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(73)专利权人 DPx控股有限公司
地址 荷兰阿姆斯特丹

(72)发明人 马丁·基尔特兹曼
赫尔马特·施瓦布
哈拉德·皮希勒
米里拉·伊万科克 奥利弗·梅
鲁道夫·吉斯波特斯·玛丽·路易坦

(74)专利代理机构 北京东方亿思知识产权代理有限责任公司 11258
代理人 肖善强 南霆

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(54)发明名称

酯酶的制备

(57)摘要

本发明涉及重组E.coli菌株,其特征在于,该生物含有编码具有酯酶活性的蛋白的基因。此类E.coli适于制备具有酯酶活性的蛋白,其中,表达在不从质粒共表达GroEL和/或GroES的情况下发生。此外,该生物在富氧条件下生长的能力已通过突变恢复。具有酯酶活性的蛋白的基因的表达不伴随编码热激伴侣蛋白的其它基因的表达。

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1.制备具有酯酶活性的蛋白的方法,所述方法包括在E.coli菌株中表达编码此类蛋白的基因,其特征在于编码具有酯酶活性的蛋白的基因是SEQ ID NO:11的多核苷酸,并且其中,所述表达在不从质粒共表达GroEL和/或GroES的情况下进行。

2.适于制备具有酯酶活性的蛋白的重组E.coli菌株,其特征在于,该菌株含有编码具有酯酶活性的蛋白的SEQ ID NO:11的多核苷酸所示的基因,并且其中,所述基因的表达在不从质粒共表达GroEL和/或GroES的情况下进行。

3.根据权利要求2的适于制备具有酯酶活性的蛋白的重组E.coli菌株,其特征在于,所述菌株不能表达谷胱甘肽还原酶和/或硫氧还蛋白还原酶。

4.根据权利要求2的适于制备具有酯酶活性的蛋白的重组E.coli菌株,其特征在于,所述菌株不能表达谷胱甘肽还原酶和硫氧还蛋白还原酶两者。

5.根据权利要求2的适于制备具有酯酶活性的蛋白的重组E.coli菌株,其特征在于,谷胱甘肽还原酶和/或硫氧还蛋白还原酶的表达通过突变消除。

6.根据权利要求2-5中任意一项的重组E.coli菌株,获得自E.coli Origami 1、Origami 2或Origami B菌株。

7.经分离的多核苷酸,其编码具有酯酶活性的功能性蛋白,所述多核苷酸的序列如SEQ ID NO:11所示。

8.适于在E.coli中复制和表达的载体,所述载体含有编码具有酯酶活性的蛋白的多核苷酸,所述多核苷酸是SEQ ID NO:11所示的多核苷酸。

酯酶的制备

[0001] 本发明涉及制备具有酯酶活性的蛋白的方法，所述方法包括在Escherichia coli (E.coli)菌株中表达编码此类蛋白的基因。

[0002] 具有猪肝酯酶活性的蛋白的高水平表达并不容易实现。Lange et al[1]的报道显示Pichia pastoris中对猪肝酯酶(γ-rPLE)的γ-同工酶的生产很低但可检测到。更近来，Böttcher et al.(2007)[2]已描述了一种方法。这些作者的工作表明，猪肝酯酶(γ-rPLE)的γ-同工酶在E.coli菌株中的表达并非直接简单的过程。如果不采用额外的手段，此类表达完全失败。这些手段暗示不仅要正确选择合适的E.coli菌株，还要共表达伴侣蛋白。特别地，仅可能在E.coli菌株Origami中制备功能性γ-rPLE，该菌株共表达相当大量的、被称为GroEL和GroES的伴侣蛋白。

[0003] 在γ-rPLE的正确表达中遇到的问题的至少一部分涉及蛋白中多个二硫键的存在。

[0004] 令人非常吃惊地，并且与Böttcher et al.(2007)[1]所报道的教导相悖地，本发明揭示：可以在没有Böttcher et al.(2007)[1]所公开的多种额外手段的情况下，特别是不共表达其它基因的情况下，实现具有猪肝酯酶(PLE)活性的蛋白的功能表达。

[0005] 因此，本发明涉及制备具有酯酶活性的蛋白的方法，所述方法包括在E.coli菌株中表达编码此类蛋白的基因，其特征在于编码具有酯酶活性的蛋白的基因与SEQ ID NO:11、SEQ ID NO:32、SEQ ID NO:34、SEQ ID NO:36、SEQ ID NO:38、SEQ ID NO:40、SEQ ID NO:42或SEQ ID NO:44的多核苷酸具有至少70%同一性，优选至少80%同一性，优选至少85%同一性，优选至少90%同一性，优选至少95%同一性，优选至少98%同一性，最优选至少99%同一性，并且所述基因编码分别与SEQ ID NO:12、SEQ ID NO:33、SEQ ID NO:35、SEQ ID NO:37、SEQ ID NO:39、SEQ ID NO:41、SEQ ID NO:43或SEQ ID NO:45具有至少80%同一性，优选至少85%同一性，优选至少90%同一性，优选至少95%同一性，优选至少98%同一性，更优选至少99%同一性的蛋白。本发明的优点之一是获得了经正确折叠的酯酶，而无需从质粒共表达GroEL和/或GroES。在一种优选的实施方式中，表达在不从质粒共表达GroEL和/或GroES的情况下进行。

[0006] 在本发明的上下文中，同一性按照Tatiana A.Tatusova,Thomas L.Madden (1999),"Blast 2sequences-a new tool for comparing protein and nucleotide sequences",FEMS Microbiol Lett.174:247-250所述来计算，其中使用http://www.ncbi.nlm.nih.gov/BLAST/bl2seq/wblast2.cgi的下述标准参数

[0007] 对蛋白序列而言：

[0008] Matrix:BLOSUM62

[0009] 开放缺口:5

[0010] 延伸缺口:2

[0011] 惩罚缺口x_dropoff:11

[0012] 预期:10

[0013] 字长:11

[0014] 对核苷酸序列而言：

[0015] 匹配奖励:1

[0016] 错配惩罚:-2

[0017] 开放缺口:11

[0018] 延伸缺口:1

[0019] 惩罚缺口x_dropoff:50

[0020] 预期:10

[0021] 字长:3

[0022] 更优选地，本发明涉及制备具有酯酶活性的蛋白的方法，所述方法包括在E.coli菌株中表达编码此类蛋白的基因，其特征在于编码具有酯酶活性的蛋白的基因与SEQ ID NO:11的多核苷酸具有至少70％同一性，优选至少80％同一性，优选至少85％同一性，优选至少90％同一性，优选至少95％同一性，优选至少98％同一性，最优选至少99％同一性，并且所述基因编码与SEQ ID NO:12具有至少80％同一性，优选至少85％同一性，优选至少90％同一性，优选至少95％同一性，优选至少98％同一性，更优选至少99％同一性的蛋白。

[0023] 根据另一实施方式，本发明涉及制备具有酯酶活性的蛋白的方法，所述方法包括在E.coli菌株中表达编码此类蛋白的基因，其特征在于所述基因编码与SEQ ID NO:12具有至少80％同一性，优选至少85％同一性，优选至少90％同一性，优选至少95％同一性，优选至少98％同一性，更优选至少99％同一性的蛋白。SEQ ID NO:12代表了APLE的氨基酸序列。

[0024] 根据另一实施方式，本发明涉及适于制备具有酯酶活性的蛋白的重组E.coli菌株，其特征在于，所述生物含有编码具有酯酶活性的蛋白并且与SEQ ID NO:11、SEQ ID NO:32、SEQ ID NO:34、SEQ ID NO:36、SEQ ID NO:38、SEQ ID NO:40、SEQ ID NO:42或SEQ ID NO:44的多核苷酸具有至少70％同一性，优选至少80％同一性，优选至少85％同一性，优选至少90％同一性，优选至少95％同一性，优选至少98％同一性，最优选至少99％同一性的基因，并且，所述基因编码分别与SEQ ID NO:12、SEQ ID NO:33、SEQ ID NO:35、SEQ ID NO:37、SEQ ID NO:39、SEQ ID NO:41、SEQ ID NO:43或SEQ ID NO:45具有至少80％同一性，优选至少85％同一性，优选至少90％同一性，优选至少95％同一性，优选至少98％同一性，更优选至少99％同一性的蛋白。在一种优选的实施方式中，重组E.coli菌株不包含用于共表达GroEL和/或GroES的质粒，并且能生产具有酯酶活性的蛋白。

[0025] 特别地，本发明涉及适于根据上文所述的方法制备具有酯酶活性的蛋白的重组E.coli菌株，其中，所述生物含有编码具有酯酶活性的蛋白并且与SEQ ID NO:11的多核苷酸具有至少70％同一性，优选至少80％同一性，优选至少85％同一性，优选至少90％同一性，优选至少95％同一性，优选至少98％同一性，最优选至少99％同一性的基因，并且，所述基因编码与SEQ ID NO:12具有至少80％同一性，优选至少85％同一性，优选至少90％同一性，优选至少95％同一性，优选至少98％同一性，更优选至少99％同一性的蛋白。

[0026] 特别地，本发明还涉及适于根据上文所述的方法制备具有酯酶活性的蛋白的重组E.coli菌株，其中，所述生物含有编码与SEQ ID NO:12具有至少80％同一性，优选至少85％同一性，优选至少90％同一性，优选至少95％同一性，优选至少98％同一性，更优选至少99％同一性的蛋白的基因。

[0027] 根据另一实施方式，本发明涉及适于制备具有酯酶活性的蛋白的重组E.coli菌

株，其特征在于，谷胱甘肽还原酶和/或硫氧还蛋白(thioredoxin)还原酶的表达的表达被消除，例如通过突变实现。

[0028] 根据本发明的另一实施方式，重组E.coli菌株的谷胱甘肽还原酶和硫氧还蛋白还原酶两者的表达都被消除。

[0029] Prinz et al(1997)[3]已教导，如果是在还原酶活性被消除的菌株中生产时，含有二硫桥的蛋白(特别是碱性磷酸酶)在E.coli中表达的活性较高。还已显示，如果两种还原酶都被消除的话，将发生自发突变，这使得能在需氧条件下生长。Beckwith et al.(2005)[4]鉴定了恢复生物在需氧条件下生长的能力的可能的自发突变。他们指出，AhpC基因中的突变(包括该基因的大约36-39位密码子的TCT三体富集区域中插入三个核苷酸)提供了这种作用。

[0030] Bessette et al.(1999)[5]已更详细地分析了Prinz et al.(1997)描述的表达系统，他们显示，辅助蛋白DsbC(二硫键异构酶)的共表达增强了活性组织血纤维蛋白溶酶原活化因子的表达和活性碱性磷酸酶的表达。此外，还公开了，DsbC的截短版本的细胞内表达导致产生功能性的二硫键异构酶蛋白。

[0031] 根据本发明的另一实施方式，已经对重组E.coli进行了进一步修饰，以生产低分子量的辅助蛋白，其能引入二硫键用于需要二硫键的蛋白的正确折叠，或者其能改正不适当的二硫键导致的错误折叠。

[0032] 上文提到的合适的低分子量辅助蛋白是二硫化物异构酶。在一种特别优选的实施方式中，辅助蛋白是E.coli的被称为DsbC的蛋白(Bessette et al(1999))。

[0033] 适于根据本发明使用的E.coli菌株较之野生型E.coli菌株而言具有较少还原性的细胞内环境。此类E.coli菌株的一个具体例子是E.coli Origami菌株，其在谷胱甘肽还原酶基因和硫氧还蛋白还原酶基因上具有突变(Terpe(2006)[6])。当这些基因功能性表达时，其抵消细胞质中的二硫键形成。因此，含有二硫键的蛋白的异源表达迄今为止被认为需要分别消除谷胱甘肽和硫氧还蛋白还原酶活性。

[0034] 但是，我们发现，仅一种突变就足以使得具有PLE活性的蛋白的功能性表达增加。

[0035] 令人吃惊地，合适的编码酯酶的基因是编码酯酶蛋白并且具有针对Pichia改造的密码子使用的多核苷酸。

[0036] 更特别地，本发明涉及下述基因，所述基因编码功能性酯酶蛋白，并且具有较之SEQ ID NO:11的多核苷酸而言具有至少70％同一性，优选至少80％同一性，优选至少85％同一性，优选至少90％同一性，优选至少95％同一性，优选至少98％同一性，最优选至少99％同一性的核苷酸序列，并且，所述基因编码与SEQ ID NO:12具有至少80％同一性，优选至少85％同一性，优选至少90％同一性，优选至少95％同一性，优选至少98％同一性，更优选至少99％同一性的蛋白。

[0037] 在本发明的一种实施方式中，本发明涉及经分离的多核苷酸，其编码具有酯酶活性的功能性蛋白，所述多核苷酸具有与SEQ ID NO:11、SEQ ID NO:32、SEQ ID NO:34、SEQ ID NO:36、SEQ ID NO:38、SEQ ID NO:40、SEQ ID NO:42或SEQ ID NO:44的多核苷酸具有至少70％同一性，优选至少80％同一性，优选至少85％同一性，优选至少90％同一性，优选至少95％同一性，优选至少98％同一性，最优选至少99％同一性的核苷酸序列，并且编码分别与SEQ ID NO:12、SEQ ID NO:33、SEQ ID NO:35、SEQ ID NO:37、SEQ ID NO:39、SEQ ID NO:

41、SEQ ID NO:43或SEQ ID NO:45具有至少80%同一性，优选至少85%同一性，优选至少90%同一性，优选至少95%同一性，优选至少98%同一性，更优选至少99%同一性的蛋白。

[0038] 通过在可大量发酵生产的微生物宿主(例如细菌和酵母等)中表达编码感兴趣的蛋白的基因，可实现对蛋白的大量生产。Terpe(2006)近来已对细菌蛋白表达系统进行了综述。

[0039] 根据本发明，在经转化的E.coli菌株中表达编码感兴趣的蛋白的此类基因。可通过任何合适的方法，例如通过电穿孔、通过热激转化或者通过化学转化，来用异源基因转化E.coli。

[0040] 为转化E.coli，编码具有酯酶活性的蛋白的基因可以是载体(例如质粒、噬菌体或噬粒)的一部分。

[0041] 本发明还涉及适于在E.coli中复制和表达的载体，所述载体含有编码具有酯酶活性的蛋白的多核苷酸，所述多核苷酸具有与SEQ ID NO:11、SEQ ID NO:32、SEQ ID NO:34、SEQ ID NO:36、SEQ ID NO:38、SEQ ID NO:40、SEQ ID NO:42或SEQ ID NO:44的多核苷酸具有至少70%同一性，优选至少80%同一性，优选至少85%同一性，优选至少90%同一性，优选至少95%同一性，优选至少98%同一性，最优选至少99%同一性的核苷酸序列，并且，编码分别与SEQ ID NO:12、SEQ ID NO:33、SEQ ID NO:35、SEQ ID NO:37、SEQ ID NO:39、SEQ ID NO:41、SEQ ID NO:43或SEQ ID NO:45具有至少80%同一性，优选至少85%同一性，优选至少90%同一性，优选至少95%同一性，优选至少98%同一性，更优选至少99%同一性的蛋白。

[0042] 载体应当含有适于进行例如选择、复制、基因调控转录(起始和终止)以及克隆所需的基因序列的必要的功能元件。

[0043] 对适用于本发明的E.coli菌株、载体、载体元件、转染方法、经转染生物的培养方法以及所需的多肽的收获和分离方法的选择将是本领域技术人员显而易见的。

[0044] 通过猪肝提取从动物来源获得的商业猪肝酯酶(PLE)广泛用于合成有机化学领域。

[0045] 商业酶制剂已显示出至少由可能具有不同底物特异性的若干PLE同工酶构成。

[0046] 商业PLE广泛用于多种生物催化反应，这利用了其宽广的底物特异性以及酯水解的对映异构选择性。

[0047] 例如，WO 01/09079描述了动物源的PLE用于对(4E)-5-氯-2-异丙基戊-4-烯酸甲酯的(R)-对映异构体进行选择性水解的用途。

[0048] 由于病毒和朊病毒等导致的传染性疾病方面的顾虑，对于药物生产而言，仅使用非动物源性原材料的兴趣在逐渐增加，使用重组DNA技术对酯酶进行微生物生产可针对此提供解决方案。

[0049] 基于鉴定和表达PLE主要(γ-)同工酶方面可获得的信息(Matsushima et al (1991)[7].,Lange et al.(2001))，制备来自猪肝的cDNA，并针对PLEγ-同工酶相关序列加以筛选。就此目的而言，设计能识别编码PLEγ-异构酶相关蛋白的cDNA片段的PCR引物。通过对多种这类γ-PLE相关cDNA片段进行DNA测序，如预计的那样，找到了已知的γ-PLE编码DNA序列，此外还鉴定出了第二种PLE同工酶，其与成熟的γ-PLE蛋白在548个氨基酸中有21处不同。该新的PLE同工酶被称为APLE，即，另外的猪肝酯酶(Alternative Pig Liver

Esterase)。

[0050] 为针对底物特异性对γ-PLE和APLE同工酶进行功能分析，将编码γ-PLE和APLE的DNA序列都插入进针对Pichia pastoris中分泌的蛋白表达而设计的表达盒中，类似于Lange et al.所述。但与后者文献相反，甚至在编码蛋白中存在C-末端氨基酸序列HAEL时，也实现了两种蛋白的成功表达。通过使用α-乙酸萘酯，通过通常的酯酶检验，进行活性测定，来鉴定酯酶活性。

[0051] 令人吃惊地，虽然氨基酸序列具有超过95％的同一性，在对5-卤-2-烷基戊-4-烯酸酯的水解方面观察到γ-PLE和APLE之间有明显的不同。APLE能非常高效地水解外消旋(4E)-5-氯-2-异丙基戊-4-烯酸甲酯，而γ-PLE根本不能水解该化合物。此外，还显示，外消旋(4E)-5-氯-2-异丙基戊-4-烯酸甲酯的水解是通过仅对R-对映异构体的选择性水解来进行：APLE则对(2S,4E)-5-氯-2-异丙基戊-4-烯酸甲酯没有反应活性。可推断：如WO 01/09079所述的动物源性的PLE对5-氯-2-异丙基戊-4-烯酸甲酯的已知的对映异构选择性水解可归功于商业猪肝提取物中存在的次要的同工酶APLE。

[0052] 对于大量生产非动物源性的能对映异构选择性水解5-氯-2-异丙基戊-4-烯酸甲酯的酯酶制备物，APLE基于Pichia pastoris的表达水平是不足的。因此，考虑到经济性生产必须要以工业规模快速可靠地进行生产，我们提出了另外的蛋白生产系统。

[0053] PLE同工酶结构上非常相关，已知蛋白需要细胞内二硫键来保持其结构完整性和活性。很多有吸引力的蛋白表达系统仅在蛋白靶向细胞外环境时允许二硫键形成，基本上如上文针对Pichia pastoris所述。大多数细菌，特别是Escherichia coli，保持细胞内的还原性环境，但是已经描述了可在细胞质中表达的蛋白内形成二硫键的E.coli突变体(Prinz et al.(1997))，多种菌株可商业获得(E.coli Origami,Novagen)。

[0054] Böttcher et al.已使用此类E.coli菌株，其显示只要通过过量表达热激蛋白，采取额外的手段确保γ-PLE蛋白的正确折叠，就可成功生产γ-PLE；这些热激蛋白或伴侣蛋白作为折叠或再折叠的辅助分子发挥功能，以协助蛋白获得其天然构象。Böttcher et al.(2007)报道称，没有大量伴侣蛋白的情况下，观察不到活性γ-PLE的表达。

[0055] 令人吃惊地，较之γ-PLE而言在548个氨基酸中仅有21处不同的APLE可在E.coli Origami菌株中作为活性酯酶产生，并且无须随之共表达伴侣蛋白；甚至注意到，存在若干过量表达的伴侣分子时，对APLE活性水平并无影响。

[0056] 更令人吃惊地，改变天然APLE基因(如从猪肝cDNA分离的)的密码子使用，提供了对E.coli Origami菌株中APLE表达的额外推动。进一步更令人吃惊地，特别是将密码子使用改造为近似一组Pichia pastoris基因，证明能比针对E.coli进行的“密码子优化”更为高效(关于密码子表，见http://www.kazusa.or.jp/)。该结果表明，DNA和源于它的信使RNA序列对于产生活性APLE蛋白折叠效率有直接影响，而不是优化的密码子诱导了增加的翻译效率和蛋白生产水平。

[0057] 通过过量表达E.coli内源二硫键异构酶(DsbC)来实现活性APLE酶生产的进一步改进；如Bessette et al.(1999)已所示，可构建DsbC蛋白的截短版本，这导致该蛋白定位于细胞内。组合此类截短的DsbC蛋白的表达导致多种重组E.coli宿主表达的APLE活性大大增加。

[0058] APLE的功能性表达并非绝对需要破坏trxB和gor基因两者导致的E.coli细胞中的

完全非还原性环境。在E.coli EL21Star(完全还原性环境!)中和在trxB或gor基因中仅一种被失活的E.coli菌株中,活性APLE表达也是可能的。

[0059] 优化的APLE基因——C8P的基因结构已用于构建多种酯酶异构体,这允许它们在简单的可上规模的工业E.coli发酵工艺中以高水平生产。

[0060] 附图描述

[0061] 图1:

[0062] a.经APLE或γ-PLE表达盒转化的P.pastoris菌株X-33的平板检验,其中使用外消旋(4E)-5-氯-2-异丙基戊-4-烯酸甲酯作为底物。

[0063] b.经APLE或γ-PLE表达盒转化的P.pastoris菌株X-33的平板检验,其中使用(2S,4E)-5-氯-2-异丙基戊-4-烯酸甲酯作为底物。

[0064] 图2:

[0065] a.表达质粒pMS470_C8P的功能性图谱;

[0066] b.表达质粒pMS470_dsbC_C8P的功能性图谱。

[0067] 图3:

[0068] 使用外消旋(4E)-5-氯-2-异丙基戊-4-烯酸甲酯作为底物,E.coli BL21Star菌株的无细胞提取物作为APLE的来源,进行的平板检验。

[0069] 图4:

[0070] a.使用外消旋(4E)-5-氯-2-异丙基戊-4-烯酸甲酯作为底物进行的平板检验。

[0071] 1.用0.1mM IPTG诱导的E.coli Origami[pMS470_C8E]

[0072] 2.用0.5mM IPTG诱导的E.coli Origami[pMS470_C8E]

[0073] 3.在存在编码伴侣分子的质粒pTf12时,用0.1mM IPTG诱导的E.coli Origami[pMS470_C8E]

[0074] 4.用0.1mM IPTG诱导的E.coli Origami[pMS470_C8P]

[0075] 5.在存在编码伴侣分子的质粒pTf12时,用0.1mM IPTG诱导的E.coli Origami[pMS470_C8P]

[0076] b.使用外消旋(4E)-5-氯-2-异丙基戊-4-烯酸甲酯作为底物进行的平板检验。左侧,将2μl全细胞悬浮液的样品应用到平板上;右侧则是向每种样品中加入1μl 1M磷酸钾缓冲液(pH 8.0),以突出最高效的水解。按字母顺序的各点具有下述含义:

[0077] A:E.coli Origami未转化菌株

[0078] B:E.coli Origami[pMS470_C8P],在4℃贮存1个月

[0079] C:E.coli Origami[pMS470_C8P]

