcggcc agggggactc gctgcacgag catatcgca acctcgctgg ctcgccagct 4320
[0183] atcaagaagg ggattctga gaccgtgaag gttgtggacg agctggtgaa ggtcatgggc 4380
[0184] aggcaaacg ctgagaacat cgtcattgag atggcccggg agaatcagac cacgcagaag 4440
[0185] ggccagaaga actcacgca gaggatgaag aggatcgagg agggcattaa ggagctgggg 4500
[0186] tcccagatcc tcaaggagca cccggtggag aacacgcagc tgcagaatga gaagctctac 4560
[0187] ctgtactacc tccagaatgg ccgcgatatg tatgtggacc aggagctgga tattaacagg 4620
[0188] ctcagcgatt acgacgtca tcatatcgtt ccacagtcac tcctgaagga tgactccatt 4680
[0189] gacaacaagg tcctcaccag gtcggacaag aaccggggca agtctgataa tgttccttca 4740
[0190] gaggaggtcg ttaagaagat gaagaactac tggcgccagc tcctgaatgc caagctgatc 4800
[0191] acgcagcggg agttcgataa cctcacaag gctgagagg gcgggctctc tgagctggac 4860
[0192] aaggcgggct tcatcaagag gcagctggtc gagacacggc agatcactaa gcacgttgcg 4920
[0193] cagattctcg actcacgat gaacactaag tacgatgaga atgacaagct gatccgcgag 4980
[0194] gtgaaggtea tcaccctgaa gtcaaagctc gtctccgact tcaggaagga tttccagttc 5040
[0195] tacaaggttc gggagatcaa caattaccac catgcccatt acgcgtacct gaacgcgggtg 5100
[0196] gtcgccacag ctctgatcaa gaagtacca aagctcgaga gcgagtctgt gtacggggac 5160
[0197] tacaaggttt acgatgtgag gaagatgatc gccaaagtcg agcaggagat tggcaaggct 5220
[0198] accccaagt acttctteta ctctaacatt atgaatttct tcaagacaga gatcactctg 5280
[0199] gccaatggcg agatccgaa gcgccccctc atcgagacga acggcgagac gggggagatc 5340
[0200] gtgtgggaca agggcaggga tttcgcgacc gtcaggaagg ttctctccat gccacaagtg 5400
[0201] aatatcgtca agaagacaga ggtccagact ggcgggttct ctaaggagtc aattctgcct 5460
[0202] aagcgaaca gcgacaagct catcgcccgc aagaaggact gggatccgaa gaagtacggc 5520
[0203] gggttcgaca gcccactgt ggcctactcg gtctgtgtg tggcgaaggt tgagaagggc 5580
[0204] aagtccaaga agctcaagag cgtgaaggag ctgctgggga tcacgattat ggagcgtcc 5640
[0205] agcttcgaga agaaccgat cgatttctg gaggcgaagg gctacaagga ggtgaagaag 5700
[0206] gacctgatca ttaagctccc caagtactca ctcttcgagc tggagaacgg caggaagcgg 5760
[0207] atgctggctt ccgctggcga gctgcagaag gggaacgagc tggctctgcc gtccaagtat 5820
[0208] gtgaacttcc tctacctggc ctcccactac gagaagctca agggcagccc cgaggacaac 5880
[0209] gagcagaagc agctgttctg cgagcagcac aagcattacc tcgacgagat cattgagcag 5940

[0210]	atttccgagt tctccaagcg cgtgatcctg gccgacgca atctggataa ggtcctctcc	6000
[0211]	gcgtacaaca agcaccgca caagccaatc agggagcagg ctgagaatat cattcatctc	6060
[0212]	ttcaccctga cgaacctcgg cgccccctgt gctttcaagt acttegacac aactatcgat	6120
[0213]	cgcaagaggt acacaagcac taaggaggtc ctggacgca ccctcatcca ccagtcgatt	6180
[0214]	accggcctct acgagacgcg catcgacctg tctcagctcg ggggcgacaa gcggccagcg	6240
[0215]	gcgacgaaga aggcggggca ggcaagaag aagaagtgag ctgagagctt tcgttcgtat	6300
[0216]	catcggtttc gacaacgttc gtcaagtca atgcatcagt ttattgctc acacaccaga	6360
[0217]	atcctactga gtttgagtat tatggcattg ggaaaactgt ttttcttgta ccatttgttg	6420
[0218]	tgcttgaat ttactgtgtt ttttattcgg ttttcgctat cgaactgtga aatggaaatg	6480
[0219]	gatggagaag agttaatgaa tgatatggtc cttttgttca ttctcaaatt aatattattt	6540
[0220]	gtttttctc ttatttgttg tgtgttgaat ttgaaattat aagagatag caaacatttt	6600
[0221]	gttttgagta aaaatgtgtc aaatcgtggc ctctaatac cgaagttaat atgaggagta	6660
[0222]	aaacacttgt agttgtacca ttatgcttat tcaactagca acaaatatat tttcagacct	6720
[0223]	agaaaagctg caaatgttac tgaatacaag tatgtcctct tgtgttttag acatttatga	6780
[0224]	actttccttt atgtaattt	6799
[0225]	<210>	7
[0226]	<211>	16230
[0227]	<212>	DNA
[0228]	<213>	人工序列
[0229]	<400>	7
[0230]	gtaaacgctc ttttctctta gttttaccg ccaatatatc ctgtcaaaca ctgatagttt	60
[0231]	aaactgaagg cgggaaacga caatctgac caagctcaag ctgctctagc attcgccatt	120
[0232]	caggctgcgc aactgttggg aagggcagc ggtgctggcc tcttcgctat tacgccagct	180
[0233]	ggcgaagg ggatgtgctg caaggcgatt aagttgggta acgccagggt tttcccagtc	240
[0234]	acgacgttgt aaaacgacgg ccagtccaa gcttcatgaa tccaaaccac acggagttea	300
[0235]	aattcccaca gattaagct cgtccgctgc acaaggtaat gtgtgaatat tatatctgtc	360
[0236]	gtgcaaaatt gcctggcctg cacaattgct gttatagttg gcggcagga gagttttaac	420
[0237]	attgactagc gtgctgataa tttgtgagaa ataataattg acaagtagat actgacattt	480
[0238]	gagaagagct tctgaactgt tattagtaac aaaaatggaa agctgatgca cggaaaaagg	540
[0239]	aaagaaaag ccatactttt ttttaggtag gaaaagaaa agccatacga gactgatgtc	600
[0240]	tctcagatgg gccgggatct gtctatctag caggcagcag cccaccaacc tcacgggcca	660
[0241]	gcaattacga gtcttctaa aagctcccgc cgaggggctg tggcgtgct gtgcagcagc	720
[0242]	acgtetaaca ttagtcccac ctgcagctt tacagggagc agaaccagct tataagcgga	780
[0243]	ggcgcggcac caagaagcg cggaagaaga cgctgctgtt ccgttttaga gctagaaata	840
[0244]	gcaagttaa ataaggctag tccgttatca acttgaaaa gtggcaccga gtcggtgctt	900
[0245]	ttttttctg ttttgattg agttttctc gtcgcatgtt tgcagttta ttttccgttt	960
[0246]	tgattgaaa tttctcgtc tcatgtttgc agcgtgttca aaaagtacgc agctgtattt	1020
[0247]	cacttattta cggcggcaca ttttcatgcc gtttgtgcca actatcccga gctagtgaat	1080
[0248]	acagcttggc ttacacacac actggtgacc cgctgacctg ctctgacctc gtaccgtcgt	1140
[0249]	acggcacagc atttggaatt aaagggtgtg atcgatactg cttgctgcta agcttgcag	1200
[0250]	cctgcagtc agcgtgacct ggtcgtgccc ctctctagag ataagtagca ttgcatgtct	1260
[0251]	aagttataaa aaattaccac atattttttt tgtcacactt gtttgaagtg cagtttatct	1320

