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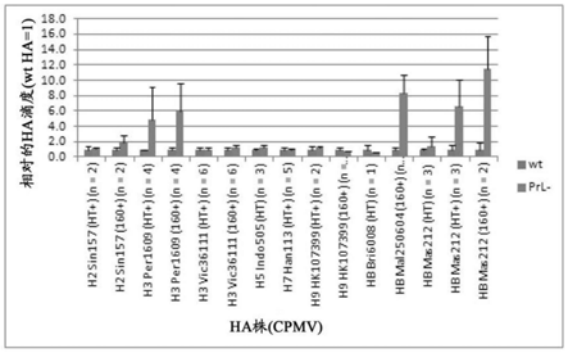
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(54)发明名称

植物中流感样病毒颗粒的产生

(57)摘要

提供了在植物中产生包含修饰的血凝素的病毒样颗粒(VLP)的方法。所述方法包括将包含调控区的核酸引入所述植物或所述植物的部分，所述调控区在植物中有活性并且与编码修饰的流感血凝素(HA)蛋白质的核苷酸序列可操作地连接，所述修饰的HA蛋白包含修饰的蛋白水解环。在允许所述核酸表达的条件下培养所述植物或所述植物的部分后，从而产生所述VLP。所述修饰的蛋白水解环可包含呈现出减少或废除的蛋白酶切割的一个或多个蛋白酶切割位点。编码所述HA的所述核苷酸序列可选自:B HA、C、H1、H2、H3、H4、H5、H6、H7、H8、H9、H10、H11、H12、H13、H14、H15和H16。也描述了通过所述方法产生的病毒样颗粒(VLP)，以及表达所述VLP的植物。所述病毒样颗粒(VLP)可包含植物-特异性N-聚糖，或修饰的N-聚糖。



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1.修饰的流感血凝素(HA),其完全缺失了位于HA1亚基和HA2亚基之间的蛋白水解环,其中:

所述修饰的流感HA是与SEQ ID NO:98的氨基酸25-576、SEQ ID NO:100的氨基酸25-576、SEQ ID NO:168的氨基酸25-577、SEQ ID NO:194的氨基酸25-576或SEQ ID NO:199的氨基酸25-576具有至少90%的序列同一性的流感B型HA,

所述修饰的流感HA是与SEQ ID NO:143的氨基酸25-559或SEQ ID NO:147的氨基酸25-559具有至少90%的序列同一性的流感A型H3亚型HA,或

所述修饰的流感HA是与SEQ ID NO:154的氨基酸25-549具有至少90%的序列同一性的流感A型H7亚型HA。

2.如权利要求1所述的修饰的流感HA,其中所述修饰的HA包含天然的HA信号肽或非天然的HA信号肽。

3.病毒样颗粒(VLP),其包含权利要求1或2所述的修饰的流感HA。

4.如权利要求3所述的VLP,其还包含一种或多种源自植物的脂质。

5.如权利要求4所述的VLP,其中所述VLP包含具有植物-特异性N-聚糖或修饰的N-聚糖的HA蛋白。

6.核酸,其编码权利要求1或2所述的修饰的流感HA。

7.组合物,其包含用于诱导免疫应答的有效剂量的权利要求3、4或5所述的VLP,以及药学上可接受的载体。

8.疫苗,其包含用于诱导免疫应答的有效剂量的权利要求7所述的组合物。

9.权利要求7所述的组合物在制备用于在对象中诱导对流感病毒感染的免疫力的疫苗中的用途。

10.如权利要求9所述的用途,其中所述疫苗用于口服施用、皮内施用、鼻内施用、肌内施用、腹膜内施用、静脉内施用或皮下施用于所述对象。

11.植物提取物,其包含权利要求3、4或5所述的VLP。

12.植物提取物,其包含权利要求1或2所述的HA。

植物中流感样病毒颗粒的产生

发明领域

[0001] 本发明涉及在植物中产生病毒样颗粒。

[0002] 发明背景

[0003] 流感是由正粘液病毒科的RNA病毒引起的。存在三种类型的这些病毒，并且它们引起三种不同类型的流感：A型、B型和C型。流感病毒的A型病毒感染哺乳动物(人、猪、雪豹、马)和禽类。该病毒类型对人类来说非常重要，因为这是引起全世界流行病的病毒类型。B型流感病毒(也被简称为流感B)仅感染人类。其偶尔引起流感的局部爆发。C型流感病毒同样仅感染人类。当它们未成熟时它们感染大多数人并且极少引起严重的疾病。

[0004] 接种疫苗通过诱导对象在感染之前建立防御，提供免受由类似病原体引起的疾病的保护。按照惯例，通过使用减毒活形式或完全灭活形式的感染性病原体作为免疫原实现接种疫苗。为了避免使用完整病毒(如杀死的或减毒的病毒)作为疫苗的危险，重组的病毒蛋白，例如亚基，已被用作疫苗。肽和亚基疫苗均为有许多潜在限制的对象。由于不正确的折叠或弱的抗原递呈，亚基疫苗可能呈现弱的免疫原性。主要的问题是难以确保被改造的蛋白的构象模仿抗原在其自然环境中的构象。合适的佐剂以及在肽的情况下的载体蛋白必须被用来促进免疫应答。此外这些疫苗主要引起体液应答，并且因此可能未能激发有效的免疫力。亚基疫苗通常对疾病是无效的，可证明完整的灭活病毒能提供保护。

[0005] 病毒样颗粒(VLP)为用于免疫原性组合物中的包含物的潜在候选物。VLP极其类似于成熟病毒粒子，但是它们不含有病毒基因组材料。因此，VLP本质上是非复制性的，这使得它们用于作为疫苗施用是安全的。此外，可改造VLP以在VLP的表面上表达病毒糖蛋白，其为它们最天然的生理性结构。而且，因为VLP类似于完整的病毒粒子并且为多价的微粒结构，所以VLP在诱导针对糖蛋白的中和抗体中，比可溶性包膜蛋白抗原更有效。

[0006] VLP已在植物(WO2009/009876;WO 2009/076778;WO 2010/003225;WO 2010/003235;WO 2011/03522;WO 2010/148511;其通过引用并入本文)，以及昆虫和哺乳动物系统中(Noad,R.and Roy,P.,2003,Trends Microbiol 11:438-44;Neumann等,2000,J.Virol.,74,547-551)产生。Latham和Galarza(2001,J.Virol.,75,6154-6165)报道了，在感染有共表达血凝素(HA)、神经氨酸酶(NA)、M1和M2基因的重组杆状病毒的昆虫细胞中，流感VLP的形成。该研究证明，在真核细胞中经共表达的流感病毒粒子蛋白的自我组装，并且M1基质蛋白是用于VLP的产生所必需的。Gomez-Puertas等(1999,J.Gen.Virol,80,1635-1645)显示M2的共表达完全地阻碍了CAT RNA传送至MDCK培养基中。

[0007] 流感病毒的刺突糖蛋白血凝素(HA)对病毒颗粒被宿主细胞摄取非常重要。它负责病毒颗粒附着于包括唾液酸的细胞受体上，并且它涉及通过病毒包膜与细胞膜融合的病毒侵入。融合活性以及从而病毒的感染性，取决于将HA前体分子HA0切割为二硫键连接的多肽链HA1和HA2。切割随后pH-依赖的构象变化导致调节膜融合的跨膜多肽HA2在氨基端处的高度保守疏水肽的暴露和再定位。

[0008] HA被合成为前体蛋白HA0，其经过蛋白水解作用加工为通过二硫键连在一起的两个亚基(HA1和HA2)。两种结构特征被认为是涉及HA的可切割性：在有限的可切割性的HA中，

接头通常由单个精氨酸组成,然而较宽范围的不同细胞种类中可切割的HA在具有主要酶识别基序Arg-X-Lys/Arg-Arg(其中X为非碱性氨基酸)的该位置上具有一系列的多个碱性残基插入。具有多个碱性切割位点的HA在其到达细胞膜上的出芽位点前在细胞外转运途径中被切割,相反,具有单碱性接头的HA在细胞外间隙或如针对WSN毒株所示在病毒进入阶段,在病毒颗粒上被激活。HA切割的第二个决定因素似乎为,存在于切割位点附近并且干扰蛋白酶易接近性的碳水化合物侧链。该碳水化合物的损失导致提高HA的可切割性和病毒的致病性。

[0009] 由于分泌可细胞外切割HA0前体的蛋白酶的细胞范围有限,哺乳类和非致病性禽流感病毒株引起解剖学上的局部感染(Chen J等.1998,Cell.Vol 95:409-417)。负责在流感感染的人类中切割HA0的蛋白酶,由呼吸道的细胞或由共感染的细菌或支原体分泌,或者它们可在对感染的炎症应答中产生。主要的蛋白酶候选物为类胰蛋白酶克拉拉(Clara),其由支气管上皮的克拉拉细胞产生,并且具有有限的组织分布(上呼吸道)。蛋白酶对H1、H2、H3和H6的切割位点处发现的单碱性序列Q/E-X-R具有特异性。来自H9和B毒株的HA显示稍有不同的单碱性切割位点,分别为SSR和KER序列。针对引起水禽中所见的肠道和呼吸道感染的大多数流感病毒,还未鉴定出蛋白酶。大多数细胞系不支持多轮复制,除非加入外源蛋白酶(通常为胰蛋白酶)。

[0010] 在高致病禽流感毒株中,然而,HA0被更普遍的细胞内蛋白酶家族切割,其导致全身的流感感染。致病性中的这种差异与HA0切割位点处的结构差异相关。致病毒株在单碱性位点内或邻近单碱性位点处具有多碱性氨基酸的插入。在该情况下的切割发生于细胞内,并且所涉及的蛋白酶被鉴定为弗林蛋白酶(Furin)和其他类谷草杆菌蛋白酶,其发现于高尔基体中并涉及激素和生长因子前体的翻译后加工。弗林蛋白酶的识别序列R-X-R/K-R为H5 H7的HA0切割位点处常见的插入氨基酸。酶的广泛组织分布性以及细胞内切割的效能,有助于由这些病毒引起的广泛传播的和毒力的全身性感染。

[0011] HA切割位点是用于病毒减毒的靶标,因为通过宿主蛋白酶将HA0前体激活切割为HA1和HA2片段,是所有流感A和B病毒株的复制循环中的步骤。只有被切割的HA能在受体介导的胞吞作用后的核内体酸性环境中经历构象变化,以暴露用于介导核内膜和病毒粒子膜之间融合的HA2片段的疏水N末端。

[0012] Horimoto T等(2006,Vaccine,Vol 24:3669-3676)描述了H5中H5(RERRRKKR↓G)的多碱性切割位点的废除。所选择的突变体被应用于鼠中免疫原性的研究,其包括具有前面4个带电氨基酸(RERR)的缺失并且使多碱性切割位点失活的修饰(用TETR代替RKKR)的突变体。切割位点的废除不影响突变体H5的免疫原性特性。也已报道,废除多碱性位点(GERRRKKR↓G被RETR代替)以产生突变体NIBSC 05/240 NIBSC流感参考病毒NIBG-23。Hoffman等(2002,2002,Vaccine,Vol 20:3165-3170)用H6的单碱性位点代替H5 HA的多碱性切割位点,以便促进在卵中的表达。缺失前面4个残基,并且通过IETR代替多碱性位点的后面四个氨基酸(用IETR↓G代替RERRRKKR↓G)。该突变体H5表现出高的表达水平、潜在的蛋白水解作用,以及在宿主细胞中病毒复制和产生所需的低pH下的构象变化,免疫原性数据未被报道。这些研究表明,可利用切割位点的修饰来减少病毒颗粒的毒力(在真实病毒被复制的情况下),其允许病毒在不杀死寄生卵的情况下复制。如果没有这样的突变,病毒在到达高滴度前杀死卵。

[0013] Sirko等的WO2013043067描述了用于鸡的DNA疫苗，其含有编码修饰的H5血凝素(HA)蛋白的cDNA，其中HA亚基之间的蛋白水解切割位点被缺失。Sirko等陈述了这种缺失提供了更安全的疫苗并以长的、无加工的多肽形式表达“超级抗原”。Sirko等进一步陈述了修饰的HA的编码区，在这样的方式下，禽细胞中的蛋白的产生实现最大产量。主要的修饰是鸡的密码子优化和HA的蛋白水解敏感区的缺失。

[0014] WO 2013/044390描述了在植物中产生具有修饰的血凝素(HA)的病毒样颗粒(VLP)的方法，其中修饰的HA蛋白包含修饰的蛋白水解环。修饰的HA在调控区豇豆花叶病毒(Cowpea mosaic virus)(CPMV)HT和来自菜豆黄矮病毒(Bean Yellow Dwarf Virus)(BeYDV)的双生病毒(geminivirus)扩增元件的存在下表达。

[0015] Yang等的US 2008/0069821公开了，用于产生作为疫苗的流感病毒的流感HA的多肽和多聚核苷酸变体。通过引入一组载体获得重组流感病毒，所述载体对应于与源自流感HA的变体的互补片段结合的主流感病毒的基因组片段。通常，基于与疫苗施用相关的预期特性选择主毒株。例如，为了疫苗的产生，例如，为了减毒活疫苗的产生，可基于减毒表型、冷适应和/或温度敏感性选择主供体病毒株。

[0016] 发明概述

[0017] 本发明涉及在植物中产生病毒样颗粒和修饰的HA蛋白。

[0018] 本发明的一个目的是提供改善的在植物中产生病毒样颗粒和HA蛋白。

[0019] 本文所描述的核酸包含表达增强子，所述表达增强子在植物有活性并与编码修饰的流感血凝素(HA)的核苷酸序列可操作地连接，所述修饰的流感血凝素(HA)包含修饰的蛋白水解环。

[0020] 此外，本文所描述的是在植物中产生病毒样颗粒(VLP)的方法(A)，所述方法包括：

[0021] a)将包含表达增强子的核酸引入植物或植物的部分，所述表达增强子在植物中有活性并与编码修饰的流感血凝素(HA)的核苷酸序列可操作地连接，所述修饰的流感血凝素(HA)包含修饰的蛋白水解环；

[0022] b)在允许核酸表达的条件下培养植物或植物的部分，从而产生VLP。

[0023] 此外，本文所描述的是在植物中产生病毒样颗粒(VLP)的方法(B)，所述方法包括：

[0024] a)提供包含核酸的植物或植物的部分，所述核酸包含表达增强子，所述表达增强子在植物中有活性并与编码修饰的流感血凝素(HA)的核苷酸序列可操作地连接，所述修饰的流感血凝素(HA)包含修饰的蛋白水解环，并且

[0025] b)在允许核酸表达的条件下培养植物或植物的部分，从而产生VLP。

[0026] 此外，本文所描述的是在植物中产生包含修饰的蛋白水解环的修饰的HA蛋白的方法(C)，所述修饰蛋白水解环包含呈现出减少或废除的切割的一个或多个蛋白酶切割位点，所述方法包括，

[0027] a)将包含表达增强子的核酸引入植物或植物的部分，所述表达增强子在植物中有活性并与编码修饰的流感血凝素(HA)的核苷酸序列可操作地连接，所述修饰的流感血凝素(HA)包含修饰的蛋白水解环；

[0028] b)在允许HA蛋白表达的条件下培养植物或植物的部分，从而产生修饰的HA蛋白，

[0029] c)收集植物，并且纯化修饰的HA蛋白。

[0030] 如上所描述的方法(A)、(B)或(C)还可包括以下步骤：

[0031] c)收集植物,并且

[0032] d)纯化VLP,其中VLP的大小范围为80-300nm。

[0033] 此外,本文所描述的是增加植物中HA蛋白的产物产量的方法(D),所述方法包括,

[0034] a)将包含表达增强子的核酸引入植物或植物的部分,所述表达增强子在植物中有活性并与编码修饰的流感血凝素(HA)的核苷酸序列可操作地连接,所述修饰的流感血凝素(HA)包含修饰的蛋白水解环;

[0035] b)在允许HA蛋白表达的条件下培养植物或植物的部分,从而产生修饰的HA蛋白,

[0036] c)收集植物,并且纯化HA蛋白。

[0037] 表达增强子可为CPMVX、CPMVX+或CPMV-HT+。此外,核苷酸可不包含双生病毒扩增元件。因此核苷酸可不包含菜豆黄矮病毒长基因间隔区(BeYDV LIR)以及BeYDV短基因间隔区(BeYDV SIR)。修饰的蛋白水解环可包含呈现出减少或废除的蛋白酶切割的一个或多个蛋白酶切割位点。蛋白酶可为克拉拉(Clara)-样或弗林蛋白酶(Furin)-样。此外修饰的蛋白水解环可包含接头序列,并且接头序列可具有氨基酸序列GG、TETQ或TETR。修饰的HA可包含天然或非天然信号肽。此外编码修饰的HA的核苷酸序列可包括嵌合核苷酸序列,所述嵌合核苷酸序列按顺序编码修饰的HA胞外域、流感跨膜结构域和胞质尾区,其中修饰的HA胞外域包含修饰的蛋白水解环且来自第一流感毒株,而跨膜结构域和胞质尾区来自第二流感毒株。

[0038] 修饰的蛋白水解环可包含呈现出减少或废除的蛋白酶切割的一个或多个蛋白酶切割位点。而且,编码HA的核苷酸序列选自:B HA、C、H1、H2、H3、H4、H5、H6、H7、H8、H9、H10、H11、H12、H13、H14、H15和H16。本文还描述了通过方法(A)所产生的病毒样颗粒(VLP)。所述病毒样颗粒(VLP)可包含植物-特异性N-聚糖或修饰的N-聚糖。

[0039] 本公开也提供组合物,其包含有效剂量的用于诱导免疫应答的VLP,以及药学上可接受的载体。

[0040] 本文还描述了包含编码流感血凝素(HA)的核苷酸序列的核酸,所述核苷酸序列与在植物中有活性的调控区可操作地连接,其中HA包含修饰的蛋白水解环序列。所述核酸可编码包含修饰的蛋白水解环的HA,其中所述蛋白具有血凝素(HA)活性。也提供包含所述核酸的植物。也包括的是在植物中所产生的病毒样颗粒(VLP)、包含由所述核酸编码的流感病毒血凝素(HA)的VLP和一种或多种源自植物的脂质。

[0041] 本发明的概述不必描述本发明的所有特征。

[0042] 附图简述

[0043] 本发明的这些和其他特征从参考所附附图的以下描述中更显而易见,其中:

[0044] 图1显示用来制备A-2X35S/CPMV-HT/H5印度尼西亚(Indonesia)/NOS(构建体489号)的组件。图1A显示引物IF-H5A-I-05.s1+3c(SEQ ID NO:2)。图1B显示引物IF-H5dTm.r(SEQ ID NO:3)。图1C显示构建体1191的示意图。图1D显示构建体1191(SEQ ID NO4)。图1E显示表达盒489号(SEQ ID NO 5)。图1F显示来自流感A/印度尼西亚/5/2005(H5N1)的H5的氨基酸序列(SEQ ID NO:6)。图1G显示编码来自流感A/印度尼西亚/5/2005(H5N1)的H5的核苷酸序列(SEQ ID NO:42)。

[0045] 图2显示用来制备B-2X35S/CPMV HT/M2新喀里多尼亚(New Caledonia)/NOS(构建体1261号)的组件。图2A显示引物IF-S1-M1+M2ANC.c(SEQ ID NO:7)。图2B显示引物IF-S1-

4-M2ANC.r(SEQ ID NO:8)。图2C显示用于合成的M2基因的核苷酸序列(对应于连接到来自Genbank登录号DQ508860的715-982的nt 1-26)(SEQ ID NO:9)。图2D显示从2X35S启动子至NOS终止子的表达盒1261号。来自流感A/新喀里多尼亚/20/1999(H1N1)的M2加下划线(SEQ ID NO:10)。图2E显示来自流感A/新喀里多尼亚/20/1999(H1N1)的M2的氨基酸序列(SEQ ID NO:11)。

[0046] 图3显示用来制备C-2X35S/CPMV-HT/M2波多黎各(Puerto Rico)/NOS(构建体859号)的组件。图3A显示合成的M2基因的核苷酸序列(对应于连接到来自Genebank登录号EF467824的nt 740-1007的nt 26-51)(SEQ ID NO:12)。图3B显示从2X35S启动子至NOS终止子的表达盒859号。来自流感A/波多黎各/8/1934(H1N1)的M2加下划线(SEQ ID NO:13)。图3C显示来自流感A/波多黎各/8/1934(H1N1)的M2的氨基酸序列(SEQ ID NO:14)。

[0047] 图4显示用来制备引入BeYDV+复制酶扩增体系中的G-2X35S/CPMV-HT/PDISP/HA B布里斯班(Brisbane)/NOS(构建体1008号)的组件。图4A显示构建体1194的示意图。用于质粒线性化的SacII和StuI限制性酶切位点在示意图上注释。图4B显示从左至右的t-DNA边界的构建体1194(加下划线)。引入具有质体蓝素-P19-质体蓝素沉默抑制子表达盒的BeYDV+复制酶扩增体系的2X35S/CPMV-HT/PDISP/NOS(SEQ ID NO:31)。图4C显示从BeYDV左LIR至BeYDV右LIR的表达盒1008号。来自流感B/布里斯班/60/2008的PDISP/HA加下划线(SEQ ID NO:32)。

[0048] 图5显示用来制备引入BeYDV+复制酶扩增体系中的I-2X35S/CPMV-HT/具有缺失的蛋白水解环的PDISP/HA B布里斯班/NOS(构建体1059号)的组件。图5A显示引物1039+1059.r(SEQ ID NO:38)。图5B显示引物1039+1059.c(SEQ ID NO:39)。图5C显示从BeYDV左LIR至BeYDV右LIR的表达盒1059号。来自流感B/布里斯班/60/2008的具有缺失的蛋白水解环的PDISP/HA加下划线(SEQ ID NO:40)。图5D显示来自流感B/布里斯班/60/2008的具有缺失的蛋白水解环的PDISP/HA的氨基酸序列(SEQ ID NO:41)。图5E显示来自流感B/布里斯班/60/2008的具有缺失的蛋白水解环的PDISP/HA的核苷酸序列(SEQ ID NO:43)。

[0049] 图6显示用来制备引入BeYDV(m)+复制酶扩增体系中的B-2X35S/CPMV-HT/HA B威斯康星州(Wisconsin)/NOS(构建体1462号)的组件。图6A显示引物IF-HA B110.S1+3c(SEQ ID NO:49)。图6B显示引物IF-HA B110.s1-4r(SEQ ID NO:50)。图6C显示合成的HA B威斯康星州(Genbank登录号JN993010)的核苷酸序列(SEQ ID NO:51)。图6D显示构建体193的示意图。图6E显示从左至右的t-DNA边界的构建体193(加下划线)。引入具有质体蓝素-P19-质体蓝素沉默抑制子表达盒的BeYDV(m)+复制酶扩增体系的2X35S/CPMV-HT/NOS(SEQ ID NO:52)。图6F显示从2X35S启动子至NOS终止子的表达盒1462号的核苷酸序列。来自流感B/威斯康星州/1/2010的HA加下划线(SEQ ID NO:53)。图6G显示来自流感B/威斯康星州/1/2010的HA的氨基酸序列(SEQ ID NO:54)。图6H显示构建体1462的示意图。

[0050] 图7显示用来制备引入BeYDV(m)+复制酶扩增体系中的C-2X35S/CPMV-HT/具有缺失的蛋白水解环的HA B威斯康星州/NOS(构建体1467号)的组件。图7A显示引物HA B110(PrL-).r(SEQ ID NO:55)。图7B显示引物HA B110(PrL-).c(SEQ ID NO:56)。图7C显示从2X35S启动子至NOS终止子表达盒1467号的核苷酸序列。来自流感B/威斯康星州/1/2010的具有缺失的蛋白水解环的HA加下划线(SEQ ID NO:57)。图7D显示具有缺失的蛋白水解环的流感B/威斯康星州/1/2010的氨基酸序列(SEQ ID NO:58)。图7E显示构建体1467的示意图。

[0051] 图8显示用来制备A-2X35S/CPMV-HT/具有缺失的蛋白水解环的PDISP/HAB布里斯班/NOS(构建体1039号)的组件。图8A显示从2X35S启动子至NOS终止子的表达盒1039号的核苷酸序列。来自流感B/布里斯班/60/2008的具有缺失的蛋白水解环的HA加下划线(SEQ ID NO:15)。图8B显示构建体1039的示意图。

[0052] 图9显示构建体1008号的质粒图谱。构建体1008号针对来自流感毒株B/布里斯班/60/2008的野生型HA的表达。该构建体包含用于DNA扩增的BeYDV-来源元件。

[0053] 图10显示构建体1059号的质粒图谱。构建体1059号针对来自流感毒株B/布里斯班/60/2008的具有缺失的蛋白水解环的突变体HA的表达。该构建体包含用于DNA扩增的BeYDV-来源元件。

[0054] 图11显示构建体1261号的质粒图谱。构建体1261号针对来自流感毒株A/新喀里多尼亚(Caledonia)/20/99(H1N1)的野生型M2的表达。

[0055] 图12显示构建体859号的质粒图谱。构建体859号针对来自流感毒株A/波多黎各/8/34(H1N1)的野生型M2的表达。

[0056] 图13A显示农杆菌渗入的烟草本塞姆氏(Nicotiana benthamiana)叶中HA蛋白表达的Western印迹分析。“1008”:来自B/布里斯班/60/2008的野生型HA在存在扩增元件(BeYDV)下的表达;“1008+1261”:来自B/布里斯班/60/2008的野生型HA在存在扩增元件(BeYDV)下与来自A/新喀里多尼亚/20/99的M2的共表达;“1059”:来自B/布里斯班/60/2008的突变体HA在存在扩增元件(BeYDV)下的表达;“1059+1261”:来自B/布里斯班/60/2008的突变体HA在存在扩增元件(BeYDV)下与来自A/新喀里多尼亚/20/99的M2的共表达。分析来自三种不同渗入的植物(A、B和C)。比值指示共表达实验中所使用的农杆菌培养物的比例。图13B显示来自产生HA的植物的粗蛋白提取物的血凝能力的比较。

[0057] 图14显示农杆菌渗入的烟草本塞姆氏叶中HA蛋白表达的Western印迹分析。“1059”:来自B/布里斯班/60/2008的突变体HA在存在扩增元件(BeYDV)下的表达;“1059+1261”:来自B/布里斯班/60/2008的突变体HA在存在扩增元件(BeYDV)下与来自A/新喀里多尼亚/20/99的M2的共表达。“1059+859”:来自B/布里斯班/60/2008的突变体HA在存在扩增元件(BeYDV)下与来自A/波多黎各/8/34的M2的共表达。分析来自三种不同渗入的植物(A、B和C)。比值指示共表达实验中所使用的农杆菌培养物的比例。

[0058] 图15显示来自不同流感毒株的HA的接头的周围区域的氨基酸序列比对:H1新喀里多尼亚(New Cal)(SEQ ID NO:22);H1布里斯班(SEQ ID NO:23);H1所罗门群岛(Sol Islands)(SEQ ID NO:24);H2A新加坡(Singapore)(SEQ ID NO:25);H3A布里斯班(SEQ ID NO:26);H3A WCN(SEQ ID NO:27);H5安徽(Anhui)(SEQ ID NO:28);H5 Indo(SEQ ID NO:29);H5越南(Vietnam)(SEQ ID NO:30);H6野鸭(Teal)HK(SEQ ID NO:33);H7 Eq布拉格(Prague)(SEQ ID NO:34);H9A HK(SEQ ID NO:35);B弗罗里达(Florida)(SEQ ID NO:36);B马来西亚(Malaysia)(SEQ ID NO:37)。通过箭头指示前体HA0的切割位点。

[0059] 图16A显示农杆菌渗入的烟草本塞姆氏叶中HA蛋白表达的Western印迹分析。来自B/威斯康星州/1/2010的HA与来自A/新喀里多尼亚/20/99的M2共表达。每个泳道加载10μg的蛋白。“C+”:阳性对照,来自英国国家生物标准和控制学会(the National Institute for Biological Standards and Control,United Kingdom)的半-纯化的B/威斯康星州/1/2010病毒;“1462”:来自B/威斯康星州/1/2010的野生型HA在存在扩增元件(BeYDV)下的

表达;“1467”:来自B/威斯康星州/1/2010的突变体HA在存在扩增元件(BeYDV)下的表达;“1462+1261”:来自B/威斯康星州/1/2010的野生型HA在存在扩增元件(BeYDV)下与M2的共表达;“1467+1261”:来自B/威斯康星州/1/2010的突变体HA在存在扩增元件(BeYDV)下与M2的共表达。比值指示了表达和共表达实验中所使用的每个农杆菌培养物的光密度。图16B显示来自转化有AGL1/1462、AGL1/1467、AGL1/1462+AGL1/1261和AGL1/1467+AGL1/1261的植物的粗蛋白提取物的血凝能力的比较。

[0060] 图17A和17B显示农杆菌渗入的烟草本塞姆氏叶中HA蛋白表达的Western印迹分析。“1008”:来自B/布里斯班/60/2008的野生型HA在存在扩增元件(BeYDV)下的表达;“1008+1261”:来自B/布里斯班/60/2008的野生型HA在存在扩增元件(BeYDV)下与来自A/新喀里多尼亚/20/99的M2的共表达;“1039”:来自B/布里斯班/60/2008的突变体HA在不存在扩增元件(BeYDV)下的表达。“1039+1261”:来自B/布里斯班/60/2008的突变体HA在不存在扩增元件(BeYDV)下与来自A/新喀里多尼亚/20/99的M2的共表达。来自A/布里斯班/59/2007(H1N1)的HA。“1059”:来自B/布里斯班/60/2008的突变体HA在存在扩增元件(BeYDV)下的表达;“1059+1261”:来自B/布里斯班/60/2008的突变体HA在存在扩增元件(BeYDV)下与来自A/新喀里多尼亚/20/99的M2的共表达。

[0061] 图18A显示通过克拉拉-样和/或弗林蛋白酶-样的蛋白酶将HA0切割为HA1和HA2的示意图。图18B显示来自不同流感毒株的HA的序列比对:H1新喀里多尼亚(SEQ ID NO:22);H1布里斯班(SEQ ID NO:23);H1所罗门群岛(SEQ ID NO:24);H2A新加坡(SEQ ID NO:25);H3A布里斯班(SEQ ID NO:26);H3AWCN(SEQ ID NO:27);H5安徽(SEQ ID NO:28);H5 Indo(SEQ ID NO:29);H5越南(SEQ ID NO:30);H6野鸭(Teal)HK(SEQ ID NO:33);H7 Eq布拉格(SEQ ID NO:34);H9A HK(SEQ ID NO:35);B弗罗里达(SEQ ID NO:36);B马来西亚(SEQ ID NO:37)。图18C显示H5毒株(A/安徽/1/2005(H5N1)SEQ ID NO:69、A/印度尼西亚(Indonesia)/5/2005(H5N1)SEQ ID NO:70、A/越南/1194/2004(H5N1)SEQ ID NO:71)和B型毒株(B/弗罗里达/4/2006SEQ ID NO:72和B/马来西亚/2506/2004SEQ ID NO:73)中的切割位点的部分缺失。

[0062] 图19显示H5/Indo中的切割位点的突变。天然的序列(SEQ ID NO:44);包含TETR的H5/Indo修饰的切割位点(SEQ ID NO:45);包含TETQ的H5/Indo修饰的切割位点(SEQ ID NO:46)。

[0063] 图20显示包含蛋白水解环内突变的不同的修饰的H5/Indo HA's在酶提取后,最初生物质中存在的滴度。H5/Indo对照(构建体489);GG接头所代替的H5/Indo蛋白水解环(构建体928);TETR接头所代替的H5/Indo蛋白水解环(构建体676);TETQ接头所代替的H5/Indo蛋白水解环(构建体766)。

[0064] 图21显示修饰B型HA的蛋白水解环的多种方法。图21A显示天然的B/布里斯班/60/2008的氨基酸序列(SEQ ID NO:16)。下划线部分是蛋白水解环和HA2结构域。图21B显示具有修饰的蛋白水解环的B/布里斯班/60/2008的氨基酸序列(SEQ ID NO:17),其中构成序列AKLLKERGFFGAIAGFLEG的19个氨基酸残基被GG接头(斜体)代替。图21C显示B/布里斯班/60/2008的氨基酸序列(SEQ ID NO:18),其中构成序列PPAKLLKER的9个氨基酸被-GSSSGSSSG-接头(斜体)代替。图21D显示天然的H3 A/珀斯/16/2009的氨基酸序列(SEQ ID NO:19)。图21E显示具有构成序列RNVPEKQTRGIF的12个氨基酸残基被GS接头(斜体)代替的H3 A/珀斯

(Perth)/16/2009的氨基酸序列(SEQ ID NO:20)。图21F显示具有构成序列RNVPEKQTR的9个氨基酸残基被GSSGSSGSS-接头(斜体)代替的H3 A/珀斯/16/2009的氨基酸序列(SEQ ID NO:21)。

[0065] 图22显示在农杆菌渗入的烟草本塞姆氏叶中HA蛋白表达的Western印迹分析。来自H5/Indo的HA。上图列出了每个泳道中所加载的组分;下图为蛋白质印迹。C:重组的H5印度尼西亚/5/05 S-STD-0002;一抗:抗-HA A/Indo/05/2005 CBER#S-BIO-0003 1/50 000;印迹。泳道1和2:H5/Indo对照(构建体489);泳道3和4:GG接头所代替的H5/Indo的蛋白水解环(构建体928);泳道5和6:TETR接头所代替的H5/Indo蛋白水解环(构建体676);泳道7和8:TETQ接头所代替的H5/Indo蛋白水解环(构建体766);泳道9:MW标记物;泳道10:H5对照。

[0066] 图23显示用来制备来自A/印度尼西亚/5/2005的具有TETR切割位点突变的B-2X35S/CPMV-HT/H5(构建体676号)的组件。图23A显示引物序列MutCleavage-H5(Indo).r(SEQ ID NO:74)。图23B显示引物序列MutCleavage-H5(Indo).c(SEQ ID NO:75)。图23C显示从2X35S启动子至NOS终止子的表达盒676的核苷酸序列(SEQ ID NO:76)。来自流感A/印度尼西亚/5/2005(H5N1)的H5的TETR切割位点突变加下划线。图23D显示来自流感A/印度尼西亚/5/2005(H5N1)的H5的TETR切割位点突变体的氨基酸序列(SEQ ID NO:77)。图23E显示构建体676号的示意图。

[0067] 图24显示用来制备来自A/印度尼西亚/5/2005的具有TETQ切割位点突变的B-2X35S/CPMV-HT/H5(构建体766号)的组件。图24A显示引物序列H5I505_TETQ.r(SEQ ID NO:78)。图24B显示引物序列H5I505_TETQ.c(SEQ ID NO:79)。图24C显示从2X35S启动子至NOS终止子的表达盒766的核苷酸序列(SEQ ID NO:80)。来自流感A/印度尼西亚/5/2005(H5N1)的H5的TETQ切割位点突变体加下划线。图24D显示来自流感A/印度尼西亚/5/2005(H5N1)的H5的TETQ切割位点突变体的氨基酸序列(SEQ ID NO:81)。图24E显示构建体766号的示意图。

[0068] 图25显示用来制备来自A/印度尼西亚/5/2005的具有缺失的蛋白水解环的B-2X35S/CPMV-HT/H5(构建体928号)的组件。图25A显示引物序列H5I505(PrL-).r(SEQ ID NO:82)。图25B显示引物序列H5I505(PrL-).c(SEQ ID NO:83)。图25C显示从2X35S启动子至NOS终止子的表达盒928的核苷酸序列(SEQ ID NO:84)。来自流感A/印度尼西亚/5/2005(H5N1)的具有缺失的蛋白水解环的H5加下划线。图25D显示来自流感A/印度尼西亚/5/2005(H5N1)的具有缺失的蛋白水解环的H5突变体的氨基酸序列(SEQ ID NO:85)。图25E显示构建体928号的示意图。