[0080] D:E.coli Origami[pMS470_dsbC_C8P]

[0081] E:技术PLE(商业猪肝酯酶,Boehringer)

[0082] 图5:

[0083] a.Coomassie染色的SDS-PAGE

[0084] 1=技术PLE

[0085] 2=E.coli Origami未转化菌株

[0086] 3=E.coli Origami[pMS470_C8P]

[0087] 4=E.coli Origami[pMS470_dsbC_C8P]

[0088] 5＝PageRuler预染色蛋白标准物

[0089] b.Western杂交印迹，其中使用针对PLE的多克隆抗体。

[0090] 1＝技术PLE

[0091] 2＝E.coli Origami未转化菌株

[0092] 3＝E.coli Origami[pMS470_C8P]

[0093] 4＝E.coli Origami[pMS470_dsbC_C8P]

[0094] 图6。

[0095] 使用甲基琥珀酸二甲酯作为底物，对多种酯酶基因构建体进行的定量平板检验(γ-PLE和APLE都对该底物有活性)。

[0096] 1＝E.coli Origami B[pMS470_dsbC_γ-PLE](天然γ-PLE)

[0097] 2＝E.coli Origami B[p MS470_dsbC_APLE](天然APLE)

[0098] 3＝E.coli Origami B[p MS470_dsbC_APLE-C8A](APLE C8A基因)

[0099] 4＝E.coli Origami B[p MS470_dsbC_APLE-C8Cp0](APLE C8Cp0基因)

[0100] 5＝E.coli Origami B[p MS470_dsbC_APLE-C8P](APLE C8P基因)

[0101] 6＝E.coli Origami B[p MS470_dsbC_APLE-C8E2](APLE C8E2基因)

[0102] 7＝E.coli Origami B[p MS470_dsbC_γ-PLE-C8P](γ-PLE C8A基因)

[0103] 8＝E.coli Origami B[p MS470_dsbC_APLE-C8E](APLE C8E基因)

[0104] 9＝E.coli Origami B[p MS470_dsbC_BosTaurus](BosTaurusγ-PLE样基因)

[0105] 10＝阴性对照

实施例

[0106] 实施例1分离mRNA和cDNA合成；鉴定另外的猪肝酯酶(APLE)

[0107] 将来自当地屠宰场的0.7g新鲜猪肝冷冻在液氮中，使用研钵和杵将其均质化。使用Fast Track®2.0mRNA分离试剂盒(Invitrogen,Carlsbad,USA)按照厂商指导，从均质物提取mRNA。该提取方案产生了13μg mRNA。取0.26μg mRNA作为模板用于cDNA合成，其中使用用于RT-PCT的SuperScript™III第一链合成系统(Invitrogen,Carlsbad,USA)按照厂商指导来进行。

[0108] 将获得的cDNA用作为模板，用于PCR反应，其中使用特异性引物fw-PLE和rv-PLE，它们被设计来扩增与Matsushima et al.(GenBank Accession No X63323;SEQ ID NO 1)所述的基因相关的猪肝酯酶/酰胺酶序列。

[0109] fw-cPLE:5'-CAGAATTCATGGCTATCGGGCAGCCAGCCTCGC-3'(SEQ ID NO:3)

[0110] rv-cPLE:5'-CCGGAATTCAGCCTCCCCTTCACAGCTCAG-3'(SEQ ID NO:4)

[0111] 为了克隆的目的引入了EcoRI限制性位点(斜体)。与已知的猪肝酯酶/酰胺酶序列同源的序列被下划线示出。

[0112] 使用1U Phusion DNA聚合酶(Finnzymes,Espoo,Finland)、500ng cDNA作为模板、20μmol的各正反引物，根据Phusion高保真DNA聚合酶手册(Finnzymes)来进行扩增。PCR条件为:98℃变性30秒，接着是30个循环(98℃10秒，68℃20秒，72℃1分钟)用于扩增，最后在72℃孵育8分钟，以确保全长扩增产物。

[0113] 使用QIAquick Gel Extraction试剂盒(QIAGEN,Hilden,Germany)来清洁得到的

1.7kbp DNA片段，用EcoRI限制性内切核酸酶消化，并插入质粒载体pHILZ和pHIL-D2(Invitrogen,Carlsbad,USA)中，将其转入电感受态的E.coli TOP10细胞。

[0114] 对来自随机选择的转化子的质粒DNA的DNA测序揭示了两条不同的DNA片段，其中一条与Matsushima et al.所述的基因完全相同，它后来还被Böttcher et al.鉴定为γ-PLE编码基因；第二条片段具有核苷酸序列SEQ ID NO:5，其编码的蛋白(APLE；SEQ ID NO:6)与γ-PLE在成熟蛋白序列在548个氨基酸中的21处有所不同。

[0115] 实施例2APLE的功能性表达以及对APLE活性的分析

[0116] 针对在Pichia pastori中的分泌性表达，通过与载体pPICZα(Invitrogen,Carlsbad,USA)中存在的α-交配型因子分泌信号序列融合，对γ-PLE和APLE基因二者都进行改造。

[0117] 首先单独扩增α-交配型因子分泌信号序列以及γ-PLE和APLE基因：

[0118] PCR 1:α-交配型因子分泌信号序列，使用引物：

[0119] fw-α：

[0120] 5'-TCTTCGAAGAATTCACGATGAGATTTCCTTCAATTTTTACTGC-3'(SEQ ID NO:7)

[0121] rv-α:

[0122] 5'-GAGGCTGGCTGCCCAGCTTCAGCCTCTCTTTTCTCG-3'(SEQ ID NO:8)

[0123] PCR 2(γ-PLE)和PCR 3(APLE)，使用引物：

[0124] fw-PLE：

[0125] 5'-AGAGAGGCTGAAGCTGGGCAGCCAGCCTCGCCG-3'(SEQ ID NO:9)

[0126] rv-PLE：

[0127] 5'-ATGGTACCGAATTCTCACTTTATCTTGGGTGGCTTCTTTG-3'(SEQ ID NO:10)

[0128] 用于克隆目的的EcoRI限制性位点以斜体示出，与模板同源的序列则以下划线示出。

[0129] 用于所有反应的PCR条件：50μl反应混合物，其具有2ng模板DNA，0.5μM各引物，0.2mM dNTPs，1x Phusion HF缓冲液和1U Phusion DNA-聚合酶，反应按照Phusion高保真DNA聚合酶手册(Finnzymes)来进行，85℃变性3分钟，以30个循环(95℃30秒，57℃30秒，72℃15秒)扩增，最后72℃孵育7分钟。

[0130] 用于α-交配型因子分泌信号扩增(PCR1)的模板是质粒pPICZα；用于γ-PLE和APLE基因扩增(分别是PCR2和PCR3)的模板是实施例1所述的pHILZ载体中的cDNA。

[0131] 随后，在α-交配型因子片段和每种猪肝酯酶基因之间进行的融合反应中组合获得的各片段，如下所述：α-交配型因子片段(PCR1)+γ-PLE片段(PCR2)；α-交配型因子片段(PCR1)+APLE片段(PCR3)。

[0132] 反应以45μl的总体积进行，其具有来自PCR1和(分别来自)PCR2或PCR3的每种3μl，0.2mM dNTPs，1x Phusion HF缓冲液和1U of Phusion DNA-聚合酶，95℃3分钟，接着是10个循环——95℃30秒和72℃45秒。随后，将引物fw-α和rv-PLE加至0.5μM的终浓度，通过95℃3分钟变性、30个循环的扩增(95℃30秒，57℃30秒，72℃15秒)以及72℃最后孵育7分钟来实现全长产物扩增。

[0133] 使用QIAquick PCR纯化试剂盒(QIAGEN,Hilden,Germany)来纯化得到的片段，用EcoRI消化之后，插入载体pGAPZ A(Invitrogen,Carlsbad,USA)中，得到质粒pGAPZA_γ-

PLE和pGAPZA_APLE。

[0134] 将质粒pGAPZA_γ-PLE和pGAPZA_APLE的DNA线性化，按照Pichia表达试剂盒手册(Invitrogen,Carlsbad,USA)，将其引入Pichia pastoris X-33。在含100mg/l Zeocin的YPD琼脂上选择转化子。

[0135] 将带有pGAPZA_γ-PLE和pGAPZA_APLE的Pichia pastoris转化子划线到补充有100mg/l Zeocin的YPD琼脂上，在30℃培养48小时。然后将细胞材料上样到Whatman 541加硬无尘70mmØ过滤器(Whatman International Ltd.,Maidstone,Great Britain)上，并干燥。然后，将过滤器浸泡进6mgα-乙酸萘酯(Sigma，溶于500μl丙酮中)、2.5mg Fast Blue Salt BN(Sigma，溶于125μl水中)和5ml 0.1M磷酸钾缓冲液(pH 7.0)中，孵育到通过α-乙酸萘酯水解产生有色产物观察到酯酶活性。较之未经转化的Pichia pastoris X-33亲本菌株，经γ-PLE和APLE表达盒转化的所有Pichia pastoris菌株都显示了增加的酯酶活性。

[0136] 开发与通常的酯酶检验所述类似的设置，来测定γ-PLE和APLE针对5-卤-2-烷基戊-4-烯酸酯的活性。现在将过滤器浸泡于由14mM磷酸钾缓冲液(pH 8.0)、10%(v/v)外消旋(4E)-5-氯-2-异丙基戊-4-烯酸甲酯(DSM Fine Chemicals Austria Nfg GmbH&Co KG,Linz,Austria)、1%(v/v)Emulgen 913去垢剂(Kao Corporation,Tokyo,Japan)、2mg/ml酚红构成的检验混合物中。酶活性通过(4E)-5-氯-2-异丙基戊-4-烯酸甲酯水解以及相关的酸性基团释放导致的由红(碱性和中性pH)至黄(酸性pH)的颜色变化来显示。

[0137] 如图1a所示，仅APLE产生了阳性信号，这表明该酶能水解外消旋(4E)-5-氯-2-异丙基戊-4-烯酸甲酯；γ-PLE不能将该底物水解至可检测到的程度。更重要地，可推断出，APLE选择性水解(2R,4E)-5-氯-2-异丙基戊-4-烯酸甲酯：仅向过滤器应用底物的S-对映异构体的平板检验中，没有检测到S-形式的水解(图1b)。

[0138] 实施例3对合成的APLE基因的设计

[0139] 基于源于APLE编码基因(SEQ ID NO:5)的成熟APLE的氨基酸序列(SEQ ID NO:6)，设计并化学合成具有改变的密码子使用并且缺乏天然分泌信号序列的合成基因(供应商：DNA2.0,Menlo Park,USA)。合成的APLE基因变体C8P(SEQ ID NO 11)、C8A(SEQ ID NO 13)、C8Cp0(SEQ ID NO 14)和C8E(SEQ ID NO 15)都编码具有额外N-末端甲硫氨酸(作为必需的翻译起始密码子)的APLE蛋白(SEQ ID NO:12)。

[0140] 为在E.coli中进行表达研究，将合成的APLE基因插入质粒pMS470(Balzer et al.(1992)[8])中。使用PCR扩增(条件见实施例1)，分别向5'末端添加NdeI限制性位点(包括ATG起始密码子)和向3'末端添加HindIII限制性位点，其中使用下述引物：

[0141] 对基因APLE C8P而言，设计下述PCR引物

[0142] Fw-C8P(SEQ ID NO:16)：

[0143] CTTTAAGAAGGAGATATACATATGGGACAACCAGCTTCGCCGCC

[0144] Rv-C8P(SEQ ID NO:17)：

[0145] CCCCCCCCCCCCCAAGCTTATTACAATTCGGCGTGCTTTATCTTAGG

[0146] 对基因APLE C8A而言，设计下述PCR引物

[0147] Fw-C8A(SEQ ID NO:18)：

[0148] ATTTATACATATGGGACAACCAGCTTCGCCGCCTGTCG

[0149] Rv-C8A(SEQ ID NO:19)：

[0150] CCGCCAAGCTTATTACAATTCAGCGTGCTTAATCTTTGGAGGC

[0151] 对基因APLE C8CpO而言，设计下述PCR引物

[0152] Fw-C8CpO(SEQ ID NO:20)：

[0153] ATTTATACATATGGGCCAACCTGCTTCTCCACCTGTTG

[0154] Rv-C8CpO(SEQ ID NO:21)：

[0155] CCGCCAAGCTTATTACAATTCAGCATGCTTGATCTTTGGTGGC

[0156] 对基因APLE C8E而言，设计下述PCR引物

[0157] Fw-C8E(SEQ ID NO:22)：

[0158] ATTTATACATATGGGACAACCAGCTTCGCCGCCTGTCG

[0159] Rv-C8E(SEQ ID NO:23)：

[0160] CCGCCAAGCTTATTACAATTCGGCGTGCTTTATCTTAGGTGGC

[0161] 用于克隆目的的NdeI和HindIII限制性位点以斜体示出，与基因模板同源的序列则以下划线示出。

[0162] 将得到的片段插入经NdeI/HindIII消化的pMS470中，产生质粒pMS470_C8P、pMS470_C8A、pMS470_C8CpO和pMS470_C8E。质粒pMS470_C8P的图谱描绘于图2a中。

[0163] APLE和γ-PLE的天然序列作为来自猪肝的cDNA获得，如实施例1所述。扩增这些天然基因，并使用下述引物将其转入E.coli表达载体：

[0164] Fw-PLEnat(SEQ ID NO:24)

[0165] ATTTATACATATGGGGCAGCCAGCCTCGCCGCCTG

[0166] Rv-PLEnat(SEQ ID NO:25)

[0167] CCGCCAAGCTTATCACAGCTCAGCATGCTTTATCTTGGGTGG

[0168] 用于克隆目的的NdeI和HindIII限制性位点以斜体示出，与模板同源的序列则以下划线示出。

[0169] 因为这些天然基因具有内在的HindIII限制性位点，必须要进行两步连接：首先，将具有NdeI和HindIII的较长片段插入pMS470中，随后，通过添加3'HindIII片段来完成每个基因，这分别产生了最终的表达载体pMS470_γ-PLE和pMS470_APLE。

[0170] 实施例4将APLE表达载体转化进合适的E.coli宿主：APLE在E.coli中的功能性表达

[0171] 为分析E.coli中的酯酶表达，使用下述流程来培养和制备用于活性分析的细胞。

[0172] 为进行平板检验，将携带多种表达质粒的E.coli菌株划线到含有100μg/ml l氨苄青霉素和0.1mM IPTG的LB-琼脂平板上，37℃孵育16小时。

[0173] 为进行液体培养检验，将携带有各表达质粒的E.coli菌株接种进5ml具有100μg/ml氨苄青霉素的Luria-Bertani(LB)培养基，于28℃在持续振荡下孵育16小时。然后用该培养物接种1L带盖摇瓶中250ml具有100μg/ml氨苄青霉素的LB培养基。当培养物达到600nm下0.6-0.8的光密度时，加入IPTG至终浓度为0.1mM，以诱导基因表达。28℃下16至20小时孵育后收获细胞。

[0174] 平板检验：实施例2中已经用多种酯酶底物描述了对琼脂平板上生长的细胞的检验。

[0175] 在液体E.coli培养物上的活性分析：在具有5mM乙酸对硝基苯酯作为底物的MOPS

缓冲液(100mM)中,在细胞悬浮液上定量测定酯酶活性。释放的对硝基苯酚的量在405nm处用分光光法度测定。1个单位的酯酶活性被定义为:在测试条件(pH 7.5,37℃)下每分钟释放1微摩尔对硝基苯酚的酶的量。

[0176] 将针对不同APLE编码基因的表达盒转化进多种E.coli菌株:常规基因表达菌株(例如E.coli BL21菌株)和经特别工程改造的、允许需要分子内二硫键用于正确折叠和酶促活性的蛋白的功能性细胞内表达的E.coli菌株(Prinz et al.,Bessette et al.)两者都用作为表达宿主菌株。就此目的而言,使用商业可获得的Origami家族的E.coli菌株(Novagen),特别是Origami 1、Origami 2和Origami B。

[0177] 在E.coli BL21Star中观察到了APLE(SEQ ID NO:12)的功能性表达(图3),但是活性远低于使用E.coli Origami菌株所获得的活性。

[0178] 用各表达载体转化E.coli Origami菌株,随后通过在底物特异性检验平板上涂板或通过摇瓶培养,针对酯酶表达对选择的转化子加以评价。

[0179] 若干APLE基因变体的表达水平被概括于图4a和表1中;基因C8E较之天然APLE基因仅具有较少改变。图4a显示了C8E和C8P原样的以及在存在伴侣分子pTf16时的表达差别;通过表1所示的摇瓶结果验证了平板结果。

[0180]

表 1

E.coli 宿主菌株	APLE 表达质粒	酯酶活性（u/ml）
Origami 1/2/B	-	0.8
Origami 1	pMS470_C8E	1.2
Origami 1	pMS470_C8P	9.1
Origami 2	pMS470_C8P	9.6
Origami B	pMS470_C8P	10.7

[0181] 进行了一系列实验,来评估若干热激伴侣分子的共表达是否作用于APLE表达。结果(概括于表2中)显示,热激蛋白/伴侣分子表达没有显著影响APLE生产。

[0182]

表 2

E.coli 宿主	APLE 表达质粒	热激蛋白表达质粒	
Origami 1	-		0.8
Origami 1	pMS470_C8P		9.2
Origami 1	pMS470_C8P	pG-KJE8	8.1
Origami 1	pMS470_C8P	pGro7	9.3
Origami 1	pMS470_C8P	pKJE7	10.1
Origami 1	pMS470_C8P	pG-Tf2	9.2
Origami 1	pMS470_C8P	pTf16	8.8

[0183] 实施例5通过添加DsbC来增加APLE表达

[0184] 没有观察到共表达的热激蛋白的作用，我们研究了是否有其它辅助因子(例如，E.coli内源二硫异构酶基因dsbC(Bessette et al.))将影响APLE在E.coli中的表达。

[0185] 用E.coli Top10F'染色体DNA作为模板，通过聚合酶链式反应(PCR)，使用Phusion™ High-Fidelity DNA聚合酶(Finnzymes,Espoo,Finland)，使用Phusion HF-缓冲液，来扩增E.coli dsbC基因的截短版本(天然DsbC蛋白分泌进周质，截短的DsbC蛋白保留在细胞内区室中)，其中使用下述条件：95℃变性5分钟，扩增进行30个循环(98℃10秒、66℃30秒、72℃30秒)，最后在72℃孵育8分钟。设计使用的引物，使其在截短的DsbC编码序列前包括Shine-Dalgarno序列：

[0186] Fw-dsbC(SEQ ID NO:26)：

[0187] 5'-CGGATCCTTTAACTTTAAGAAGGAGATATAATGGATGACGCGGCAATTCAACAAACG-3'

[0188] Rv-dsbC(SEQ ID NO:27)：

[0189] 5'-CGGATCCTTATTTACCGCTGGTCATTTTTTGGTGTTCG-3'

[0190] 用BamHI消化PCR产物，将其插入APLE表达质粒pMS470_C8P的BamHI限制性位点。DsbC和C8P之间隔开49bp。通过测序验证了构建体。构建体被命名为pMS470_dsbC_C8P，其大大增加了APLE的表达(平板检验见图4，还有表3)。质粒pMS470_dsbC_C8P的功能性图谱示于图2b中。

[0191]

表 3

E.coli 宿主菌株	质粒	酯酶活性（u/ml）
Origami B	-	0.8
Origami 1	-	0.8
Origami 1	pMS470_C8P	9.0
Origami 1	pMS470_dsbC_C8P	47
Origami B	pMS470_C8P	10.5
Origami B	pMS470_dsbC_C8P	59

[0192] 为验证观察到的酯酶活性确实是由于APLE编码基因的功能表达，进行了Western杂交印迹实验。发酵之后，在5000g对E.coli细胞离心10分钟。将得到的沉淀团重新悬浮于4倍体积的20mM磷酸钾缓冲液(pH8.0)，将2-2.5μl的细胞悬浮液与17.5-18μl SDS上样缓冲液合并，在95℃加热10-15分钟，并上样到12.5％SDS-PAGE凝胶上。使用针对猪肝酯酶的兔多克隆抗体(abcam,Cambridge,UK)作为一抗，缀合有碱性磷酸酶的山羊抗兔多克隆抗体(Leinco Technologies Inc.,St.Louis,USA)作为二抗，通过Western杂交印迹分析来检测APLE。Western杂交印迹检测通过Lumi-Phos™ WB化学发光底物(AP)(Pierce,Rockford,USA)和G:Box HR(Syngene,Cambridge,UK)中的化学发光检测来进行，或者通过BCIP/NBT检测溶液(CALBIOCHEM;La Jolla;USA)和对硝酸纤维素膜(Hybond-ECL™,Amersham Biosciences,Uppsala,Sweden)的直接染色来进行。

[0193] 图5显示了经Coomassie染色的SDS-PAGE凝胶(图5a)和Western杂交印迹实验的结果(图5b)，其表明蛋白表达水平对应于活性的差异。图5a还显示截短的DsbC非常好地表达。

[0194] 实施例6新的E.coli宿主菌株的制备和对这些宿主的APLE生产性质的分析

[0195] Escherichia coli K12菌株RV308ΔtrxB；Δgor从E.coli菌株RV308(ATCC 31608)开始构建。类似Prinz et al.所示，参与细胞内二硫化物还原的两个基因被失活。根据Datsenko et al.(2000)[9]描述的流程，使用定点重组技术，通过缺失来失活分别编码硫氧还蛋白还原酶和谷胱甘肽氧化还原酶的这两个基因trxB和gor。

[0196] 这种修饰的最初结果导致除了在还原剂存在的情况下之外E.coli菌株不再能有氧生长；但是，容易选择出能恢复不存在还原剂时的有氧生长的阻遏子突变。这些性质和表型改变以前已在使用不同的手段使基因trxB和gor失活的E.coli菌株中描述过。

[0197] 详细描述：通过PCR获得缺失盒，其中使用质粒pKD3作为模板，使用下述引物：

[0198] fw-trxB(SEQ ID NO:28):5'-GTAAATTCCCTACAATCCTGCCCATTGTCTGCCAACAACTATGGGGATCTTGTGTAGGCTGGAGCTGCTTC-3'和rv-trxB(SEQ ID NO:29):5'-CCCATAGTCGCATGGTGTCGCCTTCTTTACTTTTGTTACTGATTTGTAAAACATATGAATATCCTCCTTAG-3'用于该反应，产生了针对基因trxB的缺失盒。

[0199] fw-gor(SEQ ID NO 30):5'-CCTATTACGTCTCGCGCTACAATCGCGGTAATCAACGATAAGGACACTTTGTCTGTGTAGGCTGGAGCTGCTTC-3'和rv-gor(SEQ ID NO):5'-CTGATAGCGGAAACGTAATTAA

GGGCTAAGAGCACACTACTCTTAGCCCTTTAACCATATGAATATCCTCCTTAG-3'用于该反应,产生了针对基因gor的缺失盒。

[0200] 将trxB和gor缺失盒分别转化进已经含有质粒pKD46的E.coli RV308菌株,基于其获得的针对抗生素氯霉素的抗性选择成功的转化子。通过PCR对照或Southern杂交印迹来验证各缺失盒对trxB基因或gor基因的正确交换。随后通过用质粒pCP20(编码FLP重组酶[参考文献6])转化来除去氯霉素抗性基因。使用PCR和Southern杂交印迹,针对trxB的干净缺失,再次检查得到的E.coli菌株RV308ΔtrxB。类似地,验证了E.coli菌株RV308中gor基因的干净缺失。