[0252]	atctttatac	atatatntaa	actttactct	acgaataata	taatctatag	tactacaata	1380
[0253]	atatcagtgt	tttagagaat	catataaatg	aacagttaga	catggtctaa	aggacaattg	1440
[0254]	agtattttga	caacaggact	ctacagtttt	atcttttttag	tgtgcatgtg	ttctcctttt	1500
[0255]	tttttgcaaa	tagcttcacc	tatataatac	ttcatccatt	ttattagtag	atccatttag	1560
[0256]	ggtttagggt	taatggtttt	tatagactaa	tttttttagt	acatctatnt	tattctatnt	1620
[0257]	tagcctctaa	attaagaaaa	ctaaaactct	atnttagttt	ttttatntaa	taatnttagat	1680
[0258]	ataaaaataga	ataaaaataa	gtgactaaaa	attaacaaaa	taccctntaa	gaaatntaaa	1740
[0259]	aaactaagga	aacatntttc	ttgnttcgag	tagataatgc	cagcctgtta	aacgccgtcg	1800
[0260]	acgagtctaa	cggacaccaa	ccagcgaacc	agcagcgtcg	cgtcgggcca	agcgaagcag	1860
[0261]	acggcacggc	atctctgtcg	ctgcctctgg	accctctcg	agagttccgc	tccaccgttg	1920
[0262]	gacttgctcc	gctgtcggca	tccagaaatt	gctggtcgga	gcggcagacg	tgagccggca	1980
[0263]	cggcaggcgg	cctcctcctc	ctctcacggc	acggcagcta	cgggggatc	ctttcccacc	2040
[0264]	gctccttcgc	ttcctctcc	tcgcccgcg	taataaatag	acacccctc	cacaccctct	2100
[0265]	ttcccaacc	tcgtgtgtt	cggagcgcac	acacacacaa	ccagatctcc	cccaaatcca	2160
[0266]	cccgtcggca	cctccgcttc	aaggtacgcc	gctcgtcctc	ccccccccc	cctctctacc	2220
[0267]	ttctctagat	cgcgctccg	gtccatggt	agggcccggt	agttctactt	ctgttcatgt	2280
[0268]	ttgtgttaga	tccgtgtttg	tgtagatcc	gtgctgctag	cgttcgtaca	cggatgcgac	2340
[0269]	ctgtacgtca	gacagttct	gattgctaac	ttgccagtgt	ttctctttgg	ggaatcctgg	2400
[0270]	gatggctcta	gccgttccgc	agacgggatc	gatttcatga	ttttttttgt	ttcgttgcac	2460
[0271]	agggtttggt	ttgccctttt	cctttatntc	aatatatgcc	gtgcacttgt	ttgtcgggtc	2520
[0272]	atctnttcat	gctntttttt	gtcttggttg	tgatgatgtg	gtctggttgg	gcggtcgttc	2580
[0273]	tagatcggag	tagaattctg	ttcaaaacta	cctggtggat	ttattaatnt	tggatctgta	2640
[0274]	tgtgtgtgcc	atacatatc	atagttacga	attgaagatg	atggatggaa	atatcgatct	2700
[0275]	aggataggta	tacatgttga	tgcggtttt	actgatgcat	atacagagat	gctntttgtt	2760
[0276]	cgcttggttg	tgatgatgtg	gtgtggttgg	gcggtcgttc	attcgttcta	gatcggagta	2820
[0277]	gaactagtnt	tcaaacctac	tggtgtatnt	attaatnttg	gaactgtatg	tgtgtgtcat	2880
[0278]	acatcttcat	agttacagat	ttaagatgga	tggaaatc	gatctaggat	aggtatacat	2940
[0279]	gntgatgtgg	gtnttactga	tgcatataca	tgatggcata	tgacagatct	atntcatatgc	3000
[0280]	tctaaccttg	agtacctatc	tattataata	aacaagtatg	ttttataatnt	atntttgatct	3060
[0281]	tgatatactt	ggatgatggc	atatgcagca	gctatatgtg	gattntttta	gccctgcctt	3120
[0282]	catacgtat	ttatntgctt	ggtactgntt	ctnttgcga	tgctcaccct	gntgnttggnt	3180
[0283]	gntacttctg	cagccctagg	cctactagat	ggattacaag	gaccacgacg	gggattacaa	3240
[0284]	ggaccacgac	atntgattaca	aggatgatga	tgacaagatg	gctccgaaga	agaagaggaa	3300
[0285]	gnttggtcgc	caggggtgc	cagctgctga	caagaagtag	tcgatcggcc	tcgatattgg	3360
[0286]	gactaacctc	gntggctggg	ccgtgatcac	cgacagtag	aaggtgccct	caaagaagnt	3420
[0287]	caaggtcctg	ggcaacaccg	atcggcattc	catcaagaag	aatctcattg	gcgctctcct	3480
[0288]	gntcgcacgc	ggcgagacgg	ctgaggctac	gcggtcaag	cgacccgcc	gcaggcggta	3540
[0289]	cagcgcagg	aagaatcga	tctgctacct	gcaggagat	ttctccaacg	agatggcgaa	3600
[0290]	gnttgacgat	tctntcttcc	acaggctgga	ggagtcattc	ctcgtggagg	aggataagaa	3660
[0291]	gcacgacgg	catccaatct	tcggcaacat	tgctgacgag	gntgcctacc	acgagaagta	3720
[0292]	ccctacgatc	taccatctgc	ggaagaagct	cgtggactcc	acagataag	cggacctccg	3780
[0293]	cctgatctac	ctcgtctctg	cccacatgat	taagntcagg	ggccatntcc	tgatcggagg	3840

[0294]	ggatctcaac	ccggacaata	gcgatgttga	caagctgttc	atccagctcg	tgcagacgta	3900
[0295]	caaccagctc	ttcgaggaga	acccattaa	tgcgtcaggc	gtcgacgcga	aggctatcct	3960
[0296]	gtccgctagg	ctctcgaagt	ctcggcgct	cgagaacctg	atcgcccagc	tgccgggcga	4020
[0297]	gaagaagaac	ggcctgttcg	ggaatctcat	tgcgtcagc	ctggggctca	cgcccaactt	4080
[0298]	caagtcgaat	ttgatctcg	ctgaggacgc	caagctgcag	ctctccaagg	acacatacga	4140
[0299]	cgatgacctg	gataacctcc	tggcccagat	cgcgatcag	tacgggacc	tgttcctcgc	4200
[0300]	tgccaagaat	ctgtcggacg	ccatctcct	gtctgatatt	ctcagggtga	acaccgagat	4260
[0301]	tacgaaggct	ccgctctcag	cctccatgat	caagcgtac	gacgagcacc	atcaggatct	4320
[0302]	gaccctcctg	aaggcgtgg	tcaggcagca	gctccccgag	aagtacaagg	agatcttctt	4380
[0303]	cgatcagctg	aagaacggct	acgctgggta	cattgacggc	ggggcctctc	aggaggagt	4440
[0304]	ctacaagttc	atcaagccga	ttctggagaa	gatggacggc	acggaggagc	tgctggtgaa	4500
[0305]	gctcaatgc	gaggacctcc	tgaggaagca	gcggacattc	gataacggca	gcatcccaca	4560
[0306]	ccagattcat	ctcggggagc	tgcacgctat	cctgaggagg	caggaggact	tctacccttt	4620
[0307]	cctcaaggat	aaccgcgaga	agatcgagaa	gattctgact	ttcaggatcc	cgtactacgt	4680
[0308]	cggcccactc	gctaggggca	actcccgtt	cgcttggatg	acccgcaagt	cagaggagac	4740
[0309]	gatcacgccg	tggaacttcg	aggaggtggt	cgacaagggc	gctagcgtc	agtcgttcat	4800
[0310]	cgagaggatg	acgaatttcg	acaagaacct	gccaatgag	aaggtgctcc	ctaagcactc	4860
[0311]	gctcctgtac	gagtacttca	cagtctacaa	cgagctgact	aaggtgaagt	atgtgaccga	4920
[0312]	gggcatgagg	aagccggctt	tcctgtctgg	ggagcagaag	aaggccatcg	tggacctcct	4980
[0313]	gttcaagacc	aaccggaagg	tcacggtaa	gcagctcaag	gaggactact	tcaagaagat	5040
[0314]	tgagtcttc	gattcggteg	agatctctgg	cgttgaggac	cgcttcaacg	cctccctggg	5100
[0315]	gacctaccac	gatctcctga	agatcattaa	ggataaggac	ttcctggaca	acgaggagaa	5160
[0316]	tgaggatatac	ctcgaggaca	ttgtgctgac	actcactctg	ttcgaggacc	gggagatgat	5220
[0317]	cgaggagcgc	ctgaagactt	acgcccattc	cctcgatgac	aaggtcatga	agcagctcaa	5280
[0318]	gaggaggagg	tacaccggt	gggggaggct	gagcaggaag	ctcatcaacg	gcattcggga	5340
[0319]	caagcagtcc	gggaagacga	tcctcgactt	cctgaagagc	gatggcttcg	cgaaccgcaa	5400
[0320]	tttcatgcag	ctgattcacg	atgacagcct	cacattcaag	gaggatatcc	agaaggctca	5460
[0321]	ggtgagcggc	cagggggact	cgctgcacga	gcataatcgcg	aacctcgtg	gctcgccagc	5520
[0322]	tatcaagaag	gggattctgc	agaccgtgaa	ggttgtggac	gagctggtga	aggctatggg	5580
[0323]	caggcacaag	cctgagaaca	tcgtcattga	gatggcccgg	gagaatcaga	ccacgcagaa	5640
[0324]	gggccagaag	aactcacgcg	agaggatgaa	gaggatcgag	gagggcatta	aggagctggg	5700
[0325]	gtcccagatc	ctcaaggagc	acccggtgga	gaacacgcag	ctgcagaatg	agaagctcta	5760
[0326]	cctgtactac	ctccagaatg	gccgcgat	gtatgtggac	caggagctgg	atattaacag	5820
[0327]	gctcagcgat	tacgacgtcg	atcatatcgt	tccacagtca	ttcctgaagg	atgactccat	5880
[0328]	tgacaacaag	gtcctacca	ggtcggacaa	gaaccggggc	aagtctgata	atgttccttc	5940
[0329]	agaggaggtc	gttaagaaga	tgaagaacta	ctggcgccag	ctcctgaatg	ccaagctgat	6000
[0330]	cacgcagcgg	aagttcgata	acctcacaaa	ggctgagagg	ggcgggctct	ctgagctgga	6060
[0331]	caaggcgggc	ttcatcaaga	ggcagctggt	cgagacacgg	cagatcacta	agcacgttgc	6120
[0332]	gcagattctc	gactcacgga	tgaactacta	gtacgatgag	aatgacaagc	tgatccgcga	6180
[0333]	ggtgaaggtc	atcacctga	agtcaaagct	cgtctccgac	ttcaggaagg	atttccagtt	6240
[0334]	ctacaagggt	cgggagatca	acaattacca	ccatgcccat	gacgcgtacc	tgaacgcggt	6300
[0335]	ggtcggcaca	gctctgatca	agaagtacc	aaagctcgag	agcgagttcg	tgtacgggga	6360