[0069] 图26A显示数个增强子序列的实例的总示意图,如本文所描述的CPMVX和CPMVX+(包含CPMVX和填充片段,在该非限制性实例中填充片段包含多克隆位点和植物kozak序列)。CPMCX和CPMVX+各自显示为在其5'端与植物调控区可操作地连接,以及在其3'端连续地为目标核苷酸序列(包括ATG起始位点和终止位点)、3'UTR和终止子序列。本文所描述的构建体CPMVX的实例为CPMV160。本文所描述的构建体CPMVX+的实例为CPMV 160+。图26B显示植物中所产生的包含CPMV-HT表达构建体,以及基于CPMV 160+的表达构建体的修饰的HA蛋白的粗蛋白提取物中的相对血凝滴度(HMG)。显示了用于以下构建体的表达的数据:具有缺失的蛋白水解环且具有PDI信号肽的HA B布里斯班/60/08(构建体1039号,5'UTR:CMPV HT;和构建体1937号,5'UTR:CMPV160+;参见实施例5.7);具有缺失的蛋白水解环、具有跨膜

结构域和胞质尾区(其被具有缺失的蛋白水解环的H1,B马萨诸塞州(Massachusetts)/2/2012 2012的跨膜结构域和胞质尾区代替)且具有PDI信号肽的B布里斯班/60/08+H1Tm(构建体2072号,5'UTR:CMPV HT;和构建体2050号,5'UTR:CMPV160+;参见实施例5.14);具有缺失的蛋白水解环、具有跨膜结构域和胞质尾区(其被H1 A/加利福尼亚/07/2009的跨膜结构域和胞质尾区代替)且具有PDI信号肽的B马萨诸塞州/2/2012+H1Tm(构建体2074号,5'UTR:CMPV HT;和构建体2060号,5'UTR:CMPV160+;参见实施例5.15);具有缺失的蛋白水解环且具有天然信号肽的B威斯康星州/1/2010(构建体1445号,5'UTR:CMPV HT;和构建体1975号,5'UTR:CMPV160+;参见实施例5.16);和具有缺失的蛋白水解环、具有跨膜结构域和胞质尾区(其被H1 A/加利福尼亚/07/2009的跨膜结构域和胞质尾区代替)且具有天然信号肽的B威斯康星州/1/2010+H1Tm(构建体1454号,5'UTR:CMPV HT;和构建体1893号,5'UTR:CMPV160+;参见实施例5.18)。

[0070] 图27A显示与目标核苷酸序列融合的CPMV HT和CPMV HT+的增强子序列的总示意图。增强子序列内并非需要该图中所示的所有元件。目标核苷酸序列的3'端也可包括另外的元件(未显示),包括编码豇豆花叶病毒3'非翻译区(UTR)、质体蓝素3'UTR或豇豆花叶病毒3'UTR和质体蓝素3'UTR的组合的序列。图27B显示植物中所产生的包含与目标核苷酸序列可操作地连接的CPMV-HT表达构建体和基于CPMV HT+的表达构建体的蛋白的粗蛋白提取物中的相对血凝滴度(HMG)。显示用于以下构建体的表达的数据:具有缺失的蛋白水解环且具有PDI信号肽的HA B布里斯班/60/08(构建体1039号:CPMV HT;参见实施例5.7和构建体1829号:CPMV HT+;参见实施例5.12);具有缺失的蛋白水解环、具有跨膜结构域和胞质尾区(其被H1 A/加利福尼亚/07/2009的跨膜结构域和胞质尾区代替)且具有PDI信号肽的B布里斯班/60/08+H1TM(构建体1067号:CPMV HT;参见实施例5.14和构建体1875号:CPMV HT+;参见实施例5.19);具有缺失的蛋白水解环且具有PDI信号肽的B马萨诸塞州/2/2012(构建体2072号:CMPV HT;参见实施例5.15和构建体2052号:CMPV HT+;参见实施例5.20);具有缺失的蛋白水解环、具有跨膜结构域和胞质尾区(其被H1 A/加利福尼亚/07/2009的跨膜结构域和胞质尾区代替)且具有PDI信号肽的B马萨诸塞州/2/2012+H1Tm(构建体2074号:CMPV HT;参见实施例5.16和构建体2062号:CMPV HT+;参见实施例5.21);具有缺失的蛋白水解环且具有天然信号肽的B威斯康星州/1/2010(构建体1445号:CMPV HT;参见实施例5.17和构建体1839号:CMPV HT+;参见实施例5.22);和具有缺失的蛋白水解环、具有跨膜结构域和胞质尾区(其被H1 A/加利福尼亚/07/2009的跨膜结构域和胞质尾区代替)且具有天然信号肽的B威斯康星州/1/2010+H1Tm(构建体1454号:CMPV HT;参见实施例5.18和构建体1860号:CMPV HT+;参见实施例5.23)。

[0071] 图28A显示在农杆菌渗入的烟草本塞姆氏叶中H3珀斯蛋白表达的Western印迹分析。泳道1:(2019+1261)来自H3珀斯-16-09的天然(野生型)HA在表达增强子(CPMV-HT+)存在下与来自A/新喀里多尼亚/20/99的M2的共表达;泳道2:(2139+1261)来自H3珀斯-16-09的天然(野生型)HA在表达增强子(CPMV 160+)存在下与来自A/新喀里多尼亚/20/99的M2的共表达;泳道3:(2039+1261)来自H3珀斯-16-09的突变体(修饰的)HA在表达增强子(CPMV HT+)存在下与来自A/新喀里多尼亚/20/99的M2的共表达;泳道4:(2159+1261)来自H3珀斯-16-09的突变体(修饰的)HA在表达增强子(CPMV 160+)存在下与来自A/新喀里多尼亚/20/99的M2的共表达。

[0072] 图28B显示在农杆菌渗入的烟草本塞姆氏叶中B马来西亚蛋白表达的Western印迹分析。泳道2:(2013+1261)来自B马来西亚2506-04的天然(野生型)HA在表达增强子(CPMV-160+)存在下与来自A/新喀里多尼亚/20/99的M2的共表达;泳道2:(2014+1261)来自B马来西亚2506-04的突变体(修饰的)HA在表达增强子(CPMV 160+)存在下与来自A/新喀里多尼亚/20/99的M2的共表达。

[0073] 图28C显示在农杆菌渗入的烟草本塞姆氏叶中H9香港蛋白表达的Western印迹分析。泳道1:(1610+1261)来自H9香港-1037-99的天然(野生型)HA在表达增强子(CPMV-HT)存在下与来自A/新喀里多尼亚/20/99的M2的共表达;泳道2:(1630+1261)来自H9香港-1037-99的天然(野生型)HA在表达增强子(CPMV-HT+)和扩增元件BEYDV存在下与来自A/新喀里多尼亚/20/99的M2的共表达;泳道3:(2244+1261)来自H9香港-1037-99的天然(野生型)HA在表达增强子(CPMV-HT+)存在下与来自A/新喀里多尼亚/20/99的M2的共表达;泳道4:(2226+1261):来自H9香港-1037-99的天然(野生型)HA在表达增强子(CPMV 160+)存在下与来自A/新喀里多尼亚/20/99的M2的共表达。泳道6:(2246+1261)来自H9香港-1037-99的天然(野生型)HA在表达增强子(CPMV-160+)和扩增元件BeYDV存在下与来自A/新喀里多尼亚/20/99的M2的共表达;泳道7:(2225+1261)来自H9香港-1037-99的突变体(修饰的)HA在表达增强子(CPMV-HT+)存在下与来自A/新喀里多尼亚/20/99的M2的共表达;泳道8:(2245+1261)来自H9香港-1037-99的突变体(修饰的)HA在表达增强子(CPMV HT+)和扩增元件BeYDV存在下与来自A/新喀里多尼亚/20/99的M2的共表达。泳道9:(2227+1261)来自H9香港-1037-99的突变体(修饰的)HA在表达增强子(CPMV 160+)存在下与来自A/新喀里多尼亚/20/99的M2的共表达。泳道10:(2247+1261)来自H9香港-1037-99的突变体(修饰的)HA在表达增强子(CPMV 160+)和扩增元件BeYDV存在下与来自A/新喀里多尼亚/20/99的M2的共表达。

[0074] 图28D显示在农杆菌渗入的烟草本塞姆氏叶中B马萨诸塞州蛋白表达的Western印迹分析。泳道1:(2070+1261)来自B马萨诸塞州-2-12的天然(野生型)HA在表达增强子(CPMV-HT)存在下与来自A/新喀里多尼亚/20/99的M2的共表达;泳道2:(2080+1261)来自B马萨诸塞州-2-12的天然(野生型)HA在表达增强子(CPMV-HT+)存在下与来自A/新喀里多尼亚/20/99的M2的共表达;泳道3:(2090+1261)来自B马萨诸塞州-2-12的天然(野生型)HA在表达增强子(CPMV-160+)存在下与来自A/新喀里多尼亚/20/99的M2的共表达;泳道4:(2072+1261)来自B马萨诸塞州-2-12的突变体(修饰的)HA在表达增强子(CPMV HT)存在下与来自A/新喀里多尼亚/20/99的M2共表达;泳道5:(2052+1261)来自B马萨诸塞州-2-12的突变体(修饰的)HA在表达增强子(CPMV HT+)存在下与来自A/新喀里多尼亚/20/99的M2的共表达;泳道6:(2050+1261)来自B马萨诸塞州-2-12的突变体(修饰的)HA在表达增强子(CPMV 160+)存在下与来自A/新喀里多尼亚/20/99的M2的共表达。

[0075] 图28E显示在农杆菌渗入的烟草本塞姆氏叶中的H2新加坡蛋白表达的Western印迹分析。泳道1:(2220+1261)来自H2新加坡-1-57的天然(野生型)HA在表达增强子(CPMV-HT+)存在下与来自A/新喀里多尼亚/20/99的M2的共表达;泳道2:(2222+1261)来自H2新加坡-1-57的天然(野生型)HA在表达增强子(CPMV 160+)存在下与来自A/新喀里多尼亚/20/99的M2的共表达。泳道3:(2221+1261)来自H2新加坡-1-57的突变体(修饰的)HA在表达增强子(CPMV-HT+)存在下与来自A/新喀里多尼亚/20/99的M2的共表达;泳道4:(2223+1261)来自H2新加坡-1-57的突变体(修饰的)HA在表达增强子(CPMV 160+)存在下与来自A/新喀里多

尼亚/20/99的M2的共表达。

[0076] 图28F显示在农杆菌渗入的烟草本塞姆氏叶中B/弗罗里达蛋白表达的Western印迹分析。泳道1:(1004+1261)来自B/弗罗里达的天然(野生型)HA在表达增强子(CPMV-HT)存在下与来自A/新喀里多尼亚/20/99的M2的共表达;泳道2:(1003+1261)来自B/弗罗里达的天然(野生型)HA在表达增强子(CPMV HT)和扩增元件BeYDV存在下与来自A/新喀里多尼亚/20/99的M2的共表达。泳道3:(2102+1261)来自B/弗罗里达的突变体(修饰的)HA在表达增强子(CPMV-HT+)存在下与来自A/新喀里多尼亚/20/99的M2的共表达;泳道4:(2104+1261)来自B/弗罗里达的突变体(修饰的)HA在表达增强子(CPMV HT+)和扩增元件BeYDV存在下与来自A/新喀里多尼亚/20/99的M2的共表达。泳道5:(2106+1261)来自B/弗罗里达+H1加利福尼亚TMCT的突变体(修饰的)HA在表达增强子(CPMV HT+)存在下与来自A/新喀里多尼亚/20/99的M2的共表达。泳道6:(2108+1261)来自B/弗罗里达+H1加利福尼亚TMCT的突变体(修饰的)HA在表达增强子(CPMV HT+)和扩增元件BeYDV存在下与来自A/新喀里多尼亚/20/99的M2的共表达。

[0077] 图29A显示在增强子元件CPMV HT、CPMV HT+或CPMV 160+存在下表达来自不同流感毒株的修饰的HA的相对HA滴度。与用相同的增强子元件所表达的天然HA蛋白比较其活性。H3 A珀斯/16/09(H3 Per1609)、H3维多利亚(Victoria)/361/11(H3 Vic26111)、B布里斯班60/2008(HB Bris60008)、B马来西亚2506/04(HB Mal 250604)和B马萨诸塞州2/12(Ma212)与流感M2蛋白共表达。

[0078] 图30A显示引物IF-S2+S4-B Bris.c(SEQ ID NO:86)。图30B显示引物IF-S1a4-B Bris.r(SEQ ID NO:87)。图30C显示合成的HA B布里斯班基因的核苷酸序列(对应于来自Genbank登录号FJ766840的nt 34-1791)(SEQ ID NO:88)。图30D显示从2X35S启动子至NOS终止子的表达盒1029号的核苷酸序列。来自流感B/布里斯班/60/2008的PDISP/HA加下划线(SEQ ID NO:89)。图30E显示来自流感B/布里斯班/60/2008的PDISP/HA的氨基酸序列(SEQ ID NO:90)。图30F显示构建体1029的示意图。用于质粒线性化的SacII和StuI限制性酶切位点在示意图上注释。

[0079] 图31显示用来制备构建体1039号和1829号(分别为2X35S/CPMV HT PDISP/HA B布里斯班(PrL-)NOS和2X35S/CPMV HT+PDISP/HA B布里斯班(PrL-)NOS;参见实施例5.12)的序列组件。构建体1039号合并现有技术的CPMV-HT序列(与编码不完整M蛋白的序列融合的在位置161处具有突变的起始密码子的CMPV 5'UTR),并且在5'UTR和目标核苷酸序列(PDISP/HA B布里斯班(PrL-)之间不包含异源kozak序列。构建体1829号包括包含160个核苷酸的CPMV 5'UTR、包含不完整M蛋白的填充片段、多克隆位点和植物kozak序列,并且其为基于CPMV HT+构建体的实例。PDISP:蛋白二硫键异构酶信号肽;NOS:胭脂碱合酶终止子;PrL-:缺失的蛋白水解环。图31A显示PDISP/HA B布里斯班(PrL-)的核苷酸序列(SEQ ID NO:91)。图31B显示PDISP/HA B布里斯班(PrL-)的氨基酸序列(SEQ ID NO:92)。图31C显示构建体1829号(2X35S/CPMV HT+)的示意图。

[0080] 图32显示用来制备构建体1039号和1937号(分别为2X35S/CPMV HT PDISP/HA B布里斯班(PrL-)NOS和2X35S/CPMV 160+PDISP/HA B布里斯班(PrL-)NOS;参见实施例5.7)的序列组件。构建体1039号合并现有技术的CPMV-HT序列(与编码不完整M蛋白的序列融合的在位置161处具有突变的起始密码子的CMPV 5'UTR),并且在5'UTR和目标核苷酸序列

(PDISP/HA B布里斯班(PrL-))之间不包含异源kozak序列。构建体1937号包括包含160个核苷酸的CPMV 5'UTR、填充片段(多克隆位点)和植物kozak序列(该构建体不包含编码不完整M蛋白的序列),并且其为基于CPMV 160+(CPMVX+,其中X=160)构建体的实例。PDISP:蛋白二硫键异构酶信号肽;NOS:胭脂碱合酶终止子;PrL-:缺失的蛋白水解环。图32A显示构建体1937号(2X35S/CPMV 160+;基于CPMVX+的构建体,其中X=160)的示意图。

[0081] 图33显示用来制备构建体1067号和1977号(分别为2X35S/CPMV HT PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT NOS和2X35S/CPMV 160+PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT NOS;参见实施例5.14)的序列组件。构建体1067号合并现有技术CPMV-HT序列(与编码不完整M蛋白的序列融合的在位置161处具有突变的起始密码子的CMPV 5'UTR),并且在5'UTR和目标核苷酸序列(PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT)之间不包含异源kozak序列。构建体1977号包括包含160个核苷酸的CPMV 5'UTR、填充片段(多克隆位点)和植物kozak序列(该构建体不包含编码不完整M蛋白的序列),并且其为基于CPMV 160+(CPMVX+,其中X=160)构建体的实例。PDISP:蛋白二硫键异构酶信号肽;NOS:胭脂碱合酶终止子;PrL-:缺失的蛋白水解环;TMCT:跨膜结构域胞质尾区。图33A显示PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT的核苷酸序列(SEQ ID NO:95)。图33B显示PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT的氨基酸序列(SEQ ID NO:96)。图33C显示构建体1067号(2X35S/CPMV HT;参考构建体)的示意图。图33D显示构建体1977号(2X35S/CPMV 160+;基于CPMVX+的构建体,其中X=160)的示意图。

[0082] 图34显示用来制备构建体2072号和2050号(分别为2X35S/CPMV HT PDISP/HA B马萨诸塞州(PrL-)NOS和2X35S/CPMV 160+PDISP/HA B马萨诸塞州(PrL-)NOS;参见实施例5.15)的序列组件。构建体2072号合并现有技术的CPMV-HT序列(与编码不完整M蛋白的序列融合的在位置161处具有突变的起始密码子的CMPV 5'UTR),并且在5'UTR和目标核苷酸序列(PDISP/HA B马萨诸塞州(PrL-))之间不包含异源kozak序列。构建体2050号包括包含160个核苷酸的CPMV 5'UTR、填充片段(多克隆位点)和植物kozak序列(该构建体不包含编码不完整M蛋白的序列),并且其为基于CPMV 160+(CPMVX+,其中X=160)的构建体的实例。PDISP:蛋白二硫键异构酶信号肽;NOS:胭脂碱合酶终止子;PrL-:缺失的蛋白水解环。图34A显示PDISP/HA B马萨诸塞州(PrL-)的核苷酸序列(SEQ ID NO:97)。图34B显示PDISP/HA B马萨诸塞州(PrL-)的氨基酸序列(SEQ ID NO:98)。图34C显示构建体2072号(2X35S/CPMV HT;参考构建体)的示意图。图34D显示构建体2050号(2X35S/CPMV 160+;基于CPMVX+的构建体,其中X=160)的示意图。

[0083] 图35显示用来制备构建体2074号和2060号(分别为2X35S/CPMV HT PDISP/HA B马萨诸塞州(PrL-)+H1加利福尼亚TMCT NOS和2X35S/CPMV 160+PDISP/HA B马萨诸塞州(PrL-)+H1加利福尼亚TMCT NOS;参见实施例5.16)的序列组件。构建体2074号合并现有技术的CPMV-HT序列(与编码不完整M蛋白的序列融合的在位置161处具有突变的起始密码子的CMPV 5'UTR),并且在5'UTR和目标核苷酸序列(PDISP/HA B马萨诸塞州(PrL-)+H1加利福尼亚TMCT)之间不包含异源kozak序列。构建体2060号包括包含160个核苷酸的CPMV 5'UTR、填充片段(多克隆位点)和植物kozak序列(该构建体不包含编码不完整M蛋白的序列),并且其为基于CPMV 160+(CPMVX+,其中X=160)构建体的实例。PDISP:蛋白二硫键异构酶信号肽;NOS:胭脂碱合酶终止子;PrL-:缺失的蛋白水解环;TMCT:跨膜结构域胞质尾区。图35A显

示PDISP/HA B马萨诸塞州(PrL-)+H1加利福尼亚TMCT的核苷酸序列(SEQ ID NO:99)。图35B显示PDISP/HA B马萨诸塞州(PrL-)+H1加利福尼亚TMCT的氨基酸序列(SEQ ID NO:100)。图35C显示构建体2074号(2X35S/CPMV HT;参考构建体)的示意图。图35D显示构建体2060号(2X35S/CPMV 160+;基于CPMVX+的构建体,其中X＝160)的示意图。

[0084] 图36显示用来制备构建体1445号、1820号和1975号(分别为2X35S/CPMV HT HA B威斯康星州(PrL-)NOS、2X35S/CPMV 160+HA B威斯康星州(PrL-)NOS和2X35S/CPMV160 HA B威斯康星州(PrL-)NOS;参见实施例15.17)的序列组件。构建体1445号合并现有技术的CPMV-HT序列(与编码不完整M蛋白的序列融合的在位置161处具有突变的起始密码子的CMPV 5'UTR),并且在5'UTR和目标核苷酸序列(HA B威斯康星州(PrL-))之间不包含异源kozak序列。构建体1820号包括包含160个核苷酸的CPMV 5'UTR、填充片段(多克隆位点)和植物kozak序列(该构建体不包含编码不完整M蛋白的序列),并且其为基于CPMV 160+(CPMVX+,其中X＝160)的构建体的实例。构建体1975号包括包含160个核苷酸的CPMV 5'UTR,并且其不包括填充片段(多克隆位点)或植物kozak序列(该构建体也不包含编码不完整M蛋白的序列),并且其为基于“CPMV160”(CPMVX)的构建体的实例。PrL-:缺失的蛋白水解环;NOS:胭脂碱合酶终止子。图36A显示HA B威斯康星州(PrL-)的核苷酸序列(SEQ ID NO:101)。图36B显示HA B威斯康星州(PrL-)的氨基酸序列(SEQ ID NO:102)。图36C显示构建体1445号的示意图(2X35S/CPMV HT;参考构建体)。图36D显示构建体1820号(2X35S/CPMV 160+;基于CPMVX+的构建体)的示意图。图36E显示构建体1975号(2X35S/CPMV160;基于CPMVX的构建体,其中X＝160)的示意图。

[0085] 图37显示用来制备构建体1454号和1893号(分别为2X35S/CPMV HT HA B威斯康星州(PrL-)+H1加利福尼亚TMCT NOS和2X35S/CPMV 160+HA B威斯康星州(PrL-)+H1加利福尼亚TMCT NOS;参见实施例5.18)的序列组件。构建体1454号合并现有技术的CPMV-HT序列(与编码不完整M蛋白的序列融合的在位置161处具有突变的起始密码子的CMPV 5'UTR),并且在5'UTR和目标核苷酸序列(HA B威斯康星州(PrL-)+H1加利福尼亚TMCT)之间不包含异源kozak序列。构建体1893号包括包含160个核苷酸的CPMV 5'UTR、填充片段(多克隆位点)和植物kozak序列(该构建体不包含编码不完整M蛋白的序列),并且其为基于CPMV 160+(CPMVX+,其中X＝160)的构建体的实例。NOS:胭脂碱合酶终止子;PrL-:缺失的蛋白水解环;TMCT:跨膜结构域胞质尾区。图37A显示HA B威斯康星州(PrL-)+H1加利福尼亚TMCT的核苷酸序列(SEQ ID NO:103)。图37B显示PDISP/HA B威斯康星州(PrL-)+H1加利福尼亚TMCT的氨基酸序列(SEQ ID NO:104)。图37C显示构建体1454号(2X35S/CPMV HT;参考构建体)的示意图。图37D显示构建体1893号(2X35S/CPMV 160+;基于CPMVX+的构建体,其中X＝160)的示意图。

[0086] 图38显示用来制备构建体1067号和1875号(分别为2X35S/CPMV HT PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT NOS和2X35S/CPMV HT+PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT NOS;参见实施例5.19)的序列组件。构建体1067号合并现有技术的CPMV-HT序列(与编码不完整M蛋白的序列融合的在位置161处具有突变的起始密码子的CMPV 5'UTR),并且在5'UTR和目标核苷酸序列(PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT)之间不包含异源kozak序列。构建体1875号包括包含160个核苷酸的CPMV 5'UTR、包含不完整M蛋白的填充片段、多克隆位点和植物kozak序列,并且其为基于CPMV HT+的构建体的实例。

PDISP:蛋白二硫键异构酶信号肽;NOS:胭脂碱合酶终止子;PrL-:缺失的蛋白水解环;TMCT:跨膜结构域胞质尾区。图38A显示PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT的核苷酸序列(SEQ ID NO:105)。图38B显示PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT的氨基酸序列(SEQ ID NO:106)。图38C显示构建体1875号(2X35S/CPMV 160+)的示意图。

[0087] 图39显示用来制备构建体2072号和2052号(分别为2X35S/CPMV HT PDISP/HA B马萨诸塞州(PrL-)NOS和2X35S/CPMV HT+PDISP/HA B马萨诸塞州(PrL-)NOS;参见实施例5.20)的序列组件。构建体2072号合并现有技术的CPMV-HT序列(与编码不完整M蛋白的序列融合的在位置161处具有突变的起始密码子的CMPV 5'UTR),并且在5'UTR和目标核苷酸序列(PDISP/HA B马萨诸塞州(PrL-))之间不包含异源kozak序列。构建体2052号包括包含160个核苷酸的CPMV 5'UTR、包含不完整M蛋白的填充片段、多克隆位点和植物kozak序列,并且其为基于CPMV HT+的构建体的实例。PDISP:蛋白二硫键异构酶信号肽;NOS:胭脂碱合酶终止子;PrL-:缺失的蛋白水解环。图39A显示PDISP/HA B马萨诸塞州(PrL-)的核苷酸序列(SEQ ID NO:107)。图39B显示PDISP/HA B马萨诸塞州(PrL-)的氨基酸序列(SEQ ID NO:108)。图39C显示构建体2052号(2X35S/CPMV HT+)的示意图。

[0088] 图40显示用来制备构建体2074号和2062号(分别为2X35S/CPMV HT PDISP/HA B马萨诸塞州(PrL-)+H1加利福尼亚TMCT NOS和2X35S/CPMV HT+PDISP/HA B马萨诸塞州(PrL-)+H1加利福尼亚TMCT NOS;参见实施例5.21)的序列组件。构建体2074号合并现有技术的CPMV-HT序列(与编码不完整M蛋白的序列融合的在位置161处具有突变的起始密码子的CMPV 5'UTR),并且在5'UTR和目标核苷酸序列(PDISP/HA B马萨诸塞州(PrL-)+H1加利福尼亚TMCT)之间不包含异源kozak序列。构建体2062号包括包含160个核苷酸的CPMV 5'UTR、包含不完整M蛋白的填充片段、多克隆位点和植物kozak序列,并且其为基于CPMV HT+的构建体的实例。PDISP:蛋白二硫键异构酶信号肽;NOS:胭脂碱合酶终止子;PrL-:缺失的蛋白水解环;TMCT:跨膜结构域胞质尾区。图40A显示PDISP/HA B马萨诸塞州(PrL-)+H1加利福尼亚TMCT的核苷酸序列(SEQ ID NO:109)。图40B显示PDISP/HA B马萨诸塞州(PrL-)+H1加利福尼亚TMCT的氨基酸序列(SEQ ID NO:110)。图40C显示构建体2062号(2X35S/CPMV HT+)的示意图。

[0089] 图41显示用来制备构建体1445号和1839号(分别为2X35S/CPMV HT HA B威斯康星州(PrL-)NOS和2X35S/CPMV HT+HA B威斯康星州(PrL-)NOS;参见实施例5.22)的序列组件。构建体1445号合并现有技术的CPMV-HT序列(与编码不完整M蛋白的序列融合的在位置161处具有突变的起始密码子的CMPV 5'UTR),并且在5'UTR和目标核苷酸序列(HA B威斯康星州(PrL-))之间不包含异源kozak序列。构建体1839号包括包含160个核苷酸的CPMV 5'UTR、包含不完整M蛋白的填充片段、多克隆位点和植物kozak序列,并且其为基于CPMV HT+的构建体的实例。PrL-:缺失的蛋白水解环;NOS:胭脂碱合酶终止子。图41A显示HA B威斯康星州(PrL-)的核苷酸序列(SEQ ID NO:111)。图41B显示HA B威斯康星州(PrL-)的氨基酸序列(SEQ ID NO:112)。图41C显示构建体1839号(2X35S/CPMV HT+)的示意图。

[0090] 图42显示用来制备构建体1454号和1860号(分别为2X35S/CPMV HT HA B威斯康星州(PrL-)+H1加利福尼亚TMCT NOS和2X35S/CPMV HT+HA B威斯康星州(PrL-)+H1加利福尼亚TMCT NOS;参见实施例5.23)的序列组件。构建体1454号合并现有技术的CPMV-HT序列(与编码不完整M蛋白的序列融合的在位置161处具有突变的起始密码子的CMPV 5'UTR),并且

在5'UTR和目标核苷酸序列(HA B威斯康星州(PrL-)+H1加利福尼亚TMCT)之间不包含异源kozak序列。构建体1860号包括包含160个核苷酸的CPMV 5'UTR、包含不完整M蛋白的填充片段、多克隆位点和植物kozak序列,并且其为基于CPMV HT+的构建体的实例。NOS:胭脂碱合酶终止子;PrL-:缺失的蛋白水解环;TMCT:跨膜结构域胞质尾区。图42A显示HA B威斯康星州(PrL-)+H1加利福尼亚TMCT的核苷酸序列(SEQ ID NO:113)。图42B显示PDISP/HA B威斯康星州(PrL-)+H1加利福尼亚TMCT的氨基酸序列(SEQ ID NO:114)。图42C显示构建体1893号(2X35S/CPMV HT+)的示意图。

[0091] 图43显示用来制备构建体489号(2X35S/CPMV HT H5印度尼西亚NOS,参见实施例5.24)的序列组件。构建体489号包含CPMV-HT序列(与编码不完整M蛋白的序列融合的在位置161处具有突变的起始密码子的CMPV 5'UTR),并且在5'UTR和目标核苷酸序列(PDISP/H1加利福尼亚)之间不包含异源kozak序列。图43A显示天然H5印度尼西亚的核苷酸序列(SEQ ID NO:115)。图43B显示天然H5印度尼西亚的氨基酸序列(SEQ ID NO:116)。图43C显示构建体489号(2X35S/CPMV HT;参考构建体)的示意图。

[0092] 图44显示用来制备构建体1800号(A-2X35S CPMV 160+PDISPH3维多利亚NOS;参见实施例5.25)的序列组件。构建体1800号包括包含160个核苷酸的CPMV 5'UTR、填充片段(多克隆位点)和植物kozak序列(该构建体不包含编码不完整M蛋白的序列),并且为基于CPMV 160+(CPMVX+,其中X=160)的构建体的实例。PDISP:蛋白二硫键异构酶信号肽。NOS:胭脂碱合酶终止子。图44A显示引物序列IF**(SacII)-PDI.s1+4c(SEQ ID NO:117)。图44B显示引物序列IF-H3V36111.s1-4r(SEQ ID NO:118)。图44C显示PDISP/H3维多利亚的序列(SEQ ID NO:119)。图44D显示构建体2171(指示了用于质粒线性化的SacII和StuI限制性酶切位点)的示意图。图44E显示从左至右t-DNA边界的构建体2171(加下划线)、具有质体蓝素-P19-质体蓝素沉默抑制子表达盒的2X35S/CPMV 160+/NOS、H1加利福尼亚跨膜胞质尾区和CPMV 3'UTR(SEQ ID NO:120)。图44F显示从2X35S启动子至NOS终止子的表达盒1800号。PDISP/H3维多利亚核苷酸序列加下划线;5'UTR用粗体显示;植物kozak序列双下划线;位于5'UTR和植物kozak序列之间的16个碱基对的填充片段(多克隆位点)(SEQ ID NO:121)。图44G显示PDISP/H3维多利亚的氨基酸序列(SEQ ID NO:122)。图44H显示构建体1800号(基于CPMVX+的构建体,其中X=160)的示意图。

[0093] 图45显示用来制备构建体1819号(2X35S CPMV-HT+PDISPH3维多利亚NOS)的序列组件。构建体1819号包含CPMV-HT+序列(位置161处具有突变的起始密码子的融合的编码不完整M蛋白的填充片段的CMPV 5'UTR、多克隆位点,并且多克隆位点和目标核苷酸序列(PDISP/H3维多利亚)之间包含植物kozak序列)。PDISP:蛋白二硫键异构酶信号肽。NOS:胭脂碱合酶终止子。图45A显示引物序列IF(SacII)-Kozac_PDI.c(SEQ ID NO:123)。图45B显示引物序列IF-H3V36111.s1-4r(SEQ ID NO:124)。图45C显示构建体2181的示意图。图45D显示构建体2181(从左至右t-DNA边界,加下划线;具有质体蓝素-P19-质体蓝素沉默抑制子表达盒的2X35S/CPMV-HT+/NOS;SEQ ID NO:126)的序列。图45E显示从2X35S启动子至NOS终止子的表达盒1819号。PDISP/H3维多利亚核苷酸序列加下划线(SEQ ID NO:127)。图45F显示构建体1819的示意图。

[0094] 图46A显示天然H7杭州HA和具有缺失的蛋白水解环的修饰H7杭州HA,与M2共表达时的相对血凝活性。在M2(构建体#1261参见实施例5.1)存在下时,表达天然和修饰的H7杭

州HA(构建体#2142和2152,参见实施例5.33和5.34)并且从植物中纯化。图46B显示天然H7杭州HA(构建体#2142)和具有缺失的蛋白水解环的修饰H7杭州HA(构建体#2152)的蛋白产量。图46C显示具有泳道2和泳道3的SDS-PAGE分析,所述泳道2显示纯化的具有去除的蛋白水解环的修饰H7杭州HA(构建体#2152),所述泳道3显示纯化的天然H7杭州HA(构建体#2142)。对于每个泳道,在凝胶上加2μg总蛋白。对于这两个构建体,蛋白谱的纯度是相似的。

[0095] 图47A显示天然HA蛋白和蛋白水解环被GG接头(prl-)代替的修饰的HA、蛋白水解环被TETQ接头(TETQ)代替的修饰的HA,和蛋白水解环被TETR接头(TETR)代替的修饰的HA之间的胰蛋白酶抗性。纯化了天然的(#489)、PRL-(#928)、TETQ(#766)和TETR(#676)的H5印度尼西亚HA VLP构建体。对于每种构建体,用pH 7.4的缓冲液(100mM Na/KPO_4,150mM NaCl,0.01%吐温80)重悬两份HA VLP样品,目标浓度为150μg/mL。将胰蛋白酶以1:100蛋白比值加入一个重悬的样品中。样品在室温下孵育30、60和120分钟。SDS-PAGE凝胶上加载30μL非消化的提取物(对照)和30μL消化的提取物,其用考马斯亮蓝染色。图47B显示两个剂量后小鼠中的天然H5 VLP和其突变体对应物(prl-、TETQ和TETR)的免疫原性(HI滴度)。柱状代表每种H5突变体VLP与天然H5 VLP相比的相对(%)HI滴度。

[0096] 图48显示用来制备构建体2220号(2X35S/CPMV HT+/PDISP/H2新加坡/NOS,参见实施例5.27)的序列组件。图48A显示引物IF**-H2S157.s1-6r的核苷酸序列(SEQ ID NO:127)。图48B显示PDISP/H2新加坡的核苷酸序列(SEQ ID NO:128)。图48C显示从2X35S启动子至NOS终止子的表达盒2220号的核苷酸序列。PDISP/H2新加坡核苷酸序列加下划线。图48D显示PDISP/H2新加坡的氨基酸序列。图48E显示构建体2220号的示意图。

[0097] 图49显示用来制备构建体2221号(2X35S/CPMVHT+/具有缺失的蛋白水解环的PDISP/H2新加坡/NOS,参见实施例5.28)的序列组件。图49A显示引物H2S157(Prl-).r的核苷酸序列(SEQ ID NO:131)。图49B显示引物H2S157(Prl-).c的核苷酸序列(SEQ ID NO:132)。图49C显示从2X35S启动子至NOS终止子的表达盒2221号的核苷酸序列。PDISP/H2新加坡核苷酸序列加下划线(SEQ ID NO:133)。图49D显示具有缺失的蛋白水解环的PDISP/H2新加坡的氨基酸序列(SEQ ID NO:134)。图49E显示构建体2221号的示意图。

[0098] 图50显示用来制备构建体2222号(2X35S/CPMV 160+/PDISP/H2新加坡)和2223号(2X35S/CPMV 160+/具有缺失的蛋白水解环的PDISP/H2新加坡/NOS)(参见实施例5.29)的序列组件。图50A显示从2X35S启动子至NOS终止子的表达盒2222号的核苷酸序列。PDISP/H2新加坡核苷酸序列加下划线(SEQ ID NO:135)。图50B显示从2X35S启动子至NOS终止子的表达盒2223号的核苷酸序列。具有缺失的蛋白水解环的PDISP/H2新加坡的核苷酸序列加下划线(SEQ ID NO:136)。图50C:构建体2222号的示意图。图50D:构建体2223号的示意图。

[0099] 图51显示用来制备构建体2219号(2X35S/CPMV HT+PDISP/H3珀斯)和2139(2X35S/CPMV 160+/PDISP/H3珀斯)(参见实施例5.30)的序列组件。图51A显示PDISP/H3珀斯的核苷酸序列(SEQ ID NO:137)。图51B显示引物IF**-H3P1609.S1-6r的核苷酸序列(SEQ ID NO:138)。图51C显示PDISP/H3珀斯的氨基酸序列(SEQ ID NO:139)。图51D显示构建体2219号的示意图。图51E显示构建体2139号的示意图。