[0201] 从菌株E.coli菌株RV308ΔtrxB开始,进行完全相同的反应组,来开展对基因gor的干净缺失。因为该第二种修饰的最初结果(如Prinz et al.所述)是trxB和gor都缺失的该E.coli RV308菌株除了在还原剂存在的情况下之外不再能有氧生长,在存在还原剂DTT时培养假设也具有gor缺失的E.coli菌株。最后,因为有氧生长不再依赖DTT的存在,可选出自发的RV308ΔtrxB;Δgor突变体。

[0202] 用选择的APLE表达质粒转化E.coli菌株RV308ΔtrxB;Δgor和仅具有单种还原酶缺失的中间产物菌株,并针对APLE生产在摇瓶中加以评估(表4)。

[0203]

表4

E.coli 宿主	质粒	
Origami B	-	0.7
RV308 ΔtrxB; Δgor		0.6
Origami B	pMS470_C8P	11.5
RV308 ΔtrxB	pMS470_C8P	2.2
RV308 Δgor	pMS470_C8P	2.8
RV308 ΔtrxB; Δgor	pMS470_C8P	8.0
Origami B	pMS470_dsbC_C8P	56
RV308 ΔtrxB	pMS470_dsbC_C8P	1.5
RV308 Δgor	pMS470_dsbC_C8P	10.2
RV308 ΔtrxB; Δgor	pMS470_dsbC_C8P	42

[0204] 实施例7

[0205] 化学合成编码猪肝酯酶蛋白的多种天然异构体的其它合成基因。为生产新的酯酶蛋白,合成Bos taurus γ-PLE样基因。

[0206] 其它新的基因编码已知的PLE酯酶γ-PLE和PICE;此外还设计了APLE和γ-PLE异构体之间的杂交体。后一组的共同特征是所有都基于APLE C8P模板。从APLE C8P开始,仅改变了获得异构体或杂交体蛋白所需的密码子。

[0207] 基因及其编码蛋白由下述代表:

[0208] 天然异构体:新的可能的Bos taurus γ-PLE样基因(SEQ ID NO:32)

[0209] C8P编码的天然酯酶:C8P-γ-PLE(SEQ ID NO:34)和C8P-PICE(SEQ ID NO:36)

[0210] C8P编码的杂交体酯酶:C8P-H1(SEQ ID NO:38)、C8P-H2(SEQ ID NO:40)、C8P-H3(SEQ ID NO:42)和C8P-H4(SEQ ID NO:44)。

[0211] 所有序列都插入图2b的E.coli表达载体(pMS470_dsbC_APLE),有效替换了C8P基因。

[0212] 使用甲基琥珀酸二甲酯作为底物的定量平板检验(见实施例2)的结果证实:每个设计的基因都编码活性酯酶(图6)。但是该平板检验并不能得出定量结论,因为酶变体针对甲基琥珀酸二甲酯的比活性未知。

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<120>酯酶的制备

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<400>2

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Met Trp Leu Leu Pro Leu Val Leu Thr Ser Leu Ala Ser Ser Ala Thr
1               5                   10                  15
Trp Ala Gly Gln Pro Ala Ser Pro Pro Val Val Asp Thr Ala Gln Gly
            20                  25                  30
Arg Val Leu Gly Lys Tyr Val Ser Leu Glu Gly Leu Ala Gln Pro Val
        35                  40                  45
Ala Val Phe Leu Gly Val Pro Phe Ala Lys Pro Pro Leu Gly Ser Leu
    50                  55                  60
Arg Phe Ala Pro Pro Gln Pro Ala Glu Pro Trp Ser Phe Val Lys Asn
65                  70                  75                  80
Thr Thr Ser Tyr Pro Pro Met Cys Cys Gln Asp Pro Val Val Glu Gln
                85                  90                  95
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Met Thr Ser Asp Leu Phe Thr Asn Gly Lys Glu Arg Leu Thr Leu Glu
100 105 110
Phe Ser Glu Asp Cys Leu Tyr Leu Asn Ile Tyr Thr Pro Ala Asp Leu
115 120 125
Thr Lys Arg Gly Arg Leu Pro Val Met Val Trp Ile His Gly Gly Gly
130 135 140
Leu Val Leu Gly Gly Ala Pro Met Tyr Asp Gly Val Val Leu Ala Ala
145 150 155 160
His Glu Asn Val Val Val Val Ala Ile Gln Tyr Arg Leu Gly Ile Trp
165 170 175
Gly Phe Phe Ser Thr Gly Asp Glu His Ser Arg Gly Asn Trp Gly His
180 185 190
Leu Asp Gln Val Ala Ala Leu His Trp Val Gln Glu Asn Ile Ala Asn
195 200 205
Phe Gly Gly Asp Pro Gly Ser Val Thr Ile Phe Gly Glu Ser Ala Gly
210 215 220
Gly Glu Ser Val Ser Val Leu Val Leu Ser Pro Leu Ala Lys Asn Leu
225 230 235 240
Phe His Arg Ala Ile Ser Glu Ser Gly Val Ala Leu Thr Val Ala Leu
245 250 255
Val Arg Lys Asp Met Lys Ala Ala Ala Lys Gln Ile Ala Val Leu Ala
260 265 270
Gly Cys Lys Thr Thr Thr Ser Ala Val Phe Val His Cys Leu Arg Gln
275 280 285
Lys Ser Glu Asp Glu Leu Leu Asp Leu Thr Leu Lys Met Lys Phe Leu
290 295 300
Thr Leu Asp Phe His Gly Asp Gln Arg Glu Ser His Pro Phe Leu Pro
305 310 315 320
Thr Val Val Asp Gly Val Leu Leu Pro Lys Met Pro Glu Glu Ile Leu
325 330 335
Ala Glu Lys Asp Phe Asn Thr Val Pro Tyr Ile Val Gly Ile Asn Lys
340 345 350
Gln Glu Phe Gly Trp Leu Leu Pro Thr Met Met Gly Phe Pro Leu Ser
355 360 365
Glu Gly Lys Leu Asp Gln Lys Thr Ala Thr Ser Leu Leu Trp Lys Ser
370 375 380
Tyr Pro Ile Ala Asn Ile Pro Glu Glu Leu Thr Pro Val Ala Thr Asp
385 390 395 400
Lys Tyr Leu Gly Gly Thr Asp Asp Pro Val Lys Lys Lys Asp Leu Phe

405 410 415
Leu Asp Leu Met Gly Asp Val Val Phe Gly Val Pro Ser Val Thr Val
420 425 430
Ala Arg Gln His Arg Asp Ala Gly Ala Pro Thr Tyr Met Tyr Glu Phe
435 440 445
Gln Tyr Arg Pro Ser Phe Ser Ser Asp Lys Lys Pro Lys Thr Val Ile
450 455 460
Gly Asp His Gly Asp Glu Ile Phe Ser Val Phe Gly Phe Pro Leu Leu
465 470 475 480
Lys Gly Asp Ala Pro Glu Glu Glu Val Ser Leu Ser Lys Thr Val Met
485 490 495
Lys Phe Trp Ala Asn Phe Ala Arg Ser Gly Asn Pro Asn Gly Glu Gly
500 505 510
Leu Pro His Trp Pro Met Tyr Asp Gln Glu Glu Gly Tyr Leu Gln Ile
515 520 525
Gly Val Asn Thr Gln Ala Ala Lys Arg Leu Lys Gly Glu Glu Val Ala
530 535 540
Phe Trp Asn Asp Leu Leu Ser Lys Glu Ala Ala Lys Lys Pro Pro Lys
545 550 555 560
Ile Lys His Ala Glu Leu
565

<210>3
<211>33
<212>DNA
<213>人工序列

<220>
<223>引物

<400>3
cagaattcat ggctatcggg cagccagcct cgc 33

<210>4
<211>30
<212>DNA
<213>人工序列

<220>

<223>引物

<400>4

ccggaattca gcctcccctt cacagctcag 30

<210>5

<211>1698

<212>DNA

<213>Sus scrofa

<220>

<221>CDS

<222>(1)..(1698)

<223>另外的猪肝酯酶

<400>5

atg tgg ctt ctc ccg ctg gtc ctg acc tcc ctc gcc tct tct gca act 48
Met Trp Leu Leu Pro Leu Val Leu Thr Ser Leu Ala Ser Ser Ala Thr
1 5 10 15

tgg ggg cag cca gcc tcg ccg cct gtt gtg gac act gcc cag ggc cga 96
Trp Gly Gln Pro Ala Ser Pro Pro Val Val Asp Thr Ala Gln Gly Arg
20 25 30

gtc ctg ggg aag tac gtc agc tta gaa ggc ctg gca cag ccg gtg gcc 144
Val Leu Gly Lys Tyr Val Ser Leu Glu Gly Leu Ala Gln Pro Val Ala
35 40 45

gtc ttc ctg gga gtc cct ttt gcc aag ccc cct ctc gga tcc ttg agg 192
Val Phe Leu Gly Val Pro Phe Ala Lys Pro Pro Leu Gly Ser Leu Arg
50 55 60

ttt gct ccg ccg cag cct gca gaa cca tgg agc ttc gtg aag aac acc 240
Phe Ala Pro Pro Gln Pro Ala Glu Pro Trp Ser Phe Val Lys Asn Thr
65 70 75 80

acc tcc tac cct ccc atg tgc tgc caa gag cca att ggg gga cag atg 288
Thr Ser Tyr Pro Pro Met Cys Cys Gln Glu Pro Ile Gly Gly Gln Met
85 90 95

ctc tca gat cta ttt acc aac aga aag gag agg ctc att ccg gag ttt 336
Leu Ser Asp Leu Phe Thr Asn Arg Lys Glu Arg Leu Ile Pro Glu Phe
100 105 110

tct gaa gac tgt ctc tac cta aat att tac acc cct gct gac ctg aca 384
Ser Glu Asp Cys Leu Tyr Leu Asn Ile Tyr Thr Pro Ala Asp Leu Thr
115 120 125

aag agg ggc aga ctg ccg gtg atg gtg tgg atc cac gga gga ggt ctg 432
Lys Arg Gly Arg Leu Pro Val Met Val Trp Ile His Gly Gly Gly Leu
130 135 140
gtg gtg ggc ggg gct tcc acc tat gat gga ctg gcc ctc gct gcg cat 480
Val Val Gly Gly Ala Ser Thr Tyr Asp Gly Leu Ala Leu Ala Ala His
145 150 155 160
gaa aac gtg gtg gtg gtg gcc atc cag tac cgc ctg ggc atc tgg gga 528
Glu Asn Val Val Val Val Ala Ile Gln Tyr Arg Leu Gly Ile Trp Gly
165 170 175
ttc ttc agc aca ggg gac gaa cac agc cgg ggc aac tgg ggt cac ttg 576
Phe Phe Ser Thr Gly Asp Glu His Ser Arg Gly Asn Trp Gly His Leu
180 185 190
gac cag gtg gcc gca ctg cac tgg gtc cag gag aac atc gcc aac ttt 624
Asp Gln Val Ala Ala Leu His Trp Val Gln Glu Asn Ile Ala Asn Phe
195 200 205
gga ggc gac cca ggc tct gtg acc atc ttt gga gag tca gca gga ggg 672
Gly Gly Asp Pro Gly Ser Val Thr Ile Phe Gly Glu Ser Ala Gly Gly
210 215 220
gaa agt gtc tct gtt ctg gtg ttg tct ccc ttg gcc aag aac ctc ttc 720
Glu Ser Val Ser Val Leu Val Leu Ser Pro Leu Ala Lys Asn Leu Phe
225 230 235 240
cac cgg gcc atc tct gag agt ggc gtg gcc ttc act gct ggc ctg gtc 768
His Arg Ala Ile Ser Glu Ser Gly Val Ala Phe Thr Ala Gly Leu Val
245 250 255
agg aag gac atg aag gct gca gct aag caa att gct gtc ctt gct ggg 816
Arg Lys Asp Met Lys Ala Ala Ala Lys Gln Ile Ala Val Leu Ala Gly
260 265 270
tgt aaa acc acc acc tcg gct gtc ttt gtt cac tgc ctg cgc cag aag 864
Cys Lys Thr Thr Thr Ser Ala Val Phe Val His Cys Leu Arg Gln Lys
275 280 285
tcg gag gac gag ctc ttg gac tta acg ctg aag atg aaa ttt ttc gct 912
Ser Glu Asp Glu Leu Leu Asp Leu Thr Leu Lys Met Lys Phe Phe Ala
290 295 300
ctt gat ttg cat gga gac ccc aga gag agc cat ccc ttc ctg acc act 960
Leu Asp Leu His Gly Asp Pro Arg Glu Ser His Pro Phe Leu Thr Thr
305 310 315 320
gtg gtg gat gga gtg ctg ctg ccc aag atg cct gaa gag att ctg gct 1008
Val Val Asp Gly Val Leu Leu Pro Lys Met Pro Glu Glu Ile Leu Ala
325 330 335

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gaa aag gat ttc aac act gtc ccc tac atc gtg gga atc aac aag caa      1056
Glu Lys Asp Phe Asn Thr Val Pro Tyr Ile Val Gly Ile Asn Lys Gln
            340                 345                 350
gag ttt ggc tgg ctt ctg cca acg atg atg ggc ttc ccc ctc tct gaa      1104
Glu Phe Gly Trp Leu Leu Pro Thr Met Met Gly Phe Pro Leu Ser Glu
        355                 360                 365
ggc aag ctg gac cag aag acg gcc acg tca ctc ctg tgg aag tcc tac      1152
Gly Lys Leu Asp Gln Lys Thr Ala Thr Ser Leu Leu Trp Lys Ser Tyr
    370                 375                 380
ccc atc gct aac atc cct gag gaa ctg act cca gtg gcc act gac aag      1200
Pro Ile Ala Asn Ile Pro Glu Glu Leu Thr Pro Val Ala Thr Asp Lys
385                 390                 395                 400
tat ttg ggg ggg aca gac gac ccc gtc aaa aag aaa gac ctg ttc ctg      1248
Tyr Leu Gly Gly Thr Asp Asp Pro Val Lys Lys Lys Asp Leu Phe Leu
                405                 410                 415
gac ttg atg ggg gat gtg gtg ttt ggt gtc cca tct gtg acg gtg gcc      1296
Asp Leu Met Gly Asp Val Val Phe Gly Val Pro Ser Val Thr Val Ala
            420                 425                 430
cgt caa cac aga gat gca gga gcc ccc acc tac atg tat gag ttt cag      1344
Arg Gln His Arg Asp Ala Gly Ala Pro Thr Tyr Met Tyr Glu Phe Gln
        435                 440                 445
tat cgc cca agc ttc tca tcg gac aag aaa ccc aag acg gtg atc ggg      1392
Tyr Arg Pro Ser Phe Ser Ser Asp Lys Lys Pro Lys Thr Val Ile Gly
    450                 455                 460
gac cac ggg gat gag atc ttc tcc gtc ttt ggt ttt cca ctg tta aaa      1440
Asp His Gly Asp Glu Ile Phe Ser Val Phe Gly Phe Pro Leu Leu Lys
465                 470                 475                 480
ggc gat gcc cca gaa gag gag gtc agt ctc agc aag acg gtg atg aaa      1488
Gly Asp Ala Pro Glu Glu Glu Val Ser Leu Ser Lys Thr Val Met Lys
                485                 490                 495
ttc tgg gcc aac ttt gct cgc agt ggg aac ccc aat ggg gag ggg ctg      1536
Phe Trp Ala Asn Phe Ala Arg Ser Gly Asn Pro Asn Gly Glu Gly Leu
            500                 505                 510
ccc cat tgg ccg atg tac gac cag gaa gaa ggg tac ctt cag atc ggc      1584
Pro His Trp Pro Met Tyr Asp Gln Glu Glu Gly Tyr Leu Gln Ile Gly
        515                 520                 525
gtc aac acc cag gca gcc aag agg ctg aaa ggt gaa gaa gtg gcc ttc      1632
Val Asn Thr Gln Ala Ala Lys Arg Leu Lys Gly Glu Glu Val Ala Phe
    530                 535                 540
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tgg aac gat ctc ctg tcc aag gag gca gca aag aag cca ccc aag ata 1680
Trp Asn Asp Leu Leu Ser Lys Glu Ala Ala Lys Lys Pro Pro Lys Ile
545 550 555 560
aag cat gct gag ctg tga 1698
Lys His Ala Glu Leu
565

<210>6
<211>565
<212>PRT
<213>Sus scrofa

<400>6

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

195 200 205

Gly Gly Asp Pro Gly Ser Val Thr Ile Phe Gly Glu Ser Ala Gly Gly

210 215 220

Glu Ser Val Ser Val Leu Val Leu Ser Pro Leu Ala Lys Asn Leu Phe

225 230 235 240

His Arg Ala Ile Ser Glu Ser Gly Val Ala Phe Thr Ala Gly Leu Val

245 250 255

Arg Lys Asp Met Lys Ala Ala Ala Lys Gln Ile Ala Val Leu Ala Gly

260 265 270

Cys Lys Thr Thr Thr Ser Ala Val Phe Val His Cys Leu Arg Gln Lys

275 280 285

Ser Glu Asp Glu Leu Leu Asp Leu Thr Leu Lys Met Lys Phe Phe Ala

290 295 300

Leu Asp Leu His Gly Asp Pro Arg Glu Ser His Pro Phe Leu Thr Thr

305 310 315 320

Val Val Asp Gly Val Leu Leu Pro Lys Met Pro Glu Glu Ile Leu Ala

325 330 335

Glu Lys Asp Phe Asn Thr Val Pro Tyr Ile Val Gly Ile Asn Lys Gln

340 345 350

Glu Phe Gly Trp Leu Leu Pro Thr Met Met Gly Phe Pro Leu Ser Glu

355 360 365

Gly Lys Leu Asp Gln Lys Thr Ala Thr Ser Leu Leu Trp Lys Ser Tyr

370 375 380

Pro Ile Ala Asn Ile Pro Glu Glu Leu Thr Pro Val Ala Thr Asp Lys

385 390 395 400

Tyr Leu Gly Gly Thr Asp Asp Pro Val Lys Lys Lys Asp Leu Phe Leu

405 410 415

Asp Leu Met Gly Asp Val Val Phe Gly Val Pro Ser Val Thr Val Ala

420 425 430

Arg Gln His Arg Asp Ala Gly Ala Pro Thr Tyr Met Tyr Glu Phe Gln

435 440 445

Tyr Arg Pro Ser Phe Ser Ser Asp Lys Lys Pro Lys Thr Val Ile Gly

450 455 460

Asp His Gly Asp Glu Ile Phe Ser Val Phe Gly Phe Pro Leu Leu Lys

465 470 475 480

Gly Asp Ala Pro Glu Glu Glu Val Ser Leu Ser Lys Thr Val Met Lys

485 490 495

Phe Trp Ala Asn Phe Ala Arg Ser Gly Asn Pro Asn Gly Glu Gly Leu

500 505 510

Pro His Trp Pro Met Tyr Asp Gln Glu Glu Gly Tyr Leu Gln Ile Gly
515 520 525
Val Asn Thr Gln Ala Ala Lys Arg Leu Lys Gly Glu Glu Val Ala Phe
530 535 540
Trp Asn Asp Leu Leu Ser Lys Glu Ala Ala Lys Lys Pro Pro Lys Ile
545 550 555 560
Lys His Ala Glu Leu
565

<210>7
<211>43
<212>DNA
<213>人工序列

<220>
<223>引物

<400>7
tcttcgaaga attcacgatg agatttcctt caatttttac tgc 43

<210>8
<211>36
<212>DNA
<213>人工序列

<220>
<223>引物

<400>8
gaggctggct gcccagcttc agcctctctt ttctcg 36

<210>9
<211>33
<212>DNA
<213>人工序列

<220>
<223>引物
<400>9

agagaggctg aagctgggca gccagcctcg ccg 33

<210>10
<211>40
<212>DNA
<213>人工序列

<220>
<223>引物

<400>10
atggtaccga attctcactt tatcttgggt ggcttctttg 40

<210>11
<211>1650
<212>DNA
<213>人工序列

<220>
<223>编码酯酶活性的蛋白

<220>
<221>CDS
<222>(1).(1650)

<400>11

atg	gga	caa	cca	gct	tcg	ccg	cct	gtc	gtt	gat	acc	gct	caa	gga	cga	48
Met	Gly	Gln	Pro	Ala	Ser	Pro	Pro	Val	Val	Asp	Thr	Ala	Gln	Gly	Arg	
1				5				10					15			
gtc	ttg	ggt	aag	tac	gtc	tct	tta	gag	gga	ttg	gca	caa	ccg	gtt	gct	96
Val	Leu	Gly	Lys	Tyr	Val	Ser	Leu	Glu	Gly	Leu	Ala	Gln	Pro	Val	Ala	
			20					25					30			
gtc	ttc	ttg	gga	gtc	cct	ttt	gct	aag	cca	cct	ctt	gga	tct	ttg	agg	144
Val	Phe	Leu	Gly	Val	Pro	Phe	Ala	Lys	Pro	Pro	Leu	Gly	Ser	Leu	Arg	
		35					40					45				
ttt	gcc	ccg	ccg	caa	cca	gca	gag	cca	tgg	tct	ttc	gtt	aag	aac	act	192
Phe	Ala	Pro	Pro	Gln	Pro	Ala	Glu	Pro	Trp	Ser	Phe	Val	Lys	Asn	Thr	
	50					55					60					
act	tcc	tac	cct	cca	atg	tgt	tgt	caa	gaa	cca	atc	gga	gga	caa	atg	240