[0336] ctacaagggtt tacgatgtga ggaagatgat cgccaagtcg gagcaggaga ttggcaaggc 6420
[0337] taccgccaag tacttcttct actctaacat tatgaatttc ttcaagacag agatcactct 6480
[0338] ggccaatggc gagatccgga agcgccccct catcgagacg aacggcgaga cgggggagat 6540
[0339] cgtgtgggac aagggcaggg atttcgcgac cgtcaggaag gttctctcca tgccacaagt 6600
[0340] gaatatcgtc aagaagacag aggtccagac tggcgggttc tctaaggagt caattctgcc 6660
[0341] taagcggaac agcgacaagc tcatcgcccg caagaaggac tgggatccga agaagtacgg 6720
[0342] cgggttcgac agccccactg tggcctactc ggtcctgggtt gtggcgaagg ttgagaaggg 6780
[0343] caagtccaag aagctcaaga gcgtgaagga gctgctgggg atcacgatta tggagcgcctc 6840
[0344] cagcttcgag aagaaccga tcgatttctt ggaggcgaag ggctacaagg aggtgaagaa 6900
[0345] ggacctgatc attaagctcc ccaagtactc actcttcgag ctggagaacg gcaggaagcg 6960
[0346] gatgctggct tccgctggcg agctgcagaa ggggaacgag ctggctctgc cgtccaagta 7020
[0347] tgtgaacttc ctctacctgg cctcccacta cgagaagctc aagggcagcc ccgaggacaa 7080
[0348] cgagcagaag cagctgttcg tcgagcagca caagcattac ctcgacgaga tcattgagca 7140
[0349] gatttccgag ttctccaagc gcgtgatcct ggccgacgcg aatctggata aggtcctctc 7200
[0350] cgcgtacaac aagcaccgcg acaagccaat cagggagcag gctgagaata tcattcatct 7260
[0351] cttcaccctg acgaacctcg gcgccccctg tgctttcaag tacttcgaca caactatcga 7320
[0352] tcgcaagagg tacacaagca ctaaggaggt cctggacgcg accctcatcc accagtcgat 7380
[0353] taccggcctc tacgagacgc gcatcgacct gtctcagctc gggggcgaca agcggccagc 7440
[0354] ggcgacgaag aaggcggggc aggcgaagaa gaagaagtga gctcagagct ttcgttcgta 7500
[0355] tcatcggttt cgacaacgtt cgtcaagttc aatgcatcag tttcattgag cacacaccag 7560
[0356] aatcctactg agtttgagta ttatggcatt gggaaaactg tttttcttgt accatttgtt 7620
[0357] gtgcttghaa tttactgtgt tttttattcg gttttcgcta tcgaactgtg aaatggaaat 7680
[0358] ggatggagaa gagttaatga atgatatggt cttttgttc attctcaaat taatattatt 7740
[0359] tgttttttct cttatttgtt gtgtgttgaa tttgaaatta taagagatat gcaaacattt 7800
[0360] tgttttgagt aaaaatgtgt caaatcgtgg cctctaataa ccgaagttaa tatgaggagt 7860
[0361] aaaacacttg tagttgtacc attatgctta ttcactagc aacaaatata ttttcagacc 7920
[0362] tagaaaagct gcaaatgtta ctgaatacaa gtatgtcctc ttgtgtttta gacatttatg 7980
[0363] aactttcctt tatgtaattt tccagaatcc ttgtcagatt ctaatcattg ctttataatt 8040
[0364] atagtatac tcatggattt gtagttgagt atgaaaatat tttttaatgc attttatgac 8100
[0365] ttgccaattg attgacaacg aattcgtaat catggtcata gctgtttcct gtgtgaaatt 8160
[0366] gttatccgct cacaattcca cacaacatac gagccggaag cataaagtgt aaagcctggg 8220
[0367] gtgcctaata agtgagctaa ctcacattaa ttgcgttcg ctcactgcc gctttccagt 8280
[0368] cgggaaacct gtcgtgccag ctgcattaat gaatcggcca acgcgcgggg agaggcggtt 8340
[0369] tgcgtattgg ctagagcagc ttgccaacat ggtggagcac gacactctcg tctactccaa 8400
[0370] gaatatcaaa gatacagtct cagaagacca aagggtatt gagacttttc aacaaagggt 8460
[0371] aatatcgga aacctcctcg gattccattg cccagctatc tgtcacttca tcaaaaggac 8520
[0372] agtagaaaag gaaggtggca cctacaaatg ccatcattgc gataaaggaa aggctatcgt 8580
[0373] tcaagatgcc tctgccgaca gtggtcccaa agatggaccc ccaccacga ggagcatcgt 8640
[0374] ggaaaaagaa gacgttccaa ccacgtcttc aaagcaagtg gattgatgtg ataacatggt 8700
[0375] ggagcacgac actctcgtct actccaagaa tatcaaagat acagtctcag aagaccaaag 8760
[0376] ggctattgag acttttcaac aaagggtaat atcgggaaac ctcctcgat tccattgcc 8820
[0377] agctatctgt cacttcatca aaaggacagt agaaaaggaa ggtggcacct acaaatgcc 8880

[0378]	tcattgcgat aaaggaaagg ctatcgttca agatgcctct gccgacagtg gtcccaaaga	8940
[0379]	tggaccccca cccacgagga gcatcgtgga aaaagaagac gttccaacca cgtcttcaaa	9000
[0380]	gcaagtggat tgatgtgata tctccactga cgtaagggat gacgcacaat cccactatcc	9060
[0381]	ttcgcaagac cttcctctat ataaggaagt tcatttcatt tggagaggac acgctgaaat	9120
[0382]	caccagtctc tctctacaaa tctatctctc tcgagtctac catgagccca gaacgacgcc	9180
[0383]	cggccgacat ccgccgtgcc accgaggcgg acatgccggc ggtctgcacc atcgtcaacc	9240
[0384]	actacatcga gacaagcacg gtcaacttcc gtaccgagcc gcaggaaccg caggagtgga	9300
[0385]	cggacgacct cgtccgtctg cgggagcgt atccctggct cgtcgccgag gtggacggcg	9360
[0386]	aggtcgccgg catgcctac gcgggccct ggaaggcacg caacgcctac gactggacgg	9420
[0387]	ccgagtcgac cgtgtacgtc tccccccgc accagcggac gggactgggc tccacgctct	9480
[0388]	acaccacct gctgaagtcc ctggaggcac agggcttcaa gagcgtggtc gctgtcatcg	9540
[0389]	ggctgcccaa cgaccgagc gtgcgatgc acgaggcgt cggatatgcc ccccgcgga	9600
[0390]	tgctcgccgg cgtgcctac gcgggccct ggaaggcacg caacgcctac gactggacgg	9660
[0391]	acttcagcct gccggtaccg ccccgctccg tctgcccgt caccgagatt tgactcgagt	9720
[0392]	ttctccataa taatgtgtga gtagttcca gataaggaa ttagggttcc tatagggttt	9780
[0393]	cgctcatgtg ttgagcatat aagaaacct tagtatgtat ttgtatattg aaaatacttc	9840
[0394]	tatcaataaa atttctaatt cctaaaacca aaatccagta ctaaaatcca gatccccga	9900
[0395]	attaattcgg cgtaattca gtacattaaa aacgtccgca atgtgttatt aagttgtcta	9960
[0396]	agcgtcaatt tgtttacacc acaatatac ctgccaccag ccagccaaca gctccccgac	10020
[0397]	cggcagctcg gcacaaaatc accactcgat acaggcagcc catcagtcgg ggacggcgtc	10080
[0398]	agcgggagag ccgttgaag gcggcagact ttgctcatgt taccgatgct attcggaaga	10140
[0399]	acggcaacta agctgccggg tttgaaacac ggatgatctc gcggagggta gcatgttgat	10200
[0400]	tgtaacgatg acagagcgtt gctgcctgtg atcaccgagg tttcaaaatc ggctccgtcg	10260
[0401]	atactatggt atacgccaac tttgaaaaca actttgaaaa agctgttttc tggatattaa	10320
[0402]	ggttttagaa tgcaaggaa agtgaaattg agttcgtctt gttataatta gcttcttggg	10380
[0403]	gtatctttaa atactgtaga aaagaggaa gaaataata atggctaaaa tgagaatatac	10440
[0404]	accggaattg aaaaaactga tcgaaaaata ccgctgcgta aaagatacgg aaggaatgtc	10500
[0405]	tctgctaag gtatataagc tgggtgggaga aaatgaaaac ctatatataa aaatgacgga	10560
[0406]	cagccggtat aaagggacca cctatgatgt ggaacgggaa aaggacatga tgctatgget	10620
[0407]	ggaaggaaag ctgcctgttc caaagtcct gcactttgaa cggcatgatg gctggagcaa	10680
[0408]	tctgctcatg agtgaggccg atggcgtcct ttgctcggaa gagtatgaag atgaacaaag	10740
[0409]	ccctgaaaag attatcgagc tgtatgcgga gtgcatcagg ctctttcact ccatcgacat	10800
[0410]	atcggattgt ccctatacga atagcttaga cagccgctta gccgaattgg attacttact	10860
[0411]	gaataacgat ctggccgatg tggattgcga aaactgggaa gaagacactc catttaaaga	10920
[0412]	tccgcgcgag ctgtatgatt ttttaaagac ggaaaagccc gaagaggaaac ttgtcttttc	10980
[0413]	ccacggcgac ctgggagaca gcaacatctt tgtgaaagat ggcaaagtaa gtggctttat	11040
[0414]	tgatcttggg agaagcggca gggcggacaa gtggtatgac attgccttct gcgtccggtc	11100
[0415]	gatcagggag gatatcgggg aagaacagta tgtcgagcta ttttttgact tactggggat	11160
[0416]	caagcctgat tgggagaaaa taaaatatta tattttactg gatgaattgt tttagtacct	11220
[0417]	agaatgcatg accaaaatcc cttaacgtga gttttcgttc cactgagcgt cagaccccg	11280
[0418]	agaaaagatc aaaggatctt cttgagatcc ttttttctg cgcgtaatct gctgcttgca	11340
[0419]	aacaaaaaaa ccaccgctac cagcgggtgt ttgtttgccg gatcaagagc taccaactct	11400