[0100] 图52显示用来制备构建体2039号(2X35S/CPMVHT+具有缺失的蛋白水解环的PDISP/H3珀斯)和2159号(2X35S/CPMV 160+具有缺失的蛋白水解环的PDISP/H3珀斯)(参见实施例5.31)的序列组件。图52A显示具有缺失的蛋白水解环的PDISP/H3珀斯的核苷酸序列

(SEQ ID NO:140)。图52B显示引物H3P1609(Prl-)#2.r的核苷酸序列(SEQ ID NO:141)。图52C显示引物H3P1609(Prl-)#2.c的核苷酸序列(SEQ ID NO:142)。图52D显示具有缺失的蛋白水解环的PDISP/H3珀斯的氨基酸序列(SEQ ID NO:143)。图52E显示构建体2039号的示意图。图52F显示构建体2159号的示意图。

[0101] 图53显示用来制备构建体2230号(2X35S/CPMVHT+具有缺失的蛋白水解环的PDISP/H3维多利亚)和2250(2X35S/CPMV 160+具有缺失的蛋白水解环的PDISP/H3维多利亚)(参见实施例5.32)的序列组件。图53A显示具有缺失的蛋白水解环的PDISP/H3维多利亚的核苷酸序列(SEQ ID NO:144)。图53B显示引物H3V36111(Prl-).r的核苷酸序列(SEQ ID NO:145)。图53C显示引物H3V36111(Prl-).c的核苷酸序列(SEQ ID NO:146)。图53D显示具有缺失的蛋白水解环的PDISP/H3维多利亚的氨基酸序列(SEQ ID NO:147)。图53E显示构建体2230号的示意图。图53F显示构建体2250号的示意图。

[0102] 图54显示用来制备构建体2142号(2X35S/CPMV HT+/PDISP/H7杭州/NOS)(参见实施例5.33)的序列组件。图54A显示PDISP/H7杭州的核苷酸序列(SEQ ID NO:148)。图54B显示引物IF*-H7H113.s1-6r的核苷酸序列(SEQ ID NO:149)。图54C显示PDISP/H7杭州的氨基酸序列(SEQ ID NO:150)。图53D显示构建体2142号的示意图。

[0103] 图55显示用来制备构建体2152号(2X35S/CPMVHT+/具有缺失的蛋白水解环的PDISP/H7杭州/NOS)(参见实施例5.34)的序列组件。图55A显示具有缺失的蛋白水解环的PDISP/H7杭州的核苷酸序列(SEQ ID NO:151)。图55B显示引物H7H113(PrL-).r的核苷酸序列(SEQ ID NO:152)。图55C显示引物H7H113(PrL-).C的核苷酸序列(SEQ ID NO:153)。图55D显示具有缺失的蛋白水解环的PDISP/H7杭州的氨基酸序列(SEQ ID NO:154)。图53E显示构建体2152号的示意图。

[0104] 图56显示用来制备构建体2224号(2X35S/CPMV HT+PDISP/H9香港)和2226号(2X35S/CPMV 160+PDISP/H9香港)(参见实施例5.35)的序列组件。图56A显示PDISP/H9香港的核苷酸序列(SEQ ID NO:155)。图56B显示引物IF**-H9HK107399.S1-6r的核苷酸序列(SEQ ID NO:156)。图56C显示PDISP/H9香港的氨基酸序列(SEQ ID NO:157)。图56D显示构建体2224号的示意图。图56E显示构建体2226号的示意图。

[0105] 图57显示用来制备构建体2225号(2X35S/CPMVHT+具有缺失的蛋白水解环的PDISP/H9香港)和2227号(2X35S/CPMV 160+具有缺失的蛋白水解环的PDISP/H9香港)(参见实施例5.36)的序列组件。图57A显示具有缺失的蛋白水解环的PDISP/H9香港的核苷酸序列(SEQ ID NO:158)。图57B显示引物H9HK107399(Prl-).r的核苷酸序列(SEQ ID NO:159)。图57C显示引物H9HK107399(Prl-).c的核苷酸序列(SEQ ID NO:160)。图57D显示具有缺失的蛋白水解环的PDISP/H9香港的氨基酸序列(SEQ ID NO:161)。图57E显示构建体2225号的示意图。图57F显示构建体2227号的示意图。

[0106] 图58显示用来制备构建体2013号(2X35S/CPMV 160+/PDISP/HA B马来西亚/NOS)(参见实施例5.37)的序列组件。图58A显示PDISP/HA B马来西亚的核苷酸序列(SEQ ID NO:162)。图58B显示引物IF**-HBM250604.S1-6r的核苷酸序列(SEQ ID NO:163)。图58C显示PDISP/HA B马来西亚的氨基酸序列(SEQ ID NO:164)。图58D显示构建体2013号的示意图。

[0107] 图59显示用来制备构建体2014号(2X35S/CPMV 160+具有缺失的蛋白水解环的/PDISP/HA B马来西亚/NOS)(参见实施例5.38)序列组件。图59A显示具有缺失的蛋白水解环

的PDISP/HA B马来西亚的核苷酸序列(SEQ ID NO:165)。图59B显示引物HBM250604(PrL-).r的核苷酸序列(SEQ ID NO:166)。图59C显示引物HBM250604(PrL-).c的核苷酸序列(SEQ ID NO:167)。图59D显示具有缺失的蛋白水解环的PDISP/HA B马来西亚的氨基酸序列(SEQ ID NO:168)。图59E显示构建体2014号的示意图。

[0108] 图60显示用来制备构建体2070号(2X35S/CPMV HT PDISP/HA B马萨诸塞州)、2080号(2X35S/CPMV HT+PDISP/HA B马萨诸塞州)和2090号(2X35S/CPMV 160+PDISP/HA B马萨诸塞州)(参见实施例5.39)的序列组件。图60A显示PDISP/HA B马萨诸塞州的核苷酸序列(SEQ ID NO:169)。图60B显示PDISP/HA B马萨诸塞州的氨基酸序列(SEQ ID NO:170)。图60C显示构建体2070号的示意图。图60D显示构建体2080号的示意图。图60E显示构建体2090号的示意图。

[0109] 图61显示用来制备构建体2102号(2X35S/CPMVHT+具有缺失的蛋白水解环的PDISP/HA B弗罗里达)和2104号(2X35S/CPMVHT+具有缺失的蛋白水解环的/BeYDV/PDISP/HA B弗罗里达)(参见实施例5.40)的序列组件。图61A显示引物HBF406(PrL-).r的核苷酸序列(SEQ ID NO:190)。图61B显示引物HBF406(PrL-).c的核苷酸序列(SEQ ID NO:191)。图61C显示引物IF*-HBF406.s1-6r的核苷酸序列(SEQ ID NO:192)。图61D显示具有缺失的蛋白水解环的PDISP/HA B弗罗里达的核苷酸序列。图61E显示具有缺失的蛋白水解环的PDISP/HA B弗罗里达的氨基酸序列。图61F显示表达盒2102号的核苷酸序列。图61G显示构建体2102号的示意图。图61H显示表达盒2104号的核苷酸序列。图61I显示构建体2104号的示意图。

[0110] 图62显示用来制备构建体2106号(2X35S/CPMV HT+/PDISP/B弗罗里达+具有缺失的蛋白水解环的H1加利福尼亚TMCT/NOS)和2108号(2X35S/CPMV HT+/BeYDV/PDISP/B弗罗里达+具有缺失的蛋白水解环的H1加利福尼亚TMCT/NOS)(参见实施例5.41)的序列组件。图62A显示引物IF-H1cTMCT.S1-4r的核苷酸序列(SEQ ID NO:197)。图62B显示PDISP/HA B弗罗里达+具有缺失的蛋白水解环的H1 Cal TMCT的核苷酸序列(SEQ ID NO:198)。图62C显示PDISP/HA B弗罗里达+具有缺失的蛋白水解环的H1 Cal TMCT氨基酸序列。图62D显示表达盒2106号的核苷酸序列。图62E显示构建体2106号的示意图。图62F显示表达盒2108号的核苷酸序列。图62G显示构建体2108号的示意图。

[0111] 发明详述

[0112] 以下的描述是优选实施方案的描述。

[0113] 本发明涉及病毒样颗粒(VLP)以及在植物中产生和增加VLP产量、累积和生产的方法。

[0114] 本发明部分地,提供在植物或植物的部分中产生病毒样颗粒(VLP)的方法。所述方法包括将核酸引入植物或植物的部分。所述核酸包含调控区,所述调控区在植物中有活性并与编码流感血凝素(HA)的核苷酸序列操作性地连接到。所述HA包含修饰的蛋白水解环或切割位点。在允许所述核酸表达的条件下培养植物或植物的部分,从而产生VLP。如果需要,可收集植物或植物的部分,并且纯化VLP。

[0115] 本发明也提供通过该方法所产生的VLP。VLP可包含一种或多种源自植物的脂质。

[0116] VLP可被用来制备包含有效剂量的用于诱导免疫应答的VLP以及药学上可接受的载体的组合物。

[0117] 本文也提供修饰的血凝素,其中蛋白水解环或切割位点被修饰。

[0118] 本发明也提供包含通过表达如上所述的核酸所产生的VLP的植物物质。所述植物物质可被用于在对象中诱导对流感病毒感染的免疫力。所述植物物质也可作为食品补充剂被掺入。

[0119] 也可通过如下来产生本发明的VLP:提供包含如上所定义的核酸的植物或植物的部分,并且在允许所述核酸表达的条件下培养植物或植物的部分,从而产生VLP。VLP可包含一种或多种源自植物的脂质。VLP可被用来制备包含有效剂量的用于诱导免疫应答的VLP以及药学上可接受的载体的组合物。本发明也提供包含通过表达第一和第二核酸所产生的VLP的植物物质。所述植物物质可被用于在对象中诱导对流感病毒感染的免疫力。所述植物物质也可作为食品补充剂被掺入。

[0120] 本发明的VLP包含一个或多个修饰的流感血凝素(HA)。修饰的HA可源自任何HA,例如在WO 2009/009876;WO 2009/076778;WO 2010/003225;WO 2010/003235;WO 2011/03522中所描述的H1、H2、H3、H4、H5、H6、H7、H8、H9、H10、H11、H12、H13、H14、H15、H16或B型HA;其通过引用并入本文。

[0121] 本发明包括包含流感毒株的HA序列的VLP,其中HA序列包含修饰的多碱性切割位点,其包括例如本文所述的修饰。

[0122] HA蛋白

[0123] 本文所使用的术语“血凝素”或“HA”指存在于流感病毒颗粒的外部的糖蛋白。HA是同源三聚体I型膜糖蛋白,其通常包含信号肽、HA1结构域和HA2结构域以及小胞质尾区,所述HA2结构域包含C-端跨膜锚定位点。编码HA的核苷酸序列是公知的并且是可获得的--参见,例如,生物预防公共健康基础(BioDefence Public Health base)(流感病毒;参见URL:biohealthbase.org)或生物技术信息国家中心(National Center for Biotechnology Information)(参见URL:ncbi.nlm.nih.gov),二者均通过引用并入本文。HA可包括任何HA,例如WO 2009/009876;WO 2009/076778;WO 2010/003225;WO 2010/003235;WO 2011/03522中所描述的H1、H2、H3、H4、H5、H6、H7、H8、H9、H10、H11、H12、H13、H14、H15、H16或B型HA,其通过引用并入本文)。此外,HA可以分离自一种或多种新兴的或最新鉴定的流感病毒的血凝素的序列为基础。本发明也包括包含获自一种或多种流感亚型的修饰的HA的VLP。

[0124] HA单体可被细分为三个功能结构域—茎结构域或茎结构域簇(SDC)、球状头部结构域或头部结构域簇(HDC)以及跨膜结构域簇(TDC)。SDC和HDC可被统称为‘胞外域’。Ha等2002的出版物(EMBO J.21:865-875;其通过引用并入本文)基于X射线晶体结构阐明了一些流感亚型中SDC和HDC的多个亚结构域的相对取向。

[0125] HA蛋白被合成为约75kDa的前体蛋白(HA0),其在表面组装为延长的三聚体蛋白。前体蛋白在保守的激活切割位点处被切割为通过二硫键连接的2条多肽链,HA1和HA2(其包含跨膜区)。图15提供一些HA的接头区域的氨基酸序列的非限制性实例。

[0126] 术语“同源三聚体(homotrimer)”或“同源三聚的(homotrimeric)”指通过三个HA蛋白分子形成寡聚物。不希望受理论束缚,HA蛋白被合成为约75kDa的单体前体蛋白(HA0),其在表面组装为延长的三聚体蛋白。在三聚作用发生前,前体蛋白在保守的激活切割位点(也被称为融合肽)处被切割为通过二硫键连接的2条多肽链,HA1和HA2(其包含跨膜区)。HA1片段可为328个长度的氨基酸,以及HA2片段可为221个长度的氨基酸。尽管这种切割对

病毒的感染性很重要,但是对于蛋白的三聚作用或对于免疫原性它不是必须的。在宿主细胞内质网(ER)膜内HA的插入、信号肽的切割以及蛋白的糖基化是共翻译事件。HA的正确再折叠需要蛋白的糖基化和5-6个链内二硫键的形成。HA三聚体在顺式-和反式-高尔基复合体内组装,跨膜结构域在三聚作用过程中发挥作用。菠萝蛋白酶-处理的HA蛋白的晶体结构(其缺乏跨膜结构域)在流感毒株之间表现出高度保守的结构。也已确定,HA在感染过程期间经历主要的构象变化,其需要前体HA0被切割为2条多肽链HA1和HA2。HA蛋白可为加工的(即,包含HA1和HA2结构域),或可为未加工的(即包含HA0结构域)。HA的未加工的前体蛋白被合成为约75kDa的前体蛋白(HA0),其在表面组装为延长的三聚体蛋白。在保守的切割位点处(也被称为蛋白水解环)将前体蛋白切割为通过二硫键连接的2条多肽链,HA1和HA2(其包含跨膜区)。

[0127] 本文所描述的HA蛋白还可为修饰的HA(也被称为"突变体HA")蛋白,例如修饰的前体蛋白(HA0),其中,蛋白水解环或切割位点被修饰。

[0128] 修饰的HA/切割位点

[0129] HA0的切割后,HA变得对pH敏感,其在核内体的pH(<pH 6.0)下经历不可逆的构象变化。前体HA0的构象在低pH下是稳定的,但是切割的HA1-HA2形式是亚稳定的(Bullough PA等,1994,Nature.Vol 371:37-43)。对于B毒株,诱导不同HA的构象变化的pH阈值大约为pH 5.8-5.9,然而对于A型HA,pH阈值更酸,其为pH 5.1至5.8(Beyer WEP等,1986,Archives Virol,vol 90:173)。切割后,HA2的氨基末端为23个氨基酸的非极性序列,然后其变为扭跨宿主细胞膜的跨膜结构域(被称为融合肽;图15)。HA的切割位点位于HA表面的突出的环上,并且该位点易被蛋白酶接近。

[0130] 为了优化疫苗在卵中的产生,并且维持有活性但减毒的病毒,研究了H5的多碱性切割位点(RERRRKKR↓G)的修饰(Horimoto T等,2006,Vaccine,Vol 24:3669-3676)。目标突变体含有前面4个带电氨基酸(RERR)的缺失,并且用TETR代替氨基酸RKKR,其使多碱性切割位点失活,但是通过TETR基序(motif)的精氨酸残基维持了将HA0加工为HA1-HA2的可能性(参见图19)。通过NIBSC采用产生减毒病毒的类似策略以废除多碱性位点,其允许在不杀死卵的情况下产生高产量的A/火鸡(Turkey)/火鸡/1/2005H5N1毒株。多碱性位点序列(GERRRKKR↓G)在它们的突变体中被RETR代替(NIBSC 05/240 NIBSC流感参考病毒NIBG-23)。H5 HA的多碱性切割位点也被H6的单碱性位点代替以用于在卵中表达。在该实例中,多碱性位点的前面4个残基和后面四个氨基酸被IETR代替(用IETR↓G代替RERRRKKR↓G;Hoffman E等,2002,Vaccine,Vol 20:3165-3170)。在上文提供各个实例中,进行修饰以使病毒减毒同时维持卵内HA的产生。就是说,HA0前体的切割不完全失活,以便允许HA0被加工为HA1-HA2并且经历pH构象变化,从而允许宿主细胞中的病毒复制。

[0131] 本文所使用的术语"修饰的血凝素"或"修饰的HA"、"突变的血凝素"或"突变的HA"指HA,其中HA具有修饰或突变,例如取代、插入、缺失或其组合,其导致HA蛋白的蛋白水解环中或切割位点处的氨基酸序列的改变。

[0132] 已确定来自A/香港/68的HA0的晶体结构(Chen,J.,1998.Cell 95:409-417;其通过引用并入本文)。暴露于溶剂的残基通常被认为是切割位点的部分,其形成延伸的、高度暴露的表面环。在该选定区域确定共有序列,例如,但不限于:

[0133] A/H3/HA0共有区:NVPEKQTR/GIFGAIAGFIE(SEQ ID NO:66)

[0134] A/H1/HA0共有区:NIPSIQSR/GLFGAIAGFIE(SEQ ID NO:67)

[0135] 禽类H5共有区:QRESRRKKR/GLFGAIAGFIEG(SEQ ID NO:1)

[0136] B/HA0共有区:PAKLLKER/GFFGAIAGFLE(SEQ ID NO:68)

[0137] 其中HA1和HA2之间的切割通过“/”指示(参见Bianchi等,2005,Journal of Virology,79:7380-7388;其通过引用并入本文),以及也参见图15和18A。

[0138] HA蛋白可为B型流感血凝素或A型流感血凝素蛋白,其在蛋白水解环区具有修饰,例如蛋白水解环(切割位点)的缺失、插入、取代或其组合。不希望受理论束缚,蛋白水解环的修饰可确保HA分子被维持为HA0前体。从而产生包含HA0蛋白的更均匀和一致的VLP。

[0139] “蛋白水解环”或“切割位点”意指在前体HA0切割中所涉及的蛋白水解位点的共有序列。本文所使用的“共有区”或“共有序列”指包含相关序列的序列变异性的序列(氨基酸或核苷酸序列),其基于多重序列(例如,具体流感亚型的HA0序列)的比对分析。流感HA0切割位点的共有序列可包括流感A共有血凝素氨基酸序列(其包括例如共有H1、共有H3、共有H5),或流感B共有血凝素氨基酸序列,例如但不限于B佛罗里达和B马来西亚。蛋白水解环区域的序列的非限制性实例如图15和18B中所示(并且参见Bianchi等,2005,Journal of Virology,79:7380-7388;其通过引用并入本文)。

[0140] 蛋白水解环或切割位点处的残基可被突变,例如但不限于:点突变、取代、插入或缺失。本文所使用的术语“氨基酸突变”或“氨基酸修饰”意指涵盖氨基酸取代、缺失、插入和修饰。可做出取代、缺失、插入和修饰的任意组合以实现最终的构建体,条件是最终的构建体拥有期望的性质,例如,减少或废除的蛋白水解环或切割位点的蛋白酶切割。

[0141] “修饰的蛋白水解环”,其意指蛋白水解环可包括一个或多个点突变,被部分地缺失、完全地缺失、用接头序列部分地代替、用接头序列完全地代替、包含用一个或多个非-蛋白氨基酸来部分或完全代替切割位点内的氨基酸,或上述项的组合。类似地,“修饰的切割位点”,其意指蛋白水解环内的切割位点可包括一个或多个点突变,被部分地缺失、完全地缺失、用接头序列部分地代替、用接头序列完全地代替、包含用一个或多个非-蛋白氨基酸来部分或完全代替切割位点内的氨基酸,或上述项的组合。蛋白水解环和/或切割位点的修饰,也可涉及位于蛋白水解环或切割位点序列的外部或与其邻近的一个或多个氨基酸的缺失、代替或取代。“接头”意指包含一个或多个氨基酸的氨基酸序列,其可被引入蛋白水解环或切割位点内,或者其可代替蛋白水解环或切割位点处一些或全部氨基酸。可设计接头以确保蛋白水解环或切割位点内的任意氨基酸的缺失不破坏修饰的HA的表达或随后的活性。

[0142] 在植物中表达修饰的HA时,与相同条件下植物中所表达的天然HA相比,通过修饰或缺失蛋白水解环使HA蛋白稳定,可实现产物或蛋白产量的增加。此外,与相同条件下植物中所表达的天然HA相比时,通过修饰或缺失蛋白水解环,降低了被表达的修饰HA的表达变异性,以及增加了产生的修饰HA的一致性。

[0143] 因此,本发明也包括增加植物中HA蛋白的产物产量的方法。不希望受理论束缚,据信通过修饰或缺失HA蛋白中的蛋白水解环,提供了改善的抵抗植物中蛋白水解的稳定性,高尔基体分泌过程中HA传代期间以及纯化过程期间的稳定性。

[0144] 此外,本发明也包括提高植物中所表达的HA蛋白的产物质量的方法。产物质量意指,例如增加的在植物中所表达的HA的产物产量、产物稳定性(例如增加的植物中所表达的HA的稳定性)、产物的一致性(例如产生同质产物,例如HA0),或上述项的组合。

[0145] 产物或蛋白产量的增加,其意指与不具有去除的蛋白水解环的相同HA蛋白的产物或蛋白产量相比时,使用本领域标准技术所测定的约20%至约100%或其间的任意量,例如,约40%至约70%或其间的任意值,例如约20%、22%、24%、25%、26%、28%、30%、32%、34%、35%、36%、38%、40%、42%、44%、45%、46%、48%、50%、52%、54%、55%、56%、58%、60%、65%、70%、75%、80%、85%、90%、95%或100%,或其间的任意量的相对蛋白产量的增加。

[0146] 如图13A和图14中所示,来自B/布里斯班/60/2008的HA在农杆菌渗入的烟草本塞姆氏(Nicotiana benthamiana)叶中表达很弱(参见条带1008)。然而,经修饰以缺失蛋白水解环的B型HA的表达(参见条带1059,图13A、图14)导致表达增加。此外,HA-B型与来自A/新喀里多尼亚/20/99的M2的共表达,导致HA表达的增加(参见条带"1008+1261";和"1059+1261")。包含蛋白水解环的缺失的B型HA与来自A/波多黎各/8/34的M2的共表达,也导致表达增加(1059+859;图14)。

[0147] 图46B中进一步显示,经修饰HA以缺失或修饰蛋白水解环时,增加了在农杆菌渗入的烟草本塞姆氏中所表达的来自H7 A/杭州/1/13的HA蛋白的蛋白产量。天然(野生型)HAH7 A/杭州/1/13与来自A/新喀里多尼亚/20/99的M2的共表达,导致100%相对蛋白产量,然而,包含蛋白水解环缺失的HA H7 A/杭州/1/13与来自A/新喀里多尼亚/20/99的M2的共表达导致182%相对蛋白产量(图46B)。然而,包含蛋白水解环缺失的HA的相对蛋白产量的增加不取决于M2。例如图29A中所示,与天然的H7 A/杭州/1/13相比时,包含蛋白水解环缺失的H7 A/杭州/1/13表现出表达增加(通过相对HA滴度的测量)。

[0148] 评估一些策略以便使A毒株和B毒株的HA0的切割失活。被蛋白酶识别的共有序列被附在延伸环上、暴露于溶剂,并且靠近蛋白的膜远侧部分。在B毒株中,该环含有被蛋白酶识别的2个序列基序,以及HA2结构域的前面的N-端氨基酸。使HA0前体的切割失活的点突变方法(例如参见下面的表2),在没有增加B毒株VLP累积的情况下导致HA0产生。包含2个蛋白酶切割基序(7个氨基酸)的序列基序的缺失,废除了B HA的累积。去除来自B毒株的HA蛋白的全部18-氨基酸的环,并且插入接头以维持蛋白结构的结构特征(β链)是有效的(参见下面;图13A、14、16A、17B)。A毒株的HA蛋白中蛋白水解环的去除或代替也是有效的(参见图20、22)。

[0149] 氨基酸序列缺失和插入包括氨基酸的缺失和插入氨基酸。流感B中缺失的非限制性实例为,例如,在图18C所示的流感B弗罗里达和流感B马来西亚所示的,成熟HA蛋白的位置340至357的17个氨基酸(AKLLKERGFFGAIAGFLE)的缺失。该缺失可被适当的接头代替以连接正确表达的多肽链,例如但不限于,使用图21B所示的序列"GG"(SEQ ID NO:17;修饰的B/布里斯班/60/2008;用GG代替AKLLKERGFFGAIAGFLEG;例如构建体1059,图5D,10;构建体1039,图8B或构建体1467;图7D、7E)。可选的代替使得包括如图21C所示(SEQ ID NO:18)的用"GSSSGSSSG"代替"PPAKLLKER"。此外,如图19所示,对于流感H5/印度尼西亚,序列"RESRRKKR"可被"TETR"或"TETQ"代替。

[0150] 用于来自A毒株的HA的可选的氨基酸突变包括氨基酸取代、插入和缺失,例如但不限于H5安徽的蛋白水解环区域的氨基酸序列"RERRRKRGLFGAIAGFIE"的缺失、包含"RESRRKKRGLFGAIAGFIE"的H5 Indo的蛋白水解环区域的氨基酸序列的缺失,或H5越南的蛋白水解环区域的氨基酸序列"RERRRKKRGLFGAIAGFIE"的缺失。对于H3,序列"RNVPEKQTRGIF"

可被缺失或被适当的接头序列代替，例如但不限于图21E所示的“GS”(SEQ ID NO:20)。可选地，H3中的序列“RNVPEKQTR”可被图21F所示的“GSSGSSGSS”代替(SEQ ID NO:21;修饰的H3A/珀斯/16/2009)。

[0151] 此外，修饰或改变HA的蛋白水解环或切割位点以减少或废除蛋白水解环或切割位点的蛋白酶切割，也可包含非-保守氨基酸的取代，即用具有不同结构和/或化学特性的另一种氨基酸代替一种氨基酸。非-蛋白氨基酸也可被用来取代。例如，氨基酸的取代可包括通过亲水性氨基酸代替疏水性氨基酸。氨基酸的取代可包括通过蛋白质氨基酸的非自然发生的氨基酸或通过其自然发生的氨基酸的衍生物的代替。

[0152] 用于来自B毒株和/或A毒株的HA的氨基酸突变可包括氨基酸缺失。例如为了减少或废除蛋白水解环或切割位点的蛋白酶切割，在蛋白水解环或切割位点序列内缺失或去除一个或多个氨基酸。缺失的非限制性实例包括去除天然HA H5蛋白的氨基酸323至341，例如图18C所示的H5安徽(RERRRKRGLFGAIAGFIE)、H5Indo(RESRRKKRGLFGAIAGFIE)，或H5越南(RERRRKKRGLFGAIAGFIE)。对于H3，序列“RNVPEKQTRGIF”可被“GS”代替(图21E;SEQ ID NO:20)，或H3序列“RNVPEKQTR”可被“GSSGSSGSS”代替(图21F;SEQ ID NO:21)。对于B毒株，序列“AKLLKERGFFGAIAGFLE”可被缺失和/或被序列“GG”代替，如图21B所示(SEQ ID NO:17)，序列“AKLLKERGFFGAIAGFLEG”可被“GG”代替，或序列“PPAKLLKER”被“GSSSGSSSG”代替(图21C;SEQ ID NO:18)。

[0153] 可使用本领域已知的遗传或化学方法生成氨基酸突变。遗传方法可包括定点诱变、PCR、基因合成等等。考虑了通过除遗传改造以外的方法来改变氨基酸侧链基团的方法(如化学修饰)，也是有用的。

[0154] 因此，本发明的血凝素(HA)序列可包含修饰的蛋白水解环序列或切割位点，从而减少或废除蛋白水解环或切割位点的蛋白酶切割。血凝素多肽序列可包含修饰的蛋白水解环或修饰的切割位点序列，例如图5D、7D、8A、18C、19、21B、21C、21E、21F、24D、25D和26D所述的。使用本领域已知的许多方法，包括序列比对(参见例如图15)，可确定或预测任何流感毒株的任何血凝素多肽序列的切割位点。

[0155] 来自H1、H3和B HA的序列分析揭示，H1具有直接在融合肽之前的一个单碱性蛋白水解位点(克拉拉(Clara)型单碱性:Q/EXR)，然而H3和B HA具有2个蛋白水解位点，其中一个位点被克拉拉-样蛋白酶识别(如H1中发现的)，以及另一个位点被胰蛋白酶和胰凝乳蛋白酶-样蛋白酶(P-E/A-K)识别。关于这些HA的切割的共有序列如表1中所示。

[0156] 表1:关于前体HA_0切割的蛋白水解位点的共有序列。被克拉拉(Clara)类胰蛋白酶或胰蛋白酶/胰凝乳蛋白酶(chimotrypsine)识别的序列分别斜体和黑体显示。一些HA毒株包含多碱性的弗林型切割位点(RKKR;纯文本，加下划线)。

[0157]

H1	NIPSI*QSR*↓GLF	SEQ ID NO: 47
H3	NV**PEK***QTR*↓GIF	SEQ ID NO: 48
H5	TGLRNSPQRESRRKKR↓GLF	SEQ ID NO: 60
B	**PAK**LL*KER*↓GFF	SEQ ID NO: 59

[0158] 为了避免HA的HA0前体潜在的蛋白水解切割，只有一个蛋白水解位点可能需要从H1的序列中修饰，然而，在H3和B的情况下，两个单碱性位点可能需要被修饰。

[0159] 例如，通过用Ile代替Lys341(成熟蛋白编号)，可消除B/弗罗里达和B/布里斯班的HA_0的第一个切割位点(参见表2)。通过用NIQ代替融合肽之前的三个氨基酸KER(344-346)，可废除第二个单碱性位点。表2中提供了HA的一些修饰的蛋白水解环的序列。

[0160] 表2：破坏前体HA_0的切割的突变的实例描述。单碱性的位点斜体(克拉拉-样蛋白酶识别)且黑体(非下划线；胰蛋白酶/胰凝乳蛋白酶-样)显示。突变被显示为黑体且加下划线。箭头代表用于将HA_0转化为HA1-HA2的切割位点。

[0161]

毒株	天然序列	前体切割位点的废除
H5/Indo	TGLRNSPQ**RESRRKK** **R**↓GLF SEQ ID NO: 60	TGLRNSPQ***TETR***G LF SEQ ID NO: 61 TGLRNSPQ***TETQ***G LF SEQ ID NO: 62
H1/布里斯班	NIPSI*QSR*↓GLF SEQ ID NO: 47	NIPSIQS***Q***GLF SEQ ID NO: 63
H3/布里斯班	NV**PEK***QTR*↓GIF	NVPE***I***QT***Q***GIF

[0162]

	SEQ ID NO: 48	SEQ ID NO: 64
B/弗罗里达， B/布里斯班	**PAK**LL*KER*↓GFF SEQ ID NO: 59	PA***I***LL***NIQ***GFF SEQ ID NO: 65

[0163] 在又一个实施例中，HA0的包含蛋白水解环的序列可被代替或缺失。例如，图21E中提供H3变体，其含有除了HA2的N-端氨基酸GIFGIA的缺失之外，HA1的C-端的序列RNVPEKQT的缺失。缩短的HA1-HA2可通过GS接头连在一起。

[0164] 在另一个实施例中，例如在H3中，含有蛋白水解切割位点的环可被柔性接头代替，并且HA2部分可保持完整。可设计(GSS)3接头以便适应缩短的HA1与HA2(参见图21F)。

[0165] 在另一个实施例中，来自流感B的HA可含有除HA2的N-端氨基酸GFFGAIAGFLEG的缺失之外的，HA1的C-端处的序列ALKLLKER的缺失。缩短的HA1-HA2可被GG接头连在一起(参见例如图21B；构建体1008)。图13A和B中显示该构建体的表达。

[0166] 在另一个实施例中，含有蛋白水解位点的环的来自流感B的HA可被柔性接头代替，并且HA2部分保持完整。可设计较长的GSSS接头以便适应缩短的HA1与HA2(参见例如图21C)。

[0167] 如图13A和14所示，来自B/布里斯班/60/2008的HA在农杆菌渗入的烟草本塞姆氏叶中表达很弱(参见条带1008)。然而，经修饰以缺失蛋白水解环的HA B型的表达(参见条带1059，图13A，图14)导致表达增加。此外，HA-B型与来自A/新喀里多尼亚/20/99的M2的共表达，导致HA表达的增加(参见条带“1008+1261”；和“1059+1261”)。包含蛋白水解环缺失的HA B型与来自A/波多黎各/8/34的M2的共表达，也导致表达增加(1059+859；图14)。

[0168] 在类似的方式中，H5/Indo的蛋白水解环的缺失，并且用“GG”(构建体928；参见图46D)、“TETR”(构建体676；也参见图19、24D)或“TETQ”(构建体766；也参见图19、25D)序列的代替，导致表达水平比得上或增加超过天然H5/Indo(构建体489；参见图20和23)所观察到的表达水平。

[0169] 如图13B所示，与不具有去除的蛋白水解环的HA蛋白相比时，通过缺失HA0的蛋白水解环(如图21B所示的序列)，得到的HA0蛋白呈现出以较强的血凝能力所显示的增加的活性。

[0170] 活性增加，其意指与不具有去除的蛋白水解环的相同HA蛋白的活性相比时，使用本领域的标准技术所测定的约2％至约100％，或其间的任意量，例如，约10％至约50％或其间的任意值，例如约2、5、8、10、12、15、18、20、22、24、25、26、28、30、32、34、35、36、38、40、42、44、45、46、48、50、52、54、55、56、58、60、65、70、75、80、85、90、95或100％，或者其间的任意量的血凝能力的增加。

[0171] 本发明也包括编码来自例如修饰的H1、H2、H3、H4、H5、H6、H7、H8、H9、H10、H11、H12、H13、H14、H15、H16或B型HA的修饰的HA的核苷酸序列，或者在严格的条件下与H1、H2、H3、H4、H5、H6、H7、H8、H9、H10、H11、H12、H13、H14、H15、H16或B型HA杂交的任何核苷酸序列，或者在严格的杂交条件下与H1、H2、H3、H4、H5、H6、H7、H8、H9、H10、H11、H12、H13、H14、H15、H16或B型HA的互补物杂交的核苷酸序列，其中所述核苷酸序列编码血凝素蛋白，其表达形成VLP，并且VLP诱导抗体的产生。例如，植物细胞内核苷酸序列的表达形成VLP，并且VLP可被用来产生能够结合HA的抗体，所述HA包括来自B或H3的成熟HA。当被施用于对象时，VLP诱导免疫应答。优选地，VLP诱导抗体的产生，并且当被施用于对象时，VLP诱导免疫应答。

[0172] 例如，植物细胞内核苷酸序列的表达形成VLP，并且VLP可被用来产生能够结合诸如HA的病毒蛋白的抗体，例如，所述HA包括但不限于HA0、具有蛋白水解环缺失的或修饰的HA0蛋白、一种或多种流感型或亚型的HA1或HA2，例如但不限于亚型H1、H2、H3、H4、H5、H6、H7、H8、H9、H10、H11、H12、H13、H14、H15、H16、B型HA。当被施用于对象时，VLP诱导免疫应答。

[0173] 严格的杂交条件下的杂交是本领域已知的(参见例如Current Protocols in Molecular Biology，Ausubel等，编著.1995和supplements；Maniatis等，in Molecular Cloning(A Laboratory Manual)，Cold Spring Harbor Laboratory，1982；Sambrook和Russell，in Molecular Cloning:A Laboratory Manual，第三版2001；其各自通过引用并入本文)。该严格杂交条件的一个实例可为：在65℃下于4 X SSC中约16-20小时的杂交，随后在65℃下用0.1 X SSC冲洗1小时，或在65℃下用0.1 X SSC二次冲洗，每次20或30分钟。可选地，示例性严格杂交条件可为：42℃下于50％甲酰胺，4 X SSC中过夜(16-20小时)，随后在65℃下用0.1 X SSC冲洗1小时，或65℃下用0.1 X SSC二次冲洗，每次20或30分钟，或过夜(16-20小时)，或者在65℃下于Church磷酸盐缓冲液(7％SDS；0.5M $NaPO_4$缓冲液pH 7.2；10mM EDTA)中杂交，50℃下用0.1 X SSC、0.1％SDS二次冲洗，每次20或30分钟，或者在