Thr Ser Tyr Pro Pro Met Cys Cys Gln Glu Pro Ile Gly Gly Gln Met
65 70 75 80

ctt tca gac cta ttc act aac aga aag gaa agg ctt atc ccg gag ttc 288
Leu Ser Asp Leu Phe Thr Asn Arg Lys Glu Arg Leu Ile Pro Glu Phe
85 90 95

tct gag gat tgc ctt tac cta aat att tac act cct gcc gat ttg aca 336
Ser Glu Asp Cys Leu Tyr Leu Asn Ile Tyr Thr Pro Ala Asp Leu Thr
100 105 110

aag agg ggt agg ttg ccg gtt atg gtt tgg att cat gga gga ggt ttg 384
Lys Arg Gly Arg Leu Pro Val Met Val Trp Ile His Gly Gly Gly Leu
115 120 125

gtt gtt ggc gga gca tcc act tat gac gga ttg gct ctt gcc gcg cac 432
Val Val Gly Gly Ala Ser Thr Tyr Asp Gly Leu Ala Leu Ala Ala His
130 135 140

gag aac gtt gtt gtt gtt gct att caa tac cgt ttg ggt att tgg gga 480
Glu Asn Val Val Val Val Ala Ile Gln Tyr Arg Leu Gly Ile Trp Gly
145 150 155 160

ttt ttc tcc aca gga gat gag cat tcc cgt gga aac tgg ggc cat tta 528
Phe Phe Ser Thr Gly Asp Glu His Ser Arg Gly Asn Trp Gly His Leu
165 170 175

gat caa gtt gct gca ttg cat tgg gtc caa gaa aac att gct aac ttc 576
Asp Gln Val Ala Ala Leu His Trp Val Gln Glu Asn Ile Ala Asn Phe
180 185 190

gga ggt gat cca ggt tct gtt act att ttc gga gaa tca gca ggc gga 624
Gly Gly Asp Pro Gly Ser Val Thr Ile Phe Gly Glu Ser Ala Gly Gly
195 200 205

gag agt gtc tct gta ttg gtt tta tca cca tta gct aag aac ctt ttt 672
Glu Ser Val Ser Val Leu Val Leu Ser Pro Leu Ala Lys Asn Leu Phe
210 215 220

cat cgt gct att tcc gaa agt ggt gtt gct ttt acc gcc ggt ttg gtc 720
His Arg Ala Ile Ser Glu Ser Gly Val Ala Phe Thr Ala Gly Leu Val
225 230 235 240

agg aag gat atg aag gcc gca gcc aag cag atc gct gtc ctt gca gga 768
Arg Lys Asp Met Lys Ala Ala Ala Lys Gln Ile Ala Val Leu Ala Gly
245 250 255

tgc aaa act act act tcg gca gtc ttc gtg cat tgt ttg cgt caa aag 816
Cys Lys Thr Thr Thr Ser Ala Val Phe Val His Cys Leu Arg Gln Lys
260 265 270

tcg gaa gat gaa ctt tta gac ctc acg ttg aag atg aaa ttc ttt gcc 864

Ser Glu Asp Glu Leu Leu Asp Leu Thr Leu Lys Met Lys Phe Phe Ala
275 280 285

ctt gac tta cac gga gat cca agg gaa tct cac cct ttt ttg acc act 912
Leu Asp Leu His Gly Asp Pro Arg Glu Ser His Pro Phe Leu Thr Thr
290 295 300

gtt gtt gac gga gtt ttg ttg cct aag atg cct gag gaa atc ttg gcc 960
Val Val Asp Gly Val Leu Leu Pro Lys Met Pro Glu Glu Ile Leu Ala
305 310 315 320

gag aag gac ttt aac acc gtc cca tac att gtt gga att aac aag cag 1008
Glu Lys Asp Phe Asn Thr Val Pro Tyr Ile Val Gly Ile Asn Lys Gln
325 330 335

gag ttc gga tgg ctt ttg cca acg atg atg gga ttt cct ctt tcc gag 1056
Glu Phe Gly Trp Leu Leu Pro Thr Met Met Gly Phe Pro Leu Ser Glu
340 345 350

gga aag ttg gat caa aag acg gct acg tca ctt ttg tgg aag tcc tac 1104
Gly Lys Leu Asp Gln Lys Thr Ala Thr Ser Leu Leu Trp Lys Ser Tyr
355 360 365

cca att gcc aac att cct gaa gag ttg acc cca gtt gct acc gat aag 1152
Pro Ile Ala Asn Ile Pro Glu Glu Leu Thr Pro Val Ala Thr Asp Lys
370 375 380

tat tta gga gga aca gat gat cct gtc aaa aag aaa gat ttg ttt ttg 1200
Tyr Leu Gly Gly Thr Asp Asp Pro Val Lys Lys Lys Asp Leu Phe Leu
385 390 395 400

gat ctg atg gga gac gtt gtt ttc ggc gtc cca tca gtt acg gtt gct 1248
Asp Leu Met Gly Asp Val Val Phe Gly Val Pro Ser Val Thr Val Ala
405 410 415

cgt cag cat agg gac gca gga gct cca act tac atg tat gag ttc caa 1296
Arg Gln His Arg Asp Ala Gly Ala Pro Thr Tyr Met Tyr Glu Phe Gln
420 425 430

tat cgt cca tct ttt tca tcg gat aag aaa cct aag acg gtt att gga 1344
Tyr Arg Pro Ser Phe Ser Ser Asp Lys Lys Pro Lys Thr Val Ile Gly
435 440 445

gat cat gga gac gaa att ttt tcc gtc ttc ggc ttc cca ttg ctc aaa 1392
Asp His Gly Asp Glu Ile Phe Ser Val Phe Gly Phe Pro Leu Leu Lys
450 455 460

ggt gac gct cca gag gaa gaa gtc agt ctt tct aag acg gtt atg aaa 1440
Gly Asp Ala Pro Glu Glu Glu Val Ser Leu Ser Lys Thr Val Met Lys
465 470 475 480