[0420] ttttccgaag gtaactggct tcagcagagc gcagatacca aatactgtcc ttctagtgtg 11460
[0421] gccgtagtta ggccaccact tcaagaactc tgtagcaccg cctacatacc tcgctctgct 11520
[0422] aatcctgtta ccagtggtg ctgccagtgg cgataagtgc tgtcttaccg ggttggaactc 11580
[0423] aagacgatag ttaccggata aggcgcagcg gtcgggctga acggggggtt cgtgcacaca 11640
[0424] gccagcttg gagcgaacga cctacaccga actgagatac ctacagcgtg agctatgaga 11700
[0425] aagcggcacg cttcccgaag ggagaaaggc ggacaggtat ccggtaaagc gcagggtcgg 11760
[0426] aacaggagag cgcacgagg agcttccagg gggaaacgcc tggtatctt atagtctctg 11820
[0427] cgggtttcgc cacctctgac ttgagcgtc atttttgtga tgctcgtcag gggggcggag 11880
[0428] cctatgaaa aacgccagca acgcggcctt tttacggtc ctggcctttt gctggccttt 11940
[0429] tgctcacatg ttctttctg cgttatccc tgattctgtg gataaccgta ttaccgcctt 12000
[0430] tgagtgagct gataccgctc gccgcagccg aacgaccgag cgcagcaggt cagtgagega 12060
[0431] ggaagcggaa gagcgctga tgcggtattt tctccttac catctgtgcg gtatttcaca 12120
[0432] ccgcatatgg tgcactctca gtacaatctg ctctgatgcc gcatagttaa gccagtatac 12180
[0433] actccgctat cgctacgtga ctgggtcatg gctgcgccc gacaccgcc aacaccgct 12240
[0434] gacgcgccct gacgggcttg tctgctccc gcatccgctt acagacaagc tgtgaccgtc 12300
[0435] tccgggagct gcatgtgtca gaggttttca ccgtcatcac cgaaacgcgc gaggcagggt 12360
[0436] gccttgatgt gggcgccggc ggtcagtggt cgacggcgcg gcttgtccgc gccctggtag 12420
[0437] attgcctggc cgtaggccag ccatttttga gcggccagcg gccgcgatag gccgacgca 12480
[0438] agcggcgggg cgtagggagc gcagcgaccg aagggtagc gctttttgca gctcttcggc 12540
[0439] tgtgcgctgg ccagacagtt atgcacaggc caggcgggtt ttaagagtt taataagttt 12600
[0440] taaagagttt taggcggaaa aatgccttt tttctctttt atatcagtca cttacatgtg 12660
[0441] tgaccggttc ccaatgtacg gctttgggtt cccaatgtac gggttccggt tcccaatgta 12720
[0442] cggctttggg ttcccaatgt acgtgctatc cacaggaaac agacctttc gaccttttc 12780
[0443] ccctgctagg gcaatttgc ctageatctg ctccgtacat taggaaccg cgatgcttc 12840
[0444] gccctcgatc aggttcggt agcgcgatgac taggatcggg ccagcctgcc ccgctctc 12900
[0445] cttcaaatcg tactccgca ggtcatttga cccgatcagc ttgcgcacgg tgaacagaa 12960
[0446] cttctgaac tctccggcg tgccactgcg ttcgtagatc gtcttgaaca accatctggc 13020
[0447] ttctgccttg cctgcggcg ggcgtgccag gcggtagaga aaacggcca tgcgggac 13080
[0448] gatcaaaaag taatcgggt gaaccgtcag cacgtccggg ttcttgctt ctgtgatctc 13140
[0449] gcggtacatc caatcagcta gctcgatctc gatgtactcc ggccgcccg tttcgtctt 13200
[0450] tacgatcttg tagcgctaa tcaaggctc accctcgat accgtacca ggcggcctt 13260
[0451] cttggccttc ttcgtacgct gcatggcaac gtgcgtggtg tttaccgaa tgcaggttc 13320
[0452] taccaggtcg tctttctgct ttccgccatc ggctcgccg cagaacttga gtacgtccgc 13380
[0453] aacgtgtgga cggaacacgc ggccgggctt gtctccctc cttcccgtt atcggttcat 13440
[0454] ggattcgggt agatgggaaa ccgcatcag taccaggtcg taatcccaca cactggccat 13500
[0455] gccggccggc cctgcggaaa cctctacgtg cccgtctgga agctcgtagc ggatcacctc 13560
[0456] gccagctcgt cggtcacgct tcgacagacg gaaaacggcc acgtccatga tgctgcgact 13620
[0457] atcgcgggtg cccacgtcat agagcatcgg aacgaaaaa tctggttget cgtcgcctt 13680
[0458] gggcgcttc ctaatcgacg gcgcaccggc tgccggcggg tgccgggatt ctttgcggat 13740
[0459] tcgatcagcg gccgcttgc acgattacc gggcgtgct tctgcctcga tgcgttgccg 13800
[0460] ctggcgccg tgcgcgccct tcaactctc caccaggtca tccccagcg ccgcgccgat 13860
[0461] ttgtaccggg ccgatgggt tgcgaccgct cacgccgatt cctcgggctt gggggttcca 13920

[0462]	gtgccattgc agggccggca gacaaccag ccgcttacgc ctggccaacc gcccgttcct	13980
[0463]	ccacacatgg ggcatccac ggcgtcggg cctggttgtt cttgattttc catgccgect	14040
[0464]	cctttagcgg ctaaaattca tctactcatt tattcatttg ctcattttact ctggtagctg	14100
[0465]	cgcgatgat tcagatagca gctcggtaat ggtcttgect tggcgtaccg cgtacatctt	14160
[0466]	cagcttggtg tgatctccg ccggcaactg aaagttgacc cgcttcatgg ctggcgtgtc	14220
[0467]	tgccaggctg gccaacgttg cagccttgct gctgcgtgcg ctcggacggc cggcacttag	14280
[0468]	cgtgtttgtg cttttgctca ttttctcttt acctcattaa ctcaaatgag ttttgattta	14340
[0469]	atctcagcgg ccagcgcctg gacctcggg gcagcgtcgc cctcgggttc tgattcaaga	14400
[0470]	acggttgtgc cggcggcggc agtgccctggg tagctcacgc gctgcgtgat acgggactca	14460
[0471]	agaatgggca gctcgtaccc ggccagcgc tcggcaacct caccgccgat gcgcgtgect	14520
[0472]	ttgatcgccc gcgacacgac aaaggccgct tgtagccttc catccgtgac ctcaatgcgc	14580
[0473]	tgcttaacca gctccaccag gtcggcggtg gcccatatgt cgtaagggtc tggctgcacc	14640
[0474]	ggaatcagca cgaagtggc tgccttgatc gcggacacag ccaagtccgc cgcctggggc	14700
[0475]	gctccgtcga tctactagaa gtcgcgccgg ccgatggcct tcacgtcgcg gtcaatcgtc	14760
[0476]	ggcggtcga tgccgacaac ggttagcggg tgatcttccc gcacggccgc ccaatcgcgg	14820
[0477]	gcactgccct ggggatcggg atcgactaac agaacatcgg ccccggcgag ttgcaggggc	14880
[0478]	cgggctagat gggttgcgat ggtcgtcttg cctgaccgc ctttctggtt aagtacagcg	14940
[0479]	ataaccttca tgcgttcccc ttgcgtattt gtttatttac tcatcgcac atatacgag	15000
[0480]	cgaccgcatg acgcaagctg ttttactcaa atacacatca cttttttaga cggcggcgt	15060
[0481]	cggtttcttc agcggccaag ctggccggcc aggccgccag cttggcatca gacaaaccgg	15120
[0482]	ccaggatttc atgcagccgc acggttgaga cgtgcgcggg cggctcgaac acgtaccggg	15180
[0483]	ccgcgatcat ctccgcctcg atctcttcgg taatgaaaaa cggttcgtcc tggccgtcct	15240
[0484]	ggtgcggttt catgcttggt cctcttgcg ttattctcg gcggccgcca gggcgtcggc	15300
[0485]	ctcgttcaat gcgtctcac ggaaggcacc gcgccgctg gcctcgggtg gcgtcacttc	15360
[0486]	ctcgtcgcgc tcaagtgcgc ggtacagggt cgagcgtgc acgccaagca gtgcagccgc	15420
[0487]	ctctttcag gtgcggcctt cctggtcgat cagctcgcgg gcgtgcgcga tctgtgccgg	15480
[0488]	ggtgagggtg gggcgggggc caaactcac gcctcgggc ttggcggcct cgcgccgct	15540
[0489]	ccgggtcggg tcgatgatta gggaacgctc gaactcggca atgccggcga acacggtcaa	15600
[0490]	caccatcggg ccggccggcg tgggtggtgc ggcccacggc tctgccaggc tacgcaggcc	15660
[0491]	cgcccgccgc tcttgatgc gctcggcaat gtccagtagg tcgcgggtgc tgcgggccag	15720
[0492]	gcggtctagc ctggtcactg tcacaacgtc gccaggcgt aggtggtcaa gcatcctggc	15780
[0493]	cagctccggg cggtcgcgcc tgggtccggt gatcttctcg gaaaacagct tgggtcagcc	15840
[0494]	ggcccgctgc agttcggccc gttggttgg caagtctgg tcgtcgggtc tgacgcgggc	15900
[0495]	atagcccagc aggccagcgg cggcgtctt gttcatggcg taatgtctcc gtttctagtc	15960
[0496]	gcaagtattc tactttatgc gactaaaaca cgcgacaaga aaacgccagg aaaaggcag	16020
[0497]	ggcggcagcc tgcgcgtaa cttaggactt gtgcgacatg tcgttttcag aagacggctg	16080
[0498]	caactgaacg cagaagccga ctgcactata gcagcggagg ggttgatca aagtactttg	16140
[0499]	atcccagggg gaacctgtg gttggcatgc acatacaaat ggacgaacgg ataaaccttt	16200
[0500]	tcacgccctt ttaaataacc gttattctaa	16230
[0501]	<210> 8	
[0502]	<211> 24	
[0503]	<212> DNA	