65℃下用2 X SSC、0.1%SDS二次冲洗，每次20或30分钟。

[0174] 此外，本发明包括这样的核苷酸序列，其特点为与编码H1、H2、H3、H4、H5、H6、H7、H8、H9、H10、H11、H12、H13、H14、H15、H16或B型HA的核苷酸序列具有约70%、75%、80%、85%、87%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%、100%或其间任意量的序列同一性或序列相似性，其中所述核苷酸序列编码具有修饰的蛋白水解环序列或切割位点的血凝素蛋白(修饰的HA)，其减少或废除了蛋白水解环或切割位点的蛋白酶切割。当表达编码修饰的HA的核苷酸序列时，其形成VLP，并且所述VLP诱导抗体的产生。例如，植物细胞内核苷酸序列的表达形成VLP，并且所述VLP可被用来产生能够结合HA的抗体，所述HA包括未加工的HA(HA0)或未被加工，其中蛋白水解环已被缺失。当被施用于对象时，VLP诱导免疫应答。

[0175] 此外，本发明包括这样的核苷酸序列，其特点为与核苷酸序列SEQ ID NO:43、91、95、97、99、101、103、105、107、109、111、113、137、140、144、151、158、165具有约70%、75%、80%、85%、87%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%、100%或其间任意量的序列同一性或序列相似性，其中所述核苷酸序列编码修饰的HA蛋白，当其表达时形成VLP，并且所述VLP诱导产生能够结合HA的抗体，所述HA包括未加工的HA(HA0)或未被加工，其中蛋白水解环已被缺失或修饰。当被施用于对象时，VLP诱导免疫应答。

[0176] 此外，本发明包括这样的氨基酸序列，其特点为与氨基酸序列SEQ ID NO:17、18、20、21、41、58、77、81、85、92、96、98、100、102、104、106、108、110、112、114、134、143、147、154、161、168、194和199具有约70%、75%、80%、85%、87%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%、100%或其间任意量的序列同一性或序列相似性。其中所述氨基酸序列编码修饰的HA蛋白，当其表达时形成VLP，并且所述VLP诱导产生能够结合HA的抗体，所述HA包括未加工的HA(HA0)或未被加工的，其中蛋白水解环已被缺失或修饰。当被施用于对象时，VLP诱导免疫应答。

[0177] 可使用如DNASIS(例如，使用，但不限于以下的参数：GAP罚分5，顶部对角的#5，固定的GAP罚分10，k-串2，浮动缺口10，和窗口大小5)内所提供的核苷酸序列比对程序，测定序列同一性或序列相似性。然而，用于比较的序列对比的其他方法是本领域公知的，例如Smith&Waterman的算法(1981，Adv.Appl.Math.2:482)、Needleman&Wunsch的算法(J.Mol.Biol.48:443，1970)、Pearson&Lipman的算法(1988，Proc.Nat'l.Acad.Sci.USA 85:2444)，并且通过计算机实施这些算法(例如GAP、BESTFIT、FASTA和BLAST)，或通过手工比对和目视检查。来自不同流感毒株的HA的序列比对的实例见于图24。

[0178] 例如，但不限于，编码以下的核苷酸序列：

[0179] -具有如SEQ ID NO:17、SEQ ID NO:18、SEQ ID NO:41、SEQ ID NO:43、SEQ ID NO:58、SEQ ID NO:96、SEQ ID NO:98、SEQ ID NO:100、SEQ ID NO:102、SEQ ID NO:104、SEQ ID NO:106、SEQ ID NO:108、SEQ ID NO:110、SEQ ID NO:112、SEQ ID NO:114和SEQ ID NO:168所定义的修饰的蛋白水解环的B型HA；或者编码以下的核苷酸序列：具有如SEQ ID NO:65、SEQ ID NO:72、SEQ ID NO:73、SEQ ID NO:95、SEQ ID NO:97、SEQ ID NO:99、SEQ ID NO:101、SEQ ID NO:103、SEQ ID NO:105、SEQ ID NO:107、SEQ ID NO:109、SEQ ID NO:111、SEQ ID NO:113和SE ID NO:165所定义的修饰的蛋白水解环区域的B型HA。

[0180] -具有修饰的蛋白水解环的H1包括含有如SEQ ID NO:63所定义的修饰的切割位点

的序列。

[0181] -具有修饰的蛋白水解环的H2包括含有如SEQ ID NO:134所定义的修饰的切割位点的序列。

[0182] -具有修饰的蛋白水解环的H3包括如SEQ ID NO:20、SEQ ID NO:21、SEQ ID NO 143、SEQ ID NO:147所定义的序列或含有如SEQ ID NO:64所定义的修饰的切割位点的序列。

[0183] -具有缺失的蛋白水解环的H5包括含有如SEQ ID NO:61、SEQ ID NO:62、SEQ ID NO:69、SEQ ID NO:70、SEQ ID NO:71所定义的修饰的切割位点的序列。

[0184] -具有修饰的蛋白水解环的H7包括含有如SEQ ID NO:154所定义的修饰的切割位点的序列,或者编码含有如SEQ ID NO:151所定义的修饰的蛋白水解环区域的H7型HA的核苷酸序列。

[0185] -具有修饰的蛋白水解环的H9包括含有如SEQ ID NO:161所定义的修饰的切割位点的序列,或编码含有如SEQ ID NO:158中所定义的修饰的蛋白水解环区域的H9型HA的核苷酸序列。

[0186] 本发明关于包含跨膜结构域并且包括HA1和HA2结构域的HA蛋白的用途,例如所述HA蛋白可为HA0,或包含HA1和HA2的加工的HA。使用植物或植物细胞表达系统,HA蛋白可被用于产生或形成VLP。

[0187] 扩增元件和增强子元件/调控元件

[0188] 在另一个实施例中,修饰HA蛋白可在包含扩增元件和/或调控元件或区域(本文也被称为增强子元件)的表达系统中表达。例如来自双粒病毒组的扩增元件,如例如,来自菜豆黄矮病毒(BeYDV)的扩增元件可被用来表达修饰的HA。BeYDV属于适合于双子叶植物的玉米线条虫属(Mastreviruses)。BeYDV是具有单链环状DNA基因组的单组分,并且其可通过滚环机制复制至十分高的拷贝数。BeYDV-来源的DNA复制子载体系统,已被用于在植物中快速高产量的蛋白产生。

[0189] 本文所使用的短语“扩增元件”指包含至少一部分的双粒病毒组基因组的一个或多个长基因间隔区(LIR)的核酸节段。本文所使用的“长基因间隔区”指含有能够通过双粒病毒组Rep蛋白调节切除和复制的rep结合位点的长基因间隔区的区域。在一些方面中,包含一个或多个LIRs的核酸节段,还可包含双粒病毒组基因组的短基因间隔区(SIR)。如本文所使用的,“短基因间隔区”指互补链(玉米线条虫属的短IR(SIR))。本文可使用任何适合的双粒病毒组-来源的扩增元件。参见,例如,WO2000/20557;WO2010/025285;Zhang X等(2005,Biotechnology and Bioengineering,Vol.93,271-279),Huang Z等(2009,Biotechnology and Bioengineering,Vol.103,706-714),Huang Z等(2009,Biotechnology and Bioengineering,Vol.106,9-17);其通过引用并入本文)。如果在所述构建体中使用多于一个LIR,例如两个LIRs,那么对于两个LIRs的每一个来说,启动子、CMPV-HT区域和目标核酸序列以及终止子是相等的。

[0190] 如本文所描述的,通过烟草本塞姆氏叶的农杆菌渗入,菜豆黄矮病毒(BeYDV)-来源的载体和Rep/RepA-供应载体的共递呈,导致有效的复制子扩增和大量的蛋白产生。来自转化有驱动具有或没有蛋白水解环去除的修饰的流感B HA(来自B/布里斯班/60/2008)(参见图17A的构建体)在存在或不存在扩增元件BeYDV(构建体1059号和1039号)时的表达的基

因构建体的植物的蛋白提取物的Western印迹分析表明，当调控元件是CPMV-HT，在不存在BeYDV时，可检测到流感B HA无累积(图17B)，。

[0191] 如图17B所示，通过Western印迹分析，来自B/布里斯班/60/2008的具有蛋白水解环去除的HA，在不存在BeYDV时的表达不会导致可检测的表达(参见图17B中的条带1039)。然而，具有蛋白水解环去除的HA B型，在存在扩增元件BeYDV时的表达，导致增加的表达(参见条带1059)。同样地，在不存在BeYDV时，包含蛋白水解环缺失的突变体HA-B型与来自A/新喀里多尼亚/20/99的M2的共表达，不会导致可检测的HA表达(参见图17B中的条带“1039+1261”)。另一方面，包含蛋白水解环缺失的突变体HA-B型，在存在BeYDV时，与来自A/新喀里多尼亚/20/99的M2的共表达导致增加的表达(参见条带“1059+1261”;图17B)。

[0192] 然而，当增强子元件存在于表达系统中，且当增强元件不是CPMV-HT时，BeyDV的存在不是必须的。例如，如图29A所示，在不存在BeYDV时，在诸如CPMV 160、CPMV 160+或CPMV HT+的增强子元件的控制下不同B HA毒株的表达导致HA蛋白的产生，其表现出增加的血凝滴度(HMG)。

[0193] 因此，突变体(修饰的)HA蛋白可在不存在扩增元件，如基于双粒病毒组的扩增元件(例如BeYDV)，但在存在诸如CPMV160、CPMV 160+或CPMV HT+的增强子元件下表达。

[0194] 突变体(修饰的)HA可在存在诸如CPMV160、CPMV 160+或CPMV HT+的增强子元件，但在不存在或存在诸如BeYDV的扩增元件下表达。如图28B、28C和28F所示，突变体(修饰的)HA可在增强子元件的存在下，有或无扩增元件存在下表达。因此本发明也涉及突变体(修饰的)HA在增强子元件和任选的扩增元件的存在下表达。

[0195] 当与野生型或包含CPMV HT的HA构建体相比时，包含增强子元件(CMPVHT+或CMPV160+)和用GG接头代替蛋白水解环(缺失的蛋白水解环)的HA构建体，呈现出增加的表达(图28A，H3珀斯(Per)；图28B，B马来西亚；图28C，H9 HK(香港)；图29D，B马萨诸塞州(Mass)；图28E，H2新加坡(Sin))。

[0196] 图29A呈现在植物中所产生的包含基于CPMV HT、CPMV HT+、CPMV 160或CPMV 160+的增强子元件的修饰HA蛋白质或天然HA的血凝滴度的总结数据，所述增强子元件与编码具有缺失的蛋白水解环(GG接头)的修饰HA或天然HA的核苷酸序列可操作地连接。在大多数的情况下，基于CPMV HT+、CPMV 160或CPMV 160+构建体的表达较高(测定为血凝滴度)，表明显著的表达水平。

[0197] 增强子元件可被用来实现具有修饰的蛋白水解环的突变体(修饰的)HA蛋白的高水平的瞬转表达。增强子元件可基于RNA植物病毒，包括豇豆花叶病毒组，如豇豆花叶病毒(CPMV;参见，例如，WO2007/135480;WO2009/087391;US 2010/0287670,Sainsbury F等，2008,plant Physiology;148:121-1218;Sainsbury F等，2008,plant Biotechnology Journal;6:82-92;Sainsbury F等，2009,plant Biotechnology Journal;7:682-693;Sainsbury等，2009,Methods in Molecular Biology,Recombinant Proteins From plant,vol.483:25-39)。

[0198] CPMV 160(CPMVX)和CPMV 160+(CPMVX+)

[0199] 在一个实施方案中，增强子元件为US 61/925,852中所描述的“CPMVX”(也被称为“CPMV 160”)和/或“CPMVX+”(也被称为“CPMV 160+”)，其通过引用并入本文。

[0200] 表达增强子“CPMVX”包含豇豆花叶病毒组的豇豆花叶病毒(CPMV)5’非翻译区

(UTR)。来自CPMV RNA-2序列(SEQ ID NO:93)的核苷酸1-160的5'UTR,起始于转录起始位点至第一个框内起始密码子(在位置161),其作为用于产生由野生型豇豆花叶病毒基因组片段编码的两个羧基共末端蛋白的较长者的起始位点。此外,CPMV RNA-2基因组序列中在(或对应于)位置115处的'第三个'起始位点也可被突变、缺失或否则改变。已表明,当与不完整M蛋白结合时,除了AUG 161的去除之外,AUG 115的去除提高了表达(Sainsbury and Lomonossoff,2008,plant Physiology;148:1212-1218;WO 2009/087391;其通过引用并入本文)。

[0201] CPMVX包含SEQ ID NO:93的X个核苷酸,其中SEQ ID NO:93的X=160、155、150或114,或者包含与CPMVX具有80%至100%序列相似性的序列,其中SEQ ID NO:93的X=160、155、150或114。该表达增强子通常被称为CPMVX(参见图26A)。

[0202] 表达增强子CPMVX,其中X=160,由SEQ ID NO:93的核苷酸1-160组成:

[0203] 1 tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc

[0204] 61 ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgcgtgagc

[0205] 121 gatcttcaac gttgtcagat cgtgcttcgg caccagtaca(SEQ ID NO:93)

[0206] CPMVX增强子序列还可与填充序列融合,其中CMPVX包含SEQ ID NO:1的X个核苷酸,其中SEQ ID NO:1的X=160、155、150或114,或者包含与CPMVX具有80至100%序列相似性的序列,其中SEQ ID NO:93的X=160、155、150或114,并且填充序列包含与CMPVX序列3'端融合的1-100个核苷酸。例如,填充序列可包含约1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、30、35、40、45、50、55、60、65、70、75、80、85、90、95或100个核苷酸,或其间任意数量的核苷酸。

[0207] 如果CMPVX序列包含填充片段,那么该表达增强子可被称为CPMVX+(参见图26A),其中SEQ ID NO:1的X=160、155、150、114,其也可被称为包含填充序列的CMPVX,或其可被分别地称为CPMV 160+;CPMV155+;CPMV150+;CPMV114+,此时X=160、155、50或114。包含CPMVX(其不包含填充序列)的构建体被称为CPMVX+,其中SEQ ID NO:93的X=160、155、150、114,并且其中填充序列为0个长度的核苷酸。

[0208] 可通过位于核苷酸160的3'端的天然CMPV5'UTR序列的截短、缺失或代替来修饰填充序列。当与连接有5'UTR的原始的或未修饰的(即天然的)填充序列相比时,修饰的填充序列可被去除、代替、截短或缩短(如在Sainsbury F.and Lomonossoff G.P.,2008,plant Physiol.148:pp.1212-1218所述)。填充序列可包含一个或多个限制性位点(多接头、多克隆位点、一个或多个克隆位点),一个或多个植物kozak序列,一个或多个接头序列,一个或多个重组位点或其组合。例如,其不应被认为是限制,填充序列可连续地包含与植物kozak序列融合的预期长度的多克隆位点。填充序列不包含来自位于天然CPMV 5'UTR的核苷酸160的3'的天然5'UTR序列的核苷酸序列,例如Sainsbury F.,and Lomonossoff G.P.的图1中所示的核苷酸161至512(2008,Plant Physiol.148:pp.1212-1218;其通过引用并入本文),或SEQ ID NO:4的核苷酸161-509。就是说,在本发明中,存在于现有技术的CPMV HT序列中的不完整M蛋白(Sainsbury F.,and Lomonossoff G.P.,2008的图1中)从5'UTR被去除。

[0209] 植物Kozak共有序列是本领域已知的(参见,例如Rangan等.Mol.Biotechnol.,2008,July 39(3),pp.207-213)。自然发生的和合成的Kozak序列均可被用于表达增强子

中,或可与本文所描述的目标核苷酸序列融合。

[0210] 植物kozak序列可为任何已知的植物kozak序列(参见,例如L.Rangan等.Mol.Biotechnol.2008),其包括但不限于以下的植物共有序列:

[0211] caA(A/C)a(SEQ ID NO:174;植物界)

[0212] aaA(A/C)a(SEQ ID NO:175;双子叶植物)

[0213] aa(A/G)(A/C)a(SEQ ID NO:176;拟南芥)

[0214] 植物kozak序列也可选自:

[0215] AGAAA(SEQ ID NO:177)

[0216] AGACA(SEQ ID NO:178)

[0217] AGGAA(SEQ ID NO:179)

[0218] AAAAA(SEQ ID NO:180)

[0219] AAACA(SEQ ID NO:181)

[0220] AAGCA(SEQ ID NO:182)

[0221] AAGAA(SEQ ID NO:183)

[0222] AAAGAA(SEQ ID NO:184)

[0223] AAAGAA(SEQ ID NO:185)

[0224] (A/-)A(A/G)(A/G)(A/C)A.(SEQ ID NO:186;共有序列)

[0225] 表达增强子CPMVX或CPMVX+,在增强子序列的5'端与在植物中有活性的调控区可操作地连接,并且在表达增强子的3'端与目标核苷酸序列可操作地连接(图26A),以便促使目标核苷酸序列在植物宿主内的表达。

[0226] CPMV HT+、CPMV HT+[WT115]、CPMV HT+[511]

[0227] 在另一个实施方案中,增强子元件为US 61/971,274中所描述的"CPMV HT+",其通过引用并入本文。表达增强子"CPMV HT+"(参见图27A)包含豇豆花叶病毒5'非翻译区(UTR)和修饰的、延长的或截短的填充序列。

[0228] 也提供植物表达系统,其包括包含调控区的第一核苷酸序列和编码修饰的HA的核苷酸序列,所述调控区与本文所描述的一个或多于一个表达增强子(例如CPMV HT+、CPMV HT+[WT115]、CPMV HT+[511])可操作地连接。此外,描述了包含启动子(调控区)序列、表达增强子(例如CPMV HT+或CPMV HT+[WT115])的核酸,所述表达增强子包含豇豆花叶病毒5'UTR和与编码修饰的HA的一个或多个核酸序列融合的具有植物kozak序列的填充序列。核酸还可包含含有豇豆花叶病毒3'非翻译区(UTR)(例如,质体蓝素3'UTR或在植物中有活性的其他3'UTR)和终止子序列(例如NOS终止子)的序列,所述序列与编码修饰的HA的核苷酸序列(在图27A中被称为目标核苷酸)的3'端可操作地连接,以便将编码修饰HA的核苷酸序列插入豇豆花叶病毒3'非翻译区(UTR)、质体蓝素3'UTR或其他3'UTR序列上游。

[0229] SEQ ID NO:173包含现有技术中已知的"CPMV HT"表达增强子(例如Sainsbury and Lomonossoff 2008,plant Physiol.148:pp.1212-1218的图1;其通过引用并入本文)。CPMV HT包括来自在位置115(cgt)处具有修饰的核苷酸的SEQ ID NO:173的核苷酸1-160的5'UTR序列以及在位置162(acg)处具有修饰的核苷酸的不完整M蛋白,并且缺少植物kozak序列(5'UTR:核苷酸1-160;加下划线的不完整M蛋白,核苷酸161-509)。SEQ ID NO:173也包括不存在于现有技术CPMV HT序列中的多克隆位点(斜体,核苷酸510-528):

1 tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc

61 ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgcgtgagc

121 gatcttcaac gttgtcagat cgtgcttcgg caccagtaca ***acg***ttttctt tcactgaagc

181 gaaatcaaag atctctttgt ggacacgtag tgcggcgcca ttaaataacg tgtacttgtc

[0230]

241 ctattcttgt cggtgtggtc ttgggaaaag aaagcttgct ggaggctgct gttcagcccc

301 atacattact tgttacgatt ctgctgactt tcggcgggtg caatatctct acttctgctt

361 gacgaggtat tgttgcctgt acttctttct tcttcttctt gctgattggt tctataagaa

421 atctagtatt ttctttgaaa cagagttttc ccgtggtttt cgaacttgga gaaagattgt

[0231] 481 taagcttctg tatattctgc ccaaatttg*t cgggccc* SEQ ID NO: 173

[0232] SEQ ID NO:187中提供了具有植物kozak共有序列的CPMV HT+(核苷酸1-160,5'UTR,其包含位置115(GTG)处小写黑体且斜体的修饰的ATG;填充片段包含:加下划线的不完整M蛋白,核苷酸161-509,在162(ACG)处具有修饰的核苷酸;多克隆位点,斜体,核苷酸510-528;以及共有植物kozak序列,大写且黑体,核苷酸529-534)。

1 tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc

61 ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgc***gtg***agc

121 gatcttcaac gttgtcagat cgtgcttcgg caccagtaca ***acg***ttttctt tcactgaagc

181 gaaatcaaag atctctttgt ggacacgtag tgcggcgcca ttaaataacg tgtacttgtc

241 ctattcttgt cggtgtggtc ttgggaaaag aaagcttgct ggaggctgct gttcagcccc

[0233]

301 atacattact tgttacgatt ctgctgactt tcggcgggtg caatatctct acttctgctt

361 gacgaggtat tgttgcctgt acttctttct tcttcttctt gctgattggt tctataagaa

421 atctagtatt ttctttgaaa cagagttttc ccgtggtttt cgaacttgga gaaagattgt

481 taagcttctg tatattctgc ccaaatttgt *tcgggcccaa taccgcgg****(A/-)A(A/G)***

(A/G)(A/C)A (SEQ ID NO: 187)

[0234] SEQ ID NO:188("CPMV HT+511")包含核苷酸1-154的CPMV RNA 2基因组的天然序列的节段。来自SEQ ID NO:188的核苷酸1-511的5'UTR序列包含在位置115("g"代替"a";斜体粗体)和162("c"代替"t";斜体粗体)处修饰的"atg"序列,和来自核苷酸161-511的不完整M蛋白(加下划线)。CPMV HT+511包含天然M蛋白的kozak共有序列(核苷酸508-511;粗体):

1 tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc

[0235] 61 ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgc***gtg***agc

121 gatcttcaac gttgtcagat cgtgcttcgg caccagtaca ***acg***ttttctt tcactgaagc

181 gaaatcaaag atctctttgt ggacacgtag tgcggcgcca ttaaataacg tgtacttgtc

241 ctattcttgt cggtgtggtc ttgggaaaag aaagcttgct ggaggctgct gttcagcccc

301 atacattact tgttacgatt ctgctgactt tcggcgggtg caatatctct acttctgctt

[0236]

361 gacgaggtat tgttgcctgt acttctttct tcttcttctt gctgattggt tctataagaa

421 atctagtatt ttctttgaaa cagagttttc ccgtggtttt cgaacttgga gaaagattgt

481 taagcttctg tatattctgc ccaaatt**tga** **a**… SEQ ID NO: 188

[0237] 通过SEQ ID NO:189(CPMV HT+[WT115])的序列提供CPMV HT+增强子序列的另一个非限制性实例。表达盒或载体也是本发明的部分,所述表达盒或载体包含CPMV HT+并且包括与SEQ ID NO:189的表达增强子序列可操作地结合的植物调控区以及与编码修饰的HA的核苷酸序列融合的3'端转录起始位点(ATG)。

[0238] SEQ ID NO:189(CPMV HT+[WT115])的核苷酸1-160,5'UTR,在位置115-117处具有ATG,小写粗体;填充片段包含:加下划线的不完整M蛋白,核苷酸161-509,在位置161-163(161-153)处的小写粗体且加下划线的修饰的ATG,多克隆位点,斜体,核苷酸510-528;以及植物kozak序列,大写且粗体,核苷酸529-534)。

1 tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc

61 ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgc***atg***agc

121 gatcttcaac gttgtcagat cgtgcttcgg caccagtaca ***acg***ttttctt tcactgaagc

181 gaaatcaaag atctctttgt ggacacgtag tgcggcgcca ttaaataacg tgtacttgtc

241 ctattcttgt cggtgtggtc ttgggaaaag aaagcttgct ggaggctgct gttcagcccc

[0239]

301 atacattact tgttacgatt ctgctgactt tcggcgggtg caatatctct acttctgctt

361 gacgaggtat tgttgcctgt acttctttct tcttcttctt gctgattggt tctataagaa

421 atctagtatt ttctttgaaa cagagttttc ccgtggtttt cgaacttgga gaaagattgt

481 taagcttctg tatattctgc ccaaatttg*t tcgggcccaa taccgcgg****AG AAAA***

(SEQ ID NO: 189)

[0240] SEQ ID NO:189的植物kozak序列可为任何植物kozak序列,包括但不限于SEQ ID NO's:174-186的序列之一。

[0241] “嵌合蛋白”

[0242] 修饰HA还可为嵌合蛋白。“嵌合病毒蛋白”或“嵌合病毒多肽”，也被称为“嵌合蛋白”或“嵌合多肽”，或者“嵌合HA”，其意指包含来自两种或更多种来源例如但不限于两种或更多种流感型或流感亚型，或者不同起源的流感的氨基酸序列的蛋白或多肽，其被融合为单一多肽。嵌合蛋白或多肽可包括与所述多肽或蛋白的剩余物相同或与其异源的信号肽。嵌合蛋白或嵌合多肽可以作为嵌合核苷酸序列的转录物并且随后合成而产生，并且根据需要可结合形成多亚基蛋白。因此，嵌合蛋白或嵌合多肽也包括包含经二硫键结合的亚基的蛋白或多肽(即多亚基蛋白)。例如，包含来自两种或更多种来源的氨基酸序列的嵌合多肽可被加工成亚基，且亚基经二硫键结合以产生嵌合蛋白或嵌合多肽。嵌合HA蛋白也可包含第一流感病毒的抗原蛋白或其片段，和来自第二病毒流感HA的跨膜结构域复合体(TDC)，所述复合体(TDC)包括跨膜结构域和胞浆尾结构域(TM/CT)。多肽可为修饰的HA，并且组成多肽的两种或更多种的氨基酸序列的每一种可获自不同的HA的氨基酸序列，以产生嵌合HA、嵌合流感HA、嵌合修饰的HA或嵌合修饰的流感HA。嵌合HA也可包括包含异源信号肽的氨基酸序列(嵌合HA前蛋白)，所述信号肽在蛋白合成之后或期间被切除。优选地，嵌合多肽或嵌合的流感HA不是自然发生的。编码嵌合多肽的核酸可被描述为“嵌合核酸”或“嵌合核苷酸序列”。例如嵌合核酸可为包含编码修饰的HA的核苷酸序列，所述嵌合核酸包含按顺序编码修饰的HA胞外域、流感跨膜结构域和胞质尾区的嵌合核苷酸序列，其中修饰的HA胞外域包含修饰的蛋白水解环且来自第一流感毒株，而跨膜结构域和胞质尾区来自第二流感毒株。实施例5.14、5.16、5.18、5.19、5.21和5.23中给出了嵌合核苷酸的实例，其中修饰的HA胞外域来自第一流感毒株，并且跨膜结构域和胞质尾区来自第二流感毒株。包含嵌合HA的病毒样颗粒可被描述为“嵌合VLP”。

[0243] 如上所述，嵌合蛋白、嵌合多肽或嵌合HA可包括与所述多肽或蛋白的剩余物相同或与其异源的信号肽。术语“信号肽”是本领域已知的，并且通常指通常存在于多肽的N-端的短(约5-30个氨基酸)序列的氨基酸，其可引导最新翻译的多肽迁移至具体的细胞器，或协助多肽链的具体结构域相对于其它结构域的定位。作为非限制性实例，信号肽可靶向蛋白迁移至内质网，和/或协助N-端近侧结构域相对于新生多肽的膜-锚定结构域的定位，以协助诸如修饰的HA或嵌合修饰的HA的成熟蛋白的切割和折叠。

[0244] HA也可为嵌合HA或嵌合修饰的HA，其中HA或修饰的HA的天然跨膜结构域被异源跨膜结构域代替。HA蛋白的跨膜结构域是高度保守的(参见，例如WO 2010/148511的图1C；其通过引用并入本文)。异源跨膜结构域可获自任何HA的跨膜结构域，例如但不限于来自以下的跨膜结构域：H1加利福尼亚、B/弗罗里达/4/2006(GenBank登录号ACA33493.1)、B/马来西亚/2506/2004(GenBank登录号ABU99194.1)、H1/Bri(GenBank登录号ADE28750.1)、H1 A/所罗门群岛/3/2006(GenBank登录号ABU99109.1)、H1/NC(GenBank登录号AAP34324.1)、H2 A/新加坡/1/1957(GenBank登录号AAA64366.1)、H3 A/布里斯班/10/2007(GenBank登录号ACI26318.1)、H3 A/威斯康星州/67/2005(GenBank登录号ABO37599.1)、H5 A/安徽/1/2005(GenBank登录号ABD28180.1)、H5 A/越南/1194/2004(GenBank登录号ACR48874.1)、H5-Indo(GenBank登录号ABW06108.1)。跨膜结构域也可通过以下的共有氨基酸序列定义：

[0245] iLXiYystvAiSslXlXXmlagXsXwmcs(SEQ ID NO:94)

[0246] 包含具有异源跨膜结构域的嵌合HA的构建体实例包括：构建体1875号(CPMV-HT+

具有缺失的蛋白水解环的B布里斯班/60/08+H1TM,具有被H1 A/加利福尼亚/07/2009所代替的跨膜结构域和胞质尾区;参见实施例5.19),构建体1977号(CPMV-160+具有缺失的蛋白水解环的B布里斯班/60/08+H1TM,具有被H1 A/加利福尼亚/07/2009所代替的跨膜结构域和胞质尾区;参见实施例5.14),构建体1067号(CPMV-HT+具有缺失的蛋白水解环的B布里斯班/60/08+H1TM,具有被H1 A/加利福尼亚/07/2009所代替的跨膜结构域和胞质尾区;参见实施例5.14),构建体2074号(CPMV HT B马萨诸塞州/2/2012+H1Tm,跨膜结构域和胞质尾区被H1 A/加利福尼亚/07/2009的跨膜结构域和胞质尾区代替;参见实施例5.16),构建体2060号(CPMV HT160+马萨诸塞州/2/2012+H1Tm,跨膜结构域和胞质尾区被H1 A/加利福尼亚/07/2009的跨膜结构域和胞质尾区代替;参见实施例5.16),构建体2062号(CPMV 160+B马萨诸塞州/2/2012+H1Tm,跨膜结构域和胞质尾区被H1 A/加利福尼亚/07/2009的跨膜结构域和胞质尾区;参见实施例5.21),构建体1860号(CPMV HT+B威斯康星州/1/2010+H1Tm,跨膜结构域和胞质尾区被H1 A/加利福尼亚/07/2009的跨膜结构域和胞质尾区代替;参见实施例5.23),构建体1454号(CPMV HT B威斯康星州/1/2010+H1Tm,跨膜结构域和胞质尾区被H1 A/加利福尼亚/07/2009的跨膜结构域和胞质尾区代替,参见实施例5.18)和构建体1893号(CPMV 160+B威斯康星州/1/2010+H1Tm,跨膜结构域和胞质尾区被H1 A/加利福尼亚/07/2009的跨膜结构域和胞质尾区代替,参见实施例5.18)。图26B和27B中显示这些嵌合修饰的HA's的活性。

[0247] 信号肽

[0248] 信号肽(SP)可源于修饰的HA或嵌合修饰的HA,或者信号肽可与被表达的修饰HA的初级序列异源。修饰的HA可包含与来自一种或多种不同流感型、流感亚型或流感毒株的HA平衡的来自第一流感型、流感亚型或流感毒株的信号肽。例如HA的H1亚型、H2亚型、H3亚型、H5亚型、H6亚型、H7亚型、H9亚型或B型流感的天然信号肽可被用来在植物体系中表达修饰的HA。在本发明的一些实施方案中,SP可为流感B型、H1型、H3型或H5型;或为H1/Bri亚型、H1/NC亚型、H5/Indo亚型、H3/Bri亚型或B/Flo亚型。

[0249] 此外,修饰的HA或嵌合修饰的HA可包含天然或非-天然信号肽;非-天然信号肽可为植物来源或获自动物或细菌多肽。天然信号肽可对应于被表达的HA或修饰HA的信号肽,此外,信号肽可来自除流感外的病毒的结构蛋白或血凝素。可被使用的信号肽的非限制性实例为紫花苜蓿蛋白二硫键异构酶的信号肽(PDI SP;登录号Z11499的核苷酸32-103,也参见WO 2009/076778;WO 2010/148511或WO 2010/003235),或马铃薯糖蛋白(patatin)信号肽(PatA SP;位于GenBank登录号A08215的核苷酸1738-1806)。该登录号的PatA SP的核苷酸序列为:

[0250] ATGGCAACTACTAAAACTTTTTTAATTTTATTTTTTATGATATTAGCAACTACTAGTTCAACATGTGCT (SEQ ID NO:171)

[0251] 马铃薯糖蛋白A信号肽的氨基酸序列为:

[0252] MATTKTFLILFFMILATTSSTCA(SEQ ID NO:172)

[0253] 因此本发明提供包含天然或非-天然信号肽的修饰的HA或嵌合修饰的HA,以及编码这样的嵌合修饰的HA蛋白的核酸。

[0254] 与通道蛋白的共表达

[0255] 可通过共表达编码修饰的HA的第一核酸与编码通道蛋白例如但不限于质子通道

蛋白的第二核酸，在植物中产生突变的(修饰的)HA。第一和第二核酸可在相同的步骤中被引入植物，或它们可被连续地引入植物。可以瞬转的方式或以稳定的方式将第一和第二核酸引入植物。此外，表达编码修饰的HA的第一核酸的植物可转化有通道蛋白，例如但不限于质子通道蛋白(第二核酸)，以便第一和第二核酸在植物中共表达。可选地，表达通道蛋白例如但不限于质子通道蛋白(第二核酸)的植物可转化有编码修饰HA的第一核酸，以便第一和第二核酸在植物中共表达。此外，表达编码修饰的HA的第一核酸的第一植物可与表达编码通道蛋白例如但不限于质子通道蛋白的第二核酸的第二植物杂交，以产生共表达各自编码修饰的HA和通道蛋白例如但不限于质子通道蛋白的第一和第二核酸的后代植物。

[0256] 不希望受理论束缚，包含修饰的HA的细胞区室(包括高尔基体)的pH，可对HA的折叠、稳定性和/或蛋白水解作用是重要的。质子通道蛋白，例如流感M2和BM2蛋白可调节细胞区室中的pH。例如，通过缓冲流感病毒复制后期和早期阶段的细胞内区室，M2可调节膜融合的增强作用。

[0257] 通过与修饰的HA一起共表达通道蛋白例如但不限于质子通道蛋白，高尔基体内的pH可增加，并且导致稳定性的增加、降解的减少或其组合，并且增加修饰的HA和/或VLP的表达水平和产量。

[0258] 当与表达修饰的HA但不共表达通道蛋白例如但不限于质子通道蛋白的植物相比，通过在植物中与修饰的HA一起共表达通道蛋白例如但不限于质子通道蛋白，观察到HA和/或VLP的产量增加(参见图13A和14)。例如如图13A所示，M2与修饰的流感B HA的共表达增加了HA的累积水平(图13A，1059vs 1059+1261)。

[0259] 此外，将来自流感A/波多黎各/8/1934的M2增加修饰的流感B HA和H3的累积的功效，与来自流感A/新喀里多尼亚/20/1999的M2增加修饰的流感B HA和H3的累积的功效相比较。对于修饰的流感B HA，通过对来自转化有构建体1059、1059+1261和1059+859的植物的蛋白提取物的Western印迹分析进行比较。获得的结果表明，来自流感A/波多黎各/8/1934的M2(由构建体859号编码)的共表达与来自流感A/新喀里多尼亚/20/1999的M2(由构建体1261号编码)的共表达，在增加修饰的流感B HA的累积方面功效相同(图14)。

[0260] 本文所使用的术语“M2”、“M2蛋白”、“M2序列”和“M2结构域”指分离自、基于或存在于任何自然发生的或人工产生的流感病毒株或分离物的M2蛋白序列的全部或部分。因此，术语M2等等包括在病毒的生命周期期间通过突变所产生的或在对选择性压力(例如，药物治疗、宿主细胞趋性的扩张或感染性等等)的应答中所产生的自然发生的M2序列变体，以及重组或合成所产生的M2序列。可在本发明使用的序列的非限制性实例包括来自A/波多黎各/8/1934的M2和来自A/新喀里多尼亚/20/1999的M2。

[0261] 免疫应答

[0262] “免疫应答”通常指适应性免疫系统的应答。适应性免疫系统通常包含体液应答，以及细胞介导的应答。体液应答是由在B淋巴细胞谱系(B细胞)的细胞中所产生的分泌抗体所介导的免疫力方面。分泌抗体结合入侵微生物(如病毒或细菌)表面上的抗原，其标记入侵微生物用于消灭。体液免疫通常被用来指抗体的产生和伴随抗体产生的过程，以及抗体的效应子功能，其包括Th2细胞激活和细胞因子的产生、记忆细胞增殖、调理素促进吞噬作用、病原体的清除等等。术语“调节”或“调整”等指具体的应答或参数的增加或减少，通过通常已知的或使用的任何一些检测(assay)来测定，本文例示了其中一些检测。