ttt tgg gct aac ttc gcc cgt agt gga aac cct aat gga gaa gga ttg 1488

```
Phe Trp Ala Asn Phe Ala Arg Ser Gly Asn Pro Asn Gly Glu Gly Leu
                485                 490                 495
cct cac tgg ccg atg tac gat caa gag gag gga tac ctt caa att ggt      1536
Pro His Trp Pro Met Tyr Asp Gln Glu Glu Gly Tyr Leu Gln Ile Gly
            500                 505                 510
gtc aac act caa gca gct aag agg ttg aaa ggc gag gag gtt gct ttt      1584
Val Asn Thr Gln Ala Ala Lys Arg Leu Lys Gly Glu Glu Val Ala Phe
        515                 520                 525
tgg aac gac ctg ttg tcc aag gaa gca gca aag aag cca cct aag ata      1632
Trp Asn Asp Leu Leu Ser Lys Glu Ala Ala Lys Lys Pro Pro Lys Ile
    530                 535                 540
aag cac gcc gaa ttg taa                                             1650
Lys His Ala Glu Leu
545
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<210>12
<211>549
<212>PRT
<213>人工序列

<220>
<223>合成构建体

<400>12

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Met Gly Gln Pro Ala Ser Pro Pro Val Val Asp Thr Ala Gln Gly Arg
1               5                  10                  15
Val Leu Gly Lys Tyr Val Ser Leu Glu Gly Leu Ala Gln Pro Val Ala
            20                  25                  30
Val Phe Leu Gly Val Pro Phe Ala Lys Pro Pro Leu Gly Ser Leu Arg
        35                  40                  45
Phe Ala Pro Pro Gln Pro Ala Glu Pro Trp Ser Phe Val Lys Asn Thr
    50                  55                  60
Thr Ser Tyr Pro Pro Met Cys Cys Gln Glu Pro Ile Gly Gly Gln Met
65                  70                  75                  80
Leu Ser Asp Leu Phe Thr Asn Arg Lys Glu Arg Leu Ile Pro Glu Phe
                85                  90                  95
Ser Glu Asp Cys Leu Tyr Leu Asn Ile Tyr Thr Pro Ala Asp Leu Thr
            100                 105                 110
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Lys Arg Gly Arg Leu Pro Val Met Val Trp Ile His Gly Gly Gly Leu
115 120 125
Val Val Gly Gly Ala Ser Thr Tyr Asp Gly Leu Ala Leu Ala Ala His
130 135 140
Glu Asn Val Val Val Val Ala Ile Gln Tyr Arg Leu Gly Ile Trp Gly
145 150 155 160
Phe Phe Ser Thr Gly Asp Glu His Ser Arg Gly Asn Trp Gly His Leu
165 170 175
Asp Gln Val Ala Ala Leu His Trp Val Gln Glu Asn Ile Ala Asn Phe
180 185 190
Gly Gly Asp Pro Gly Ser Val Thr Ile Phe Gly Glu Ser Ala Gly Gly
195 200 205
Glu Ser Val Ser Val Leu Val Leu Ser Pro Leu Ala Lys Asn Leu Phe
210 215 220
His Arg Ala Ile Ser Glu Ser Gly Val Ala Phe Thr Ala Gly Leu Val
225 230 235 240
Arg Lys Asp Met Lys Ala Ala Ala Lys Gln Ile Ala Val Leu Ala Gly
245 250 255
Cys Lys Thr Thr Thr Ser Ala Val Phe Val His Cys Leu Arg Gln Lys
260 265 270
Ser Glu Asp Glu Leu Leu Asp Leu Thr Leu Lys Met Lys Phe Phe Ala
275 280 285
Leu Asp Leu His Gly Asp Pro Arg Glu Ser His Pro Phe Leu Thr Thr
290 295 300
Val Val Asp Gly Val Leu Leu Pro Lys Met Pro Glu Glu Ile Leu Ala
305 310 315 320
Glu Lys Asp Phe Asn Thr Val Pro Tyr Ile Val Gly Ile Asn Lys Gln
325 330 335
Glu Phe Gly Trp Leu Leu Pro Thr Met Met Gly Phe Pro Leu Ser Glu
340 345 350
Gly Lys Leu Asp Gln Lys Thr Ala Thr Ser Leu Leu Trp Lys Ser Tyr
355 360 365
Pro Ile Ala Asn Ile Pro Glu Glu Leu Thr Pro Val Ala Thr Asp Lys
370 375 380
Tyr Leu Gly Gly Thr Asp Asp Pro Val Lys Lys Lys Asp Leu Phe Leu
385 390 395 400
Asp Leu Met Gly Asp Val Val Phe Gly Val Pro Ser Val Thr Val Ala
405 410 415
Arg Gln His Arg Asp Ala Gly Ala Pro Thr Tyr Met Tyr Glu Phe Gln

420 425 430

Tyr Arg Pro Ser Phe Ser Ser Asp Lys Lys Pro Lys Thr Val Ile Gly

435 440 445

Asp His Gly Asp Glu Ile Phe Ser Val Phe Gly Phe Pro Leu Leu Lys

450 455 460

Gly Asp Ala Pro Glu Glu Glu Val Ser Leu Ser Lys Thr Val Met Lys

465 470 475 480

Phe Trp Ala Asn Phe Ala Arg Ser Gly Asn Pro Asn Gly Glu Gly Leu

485 490 495

Pro His Trp Pro Met Tyr Asp Gln Glu Glu Gly Tyr Leu Gln Ile Gly

500 505 510

Val Asn Thr Gln Ala Ala Lys Arg Leu Lys Gly Glu Glu Val Ala Phe

515 520 525

Trp Asn Asp Leu Leu Ser Lys Glu Ala Ala Lys Lys Pro Pro Lys Ile

530 535 540

Lys His Ala Glu Leu

545

<210>13

<211>1650

<212>DNA

<213>人工序列

<220>

<223>具有酯酶活性的蛋白

<400>13

atgggacaac cagcttcgcc gcctgtcgtt gacaccgctc aaggtcgtgt cttgggtaag 60

tacgtctctc ttgagggttt ggcacaacct gttgctgtct tcttgggtgt ccctttcgct 120

aagcctccac ttggatcttt gcgtttcgcc cctcctcaac cagcagagcc atggtctttc 180

gttaagaaca ctacttccta ccctccaatg tgttgtcaag aaccaattgg aggtcaaatg 240

ctttccgatc ttttcactaa cagaaaggaa cgtcttattc ctgagttctc tgaggactgc 300

ctttacctta acatctacac tcctgccgac ttgaccaaga gaggtagatt gcctgttatg 360

gtttggatcc acggaggtgg tttggttgtt ggtggagcat ccacttacga tggattggct 420

cttgccgcac acgagaacgt tgttgttgtt gctatccaat acagattggg tatctgggga 480

ttcttctcca ccggtgacga acactcccgt ggtaactggg gacaccttga ccaagttgct 540

gcattgcatt gggtccaaga aaacatcgct aacttcggag gtgacccagg ttctgttact 600

atcttcggtg aaagtgcagg aggtgagagt gtctctgtct tggttttatc cccacttgct 660

aagaaccttt tccaccgtgc tatctccgaa tccggtgttg ctttcaccgc cggtttggtc 720

agaaaggaca tgaaggccgc agccaagcag attgccgtcc ttgccggatg caagactact 780
actagtgccg tcttcgtcca ctgtttgaga caaaagagtg aagacgaact tttggacctc 840
acattgaaga tgaagttttt cgcccttgac cttcacggag acccaagaga atctcaccca 900
ttcttgacca ctgttgttga cggagttttg ttgccaaaga tgcctgagga aattttggcc 960
gagaaggact tcaacaccgt cccatacatc gttggaatca acaagcagga gttcggttgg 1020
cttttgccaa caatgatggg tttcccactt tccgaaggta agttggacca aaagacagct 1080
acatcccttt tgtggaagtc ctacccaatc gccaacatcc ctgaagagtt gaccccagtt 1140
gctaccgaca agtaccttgg tggtaccgac gacccagtca aaaagaagga cttgttcttg 1200
gaccttatgg gtgatgttgt tttcggtgtc ccatctgtta cagttgctcg tcagcacaga 1260
gatgcaggag ctccaactta catgtacgag ttccaataca gaccatcttt ctcaagtgac 1320
aagaagccaa agaccgttat cggagaccac ggtgacgaaa tcttctccgt cttcggattc 1380
ccattgctta agggtgacgc tccagaggaa gaagtctccc tttctaagac cgttatgaag 1440
ttctgggcta acttcgcccg ttctggtaac ccaaacggag aaggtttgcc acactggcct 1500
atgtacgacc aagaggaggg ataccttcaa atcggtgtca acactcaagc cgctaagcgt 1560
ttgaagggtg aggaagttgc tttctggaac gacctttgt ccaaggaagc agcaaagaag 1620
cctccaaaga ttaagcacgc tgaattgtaa 1650

<210>14
<211>1650
<212>DNA
<213>人工序列

<220>
<223>具有酯酶活性的蛋白

<400>14
atgggccaac ctgcttctcc acctgttgtt gacaccgctc aaggtcgtgt cttgggtaag 60
tacgtttctt tggaaggttt ggctcaacct gttgctgtct tcttgggtgt tccatttgcc 120
aagccaccat taggttcttt aagatttgct ccaccacagc ctgctgaacc atggtctttc 180
gtcaagaaca ctacttctta ccctccaatg tgttgtcagg aaccaatcgg tggtcaaatg 240
ctttccgatt tattcaccaa cagaaaggaa agattgattc cagagttcag tgaggactgt 300
ctatatttga acatttacac tccagctgat ttgaccaaga gaggtcgtct gcccgtcatg 360
gtttggattc acggtggtgg tttggttgtc ggtggtgctt ccacttacga tggtttggct 420
ttggctgctc acgagaatgt tgttgttgtt gccattcaat acagattagg tatctggggt 480
ttcttctcca ccggtgacga acactccaga ggtaactggg gtcatttgga ccaagttgct 540
gctttgcact gggttcaaga aaacattgcc aacttcggtg gtgacccagg ttccgtcact 600
atctttggtg aatctgctgg tggtgaatcc gtctccgtct tggtcttgtc tccattggcc 660
aagaacttat tccatcgtgc catctctgaa tccggtgttg ccttcaccgc tggtttagtg 720
agaaaggaca tgaaggctgc tgccaagcaa attgctgtct tggctggttg taagaccacc 780

acctctgctg tctttgtcca ttgtttgaga caaaagtctg aagatgagct actagatttg 840
actttgaaaa tgaaattctt tgctttggat ttgcacggtg acccaagaga atctcatcct 900
ttcttgacca ccgttgttga tggtgtcttg ttgccaaaga tgccagaaga aatcttggct 960
gaaaaggatt tcaacactgt tccatacatt gtcggtatta acaagcaaga gtttggctgg 1020
ttactaccaa ccatgatggg tttcccatta tctgaaggta aactggacca aaagactgcc 1080
acttcgctgc tatggaaatc ttacccaatt gccaacattc cagaagaatt gactccagtt 1140
gccactgaca agtacttggg tggtactgat gatcctgtca agaagaagga cttgttcttg 1200
gatttgatgg gtgatgttgt tttcggtgtt ccatctgtca ccgttgccag acaacaccgt 1260
gatgctggtg ctccaactta catgtatgag ttccaataca gaccttcctt ctcctctgac 1320
aagaagccaa agactgtcat cggtgaccac ggtgatgaaa tcttctccgt ctttggtttc 1380
ccattattga agggtgacgc tccagaagaa gaagtttctt tatccaagac cgtcatgaaa 1440
ttctgggcca actttgccag atccggtaac ccaaatggtg aaggtttacc tcactggcca 1500
atgtatgacc aagaagaagg ttacttgcaa atcggtgtca acactcaagc tgccaagaga 1560
ttgaagggtg aagaagttgc tttctggaat gacttgttgt ccaaggaagc tgccaagaag 1620
ccaccaaaga tcaagcatgc tgaattgtaa 1650

<210>15
<211>1650
<212>DNA
<213>人工序列

<220>
<223>引物

<400>15
atggggcagc cagcctcgcc gcctgttgtg gacactgccc agggccgtgt cctggggaag 60
tacgtcagct tagaaggcct ggcacagccg gtggccgtct tcctgggagt ccctttgcc 120
aagccccctc tcggatcttt gcgttttgct ccgccgcagc ctgcagaacc atggagcttc 180
gtgaagaaca ccacctccta ccctcccatg tgctgccaag agccaattgg gggacagatg 240
ctctctgatc tgtttaccaa ccgtaaggag cgtctcattc cggagtttc tgaagactgt 300
ctctacctga atatttacac ccctgctgac ctgacaaagc gtggccgtct gccggtgatg 360
gtgtggattc acggaggagg tctggtggtg ggcggggctt ccacctatga tggactggcc 420
ctcgctgcgc atgaaaacgt ggtggtggtg gccatccagt accgcctggg catctgggga 480
ttcttcagca caggggacga acacagccgg ggcaactggg gtcacttgga ccaggtggcc 540
gcactgcact gggtccagga gaacatcgcc aactttggag gcgacccagg ctctgtgacc 600
atctttggag agtcagcagg aggggaaagt gtctctgttc tggtgttgtc tcccttggcc 660
aagaacctct tccaccgggc catctctgag agtggcgtgg ccttcactgc tggcctggtc 720
cgtaaggaca tgaaggctgc agctaagcaa attgctgtcc ttgctgggtg taaaaccacc 780
acctcggctg tctttgttca ctgcctgcgc cagaagtcgg aggacgagct cttggactta 840

acgctgaaga tgaaattttt cgctcttgat ttgcatggag acccccgtga gagccatccc 900
ttcctgacca ctgtggtgga tggagtgctg ctgcccaaga tgcctgaaga gattctggct 960
gaaaaggatt tcaacactgt cccctacatc gtgggaatca acaagcaaga gtttggctgg 1020
cttctgccaa cgatgatggg cttccccctc tctgaaggca agctggacca gaagacggcc 1080
acgtcactcc tgtggaagtc ctaccccatc gctaacatcc ctgaggaact gactccagtg 1140
gccactgaca agtatttggg ggggacagac gaccccgtca aaaagaaaga cctgttcctg 1200
gacttgatgg gggatgtggt gtttggtgtc ccatctgtga cggtggcccg tcaacaccgc 1260
gatgcaggag cccccaccta catgtatgag tttcagtatc gcccgagctt ctcatcggac 1320
aagaaaccca agacggtgat cggggaccac ggggatgaaa tcttctccgt ctttggtttt 1380
ccactgttaa aaggcgatgc cccagaagag gaggtcagtc tcagcaagac ggtgatgaaa 1440
ttctgggcca actttgctcg cagtgggaac cccaatgggg aggggctgcc ccattggccg 1500
atgtacgacc aggaagaagg gtaccttcag atcggcgtca acacccaggc agccaagcgc 1560
ctgaaaggtg aagaagtggc cttctggaac gatctcctgt ccaaggaggc agcaaagaag 1620
ccacccaaga taaagcatgc tgagctgtga 1650

<210>16
<211>44
<212>DNA
<213>人工序列

<220>
<223>引物

<400>16
ctttaagaag gagatataca tatgggacaa ccagcttcgc cgcc 44

<210>17
<211>46
<212>DNA
<213>人工序列

<220>
<223>引物

<400>17
ccccccccccc ccaagcttat tacaattcgg cgtgctttat cttagg 46

<210>18
<211>38

<212>DNA
<213>人工序列

<220>
<223>引物

<400>18
atttatacat atgggacaac cagcttcgcc gcctgtcg 38

<210>19
<211>43
<212>DNA
<213>人工序列

<220>
<223>引物

<400>19
ccgccaagct tattacaatt cagcgtgctt aatctttgga ggc 43

<210>20
<211>38
<212>DNA
<213>人工序列

<220>
<223>引物

<400>20
atttatacat atgggccaac ctgcttctcc acctgttg 38

<210>21
<211>43
<212>DNA
<213>人工序列

<220>
<223>引物

<400>21
ccgccaagct tattacaatt cagcatgctt gatctttggt ggc 43

<210>22
<211>38
<212>DNA
<213>人工序列

<220>
<223>引物

<400>22
atttatacat atgggacaac cagcttcgcc gcctgtcg 38

<210>23
<211>43
<212>DNA
<213>人工序列

<220>
<223>引物

<400>23
ccgccaagct tattacaatt cggcgtgctt tatcttaggt ggc 43

<210>24
<211>35
<212>DNA
<213>人工序列

<220>
<223>引物

<400>24
atttatacat atggggcagc cagcctcgcc gcctg 35

<210>25
<211>42
<212>DNA

<213>人工序列

<220>
<223>引物

<400>25
ccgccaagct tatcacagct cagcatgctt tatcttgggt gg 42

<210>26
<211>57
<212>DNA
<213>人工序列

<220>
<223>引物

<400>26
cggatccttt aactttaaga aggagatata atggatgacg cggcaattca acaaacg 57

<210>27
<211>38
<212>DNA
<213>人工序列

<220>
<223>引物

<400>27
cggatcctta tttaccgctg gtcatttttt ggtgttcg 38

<210>28
<211>71
<212>DNA
<213>人工序列

<220>
<223>引物

<400>28

gtaaattccc tacaatcctg cccattgtct gccaacaact atggggatct tgtgtaggct 60
ggagctgctt c 71

<210>29
<211>71
<212>DNA
<213>人工序列

<220>
<223>引物

<400>29
cccatagtcg catggtgtcg ccttctttac ttttgttact gatttgtaaa acatatgaat 60
atcctcctta g 71
<210>30
<211>74
<212>DNA
<213>人工序列

<220>
<223>引物

<400>30
cctattacgt ctcgcgctac aatcgcggta atcaacgata aggacacttt gtctgtgtag 60
gctggagctg cttc 74

<210>31
<211>75
<212>DNA
<213>人工序列

<220>
<223>引物

<400>31
ctgatagcgg aaacgtaatt aagggctaag agcacactac tcttagccct ttaaccatat 60
gaatatcctc cttag 75

<210>32

<211>1629
<212>DNA
<213>人工序列

<220>
<223>具有酯酶活性的蛋白

<220>
<221>CDS
<222>(1)..(1629)
<223>牛肝酯酶

<400>32

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atg ggt ctg gca ccg tcg cca cct atc gtg gat acc gca cag ggc cgt      48
Met Gly Leu Ala Pro Ser Pro Pro Ile Val Asp Thr Ala Gln Gly Arg
1               5                  10                  15
gtt ctg ggg aag cac gtc agc cta aaa ggt ttc gca cag ccg gtg gcg      96
Val Leu Gly Lys His Val Ser Leu Lys Gly Phe Ala Gln Pro Val Ala
            20                  25                  30
gtt ttt ctg ggt gtc cca ttc gcc aaa ccg cct tta ggg tcc ttg cgt     144
Val Phe Leu Gly Val Pro Phe Ala Lys Pro Pro Leu Gly Ser Leu Arg
        35                  40                  45
ttc gcc cca cct cag ccg gct gag ccg tgg acc ttt gtc aag aat acc     192
Phe Ala Pro Pro Gln Pro Ala Glu Pro Trp Thr Phe Val Lys Asn Thr
    50                  55                  60
att agc tat cca ccg atg tgc tcc caa gat cca gtg ggt gct cag ttg     240
Ile Ser Tyr Pro Pro Met Cys Ser Gln Asp Pro Val Gly Ala Gln Leu
65                  70                  75                  80
tta tcg gac ctg ttc acc aac cgc aaa gaa aac atc agt ctg act ttc     288
Leu Ser Asp Leu Phe Thr Asn Arg Lys Glu Asn Ile Ser Leu Thr Phe
                85                  90                  95
agc gag gat tgc ttg tat ctc aat atc tat acc ccg gca gat tta act     336
Ser Glu Asp Cys Leu Tyr Leu Asn Ile Tyr Thr Pro Ala Asp Leu Thr
            100                 105                 110
aaa cgt agc cgc ctg ccg gtg atg gtg tgg att cat ggt ggt gga ctg     384
Lys Arg Ser Arg Leu Pro Val Met Val Trp Ile His Gly Gly Gly Leu
        115                 120                 125
atg gtg ggc ggc gct tct acg tac gac ggg ctg gtc tta tcg gcg cac     432
Met Val Gly Gly Ala Ser Thr Tyr Asp Gly Leu Val Leu Ser Ala His
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130 135 140

gag aac gtg gtg gtg gtg acc atc cag tat cgt ctg ggc att tgg ggt 480
Glu Asn Val Val Val Val Thr Ile Gln Tyr Arg Leu Gly Ile Trp Gly
145 150 155 160

ttc ttt agc acg ggc gat gaa cat agt cgc ggg aac tgg ggc cat ttg 528
Phe Phe Ser Thr Gly Asp Glu His Ser Arg Gly Asn Trp Gly His Leu
165 170 175

gat cag gtg gcg gct ctg cat tgg gtc cag gaa aac atc gcg aac ttc 576
Asp Gln Val Ala Ala Leu His Trp Val Gln Glu Asn Ile Ala Asn Phe
180 185 190

ggt ggc gac cca ggc agc gtg acc atc ttt ggt gaa tcc gcc ggt gca 624
Gly Gly Asp Pro Gly Ser Val Thr Ile Phe Gly Glu Ser Ala Gly Ala
195 200 205

gag agt gtt agc atc tta gtg ctt tcc ccg ctg gcg cgt aat ttg ttt 672
Glu Ser Val Ser Ile Leu Val Leu Ser Pro Leu Ala Arg Asn Leu Phe
210 215 220

cat cgc gcg att agc gaa agc ggc gtg gcg ctg atc tcg acc ctg gtt 720
His Arg Ala Ile Ser Glu Ser Gly Val Ala Leu Ile Ser Thr Leu Val
225 230 235 240

aaa cgt gat tca aaa gcc gca gct gag caa atc gcc gtc atc gca ggc 768
Lys Arg Asp Ser Lys Ala Ala Ala Glu Gln Ile Ala Val Ile Ala Gly
245 250 255

tgt aag acc acc acc tct gcc gtc tta gta cat tgc ctg cgc cag aaa 816
Cys Lys Thr Thr Thr Ser Ala Val Leu Val His Cys Leu Arg Gln Lys
260 265 270

acg gaa gat gaa ttg ctg gaa atc acg ctg aag atg aaa ttc ttt gca 864
Thr Glu Asp Glu Leu Leu Glu Ile Thr Leu Lys Met Lys Phe Phe Ala
275 280 285

ctc gac ctt cat aag gat agt acg gaa agc cac ccg ttt ctg ccg acc 912
Leu Asp Leu His Lys Asp Ser Thr Glu Ser His Pro Phe Leu Pro Thr
290 295 300

gtg gtt gac ggc atg ctg ctg cct aaa atg ccg gaa gag atg ctg gcc 960
Val Val Asp Gly Met Leu Leu Pro Lys Met Pro Glu Glu Met Leu Ala
305 310 315 320

gag aaa aat ttt aac aac gtc ccg tat atg gtg ggt att aac aaa caa 1008
Glu Lys Asn Phe Asn Asn Val Pro Tyr Met Val Gly Ile Asn Lys Gln
325 330 335

gaa ttc ggc tgg atc atc cca ctg ttt atg agc tat ccg ctg ccg gag 1056
Glu Phe Gly Trp Ile Ile Pro Leu Phe Met Ser Tyr Pro Leu Pro Glu

340 345 350

gat aaa ctg gat cag aaa act gcg acg agt ctg ctg tgg cag agc tat 1104
Asp Lys Leu Asp Gln Lys Thr Ala Thr Ser Leu Leu Trp Gln Ser Tyr
355 360 365

tcc tta ttg agc atc cca gaa gaa ctg tcg ccg gtg gcc acg gat aaa 1152
Ser Leu Leu Ser Ile Pro Glu Glu Leu Ser Pro Val Ala Thr Asp Lys
370 375 380

tac cta ggt ggg act gat gat cct gtt aaa aag aaa gat ctg ttt ctg 1200
Tyr Leu Gly Gly Thr Asp Asp Pro Val Lys Lys Lys Asp Leu Phe Leu
385 390 395 400

gat ctg atc gct gac gtt ctc ttc ggc gtc ccg tct gtc aat gtg gcg 1248
Asp Leu Ile Ala Asp Val Leu Phe Gly Val Pro Ser Val Asn Val Ala
405 410 415