[0504] <213> 人工序列
 [0505] <400> 8
 [0506] ggcggaagaa gacgctgctg ttcc 24
 [0507] <210> 9
 [0508] <211> 25
 [0509] <212> DNA
 [0510] <213> 人工序列
 [0511] <400> 9
 [0512] aaacggaaca gcagcgtctt cttct 25
 [0513] <210> 10
 [0514] <211> 20
 [0515] <212> DNA
 [0516] <213> 人工序列
 [0517] <400> 10
 [0518] gaagaagacg ctgctgttcc 20
 [0519] <210> 11
 [0520] <211> 1734
 [0521] <212> DNA
 [0522] <213> 序列
 [0523] <400> 11
 [0524] atggagagca gtcgcatgcc actgagtgcc acccctcgca tgccatgcag cctccaaacc 60
 [0525] cttgcgcgcc gcaaccttct ccgtggcctt cacctccgga aggacgcgag gcagccacgg 120
 [0526] cgtctctcag tctcatgcga ggcgaccggc ggctgcccgcg tcgaccgccg tgagggtgctc 180
 [0527] ctcggtctcg gcggcgccgc ggctgccggc ctggccacgg acaaaggctc aggcgcgatc 240
 [0528] gccgcgcca tccaggcccc ggacctccgc aactgceaaa cgccccgagc cccgaacacg 300
 [0529] ccgcccgaca ccaactgctg cccgacgcc ggcaccggca tcaccgactt cgtgctgccg 360
 [0530] cccgtctcct cgccgctccg cgtgctccg gcagcgcacc tgggtggacgc ggggtacctg 420
 [0531] gccaaagtag agagggccgt ggcgctcatg aagcagctgc ccgcccacga cccgcgcagc 480
 [0532] ttcgagcagc agtggcacgt gcaactgcgc tactgagacg ccgectacga ccaggctcggg 540
 [0533] ttcccggacc tggagtcca gatacacaac tgctggtctt tcttcccatg gcacaggttc 600
 [0534] taccttact tccacgagag gatcctcggc aagctcatcg gcgacgacac cttcgcgctg 660
 [0535] cccttctgga actgggacgc gccggccggc atgacgctgc cggccatcta cgccgacagg 720
 [0536] tcgtcgccgc tctacgacga gaggcgcgac cccgcgcacc agccgcccgt gctggtcgac 780
 [0537] cttgactcca gtgggtccga caccaatata ccaagagacc agcagatcga cgagaacctc 840
 [0538] aagatcatgt accgccagat gatttcgaac gcgaagaaga cgctgctggt cctgggacag 900
 [0539] ccgtaccgcg ccggcgacca gccggaccgg ggcgccggct ccctggagaa cgtgccgcac 960
 [0540] ggcacggtcc acgtctggac tggcgaccca aggacgccc acttgccgga catgggcaac 1020
 [0541] ttcttctcgg cggcgcgca ccccatctt ttcgcgcacc acggcaacat cgaccgctg 1080
 [0542] tggcacgtct ggcgcggcct ccgcccagc aacctgact tcaccgacc cgactggtc 1140
 [0543] gacgccgctt tcctcttcta cgacgaggag gcccgcccc tgccgctgcg cgtccgggac 1200
 [0544] tgctctgacc cggccgcgct gcggtacatg taccaggacg tcggcctgcc gtggctcaac 1260
 [0545] gccaggccgg ccaaggcgtc cggcgggacg ccggcgcccc ccaccaccgg taccctcct 1320

[0546]	gccacctg acagcacat acgggtgacc gtgacgagg ccagggtgtc gaggagccgc	1380
[0547]	cgggaaaagg acgaggagga ggaggtgtc gtcgtggagg ggatcgagat cgccgacat	1440
[0548]	ttcaacaagt tcatcaagtt cgacgtgtc gtgaacgagc ccgagggagg ggtggacggc	1500
[0549]	acgccggcga cggcgacggg gtactgcgc gggagcttc cgcacacgc gcacatggtc	1560
[0550]	cggcccagg agacaggaa ggggtcgtc aagacgtgg cgaggttcgg cgtgtgcgac	1620
[0551]	ctgatggac acatcggagc ggacggcgc cagacgtgg tcgtgtcgt cgtaccagg	1680
[0552]	tgcggcgtg agctggtcac cgtaggcgc gtcagcatca gctacctcaa gtga	1734
[0553]	<210> 12	
[0554]	<211> 1732	
[0555]	<212> DNA	
[0556]	<213> 序列	
[0557]	<400> 12	
[0558]	atggagagca gtcgatgcc actgagtgc acccctcga tgccatgcag cctccaaacc	60
[0559]	cttgccgcc gcaaccttct ccgtggcctt cacctccga aggacgcgag gcagccacgg	120
[0560]	cgtctctcag tctcatgca ggcgaccgc ggctgccgc tcgaccgcc tgaggtgctc	180
[0561]	ctcggctctc ggcggcccgc ggctgccgt ctggccacgg acaaaggtc aggcgcgatc	240
[0562]	gccgcgcca tccaggccc ggacctcgc aactgcaaaa cgcccagct cccgaacacg	300
[0563]	ccgccggaca ccaactgtc cccgacgcc ggcaccgca tcaccgactt cgtgtgccg	360
[0564]	cccgtctct cgccgtccg cgtgctccg gcagcgcacc tgggtggacgc ggggtacctg	420
[0565]	gccaaagtac agaggccgt ggcgctcatg aagcagctc ccgccgacga cccgcgcagc	480
[0566]	ttcagcagc agtggcacgt gactgcgcc tactgcgac ccgcctacga ccaggtcggg	540
[0567]	ttcccggacc tggagtcca gatacacaac tgctggctt tcttccatg gcacaggttc	600
[0568]	taccttact tccacgagag gatcctcggc aagctcatc gcgacgacac cttcgcgctg	660
[0569]	cccttctgga actgggacgc gccggccgc atgacgtgc cggccatcta cgccgacagg	720
[0570]	tcgtgccgc tctacgacga gagcgcgac ccgcgcacc agccgccgt gctggtcgc	780
[0571]	cttgactcca gtgggtcca caccaatc ccaagagacc agcagatcga cgagaacctc	840
[0572]	aagatcatgt accgccagat gatttcgaa gcgaagaaga cgctgcttc tgggacagcc	900
[0573]	gtaccgcgcc ggcgaccagc cggaccggg cgccggctc ctggagaacg tgccgcacgg	960
[0574]	cacggtccac gtctggactg gcgacccaag gcagcccaac ttggcggaca tgggcaactt	1020
[0575]	cttctcggc gcgcgcgacc ccatcttct cgcgcaccac ggcaacatc accgcctgtg	1080
[0576]	gcagctctg cgcgccctc gcccgagca cactgactc accgacccc actggctcga	1140
[0577]	cgccgcctc ctcttctac acgaggagg ccgccccgt cgcgtgcgc tccgggactg	1200
[0578]	cctcgaccg gccgcgctc ggtacatgta ccaggacgc ggctgcccgt ggctcaacgc	1260
[0579]	caggccggc aaggctccg gcgggacgc ggcgccgcc accaccgta cctcctcgc	1320
[0580]	caccctggac agcaccatac gggtgaccgt gacgagccc aggtgtcga ggagccgcc	1380
[0581]	ggaaaaggac gaggaggagg aggtgtggt cgtggaggg atcgagatc ccgaccattt	1440
[0582]	caacaagttc atcaagttc acgtgtggt gaacgagccc gagggagggg tggacggc	1500
[0583]	gccggcgac gcgacgggt actgcgccg gagcttcgc cacacgccgc acatggtccg	1560
[0584]	gcccgaggag acgaggaagg ggtcgtcaa gacggtggc aggttcggc tgtgcgacct	1620
[0585]	gatggacgac atcggagcgc acggcgacca gacggtggtc gtgtcgtcgc taccaggtg	1680
[0586]	cggcgtgag ctggtcacc taggcggcgt cagcatcagc tacctcaagt ga	1732
[0587]	<210> 13	

[0588] <211> 4107
 [0589] <212> DNA
 [0590] <213> 序列
 [0591] <400> 13
 [0592] atggacaaga agtactcgat cggcctcgat attgggacta actctgttgg ctgggccgtg 60
 [0593] atcaccgacg agtacaaggt gccctcaaag aagttcaagg tcctgggcaa caccgatcgg 120
 [0594] cattccatca agaagaatct cattggcgct ctctgttctg acagcggcga gacggctgag 180
 [0595] gctacgcggc tcaagcgcac cgcccgcagg cggtagacgc gcaggaagaa tcgcatctgc 240
 [0596] tacctgcagg agattttctc caacgagatg gcgaaggtg acgattcttt cttccacagg 300
 [0597] ctggaggagt cattcctcgt ggaggaggat aagaagcacg agcggcatcc aatcttcggc 360
 [0598] aacattgtcg acgaggttgc ctaccacgag aagtacccta cgatctacca tctgcggaag 420
 [0599] aagctcgtgg actccacaga taaggcggac ctccgcctga tctacctcgc tctggcccac 480
 [0600] atgattaagt tcaggggcca tttcctgatc gagggggatc tcaaccggga caatagcgat 540
 [0601] gttgacaagc tgttcatcca gctcgtgcag acgtacaacc agctcttctg ggagaacccc 600
 [0602] attaatgcgt caggcgtcga cggaaggct atcctgtccg ctaggctctc gaagtctcgg 660
 [0603] cgctcgcgag acctgatcgc ccagctgccg ggcgagaaga agaacggcct gttcgggaat 720
 [0604] ctattgcgc tcagcctggg gctcacgcc aacttcaagt cgaatttctg tctcgtctgag 780
 [0605] gacccaagc tgcagctctc caaggacaca tacgacgatg acctggataa cctcctggcc 840
 [0606] cagatcggcg atcagtacgc ggacctgttc ctgcgtgcca agaattctgtc ggacgccatc 900
 [0607] ctctgtctg atattctcag ggtgaacacc gagattacga aggtctcctc ctcagcctcc 960
 [0608] atgatcaagc gctacgacga gcaccatcag gatctgacct tcctgaaggc gctggtcagg 1020
 [0609] cagcagctcc ccgagaagta caaggagatc ttcttcgatc agtcgaagaa cggctacgct 1080
 [0610] gggtagattg acggcggggc ctctcaggag gagttctaca agttcatcaa gccgattctg 1140
 [0611] gagaagatgg acggcacgga ggagctgctg gtgaagctca atcgcgagga cctcctgagg 1200
 [0612] aagcagcggg cattcgataa cggcagcatc ccacaccaga ttcatctcgg ggagctgcac 1260
 [0613] gctatcctga ggagcagga ggacttctac ctttctctca aggataaccg cgagaagatc 1320
 [0614] gagaagattc tgactttcag gatcccgtac tacgtcggcc cactcgttag gggcaactcc 1380
 [0615] cgcttcgctt ggatgaccgc caagtcagag gagacgatca cgccgtggaa cttcagaggag 1440
 [0616] gtggtcgaca agggcgttag cgctcagtcg ttcatcgaga ggatgacgaa tttcgacaag 1500
 [0617] aacctgcaa atgagaaggt gctccctaag cactcgtctc tgtacgagta cttcacagtc 1560
 [0618] tacaacgagc tgactaaggt gaagtatgtg accgagggca tgaggaagcc ggctttcctg 1620
 [0619] tctggggagc agaagaaggc catcgtggac ctctgttca agaccaaccg gaaggtcacg 1680
 [0620] gtttaagcagc tcaaggagga ctacttcaag aagattgagt gcttcgattc ggtcagatc 1740
 [0621] tctggcgttg aggaccgctt caacgcctcc ctggggacct accacgatct cctgaagatc 1800
 [0622] attaaggata aggacttctt ggacaacgag gagaatgagg atatcctcga ggacattgtg 1860
 [0623] ctgacactca ctctgttctg ggaccgggag atgatcagag agcgcctgaa gacttacgcc 1920
 [0624] catctctctg atgacaaggt catgaagcag ctcaagagga ggaggtacac cggctggggg 1980
 [0625] aggetgagca ggaagctcat caacggcatt cgggacaagc agtccgggaa gacgatctc 2040
 [0626] gacttctgag agagcgatgg cttcgcgaac cgcaatttca tgcagctgat tcacgatgac 2100
 [0627] agcctcacat tcaaggagga tatccagaag gctcaggtga gcggccaggg ggactcgtctg 2160
 [0628] cacgagcata tcgcaacct cgctggctcg ccagctatca agaaggggat tctgcagacc 2220
 [0629] gtgaaggttg tggacgagct ggtgaaggtc atgggcaggc acaagcctga gaacatcgtc 2280