[0263] 细胞介导的应答为免疫应答，其不涉及抗体但涉及巨噬细胞、自然杀伤细胞(NK)、抗原-特异性细胞毒素T-淋巴细胞的活化，以及在对抗原应答中的多种细胞因子的释放。细胞介导的免疫通常被用来指一些Th细胞激活、Tc细胞激活和T-细胞介导的应答。细胞介导的免疫在对病毒性感染的应答中尤为重要。

[0264] 例如，可使用ELISPOT检测来测定抗原特异性CD8阳性T淋巴细胞的诱导；可使用增殖检测来测定CD4阳性T-淋巴细胞的刺激。可使用ELISA检测来定量抗-流感的抗体滴度；也可使用抗-同型抗体(例如抗-IgG、IgA、IgE或IgM)来测定抗原-特异性或交叉反应抗体的同型。用于进行这样的检测的方法和技术是本领域公知的。

[0265] 交叉反应性的HAI滴度也可被用来证明对与疫苗亚型相关的其他病毒株的免疫应答的功效。例如，来自被第一毒株(例如A/印度尼西亚5/05的VLP)的疫苗组合物免疫的对象的血清，可被用于具有完整的病毒或病毒颗粒的第二种毒株(例如A/越南/1194/2004)的HAI检测中，并且测定HAI滴度。

[0266] 也可定量细胞因子的存在或水平。例如通过使用ELISA(例如BD Biosciences OptEIA试剂盒)来测量分泌IFN-γ和IL-4的细胞来表征辅助性T细胞的应答(Th1/Th2)。可培养获自对象的外周血单核细胞(PBMC)或脾细胞，并且分析上清液。也可通过荧光-激活细胞分选法(FACS)，使用标记物特异性荧光标签定量T淋巴细胞，并且方法是本领域已知的。

[0267] 也可进行微量中和检测以表征对象中的免疫应答，参见例如Rowe等，1973的方法。可通过几种方式获得病毒的中和滴度，其包括：1)细胞的结晶紫固定/着色后溶菌噬斑(噬斑检测)的计数；2)培养物中细胞溶菌的显微镜观察；3)NP病毒蛋白(与宿主细胞的病毒感染性相关)的ELISA和分光光度检测。

[0268] 术语“病毒样颗粒”(VLP)”或“病毒-样颗粒”或“VLP”指自我组装的结构并且包含病毒蛋白，例如流感HA蛋白或修饰的HA蛋白(例如HA0蛋白)，其中蛋白水解环已被修饰。VLP通常形态学上和抗原性上类似于感染所产生的病毒粒子，但是缺乏足以复制的遗传信息，并且因此为非-感染性的。在一些实例中，VLP可包含单一的蛋白种类，或多于一种蛋白种类。对于包含多于一种蛋白种类的VLP，蛋白种类可来自相同种类的病毒，或可包含来自不同种、属、亚科或科的病毒(通过ICTV命名法命名)的蛋白。在其他实施例中，构成VLP的蛋白种类的一种或多种可来自对自然发生的序列进行修饰，例如本文所描述的修饰的HA。VLP可在适合的宿主细胞中产生，其包括植物和昆虫宿主细胞。来自宿主细胞的取提物并且在适合的条件下经分离和进一步纯化后，VLP可被纯化为完整的结构。

[0269] 此外，可产生包含HA亚型的组合的VLP。例如，VLP可包含来自下述的一种或多种HA或一种或多种修饰的HA：H1亚型、H2亚型、H3亚型、H4亚型、H5亚型、H6亚型、H7亚型、H8亚型、H9亚型、H10亚型、H11亚型、H12亚型、H13亚型、H14亚型、H15亚型、H16亚型、B亚型HA或其组合。可通过由VLP所制备的疫苗的预期用途来确定选择HA或修饰的HA的组合。例如，用于在接种禽类中使用的疫苗，可包含HA亚型或修饰HA亚型的任意组合，而用于接种人类的VLP可包含一种或多种H1亚型、H2亚型、H3亚型、H4亚型、H5亚型、H6亚型、H7亚型、H8亚型、H9亚型、H10亚型、H11亚型、H12亚型、H13亚型、H14亚型、H15亚型、H16亚型、B亚型HA，或包含一种或多种修饰的H1亚型、H2亚型、H3亚型、H4亚型、H5亚型、H6亚型、H7亚型、H8亚型、H9亚型、H10亚型、H11亚型、H12亚型、H13亚型、H14亚型、H15亚型、H16亚型、B亚型HA。然而，可根据VLP的用途制备其他HA亚型或修饰的HA亚型的组合。为了产生包含HA亚型或修饰的亚型HA的组合

的VLP,可在相同的细胞例如植物细胞内共表达期望的HA亚型或修饰的HA亚型。

[0270] 根据本发明,产生自流感来源的蛋白的VLP不包含M1蛋白。已知M1蛋白结合RNA(Wakefield和Brownlee,1989),RNA为VLP制备的污染物。当获得用于VLP产物的监管批准时,RNA的存在是不理想的,因此缺乏RNA的VLP制剂可为有益的。

[0271] 如本文所述产生的VLP通常不包含神经氨酸苷酶(NA)。然而,如果包含HA和NA的VLP是理想的,可将NA与HA共表达。

[0272] 本发明也包括但不限于病毒来源VLP,其从表达VLP蛋白的细胞的质膜获得脂质包膜。例如,如果VLP在基于植物的系统中表达,那么所述VLP可获得来自所述细胞的质膜的脂质包膜。

[0273] 通常,术语“脂质”指脂溶性(亲脂的)自然发生的分子。该术语也被用来更具体地指脂肪酸和其衍生物(包括甘油三酯、甘油二酯和甘油单酯以及磷脂),以及其他脂溶性含有甾醇的代谢物或甾醇类。磷脂连同糖脂类、甾醇类和蛋白是所有生物膜的主要组分。磷脂的实例包括磷脂酰乙醇胺、磷脂酰胆碱、磷脂酰肌醇、磷脂酰丝氨酸等等。甾醇类的实例包括动物甾醇(例如,胆固醇)和植物甾醇。超过200种植物甾醇已在多种植物种类中鉴定,最常见的为菜油甾醇、豆甾醇、麦角甾醇、菜子甾醇、δ-7-豆甾醇、δ-7-燕麦甾醇、daunosterol、谷甾醇、24-甲基胆甾醇、胆固醇或β-谷甾醇。本领域技术人员理解,细胞质膜的脂质组成可随着细胞或获得细胞的有机体的培养或生长条件而变化。

[0274] 细胞膜通常包含脂质双层,以及用于多种功能的蛋白。脂质双层中可发现局部浓度的特殊脂质,被称为‘脂质筏’。不希望受理论束缚,脂质筏可在胞吞作用和胞吐作用、病毒或其他感染原的进入或排出、细胞内信号转导、细胞或有机体的其他结构组分如细胞内和细胞外基质的相互作用中发挥重大作用。

[0275] 在植物中,流感VLP从质膜出芽,因此VLP的脂质组成反映了它们的来源。根据本发明所产生的VLP包含与植物来源的脂质复合的一种或多种流感型或流感亚型的HA。植物脂质能刺激特异性免疫细胞并且提高诱导的免疫应答。植物膜由脂质、磷脂酰胆碱(PC)和磷脂酰乙醇胺(PE)组成,并且也含有鞘糖脂、皂苷和植物甾醇。此外,脂质筏也发现于植物质膜中—这些微区富含鞘脂类和甾醇类。在植物中,已知存在多种植物甾醇,其包括豆甾醇、谷甾醇、24-甲基胆甾醇和胆固醇(Mongrand等,2004)。

[0276] PC和PE,以及鞘糖脂能结合由哺乳免疫细胞如抗原-递呈细胞(APCs)样树突细胞和巨噬细胞以及其他细胞(包括胸腺和肝脏中的B淋巴细胞和T淋巴细胞)所表达的CD1分子(Tsuji M,.2006)。CD1分子结构上类似于I型的主要组织相容性复合体(MHC)分子,并且其作用是将糖脂类抗原递呈至NKT细胞(自然杀伤T细胞)。一旦激活,NKT细胞激活天然免疫细胞,如NK细胞和树突状细胞,并且也激活适应性免疫细胞,如产生抗体的B细胞和T-细胞。

[0277] 质膜中可发现多种植物甾醇—具体的补体可根据种类、生长条件、营养资源或病原体状态(列举几个因素)变化。通常,β-谷甾醇是最丰富的植物甾醇。

[0278] 存在于复合有脂质双层(如质膜来源的包膜)的流感VLP中的植物甾醇,可提供有益的疫苗组合物。不希望受理论束缚,复合有脂质双层(如质膜来源的包膜)植物-产生的VLP,可诱导比其他表达系统中产生的VLP更强的免疫反应,并且可类似于由活或减毒的完整病毒疫苗所诱导的免疫反应。

[0279] 本文所描述的VLP可复合有植物-来源的脂质双层。在一些实施方案中,植物-来源

的脂质双层可包含VLP的包膜。植物来源的脂质可包含产生VLP的植物的质膜的脂质成分，其包括但不限于：磷脂酰胆碱(PC)、磷脂酰乙醇胺(PE)、鞘糖脂、植物甾醇或其组合。植物-来源的脂质可选择性地被称为‘植物脂质’。植物甾醇的实例是本领域已知的，并且其包括，例如，豆甾醇、谷甾醇、24-甲基胆甾醇和胆固醇-参见，例如，Mongrand等，2004。

[0280] 可通过例如凝集检测、电子显微镜或通过尺寸排阻色谱评估VLP的结构和尺寸。

[0281] 对于尺寸排阻色谱，可通过在提取缓冲液中匀浆(Polytron)冻碎的植物材料样品，从植物组织提取总的可溶性蛋白，并且通过离心去除不可溶材料。可使用PEG沉淀。定量可溶性蛋白，并且提取物通过分子排阻基质，例如但不限于Sephacryl™。层析后，通过免疫印迹进一步分析级分以确定级分的蛋白补体。

[0282] 不希望受理论束缚，通过HA对唾液酸α2,3或α2,3以及RBC的表面上存在的这些唾液酸的亲和力，驱动HA结合来自不同动物的RBC的能力。来自流感病毒的马和禽HA使来自所有几个物种的红细胞凝集，所述物种包括火鸡、鸡、鸭、豚鼠、人、羊、马和牛；然而人HA结合火鸡、鸡、鸭、豚鼠、人和羊的红细胞(也参见Ito T.等，1997，Virology，vol 227，p493-499；和Medeiros R等，2001，Virology，vol 289，p74-85)。

[0283] 所表达的病毒蛋白的正确折叠，对蛋白的稳定性、多聚体的形成、VLP的形成、病毒蛋白的功能和通过抗体识别病毒蛋白，及其他特性是重要的。蛋白的折叠和累积可受一个或多个因素影响，所述因素包括但不限于：蛋白的序列、蛋白的相对丰度、细胞内聚集的程度、细胞室中的pH、可结合折叠的、部分折叠的或未折叠的蛋白或瞬间与其联合的辅因子的可利用性、一个或多个伴侣蛋白的存在等。

[0284] 热激蛋白(Hsp)或应激蛋白是伴侣蛋白的实例，其可参与多种细胞过程，包括蛋白合成、细胞内运输、错误折叠的预防、蛋白聚集的预防、蛋白复合体的组装和解装配、蛋白折叠和蛋白解聚。这样的伴侣蛋白的实例包括但不限于：Hsp60、Hsp65、Hsp 70、Hsp90、Hsp100、Hsp20-30、Hsp10、Hsp100-200、Hsp100、Hsp90、Lon、TF55、FKBPs、亲环素、ClpP、GrpE、泛素、钙联结蛋白和蛋白二硫化物异构酶(参见，例如，Macario，A.J.L.，Cold Spring Harbor Laboratory Res.25：59-70.1995；Parsell，D.A.&Lindquist，S.Ann.Rev.Genet.27：437-496(1993)；美国专利第5,232,833号)。本文所描述的伴侣蛋白，例如但不限于Hsp40和Hsp70，可被用来确保病毒蛋白的折叠。

[0285] Hsp70的实例包括来自哺乳动物细胞的Hsp72和Hsc73、来自细菌，特别是分枝细菌，如麻风分枝杆菌(Mycobacterium leprae)、结核分枝杆菌(Mycobacterium tuberculosis)和牛分枝杆菌(Mycobacterium bovis)的DnaK(如杆菌-卡介苗：本文被称为Hsp71)。来自大肠杆菌(Escherichia coli)、酵母和其他原核生物的DnaK，以及来自真核生物如拟南芥的BiP和Grp78(Lin等2001(Cell Stress and Chaperones 6:201-208))。Hsp70的具体实例为拟南芥Hsp70(由Genbank参考号：AY120747.1编码)。Hsp70能够特异性地结合ATP以及未折叠的多肽和肽，从而参与蛋白的折叠和去折叠，以及参与蛋白复合体的组装和解装配。

[0286] Hsp40的实例包括来自原核生物(如大肠杆菌和分枝细菌)的DnaJ和来自真核生物(如紫花苜蓿)的HSJ1、HDJ1和Hsp40(Frugis等，1999.Plant Molecular Biology 40:397-408)。Hsp40的具体实例为紫花苜蓿的MsJ1(Genbank参考号：AJ000995.1)。Hsp40作为分子伴侣在蛋白折叠、耐热性和DNA复制及其他细胞活性中发挥作用。

[0287] 在合成完全之前，翻译和新合成的多肽的稳定性涉及Hsp中的Hsp70和其辅伴侣分子Hsp40。不希望受理论束缚，Hsp40结合未折叠(初生的或新翻译的)多肽的疏水斑区，因此促进Hsp70-ATP复合体与所述多肽的相互作用。ATP的水解导致多肽、Hsp70和ADP稳定复合体的形成，以及Hsp40的释放。Hsp70-ADP复合体与所述多肽的疏水斑区的结合防止其与其他疏水斑区的相互作用，而防止不正确折叠和与其他蛋白的聚集物的形成(Hartl，FU.1996.Nature 381:571-579中论述)。

[0288] 虽然天然的伴侣蛋白能够促进低水平的重组蛋白的正确折叠，但是随着表达水平增加，天然的分子伴侣的丰度可变成限制因素。病毒蛋白在农杆菌渗入的叶中的高水平表达，可导致病毒蛋白在细胞溶胶中的累积，并且一种或多种伴侣蛋白如Hsp70、Hsp40或Hsp70和Hsp40的共表达可降低错误折叠的或聚集的蛋白的水平，并且增加显示三级和四级结构特性的蛋白的数量，其允许病毒样颗粒的形成。

[0289] 因此，本发明也提供在植物中产生病毒蛋白VLP的方法，其中编码病毒蛋白的第一核酸与编码通道蛋白例如但不限于质子通道蛋白的第二核酸，以及编码分子伴侣的第三核酸共表达。可在相同的步骤中将第一、第二和第三核酸引入植物，或可将其连续地引入植物。

[0290] 植物内所产生的VLP可诱导包含植物-特异性N-聚糖的病毒蛋白。因此，本发明也提供包含具有植物特异性N-聚糖的病毒蛋白的VLP。

[0291] 此外，植物中N-聚糖的修饰是已知的(参见，例如WO 2008/151440;WO 2010/006452;或U.S.60/944,344;其通过引用并入本文)，并且可产生具有修饰的N-聚糖的病毒蛋白。可获得包含修饰的糖基化形式的病毒蛋白，例如具有降低的盐藻糖基化(fucosylated)和/或木糖基化(xylosylated)、盐藻糖基化(fucosylated)和木糖基化的(xylosylated)的N-聚糖，或可获得具有修饰的糖基化形式的病毒蛋白，其中蛋白缺乏盐藻糖基化和/或木糖基化(xylosylation)，并且包含增加的半乳糖基化。此外，当与野生型植物表达的病毒蛋白相比，翻译后修饰的调节，例如末端半乳糖的加入可导致所表达的病毒蛋白的盐藻糖基化和木糖基化(xylosylation)的降低。

[0292] 例如，其不应理解为限制，可通过与编码β-1.4半乳糖基转移酶(GalT)的核苷酸序列一起共表达目标蛋白来实现具有修饰的糖基化形式的病毒蛋白的合成，所述GalT例如但不限于哺乳动物GalT或人GalT，然而也可使用来自另外来源的GalT。GalT的催化结构域也可与N-乙酰葡糖胺转移酶(GNT1)的CTS结构域(即胞质尾区、跨膜结构域、茎区)融合，以产生GNT1-GalT杂合酶，并且所述杂合酶可与病毒蛋白共表达。也可与编码N-乙酰葡糖胺转移酶III(GnT-III)的核苷酸序列一起共表达病毒蛋白，所述GnT-III例如但不限于哺乳动物GnT-III或人GnT-III，也可使用来自其他来源的GnT-III。此外，也可使用GNT1-GnT-III杂合酶，其包含与GnT-III融合的GNT1的CTS。

[0293] 因此，本发明也包括包含具有修饰的N-聚糖的一种或多种病毒蛋白的VLP's。

[0294] 可在本发明使用以产生修饰的HA's的序列的非限制性实例，也包括WO 2009/009876;WO 2009/076778;WO 2010/003225;WO 2010/148511;WO 2010/003235;WO 2010/006452(其通过引用并入本文)中所描述的序列，例如但不限于：

[0295] 由例如来自A/布里斯班/59/2007(H1N1)毒株、A/新喀里多尼亚/20/99(H1N1)毒株、A/所罗门群岛3/2006(H1N1)毒株、/波多黎各/8/34(H1N1)毒株、A/布里斯班/59/2007

(H1N1)毒株的核酸分子所编码的H1蛋白；

[0296] 由核酸分子所编码的H2蛋白可来自A/新加坡/1/57(H2N2)毒株；

[0297] 由核酸分子所编码的H3蛋白可来自A/布里斯班10/2007(H3N2)、A/威斯康星州/67/2005(H3N2)毒株、A/维多利亚/361/2011(H3N2)或A/珀斯/16/2009(H3N2)；

[0298] 由核酸分子所编码的H5蛋白可来自A/安徽/1/2005(H5N1)、A/印度尼西亚/5/2005(H5N1)、A/越南/1194/2004(H5N1)；

[0299] 由核酸分子所编码的H6蛋白可来自A/野鸭/香港/W312/97(H6N1)毒株；

[0300] 由核酸分子所编码的H7蛋白也可来自A/杭州/1/13(H7N9)、A/马/布拉格/56(H7N7)毒株；

[0301] 由核酸分子所编码的H9蛋白可来自A/香港/1073/99(H9N2)毒株；

[0302] 由核酸所编码的来自B亚型的HA蛋白可来自B/弗罗里达/4/2006、B/马萨诸塞州/2/12、B/马来西亚/2506/2004、B/威斯康星州/1/2010或B/布里斯班/60/2008毒株。

[0303] 表3：本文所描述的已制备的构建体的实例：

[0304]

构建体#	表达增强子	扩增元件	描述	实施例
1261	CPMV-HT		A/新喀里多尼亚/20/1999(H1N1)	实施例 5.1
859	CPMV-HT		A/波多黎各/8/1934(H1N1)	实施例 5.2
1008	CPMV-HT	BeYDV	B/布里斯班/60/2008	实施例 5.3
1059	CPMV-HT	BeYDV	具有缺失的蛋白水解环的 B/布里斯班/60/2008	实施例 5.4
1462	CPMV-HT	BeYDV	B/威斯康星州/1/2010	实施例 5.5
1467	CPMV-HT	BeYDV	具有缺失的蛋白水解环的 B/威斯康星州/1/2010	实施例 5.6
1039	CPMV-HT		具有缺失的蛋白水解环的 B/布里斯班/60/2008	实施例 5.7
1029	CPMV-HT		B/布里斯班/60/2008	实施例 5.11
1829	CPMV-HT+		具有缺失的蛋白水解环的 B/布里斯班/60/2008	实施例 5.12
1937	CPMV-160+		具有缺失的蛋白水解环的 B/布里斯班/60/2008	实施例 5.13
1067	CPMV-HT		具有缺失的蛋白水解环的 B/布里斯班/60/2008 +H1 加利福尼亚 TMCT	实施例 5.14
1977	CPMV-160+		具有缺失的蛋白水解环的 B/布里斯班/60/2008 +H1 加利福尼亚 TMCT	实施例 5.14
1875	CPMV-HT+		具有缺失的蛋白水解环的 B/布里斯班/60/2008 +H1 加利福尼亚 TMCT	实施例 5.19
676	CPMV-HT		具有 TETR 切割位点的 H5 A/印度尼西亚/5/2005	实施例 5.8

[0305]

766	CPMV-HT		具有TETQ切割位点的H5 A/印度尼西亚/5/2005	实施例 5.9
928	CPMV-HT		具有缺失的蛋白水解环的 H5 A/印度尼西亚/5/2005	实施例 5.10
489	CPMV-HT		H5 A/印度尼西亚/5/2005(天然的)	实施例 5.24
2220	CPMV-HT+		H2 A/新加坡/1/57(天然的)	实施例 5.27
2221	CPMV-HT+		具有缺失的蛋白水解环的 H2 A/新加坡/1/57	实施例 5.28
2222	CPMV-160+		H2 A/新加坡/1/57(天然的)	实施例 5.29
2223	CPMV-160+		具有缺失的蛋白水解环的 H2 A/新加坡/1/57	实施例 5.29
2019	CPMV-HT+		H3 A/珀斯/16/09(天然的)	实施例 5.30
2039	CPMV-HT+		具有缺失的蛋白水解环的 H3 A/珀斯/16/09	实施例 5.31
2139	CPMV-160+		H3 A/珀斯/16/09(天然的)	实施例 5.30
2159	CPMV-160+		具有缺失的蛋白水解环的 H3 A/珀斯/16/09	实施例 5.31
1819	CPMV-HT+		H3 A/维多利亚/361/11(天然的)	实施例 5.26
2230	CPMV-HT+		具有缺失的蛋白水解环的 H3 A/维多利亚 /361/11	实施例 5.32
1800	CPMV-160+		H3 A/维多利亚 /361/11(天然的)	实施例 5.25
2250	CPMV-160+		具有缺失的蛋白水解环的 H3 A/维多利亚/361/11	实施例 5.32
2142	CPMV-HT+		H7 A/杭州/1/13(天然的)	实施例 5.33
2152	CPMV-HT+		具有缺失的蛋白水解环的 H7 A/杭州/1/13	实施例 5.34
2224	CPMV-HT+		H9 A/香港/1073/99(天然的)	实施例 5.35
2225	CPMV-HT+		具有缺失的蛋白水解环的 H9 A/香港/1073/99	实施例 5.36
2226	CPMV-160+		H9 A/香港/1073/99(天然的)	实施例 5.35
2227	CPMV-160+		具有缺失的蛋白水解环的 H9 A/香港/1073/99	实施例 5.36
2013	CPMV-160+		B/马来西亚 /2506/04(天然的)	实施例 5.37
2014	CPMV-160+		具有缺失的蛋白水解环的 B/马来西亚/2506/04	实施例 5.38
2070	CPMV-HT		B/马萨诸塞州/2/12(天然的)	实施例 5.39
2072	CPMV-HT		具有缺失的蛋白水解环的 B/马萨诸塞州/2/12	实施例 5.15
2080	CPMV-HT+		B/马萨诸塞州/2/12(天然的)	实施例 5.39
2052	CPMV-HT+		具有缺失的蛋白水解环的 B/马萨诸塞州/2/12	实施例 5.20
2090	CPMV-160+		B/马萨诸塞州/2/12(天然的)	实施例 5.39
2050	CPMV-160+		具有缺失的蛋白水解环的 B/马萨诸塞州/2/12	实施例 5.15
2074	CPMV HT		HA B/马萨诸塞州/2/12(PrL-)+H1 加利福尼亚 TMCT	实施例 5.16
2060	CPMV-160+		HA B/马萨诸塞州/2/12(PrL-)+H1 加利福尼亚 TMCT	实施例 5.16

[0306]

2062	CPMV-HT+		HA B/马萨诸塞州/2/12(PrL-)+H1 加利福尼亚 TMCT	实施例 5.21
1445	CPMV HT		具有缺失的蛋白水解环(PrL-)的 B/威斯康星州/1/2010	实施例 5.17
1839	CPMV-HT+		具有缺失的蛋白水解环(PrL-)的 B/威斯康星州/1/2010	实施例 5.22
1820	CPMV160+		具有缺失的蛋白水解环(PrL-)的 B/威斯康星州/1/2010	实施例 5.17
1975	CPMV160		具有缺失的蛋白水解环(PrL-)的 B/威斯康星州/1/2010	实施例 5.17
1454	CPMV-HT		具有缺失的蛋白水解环(PrL-)的 HA B 威斯康星州+H1 加利福尼亚 TMCT	实施例 5.18
1893	CPMV-160+		具有缺失的蛋白水解环(PrL-)的 HA B 威斯康星州+H1 加利福尼亚 TMCT	实施例 5.18
1860	CPMV-HT+		具有缺失的蛋白水解环(PrL-)的 HA B 威斯康星州+H1 加利福尼亚 TMCT	实施例 5.23
2102	CPMV-HT+		具有缺失的蛋白水解环(PrL-)的 B 弗罗里达	实施例 5.40
2104	CPMV-HT+	BeYDV	具有缺失的蛋白水解环(PrL-)的 B 弗罗里达	实施例 5.40
2016	CPMV-HT+		具有缺失的蛋白水解环(PrL-)的 B 弗罗里达+ H1 加利福尼亚 TMCT	实施例 5.41
2108	CPMV-HT+	BeYDV	具有缺失的蛋白水解环(PrL-)的 B 弗罗里达+ H1 加利福尼亚 TMCT	实施例 5.41

[0307] 表:4:序列描述

[0308]

SEQ ID NO:	描述	图	SEQ ID NO:	描述	图
1	禽类 H5 蛋白水解环共有序列		102	HA B 威斯康星州(PrL-)的氨基酸序列	36B
2	IF-H5A-I-05. si+3c	1A	103	HA B 威斯康星州(PrL-)+H1 加利福尼亚 TMCT 的核苷酸序列	37A
3	IF-H5dTm. r	1B	104	HA B 威斯康星州(PrL-)+H1 加利福尼亚 TMC 的氨基酸序列	37B
4	构建体 1191	1D	105	PDISP/HA B 布里斯班(PrL-)+H1 加利福尼亚 TMCT 的核苷酸序列	38A
5	盒 489	1E	106	PDISP/HA B 布里斯班(PrL-)+H1 加利福尼亚	38B

[0309]

SEQ ID NO：	描述	图	SEQ ID NO：	描述	图
				TMCT 的氨基酸序列	
6	氨基酸序列 H5 A/印度尼西亚 /5/2005(H5N1)	1F	107	PDISP/HA B 马萨诸塞 (PrL-)的核苷酸序列	39A
7	IF-S1-M1+M2ANC. c	2A	108	PDISP/HA B 马萨诸塞 (PrL-)的氨基酸序列	39B
8	IF-S1-4-M2ANC. r	2B	109	PDISP/HA B 马萨诸塞 (PrL-)+H1 加利福尼亚 TMCT 的核苷酸序列	40A
9	合成 M2(连接到来自 DQ508860 的 nt 715-982 的 nt 1-26)	2C	110	PDISP/HA B 马萨诸塞 (PrL-)+H1 加利福尼亚 TMCT 的氨基酸序列	40B
10	盒(cassette)1261	2D	111	HA B 威斯康星州(PrL-)的 核苷酸序列	41A
11	来自流感 A/新喀里 多尼亚 /20/1999(H1N1)的 M2	2E	112	HA B 威斯康星州(PrL-)的 氨基酸序列	41B
12	合成 M2(连接到来自 EF467824 的 nt 740-1007 的 nt 26-51)	3A	113	HA B 威斯康星州 (PrL-)+H1 加利福尼亚 TMCT 的核苷酸序列	42A
13	盒 859	3B	114	HA B 威斯康星州 (PrL-)+H1 加利福尼亚 TMC 的氨基酸序列	42B
14	氨基酸序列 M2 流感 A/波多黎各 /8/1934(H1N1)	3C	115	天然 H5 印度尼西亚的核 苷酸序列	43A
15	盒 1039	8A	116	天然 H5 印度尼西亚的氨基 酸序列	43B
16	氨基酸序列 B/布里斯班/60/2008	21A	117	IF**(SacII)-PDI. s1+4c	44A
17	氨基酸序列 B 型 HA(具有接头 GG)的 δ-蛋白水解环	21B	118	IF-H3V36111. s1-4r	44B
18	氨基酸序列 用接头代替 B HA 的 切割位点	21C	119	PDISP/H3 维多利亚的核 苷酸序列	44C
19	氨基酸序列 H3 A/珀斯/16/2009	21D	120	构建体 2171	44E

[0310]

SEQ ID NO：	描述	图	SEQ ID NO：	描述	图
20	氨基酸序列 δ-蛋白水解环 H3(具有接头 GS)	21E	121	构建体 1800	44F
21	氨基酸序列 用接头代替 H3 的切割位点	21F	122	PDISP/H3 维多利亚的氨基酸序列	44G
22	H1 新喀里多尼亚接头区	15	123	IF(SacII)-Kozac_PDI. c	45A
23	H1 布里斯班接头区	15	124	IF-H3V36111. s1-4r	45B
24	H1 所罗门群岛接头区	15	125	构建体 2181	45D
25	H2A 新加坡接头区	15	126	构建体 1819	45E
26	H3A 布里斯班接头区	15	127	IF**-H2S157. s1-6r	48A
27	H3A WCN 接头区	15	128	PDISP/H2 新加坡的核苷酸序列	48B
28	H5 安徽接头区	15	129	表达盒号 2220	48C
29	H5 Indo 接头区	15	130	PDISP/H2 新加坡的氨基酸序列	48D
30	H5 越南接头区	15	131	H2S157(Prl-).r	49A
31	构建体 1194	4B	132	H2S157(Prl-).c	49B
32	盒 1008	4C	133	表达盒号 2221	49C
33	H6 野鸭 HK 接头区	15	134	具有缺失的蛋白水解环的 PDISP/H2 新加坡的氨基酸序列	49D
34	H7 Eq 布拉格接头区	15	135	表达盒号 2222	50A
35	H9A HK 接头区	15	136	表达盒号 2223	50B
36	B 弗罗里达接头区	15	137	PDISP/H3 珀斯的核苷酸序列	51A
37	B 马来西亚	15	138	IF**-H3P1609.S1-6r	51B
38	1039+1059. r	5A	139	PDISP/H3 珀斯的氨基酸序列	51C
39	1039+1059. c	5B	140	具有缺失的蛋白水解环的	52A

[0311]

SEQ ID NO：	描述	图	SEQ ID NO：	描述	图
				PDISP/H3 珀斯的核苷酸序列	
40	盒 1059	5C	141	H3P1609(Prl-)#2.r	52B
41	氨基酸序列 PDISP/HA 流感 B/布里斯班/60/2008(缺失的蛋白水解环)	5D	142	H3P1609(Prl-)#2.c	52C
42	核苷酸序列 H5 A/印度尼西亚/5/2005(H5N1)	1G	143	具有缺失的蛋白水解环的 PDISP/H3 珀斯的氨基酸序列	52D
43	核苷酸序列 PDISP/HA 流感 B/布里斯班/60/2008(缺失的蛋白水解环)	5E	144	具有缺失的蛋白水解环的 PDISP/H3 维多利亚的核苷酸序列	53A
44	H5/Indo 切割位点的天然序列	19	145	H3V36111(Prl-).r	53B
45	H5/Indo 修饰的切割位点(TETR)	19	146	H3V36111(Prl-).c	53C
46	H5/Indo 修饰的切割位点(TETQ)	19	147	具有缺失的蛋白水解环的 PDISP/H3 维多利亚的氨基酸序列	53D
47	H1 切割位点	表 1	148	PDISP/H7 杭州的核苷酸序列	54A
48	H3 切割位点	表 1	149	IF*-H7H113.s1-6r	54B
49	IF-HA B110. S1+3c	6A	150	PDISP/H7 杭州的氨基酸序列	54C
50	IF-HA B110. s1-4r	6B	151	具有缺失的蛋白水解环的 PDISP/H7 杭州的核苷酸序列	55A
51	合成的 HA B 威斯康星州	6C	152	H7H113(PrL-).r	55B
52	构建体 193	6E	153	H7H113(PrL-).c	55C
53	盒 1462	6F	154	具有缺失的蛋白水解环的 PDISP/H7 杭州的氨基酸序列	55D
54	氨基酸序列 HA 流感 B/威斯康星	6G	155	PDISP/H9 香港的核苷酸序	56A

[0312]

SEQ ID NO：	描述	图	SEQ ID NO：	描述	图
	州/1/2010			列	
55	HA B110(PrL-). r	7A	156	IF**-H9HK107399.S1-6r	56B
56	HA B110(PrL-). c	7B	157	PDISP/H9 香港的氨基酸序列	56C
57	盒 1467	7C	158	具有缺失的蛋白水解环的 PDISP/H9 香港的核苷酸序列	57A
58	氨基酸序列 HA 流感 B/威斯康星州/1/2010(缺失 PL)	7D	159	H9HK107399(Prl-).r	57B
59	B 切割位点	表 1	160	H9HK107399(Prl-).c	57C
60	H5/Indo 天然切割位点	表 2	161	具有缺失的蛋白水解环的 PDISP/H9 香港的氨基酸序列	57D
61	H5/Indo 修饰的切割位点	表 2	162	PDISP/HA B 马来西亚的核苷酸序列	58A
62	H5/Indo 修饰的切割位点	表 2	163	IF**-HBM250604.S1-6r	58B
63	H1/布里斯班修饰的切割位点	表 2	164	PDISP/HA B 马来西亚的氨基酸序列	58C
64	H3/布里斯班修饰的切割位点	表 2	165	具有缺失的蛋白水解环的 PDISP/HA B 马来西亚的核苷酸序列	59A
65	B/弗罗里达、B/布里斯班修饰的切割位点	表 2	166	HBM250604(PrL-).r	59B
66	A/H3/HA0 共有序列		167	HBM250604(PrL-).c	59C
67	A/H1/HA0 共有序列		168	具有缺失的蛋白水解环的 PDISP/HA B 马来西亚的氨基酸序列	59D
68	B/HA0 共有序列		169	PDISP/HA B 马萨诸塞州的核苷酸序列	60A
69	H5 安徽蛋白水解环缺失	18C	170	PDISP/HA B 马萨诸塞州的氨基酸序列	60B
70	H5 Indo 蛋白水解环	18C	171	PatA SP 的核苷酸序列	

[0313]

SEQ ID NO：	描述	图	SEQ ID NO：	描述	图
	缺失				
71	H5 越南蛋白水解环缺失	18C	172	马铃薯糖蛋白 A 信号肽的氨基酸序列	
72	B 弗罗里达蛋白水解环缺失	18C	173	CPMV HT 序列	
73	B 马来西亚蛋白水解环缺失	18C	174	植物共有 kozak 序列- 植物界	
74	MutCleavage -H5(Indo). r	23A	175	植物共有 kozak 序列-双子叶植物	
75	MutCleavage -H5(Indo). c	23B	176	植物共有 kozak 序列-拟南芥	
76	盒 676	23C	177	植物共有 kozak 序列	
77	氨基酸序列 流感 A/印度尼西亚/5/2005(H5N1)TETR 切割位点突变	23D	178	植物共有 kozak 序列	
78	H5I505_TETQ. r	24A	179	植物共有 kozak 序列	
79	H5I505_TETQ. c	24B	180	植物共有 kozak 序列	
80	盒 766	25C	181	植物共有 kozak 序列	
81	氨基酸序列 流感 A/印度尼西亚/5/2005(H5N1)TETQ 切割位点突变	25D	182	植物共有 kozak 序列	
82	H5I505(PrL-). r	26A	183	植物共有 kozak 序列	
83	H5I505(PrL-). c	26B	184	植物共有 kozak 序列	
84	盒 928	26C	185	植物共有 kozak 序列	
85	氨基酸序列 具有缺失的蛋白水解环的流感 A/印度尼西亚/5/2005(H5N1)	26D	186	Kozak 共有序列	
86	IF-S2+S4-B Bris. c	30A	187	CPMV HT+的核苷酸序列	
87	IF-S1a4-B Bris. r	30B	188	CPMV HT+ 511 的核苷酸序列	
88	合成的 HA B 布里斯	30C	189	CPMV HT+[WT115]的核	