cgg cgc cat cgt gac gct ggg gcg ccg acc tat atg tac gaa ttc cag 1296
Arg Arg His Arg Asp Ala Gly Ala Pro Thr Tyr Met Tyr Glu Phe Gln
420 425 430

tac cgc ccg agc ttt agt tca gaa ctg aag ccg aaa act gtg atc ggt 1344
Tyr Arg Pro Ser Phe Ser Ser Glu Leu Lys Pro Lys Thr Val Ile Gly
435 440 445

gat cac ggt gac gaa ctg ttt tcc gtc ttt ggg gca cca ttc cta aag 1392
Asp His Gly Asp Glu Leu Phe Ser Val Phe Gly Ala Pro Phe Leu Lys
450 455 460

gac ggt gcg tcg gaa gag gaa att aat ctg agc aaa atg gtg atg aag 1440
Asp Gly Ala Ser Glu Glu Glu Ile Asn Leu Ser Lys Met Val Met Lys
465 470 475 480

ttt tgg gcg aac ttc gca cgc aat ggg aac ccg aat ggt gaa ggc tta 1488
Phe Trp Ala Asn Phe Ala Arg Asn Gly Asn Pro Asn Gly Glu Gly Leu
485 490 495

cct cac tgg cca gct tac gat cat aaa gag ggc tat ttg cag atc ggt 1536
Pro His Trp Pro Ala Tyr Asp Hi s Lys Glu Gly Tyr Leu Gln Ile Gly
500 505 510

gtt aac acg cgt gcg gcc gag aaa ctg aag gat aaa gag gta gca ttt 1584
Val Asn Thr Arg Ala Ala Glu Lys Leu Lys Asp Lys Glu Val Ala Phe
515 520 525

tgg aat gaa ctg ctg agc cgc gaa gtg gca cgt cat cat acc taa 1629
Trp Asn Glu Leu Leu Ser Arg Glu Val Ala Arg His His Thr
530 535 540

<210>33

<211>542
<212>PRT
<213>人工序列

<220>
<223>合成构建体

<400>33

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

Lys Arg Asp Ser Lys Ala Ala Ala Glu Gln Ile Ala Val Ile Ala Gly
245 250 255
Cys Lys Thr Thr Thr Ser Ala Val Leu Val His Cys Leu Arg Gln Lys
260 265 270
Thr Glu Asp Glu Leu Leu Glu Ile Thr Leu Lys Met Lys Phe Phe Ala
275 280 285
Leu Asp Leu His Lys Asp Ser Thr Glu Ser His Pro Phe Leu Pro Thr
290 295 300
Val Val Asp Gly Met Leu Leu Pro Lys Met Pro Glu Glu Met Leu Ala
305 310 315 320
Glu Lys Asn Phe Asn Asn Val Pro Tyr Met Val Gly Ile Asn Lys Gln
325 330 335
Glu Phe Gly Trp Ile Ile Pro Leu Phe Met Ser Tyr Pro Leu Pro Glu
340 345 350
Asp Lys Leu Asp Gln Lys Thr Ala Thr Ser Leu Leu Trp Gln Ser Tyr
355 360 365
Ser Leu Leu Ser Ile Pro Glu Glu Leu Ser Pro Val Ala Thr Asp Lys
370 375 380
Tyr Leu Gly Gly Thr Asp Asp Pro Val Lys Lys Lys Asp Leu Phe Leu
385 390 395 400
Asp Leu Ile Ala Asp Val Leu Phe Gly Val Pro Ser Val Asn Val Ala
405 410 415
Arg Arg His Arg Asp Ala Gly Ala Pro Thr Tyr Met Tyr Glu Phe Gln
420 425 430
Tyr Arg Pro Ser Phe Ser Ser Glu Leu Lys Pro Lys Thr Val Ile Gly
435 440 445
Asp His Gly Asp Glu Leu Phe Ser Val Phe Gly Ala Pro Phe Leu Lys
450 455 460
Asp Gly Ala Ser Glu Glu Glu Ile Asn Leu Ser Lys Met Val Met Lys
465 470 475 480
Phe Trp Ala Asn Phe Ala Arg Asn Gly Asn Pro Asn Gly Glu Gly Leu
485 490 495
Pro His Trp Pro Ala Tyr Asp His Lys Glu Gly Tyr Leu Gln Ile Gly
500 505 510
Val Asn Thr Arg Ala Ala Glu Lys Leu Lys Asp Lys Glu Val Ala Phe
515 520 525
Trp Asn Glu Leu Leu Ser Arg Glu Val Ala Arg His His Thr
530 535 540

<210>34
<211>1650
<212>DNA
<213>人工序列

<220>
<223>具有酯酶活性的蛋白

<220>
<221>CDS
<222>(1)..(1650)

<400>34

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atg gga caa cca gct tcg ccg cct gtc gtt gat acc gct caa gga cga    48
Met Gly Gln Pro Ala Ser Pro Pro Val Val Asp Thr Ala Gln Gly Arg
1               5                  10                  15
gtc ttg ggt aag tac gtc tct tta gag gga ttg gca caa ccg gtt gct    96
Val Leu Gly Lys Tyr Val Ser Leu Glu Gly Leu Ala Gln Pro Val Ala
            20                  25                  30
gtc ttc ttg gga gtc cct ttt gct aag cca cct ctt gga tct ttg agg    144
Val Phe Leu Gly Val Pro Phe Ala Lys Pro Pro Leu Gly Ser Leu Arg
        35                  40                  45
ttt gcc ccg ccg caa cca gca gag cca tgg tct ttc gtt aag aac act    192
Phe Ala Pro Pro Gln Pro Ala Glu Pro Trp Ser Phe Val Lys Asn Thr
    50                  55                  60
act tcc tac cct cca atg tgt tgt caa gat cca gtc gta gaa caa atg    240
Thr Ser Tyr Pro Pro Met Cys Cys Gln Asp Pro Val Val Glu Gln Met
65                  70                  75                  80
acg tca gac cta ttc act aac gga aag gaa agg ctt acc ctg gag ttc    288
Thr Ser Asp Leu Phe Thr Asn Gly Lys Glu Arg Leu Thr Leu Glu Phe
                85                  90                  95
tct gag gat tgc ctt tac cta aat att tac act cct gcc gat ttg aca    336
Ser Glu Asp Cys Leu Tyr Leu Asn Ile Tyr Thr Pro Ala Asp Leu Thr
            100                 105                 110
aag agg ggt agg ttg ccg gtt atg gtt tgg att cat gga gga ggt ttg    384
Lys Arg Gly Arg Leu Pro Val Met Val Trp Ile His Gly Gly Gly Leu
        115                 120                 125
gtt ctg ggc gga gca ccg atg tat gac gga gtg gtt ctt gcc gcg cac    432
Val Leu Gly Gly Ala Pro Met Tyr Asp Gly Val Val Leu Ala Ala His
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130 135 140

gag aac gtt gtt gtt gtt gct att caa tac cgt ttg ggt att tgg gga 480
Glu Asn Val Val Val Val Ala Ile Gln Tyr Arg Leu Gly Ile Trp Gly
145 150 155 160

ttt ttc tcc aca gga gat gag cat tcc cgt gga aac tgg ggc cat tta 528
Phe Phe Ser Thr Gly Asp Glu His Ser Arg Gly Asn Trp Gly His Leu
165 170 175

gat caa gtt gct gca ttg cat tgg gtc caa gaa aac att gct aac ttc 576
Asp Gln Val Ala Ala Leu His Trp Val Gln Glu Asn Ile Ala Asn Phe
180 185 190

gga ggt gat cca ggt tct gtt act att ttc gga gaa tca gca ggc gga 624
Gly Gly Asp Pro Gly Ser Val Thr Ile Phe Gly Glu Ser Ala Gly Gly
195 200 205

gag agt gtc tct gta ttg gtt tta tca cca tta gct aag aac ctt ttt 672
Glu Ser Val Ser Val Leu Val Leu Ser Pro Leu Ala Lys Asn Leu Phe
210 215 220

cat cgt gct att tcc gaa agt ggt gtt gct tta acc gtc gct ttg gtc 720
His Arg Ala Ile Ser Glu Ser Gly Val Ala Leu Thr Val Ala Leu Val
225 230 235 240

agg aag gat atg aag gcc gca gcc aag cag atc gct gtc ctt gca gga 768
Arg Lys Asp Met Lys Ala Ala Ala Lys Gln Ile Ala Val Leu Ala Gly
245 250 255

tgc aaa act act act tcg gca gtc ttc gtg cat tgt ttg cgt caa aag 816
Cys Lys Thr Thr Thr Ser Ala Val Phe Val His Cys Leu Arg Gln Lys
260 265 270

tcg gaa gat gaa ctt tta gac ctc acg ttg aag atg aaa ttc ctg acc 864
Ser Glu Asp Glu Leu Leu Asp Leu Thr Leu Lys Met Lys Phe Leu Thr
275 280 285

ctt gac ttt cac gga gat caa agg gaa tct cac cct ttt ttg ccg act 912
Leu Asp Phe His Gly Asp Gln Arg Glu Ser His Pro Phe Leu Pro Thr
290 295 300

gtt gtt gac gga gtt ttg ttg cct aag atg cct gag gaa atc ttg gcc 960
Val Val Asp Gly Val Leu Leu Pro Lys Met Pro Glu Glu Ile Leu Ala
305 310 315 320

gag aag gac ttt aac acc gtc cca tac att gtt gga att aac aag cag 1008
Glu Lys Asp Phe Asn Thr Val Pro Tyr Ile Val Gly Ile Asn Lys Gln
325 330 335

gag ttc gga tgg ctt ttg cca acg atg atg gga ttt cct ctt tcc gag 1056
Glu Phe Gly Trp Leu Leu Pro Thr Met Met Gly Phe Pro Leu Ser Glu

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                340                 345                 350
gga aag ttg gat caa aag acg gct acg tca ctt ttg tgg aag tcc tac    1104
Gly Lys Leu Asp Gln Lys Thr Ala Thr Ser Leu Leu Trp Lys Ser Tyr
        355                 360                 365
cca att gcc aac att cct gaa gag ttg acc cca gtt gct acc gat aag    1152
Pro Ile Ala Asn Ile Pro Glu Glu Leu Thr Pro Val Ala Thr Asp Lys
    370                 375                 380
tat tta gga gga aca gat gat cct gtc aaa aag aaa gat ttg ttt ttg    1200
Tyr Leu Gly Gly Thr Asp Asp Pro Val Lys Lys Lys Asp Leu Phe Leu
385                 390                 395                 400
gat ctg atg gga gac gtt gtt ttc ggc gtc cca tca gtt acg gtt gct    1248
Asp Leu Met Gly Asp Val Val Phe Gly Val Pro Ser Val Thr Val Ala
                405                 410                 415
cgt cag cat agg gac gca gga gct cca act tac atg tat gag ttc caa    1296
Arg Gln His Arg Asp Ala Gly Ala Pro Thr Tyr Met Tyr Glu Phe Gln
            420                 425                 430
tat cgt cca tct ttt tca tcg gat aag aaa cct aag acg gtt att gga    1344
Tyr Arg Pro Ser Phe Ser Ser Asp Lys Lys Pro Lys Thr Val Ile Gly
        435                 440                 445
gat cat gga gac gaa att ttt tcc gtc ttc ggc ttc cca ttg ctc aaa    1392
Asp His Gly Asp Glu Ile Phe Ser Val Phe Gly Phe Pro Leu Leu Lys
    450                 455                 460
ggt gac gct cca gag gaa gaa gtc agt ctt tct aag acg gtt atg aaa    1440
Gly Asp Ala Pro Glu Glu Glu Val Ser Leu Ser Lys Thr Val Met Lys
465                 470                 475                 480
ttt tgg gct aac ttc gcc cgt agt gga aac cct aat gga gaa gga ttg    1488
Phe Trp Ala Asn Phe Ala Arg Ser Gly Asn Pro Asn Gly Glu Gly Leu
                485                 490                 495
cct cac tgg ccg atg tac gat caa gag gag gga tac ctt caa att ggt    1536
Pro His Trp Pro Met Tyr Asp Gln Glu Glu Gly Tyr Leu Gln Ile Gly
            500                 505                 510
gtc aac act caa gca gct aag agg ttg aaa ggc gag gag gtt gct ttt    1584
Val Asn Thr Gln Ala Ala Lys Arg Leu Lys Gly Glu Glu Val Ala Phe
        515                 520                 525
tgg aac gac ctg ttg tcc aag gaa gca gca aag aag cca cct aag ata    1632
Trp Asn Asp Leu Leu Ser Lys Glu Ala Ala Lys Lys Pro Pro Lys Ile
    530                 535                 540
aag cac gcc gaa ttg taa                                        1650
Lys His Ala Glu Leu
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Val Phe Leu Gly Val Pro Phe Ala Lys Pro Pro Leu Gly Ser Leu Arg
35 40 45
Phe Ala Pro Pro Gln Pro Ala Glu Pro Trp Ser Phe Val Lys Asn Thr
50 55 60
Thr Ser Tyr Pro Pro Met Cys Cys Gln Asp Pro Val Val Glu Gln Met
65 70 75 80
Thr Ser Asp Leu Phe Thr Asn Gly Lys Glu Arg Leu Thr Leu Glu Phe
85 90 95
Ser Glu Asp Cys Leu Tyr Leu Asn Ile Tyr Thr Pro Ala Asp Leu Thr
100 105 110
Lys Arg Gly Arg Leu Pro Val Met Val Trp Ile His Gly Gly Gly Leu
115 120 125
Val Leu Gly Gly Ala Pro Met Tyr Asp Gly Val Val Leu Ala Ala His
130 135 140
Glu Asn Val Val Val Val Ala Ile Gln Tyr Arg Leu Gly Ile Trp Gly
145 150 155 160
Phe Phe Ser Thr Gly Asp Glu His Ser Arg Gly Asn Trp Gly His Leu
165 170 175
Asp Gln Val Ala Ala Leu His Trp Val Gln Glu Asn Ile Ala Asn Phe
180 185 190
Gly Gly Asp Pro Gly Ser Val Thr Ile Phe Gly Glu Ser Ala Gly Gly
195 200 205
Glu Ser Val Ser Val Leu Val Leu Ser Pro Leu Ala Lys Asn Leu Phe

210 215 220

His Arg Ala Ile Ser Glu Ser Gly Val Ala Leu Thr Val Ala Leu Val
225 230 235 240

Arg Lys Asp Met Lys Ala Ala Ala Lys Gln Ile Ala Val Leu Ala Gly
245 250 255

Cys Lys Thr Thr Thr Ser Ala Val Phe Val His Cys Leu Arg Gln Lys
260 265 270

Ser Glu Asp Glu Leu Leu Asp Leu Thr Leu Lys Met Lys Phe Leu Thr
275 280 285

Leu Asp Phe His Gly Asp Gln Arg Glu Ser His Pro Phe Leu Pro Thr
290 295 300

Val Val Asp Gly Val Leu Leu Pro Lys Met Pro Glu Glu Ile Leu Ala
305 310 315 320

Glu Lys Asp Phe Asn Thr Val Pro Tyr Ile Val Gly Ile Asn Lys Gln
325 330 335

Glu Phe Gly Trp Leu Leu Pro Thr Met Met Gly Phe Pro Leu Ser Glu
340 345 350

Gly Lys Leu Asp Gln Lys Thr Ala Thr Ser Leu Leu Trp Lys Ser Tyr
355 360 365

Pro Ile Ala Asn Ile Pro Glu Glu Leu Thr Pro Val Ala Thr Asp Lys
370 375 380

Tyr Leu Gly Gly Thr Asp Asp Pro Val Lys Lys Lys Asp Leu Phe Leu
385 390 395 400

Asp Leu Met Gly Asp Val Val Phe Gly Val Pro Ser Val Thr Val Ala
405 410 415

Arg Gln His Arg Asp Ala Gly Ala Pro Thr Tyr Met Tyr Glu Phe Gln
420 425 430

Tyr Arg Pro Ser Phe Ser Ser Asp Lys Lys Pro Lys Thr Val Ile Gly
435 440 445

Asp His Gly Asp Glu Ile Phe Ser Val Phe Gly Phe Pro Leu Leu Lys
450 455 460

Gly Asp Ala Pro Glu Glu Glu Val Ser Leu Ser Lys Thr Val Met Lys
465 470 475 480

Phe Trp Ala Asn Phe Ala Arg Ser Gly Asn Pro Asn Gly Glu Gly Leu
485 490 495

Pro His Trp Pro Met Tyr Asp Gln Glu Glu Gly Tyr Leu Gln Ile Gly
500 505 510

Val Asn Thr Gln Ala Ala Lys Arg Leu Lys Gly Glu Glu Val Ala Phe
515 520 525

Trp Asn Asp Leu Leu Ser Lys Glu Ala Ala Lys Lys Pro Pro Lys Ile
530 535 540
Lys His Ala Glu Leu
545

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gtc ttg ggt aag tac gtc tct tta gag gga ttg gca caa ccg gtt gct    96
Val Leu Gly Lys Tyr Val Ser Leu Glu Gly Leu Ala Gln Pro Val Ala
            20                  25                  30
gtc ttc ttg gga gtc cct ttt gct aag cca cct ctt gga tct ttg agg    144
Val Phe Leu Gly Val Pro Phe Ala Lys Pro Pro Leu Gly Ser Leu Arg
        35                  40                  45
ttt gcc ccg ccg caa cca gca gag cca tgg tct ttc gtt aag aac act    192
Phe Ala Pro Pro Gln Pro Ala Glu Pro Trp Ser Phe Val Lys Asn Thr
    50                  55                  60
act tcc tac cct cca atg tgt tgt caa gat cca gtc gca gga caa atg    240
Thr Ser Tyr Pro Pro Met Cys Cys Gln Asp Pro Val Ala Gly Gln Met
65                  70                  75                  80
acg tca gac cta ttc act aac gga aag gaa agg ctt atc ccg gag ttc    288
Thr Ser Asp Leu Phe Thr Asn Gly Lys Glu Arg Leu Ile Pro Glu Phe
                85                  90                  95
tct gag gat tgc ctt tac cta aat att tac act cct gcc gat ttg aca    336
Ser Glu Asp Cys Leu Tyr Leu Asn Ile Tyr Thr Pro Ala Asp Leu Thr
            100                 105                 110
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aag agg ggt agg ttg ccg gtt atg gtt tgg att cat gga gga ggt ttg 384
Lys Arg Gly Arg Leu Pro Val Met Val Trp Ile His Gly Gly Gly Leu
115 120 125

gtt gtt ggc gga gca tcc act tat gac gga ttg get ctt gcc gcg cac 432
Val Val Gly Gly Ala Ser Thr Tyr Asp Gly Leu Ala Leu Ala Ala His
130 135 140

gag aac gtt gtt gtt gtt gct att caa tac cgt ttg ggt att tgg gga 480
Glu Asn Val Val Val Val Ala Ile Gln Tyr Arg Leu Gly Ile Trp Gly
145 150 155 160

ttt ttc tcc aca gga gat gag cat tcc cgt gga aac tgg ggc cat tta 528
Phe Phe Ser Thr Gly Asp Glu His Ser Arg Gly Asn Trp Gly His Leu
165 170 175

gat caa gtt gct gca ttg cat tgg gtc caa gaa aac att gct aac ttc 576
Asp Gln Val Ala Ala Leu His Trp Val Gln Glu Asn Ile Ala Asn Phe
180 185 190

gga ggt gat aca ggt tct gtt act att ttc gga gaa tca gca ggc gga 624
Gly Gly Asp Thr Gly Ser Val Thr Ile Phe Gly Glu Ser Ala Gly Gly
195 200 205

gag agt gtc tct gta ttg gtt tta tca cca tta gct aag aac ctt ttt 672
Glu Ser Val Ser Val Leu Val Leu Ser Pro Leu Ala Lys Asn Leu Phe
210 215 220

cat cgt gct att tcc gaa agt ggt gtt gct tta acc gcc ggt ttg gtc 720
His Arg Ala Ile Ser Glu Ser Gly Val Ala Leu Thr Ala Gly Leu Val
225 230 235 240

agg aag gat atg aag gcc gca gcc aag cag atc gct gtc ctt gca gga 768
Arg Lys Asp Met Lys Ala Ala Ala Lys Gln Ile Ala Val Leu Ala Gly
245 250 255

tgc aaa act act act tcg gca gtc ttc gtg cat tgt ttg cgt caa aag 816
Cys Lys Thr Thr Thr Ser Ala Val Phe Val His Cys Leu Arg Gln Lys
260 265 270

tcg gaa gat gaa ctt tta gac ctc acg ttg aag atg aaa ttc ctg acc 864
Ser Glu Asp Glu Leu Leu Asp Leu Thr Leu Lys Met Lys Phe Leu Thr
275 280 285

ctt gac tta cac gga gat cca agg gaa tct cac cct ttt ttg ccg act 912
Leu Asp Leu His Gly Asp Pro Arg Glu Ser His Pro Phe Leu Pro Thr
290 295 300

gtt gtt gac gga gtt ttg ttg cct aag atg cct gag gaa atc ttg gcc 960
Val Val Asp Gly Val Leu Leu Pro Lys Met Pro Glu Glu Ile Leu Ala
305 310 315 320

gag aag gac ttt aac acc gtc cca tac att gtt gga att aac aag cag 1008
Glu Lys Asp Phe Asn Thr Val Pro Tyr Ile Val Gly Ile Asn Lys Gln
325 330 335

gag ttc gga tgg ctt ttg cca acg atg atg gga ttt cct ctt tcc gag 1056
Glu Phe Gly Trp Leu Leu Pro Thr Met Met Gly Phe Pro Leu Ser Glu
340 345 350

gga aag ttg gat caa aag acg gct acg tca ctt ttg tgg aag tcc ttc 1104
Gly Lys Leu Asp Gln Lys Thr Ala Thr Ser Leu Leu Trp Lys Ser Phe
355 360 365

cca att acc aac att cct gaa gag ttg acc cca gtt gct acc gat aag 1152
Pro Ile Thr Asn Ile Pro Glu Glu Leu Thr Pro Val Ala Thr Asp Lys
370 375 380

tat tta gga gga aca gat gat cct gtc aaa aag aaa gat ttg ttt ttg 1200
Tyr Leu Gly Gly Thr Asp Asp Pro Val Lys Lys Lys Asp Leu Phe Leu
385 390 395 400

gat ctg atg gga gac gtt gtt ttc ggc gtc cca tca gtt acg gtt gct 1248
Asp Leu Met Gly Asp Val Val Phe Gly Val Pro Ser Val Thr Val Ala
405 410 415

cgt cag cat agg gac gca gga gct cca act tac atg tat gag ttc caa 1296
Arg Gln His Arg Asp Ala Gly Ala Pro Thr Tyr Met Tyr Glu Phe Gln
420 425 430

tat cgt cca tct ttt tca tcg gat aag aaa cct aag acg gtt att gga 1344
Tyr Arg Pro Ser Phe Ser Ser Asp Lys Lys Pro Lys Thr Val Ile Gly
435 440 445

gat cat gga gac gaa att ttt tcc gtc ttc ggc ttc cca ttg ctc aaa 1392
Asp His Gly Asp Glu Ile Phe Ser Val Phe Gly Phe Pro Leu Leu Lys
450 455 460

ggt gac gct cca gag gaa gaa gtc agt ctt tct aag acg gtt atg aaa 1440
Gly Asp Ala Pro Glu Glu Glu Val Ser Leu Ser Lys Thr Val Met Lys
465 470 475 480

ttt tgg gct aac ttc gcc cgt agt gga aac cct aat gga gaa gga ttg 1488
Phe Trp Ala Asn Phe Ala Arg Ser Gly Asn Pro Asn Gly Glu Gly Leu
485 490 495

cct cac tgg ccg atg tac gat caa gag gag gga tac ctt caa att ggt 1536
Pro His Trp Pro Met Tyr Asp Gln Glu Glu Gly Tyr Leu Gln Ile Gly