[0630]	attgagatgg cccgggagaa tcagaccacg cagaagggcc agaagaactc acgcgagagg	2340
[0631]	atgaagagga tcgaggaggg cattaaggag ctgggggtccc agatcctcaa ggagcacccc	2400
[0632]	gtggagaaca cgcagctgca gaatgagaag ctctacctgt actacctcca gaatggccgc	2460
[0633]	gatatgtatg tggaccagga gctggatatt aacaggctca gcgattacga cgtcgatcat	2520
[0634]	atcgttccac agtcattcct gaaggatgac tccattgaca acaaggctct caccaggctc	2580
[0635]	gacaagaacc ggggcaagtc tgataatggt cttcagagg aggtcgtaa gaagatgaag	2640
[0636]	aactactggc gccagctcct gaatgccaag ctgatcacgc agcggaagtt cgataacctc	2700
[0637]	acaaggtc agagggcgg gctctctgag ctggacaagg cgggcttcat caagaggcag	2760
[0638]	ctggtcgaga cacggcagat cactaagcac gttgvcaga ttctcgactc acggatgaac	2820
[0639]	actaagtacg atgagaatga caagctgatc cgcgaggatga aggtcatcac cctgaagtca	2880
[0640]	aagctcgtct ccgacttcag gaaggatttc cagttctaca aggttcggga gatcaacaat	2940
[0641]	taccacatg cccatgacgc gtacctgaac gcggtggtcg gcacagctct gatcaagaag	3000
[0642]	tacccaagc tcgagagcga gttcgtgtac ggggactaca aggtttacga tgtgaggaag	3060
[0643]	atgatcgcca agtcggagca ggagattggc aaggctaccg ccaagtactt cttctactct	3120
[0644]	aacattatga atttctcaa gacagagatc actctggcca atggcgagat ccggaagcgc	3180
[0645]	ccctcatcg agacgaacgg cgagacgggg gagatcgtgt gggacaaggg cagggatttc	3240
[0646]	gcgaccgtca ggaaggttct ctccatgcca caagtgaata tcgtcaagaa gacagaggtc	3300
[0647]	cagactggcg gtttctctaa ggagtcaatt ctgcctaagc ggaacagcga caagctcatc	3360
[0648]	gcccgaaga aggactggga tccgaagaag tacggcgggt tcgacagccc cactgtggcc	3420
[0649]	tactcggtcc tggttgtggc gaaggttag aagggcaagt ccaagaagct caagagcgtg	3480
[0650]	aaggagctgc tgggatcac gattatggag cgctccagct tcgagaagaa ccgatcgat	3540
[0651]	ttctggagg cgaaggcta caaggagtg aagaaggacc tgatcattaa gctccccaag	3600
[0652]	tactcactct tcgagctgga gaacggcagg aagcggatgc tggcttccgc tggcgagctg	3660
[0653]	cagaagggga acgagctggc tctgccgtcc aagtatgtga acttctctta cctggcctcc	3720
[0654]	cactacgaga agctcaaggc cagccccgag gacaacgagc agaagcagct gttcgtcgag	3780
[0655]	cagcacaagc attacctga cgagatcatt gagcagattt ccgagttctc caagcgcgtg	3840
[0656]	atcctggccg acgcgaatct ggataaggtc ctctccgct acaacaagca ccgcgacaag	3900
[0657]	ccaatcaggg agcagctga gaatatcatt catctctca ccctgacgaa cctcgcgcc	3960
[0658]	cctgetgett tcaagtactt cgacacaact atcgatcgca agaggtacac aagcactaag	4020
[0659]	gagtcctgg acgcgacct catccaccag tcgattaccg gcctctacga gacgcgcatc	4080
[0660]	gacctgtctc agctcggggg cgactga	4107

ATGGAGAGCAGTCGCATGCCACTGAGTGCCACCCCTCGCATGCCATGCAGCCTCCAAACCCTTGCGCGCCGCAACTTCTCCGTGGCCTTACCTCCGGAAGGACGCGAGGCAGCCACGGCGTCTCTCAGTC
TCATGCGAGGCGACCGGCGGCTCCGCGTCGACCCCGTGAGGTGCTCCTGGTCTCGGCGCGCCGCGGCTGCGGCTGACCACGGACAAGGTGAGGCGCGATCGCCGCGCCATCCAGGCCCGG
ACCTCCGAACTGCCAAACGCCCGAGCTCCCGAACACGCGCCCGGACACAACTGCTCCCGACGCCCGGACCCGGCATCCGACTTCGTGTGCGCGCCGTCTCTCGCCGCTCCGCGTGCGTCCGGCAG
CGCACCTGGTGGACGCGGGTACTGGCCAAGTACGAGAGGGCCGTGGCGTCTATGAAGCAGCTGCCCGCCGACGACCCGCGCAGCTTCGAGCAGCAGTGGCACGTGCACTGCGCCTACTGCGACGCCG
CTACGACCAGTCCGGTTCCCGGACTCGAGCTCAGATACACAACCTGCTGGCTCTTCTCCATGGCACAGGTTCTACTCTACTCCACGAGAGGATCTCGGCAAGCTCATCGGCGACGACACCTTCGCG
CTGCCCTTCTGGAACTGGGACGCGCGGCGCGCATGACGCTGCCGCCATCTACGCCGACAGTCTGCGCGCTCTACGACGAGAGGCGGACCCCGCGCACCCAGCCCGCGTGCTGGTGCACCTTGACTC
CAGTGGGTCCGACACCAATATCCAAGAGACCAGCAGATCGACGAGAACCTCAAGATCATGTACCCAGATGATTTGAAACGCGAAGAAGACGCTGCTGTTCTGGGACAGCCGTACCCGCGCGGCGAC
AGCCGACCCCGGCGCCGCTCCCTGGAGAAGCTGCCGACGGCACGGTCCACGCTGGACTGCGGACCCAGGACGCCCACTTGGCGGACATGGGCAACTCTTCTCGGCGCGCGGACCCCATCTTC
TTCGCGCACACGGCAACATCGACCGCTGTGGCACGTGCGCGGCTCCGCCCAGCAACTGACTTACCAGCCCGACTGGCTGACGCCCTCTCTCTACGACGAGGAGGCCCGCCCGT
CGCGTCCGCTCCGGACTGCTCGACCCGCGCGCTGCGGTACATGTACCAGGACGTGCGCTGCGTCAACGCGCAGGCCGCAAGGCGTCCGGCGGACCGCGCGCCCGCCACCCACCGGTA
CCCTCCTGCCACCTGGACAGCACCATACGGGTGACCGTGACGAGGCCAGGGTGTGAGGAGCCCGGAAAGGACGAGGAGGAGGAGTGTGCTGTGAGGGGATCGAGATCGCCGACCAT
TTCAACAAGTTCATCAAGTTCGACGTGCTGGTGAACGAGCCCGAGGGGAGGGTGGACGGCACCGCGGACGCGGACGGGGTACTGCGCCGGAGCTTCGCGCACACCGCCACATGGTCCGCGCCGAG
GAGACGAGGAAGGGTCCGTCAGACGGTGGCGAGTTCGGCGTGTGCGACTGTGACGACATCGGAGCGGACGGCGCACAGCGGTGCTGCTGCTGCTACCCAGTGCGCGGTGAGCTGTT
CACCGTAGGCGCGTCCAGCATCAGCTACCTCAAGTGA