[0314]

SEQ ID NO：	描述	图	SEQ ID NO：	描述	图
	班基因			苷酸序列	
89	构建体 1029	30D	190	HBF406(PrL-). r	61A
90	来自流感 B/布里斯班/60/2008 的 PDISP/HA 的氨基酸序列	30E	191	HBF406(PrL-). c	61B
91	PDISP/HA B 布里斯班(PrL-)的核苷酸序列	31A	192	IF*-HBF406.s1-6r	61C
92	PDISP/HA B 布里斯班(PrL-)的氨基酸序列	31B	193	具有缺失的蛋白水解环的 PDISP/HA B 弗罗里达的核苷酸序列	61D
93	CPMVX/CPMVX+核苷酸序列		194	具有缺失的蛋白水解环的 PDISP/HA B 弗罗里达的氨基酸序列	61E
94	跨膜结构域的共有的氨基酸序列		195	表达盒号 2102	61F
95	PDISP/HA B 布里斯班(PrL-)+H1 加利福尼亚 TMCT 的核苷酸序列	33A	196	表达盒号 2104	61H
96	PDISP/HA B 布里斯班(PrL-)+H1 加利福尼亚 TMCT 的氨基酸序列	33B	197	IF-H1cTMCT.S1-4r	62A
97	PDISP/HA B 马萨诸塞(PrL-)的核苷酸序列	34A	198	具有缺失的蛋白水解环的 PDISP/HA B 弗罗里达+ H1Cal TMCT 的核苷酸序列	62B
98	PDISP/HA B 马萨诸塞(PrL-)的氨基酸序列	34B	199	具有缺失的蛋白水解环的 PDISP/HA B 弗罗里达+ H1Cal TMCT 的氨基酸序列	62C
99	PDISP/HA B 马萨诸塞(PrL-)+H1 加利福尼亚 TMCT 的核苷酸序列.	35A	200	表达盒号 2106	62D
100	PDISP/HA B 马萨诸塞(PrL-)+H1 加利福尼亚 TMCT 的氨基	35B	201	表达盒号 2108	62F

[0315]

SEQ ID NO:	描述	图	SEQ ID NO:	描述	图
	酸序列.				
101	HA B 威斯康星州 (PrL-)的核苷酸序列.	36A			

[0316] 本申请还涉及以下技术方案：

[0317] 1.核酸，其包含在植物中有活性的调控区和在植物中有活性的表达增强子，所述调控区和所述表达增强子与编码修饰的流感血凝素(HA)的核苷酸序列可操作地连接，所述修饰的流感血凝素(HA)包含修饰的蛋白水解环。

[0318] 2.如实施方案1所述的核苷酸，其中所述表达增强子选自：CPMVX、CPMVX+、CPMV-HT+、CPMV HT+[WT115]和CPMV HT+[511]。

[0319] 3.如实施方案1所述的核苷酸，其中所述表达增强子不是CPMV HT。

[0320] 4.如实施方案1所述的核苷酸，其中所述核苷酸不包含菜豆黄矮病毒长基因间隔区(BeYDV LIR)和BeYDV短基因间隔区(BeYDV SIR)。

[0321] 5.如实施方案1所述的核苷酸，其中所述修饰的蛋白水解环包含一个或多个蛋白酶切割位点，所述蛋白酶切割位点与天然HA的一个或多个切割位点的切割相比，呈现出减少或废除的蛋白酶切割。

[0322] 6.如实施方案5所述的核苷酸，其中所述蛋白酶为克拉拉-样、弗林蛋白酶-样或胰蛋白酶。

[0323] 7.如实施方案1所述的核苷酸，其中所述修饰的蛋白水解环包含接头序列。

[0324] 8.如实施方案7所述的核苷酸，其中所述接头序列具有氨基酸序列GG、TETQ或TETR。

[0325] 9.如实施方案1所述的核苷酸，其中由所述核苷酸序列编码的HA选自来自以下的HA：流感B型、C型、A型以及H1亚型、H2亚型、H3亚型、H4亚型、H5亚型、H6亚型、H7亚型、H8亚型、H9亚型、H10亚型、H11亚型、H12亚型、H13亚型、H14亚型、H15亚型和H16亚型。

[0326] 10.如实施方案1所述的核苷酸，其中编码所述流感血凝素(HA)蛋白的核苷酸序列与选自以下的序列具有至少70％的序列同一性：SEQ ID NO:17、18、20、21、41、58、77、81、85、92、96、98、100、102、104、106、108、110、112、114、134、143、147、154、161、168、194和199。

[0327] 11.如实施方案1所述的核苷酸，其中所述HA为HA0。

[0328] 12.如实施方案1所述的核酸，其中所述修饰的HA包含天然或非天然的信号肽。

[0329] 13.如实施方案1所述的核酸，其中编码所述修饰的HA的核苷酸序列包含嵌合核苷酸序列，所述嵌合核苷酸序列按顺序编码修饰的HA胞外域、流感跨膜结构域和胞质尾区，其中所述修饰的HA胞外域包含修饰的蛋白水解环且来自第一流感毒株，而所述跨膜结构域和所述胞质尾区来自第二流感毒株。

[0330] 14.在植物中产生流感病毒样颗粒(VLP)的方法，其包括：

[0331] a)将实施方案1所述的核苷酸引入所述植物或所述植物的部分；

[0332] b)在允许所述核酸表达的条件下培养所述植物或所述植物的部分，从而产生所述VLP。

[0333] 15.如实施方案14所述的方法,其中在步骤a)中,将第二核苷酸引入所述植物或所述植物的部分,所述第二核苷酸包含第二调控区,所述第二调控区在所述植物中有活性并且与编码质子通道蛋白的核苷酸序列可操作地连接。

[0334] 16.如实施方案15所述的方法,其中所述质子通道蛋白选自流感M2或流感BM2。

[0335] 17.在植物中产生流感病毒样颗粒(VLP)的方法,其包括:

[0336] a)提供植物或植物的部分,其包含实施方案1所述的核酸,以及

[0337] b)在允许所述核酸表达的条件下培养所述植物或所述植物的部分,从而产生所述VLP。

[0338] 18.如实施方案14所述的方法,其还包括以下步骤:

[0339] c)收集所述植物,以及

[0340] d)纯化所述VLP,其中所述VLP的大小范围为80-300nm。

[0341] 19.通过实施方案14的任一种方法产生的VLP。

[0342] 20.如实施方案19所述的VLP,其还包含一种或多种源自植物的脂质。

[0343] 21.如实施方案20所述的VLP,其中所述修饰的HA蛋白包含植物-特异性N-聚糖或修饰的N-聚糖。

[0344] 22.在植物中产生修饰的HA蛋白的方法,所述修饰的HA蛋白包含修饰的蛋白水解环,所述修饰的蛋白水解环包含呈现出减少或废除的蛋白酶切割的一个或多个蛋白酶切割位点,所述方法包括:

[0345] a)将实施方案1所述的核苷酸引入所述植物;

[0346] b)在允许所述HA蛋白表达的条件下培养所述植物或所述植物的部分,从而产生所述修饰的HA蛋白,

[0347] c)收集所述植物,并且纯化所述修饰的HA蛋白。

[0348] 23.通过实施方案22所述的方法产生的HA蛋白,其中所述蛋白具有血凝素(HA)活性。

[0349] 24.由实施方案1所述的核酸编码的HA。

[0350] 25.如实施方案14所述的方法,其中在所述引入步骤(步骤a)中,以瞬转的方式将所述核酸引入植物中,或者将所述核酸引入所述植物中以使其稳定。

[0351] 26.包含实施方案1所述的核酸的植物。

[0352] 28.组合物,其包含用于诱导免疫应答的有效剂量的实施方案19所述的VLP,以及药学上可接受的载体。

[0353] 29.疫苗,其包含用于诱导免疫应答的有效剂量的实施方案19所述的VLP。

[0354] 30.在对象中诱导对流感病毒感染的免疫力的方法,其包括施用实施方案19所述的VLP。

[0355] 31.如实施方案30所述的方法,其中所述VLP经口、皮内、鼻内、肌肉内、腹腔内、静脉内或皮下施用于对象。

[0356] 32.提高植物中表达的HA蛋白的产物质量的方法,其包括:

[0357] a)将实施方案1所述的核苷酸引入所述植物;

[0358] b)在允许所述HA蛋白表达的条件下培养所述植物或所述植物的部分,从而产生修饰的HA蛋白,

[0359] c)收集所述植物,并且纯化所述修饰的HA蛋白,其中所述修饰的HA蛋白与天然HA相比,具有提高的产物质量。

[0360] 33.如实施方案32所述的方法,其中所述质量包括提高的产物产量、产物稳定性、产物一致性或其组合。

实施例

[0361] 实施例1

[0362] 农杆菌(Agrobacterium)转染

[0363] 使用D'Aoust等2008(Plant Biotechnology Journal 6:930-940)所描述的方法,通过电穿孔法用DNA构建体转染农杆菌菌株AGL1。被转染的农杆菌在补充有10mM 2-(N-吗啉代)乙磺酸(MES)、20μM乙酰丁香酮、50μg/ml卡那霉素和25μg/ml羧苄青霉素的pH5.6的YEB培养基上生长至OD_{600}为0.6-1.6。使用前将农杆菌悬浮液离心,然后重悬于渗入培养基(10mM $MgCl_2$和10mM MES pH 5.6)。

[0364] 植物生物质、接种量和农杆菌渗入的制备

[0365] 本文所使用的术语"生物质"和"植物物质"意指源自植物的任何物质。生物质或植物物质可包括完整的植物、组织、细胞或其任何部分(fraction)。进一步地,生物质或植物物质可包括细胞内植物组分,细胞外植物组分,植物的液体或固体提取物或其组合。进一步地,生物质或植物物质可包括来自植物叶、茎、果实、根或其组合的植物、植物细胞、组织、液体提取物或其组合。植物的部分可包括植物物质或生物质。

[0366] 在铺满商购的泥煤苔培养基的平地上由种子生长烟草本塞姆氏(Nicotiana benthamiana)植物。允许植物生长在温室中,16/8光周期和25℃白天/20℃夜晚的温度方案。播种后三周,挑选出个别小植株,移植在盆中,并且在相同环境条件的温室中继续生长另外三周。

[0367] 转染有各个构建体的农杆菌生长在补充有10mM 2-(N-吗啉代)乙磺酸(MES)、20μM乙酰丁香酮、50μg/ml卡那霉素和25μg/ml羧苄青霉素的pH5.6的YEB培养基中直到它们达到OD_{600}为0.6-1.6。使用前将农杆菌悬浮液离心,然后重悬于渗入培养基(10mM $MgCl_2$和10mM MES,pH 5.6),并且在4℃下保存整夜。渗入的当天,将分批培养物稀释成2.5倍的培养物体积,并且在使用前允许加温。真空20-40Torr下,将烟草本塞姆氏的完整植物倒置在密封不锈钢罐中的细菌悬浮液中2分钟。将植物返回至温室中经2-6天的培养期直到收集。

[0368] 叶的收集和总蛋白提取

[0369] 培养后,收集植物的地上部分,-80℃下冷冻并且碾成碎块。通过用3倍体积的冷50mM Tris pH 8.0、0.5M NaCl、0.1%Triton X-100和1mM苯甲基磺酰氟匀浆(Polytron)冻碎的植物物质的各个样品,以提取总的可溶性蛋白。匀浆后,浆液在10,000g,4℃下离心10分钟,并且保留这些澄清的粗提取物(上清液)用于分析。

[0370] 蛋白分析和免疫印迹

[0371] 使用牛血清白蛋白作为参考标准,通过Bradford检测(Bio-Rad,Hercules,CA)测定澄清的粗提取物的总蛋白含量。通过SDS-PAGE分离蛋白,并且将其电转移至聚偏二氟乙烯(PVDF)膜上(Roche Diagnostics Corporation,Indianapolis,IN),用于免疫检测。免疫印迹之前,4℃将膜在tris-缓冲盐水溶液中的5%脱脂牛奶和0.1%吐温-20(TBS-T)中封闭

16-18小时。

[0372] 用2μg/ml的一抗(在0.1％的TBS-吐温20中的2％脱脂牛奶中)首先孵育(表4显示用于检测每个HA所使用的抗体和条件),以进行免疫印迹。用于化学发光检测所使用的二抗如表4所示,按所示用0.1％的TBS-吐温20中的2％脱脂牛奶稀释。使用鲁米诺作为底物(Roche Diagnostics Corporation),通过化学发光来检测免疫反应复合物。通过使用EZ-Link Plus®活化的过氧化物酶结合试剂盒(Pierce,Rockford,IL),实施人IgG抗体与辣根过氧化物酶的结合。

[0373] 表4:用于所表达蛋白的免疫印迹的电泳条件、抗体和稀释。

[0374]

HA亚型	流感毒株	电泳条件	一抗	稀释	二抗	稀释
B	B/布里斯班/60/2008	非还原的	TGA，AS397	1：20000	兔抗-羊(JIR 313-035-045)	1：10000
B	B/威斯康星州/1/2010	非还原的	NIBSC 07/356	1：2000	兔抗-羊(JIR 313-035-045)	1：10000
B	B/马来西亚/2506/2004	非还原的	NIBSC 07/184	1：2000	兔抗-羊(JIR 313-035-045)	1：10000
H3	A/珀斯/16/2009(H3N2)	非还原的	TGA，AS400	1：20000	兔抗-羊(JIR 313-035-045)	1：10000
H3	A/维多利亚/361/2011	非还原的	TGA，AS400	1：20000	兔抗-羊(JIR 313-035-045)	1：10000
H1	A/加利福尼亚/07/2009(H1N1)	还原的	Sino，11055-MMO1	1 μg/ml	山羊抗-鼠(JIR 115-035-146)	1：7500
H5	A/印度尼西亚/05/2005(H5N1)	还原的	CBER，S-7858	1：4000	兔抗-羊(JIR 313-035-045)	1：10000

[0375] JIR:Jackson ImmunoResearch(美国杰克逊免疫研究),West Grove,PA,USA;CBER:Center for Biologics Evaluation and Research(美国生物制品评价和研究中心),Rockville,MD,USA;

[0376] Sino:Sino Biological inc.(北京义翘神州生物技术有限公司),Beijing,China;

[0377] TGA:Therapeutic Goods Administration(澳大利亚治疗产品管理局),Australia;

[0378] NIBSC:National Institute for Biological Standards and control(英国国家生物制品检定所),United Kingdom.

[0379] 凝集检测

[0380] 凝集检测基于Nayak and Reichl(2004)所描述的方法。简单地说,在含有100μL PBS的V-形底96-孔微量滴定板中进行测试样品(100μL)的连续倍比稀释,每孔留有100μL稀释样品。将100微升0.25%鲜红色血细胞悬浮液(Bio Link Inc.,Syracuse,NY)加入各孔中,并且在室温下孵育板2小时。显示完全血凝的最高稀释倍数的倒数被记录为HA活性。并列地,重组的HA标准(A/越南/1203/2004H5N1)(Protein Science Corporation,Meriden,CT)用PBS稀释,并且将其作为每块板上的对照。

[0381] 细胞壁消化的VLP提取物

[0382] 从烟草本塞姆氏植物收集叶片组织,并且切成～1cm^{2}的片状。室温(RT)下,叶片在500mM甘露糖醇中浸泡30分钟。然后去除甘露糖醇溶液,并且更换为原生质体溶液(500mM甘露糖醇、10mM $CaCl_2$和5mM MES/KOH(pH 5.6))中的酶混合物(来自绿色木酶(Trichoderma viride)的纤维素酶的混合物(Onozuka R-10;3%v/v)和来自根霉(Rhizopus sp.)的果胶酶的混合物(MACEROZYME™;0.75%v/v;均来自Yakult Pharmaceuticals)。所用的比值为每100mL溶液20g叶片。将该制备物均匀地分散在浅的器皿(～11x18cm)中,并且在40rpm和26℃的摇床上孵育16小时。

[0383] 可选地,VLP的提取可按如下进行:植物被AGL1/#489、928、676和766农杆菌渗入。渗入后第7天,从烟草本塞姆氏植物中收集叶片组织,并且切成～1cm^{2}的片状。将果胶酶162L(Biocatalysts)、Multifect CX CG和Multifect CX B(Genencor)加入至200mM甘露糖醇、75mM柠檬酸盐、0.04%亚硫酸氢钠缓冲液(pH 6.0);消化缓冲液。两份生物质在定轨摇床上室温整夜消化。

[0384] 酶辅助提取后,通过过滤(250或400μm目的尼龙滤器)去除叶的碎片。粗滤提取物在5000xg下离心5分钟。上清液被应用于检测HA表达(血凝活性(参见图20)和蛋白质印迹(参见图22))。

[0385] 实施例2:

[0386] 修饰的蛋白水解环对HA的累积的影响

[0387] 如图13A所示,天然的B/布里斯班(构建体号:1008)的表达低于包含修饰的蛋白水解环的B/布里斯班(构建体号:1059)的表达。当与天然B/布里斯班HA(构建体号:1008;图13B)相比时,包含修饰的蛋白水解环的B/布里斯班(构建体号:1059)中也观察到增加的血凝活性。

[0388] 在包含修饰的蛋白水解环的B/威斯康星州(构建体号:1467)的累积水平方面观察到类似结果,其大于天然B/威斯康星州HA(构建体号:1462;图16A)所观察到的累积水平。当与天然的B/威斯康星州HA(构建体号:1462;图16B)相比时,包含修饰的蛋白水解环的B/威斯康星州(构建体号:1467)中也观察到增加的血凝活性,其指示了突变蛋白的更多累积。

[0389] 也观察到包含修饰的蛋白水解环的H5/Indo的表达,所述修饰包括包含GG接头(构建体号:928;SEQ ID NO:85)、TETR接头(构建体号:676;SEQ ID NO:77)或TETQ接头(构建体号:766;SEQ ID NO:8;图22)的蛋白水解环。

[0390] 流感M2的共表达对HA的累积水平的影响

[0391] 评价M2的共表达对修饰的流感B HA的累积水平的影响。构建体1059号编码流感B HA,其中蛋白水解环被2个氨基酸接头代替(GG代替aa 341-359)。来自图13A中呈现的

Western印迹分析的结果表明,蛋白水解环的去除导致增加的流感B HA累积水平(比较1008与1059),并且M2与修饰的流感B HA的共表达也增加HA累积水平(图13A,1059vs 1059+1261)。来自转化有具有或没有修饰以及与或不与M2共表达的流感B HA的植物的粗蛋白提取物的血凝活性分析证实,M2共表达对天然的流感B HA(图13B,1008vs 1008+1261)和修饰的流感B HA(图13B,1059vs 1059+1261)的累积水平有积极作用。

[0392] M2与包含修饰的蛋白水解环的A型HA的共表达也导致HA表达。例如,具有用GS接头或$(GSS)_3$接头所代替的蛋白水解环的修饰的H3(参见图21E,21F)与M2一起的共表达,也可导致植物中HA的累积。

[0393] 将来自流感A/波多黎各/8/1934的M2增加修饰的流感B HA和H3的累积的功效,与来自流感A/新喀里多尼亚/20/1999的M2增加修饰的流感B HA和H3的累积的功效相比。对于修饰的流感B HA,通过来自转化有构建体1059、1059+1261和1059+859的植物的蛋白提取物的Western印迹分析进行所述比较。获得的结果表明,来自流感A/波多黎各/8/1934(由构建体859号编码)的M2的共表达,与来自流感A/新喀里多尼亚/20/1999(由构建体1261号编码)的M2的共表达在增加修饰的流感B HA的累积方面功效相同(图14)。

[0394] 流感M2共表达对不同毒株B HA的累积水平的影响

[0395] 来自转化有基因构建体的植物的蛋白提取物的Western印迹分析显示M2共表达导致流感B HA累积的增加(图16A),所述基因构建体驱动流感B HA(来自B/威斯康星州/1/2010)(构建体1462号)在存在或不存在M2-表达构建体(构建体1261号)时表达。

[0396] 也评价了M2的共表达对修饰的流感B HA的累积水平的影响。构建体1467号编码流感B HA,其中蛋白水解环被2个氨基酸接头代替(GG代替aa 341-359)。来自图16A中所呈现的Western印迹分析的结果显示,蛋白水解环的去除导致流感B HA累积水平增加(比较1462与1467),并且M2与修饰的流感B HA的共表达也增加了HA累积水平(图16A,1467vs 1467+1261)。来自转化有具有或没有修饰并且与或不与M2共表达的流感B HA的植物的粗蛋白提取物的血凝活性的分析证实,M2共表达对天然的流感B HA(图16B,1462vs 1462+1261)和修饰的流感B HA(图16B,1467vs 1467+1261)的累积水平有积极作用。

[0397] 扩增元件BeYDV和修饰的蛋白水解环对HA累积的影响

[0398] 来自转化有基因构建体的植物的蛋白提取物的Western印迹分析显示,当调控元件为CPMV-HT,在不存在BeYDV时,可检测到流感B HA无累积(图17B),所述基因构建体驱动具有或没有蛋白水解环去除的修饰的流感B HA(来自B/布里斯班/60/2008)(参见图17A的构建体)在存在或不存在扩增元件BeYDV(构建体1059号和1039号)时表达。

[0399] 修饰的蛋白水解环对相对的HA滴度和血凝的影响

[0400] 根据图29A,显示在植物中所产生的包含基于CPMV HT、CPMV HT+、CPMV 160或CPMV 160+的增强子元件的修饰HA蛋白或天然HA的活性比较,所述增强子元件与编码具有蛋白水解环缺失(用GG接头代替)的修饰HA的或天然HA核苷酸序列可操作地连接。在大多数情况下,对于基于CPMV HT+、CPMV 160或CPMV 160+的构建体,表达较高(测定为血凝滴度或活性),表明显著的表达水平。

[0401] 表5a:相对的HA滴度(wt HA=1)(参见图29A)

[0402]

	Fct = 1					
	wt			PrL-		
描述(n=)	GM	MD	n=	GM	MD	n=
H2 Sin157(HT+)(n = 2)	1.0	0.4	2	1.2	0.1	2
H2 Sin157(160+)(n = 2)	1.0	0.3	2	1.9	0.9	2
H3 Per1609(HT+)(n = 4)	1.0	0.0	4	4.9	4.2	4
H3 Per1609(160+)(n = 4)	1.0	0.3	4	6.1	3.6	4
H3 Vic36111(HT+)(n = 6)	1.0	0.3	6	0.9	0.3	6
H3 Vic36111(160+)(n = 6)	1.0	0.3	6	1.2	0.4	6
H5 Indo505(HT)(n = 3)	1.0	0.1	3	1.2	0.3	3
H7 Han113(HT+)(n = 5)	1.0	0.2	5	1.0	0.1	5
H9 HK107399(HT+)(n = 2)	1.0	0.4	2	1.2	0.2	2
H9 HK107399(160+)(n = 2)	1.0	0.2	2	0.7	0.1	2
HB Bri6008(HT)(n = 1)	1.0	0.6	1	0.6	0.1	1
HB Mal250604(160+)(n = 2)	1.0	0.3	2	8.4	2.4	2
HB Mas212(HT)(n = 3)	1.0	0.1	3	1.4	1.3	3
HB Mas212(HT+)(n = 3)	1.0	0.6	3	6.7	3.5	3
HB Mas212(160+)(n = 2)	1.0	0.8	2	11.5	4.2	2

[0403] 实施例3

[0404] 与天然的构建体相比,当去除蛋白水解环(PrL-)时增加的H7杭州HA VLP产量

[0405] 用AGL1/#2142+1261和#2152+1261渗入烟草本塞姆氏植物,并且7天培育期后收集叶。收集叶片组织,并且将其切成～1cm^{2}片状。将果胶酶162L和果胶酶444L(Biocatalysts)、Multifect CX CG和Multifect CX B(Genencor)加入到200mM甘露糖醇、125mM柠檬酸盐、0.04%亚硫酸氢钠缓冲液(pH 6.0)。生物质在定轨摇床上室温下整夜消化。

[0406] 消化后,将非原生质体级分(fraction)滤过400μm尼龙滤器以去除粗的未消化的植物组织(<5%原始生物质)。然后将滤过的提取物在室温下5000xg离心15分钟,以去除原生质体和细胞内污染物(蛋白、DNA、膜、囊泡、色素,等等)。其次,在被施用于层析前,使用1.2μm玻璃纤维过滤器(Sartopore GF plus/Sartorius Stedim),以及0.45/0.2μm过滤器(Sartopore 2/Sartorius Stedim)深度过滤(用于澄清)上清液。

[0407] 将澄清的非原生质体级分加载至用平衡/洗脱缓冲液(50mM $NaPO_4$,100mM NaCl,0.005%吐温80pH 6.0)平衡好的阳离子交换柱上(Poros HS Applied Biosystems)。一旦UV归零,用含有渐增浓度的NaCl(500mM)的平衡/洗脱缓冲液分步洗脱提取物。纯化的VLP用TFF浓缩、配制缓冲液(100mM PO4,150mM NaCl,pH 7.4的0.01%吐温80)渗滤(diafiltered),并且通过0.22μm的过滤器。

[0408] 基于Nayak and Reichl(2004)所描述的方法进行H7的凝集检测。简单地说,在含有100μL PBS的V-形底96-孔微量滴定板中进行测试样品(100μL)的连续倍比稀释,使每孔剩余100μL稀释样品。将100微升0.25%的鲜红色血细胞悬浮液(Bio Link Inc.,Syracuse,NY)加入至各孔,并且在室温下孵育板2小时。显示完全凝集的最高稀释倍数的倒数被记录为血凝活性。

[0409] 使用牛血清白蛋白作为参考标准,测定澄清的粗提取物的总蛋白含量。通过比较PrL-构建体与用作对照的天然构建体获得相对产量。在还原条件下进行具有变性样品上样

缓冲液(0.1M Tris pH 6.8,0.05％溴酚蓝,12.5％甘油,4％SDS和5％β-巯基乙醇)的SDS-PAGE的分离,并且使用考马斯亮蓝R-250用于蛋白质染色。

[0410] 图46A显示与天然构建体(#2142+#1261,参见实施例5.33)相比,对于去除蛋白水解环的H7杭州构建体(#2152+#1261,参见实施例5.34),植物提取物中的血凝活性更大。

[0411] 图46B显示与天然的构建体(#2142+#1261)相比时,对于去除蛋白水解环的H7杭州构建体(#2152+#1261),纯化的VLP中的相对总蛋白产量较高。该实施例表明植物中所累积的VLP的改善与当进行全过程时的最终产量之间的良好相关性。

[0412] 图46C显示SDS-PAGE分析,泳道2显示具有去除的蛋白水解环的纯化的H7杭州构建体,泳道3显示纯化的天然H7杭州构建体。对于每个泳道,在凝胶上加载2μg的总蛋白。对于这两种构建体,蛋白谱的纯度是类似的,并且大于90％。

[0413] 实施例4.1

[0414] 突变体H5印度尼西亚VLP(其中蛋白水解环被修饰或去除)的胰蛋白酶抗性大于天然H5印度尼西亚。

[0415] 烟草本塞姆氏植物用农杆菌渗入实施例1中所描述的AGL1/#489、#928、#766和#676(上文)。渗入后7天从植物收集叶,切成～cm^2片状。将果胶酶162L(Biocatalysts)、Multifect CX CG和Multifect CX B(Genencor)加入至200mM甘露糖醇、75mM柠檬酸盐、0.04％亚硫酸氢钠缓冲液(pH 6.0)。生物质在定轨摇床上室温整夜消化。按照实施例3(H7杭州)所述,将消化的提取物进行粗滤、离心、澄清并且纯化。

[0416] 对于各个天然的(#489)、PRL-(#928)、TETQ(#766)和TETR(#676),H5印度尼西亚HA VLP提取物,用pH 7.4的缓冲液(100mM Na/KPO_4,150mM NaCl,0.01％吐温80)重悬两份HA VLP样品。按1:100的蛋白比例加入胰蛋白酶。室温下孵育30、60和120分钟后吸取样品,然后在上样缓冲液中煮沸以中止反应。通过实施例3所描述的SDS-PAGE凝胶分析非消化的提取物(对照)和胰蛋白酶-消化的提取物。

[0417] 图47A显示胰蛋白酶-消化样品的SDS-PAGE分析,在消化的不同时间点(0、30、60和120分钟),泳道2至5显示天然H5印度尼西亚VLP(#489)、泳道6至9显示PrL-H5印度尼西亚VLP(#928)、泳道10至13显示TETQ H5印度尼西亚VLP(#766),泳道14至17显示TETR H5印度尼西亚VLP(#676)。通过胰蛋白酶的加入,在泳道2的非消化提取物中在大约75kDa下可检测的具有对应于HA0单体的条带的天然H5印度尼西亚VPL,被快速地加工为HA1和HA2条带,泳道3至5中在胰蛋白酶消化期间分别在大约50和25kDa下可检测。通过蛋白水解位点的去除或修饰所稳定的PrL-和TETQ H5印度尼西亚VLP,表现出胰蛋白酶的抗性,因为HA0条带没有切割为HA1和HA2条带。通过蛋白水解位点的修饰使TETR H5印度尼西亚VLP部分地稳定,并且HA0单体被切割为HA1和HA2慢于天然H5印度尼西亚VLP。

[0418] 这些数据表明,通过缺失蛋白水解环(prl-)或用接头序列(TETQ)方法代替蛋白水解环,在HA1-HA2内的蛋白水解位点处成功地保护了HA0蛋白。

[0419] 实施例4.2

[0420] 在小鼠中天然H5印度尼西亚VLP的免疫原性类似于其突变体对应物(PrL-、TETQ和TETR)

[0421] 按照实施例4.1所述(上文)纯化天然的、PrL-、TETR和TETQ H5印度尼西亚VLP提取物。

[0422] 图47B显示两个剂量后小鼠中的天然H5 VLP和其突变体对应物(prl-、TETQ和TETR)的免疫原性(HI滴度)。BALB/c小鼠(n＝8/组)两次肌内注射10μg剂量的基于植物的H5 VLP疫苗(天然的、prl-、TETQ或TETR)(基于其HA含量),相隔21天。42dpv(第二次剂量后21天),将每只动物的血清进行HI滴度分析,并且H5 VLP A/印度尼西亚/5/2005(H5N1)被用作抗原。柱状代表各个H5突变体VLP与天然H5VLP相比的相对(%)HI滴度(用log2 HI滴度GMT和95%CI计算)。通过使用单因子方差分析,随后通过对Log2-转换数据(假设它们是正态分布的)的图基氏事后分析(Tukey's post-hoc analysis)比较各个剂量组之间的统计学差异。*p<0.05被认为是显著的。观察到各个剂量组之间无差异。

[0423] 实施例5.1:

[0424] B-2X35S/CPMV-HT/M2新喀里多尼亚/NOS(构建体1261号)

[0425] 使用以下基于PCR的方法,将编码来自流感A/新喀里多尼亚/20/1999(H1N1)的M2的序列,克隆进含有Plasto_pro/P19/Plasto_ter表达盒的质粒中的2X35S/CPMV-HT/NOS表达系统中。使用引物IF-S1-M1+M2ANC.c(图2A,SEQ ID NO:7)和IF-S1-4-M2ANC.r(图2B,SEQ ID NO:8),采用合成的M2基因(对应于连接到来自GenBank登录号DQ508860的nt 715-982的nt 1-26;图2C,SEQ ID NO:9)作为模板,扩增含有完整M2编码序列的片段。使用In-Fusion克隆体系(Clontech,Mountain View,CA),将PCR产物克隆进2X35S/CPMV-HT/NOS表达系统中。用SacII和StuI限制性内切酶消化构建体1191(图1C),并且线性化质粒被用于In-Fusion组装反应。构建体1191号为意图用于在基于CPMV-HT的表达盒中"In Fusion"克隆目标基因的受体质粒。它也合并了用于在紫花苜蓿质体蓝素基因启动子和终止子下TBSV P19沉默抑制子的共表达的基因构建体。骨架是pCAMBIA双元质粒,并且图1D呈现从左至右的t-DNA边界的序列(SEQ ID NO:4)。得到的构建体被命名1261号(图2D,SEQ ID NO:10)。图2E呈现来自流感A/新喀里多尼亚/20/1999(H1N1)的M2的氨基酸序列(SEQ ID NO:11)。图11呈现质粒1261的示意图。

[0426] 实施例5.2:

[0427] C-2X35S/CPMV-HT/M2波多黎各/NOS(构建体859号)

[0428] 使用以下基于PCR的方法,将编码来自流感A/波多黎各/8/1934(H1N1)的M2的序列,克隆进含有Plasto_pro/P19/Plasto_ter表达盒的质粒中的2X35S/CPMV-HT/NOS表达系统中。使用引物IF-S1-M1+M2ANC.c(图2A,SEQ ID NO:7)和IF-S1-4-M2ANC.r(图2B,SEQ ID NO:8),采用合成的M2基因(对应于连接到来自Genbank登录号EF467824的nt 740-1007的nt 26-51)(图3A,SEQ ID NO:12)作为模板,扩增含有完整的M2编码序列的片段。使用In-Fusion克隆体系(Clontech,Mountain View,CA),将PCR产物克隆进2X35S/CPMV-HT/NOS表达系统中。用SacII和StuI限制性内切酶消化构建体1191(图1C),并且线性化质粒被用于In-Fusion组装反应。构建体1191号为意图用于在基于CPMV-HT的表达盒中"In Fusion"克隆目标基因的受体质粒。它也合并了用于在紫花苜蓿质体蓝素基因启动子和终止子下TBSV P19沉默抑制子的共表达的基因构建体。载体为pCAMBIA双元质粒,并且图1D呈现从左至右的t-DNA边界的序列(SEQ ID NO:4)。得到的构建体被命名为859号(图3B,SEQ ID NO:13)。图3C呈现来自流感A/波多黎各/8/1934(H1N1)的M2的氨基酸序列(SEQ ID NO:14)。图17呈现质粒859的示意图。

[0429] 实施例5.3:

[0430] 引入BeYDV+复制酶扩增体系中的G-2X35S/CPMV-HT/PDISP/HA B布里斯班/NOS(构建体1008号)

[0431] US 61/541,780中描述了构建体1008的制备。简单地说,使用基于PCR的方法,采用合成的HA B布里斯班基因(对应于来自Genbank登录号FJ766840的nt 34-1791),将编码来自流感B的HA/布里斯班/60/2008的序列,克隆进含有Plasto_pro/P19/Plasto_ter表达盒的质粒中包含BeYDV+复制酶扩增体系的2X35S/CPMV-HT/PDISP/NOS中。将PCR产物克隆进引入BeYDV扩增体系中的2X35S/CPMV-HT/NOS表达盒中具有紫花苜蓿PDI信号肽的框中。用SacII和StuI限制性内切酶消化构建体1194(参见图4A,4B),并且线性化质粒被用于组装反应以产生构建体1008号(图4C,图9;SEQ ID NO:32)。

[0432] 构建体1194号(图4A)为意图用于在引入BeYDV扩增体系中的基于CPMV-HT的表达盒中具有紫花苜蓿PDI信号肽的框中"In Fusion"克隆目标基因的受体质粒。它也合并了用于在紫花苜蓿质体蓝素基因启动子和终止子下TBSV P19沉默抑制子的共表达的基因构建体。骨架为pCAMBIA双元质粒。

[0433] 实施例5.4:

[0434] 引入BeYDV+复制酶扩增体系中的具有缺失的蛋白水解环的I-2X35S/CPMV-HT/PDISP-HA B布里斯班(构建体1059号)

[0435] US 61/541.780中描述了构建体1059的制备。简单地说,使用基于PCR的连接方法(Darveau等,1995,Methods in Neuroscience 26:77-85),将编码来自流感B/布里斯班/60/2008的具有缺失的蛋白水解环的HA的序列,克隆进含有Plasto_pro/P19/Plasto_ter表达盒的质粒中包含BeYDV+复制酶扩增体系的2X35S/CPMV-HT/PDISP/NOS中。在第一轮PCR中,使用合成的HA B布里斯班基因(对应于来自Genebank登录号FJ766840的nt 34-1791)作为模板,扩增含有HA B布里斯班编码序列的nt 46至nt 1065的片段。使用合成的HA B布里斯班基因(对应于来自Genbank登录号FJ766840的nt 34-1791)作为模板,扩增含有HA B布里斯班编码序列的nt 1123至nt 1758的第二片段。然后将来自两次扩增的PCR产物混合并且用作模板,用于第二轮的扩增。将得到的片段(编码片段之间具有GG接头的HA B/布里斯班/60/2008Δa.a.356-374;参见图21B)克隆进包含BeYDV扩增体系的2X35S/CPMV-HT/NOS表达盒中具有紫花苜蓿PDI信号肽的框中,以产生构建体1194(图4A,4B),用SacII和StuI限制性内切酶消化构建体1194,并且线性化质粒被用于组装反应。得到的构建体被命名为1059号(图5C;SEQ ID NO:40)。