500 505 510

gtc aac act caa gca gct aag agg ttg aaa ggc gag gag gtt gct ttt 1584
Val Asn Thr Gln Ala Ala Lys Arg Leu Lys Gly Glu Glu Val Ala Phe
515 520 525

tgg aac gac ctg ttg tcc aag gaa gca gca aag aag cca cct aag ata 1632
Trp Asn Asp Leu Leu Ser Lys Glu Ala Ala Lys Lys Pro Pro Lys Ile
530 535 540
aag cac gcc gaa ttg taa 1650
Lys His Ala Glu Leu
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Val Phe Leu Gly Val Pro Phe Ala Lys Pro Pro Leu Gly Ser Leu Arg
35 40 45
Phe Ala Pro Pro Gln Pro Ala Glu Pro Trp Ser Phe Val Lys Asn Thr
50 55 60
Thr Ser Tyr Pro Pro Met Cys Cys Gln Asp Pro Val Ala Gly Gln Met
65 70 75 80
Thr Ser Asp Leu Phe Thr Asn Gly Lys Glu Arg Leu Ile Pro Glu Phe
85 90 95
Ser Glu Asp Cys Leu Tyr Leu Asn Ile Tyr Thr Pro Ala Asp Leu Thr
100 105 110
Lys Arg Gly Arg Leu Pro Val Met Val Trp Ile His Gly Gly Gly Leu
115 120 125
Val Val Gly Gly Ala Ser Thr Tyr Asp Gly Leu Ala Leu Ala Ala His
130 135 140
Glu Asn Val Val Val Val Ala Ile Gln Tyr Arg Leu Gly Ile Trp Gly
145 150 155 160
Phe Phe Ser Thr Gly Asp Glu His Ser Arg Gly Asn Trp Gly His Leu
165 170 175

Asp Gln Val Ala Ala Leu His Trp Val Gln Glu Asn Ile Ala Asn Phe
180 185 190
Gly Gly Asp Thr Gly Ser Val Thr Ile Phe Gly Glu Ser Ala Gly Gly
195 200 205
Glu Ser Val Ser Val Leu Val Leu Ser Pro Leu Ala Lys Asn Leu Phe
210 215 220
His Arg Ala Ile Ser Glu Ser Gly Val Ala Leu Thr Ala Gly Leu Val
225 230 235 240
Arg Lys Asp Met Lys Ala Ala Ala Lys Gln Ile Ala Val Leu Ala Gly
245 250 255
Cys Lys Thr Thr Thr Ser Ala Val Phe Val His Cys Leu Arg Gln Lys
260 265 270
Ser Glu Asp Glu Leu Leu Asp Leu Thr Leu Lys Met Lys Phe Leu Thr
275 280 285
Leu Asp Leu His Gly Asp Pro Arg Glu Ser His Pro Phe Leu Pro Thr
290 295 300
Val Val Asp Gly Val Leu Leu Pro Lys Met Pro Glu Glu Ile Leu Ala
305 310 315 320
Glu Lys Asp Phe Asn Thr Val Pro Tyr Ile Val Gly Ile Asn Lys Gln
325 330 335
Glu Phe Gly Trp Leu Leu Pro Thr Met Met Gly Phe Pro Leu Ser Glu
340 345 350
Gly Lys Leu Asp Gln Lys Thr Ala Thr Ser Leu Leu Trp Lys Ser Phe
355 360 365
Pro Ile Thr Asn Ile Pro Glu Glu Leu Thr Pro Val Ala Thr Asp Lys
370 375 380
Tyr Leu Gly Gly Thr Asp Asp Pro Val Lys Lys Lys Asp Leu Phe Leu
385 390 395 400
Asp Leu Met Gly Asp Val Val Phe Gly Val Pro Ser Val Thr Val Ala
405 410 415
Arg Gln His Arg Asp Ala Gly Ala Pro Thr Tyr Met Tyr Glu Phe Gln
420 425 430
Tyr Arg Pro Ser Phe Ser Ser Asp Lys Lys Pro Lys Thr Val Ile Gly
435 440 445
Asp His Gly Asp Glu Ile Phe Ser Val Phe Gly Phe Pro Leu Leu Lys
450 455 460
Gly Asp Ala Pro Glu Glu Glu Val Ser Leu Ser Lys Thr Val Met Lys
465 470 475 480
Phe Trp Ala Asn Phe Ala Arg Ser Gly Asn Pro Asn Gly Glu Gly Leu

485 490 495
Pro His Trp Pro Met Tyr Asp Gln Glu Glu Gly Tyr Leu Gln Ile Gly
500 505 510
Val Asn Thr Gln Ala Ala Lys Arg Leu Lys Gly Glu Glu Val Ala Phe
515 520 525
Trp Asn Asp Leu Leu Ser Lys Glu Ala Ala Lys Lys Pro Pro Lys Ile
530 535 540
Lys His Ala Glu Leu
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Val Leu Gly Lys Tyr Val Ser Leu Glu Gly Leu Ala Gln Pro Val Ala
20 25 30
gtc ttc ttg gga gtc cct ttt gct aag cca cct ctt gga tct ttg agg 144
Val Phe Leu Gly Val Pro Phe Ala Lys Pro Pro Leu Gly Ser Leu Arg
35 40 45
ttt gcc ccg ccg caa cca gca gag cca tgg tct ttc gtt aag aac act 192
Phe Ala Pro Pro Gln Pro Ala Glu Pro Trp Ser Phe Val Lys Asn Thr
50 55 60
act tcc tac cct cca atg tgt tgt caa gat cca gtc gta gaa caa atg 240
Thr Ser Tyr Pro Pro Met Cys Cys Gln Asp Pro Val Val Glu Gln Met
65 70 75 80
acg tca gac cta ttc act aac gga aag gaa agg ctt acc ctg gag ttc 288

Thr Ser Asp Leu Phe Thr Asn Gly Lys Glu Arg Leu Thr Leu Glu Phe
85 90 95

tct gag gat tgc ctt tac cta aat att tac act cct gcc gat ttg aca 336
Ser Glu Asp Cys Leu Tyr Leu Asn Ile Tyr Thr Pro Ala Asp Leu Thr
100 105 110

aag agg ggt agg ttg ccg gtt atg gtt tgg att cat gga gga ggt ttg 384
Lys Arg Gly Arg Leu Pro Val Met Val Trp Ile His Gly Gly Gly Leu
115 120 125

gtt gtt ggc gga gca tcc act tat gac gga ttg gct ctt gcc gcg cac 432
Val Val Gly Gly Ala Ser Thr Tyr Asp Gly Leu Ala Leu Ala Ala His
130 135 140

gag aac gtt gtt gtt gtt gct att caa tac cgt ttg ggt att tgg gga 480
Glu Asn Val Val Val Val Ala Ile Gln Tyr Arg Leu Gly Ile Trp Gly
145 150 155 160

ttt ttc tcc aca gga gat gag cat tcc cgt gga aac tgg ggc cat tta 528
Phe Phe Ser Thr Gly Asp Glu His Ser Arg Gly Asn Trp Gly His Leu
165 170 175

gat caa gtt gct gca ttg cat tgg gtc caa gaa aac att gct aac ttc 576
Asp Gln Val Ala Ala Leu His Trp Val Gln Glu Asn Ile Ala Asn Phe
180 185 190

gga ggt gat cca ggt tct gtt act att ttc gga gaa tca gca ggc gga 624
Gly Gly Asp Pro Gly Ser Val Thr Ile Phe Gly Glu Ser Ala Gly Gly
195 200 205

gag agt gtc tct gta ttg gtt tta tca cca tta gct aag aac ctt ttt 672
Glu Ser Val Ser Val Leu Val Leu Ser Pro Leu Ala Lys Asn Leu Phe
210 215 220

cat cgt gct att tcc gaa agt ggt gtt gct ttt acc gcc ggt ttg gtc 720
His Arg Ala Ile Ser Glu Ser Gly Val Ala Phe Thr Ala Gly Leu Val
225 230 235 240

agg aag gat atg aag gcc gca gcc aag cag atc gct gtc ctt gca gga 768
Arg Lys Asp Met Lys Ala Ala Ala Lys Gln Ile Ala Val Leu Ala Gly
245 250 255

tgc aaa act act act tcg gca gtc ttc gtg cat tgt ttg cgt caa aag 816
Cys Lys Thr Thr Thr Ser Ala Val Phe Val His Cys Leu Arg Gln Lys
260 265 270

tcg gaa gat gaa ctt tta gac ctc acg ttg aag atg aaa ttc ttt gcc 864
Ser Glu Asp Glu Leu Leu Asp Leu Thr Leu Lys Met Lys Phe Phe Ala
275 280 285

ctt gac tta cac gga gat cca agg gaa tct cac cct ttt ttg acc act 912

Leu Asp Leu His Gly Asp Pro Arg Glu Ser His Pro Phe Leu Thr Thr
290 295 300

gtt gtt gac gga gtt ttg ttg cct aag atg cct gag gaa atc ttg gcc 960
Val Val Asp Gly Val Leu Leu Pro Lys Met Pro Glu Glu Ile Leu Ala
305 310 315 320

gag aag gac ttt aac acc gtc cca tac att gtt gga att aac aag cag 1008
Glu Lys Asp Phe Asn Thr Val Pro Tyr Ile Val Gly Ile Asn Lys Gln
325 330 335

gag ttc gga tgg ctt ttg cca acg atg atg gga ttt cct ctt tcc gag 1056
Glu Phe Gly Trp Leu Leu Pro Thr Met Met Gly Phe Pro Leu Ser Glu
340 345 350

gga aag ttg gat caa aag acg gct acg tca ctt ttg tgg aag tcc tac 1104
Gly Lys Leu Asp Gln Lys Thr Ala Thr Ser Leu Leu Trp Lys Ser Tyr
355 360 365

cca att gcc aac att cct gaa gag ttg acc cca gtt gct acc gat aag 1152
Pro Ile Ala Asn Ile Pro Glu Glu Leu Thr Pro Val Ala Thr Asp Lys
370 375 380

tat tta gga gga aca gat gat cct gtc aaa aag aaa gat ttg ttt ttg 1200
Tyr Leu Gly Gly Thr Asp Asp Pro Val Lys Lys Lys Asp Leu Phe Leu
385 390 395 400

gat ctg atg gga gac gtt gtt ttc ggc gtc cca tca gtt acg gtt gct 1248
Asp Leu Met Gly Asp Val Val Phe Gly Val Pro Ser Val Thr Val Ala
405 410 415

cgt cag cat agg gac gca gga gct cca act tac atg tat gag ttc caa 1296
Arg Gln His Arg Asp Ala Gly Ala Pro Thr Tyr Met Tyr Glu Phe Gln
420 425 430

tat cgt cca tct ttt tca tcg gat aag aaa cct aag acg gtt att gga 1344
Tyr Arg Pro Ser Phe Ser Ser Asp Lys Lys Pro Lys Thr Val Ile Gly
435 440 445

gat cat gga gac gaa att ttt tcc gtc ttc ggc ttc cca ttg ctc aaa 1392
Asp His Gly Asp Glu Ile Phe Ser Val Phe Gly Phe Pro Leu Leu Lys
450 455 460

ggt gac gct cca gag gaa gaa gtc agt ctt tct aag acg gtt atg aaa 1440
Gly Asp Ala Pro Glu Glu Glu Val Ser Leu Ser Lys Thr Val Met Lys
465 470 475 480

ttt tgg gct aac ttc gcc cgt agt gga aac cct aat gga gaa gga ttg 1488
Phe Trp Ala Asn Phe Ala Arg Ser Gly Asn Pro Asn Gly Glu Gly Leu
485 490 495

cct cac tgg ccg atg tac gat caa gag gag gga tac ctt caa att ggt 1536

Pro His Trp Pro Met Tyr Asp Gln Glu Glu Gly Tyr Leu Gln Ile Gly
500 505 510

gtc aac act caa gca gct aag agg ttg aaa ggc gag gag gtt gct ttt 1584
Val Asn Thr Gln Ala Ala Lys Arg Leu Lys Gly Glu Glu Val Ala Phe
515 520 525

tgg aac gac ctg ttg tcc aag gaa gca gca aag aag cca cct aag ata 1632
Trp Asn Asp Leu Leu Ser Lys Glu Ala Ala Lys Lys Pro Pro Lys Ile
530 535 540

aag cac gcc gaa ttg taa 1650
Lys His Ala Glu Leu
545

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35 40 45
Phe Ala Pro Pro Gln Pro Ala Glu Pro Trp Ser Phe Val Lys Asn Thr
50 55 60
Thr Ser Tyr Pro Pro Met Cys Cys Gln Asp Pro Val Val Glu Gln Met
65 70 75 80
Thr Ser Asp Leu Phe Thr Asn Gly Lys Glu Arg Leu Thr Leu Glu Phe
85 90 95
Ser Glu Asp Cys Leu Tyr Leu Asn Ile Tyr Thr Pro Ala Asp Leu Thr
100 105 110
Lys Arg Gly Arg Leu Pro Val Met Val Trp Ile His Gly Gly Gly Leu
115 120 125
Val Val Gly Gly Ala Ser Thr Tyr Asp Gly Leu Ala Leu Ala Ala His

130 135 140

Glu Asn Val Val Val Val Ala Ile Gln Tyr Arg Leu Gly Ile Trp Gly
145 150 155 160

Phe Phe Ser Thr Gly Asp Glu His Ser Arg Gly Asn Trp Gly His Leu
165 170 175

Asp Gln Val Ala Ala Leu His Trp Val Gln Glu Asn Ile Ala Asn Phe
180 185 190

Gly Gly Asp Pro Gly Ser Val Thr Ile Phe Gly Glu Ser Ala Gly Gly
195 200 205

Glu Ser Val Ser Val Leu Val Leu Ser Pro Leu Ala Lys Asn Leu Phe
210 215 220

His Arg Ala Ile Ser Glu Ser Gly Val Ala Phe Thr Ala Gly Leu Val
225 230 235 240

Arg Lys Asp Met Lys Ala Ala Ala Lys Gln Ile Ala Val Leu Ala Gly
245 250 255

Cys Lys Thr Thr Thr Ser Ala Val Phe Val His Cys Leu Arg Gln Lys
260 265 270

Ser Glu Asp Glu Leu Leu Asp Leu Thr Leu Lys Met Lys Phe Phe Ala
275 280 285

Leu Asp Leu His Gly Asp Pro Arg Glu Ser His Pro Phe Leu Thr Thr
290 295 300

Val Val Asp Gly Val Leu Leu Pro Lys Met Pro Glu Glu Ile Leu Ala
305 310 315 320

Glu Lys Asp Phe Asn Thr Val Pro Tyr Ile Val Gly Ile Asn Lys Gln
325 330 335

Glu Phe Gly Trp Leu Leu Pro Thr Met Met Gly Phe Pro Leu Ser Glu
340 345 350

Gly Lys Leu Asp Gln Lys Thr Ala Thr Ser Leu Leu Trp Lys Ser Tyr
355 360 365

Pro Ile Ala Asn Ile Pro Glu Glu Leu Thr Pro Val Ala Thr Asp Lys
370 375 380

Tyr Leu Gly Gly Thr Asp Asp Pro Val Lys Lys Lys Asp Leu Phe Leu
385 390 395 400

Asp Leu Met Gly Asp Val Val Phe Gly Val Pro Ser Val Thr Val Ala
405 410 415

Arg Gln His Arg Asp Ala Gly Ala Pro Thr Tyr Met Tyr Glu Phe Gln
420 425 430

Tyr Arg Pro Ser Phe Ser Ser Asp Lys Lys Pro Lys Thr Val Ile Gly
435 440 445

Asp His Gly Asp Glu Ile Phe Ser Val Phe Gly Phe Pro Leu Leu Lys
450 455 460
Gly Asp Ala Pro Glu Glu Glu Val Ser Leu Ser Lys Thr Val Met Lys
465 470 475 480
Phe Trp Ala Asn Phe Ala Arg Ser Gly Asn Pro Asn Gly Glu Gly Lcu
485 490 495
Pro His Trp Pro Met Tyr Asp Gln Glu Glu Gly Tyr Leu Gln Ile Gly
500 505 510
Val Asn Thr Gln Ala Ala Lys Arg Leu Lys Gly Glu Glu Val Ala Phe
515 520 525
Trp Asn Asp Leu Leu Scr Lys Glu Ala Ala Lys Lys Pro Pro Lys Ile
530 535 540
Lys His Ala Glu Leu
545

<210>40
<211>1650
<212>DNA
<213>人工序列

<220>
<223>具有酯酶活性的蛋白

<220>
<221>CDS
<222>(1)..(1650)

<400>40

atg gga caa cca gct tcg ccg cct gtc gtt gat acc gct caa gga cga 48
Met Gly Gln Pro Ala Ser Pro Pro Val Val Asp Thr Ala Gln Gly Arg
1 5 10 15
gtc ttg ggt aag tac gtc tct tta gag gga ttg gca caa ccg gtt gct 96
Val Leu Gly Lys Tyr Val Ser Leu Glu Gly Leu Ala Gln Pro Val Ala
20 25 30
gtc ttc ttg gga gtc cct ttt gct aag cca cct ctt gga tct ttg agg 144
Val Phe Leu Gly Val Pro Phe Ala Lys Pro Pro Leu Gly Ser Leu Arg
35 40 45
ttt gcc ccg ccg caa cca gca gag cca tgg tct ttc gtt aag aac act 192
Phe Ala Pro Pro Gln Pro Ala Glu Pro Trp Ser Phe Val Lys Asn Thr
50 55 60

act tcc tac cct cca atg tgt tgt caa gaa cca atc gga gga caa atg 240
Thr Ser Tyr Pro Pro Met Cys Cys Gln Glu Pro Ile Gly Gly Gln Met
65 70 75 80
ctt tca gac cta ttc act aac aga aag gaa agg ctt atc ccg gag ttc 288
Leu Ser Asp Leu Phe Thr Asn Arg Lys Glu Arg Leu Ile Pro Glu Phe
85 90 95
tct gag gat tgc ctt tac cta aat att tac act cct gcc gat ttg aca 336
Ser Glu Asp Cys Leu Tyr Leu Asn Ile Tyr Thr Pro Ala Asp Leu Thr
100 105 110
aag agg ggt agg ttg ccg gtt atg gtt tgg att cat gga gga ggt ttg 384
Lys Arg Gly Arg Leu Pro Val Met Val Trp Ile His Gly Gly Gly Leu
115 120 125
gtt ctg ggc gga gca ccg atg tat gac gga gtg gtt ctt gcc gcg cac 432
Val Leu Gly Gly Ala Pro Met Tyr Asp Gly Val Val Leu Ala Ala His
130 135 140
gag aac gtt gtt gtt gtt gct att caa tac cgt ttg ggt att tgg gga 480
Glu Asn Val Val Val Val Ala Ile Gln Tyr Arg Leu Gly Ile Trp Gly
145 150 155 160
ttt ttc tcc aca gga gat gag cat tcc cgt gga aac tgg ggc cat tta 528
Phe Phe Ser Thr Gly Asp Glu His Ser Arg Gly Asn Trp Gly His Leu
165 170 175
gat caa gtt gct gca ttg cat tgg gtc caa gaa aac att gct aac ttc 576
Asp Gln Val Ala Ala Leu His Trp Val Gln Glu Asn Ile Ala Asn Phe
180 185 190
gga ggt gat cca ggt tct gtt act att ttc gga gaa tca gca ggc gga 624
Gly Gly Asp Pro Gly Ser Val Thr Ile Phe Gly Glu Ser Ala Gly Gly
195 200 205
gag agt gtc tct gta ttg gtt tta tca cca tta gct aag aac ctt ttt 672
Glu Ser Val Ser Val Leu Val Leu Ser Pro Leu Ala Lys Asn Leu Phe
210 215 220
cat cgt gct att tcc gaa agt ggt gtt gct ttt acc gcc ggt ttg gtc 720
His Arg Ala Ile Ser Glu Ser Gly Val Ala Phe Thr Ala Gly Leu Val
225 230 235 240
agg aag gat atg aag gcc gca gcc aag cag atc gct gtc ctt gca gga 768
Arg Lys Asp Met Lys Ala Ala Ala Lys Gln Ile Ala Val Leu Ala Gly
245 250 255
tgc aaa act act act tcg gca gtc ttc gtg cat tgt ttg cgt caa aag 816
Cys Lys Thr Thr Thr Ser Ala Val Phe Val His Cys Leu Arg Gln Lys
260 265 270

tcg gaa gat gaa ctt tta gac ctc acg ttg aag atg aaa ttc ttt gcc 864
Ser Glu Asp Glu Leu Leu Asp Leu Thr Leu Lys Met Lys Phe Phe Ala
275 280 285
ctt gac tta cac gga gat cca agg gaa tct cac cct ttt ttg acc act 912
Leu Asp Leu His Gly Asp Pro Arg Glu Ser His Pro Phe Leu Thr Thr
290 295 300
gtt gtt gac gga gtt ttg ttg cct aag atg cct gag gaa atc ttg gcc 960
Val Val Asp Gly Val Leu Leu Pro Lys Met Pro Glu Glu Ile Leu Ala
305 310 315 320
gag aag gac ttt aac acc gtc cca tac att gtt gga att aac aag cag 1008
Glu Lys Asp Phe Asn Thr Val Pro Tyr Ile Val Gly Ile Asn Lys Gln
325 330 335
gag ttc gga tgg ctt ttg cca acg atg atg gga ttt cct ctt tcc gag 1056
Glu Phe Gly Trp Leu Leu Pro Thr Met Met Gly Phe Pro Leu Ser Glu
340 345 350
gga aag ttg gat caa aag acg gct acg tca ctt ttg tgg aag tcc tac 1104
Gly Lys Leu Asp Gln Lys Thr Ala Thr Ser Leu Leu Trp Lys Ser Tyr
355 360 365
cca att gcc aac att cct gaa gag ttg acc cca gtt gct acc gat aag 1152
Pro Ile Ala Asn Ile Pro Glu Glu Leu Thr Pro Val Ala Thr Asp Lys
370 375 380
tat tta gga gga aca gat gat cct gtc aaa aag aaa gat ttg ttt ttg 1200
Tyr Leu Gly Gly Thr Asp Asp Pro Val Lys Lys Lys Asp Leu Phe Leu
385 390 395 400
gat ctg atg gga gac gtt gtt ttc ggc gtc cca tca gtt acg gtt gct 1248
Asp Leu Met Gly Asp Val Val Phe Gly Val Pro Ser Val Thr Val Ala
405 410 415
cgt cag cat agg gac gca gga gct cca act tac atg tat gag ttc caa 1296
Arg Gln His Arg Asp Ala Gly Ala Pro Thr Tyr Met Tyr Glu Phe Gln
420 425 430
tat cgt cca tct ttt tca tcg gat aag aaa cct aag acg gtt att gga 1344
Tyr Arg Pro Ser Phe Ser Ser Asp Lys Lys Pro Lys Thr Val Ile Gly
435 440 445
gat cat gga gac gaa att ttt tcc gtc ttc ggc ttc cca ttg ctc aaa 1392
Asp His Gly Asp Glu Ile Phe Ser Val Phe Gly Phe Pro Leu Leu Lys
450 455 460
ggt gac gct cca gag gaa gaa gtc agt ctt tct aag acg gtt atg aaa 1440
Gly Asp Ala Pro Glu Glu Glu Val Ser Leu Ser Lys Thr Val Met Lys
465 470 475 480

ttt tgg gct aac ttc gcc cgt agt gga aac cct aat gga gaa gga ttg 1488
Phe Trp Ala Asn Phe Ala Arg Ser Gly Asn Pro Asn Gly Glu Gly Leu
485 490 495
cct cac tgg ccg atg tac gat caa gag gag gga tac ctt caa att ggt 1536
Pro His Trp Pro Met Tyr Asp Gln Glu Glu Gly Tyr Leu Gln Ile Gly
500 505 510
gtc aac act caa gca gct aag agg ttg aaa ggc gag gag gtt gct ttt 1584
Val Asn Thr Gln Ala Ala Lys Arg Leu Lys Gly Glu Glu Val Ala Phe
515 520 525
tgg aac gac ctg ttg tcc aag gaa gca gca aag aag cca cct aag ata 1632
Trp Asn Asp Leu Leu Ser Lys Glu Ala Ala Lys Lys Pro Pro Lys Ile
530 535 540
aag cac gcc gaa ttg taa 1650
Lys His Ala Glu Leu
545

<210>41
<211>549
<212>PRT
<213>人工序列

<220>
<223>合成构建体

<400>41

Met Gly Gln Pro Ala Ser Pro Pro Val Val Asp Thr Ala Gln Gly Arg
1 5 10 15
Val Leu Gly Lys Tyr Val Ser Leu Glu Gly Leu Ala Gln Pro Val Ala
20 25 30
Val Phe Leu Gly Val Pro Phe Ala Lys Pro Pro Leu Gly Ser Leu Arg
35 40 45
Phe Ala Pro Pro Gln Pro Ala Glu Pro Trp Ser Phe Val Lys Asn Thr
50 55 60
Thr Ser Tyr Pro Pro Met Cys Cys Gln Glu Pro Ile Gly Gly Gln Met
65 70 75 80
Leu Ser Asp Leu Phe Thr Asn Arg Lys Glu Arg Leu Ile Pro Glu Phe
85 90 95
Ser Glu Asp Cys Leu Tyr Leu Asn Ile Tyr Thr Pro Ala Asp Leu Thr

100 105 110

Lys Arg Gly Arg Leu Pro Val Met Val Trp Ile His Gly Gly Gly Leu

115 120 125

Val Leu Gly Gly Ala Pro Met Tyr Asp Gly Val Val Leu Ala Ala His

130 135 140

Glu Asn Val Val Val Val Ala Ile Gln Tyr Arg Leu Gly Ile Trp Gly

145 150 155 160

Phe Phe Ser Thr Gly Asp Glu His Ser Arg Gly Asn Trp Gly His Leu

165 170 175

Asp Gln Val Ala Ala Leu His Trp Val Gln Glu Asn Ile Ala Asn Phe

180 185 190

Gly Gly Asp Pro Gly Ser Val Thr Ile Phe Gly Glu Ser Ala Gly Gly

195 200 205

Glu Ser Val Ser Val Leu Val Leu Ser Pro Leu Ala Lys Asn Leu Phe