图1

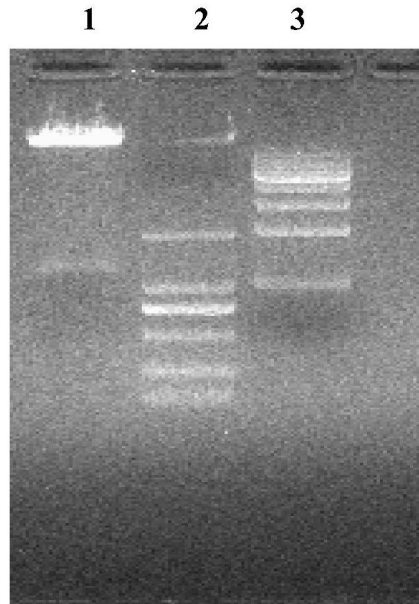


图2

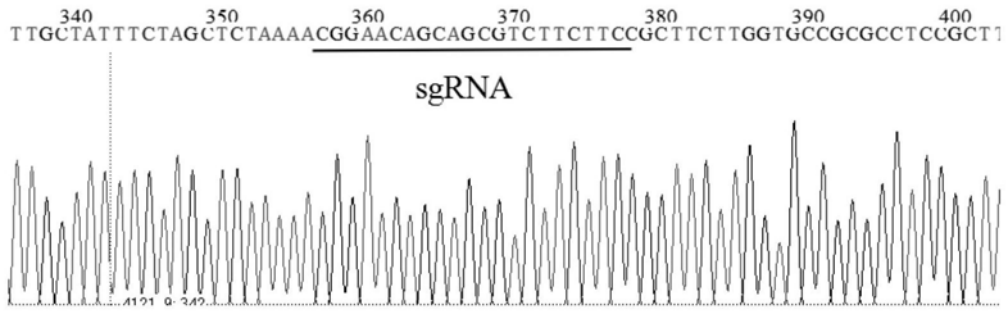


图3

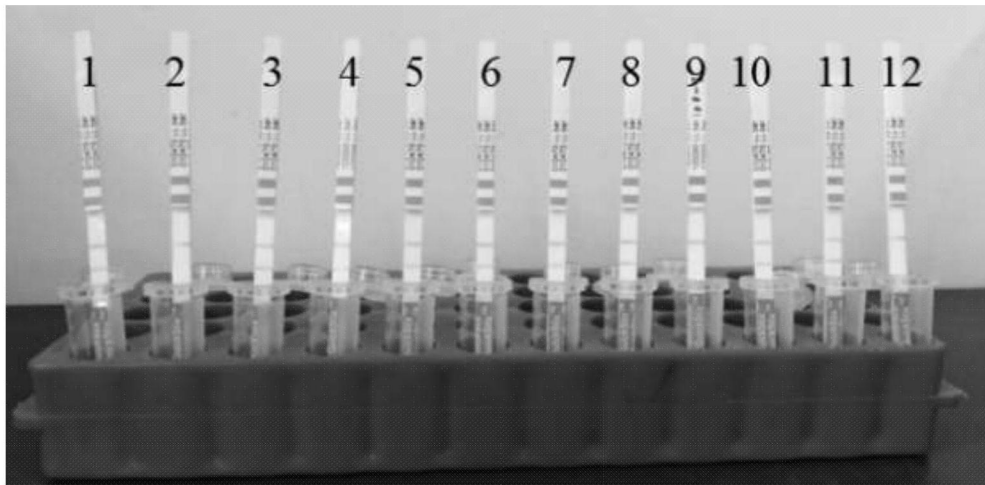


图4

MESSRMPLSATPRMPCSLQTLARRNLLRGLHLRKDARQPRRLSVSCEATGGCRVDRREVLLGLGGAAAAGLATDKGRGAIAPDL
 RNCQTPPELNPPTDNCCTPGTGITDFVLPVSSPLRVRPAAHLVDAGYLAKYERAVALMKQLPADDPRSFEQQWHVHCAYCDAAYDQ
 VGFPDLELQIHNCWLFFPWHRFYLFHERILGKLGDDTFALPFWNWDAPAGMTLPAIYADRSSPLYDERRDPAHQPPVLVDLSSGSDT
 NIPRDQQIDENLKIMYRQMISNAKKTLLFLGQPYRAGDQPDGAGSLENVPHGTVHVWTGDRQPNLADMGNFFSAARDPIFFAHHG
 NIDRLWHVWRGLRPSNTDFDPDWLDA AFLYDEEARPVRVVRDCLDPAALRYMYQDVGLPWLNARPAKASGGTPAPATTGTLPATL
 DSTIRVTVTRPRVSRREKDEEEVLVVEGIEIADHFNFKIFDVLVNEPEGVDGTPATATGYCAGSFAHTPHMVRPEETRKGSVKTVAR
 FGVCDLMDDIGADGDQTVVSVLPRCGGELVTVGGVSI SYLK

图5

MESSRMPLSATPRMPCSLQTLARRNLLRGLHLRKDARQPRRLSVSCEATGGCRVDRREVLLGLGGAAAAGLATDKGRGAIAPDL
 RNCQTPPELNPPTDNCCTPGTGITDFVLPVSSPLRVRPAAHLVDAGYLAKYERAVALMKQLPADDPRSFEQQWHVHCAYCDAAYD
 QVGFDPDLELQIHNCWLFFPWHRFYLFHERILGKLGDDTFALPFWNWDAPAGMTLPAIYADRSSPLYDERRDPAHQPPVLVDLSSGS
 DTNIPRDQQIDENLKIMYRQMISNAKKTLLPGTAVPRRRPAGPGRRLPGERAARHGPRLDWRPKAAQLGGHGQLLLGGARPHLLRAPR
 QHRPPVARLARPPPEQH

图6

```
Query 1 ATGGAGAGCAGTCGCATGCCACTGAGTGCCACCCCTCGCATGCCATGCAGCCTCCAAACC 60
|||||
Sbjct 1 ATGGAGAGCAGTCGCATGCCACTGAGTGCCACCCCTCGCATGCCATGCAGCCTCCAAACC 60
Query 61 CTTGCGCGCCGCAACCTTCTCCGTGGCCTTACCTCCGGAAGGACGCGAGGCAGCCACGG 120
|||||
Sbjct 61 CTTGCGCGCCGCAACCTTCTCCGTGGCCTTACCTCCGGAAGGACGCGAGGCAGCCACGG 120
Query 121 CGTCTCTCAGTCTCATGCGAGGCGACCGCGGCTGCCCGTCGACCGCCGTGAGGTGCTC 180
|||||
Sbjct 121 CGTCTCTCAGTCTCATGCGAGGCGACCGCGGCTGCCCGTCGACCGCCGTGAGGTGCTC 180
Query 181 CTCGGTCTCGGCGGCCCGCGGCTGCCGTCTGGCCACGGACAAAGGTCGAGGCGCGATC 240
|||||
Sbjct 181 CTCGGTCTCGGCGGCCCGCGGCTGCCGTCTGGCCACGGACAAAGGTCGAGGCGCGATC 240
```

Query 241 GCCGCGCCCATCCAGGCCCGGACCTCCGCAACTGCCAAAGCCCGAGCTCCCGAACAG 300
 |||
 Sbjct 241 GCCGCGCCCATCCAGGCCCGGACCTCCGCAACTGCCAAAGCCCGAGCTCCCGAACAG 300

Query 301 CCGCCGGACACCAACTGCTGCCGACGCCGGCACC GG CATCACC GACTTCGTGCTGCCG 360
 |||
 Sbjct 301 CCGCCGGACACCAACTGCTGCCGACGCCGGCACC GG CATCACC GACTTCGTGCTGCCG 360

Query 361 CCCGTCTCCTCGCCGCTCCGCGTGGTCCGGCAGCGCACCTGGTGGACGCGGGTACCTG 420
 |||
 Sbjct 361 CCCGTCTCCTCGCCGCTCCGCGTGGTCCGGCAGCGCACCTGGTGGACGCGGGTACCTG 420

Query 421 GCCAAGTACGAGAGGGCCGTGGCGCTCATGAAGCAGCTGCCCGCCGACGCCCGCGCAGC 480
 |||
 Sbjct 421 GCCAAGTACGAGAGGGCCGTGGCGCTCATGAAGCAGCTGCCCGCCGACGCCCGCGCAGC 480

Query 481 TTCGAGCAGCAGTGGCAGCTGCACTGCGCCTACTGCGACGCCCTACGACCAGGTCGGG 540
 |||
 Sbjct 481 TTCGAGCAGCAGTGGCAGCTGCACTGCGCCTACTGCGACGCCCTACGACCAGGTCGGG 540

Query 541 TTCCCGGACCTGGAGCTCCAGATACACAACCTGCTGGCTCTTCTCCCATGGCACAGGTTTC 600
 |||
 Sbjct 541 TTCCCGGACCTGGAGCTCCAGATACACAACCTGCTGGCTCTTCTCCCATGGCACAGGTTTC 600

Query 601 TACCTCTACTTCCACGAGAGGATCCTCGGCAAGCTCATCGCGCAGCACCTTCGCGCTG 660
 |||
 Sbjct 601 TACCTCTACTTCCACGAGAGGATCCTCGGCAAGCTCATCGCGCAGCACCTTCGCGCTG 660

Query 661 CCCTTCTGGAAGTGGGACGCGCCGGCCGCATGACGCTGCCGGCCATCTACGCCGACAGG 720
 |||
 Sbjct 661 CCCTTCTGGAAGTGGGACGCGCCGGCCGCATGACGCTGCCGGCCATCTACGCCGACAGG 720

Query 721 TCGTCGCCGCTCTACGACGAGAGGCGGACCCCGCACCAGCCCGGCTGGTTCGAC 780
 |||
 Sbjct 721 TCGTCGCCGCTCTACGACGAGAGGCGGACCCCGCACCAGCCCGGCTGGTTCGAC 780

Query 781 CTTGACTCCAGTGGTCCGACCAATATCCCAAGAGACCAGCAGATCGACGAGAACCTC 840
 |||
 Sbjct 781 CTTGACTCCAGTGGTCCGACCAATATCCCAAGAGACCAGCAGATCGACGAGAACCTC 840

Query 841 AAGATCATGTACCGCCAGATGATTTCGAACGCGAAGAAGACGCTGCTTCTCTGGGACAG 900
 |||
 Sbjct 841 AAGATCATGTACCGCCAGATGATTTCGAACGCGAAGAAGACGCTGCTTCTCTGGGACAG 898