[0436] 图5D呈现具有缺失的蛋白水解环的PDISP-HA B/布里斯班/60/2008的氨基酸序列(SEQ ID NO:41)。

[0437] 实施例5.5:

[0438] 引入BeYDV(m)+复制酶扩增体系中的B-2X35S/CPMV-HT/HA B威斯康星州/NOS(构建体1462号)

[0439] US 61/541,780中描述构建体1462的制备。简单地说,使用基于PCR的方法,将编码来自流感B/威斯康星州/1/2010的HA的序列,克隆进含有Plasto_pro/P19/Plasto_ter表达盒的质粒中包含BeYDV(m)+复制酶扩增体系的2X35S/CPMV-HT/NOS中。使用合成的HA B威斯康星州基因(Genbank登录号JN993010)作为模板,扩增含有完整的HA B威斯康星州编码序列的片段。将PCR产物克隆进引入BeYDV(m)扩增体系中的2X35S/CPMV-HT/NOS表达盒中。用

SacII和StuI限制性内切酶消化构建体193(图6D),并且线性化质粒被用于组装反应。

[0440] 构建体193号为意图用于在引入BeYDV(m)扩增体系中的基于CPMV-HT的表达盒中"In Fusion"克隆目标基因的受体质粒。它也合并了用于在紫花苜蓿质体蓝素基因启动子和终止子下TBSV P19沉默抑制子的共表达的基因构建体。骨架为pCAMBIA双元质粒,并且图6E呈现从左至右的t-DNA边界的序列(SEQ ID NO:52)。得到的构建体被命名为1462号(图6F,SEQ ID NO:53)。图6G呈现来自流感B/威斯康星州/1/2010的PDISP/HA的氨基酸序列(SEQ ID NO:54)。图6H呈现质粒1462的示意图。

[0441] 实施例5.6:

[0442] 引入BeYDV(m)+复制酶扩增体系中的具有缺失的蛋白水解环的C-2X35S/CPMV-HT/HA B威斯康星州(构建体1467号)

[0443] US 61/541,780中描述了构建体1467的制备。简单地说,使用基于PCR的连接方法(Darveau等1995,Methods in Neuroscience 26:77-85),将编码来自流感B/威斯康星州/1/2010的具有缺失的蛋白水解环的HA的序列,克隆进含有Plasto_pro/P19/Plasto_ter表达盒的质粒中包含BeYDV(m)+复制酶扩增体系的2X35S/CPMV-HT/NOS中。在第一轮PCR中,使用引物IF-HA B110.S1+3c(图6A,SEQ ID NO:49)和HA B110(PrL-).r(图7A,SEQ ID NO:55),采用合成的HA B威斯康星州基因(Genbank登录号JN993010)(图6C,SEQ ID NO:51)作为模板,扩增含有HA B威斯康星州编码序列的nt 1至nt 1062的片段。使用引物HA B110(PrL-).c(图7B,SEQ ID NO:56)和IF-HA B110.s1-4r(图6B,SEQ ID NO:50),采用合成的HA B威斯康星州基因(Genbank登录号JN993010)(图6C,SEQ ID NO:51)作为模板,扩增含有HA B威斯康星州编码序列的nt 1120至nt 1755的第二片段。然后将来自两次扩增的PCR产物混合并且用作模板,用于使用IF-HA B110.S1+3c(图6A,SEQ ID NO:49)和IF-HA B110.s1-4r(图6B,SEQ ID NO:50)作为引物的第二轮扩增。使用In-Fusion克隆体系(Clontech,Mountain View,CA),将得到的片段(编码片段之间具有接头的HA B/威斯康星州/1/2010Δa.a.340-358)克隆进包含BeYDV(m)扩增体系的2X35S/CPMV-HT/NOS表达盒中。用SacII和StuI限制性内切酶消化构建体193(图6D),并且线性化质粒被用于In-Fusion组装反应。

[0444] 构建体193号为意图用于在引入BeYDV(m)扩增体系中的基于CPMV-HT的表达盒中"In Fusion"克隆目标基因的受体质粒。它也合并了用于紫花苜蓿质体蓝素基因启动子和终止子下TBSV P19沉默抑制子的共表达的基因构建体。骨架为pCAMBIA双元质粒,并且图6E呈现从左至右的t-DNA边界的序列(SEQ ID NO:52)。得到的构建体被命名为1467号(图7C,SEQ ID NO:57)。图7D呈现来自具有缺失的蛋白水解环的流感B/威斯康星州/1/2010的HA的氨基酸序列(SEQ ID NO:58)。图7E呈现质粒1467的示意图。

[0445] 实施例5.7:

[0446] 具有缺失的蛋白水解环的A-2X35S/CPMV-HT/PDISP-HA B布里斯班(构建体1039号)

[0447] US 61/541,780中描述了构建体1192的制备。简单地说,使用以下基于PCR的连接方法(Darveau等,1995,Methods in Neuroscience 26:77-85),将编码来自流感B的HA/布里斯班/60/2008的具有缺失的蛋白水解环的序列,克隆进含有Plasto_pro/P19/Plasto_ter表达盒的质粒中的2X35S/CPMV-HT/PDISP/NOS中。在第一轮PCR中,使用合成的HA B布里斯班基因(对应于来自Genebank登录号FJ766840的nt 34-1791)作为模板,扩增含有HA B布

里斯班编码序列的nt 46至nt 1065的片段。使用合成的HA B布里斯班基因(对应于来自Genbank登录号FJ766840的nt 34-1791)作为模板,扩增含有HA B布里斯班编码序列的nt 1123至nt 1758的第二片段。然后将来自两次扩增的PCR产物混合并且用作模板,用于第二轮扩增。将得到的片段(编码片段之间具有GG接头的HA B/布里斯班/60/2008Δa.a.356-374),克隆进2X35S/CPMV-HT/NOS表达盒中具有紫花苜蓿PDI信号肽的框中。用SacII和StuI限制性内切酶消化构建体1192,并且线性化质粒被用于In-Fusion组装反应。

[0448] 构建体1192号为意图用于在基于CPMV-HT的表达盒中具有紫花苜蓿PDI信号肽的框中"In Fusion"克隆目标基因的受体质粒。它也合并了用于紫花苜蓿质体蓝素基因启动子和终止子下TBSV P19沉默抑制子的共表达的基因构建体。骨架为pCAMBIA双元质粒,得到的构建体被命名为1039号(图8B)。图5D呈现具有缺失的蛋白水解环的PDISP-HA B/布里斯班/60/2008的氨基酸序列(SEQ ID NO:41)。图8A呈现质粒1039的示意图(SEQ ID NO:15)。

[0449] 实施例5.8:

[0450] 来自A/印度尼西亚/5/2005的具有TETR切割位点突变的A-2X35S/CPMV-HT/H5(构建体676号)

[0451] 使用以下基于PCR的连接方法(Darveau等,1995,Methods in Neuroscience 26:77-85),将编码来自A/印度尼西亚/5/2005的具有TETR切割位点突变的H5的序列,克隆进含有Plasto_pro/P19/Plasto_ter表达盒的质粒中的2X35S/CPMV-HT/NOS中。在第一轮PCR中,使用引物IF-H5A-I-05.s1+3c(图1A,SEQ ID NO:2)和MutCleavage-H5(Indo).r(图23A,SEQ ID NO:74),采用来自A/印度尼西亚/5/2005的合成H5(图1G,SEQ ID NO:42)作为模板,扩增含有来自A/印度尼西亚/5/2005的H5编码序列的nt 1至nt 1015的片段。使用引物MutCleavage-H5(Indo).c(图23B,SEQ ID NO:75)和IF-H5dTm.r(图1B,SEQ ID NO:3),采用来自A/印度尼西亚/5/2005的合成H5(图1G,SEQ ID NO:42)作为模板,扩增含有来自A/印度尼西亚/5/2005的H5编码序列的nt 1038至nt 1707的第二片段。然后将来自两次扩增的PCR产物混合并且用作模板,用于使用IF-H5A-I-05.s1+3c(图1A,SEQ ID NO:2)和IF-H5dTm.r(图1B,SEQ ID NO:3)作为引物的第二轮扩增。使用In-Fusion克隆体系(Clontech,Mountain View,CA),将得到的片段(编码片段之间具有TETR接头的来自A/印度尼西亚/5/2005的H5Δa.a.339-346)克隆进2X35S/CPMV-HT/NOS表达盒中。用SacII和StuI限制性内切酶消化构建体1191(图1D),并且线性化质粒被用于In-Fusion组装反应。构建体1191号为意图用于在基于CPMV-HT的表达盒的框中"In Fusion"克隆目标基因的受体质粒。它也合并了用于在紫花苜蓿质体蓝素基因启动子和终止子下TBSV P19沉默抑制子的共表达的基因构建体。骨架为pCAMBIA双元质粒,并且图1D呈现从左至右的t-DNA边界的序列(SEQ ID NO:4)。得到的构建体被命名为676号(图23C,SEQ ID NO:76)。图23D呈现来自A/印度尼西亚/5/2005的具有TETR切割位点突变的H5的氨基酸序列(SEQ ID NO:77)。图23E呈现质粒676的示意图。

[0452] 实施例5.9:

[0453] 来自A/印度尼西亚/5/2005的具有TETQ切割位点突变的B-2X35S/CPMV-HT/H5(构建体766号)

[0454] 使用以下基于PCR的连接方法(Darveau等,1995,Methods in Neuroscience 26:77-85),将编码来自A/印度尼西亚/5/2005的具有TETQ切割位点突变的H5的序列,克隆进含

有Plasto_pro/P19/Plasto_ter表达盒的质粒中的2X35S/CPMV-HT/NOS中。在第一轮PCR中，使用引物IF-H5A-I-05.s1+3c(图1A,SEQ ID NO:2)和H5I505_TETQ.r(图24A,SEQ ID NO:78)，采用来自A/印度尼西亚/5/2005(图1G,SEQ ID NO:42)的合成H5作为模板，扩增含有来自A/印度尼西亚/5/2005的H5编码序列的nt 1至nt 1015的片段。使用引物H5I505_TETQ.c(图24B,SEQ ID NO:79)和IF-H5dTm.r(图1B,SEQ ID NO:3)，采用来自A/印度尼西亚/5/2005的合成H5(图1G,SEQ ID NO:42)作为模板，扩增含有来自A/印度尼西亚/5/2005的H5编码序列的nt 1038至nt 1707的第二片段。然后将来自两次扩增的PCR产物混合并且用作模板，使用IF-H5A-I-05.s1+3c(图1A,SEQ ID NO:2)和IF-H5dTm.r(图1B,SEQ ID NO:3)作为引物，用于第二轮扩增。使用In-Fusion克隆体系(Clontech,Mountain View,CA)，将得到的片段(编码来自片段之间具有TETQ接头的H5Δa.a.339-346)克隆进2X35S/CPMV-HT/NOS表达盒中。用SacII和StuI限制性内切酶消化构建体1191(图1D)，并且线性化质粒被用于In-Fusion组装反应。构建体1191号为意图用于在基于CPMV-HT的表达盒的框中“In Fusion”克隆目标基因的受体质粒。它也合并用于在紫花苜蓿质体蓝素基因启动子和终止子下TBSV P19沉默抑制子的共表达的基因构建体。骨架为pCAMBIA双元质粒，并且图1D呈现从左至右的t-DNA边界的序列(SEQ ID NO:4)。得到的构建体被命名为766号(图24C,SEQ ID NO:80)。图24D呈现来自A/印度尼西亚/5/2005的具有TETQ切割位点突变的H5的氨基酸序列(SEQ ID NO:81)。图24E呈现质粒766的示意图。

[0455] 实施例5.10:

[0456] 来自A/印度尼西亚/5/2005的具有缺失的蛋白水解环的C-2X35S/CPMV-HT/H5(构建体928号)

[0457] 使用由Darveau等(Methods in Neuroscience 26:77-85(1995))所呈现的以下基于PCR的连接方法，将编码来自A/印度尼西亚/5/2005的具有缺失的蛋白水解环的H5的序列，克隆进含有Plasto_pro/P19/Plasto_ter表达盒的质粒中的2X35S/CPMV-HT/NOS中。在第一轮PCR中，使用引物IF-H5A-I-05.s1+3c(图1A,SEQ ID NO:2)和H5I505(PrL-).r(图25A,SEQ ID NO:82)，采用来自A/印度尼西亚/5/2005的合成的H5(图1G,SEQ ID NO:42)作为模板，扩增含有来自A/印度尼西亚/5/2005的H5编码序列的nt 1至nt 1011的片段。使用引物H5I505(PrL-).c(图25B,SEQ ID NO:83)和IF-H5dTm.r(图1B,SEQ ID NO:3)，采用来自A/印度尼西亚/5/2005的合成的H5(图1G,SEQ ID NO:42)作为模板，扩增含有来自A/印度尼西亚/5/2005的H5编码序列的nt 1075至nt 1707的第二片段。然后将来自两次扩增的PCR产物混合并且用作模板，用于使用IF-H5A-I-05.s1+3c(图1A,SEQ ID NO:2)和IF-H5dTm.r(图1B,SEQ ID NO:3)作为引物的第二轮扩增。使用In-Fusion克隆体系(Clontech,Mountain View,CA)，将得到的片段(编码片段之间具有GG接头的来自A/印度尼西亚/5/2005的H5Δa.a.338-358)，克隆进2X35S/CPMV-HT/NOS表达盒中。用SacII和StuI限制性内切酶消化构建体1191(图1D)，并且线性化质粒被用于In-Fusion组装反应。构建体1191号为意图用于在基于CPMV-HT的表达盒的框中“In Fusion”克隆目标基因的受体质粒。它也合并了在紫花苜蓿质体蓝素基因启动子和终止子下的TBSV P19沉默抑制子的共表达的基因构建体。骨架为pCAMBIA双元质粒，并且图1D呈现从左至右的t-DNA边界的序列(SEQ ID NO:4)。得到的构建体被命名为928号(图25C,SEQ ID NO:84)。图25D呈现来自A/印度尼西亚/5/2005的具有缺失的蛋白水解环的H5的氨基酸序列(SEQ ID NO:85)。图25E呈现质粒928的示意图。

[0458] 实施例5.11

[0459] -F-2X35S/CPMV-HT/PDISP/HAB布里斯班/NOS(构建体1029号)

[0460] 使用以下基于PCR的方法,将编码来自流感B的HA/布里斯班/60/2008的序列,克隆进含有Plasto_pro/P19/Plasto_ter表达盒的质粒中的2X35S/CPMV-HT/PDISP/NOS表达系统中。使用引物IF-S2+S4-B Bris.c(图30A,SEQ ID NO:86)和IF-S1a4-B Bris.r(图30B,SEQ ID NO:87),采用合成的HA B布里斯班基因(对应于来自Genbank登录号FJ766840的nt 34-1791)(图30C,SEQ ID NO:88)作为模板,扩增含有HA B布里斯班编码序列且无其野生型信号肽的片段。使用In-Fusion克隆体系(Clontech,Mountain View,CA),将PCR产物克隆进2X35S/CPMV-HT/NOS表达系统中具有紫花苜蓿PDI信号肽的框中。用SacII和StuI限制性内切酶消化构建体1192,并且线性化质粒被用于In-Fusion组装反应。构建体1192号意图用于在基于CPMV-HT的表达盒中具有紫花苜蓿PDI信号肽的框中"In Fusion"克隆目标基因的受体质粒。它也合并了用于在紫花苜蓿质体蓝素基因启动子和终止子下TBSV P19沉默抑制子的共表达的基因构建体。骨架为pCAMBIA双元质粒,和从左至右t-DNA边界的序列。得到的构建体被命名为1029号(图30D,SEQ ID NO:89)。图30E呈现来自流感B/布里斯班/60/2008的PDISP/HA的氨基酸序列(SEQ ID NO:90)。图30F呈现质粒1029的示意图。

[0461] 实施例5.12

[0462] -用于PDISP/HA B布里斯班(PrL-)的2X35S/CPMV HT(构建体1039号)和HT+(构建体1829号)

[0463] 使用实施例5.7和5.11中所描述的相同的基于PCR的方法,但是使用为PDISP/HA B布里斯班(PrL-)特别设计的修饰的PCR引物,将对应于来自流感B/布里斯班/60/2008的具有缺失的蛋白水解环(PrL-)的HA的编码序列(PDISP/HA B布里斯班(PrL-);图31A,SEQ ID NO:91),克隆进原始的HT和修饰的HT+中,其中,天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替。图31B中呈现与PDISP融合的成熟HA B布里斯班(PrL-)的氨基酸序列(SEQ ID NO:92)。图8B和31D呈现质粒1039和1829的示意图。

[0464] 实施例5.13

[0465] -用于PDISP/HA B布里斯班(PrL-)的2X35S/CPMV HT(构建体1039号)和2X35S/CPMV 160+(构建体1937号)

[0466] 使用实施例5.7和实施例5.11中所描述的相同的基于PCR的方法,但是使用为PDISP/HA B布里斯班(PrL-)特别设计的修饰的PCR引物,将对应于来自流感B/布里斯班/60/2008的具有缺失的蛋白水解环(PrL-)的HA(对于另外的信息:HA序列中缺失的蛋白水解环区域,参见于2013年3月28日提交的美国临时申请第61/806,227号,其通过引用并入本文)的编码序列(PDISP/HA B布里斯班(PrL-))(图32A,SEQ ID NO:93),克隆进原始的CPMV-HT和CPMV 160+中,其中,天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替。图31B中呈现与PDISP融合的成熟HA B布里斯班(PrL-)的氨基酸序列(SEQ ID NO:92)。图8B和图32C呈现质粒1039和1937的示意图。

[0467] 实施例5.14

[0468] -用于PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT的2X35S/CPMV HT(构建体1067号)和2X35S/CPMV 160+(构建体1977号)

[0469] 使用实施例5.7和5.11中所描述的相同的基于PCR的方法,但是使用为PDISP/HA B

布里斯班(PrL-)+H1加利福尼亚TMCT特别设计的修饰的PCR引物,将嵌合血凝素编码序列(PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT)(图33A,SEQ ID NO:95),克隆进原始的CPMV-HT和CPMV 160+中,所述嵌合血凝素编码序列对应于与来自流感A/加利福尼亚/7/2009的H1的跨膜结构域和胞质尾区(TMCT)融合且具有紫花苜蓿蛋白二硫键异构酶的信号肽的来自流感B/布里斯班/60/08的具有缺失的蛋白水解环(PrL-)的HA的胞外域(对于另外的信息:HA序列中缺失的蛋白水解环区域,参见于2013年3月28日提交的美国临时申请第61/806,227号,其通过引用并入本文)。图33B中呈现与PDISP融合的成熟HA B布里斯班(PrL-)+H1加利福尼亚TMCT的氨基酸序列(SEQ ID NO:96)。图33C和图33D呈现质粒1067和1977的示意图。

[0470] 实施例5.15

[0471] -用于PDISP/HA B马萨诸塞(PrL-)的2X35S/CPMV HT(构建体2072号)和2X35S/CPMV 160+(构建体2050号)

[0472] 使用实施例5.7和5.11中所描述的相同的基于PCR的方法,但是使用为PDISP/HA B马萨诸塞(PrL-)特别设计的修饰的PCR引物,将对应于来自流感B/马萨诸塞/2/2012的具有缺失的蛋白水解环(PrL-)的HA(对于另外的信息:HA序列中缺失的蛋白水解环区域,参见于2013年3月28日提交的美国临时申请第61/806,227号,其通过引用并入本文)的编码序列(PDISP/HA B马萨诸塞(PrL-))(图34A,SEQ ID NO:97),克隆进原始的CPMV-HT和CPMV 160+中,其中,天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替。图34B呈现与PDISP融合的成熟HA B马萨诸塞(PrL-)的氨基酸序列(SEQ ID NO:98)。图34C和图34D中呈现质粒2072和2050的示意图。

[0473] 实施例5.16

[0474] -用于PDISP/HA B马萨诸塞(PrL-)+H1加利福尼亚TMCT的2X35S/CPMV HT(构建体2074号)和2X35S/CPMV 160+(构建体2060号)

[0475] 使用实施例5.7和5.11中所描述的相同的基于PCR的方法,但是使用为PDISP/HA B马萨诸塞(PrL-)+H1加利福尼亚TMCT特别设计的修饰的PCR引物,将嵌合血凝素编码序列(PDISP/HA B马萨诸塞(PrL-)+H1加利福尼亚TMCT)(图35A,SEQ ID NO:99),克隆进原始的CPMV-HT和CPMV 160+中,所述嵌合血凝素编码序列对应于与来自流感A/加利福尼亚/7/2009的H1的融合跨膜结构域和胞质尾区(TMCT)融合且具有紫花苜蓿蛋白二硫键异构酶信号肽的来自流感B/马萨诸塞/2/2012的具有缺失的蛋白水解环(PrL-)的HA的胞外域(对于另外的信息:HA序列中缺失的蛋白水解环区域,参见于2013年3月28日提交的美国临时申请第61/806,227号,其通过引用并入本文)。图35B呈现与PDISP融合的成熟HA B马萨诸塞(PrL-)+H1加利福尼亚TMCT的氨基酸序列(SEQ ID NO:100)。图35C和35D中呈现质粒2074和2060的示意图。

[0476] 实施例5.17

[0477] -用于HA B威斯康星州(PrL-)的2X35S/CPMV HT(构建体1445号)、2X35S/CPMV HT+(构建体1820号)和CPMV 160+(构建体1975号)

[0478] 使用实施例5.7和5.11中所描述的相同的基于PCR的方法,但是使用为HA B威斯康星州(PrL-)特别设计的修饰的PCR引物,将对应于来自流感B/威斯康星州/1/2010的具有其天然信号肽的缺失的蛋白水解环(PrL-)的HA(对于另外的信息:HA序列中缺失的蛋白水解

环区域,参见于2013年3月28日提交的美国临时申请第61/806,227号,其通过引用并入本文)的编码序列(HA B威斯康星州(PrL-))(图36AA,SEQ ID NO:101),克隆进原始的CPMV-HT、CPMV HT+和CPMV160中。图36B中呈现具有其天然信号肽的HA B威斯康星州(PrL-)的氨基酸序列(SEQ ID NO:102)。图36C、36D和36E中分别呈现质粒1445、1820和1975的示意图。

[0479] 实施例5.18

[0480] -用于HA B威斯康星州(PrL-)+H1加利福尼亚TMCT的2X35S/CPMV HT(构建体1454号)和2X35S/CPMV 160+(构建体1893号)

[0481] 使用实施例5.7和5.11中所描述的相同的基于PCR的方法,但是使用为HA B威斯康星州(PrL-)+H1加利福尼亚TMCT特别设计的修饰的PCR引物,将嵌合血凝素编码序列(HA B威斯康星州(PrL-)+H1加利福尼亚TMCT)(图37A,SEQ ID NO:103),克隆进原始的CPMV-HT和CPMV 160+中,所述嵌合血凝素编码序列对应与来自流感A/加利福尼亚/7/2009的H1的跨膜结构域和胞质尾区(TMCT)融合且具有HA B威斯康星州天然信号肽的来自流感B/威斯康星州/2/2012的具有缺失的蛋白水解环(PrL-)的HA的胞外域(对于另外的信息:HA序列中缺失的蛋白水解环区域,参见于2013年3月28日提交的美国临时申请第61/806,227号,其通过引用并入本文)。图37中呈现HA B威斯康星州(PrL-)+H1加利福尼亚TMCT的氨基酸序列(SEQ ID NO:104)。图37C和37D中呈现质粒1454和1893的示意图。

[0482] 实施例5.19:

[0483] 用于PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT的2X35S/CPMV HT(构建体1067号)和HT+(构建体1875号)

[0484] 使用实施例5.26中所描述的相同的基于PCR的方法,但是使用为PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT特别设计的修饰的PCR引物,将嵌合血凝素编码序列(PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT)(图38A,SEQ ID NO:105),克隆进原始的HT和修饰的HT+中,所述嵌合血凝素编码序列对应与来自流感A/加利福尼亚/7/2009的H1的跨膜结构域和胞质尾区(TMCT)融合且具有紫花苜蓿蛋白二硫键异构酶的信号肽的来自流感B/布里斯班/60/08的具有缺失的蛋白水解环(PrL-)的HA的胞外域。图38B中呈现与PDISP融合的成熟HA B布里斯班(PrL-)+H1加利福尼亚TMCT的氨基酸序列(SEQ ID NO:106)。图33C和39C中呈现质粒1067和1875的示意图。

[0485] 实施例5.20:

[0486] 用于PDISP/HA B马萨诸塞(PrL-)的2X35S/CPMV HT(构建体2072号)和HT+(构建体2052号)

[0487] 使用如实施例5.26中所描述的相同的基于PCR的方法,但是使用为PDISP/HA B马萨诸塞(PrL-)特别设计的修饰的PCR引物,将对应于来自流感B/马萨诸塞/2/2012的具有缺失的蛋白水解环(PrL-)的HA的编码序列(PDISP/HA B马萨诸塞(PrL-))(图39A,SEQ ID NO:107),克隆进原始的HT和修饰的HT+中,其中,天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替。图39B中呈现与PDISP融合的成熟HA B马萨诸塞(PrL-)的氨基酸序列(SEQ ID NO:108)。图34C和图39C中呈现质粒2072和2052的示意图。

[0488] 实施例5.21:

[0489] 用于PDISP/HA B马萨诸塞(PrL-)+H1加利福尼亚TMCT的2X35S/CPMV HT(构建体2074号)和HT+(构建体2062号)

[0490] 使用如实施例5.26中所描述的相同的基于PCR的方法，但是使用为PDISP/HA B马萨诸塞(PrL-)+H1加利福尼亚TMCT特别设计的修饰的PCR引物，将嵌合血凝素编码序列(PDISP/HA B马萨诸塞(PrL-)+H1加利福尼亚TMCT)(图40A，SEQ ID NO:109)，克隆进原始的HT和修饰的HT+中，所述嵌合血凝素编码序列对应于与来自流感A/加利福尼亚/7/2009的H1的跨膜结构域和胞质尾区(TMCT)融合且具有紫花苜蓿蛋白二硫键异构酶的信号肽的来自流感B/马萨诸塞/2/2012的具有缺失的蛋白水解环(PrL-)的HA的胞外域。图40B中呈现与PDISP融合的成熟HA B马萨诸塞(PrL-)+H1加利福尼亚TMCT的氨基酸序列(SEQ ID NO:110)。图35C和图40C中呈现质粒2074和2062的示意图。

[0491] 实施例5.22：

[0492] 用于HA B威斯康星州(PrL-)的2X35S/CPMV HT(构建体1445号)和HT+(构建体1839号)

[0493] 使用实施例5.26中所描述的相同的基于PCR的方法，但是使用为HA B威斯康星州(PrL-)特别设计的修饰的PCR引物，将对应于来自流感B/威斯康星州/1/2010的具有其天然信号肽且具有缺失的蛋白水解环(PrL-)的HA的编码序列(HA B威斯康星州(PrL-))(图41A，SEQ ID NO:111)，克隆进原始的HT和修饰的HT+中。图41B中呈现具有其天然信号肽的HA B威斯康星州(PrL-)的氨基酸序列(SEQ ID NO:112)。图36C和41C中呈现质粒1445和1839的示意图。

[0494] 实施例5.23：

[0495] 用于HA B威斯康星州(PrL-)+H1加利福尼亚TMCT的2X35S/CPMV HT(构建体1454号)和HT+(构建体1860号)

[0496] 使用实施例5.26中所描述的相同的基于PCR的方法，但是使用为HA B威斯康星州(PrL-)+H1加利福尼亚TMCT特别设计的修饰的PCR引物，将嵌合血凝素编码序列(HA B威斯康星州(PrL-)+H1加利福尼亚TMCT)(图42A，SEQ ID NO:113)，克隆进原始的HT和修饰的HT+中，所述嵌合血凝素编码序列对应于与来自流感A/加利福尼亚/7/2009的H1的跨膜结构域和胞质尾区(TMCT)融合且具有HA B威斯康星州的天然信号肽的来自流感B/威斯康星州/2/2012的具有缺失的蛋白水解环(PrL-)的HA的胞外域。图42B中呈现HA B威斯康星州(PrL-)+H1加利福尼亚TMCT的氨基酸序列(SEQ ID NO:114)。图37C和42C中呈现质粒1454和1860的示意图。

[0497] 实施例5.24

[0498] -用于H5印度尼西亚的2X35S/CPMV HT(构建体489号)、2X35S/CPMV 160+(构建体1880号)和2X35S/CPMV160(构建体1885号)

[0499] 使用如实施例5.25中所描述的相同的基于PCR的方法，但是使用为H5印度尼西亚特别设计的修饰的PCR引物，将对应于来自流感A/印度尼西亚/5/2005的天然H5的编码序列(图43A，SEQ ID NO:115)，克隆进原始的CPMV-HT、CPMV 160+和CPMV160中。图43B中呈现来自流感A/印度尼西亚/5/2005的天然H5的氨基酸序列(SEQ ID NO:116)。图43C中呈现质粒489的示意图。

[0500] 实施例5.25

[0501] -2X35S/CPMV 160+/PDISP/H3维多利亚/NOS(构建体1800号)

[0502] 使用以下基于PCR的方法，将编码来自流感A/维多利亚/361/2011的H3的序列(其

中,天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替)(PDISP/H3维多利亚),克隆进2X35S/CPMV 160+/NOS表达系统(CPMV 160+)中。使用引物IF**(SacII)-PDI.s1+4c(图44A,SEQ ID NO:117)和IF-H3V36111.s1-4r(图44B,SEQ ID NO:118),采用PDISP/H3维多利亚序列(图44C,SEQ ID NO:119)作为模板,扩增含有PDISP/H3维多利亚编码序列的片段。使用In-Fusion克隆体系(Clontech,Mountain View,CA),将PCR产物克隆进2X35S/CPMV160+/NOS表达系统中。用SacII和StuI限制性内切酶消化构建体2171号(图44D),并且线性化质粒被用于In-Fusion组装反应。构建体2171号为意图用于在基于CPMV 160+的表达盒中"In Fusion"克隆目标基因的受体质粒。它也合并了用于在紫花苜蓿质体蓝素基因启动子和终止子下TBSV P19沉默抑制子的共表达的基因构建体。骨架为pCAMBIA双元质粒,并且图44E中呈现从左至右的t-DNA边界的序列(SEQ ID NO:120)。得到的构建体被命名为1800号(图44F,SEQ ID NO:121)。图44G中呈现与PDISP融合的来自流感A/维多利亚/361/2011的成熟H3的氨基酸序列(SEQ ID NO:122)。图44H中呈现质粒1800的示意图。

[0503] 实施例5.26:

[0504] 2X35S/CPMV-HT+/PDISP/H3维多利亚/NOS(构建体1819号)

[0505] 使用以下基于PCR的方法,将编码来自流感A/维多利亚/361/2011的H3的序列(其中,天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替)(PDISP/H3维多利亚),克隆进2X35S-CPMV-HT+/NOS表达系统中。使用引物IF(SacII)-Kozac_PDI.c(图45A,SEQ ID NO:123)和IF-H3V36111.s1-4r(图45B,SEQ ID NO:124),采用PDISP/H3维多利亚序列(图44C,SEQ ID NO:119)作为模板,扩增含有PDISP/H3维多利亚编码序列的片段。使用In-Fusion克隆体系(Clontech,Mountain View,CA),将PCR产物克隆进2X35S/CPMV-HT+/NOS表达系统中。用SacII和StuI限制性内切酶消化构建体2181号(图45D),并且线性化质粒被用于In-Fusion组装反应。构建体2181号为意图用于在基于CPMV-HT+的表达盒中"In Fusion"克隆目标基因的受体质粒。它也合并了用于在紫花苜蓿质体蓝素基因启动子和终止子下TBSV P19沉默抑制子的共表达的基因构建体。骨架为pCAMBIA双元质粒,并且图45E中呈现从左至右的t-DNA边界的序列(SEQ ID NO:125)。得到的构建体被命名为1819号(图45E,SEQ ID NO:126)。图44G中呈现与PDISP融合的来自流感A/维多利亚/361/2011的成熟H3的氨基酸序列(SEQ ID NO:122)。图45F中呈现质粒1819的示意图。

[0506] 实施例5.27

[0507] 2X35S/CPMV HT+/PDISP/H2新加坡/NOS(构建体2220号)

[0508] 使用以下基于PCR的方法,将编码来自流感A/新加坡/1/1957的H2的序列(其中,天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替)(PDISP/H2新加坡),克隆进2X35S/CPMV HT+/NOS表达系统中。使用引物IF(SacII)-Kozac_PDI.c(如用于实施例5.26中构建体1819所描述的)和IF**-H2S157.s1-6r(图48A,SEQ ID NO:127),采用PDISP/H2新加坡序列(图48B,SEQ ID NO:128)作为模板,扩增含有PDISP/H2新加坡编码序列的片段。使用In-Fusion克隆体系(Clontech,Mountain View,CA),将PCR产物克隆进2X35S/CPMV HT+/NOS表达系统中。用SacII和StuI限制性内切酶消化构建体2181号(如用于实施例5.26中构建体1819所描述的),并且线性化质粒被用于In-Fusion组装反应。构建体2181号为意图用于在基于CPMV HT+的表达盒中"In Fusion"克隆目标基因的受体质粒。它也合并了用于在紫花苜蓿质体蓝素基因启动子和终止子下TBSV P19沉默抑制子的共表达的基因构建体。骨架为

pCAMBIA双元质粒，并且图45D中呈现从左至右的t-DNA边界的序列。得到的构建体被命名为2220号(图48C，SEQ ID NO:129)。图48D中呈现与PDISP融合的来自流感A/新加坡/1/1957的成熟H2的氨基酸序列(SEQ ID NO:130)。图48E中呈现质粒2220的示意图。

[0509] 实施例5.28

[0510] 2X35S/CPMV HT+/具有缺失的蛋白水解环的PDISP/H2新加坡/NOS(构建体2221号)

[0511] 使用由Darveau等(Methods in Neuroscience 26:77-85(1995))所呈现的以下基于PCR的连接方法，将编码来自流感A/新加坡/1/1957的具有缺失的蛋白水解环的H2的序列(其中，天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替)(具有缺失的蛋白水解环的PDISP/H2新加坡)，克隆进2X35S/CPMV HT+/NOS表达系统中。在第一轮PCR中，使用引物IF(SacII)-Kozac_PDI.c(如用于实施例5.26中构建体1819所描述的)和H2S157(Prl-).r(图49A，SEQ ID NO:131)，采用PDISP/H2新加坡序列(图48B，SEQ ID NO:128)作为模板，扩增含有来自流感A/新加坡/1/1957的H2编码序列的nt 1至nt 1032的片段。使用引物H2S157(Prl-).c(图49B，SEQ ID NO:132)和IF**-H2S157.s1-6r(图48A，SEQ ID NO:127)，采用PDISP/H2新加坡序列(图48B，SEQ ID NO:128)作为模板，扩增含有来自流感A/新加坡/1/1957的H2编码序列的nt 1084至nt 1716的第二片段。然后将来自两次扩增的PCR产物混合并且用作模板，用于使用IF(SacII)-Kozac_PDI.c(如用于实施例5.26中构建体1819所描述的)和IF**-H2S157.s1-6r(图48A，SEQ ID NO:127)作为引物的第二轮扩增。使用In-Fusion克隆体系(Clontech，Mountain View，CA)，将PCR产物(包含aa 321至337被GG接头代替的PDISP/H2新加坡编码序列)，克隆进2X35S/CPMV HT+/NOS表达系统中。用SacII和StuI限制性内切酶消化构建体2181号(如用于实施例5.26中构建体1819所描述的)，并且线性化质粒被用于In-Fusion组装反应。构建体2181号为意图用于在基于CPMV HT+的表达盒中“In Fusion”克隆目标基因的受体质粒。它也合并了用于在紫花苜蓿质体蓝素基因启动子和终止子下TBSV P19沉默抑制子的共表达的基因构建体。骨架为pCAMBIA双元质粒，并且图45D中呈现从左至右的t-DNA边界的序列(如用于实施例5.26中的构建体1819所描述的)。得到的构建体被命名为2221号(图49C，SEQ ID NO:133)。图49D中呈现与PDISP融合的来自流感A/新加坡/1/1957的具有缺失的蛋白水解环的成熟H2的氨基酸序列(SEQ ID NO:134)。图49E中呈现质粒2221的示意图。