210 215 220

His Arg Ala Ile Ser Glu Ser Gly Val Ala Phe Thr Ala Gly Leu Val

225 230 235 240

Arg Lys Asp Met Lys Ala Ala Ala Lys Gln Ile Ala Val Leu Ala Gly

245 250 255

Cys Lys Thr Thr Thr Ser Ala Val Phe Val His Cys Leu Arg Gln Lys

260 265 270

Ser Glu Asp Glu Leu Leu Asp Leu Thr Leu Lys Met Lys Phe Phe Ala

275 280 285

Leu Asp Leu His Gly Asp Pro Arg Glu Ser His Pro Phe Leu Thr Thr

290 295 300

Val Val Asp Gly Val Leu Leu Pro Lys Met Pro Glu Glu Ile Leu Ala

305 310 315 320

Glu Lys Asp Phe Asn Thr Val Pro Tyr Ile Val Gly Ile Asn Lys Gln

325 330 335

Glu Phe Gly Trp Leu Leu Pro Thr Met Met Gly Phe Pro Leu Ser Glu

340 345 350

Gly Lys Leu Asp Gln Lys Thr Ala Thr Ser Leu Leu Trp Lys Ser Tyr

355 360 365

Pro Ile Ala Asn Ile Pro Glu Glu Leu Thr Pro Val Ala Thr Asp Lys

370 375 380

Tyr Leu Gly Gly Thr Asp Asp Pro Val Lys Lys Lys Asp Leu Phe Leu

385 390 395 400

Asp Leu Met Gly Asp Val Val Phe Gly Val Pro Ser Val Thr Val Ala

405 410 415

Arg Gln His Arg Asp Ala Gly Ala Pro Thr Tyr Met Tyr Glu Phe Gln
420 425 430
Tyr Arg Pro Ser Phe Ser Ser Asp Lys Lys Pro Lys Thr Val Ile Gly
435 440 445
Asp His Gly Asp Glu Ile Phe Ser Val Phe Gly Phe Pro Leu Leu Lys
450 455 460
Gly Asp Ala Pro Glu Glu Glu Val Ser Leu Ser Lys Thr Val Met Lys
465 470 475 480
Phe Trp Ala Asn Phe Ala Arg Ser Gly Asn Pro Asn Gly Glu Gly Leu
485 490 495
Pro His Trp Pro Met Tyr Asp Gln Glu Glu Gly Tyr Leu Gln Ile Gly
500 505 510
Val Asn Thr Gln Ala Ala Lys Arg Leu Lys Gly Glu Glu Val Ala Phe
515 520 525
Trp Asn Asp Leu Leu Ser Lys Glu Ala Ala Lys Lys Pro Pro Lys Ile
530 535 540
Lys His Ala Glu Leu
545

<210>42
<211>1650
<212>DNA
<213>人工序列

<220>
<223>具有酯酶活性的蛋白

<220>
<221>CDS
<222>(1)..(1650)

<400>42
atg gga caa cca gct tcg ccg cct gtc gtt gat acc gct caa gga cga 48
Met Gly Gln Pro Ala Ser Pro Pro Val Val Asp Thr Ala Gln Gly Arg
1 5 10 15
gtc ttg ggt aag tac gtc tct tta gag gga ttg gca caa ccg gtt gct 96
Val Leu Gly Lys Tyr Val Ser Leu Glu Gly Leu Ala Gln Pro Val Ala
20 25 30
gtc ttc ttg gga gtc cct ttt gct aag cca cct ctt gga tct ttg agg 144

Val Phe Leu Gly Val Pro Phe Ala Lys Pro Pro Leu Gly Ser Leu Arg
35 40 45

ttt gcc ccg ccg caa cca gca gag cca tgg tct ttc gtt aag aac act 192
Phe Ala Pro Pro Gln Pro Ala Glu Pro Trp Ser Phe Val Lys Asn Thr
50 55 60

act tcc tac cct cca atg tgt tgt caa gaa cca atc gga gga caa atg 240
Thr Ser Tyr Pro Pro Met Cys Cys Gln Glu Pro Ile Gly Gly Gln Met
65 70 75 80

ctt tca gac cta ttc act aac aga aag gaa agg ctt atc ccg gag ttc 288
Leu Ser Asp Leu Phe Thr Asn Arg Lys Glu Arg Leu Ile Pro Glu Phe
85 90 95

tct gag gat tgc ctt tac cta aat att tac act cct gcc gat ttg aca 336
Ser Glu Asp Cys Leu Tyr Leu Asn Ile Tyr Thr Pro Ala Asp Leu Thr
100 105 110

aag agg ggt agg ttg ccg gtt atg gtt tgg att cat gga gga ggt ttg 384
Lys Arg Gly Arg Leu Pro Val Met Val Trp Ile His Gly Gly Gly Leu
115 120 125

gtt gtt ggc gga gca tcc act tat gac gga ttg gct ctt gcc gcg cac 432
Val Val Gly Gly Ala Ser Thr Tyr Asp Gly Leu Ala Leu Ala Ala His
130 135 140

gag aac gtt gtt gtt gtt gct att caa tac cgt ttg ggt att tgg gga 480
Glu Asn Val Val Val Val Ala Ile Gln Tyr Arg Leu Gly Ile Trp Gly
145 150 155 160

ttt ttc tcc aca gga gat gag cat tcc cgt gga aac tgg ggc cat tta 528
Phe Phe Ser Thr Gly Asp Glu His Ser Arg Gly Asn Trp Gly His Leu
165 170 175

gat caa gtt gct gca ttg cat tgg gtc caa gaa aac att gct aac ttc 576
Asp Gln Val Ala Ala Leu His Trp Val Gln Glu Asn Ile Ala Asn Phe
180 185 190

gga ggt gat cca ggt tct gtt act att ttc gga gaa tca gca ggc gga 624
Gly Gly Asp Pro Gly Ser Val Thr Ile Phe Gly Glu Ser Ala Gly Gly
195 200 205

gag agt gtc tct gta ttg gtt tta tca cca tta gct aag aac ctt ttt 672
Glu Ser Val Ser Val Leu Val Leu Ser Pro Leu Ala Lys Asn Leu Phe
210 215 220

cat cgt gct att tcc gaa agt ggt gtt gct tta acc gtc gct ttg gtc 720
His Arg Ala Ile Ser Glu Ser Gly Val Ala Leu Thr Val Ala Leu Val
225 230 235 240

agg aag gat atg aag gcc gca gcc aag cag atc gct gtc ctt gca gga 768

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Arg Lys Asp Met Lys Ala Ala Ala Lys Gln Ile Ala Val Leu Ala Gly
                245                 250                 255
tgc aaa act act act tcg gca gtc ttc gtg cat tgt ttg cgt caa aag    816
Cys Lys Thr Thr Thr Ser Ala Val Phe Val His Cys Leu Arg Gln Lys
            260                 265                 270
tcg gaa gat gaa ctt tta gac ctc acg ttg aag atg aaa ttc tta gcc    864
Ser Glu Asp Glu Leu Leu Asp Leu Thr Leu Lys Met Lys Phe Leu Ala
        275                 280                 285
ctt gac tta cac gga gat cca agg gaa tct cac cct ttt ttg acc act    912
Leu Asp Leu His Gly Asp Pro Arg Glu Ser His Pro Phe Leu Thr Thr
    290                 295                 300
gtt gtt gac gga gtt ttg ttg cct aag atg cct gag gaa atc ttg gcc    960
Val Val Asp Gly Val Leu Leu Pro Lys Met Pro Glu Glu Ile Leu Ala
305                 310                 315                 320
gag aag gac ttt aac acc gtc cca tac att gtt gga att aac aag cag   1008
Glu Lys Asp Phe Asn Thr Val Pro Tyr Ile Val Gly Ile Asn Lys Gln
                325                 330                 335
gag ttc gga tgg ctt ttg cca acg atg atg gga ttt cct ctt tcc gag   1056
Glu Phe Gly Trp Leu Leu Pro Thr Met Met Gly Phe Pro Leu Ser Glu
            340                 345                 350
gga aag ttg gat caa aag acg gct acg tca ctt ttg tgg aag tcc tac   1104
Gly Lys Leu Asp Gln Lys Thr Ala Thr Ser Leu Leu Trp Lys Ser Tyr
        355                 360                 365
cca att gcc aac att cct gaa gag ttg acc cca gtt gct acc gat aag   1152
Pro Ile Ala Asn Ile Pro Glu Glu Leu Thr Pro Val Ala Thr Asp Lys
    370                 375                 380
tat tta gga gga aca gat gat cct gtc aaa aag aaa gat ttg ttt ttg   1200
Tyr Leu Gly Gly Thr Asp Asp Pro Val Lys Lys Lys Asp Leu Phe Leu
385                 390                 395                 400
gat ctg atg gga gac gtt gtt ttc ggc gtc cca tca gtt acg gtt gct   1248
Asp Leu Met Gly Asp Val Val Phe Gly Val Pro Ser Val Thr Val Ala
                405                 410                 415
cgt cag cat agg gac gca gga gct cca act tac atg tat gag ttc caa   1296
Arg Gln His Arg Asp Ala Gly Ala Pro Thr Tyr Met Tyr Glu Phe Gln
            420                 425                 430
tat cgt cca tct ttt tca tcg gat aag aaa cct aag acg gtt att gga   1344
Tyr Arg Pro Ser Phe Ser Ser Asp Lys Lys Pro Lys Thr Val Ile Gly
        435                 440                 445
gat cat gga gac gaa att ttt tcc gtc ttc ggc ttc cca ttg ctc aaa   1392
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Asp His Gly Asp Glu Ile Phe Ser Val Phe Gly Phe Pro Leu Leu Lys
450 455 460

ggt gac gct cca gag gaa gaa gtc agt ctt tct aag acg gtt atg aaa 1440
Gly Asp Ala Pro Glu Glu Glu Val Ser Leu Ser Lys Thr Val Met Lys
465 470 475 480

ttt tgg gct aac ttc gcc cgt agt gga aac cct aat gga gaa gga ttg 1488
Phe Trp Ala Asn Phe Ala Arg Ser Gly Asn Pro Asn Gly Glu Gly Leu
485 490 495

cct cac tgg ccg atg tac gat caa gag gag gga tac ctt caa att ggt 1536
Pro His Trp Pro Met Tyr Asp Gln Glu Glu Gly Tyr Leu Gln Ile Gly
500 505 510

gtc aac act caa gca gct aag agg ttg aaa ggc gag gag gtt gct ttt 1584
Val Asn Thr Gln Ala Ala Lys Arg Leu Lys Gly Glu Glu Val Ala Phe
515 520 525

tgg aac gac ctg ttg tcc aag gaa gca gca aag aag cca cct aag ata 1632
Trp Asn Asp Leu Leu Ser Lys Glu Ala Ala Lys Lys Pro Pro Lys Ile
530 535 540

aag cac gcc gaa ttg taa 1650
Lys His Ala Glu Leu
545

<210>43
<211>549
<212>PRT
<213>人工序列

<220>
<223>合成构建体

<400>43

Met Gly Gln Pro Ala Ser Pro Pro Val Val Asp Thr Ala Gln Gly Arg
1 5 10 15
Val Leu Gly Lys Tyr Val Ser Leu Glu Gly Leu Ala Gln Pro Val Ala
20 25 30
Val Phe Leu Gly Val Pro Phe Ala Lys Pro Pro Leu Gly Ser Leu Arg
35 40 45
Phe Ala Pro Pro Gln Pro Ala Glu Pro Trp Ser Phe Val Lys Asn Thr
50 55 60

Thr Ser Tyr Pro Pro Met Cys Cys Gln Glu Pro Ile Gly Gly Gln Met
65 70 75 80
Leu Ser Asp Leu Phe Thr Asn Arg Lys Glu Arg Leu Ile Pro Glu Phe
85 90 95
Ser Glu Asp Cys Leu Tyr Leu Asn Ile Tyr Thr Pro Ala Asp Leu Thr
100 105 110
Lys Arg Gly Arg Leu Pro Val Met Val Trp Ile His Gly Gly Gly Leu
115 120 125
Val Val Gly Gly Ala Ser Thr Tyr Asp Gly Leu Ala Leu Ala Ala His
130 135 140
Glu Asn Val Val Val Val Ala Ile Gln Tyr Arg Leu Gly Ile Trp Gly
145 150 155 160
Phe Phe Ser Thr Gly Asp Glu His Ser Arg Gly Asn Trp Gly His Leu
165 170 175
Asp Gln Val Ala Ala Leu His Trp Val Gln Glu Asn Ile Ala Asn Phe
180 185 190
Gly Gly Asp Pro Gly Ser Val Thr Ile Phe Gly Glu Ser Ala Gly Gly
195 200 205
Glu Ser Val Ser Val Leu Val Leu Ser Pro Leu Ala Lys Asn Leu Phe
210 215 220
His Arg Ala Ile Ser Glu Ser Gly Val Ala Leu Thr Val Ala Leu Val
225 230 235 240
Arg Lys Asp Met Lys Ala Ala Ala Lys Gln Ile Ala Val Leu Ala Gly
245 250 255
Cys Lys Thr Thr Thr Ser Ala Val Phe Val His Cys Leu Arg Gln Lys
260 265 270
Ser Glu Asp Glu Leu Leu Asp Leu Thr Leu Lys Met Lys Phe Leu Ala
275 280 285
Leu Asp Leu His Gly Asp Pro Arg Glu Ser His Pro Phe Leu Thr Thr
290 295 300
Val Val Asp Gly Val Leu Leu Pro Lys Met Pro Glu Glu Ile Leu Ala
305 310 315 320
Glu Lys Asp Phe Asn Thr Val Pro Tyr Ile Val Gly Ile Asn Lys Gln
325 330 335
Glu Phe Gly Trp Leu Leu Pro Thr Met Met Gly Phe Pro Leu Ser Glu
340 345 350
Gly Lys Leu Asp Gln Lys Thr Ala Thr Ser Leu Leu Trp Lys Ser Tyr
355 360 365
Pro Ile Ala Asn Ile Pro Glu Glu Leu Thr Pro Val Ala Thr Asp Lys

370 375 380
Tyr Leu Gly Gly Thr Asp Asp Pro Val Lys Lys Lys Asp Leu Phe Leu
385 390 395 400
Asp Leu Met Gly Asp Val Val Phe Gly Val Pro Ser Val Thr Val Ala
405 410 415
Arg Gln His Arg Asp Ala Gly Ala Pro Thr Tyr Met Tyr Glu Phe Gln
420 425 430
Tyr Arg Pro Ser Phe Ser Ser Asp Lys Lys Pro Lys Thr Val Ile Gly
435 440 445
Asp His Gly Asp Glu Ile Phe Ser Val Phe Gly Phe Pro Leu Leu Lys
450 455 460
Gly Asp Ala Pro Glu Glu Glu Val Ser Leu Ser Lys Thr Val Met Lys
465 470 475 480
Phe Trp Ala Asn Phe Ala Arg Ser Gly Asn Pro Asn Gly Glu Gly Leu
485 490 495
Pro His Trp Pro Met Tyr Asp Gln Glu Glu Gly Tyr Leu Gln Ile Gly
500 505 510
Val Asn Thr Gln Ala Ala Lys Arg Leu Lys Gly Glu Glu Val Ala Phe
515 520 525
Trp Asn Asp Leu Leu Ser Lys Glu Ala Ala Lys Lys Pro Pro Lys Ile
530 535 540
Lys His Ala Glu Leu
545

<210>44
<211>1650
<212>DNA
<213>人工序列

<220>
<223>具有酯酶活性的蛋白

<220>
<221>CDS
<222>(1)..(1650)

<400>44
atg gga caa cca gct tcg ccg cct gtc gtt gat acc gct caa gga cga 48
Met Gly Gln Pro Ala Ser Pro Pro Val Val Asp Thr Ala Gln Gly Arg

1 5 10 15

gtc ttg ggt aag tac gtc tct tta gag gga ttg gca caa ccg gtt gct 96
Val Leu Gly Lys Tyr Val Ser Leu Glu Gly Leu Ala Gln Pro Val Ala
20 25 30

gtc ttc ttg gga gtc cct ttt gct aag cca cct ctt gga tct ttg agg 144
Val Phe Leu Gly Val Pro Phe Ala Lys Pro Pro Leu Gly Ser Leu Arg
35 40 45

ttt gcc ccg ccg caa cca gca gag cca tgg tct ttc gtt aag aac act 192
Phe Ala Pro Pro Gln Pro Ala Glu Pro Trp Ser Phe Val Lys Asn Thr
50 55 60

act tcc tac cct cca atg tgt tgt caa gaa cca atc gga gga caa atg 240
Thr Ser Tyr Pro Pro Met Cys Cys Gln Glu Pro Ile Gly Gly Gln Met
65 70 75 80

ctt tca gac cta ttc act aac aga aag gaa agg ctt atc ccg gag ttc 288
Leu Ser Asp Leu Phe Thr Asn Arg Lys Glu Arg Leu Ile Pro Glu Phe
85 90 95

tct gag gat tgc ctt tac cta aat att tac act cct gcc gat ttg aca 336
Ser Glu Asp Cys Leu Tyr Leu Asn Ile Tyr Thr Pro Ala Asp Leu Thr
100 105 110

aag agg ggt agg ttg ccg gtt atg gtt tgg att cat gga gga ggt ttg 384
Lys Arg Gly Arg Leu Pro Val Met Val Trp Ile His Gly Gly Gly Leu
115 120 125

gtt gtt ggc gga gca tcc act tat gac gga ttg gct ctt gcc gcg cac 432
Val Val Gly Gly Ala Ser Thr Tyr Asp Gly Leu Ala Leu Ala Ala His
130 135 140

gag aac gtt gtt gtt gtt gct att caa tac cgt ttg ggt att tgg gga 480
Glu Asn Val Val Val Val Ala Ile Gln Tyr Arg Leu Gly Ile Trp Gly
145 150 155 160

ttt ttc tcc aca gga gat gag cat tcc cgt gga aac tgg ggc cat tta 528
Phe Phe Ser Thr Gly Asp Glu His Ser Arg Gly Asn Trp Gly His Leu
165 170 175

gat caa gtt gct gca ttg cat tgg gtc caa gaa aac att gct aac ttc 576
Asp Gln Val Ala Ala Leu His Trp Val Gln Glu Asn Ile Ala Asn Phe
180 185 190

gga ggt gat cca ggt tct gtt act att ttc gga gaa tca gca ggc gga 624
Gly Gly Asp Pro Gly Ser Val Thr Ile Phe Gly Glu Ser Ala Gly Gly
195 200 205

gag agt gtc tct gta ttg gtt tta tca cca tta gct aag aac ctt ttt 672
Glu Ser Val Ser Val Leu Val Leu Ser Pro Leu Ala Lys Asn Leu Phe

210 215 220

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Arg Lys Asp Met Lys Ala Ala Ala Lys Gln Ile Ala Val Leu Ala Gly
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Cys Lys Thr Thr Thr Ser Ala Val Phe Val His Cys Leu Arg Gln Lys
260 265 270

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Ser Glu Asp Glu Leu Leu Asp Leu Thr Leu Lys Met Lys Phe Leu Thr
275 280 285

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Leu Asp Phe His Gly Asp Gln Arg Glu Ser His Pro Phe Leu Pro Thr
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Val Val Asp Gly Val Leu Leu Pro Lys Met Pro Glu Glu Ile Leu Ala
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Glu Phe Gly Trp Leu Leu Pro Thr Met Met Gly Phe Pro Leu Ser Glu
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Gly Lys Leu Asp Gln Lys Thr Ala Thr Ser Leu Leu Trp Lys Ser Tyr
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Tyr Leu Gly Gly Thr Asp Asp Pro Val Lys Lys Lys Asp Leu Phe Leu
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Arg Gln His Arg Asp Ala Gly Ala Pro Thr Tyr Met Tyr Glu Phe Gln

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Tyr Arg Pro Ser Phe Ser Ser Asp Lys Lys Pro Lys Thr Val Ile Gly
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Phe Trp Ala Asn Phe Ala Arg Ser Gly Asn Pro Asn Gly Glu Gly Leu
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Val Asn Thr Gln Ala Ala Lys Arg Leu Lys Gly Glu Glu Val Ala Phe
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35 40 45

Phe Ala Pro Pro Gln Pro Ala Glu Pro Trp Ser Phe Val Lys Asn Thr
50 55 60

Thr Ser Tyr Pro Pro Met Cys Cys Gln Glu Pro Ile Gly Gly Gln Met
65 70 75 80

Leu Ser Asp Leu Phe Thr Asn Arg Lys Glu Arg Leu Ile Pro Glu Phe
85 90 95

Ser Glu Asp Cys Leu Tyr Leu Asn Ile Tyr Thr Pro Ala Asp Leu Thr
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Lys Arg Gly Arg Leu Pro Val Met Val Trp Ile His Gly Gly Gly Leu
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Val Val Gly Gly Ala Ser Thr Tyr Asp Gly Leu Ala Leu Ala Ala His
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Glu Asn Val Val Val Val Ala Ile Gln Tyr Arg Leu Gly Ile Trp Gly
145 150 155 160

Phe Phe Ser Thr Gly Asp Glu His Ser Arg Gly Asn Trp Gly His Leu
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Asp Gln Val Ala Ala Leu His Trp Val Gln Glu Asn Ile Ala Asn Phe
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Gly Gly Asp Pro Gly Ser Val Thr Ile Phe Gly Glu Ser Ala Gly Gly
195 200 205

Glu Ser Val Ser Val Leu Val Leu Ser Pro Leu Ala Lys Asn Leu Phe
210 215 220

His Arg Ala Ile Ser Glu Ser Gly Val Ala Phe Thr Ala Gly Leu Val
225 230 235 240

Arg Lys Asp Met Lys Ala Ala Ala Lys Gln Ile Ala Val Leu Ala Gly
245 250 255

Cys Lys Thr Thr Thr Ser Ala Val Phe Val His Cys Leu Arg Gln Lys
260 265 270

Ser Glu Asp Glu Leu Leu Asp Leu Thr Leu Lys Met Lys Phe Leu Thr
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Leu Asp Phe His Gly Asp Gln Arg Glu Ser His Pro Phe Leu Pro Thr
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Val Val Asp Gly Val Leu Leu Pro Lys Met Pro Glu Glu Ile Leu Ala
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Glu Lys Asp Phe Asn Thr Val Pro Tyr Ile Val Gly Ile Asn Lys Gln
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Glu Phe Gly Trp Leu Leu Pro Thr Met Met Gly Phe Pro Leu Ser Glu
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Gly Lys Leu Asp Gln Lys Thr Ala Thr Ser Leu Leu Trp Lys Ser Tyr
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Pro Ile Ala Asn Ile Pro Glu Glu Leu Thr Pro Val Ala Thr Asp Lys
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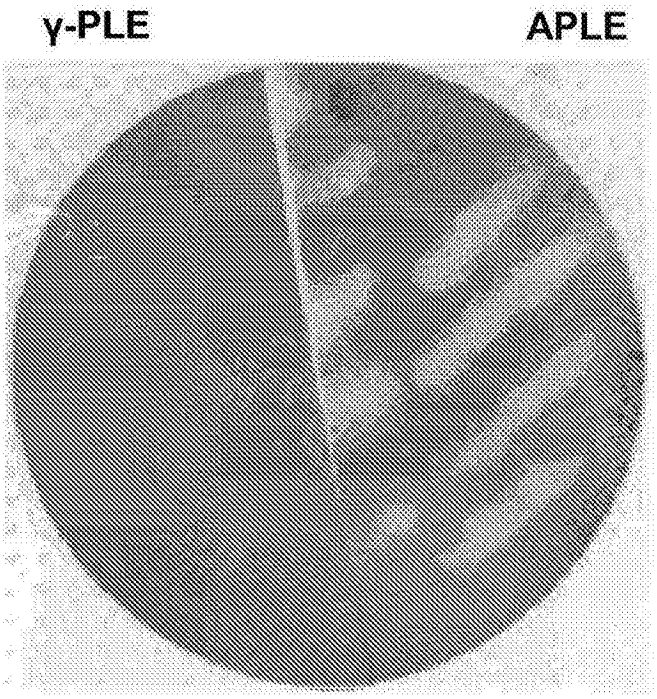

图1a

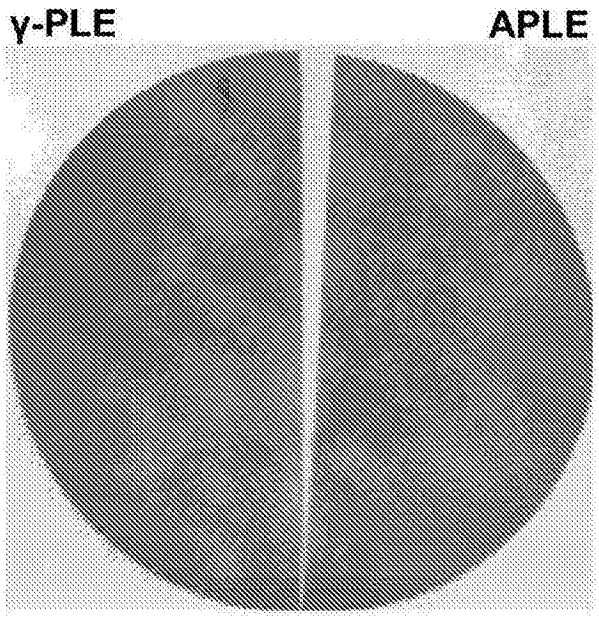

图1b

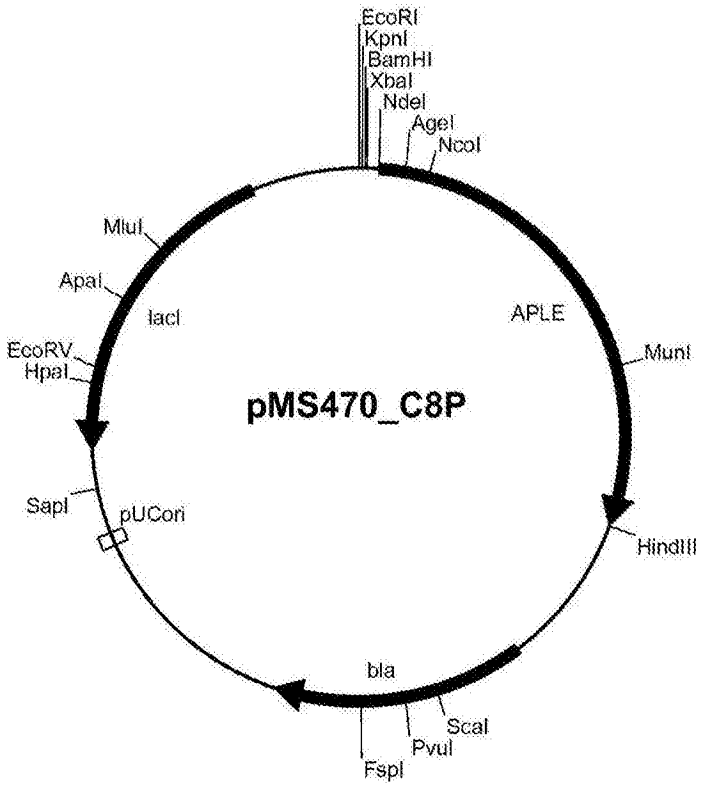
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图2a

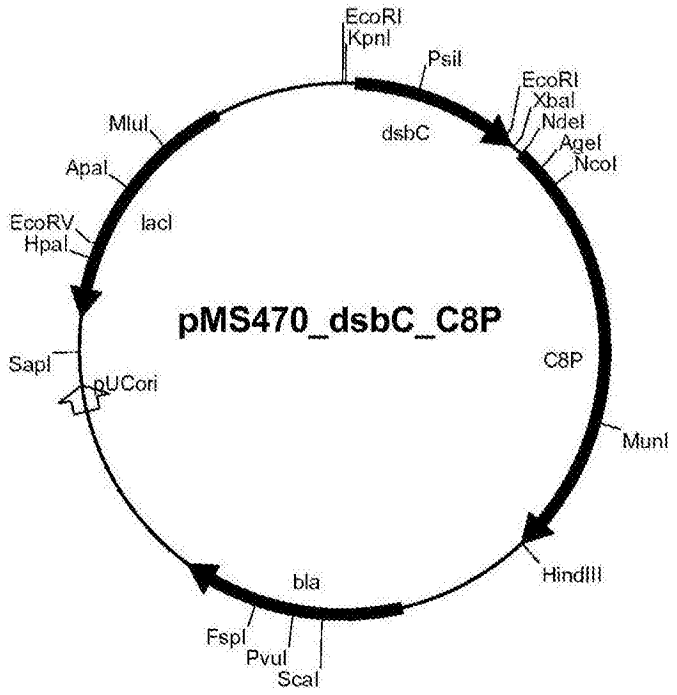
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图2b

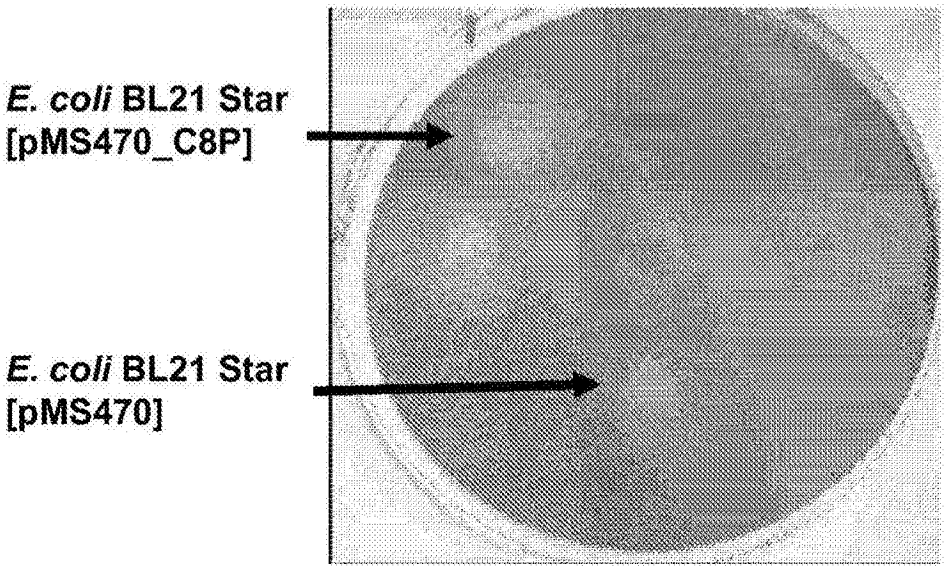

图3

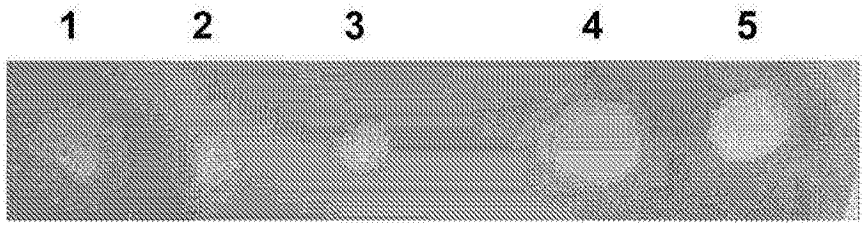

图4a

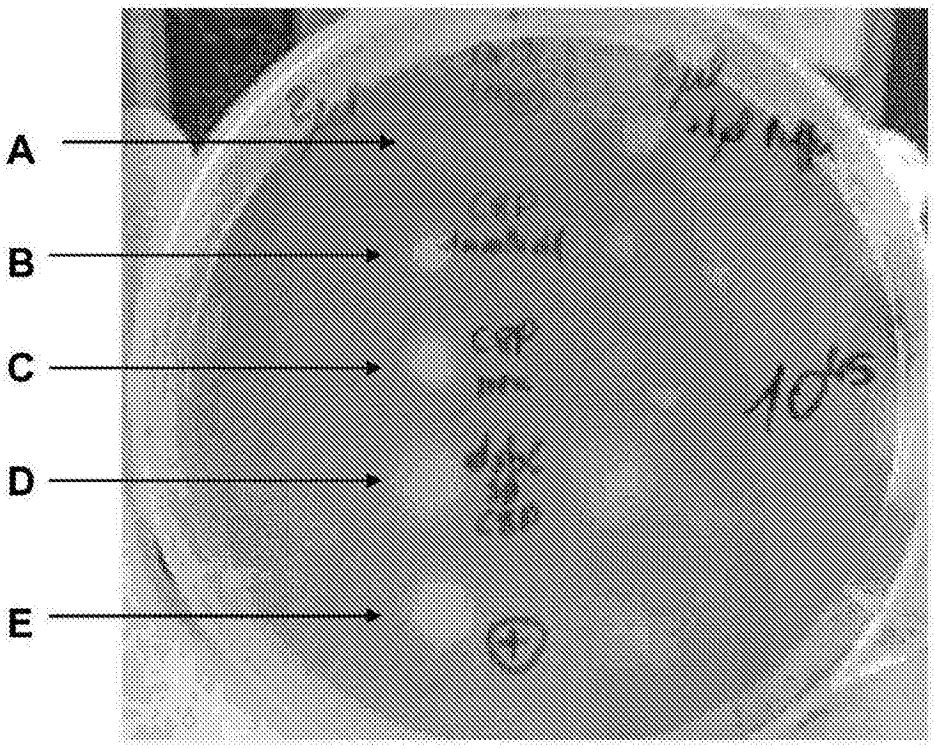

图4b

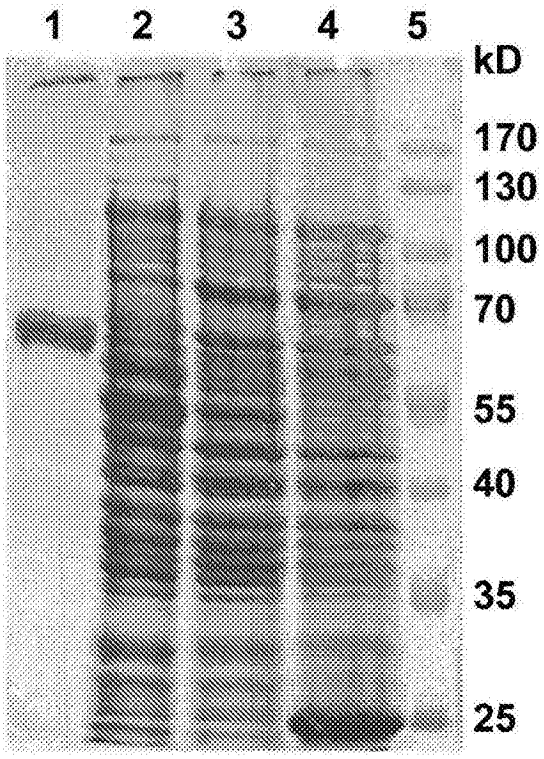

图5a

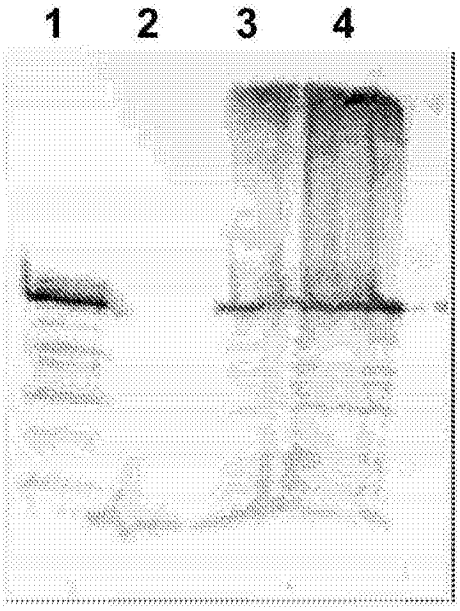

图5b

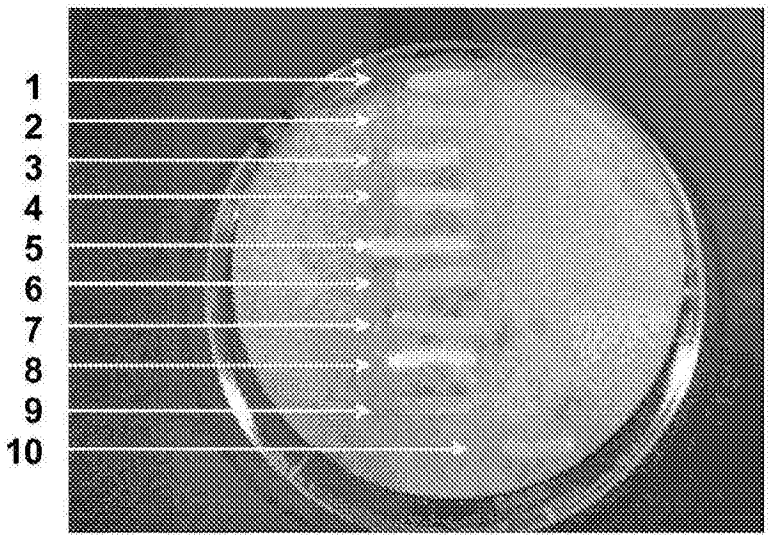

图6