Query 901 CCGTACCGCGCCGGCACCAGCCGGACCCGGCGCGGCTCCCTGGAGAAGCTGCCGCAC 960

```

|||||
Sbjct 899 CCGTACCGCGCCGGCGACCAGCCGACCCGGGGCCGGCTCCCTGGAGAACGTGCCGCAC 958
Query 961 GGCACGGTCCACGTCTGGACTGGCGACCCAAGGCAGCCCAACTTGGCGGACATGGGCAAC 1020
|||||
Sbjct 959 GGCACGGTCCACGTCTGGACTGGCGACCCAAGGCAGCCCAACTTGGCGGACATGGGCAAC 1018
Query 1021 TTCTTCTCGGGCGCGCGACCCCATCTTCTTCGCGCACCACGGCAACATCGACCGCTG 1080
|||||
Sbjct 1019 TTCTTCTCGGGCGCGCGACCCCATCTTCTTCGCGCACCACGGCAACATCGACCGCTG 1078
Query 1081 TGGCAGCTCTGGCGCGGCTCCGCCGAGCAACTGACTTCACCGACCCCGACTGGCTC 1140
|||||
Sbjct 1079 TGGCAGCTCTGGCGCGGCTCCGCCGAGCAACTGACTTCACCGACCCCGACTGGCTC 1138
Query 1141 GACGCCGCTTCTCTTCTACGACGAGGAGGCCCGCCCGTGGCGTGGCGTCCGGGAC 1200
|||||
Sbjct 1139 GACGCCGCTTCTCTTCTACGACGAGGAGGCCCGCCCGTGGCGTGGCGTCCGGGAC 1198
Query 1201 TGCCTCGACCCGGCGGCTGGGTACATGTACCAGGACGTGGCCTGCCGTGGCTCAAC 1260
|||||
Sbjct 1199 TGCCTCGACCCGGCGGCTGGGTACATGTACCAGGACGTGGCCTGCCGTGGCTCAAC 1258
Query 1261 GCCAGGCCGCCAAGGCGTCCGGCGGACGCCGGCGCCGCCACCACCGGTACCCTCCCT 1320
|||||
Sbjct 1259 GCCAGGCCGCCAAGGCGTCCGGCGGACGCCGGCGCCGCCACCACCGGTACCCTCCCT 1318
Query 1321 GCCACCCTGGACAGCACCATACGGGTGACCGTGACGAGGCCAGGGTGTGAGGAGCCGC 1380
|||||
Sbjct 1319 GCCACCCTGGACAGCACCATACGGGTGACCGTGACGAGGCCAGGGTGTGAGGAGCCGC 1378
Query 1381 CGGGAAAAGGACGAGGAGGAGGTGCTGGTGGAGGGGATCGAGATCGCCGACCAT 1440
|||||
Sbjct 1379 CGGGAAAAGGACGAGGAGGAGGTGCTGGTGGAGGGGATCGAGATCGCCGACCAT 1438
Query 1441 TTCAACAAAGTTCATCAAGTTCGACGTGCTGGTGAACGAGCCGAGGGAGGGGTGGACGGC 1500
|||||
Sbjct 1439 TTCAACAAAGTTCATCAAGTTCGACGTGCTGGTGAACGAGCCGAGGGAGGGGTGGACGGC 1498
Query 1501 ACGCCGGCGACGGCGACGGGTACTGCGCCGGGAGCTTCGCGCACACCCGCACATGGTC 1560
|||||
Sbjct 1499 ACGCCGGCGACGGCGACGGGTACTGCGCCGGGAGCTTCGCGCACACCCGCACATGGTC 1558
Query 1561 CGGCCCGAGGAGACGAGGAAGGGTCCGGTCAAGACGGTGGCGAGGTTCCGGCTGTGCGAC 1620
|||||
Sbjct 1559 CGGCCCGAGGAGACGAGGAAGGGTCCGGTCAAGACGGTGGCGAGGTTCCGGCTGTGCGAC 1618

```

Query 1621 CTGATGGACGACATCGGAGCGGACGGCGACCAGACGGTGGTCGTGCTCGTACCCAGG 1680
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
 Sbjct 1619 CTGATGGACGACATCGGAGCGGACGGCGACCAGACGGTGGTCGTGCTCGTACCCAGG 1678
 Query 1681 TCGCGCGGTGAGCTGGTCACCGTAGGCGGCGTCAGCATCAGCTACCTCAAGTGA 1734
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
 Sbjct 1679 TCGCGCGGTGAGCTGGTCACCGTAGGCGGCGTCAGCATCAGCTACCTCAAGTGA 1732

图7

WT.pro	MESSRMPLSATPRMPCSLQTLARRNLLRGLHLRKDARQPRRLSVSCEATGGCRVDRREVL	60
ppo.pro	MESSRMPLSATPRMPCSLQTLARRNLLRGLHLRKDARQPRRLSVSCEATGGCRVDRREVL	60
Consensus	messrmp lsatprmpcslqtlarrnllrglhrlkdarqprrlsvsceatggc rvdrrrevl	
WT.pro	LGLGGAAAAGLATDKGRGAIAAPIQAPDLRNCQTPPELNPPTDNCCPTFGTGITDFVLP	120
ppo.pro	LGLGGAAAAGLATDKGRGAIAAPIQAPDLRNCQTPPELNPPTDNCCPTFGTGITDFVLP	120
Consensus	lglggaaaaglatdkgrgaiaapiqapdlrncqtpelpntppdtnccptfgtgitdfvlp	
WT.pro	PVSSPLRVRPAAHLVDAGYLAKYERAVALMKQLPADDPRSFEQQWHVHCAYCDAAYDQVG	180
ppo.pro	PVSSPLRVRPAAHLVDAGYLAKYERAVALMKQLPADDPRSFEQQWHVHCAYCDAAYDQVG	180
Consensus	pvssplrivrpaahlvdagylakyeravalmkqlpaddprsfeqqwhvhcaycdaaydqvg	
WT.pro	FPDLELQIHNCWLFFPWHRFYLYFHERILGKLI GDDTFALPFWNWDAPAGMTLPAIYADR	240
ppo.pro	FPDLELQIHNCWLFFPWHRFYLYFHERILGKLI GDDTFALPFWNWDAPAGMTLPAIYADR	240
Consensus	fpdlelqihncwlffpwhrfylyfherilgkligddtfalpfnwnwdapagmtlpaiyadr	
WT.pro	SSPLYDERRDPAHQPPVLVDL DSSGSDTNIPRDQQIDENLKIMYRQMISNAKKTLLFLGQ	300
ppo.pro	SSPLYDERRDPAHQPPVLVDL DSSGSDTNIPRDQQIDENLKIMYRQMISNAKKTLL....	296
Consensus	ssplyderrdpahqppvlvlddssgsdtniprdqqidenlkimyrqm isnakktll	
WT.pro	PYRAGDQPLPGAGSLENVHGCTVHVWTC DPRQPNLADMGNFFSAARDPIFFAHGHNIDRL	360
ppo.proPGTAVPRRREACPGRRLEGE.....RAAR.....HG.....	322
Consensus	pg p g g aar hg	
WT.pro	WHVWRGLRPSNTDFTDPDWLDAAFLFYDEEARFVRVRVRDCLDPAALRYMYQDVGFHWLN	420
ppo.proFRID	326
Consensus	p l	
WT.pro	AREAKASGCTPAPATTGTLPATL DSTIRVTVTRERVSRSRREKDEEEEVLVVEGIEIADH	480
ppo.pro	WREKAAQIGG.....HGQLLLGGAREHLLRAERQHR.....	357
Consensus	rp a g rp r r	
WT.pro	FNKFIKFDVLVNEPEGGVDGTEATATGYCAGSFAHTPHMVREETRKGSVKTVARFGVCD	540
ppo.proEFVAR.....LAREPPEQH.....	371
Consensus	p a rp	
WT.pro	LMDDIGADGDQTVVVVSLVPRCGGELVTVGGVVISYSL	577
ppo.pro	371
Consensus		

图8

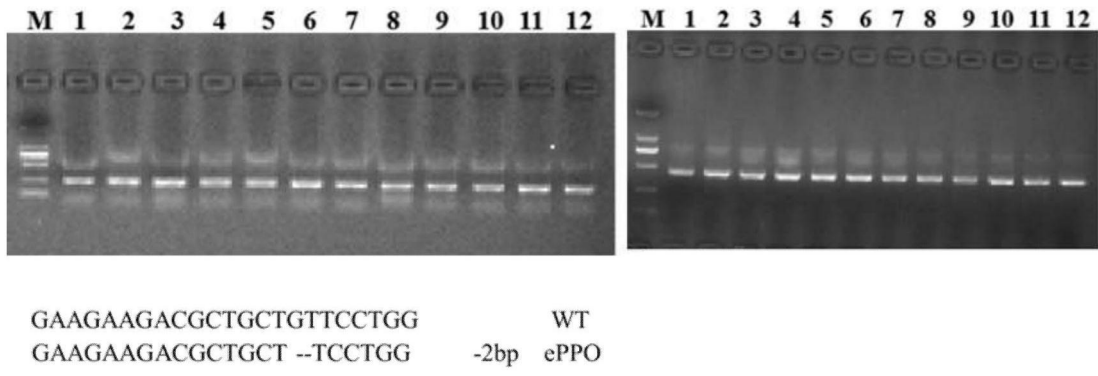


图9

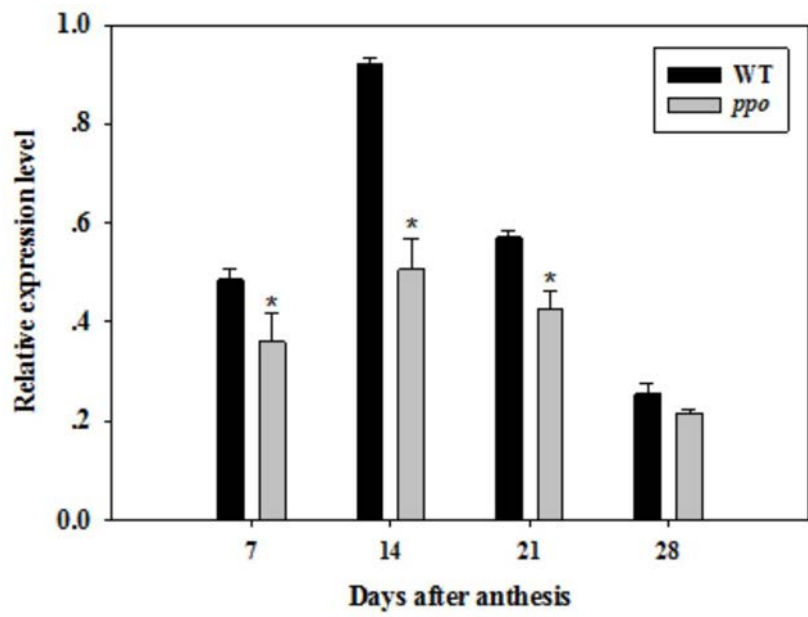


图10