[0512] 实施例5.29

[0513] 在2X35S/CPMV 160+/NOS表达系统中PDISP/H2新加坡(构建体2222号)和具有缺失的蛋白水解环的PDISP/H2新加坡(构建体2223号)

[0514] 分别使用如构建体2220和2221的相同的基于PCR的方法，但使用用于扩增的修饰的正向引物IF**(SacII)-PDI.s1+4c(如用于实施例5.25中构建体1800所描述的)和不同的受体质粒，将编码来自流感A/新加坡/1/1957的具有或没有蛋白水解环的H2的序列(其中，天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替)(PDISP/H2新加坡和具有缺失的蛋白水解环的PDISP/H2新加坡)，克隆进2X35S/CPMV160+/NOS表达系统中。使用In-Fusion克隆体系(Clontech，Mountain View，CA)，将得到的PCR产物克隆进2X35S/CPMV 160+/NOS表达系统中。用SacII和StuI限制性内切酶消化构建体2171号(如用于实施例5.25中构建体1800所描述的)，并且线性化质粒被用于In-Fusion组装反应。构建体2171号为意图用于在基于CPMV 160+的表达盒中“In Fusion”克隆目标基因的受体质粒。它也合并了用于在紫花

苜蓿质体蓝素基因启动子和终止子下TBSV P19沉默抑制子的共表达的基因构建体。骨架为pCAMBIA双元质粒，并且图中呈现从左至右的t-DNA边界的序列(如用于实施例5.25中构建体1800所描述的)。得到的构建体被命名为2222号用于PDISP/H2新加坡(图50A，SEQ ID NO:135)，和2223号用于具有缺失的蛋白水解环的PDISP/H2新加坡(图50B，SEQ ID NO:136)。图50C和50D中分别呈现质粒2222和2223的示意图。

[0515] 实施例5.30

[0516] 用于PDISP/H3珀斯的2X35S/CPMV HT+(构建体号2019)和160+(构建体2139号)

[0517] 分别使用如构建体2220和2222的相同的基于Fusion的方法，但是用为PDISP/H3珀斯特别设计的修饰的PCR引物(图51B，SEQ ID NO:138)，将对应于来自流感A/珀斯/16/2009的H3的编码序列(其中，天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替)(PDISP/H3珀斯)(图51A，SEQ ID NO:137)，克隆进修饰的CPMV HT+和160+中。图51C中呈现与PDISP融合的来自流感A/珀斯/16/2009的成熟H3的氨基酸序列(SEQ ID NO:139)。图51D和图51E中呈现质粒2019和2139的示意图。

[0518] 实施例5.31

[0519] 用于具有缺失的蛋白水解环的PDISP/H3珀斯的2X35S/CPMV HT+(构建体2039号)和160+(构建体2159号)

[0520] 分别使用如构建体2221和2223的相同的基于Fusion方法，但是使用为具有缺失的蛋白水解环的PDISP/H3珀斯特别设计的修饰的PCR引物(图51B(SEQ ID NO:138)、52B(SEQ ID NO:141)和53C(SEQ ID NO:142))，将对应于来自流感A/珀斯/16/2009的具有缺失的蛋白水解环的H3的编码序列(其中，天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替)(具有缺失的蛋白水解环的PDISP/H3珀斯)(图52，SEQ ID NO:140)，克隆进修饰的CPMV HT+和160+中。图52D中呈现与PDISP融合的来自流感A/珀斯/16/2009的具有缺失的蛋白水解环的成熟H3的氨基酸序列(SEQ ID NO:143)。图52E和图52F中呈现质粒2039和2159的示意图。

[0521] 实施例5.32

[0522] 用于具有缺失的蛋白水解环的PDISP/H3维多利亚的2X35S/CPMV HT+(构建体2230号)和160+(构建体2250号)

[0523] 分别使用如构建体2221和2223的相同的基于Fusion方法(参见实施例5.28和5.29)，但使用为具有缺失的蛋白水解环的PDISP/H3维多利亚特别设计的修饰的PCR引物(图53B(SEQ ID NO:145)和53C(SEQ ID NO:146))，将对应于来自流感A/维多利亚/361/2011的具有缺失的蛋白水解环的H3的编码序列(其中，天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替)(具有缺失的蛋白水解环的PDISP/H3维多利亚)(图53，SEQ ID NO:144)，克隆进修饰的CPMV HT+和160+中。图53D中呈现与PDISP融合的来自流感A/维多利亚/361/2011的具有缺失的蛋白水解环的成熟H3的氨基酸序列(SEQ ID NO:147)。图53E和图53F中呈现质粒2230和2250的示意图。

[0524] 实施例5.33

[0525] 2X35S/CPMV HT+/PDISP/H7杭州/NOS(构建体2142号)

[0526] 使用如构建体2220的相同的基于Fusion的方法，但使用为PDISP/H7杭州特别设计的修饰的PCR引物(图54B，SEQ ID NO:149)，将对应于来自流感A/杭州/1/2013的H7的编码序列(其中，天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替)(PDISP/H7杭州)(图

54A,SEQ ID NO:148),克隆进修饰的CPMV HT+中。图54C中呈现与PDISP融合的来自流感A/杭州/1/2013的成熟H7的氨基酸序列(SEQ ID NO:150)。图54E中呈现质粒2142的示意图。

[0527] 实施例5.34

[0528] 2X35S/CPMV HT+/具有缺失的蛋白水解环的PDISP/H7杭州/NOS(构建体2152号)

[0529] 使用如构建体2221的相同的基于Fusion的方法,但是使用为具有缺失的蛋白水解环的PDISP/H7杭州特别设计的修饰的PCR引物(图54B(SEQ ID NO:149)、图55B(SEQ ID NO:152)和图55C(SEQ ID NO:153)),将对应于来自流感A/杭州/1/2013的具有缺失的蛋白水解环的H7的编码序列(其中,天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替)(具有缺失的蛋白水解环的PDISP/H7杭州)(图55A,SEQ ID NO:151),克隆进修饰的CPMV HT+中。图55D中呈现与PDISP融合的来自流感A/杭州/1/2013的具有缺失的蛋白水解环的成熟H7的氨基酸序列(SEQ ID NO:154)。图55E中呈现质粒2152的示意图。

[0530] 实施例5.35

[0531] 用于PDISP/H9香港的2X35S/CPMV HT+(构建体2224号)和160+(构建体2226号)

[0532] 分别使用如构建体2220和2222的相同的基于Fusion的方法,但是使用为PDISP/H9香港特别设计的修饰的PCR引物(图56B,SEQ ID NO:156),将对应于来自流感A/香港/1073/1999的H9的编码序列(其中,天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替)(PDISP/H9香港)(图56A,SEQ ID NO:155),克隆进修饰的CPMV HT+和160+中。图56C中呈现与PDISP融合的来自流感A/香港/1073/1999的成熟H9的氨基酸序列(SEQ ID NO:157)。图56D和图56E中呈现质粒2224和2226的示意图。

[0533] 实施例5.36

[0534] 用于具有缺失的蛋白水解环的PDISP/H9香港的2X35S/CPMV HT+(构建体2225号)和160+(构建体2227号)

[0535] 分别使用如构建体2221和2223的相同的基于Fusion方法,但是使用为具有缺失的蛋白水解环的PDISP/H9香港特别设计的修饰的PCR引物(图56B(SEQ ID NO:156)、图57B(SEQ ID NO:159)和图57C(SEQ ID NO:160)),将对应于来自流感A/香港/1073/1999的具有缺失的蛋白水解环的H9的编码序列(其中,天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替)(具有缺失的蛋白水解环的PDISP/H9香港)(图57A,SEQ ID NO:158),克隆进修饰的CPMV HT+和160+中。图57D中呈现与PDISP融合的来自流感A/香港/1073/1999的具有缺失的蛋白水解环的成熟H9的氨基酸序列(SEQ ID NO:161)。图57E和图57F中呈现质粒2225和2227的示意图。

[0536] 实施例5.37

[0537] 2X35S/CPMV 160+/PDISP/HA B马来西亚/NOS(构建体2013号)

[0538] 使用如构建体2222的相同的基于Fusion的方法,但是用为PDISP/HA B马来西亚特别设计的修饰的PCR引物(图58B,SEQ ID NO:163),将对应于来自流感B/马来西亚/2506/2004的HA的编码序列(其中,天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替)(PDISP/HA B马来西亚)(图58A,SEQ ID NO:162),克隆进修饰的CPMV 160+中。图58C中呈现与PDISP融合的来自流感B/马来西亚/2506/2004的成熟HA的氨基酸序列(SEQ ID NO:164)。图58D中呈现质粒2013的示意图。

[0539] 实施例5.38

[0540] 2X35S/CPMV 160+/具有缺失的蛋白水解环的PDISP/HA B马来西亚/NOS(构建体2014号)

[0541] 使用如构建体2223的相同的基于Fusion的方法,但是使用为具有缺失的蛋白水解环的PDISP/HA B马来西亚特别设计的修饰的PCR引物(图58B(SEQ ID NO:163)、图59B(SEQ ID NO:166)、图59C(SEQ ID NO:167),将对应于来自流感B/马来西亚/2506/2004的具有缺失的蛋白水解环的HA的编码序列(其中,天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替)(具有缺失的蛋白水解环的PDISP/HA B马来西亚)(图59A,SEQ ID NO:165),克隆进修饰的CPMV 160+中。图59D中呈现与PDISP融合的来自流感B/马来西亚/2506/2004的具有缺失的蛋白水解环的成熟HA的氨基酸序列(SEQ ID NO:168)。图59E中呈现质粒2014的示意图。

[0542] 实施例5.39

[0543] 用于PDISP/HA B马萨诸塞州的2X35S/CPMV HT(构建体2070号)、HT+(构建体2080号)和160+(-Mprot)(构建体2090号)

[0544] 分别使用如构建体2072、2220和2222的相同的基于Fusion的方法,但是使用为PDISP/HA B马萨诸塞州特别设计的修饰的PCR引物,将对应于来自流感B/马萨诸塞州/2/2012的HA的编码序列(其中,天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替)(PDISP/HA B马萨诸塞州)(图60A,SEQ ID NO:169),克隆进原始的HT、修饰的HT+和160+中。图60B中呈现与PDISP融合的来自流感B/马萨诸塞州/2/2012的成熟HA的氨基酸序列(SEQ ID NO:170)。图60C、图60D和图60E中呈现质粒2070、2080和2090的示意图。

[0545] 实施例5.40

[0546] 用于具有缺失的蛋白水解环的PDISP/HA B弗罗里达的2X35S/CPMV HT+(构建体2102号)、具有BeYDV的HT+(构建体2104号)

[0547] 如上所述的相同的基于Fusion的方法,但是使用为PDISP/HA B/弗罗里达特别设计的修饰的PCR引物(参见图61A(SEQ ID No:190)、图61B(SEQ ID NO:191)和图61C(SEQ ID NO:192),将对应于来自具有缺失的蛋白水解环的流感B/弗罗里达并且其中天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替的HA的编码序列(PDISP/HA B弗罗里达)(图61D,SEQ ID NO:193),克隆进修饰的HT+中。图61F中给出得到的表达盒2102的核苷酸序列(SEQ ID NO:195)。同样地,将对应于来自具有缺失的蛋白水解环的流感B/弗罗里达并且其中天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替的HA的编码序列,克隆进连有扩增元件BeYDV的修饰的HT+中。图61F中给出得到的表达盒2104的核苷酸序列(SEQ ID NO:196)。图61E中呈现与PDISP融合的来自具有缺失的蛋白水解环的流感B/弗罗里达的成熟HA的氨基酸序列(SEQ ID NO:194)。图61G和61I中呈现质粒2102和2104的示意图。

[0548] 实施例5.41

[0549] 用于具有缺失的蛋白水解环的PDISP/HA B弗罗里达+H1加利福尼亚TMCT的2X35S/CPMV HT+(构建体2106号)、具有BeYDV的HT+(构建体2108号)

[0550] 如上所述的相同的基于Fusion的方法,但是使用为PDISP/HA B/弗罗里达+H1加利福尼亚TMCT特别设计的修饰的PCR引物(参见图61A(SEQ ID No:197)),将对应于来自具有缺失的蛋白水解环的流感B/弗罗里达+H1加利福尼亚TMCT并且其中天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替的HA的编码序列(PDISP/HA B弗罗里达+H1加利福尼亚

TMCT)(图62B,SEQ ID NO:198),克隆进修饰的HT+中。图62D中给出得到的表达盒2106的核苷酸序列(SEQ ID NO:200)。同样地,将对应于来自具有缺失的蛋白水解环的流感B/弗罗里达+H1加利福尼亚TMCT并且其中天然信号肽被紫花苜蓿蛋白二硫键异构酶的信号肽代替的HA的编码序列,克隆进加有扩增元件BeYDV的修饰的HT+中。图62F中给出得到的表达盒2108的核苷酸序列(SEQ ID NO:201)。图62C中呈现与PDISP融合的来自具有缺失的蛋白水解环的流感B/弗罗里达+H1加利福尼亚TMCT的成熟HA的氨基酸序列(SEQ ID NO:199)。图62E和62G中呈现质粒2106和2108的示意图。

[0551] 所有引文通过引用据此并入。

[0552] 已结合一个或多个实施方案描述了本发明。然而,对本领域技术人员显而易见的是,在不脱离权利要求书所定义的本发明的范围下可做出多种改变和修饰。

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Pro Met Cys Asp Glu Phe Ile Asn Val Pro Glu Trp Ser Tyr Ile Val
85 90 95
Glu Lys Ala Asn Pro Thr Asn Asp Leu Cys Tyr Pro Gly Ser Phe Asn
100 105 110
Asp Tyr Glu Glu Leu Lys His Leu Leu Ser Arg Ile Asn His Phe Glu
115 120 125
Lys Ile Gln Ile Ile Pro Lys Ser Ser Trp Ser Asp His Glu Ala Ser
130 135 140
Ser Gly Val Ser Ser Ala Cys Pro Tyr Leu Gly Ser Pro Ser Phe Phe
145 150 155 160
Arg Asn Val Val Trp Leu Ile Lys Lys Asn Ser Thr Tyr Pro Thr Ile
165 170 175

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

485 490 495
Ser Ile Arg Asn Gly Thr Tyr Asn Tyr Pro Gln Tyr Ser Glu Glu Ala
500 505 510
Arg Leu Lys Arg Glu Glu Ile Ser Gly Val Lys Leu Glu Ser Ile Gly
515 520 525
Thr Tyr Gln Ile Leu Ser Ile Tyr Ser Thr Val Ala Ser Ser Leu Ala
530 535 540
Leu Ala Ile Met Met Ala Gly Leu Ser Leu Trp Met Cys Ser Asn Gly
545 550 555 560
Ser Leu Gln Cys Arg Ile Cys Ile
565

<210> 7
<211> 42
<212> DNA
<213> 人工序列(Artificial Sequence)
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<222>
<223> 引物IF-S1-M1+M2ANC.c
<400> 7
aaatttgtcg ggcccatgag tcttctaacc gaggtcgaaa cg 42
<210> 8
<211> 43
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 引物IF-S1-4-M2ANC.r
<400> 8
actaaagaaa ataggccttt actccagctc tatgctgaca aaa 43
<210> 9
<211> 294
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 合成的M2基因

<400> 9
atgagtcttc taaccgaggt cgaaacgcct atcagaaacg aatgggggtg cagatgcaac 60
gattcaagtg atcctcttgt tgttgccgca agtataattg ggattgtgca cctgatattg 120
tggattattg atcgcctttt ttccaaaagc atttatcgta tctttaaaca cggtttaaaa 180
agagggcctt ctacggaagg agtaccagag tctatgaggg aagaatatcg agaggaacag 240
cagaatgctg tggatgctga cgatggtcat tttgtcagca tagagctgga gtaa 294
<210> 10
<211> 2034
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 盒1261
<400> 10
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gaagaccaaa gggcaattga gacttttcaa caaagggtaa tatccggaaa cctcctcgga 120
ttccattgcc cagctatctg tcactttatt gtgaagatag tggaaaagga aggtggctcc 180
tacaaatgcc atcattgcga taaaggaaag gccatcgttg aagatgcctc tgccgacagt 240
ggtcccaaag atggaccccc acccacgagg agcatcgtgg aaaaagaaga cgttccaacc 300
acgtcttcaa agcaagtgga ttgatgtgat aacatggtgg agcacgacac acttgtctac 360
tccaaaaata tcaaagatac agtctcagaa gaccaaaggg caattgagac ttttcaacaa 420
agggtaatat ccggaaacct cctcggattc cattgcccag ctatctgtca ctttattgtg 480
aagatagtgg aaaaggaagg tggctcctac aaatgccatc attgcgataa aggaaaggcc 540
atcgttgaag atgcctctgc cgacagtggt cccaaagatg gacccccacc cacgaggagc 600
atcgtggaaa aagaagacgt tccaaccacg tcttcaaagc aagtggattg atgtgatatc 660
tccactgacg taagggatga cgcacaatcc cactatcctt cgcaagaccc ttcctctata 720
taaggaagtt catttcattt ggagaggtat taaaatctta ataggttttg ataaaagcga 780
acgtggggaa acccgaacca aaccttcttc taaactctct ctcatctctc ttaaagcaaa 840
cttctctctt gtctttcttg cgtgagcgat cttcaacgtt gtcagatcgt gcttcggcac 900
cagtacaacg ttttctttca ctgaagcgaa atcaaagatc tctttgtgga cacgtagtgc 960
ggcgccatta ataacgtgt acttgtccta ttcttgtcgg tgtggtcttg ggaaaagaaa 1020
gcttgctgga ggctgctgtt cagccccata cattacttgt tacgattctg ctgactttcg 1080
gcgggtgcaa tatctctact tctgcttgac gaggtattgt tgcctgtact tctttcttct 1140
tcttcttgct gattggttct ataagaaatc tagtattttc tttgaaacag agttttcccg 1200
tggttttcga acttggagaa agattgttaa gcttctgtat attctgccca aatttgtcgg 1260
gcccatgagt cttctaaccg aggtcgaaac gcctatcaga aacgaatggg ggtgcagatg 1320
caacgattca agtgatcctc ttgttgttgc cgcaagtata attgggattg tgcacctgat 1380
attgtggatt attgatcgcc ttttttccaa aagcatttat cgtatcttta aacacggttt 1440

aaaaagaggg ccttctacgg aaggagtacc agagtctatg agggaagaat atcgagagga 1500
acagcagaat gctgtggatg ctgacgatgg tcattttgtc agcatagagc tggagtaaag 1560
gcctattttc tttagtttga atttactgtt attcggtgtg catttctatg tttggtgagc 1620
ggttttctgt gctcagagtg tgtttatttt atgtaattta atttctttgt gagctcctgt 1680
ttagcaggtc gtcccttcag caaggacaca aaaagatttt aattttatta aaaaaaaaaa 1740
aaaaaaagac cgggaattcg atatcaagct tatcgacctg cagatcgttc aaacatttgg 1800
caataaagtt tcttaagatt gaatcctgtt gccggtcttg cgatgattat catataattt 1860
ctgttgaatt acgttaagca tgtaataatt aacatgtaat gcatgacgtt atttatgaga 1920
tgggttttta tgattagagt cccgcaatta tacatttaat acgcgataga aaacaaaata 1980
tagcgcgcaa actaggataa attatcgcgc gcggtgtcat ctatgttact agat 2034

<210> 11
<211> 97
<212> PRT
<213> 流感M2新喀里多尼亚/20/1999(H1N1)(Influenza M2 New Caledonia/20/1999 (H1N1))
<400> 11

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

<210> 12
<211> 294
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 合成的M2基因
<400> 12

atgagtcttc taaccgaggt cgaaacgcct atcagaaacg aatgggggtg cagatgcaac 60

ggttcaagtg atcctctcac tattgccgca aatatcattg ggatcttgca cttgacattg 120
tggattcttg atcgtctttt tttcaaatgc atttaccgtc gctttaaata cggactgaaa 180
ggagggcctt ctacggaagg agtgccaaag tctatgaggg aagaatatcg aaaggaacag 240
cagagtgctg tggatgctga cgatggtcat tttgtcagca tagagctgga gtaa 294

<210> 13
<211> 2034
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 盒859
<400> 13

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gaagaccaaa gggcaattga gacttttcaa caaagggtaa tatccggaaa cctcctcgga 120
ttccattgcc cagctatctg tcactttatt gtgaagatag tggaaaagga aggtggctcc 180
tacaaatgcc atcattgcga taaaggaaag gccatcgttg aagatgcctc tgccgacagt 240
ggtcccaaag atggaccccc acccacgagg agcatcgtgg aaaaagaaga cgttccaacc 300
acgtcttcaa agcaagtgga ttgatgtgat aacatggtgg agcacgacac acttgtctac 360
tccaaaaata tcaaagatac agtctcagaa gaccaaaggg caattgagac ttttcaacaa 420
agggtaatat ccggaaacct cctcggattc cattgcccag ctatctgtca ctttattgtg 480
aagatagtgg aaaaggaagg tggctcctac aaatgccatc attgcgataa aggaaaggcc 540
atcgttgaag atgcctctgc cgacagtggt cccaaagatg gacccccacc cacgaggagc 600
atcgtggaaa aagaagacgt tccaaccacg tcttcaaagc aagtggattg atgtgatatc 660
tccactgacg taagggatga cgcacaatcc cactatcctt cgcaagaccc ttcctctata 720
taaggaagtt catttcattt ggagaggtat taaaatctta ataggttttg ataaaagcga 780
acgtggggaa acccgaacca aaccttcttc taaactctct ctcatctctc ttaaagcaaa 840
cttctctctt gtctttcttg cgtgagcgat cttcaacgtt gtcagatcgt gcttcggcac 900
cagtacaacg ttttctttca ctgaagcgaa atcaaagatc tctttgtgga cacgtagtgc 960
ggcgccatta aataacgtgt acttgtccta ttcttgtcgg tgtggtcttg ggaaaagaaa 1020
gcttgctgga ggctgctgtt cagccccata cattacttgt tacgattctg ctgactttcg 1080
gcgggtgcaa tatctctact tctgcttgac gaggtattgt tgcctgtact tctttcttct 1140
tcttcttgct gattggttct ataagaaatc tagtattttc tttgaaacag agttttcccg 1200
tggttttcga acttggagaa agattgttaa gcttctgtat attctgccca aatttgtcgg 1260
gcccatgagt cttctaaccg aggtcgaaac gcctatcaga aacgaatggg ggtgcagatg 1320
caacggttca agtgatcctc tcactattgc cgcaaatatc attgggatct tgcacttgac 1380
attgtggatt cttgatcgtc tttttttcaa atgcatttac cgtcgcttta aatacggact 1440
gaaaggaggg ccttctacgg aaggagtgcc aaagtctatg agggaagaat atcgaaagga 1500
acagcagagt gctgtggatg ctgacgatgg tcattttgtc agcatagagc tggagtaaag 1560

gcctattttc tttagtttga atttactgtt attcggtgtg catttctatg tttggtgagc 1620
ggttttctgt gctcagagtg tgtttatttt atgtaattta atttctttgt gagctcctgt 1680
ttagcaggtc gtcccttcag caaggacaca aaaagatttt aattttatta aaaaaaaaaa 1740
aaaaaaagac cgggaattcg atatcaagct tatcgacctg cagatcgttc aaacatttgg 1800
caataaagtt tcttaagatt gaatcctgtt gccggtcttg cgatgattat catataattt 1860
ctgttgaatt acgttaagca tgtaataatt aacatgtaat gcatgacgtt atttatgaga 1920
tgggttttta tgattagagt cccgcaatta tacatttaat acgcgataga aaacaaaata 1980
tagcgcgcaa actaggataa attatcgcgc gcggtgtcat ctatgttact agat 2034

<210> 14
<211> 97
<212> PRT
<213> 流感M2 A/波多黎各/8/1934(H1N1)(Influenza M2 A/Puerto Rico/8/1934 (H1N1))
<400> 14

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

<210> 15
<211> 3474
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 盒1039
<400> 15

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gaagaccaaa gggcaattga gacttttcaa caaagggtaa tatccggaaa cctcctcgga 120
ttccattgcc cagctatctg tcactttatt gtgaagatag tggaaaagga aggtggctcc 180

tacaaatgcc atcattgcga taaaggaaag gccatcgttg aagatgcctc tgccgacagt 240
ggtcccaaag atggaccccc acccacgagg agcatcgtgg aaaaagaaga cgttccaacc 300
acgtcttcaa agcaagtgga ttgatgtgat aacatggtgg agcacgacac acttgtctac 360
tccaaaaata tcaaagatac agtctcagaa gaccaaaggg caattgagac ttttcaacaa 420
agggtaatat ccggaaacct cctcggattc cattgcccag ctatctgtca ctttattgtg 480
aagatagtgg aaaaggaagg tggctcctac aaatgccatc attgcgataa aggaaaggcc 540
atcgttgaag atgcctctgc cgacagtggt cccaaagatg gacccccacc cacgaggagc 600
atcgtggaaa aagaagacgt tccaaccacg tcttcaaagc aagtggattg atgtgatatc 660
tccactgacg taagggatga cgcacaatcc cactatcctt cgcaagaccc ttcctctata 720
taaggaagtt catttcattt ggagaggtat taaaatctta ataggttttg ataaaagcga 780
acgtggggaa acccgaacca aaccttcttc taaactctct ctcatctctc ttaaagcaaa 840
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cagtacaacg ttttctttca ctgaagcgaa atcaaagatc tctttgtgga cacgtagtgc 960
ggcgccatta aataacgtgt acttgtccta ttcttgtcgg tgtggtcttg ggaaaagaaa 1020
gcttgctgga ggctgctgtt cagccccata cattacttgt tacgattctg ctgactttcg 1080
gcgggtgcaa tatctctact tctgcttgac gaggtattgt tgcctgtact tctttcttct 1140
tcttcttgct gattggttct ataagaaatc tagtattttc tttgaaacag agttttcccg 1200
tggttttcga acttggagaa agattgttaa gcttctgtat attctgccca aatttgtcgg 1260
gcccatggcg aaaaacgttg cgattttcgg cttattgttt tctcttcttg tgttggttcc 1320
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aatggcttgg gccgtcccaa aaaacgacaa aaacaaaaca gcaacaaatc cattaacaat 1860
agaagtacca tacatttgta cagaaggaga agaccaaatt accgtttggg ggttccactc 1920
tgacaacgag acccaaatgg caaagctcta tggggactca aagccccaga agttcacctc 1980
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tgggaaaaca ggaacaatta cctatcaaag gggtatttta ttgcctcaaa aggtgtggtg 2160
cgcaagtggc aggagcaagg taataaaagg atccttgcct ttaattggag aagcagattg 2220
cctccacgaa aaatacggtg gattaaacaa aagcaagcct tactacacag gggaacatgc 2280
aaaggccata ggaaattgcc caatatgggt gaaaacaccc ttgaagctgg ccaatggaac 2340
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aaacaagata acaaaaaatc tcaactcttt gagtgagctg gaagtaaaga atcttcaaag 2520

actaagcggt gccatggatg aactccacaa cgaaatacta gaactagatg agaaagtgga 2580
tgatctcaga gctgatacaa taagctcaca aatagaactc gcagtcctgc tttccaatga 2640
aggaataata aacagtgaag atgaacatct cttggcgctt gaaagaaagc tgaagaaaat 2700
gctgggcccc tctgctgtag agatagggaa tggatgcttt gaaaccaaac acaagtgcaa 2760
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ggttttctgt gctcagagtg tgtttatttt atgtaattta atttctttgt gagctcctgt 3120
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aaaaaaagac cgggaattcg atatcaagct tatcgacctg cagatcgttc aaacatttgg 3240
caataaagtt tcttaagatt gaatcctgtt gccggtcttg cgatgattat catataattt 3300
ctgttgaatt acgttaagca tgtaataatt aacatgtaat gcatgacgtt atttatgaga 3360
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<210> 16
<211> 570
<212> PRT
<213> 流感B/布里斯班/60/2008(Influenza B/Brisbane/60/2008)
<400> 16

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

Asn Ile Thr Asn Gly Asn Gly Phe Phe Ala Thr Met Ala Trp Ala Val
145 150 155 160
Pro Lys Asn Asp Lys Asn Lys Thr Ala Thr Asn Pro Leu Thr Ile Glu
165 170 175
Val Pro Tyr Ile Cys Thr Glu Gly Glu Asp Gln Ile Thr Val Trp Gly
180 185 190
Phe His Ser Asp Asn Glu Thr Gln Met Ala Lys Leu Tyr Gly Asp Ser
195 200 205
Lys Pro Gln Lys Phe Thr Ser Ser Ala Asn Gly Val Thr Thr His Tyr
210 215 220
Val Ser Gln Ile Gly Gly Phe Pro Asn Gln Thr Glu Asp Gly Gly Leu
225 230 235 240
Pro Gln Ser Gly Arg Ile Val Val Asp Tyr Met Val Gln Lys Ser Gly
245 250 255
Lys Thr Gly Thr Ile Thr Tyr Gln Arg Gly Ile Leu Leu Pro Gln Lys
260 265 270
Val Trp Cys Ala Ser Gly Arg Ser Lys Val Ile Lys Gly Ser Leu Pro
275 280 285
Leu Ile Gly Glu Ala Asp Cys Leu His Glu Lys Tyr Gly Gly Leu Asn
290 295 300
Lys Ser Lys Pro Tyr Tyr Thr Gly Glu His Ala Lys Ala Ile Gly Asn
305 310 315 320
Cys Pro Ile Trp Val Lys Thr Pro Leu Lys Leu Ala Asn Gly Thr Lys
325 330 335
Tyr Arg Pro Pro Ala Lys Leu Leu Lys Glu Arg Gly Phe Phe Gly Ala
340 345 350
Ile Ala Gly Phe Leu Glu Gly Gly Trp Glu Gly Met Ile Ala Gly Trp
355 360 365
His Gly Tyr Thr Ser His Gly Ala His Gly Val Ala Val Ala Ala Asp
370 375 380
Leu Lys Ser Thr Gln Glu Ala Ile Asn Lys Ile Thr Lys Asn Leu Asn
385 390 395 400
Ser Leu Ser Glu Leu Glu Val Lys Asn Leu Gln Arg Leu Ser Gly Ala
405 410 415
Met Asp Glu Leu His Asn Glu Ile Leu Glu Leu Asp Glu Lys Val Asp
420 425 430
Asp Leu Arg Ala Asp Thr Ile Ser Ser Gln Ile Glu Leu Ala Val Leu
435 440 445
Leu Ser Asn Glu Gly Ile Ile Asn Ser Glu Asp Glu His Leu Leu Ala

450 455 460
Leu Glu Arg Lys Leu Lys Lys Met Leu Gly Pro Ser Ala Val Glu Ile
465 470 475 480
Gly Asn Gly Cys Phe Glu Thr Lys His Lys Cys Asn Gln Thr Cys Leu
485 490 495
Asp Arg Ile Ala Ala Gly Thr Phe Asp Ala Gly Glu Phe Ser Leu Pro
500 505 510
Thr Phe Asp Ser Leu Asn Ile Thr Ala Ala Ser Leu Asn Asp Asp Gly
515 520 525
Leu Asp Asn His Thr Ile Leu Leu Tyr Tyr Ser Thr Ala Ala Ser Ser
530 535 540
Leu Ala Val Thr Leu Met Ile Ala Ile Phe Val Val Tyr Met Val Ser
545 550 555 560
Arg Asp Asn Val Ser Cys Ser Ile Cys Leu
565 570

<210> 17
<211> 554
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> B型HA的δ蛋白水解环(具有接头GG)的氨基酸序列
<400> 17
Asp Arg Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His Val Val
1 5 10 15
Lys Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Val Ile Pro Leu
20 25 30
Thr Thr Thr Pro Thr Lys Ser His Phe Ala Asn Leu Lys Gly Thr Glu
35 40 45
Thr Arg Gly Lys Leu Cys Pro Lys Cys Leu Asn Cys Thr Asp Leu Asp
50 55 60
Val Ala Leu Gly Arg Pro Lys Cys Thr Gly Lys Ile Pro Ser Ala Arg
65 70 75 80
Val Ser Ile Leu His Glu Val Arg Pro Val Thr Ser Gly Cys Phe Pro
85 90 95
Ile Met His Asp Arg Thr Lys Ile Arg Gln Leu Pro Asn Leu Leu Arg
100 105 110
Gly Tyr Glu His Ile Arg Leu Ser Thr His Asn Val Ile Asn Ala Glu

115 120 125

Asn Ala Pro Gly Gly Pro Tyr Lys Ile Gly Thr Ser Gly Ser Cys Pro

130 135 140

Asn Ile Thr Asn Gly Asn Gly Phe Phe Ala Thr Met Ala Trp Ala Val

145 150 155 160

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

165 170 175

Val Pro Tyr Ile Cys Thr Glu Gly Glu Asp Gln Ile Thr Val Trp Gly

180 185 190

Phe His Ser Asp Asn Glu Thr Gln Met Ala Lys Leu Tyr Gly Asp Ser

195 200 205

Lys Pro Gln Lys Phe Thr Ser Ser Ala Asn Gly Val Thr Thr His Tyr

210 215 220

Val Ser Gln Ile Gly Gly Phe Pro Asn Gln Thr Glu Asp Gly Gly Leu

225 230 235 240

Pro Gln Ser Gly Arg Ile Val Val Asp Tyr Met Val Gln Lys Ser Gly

245 250 255

Lys Thr Gly Thr Ile Thr Tyr Gln Arg Gly Ile Leu Leu Pro Gln Lys

260 265 270

Val Trp Cys Ala Ser Gly Arg Ser Lys Val Ile Lys Gly Ser Leu Pro

275 280 285

Leu Ile Gly Glu Ala Asp Cys Leu His Glu Lys Tyr Gly Gly Leu Asn

290 295 300

Lys Ser Lys Pro Tyr Tyr Thr Gly Glu His Ala Lys Ala Ile Gly Asn

305 310 315 320

Cys Pro Ile Trp Val Lys Thr Pro Leu Lys Leu Ala Asn Gly Thr Lys

325 330 335

Tyr Arg Pro Pro Gly Gly Gly Trp Glu Gly Met Ile Ala Gly Trp His

340 345 350

Gly Tyr Thr Ser His Gly Ala His Gly Val Ala Val Ala Ala Asp Leu

355 360 365

Lys Ser Thr Gln Glu Ala Ile Asn Lys Ile Thr Lys Asn Leu Asn Ser

370 375 380

Leu Ser Glu Leu Glu Val Lys Asn Leu Gln Arg Leu Ser Gly Ala Met

385 390 395 400

Asp Glu Leu His Asn Glu Ile Leu Glu Leu Asp Glu Lys Val Asp Asp

405 410 415

Leu Arg Ala Asp Thr Ile Ser Ser Gln Ile Glu Leu Ala Val Leu Leu

420 425 430

Ser Asn Glu Gly Ile Ile Asn Ser Glu Asp Glu His Leu Leu Ala Leu
435 440 445
Glu Arg Lys Leu Lys Lys Met Leu Gly Pro Ser Ala Val Glu Ile Gly
450 455 460
Asn Gly Cys Phe Glu Thr Lys His Lys Cys Asn Gln Thr Cys Leu Asp
465 470 475 480
Arg Ile Ala Ala Gly Thr Phe Asp Ala Gly Glu Phe Ser Leu Pro Thr
485 490 495
Phe Asp Ser Leu Asn Ile Thr Ala Ala Ser Leu Asn Asp Asp Gly Leu
500 505 510
Asp Asn His Thr Ile Leu Leu Tyr Tyr Ser Thr Ala Ala Ser Ser Leu
515 520 525
Ala Val Thr Leu Met Ile Ala Ile Phe Val Val Tyr Met Val Ser Arg
530 535 540
Asp Asn Val Ser Cys Ser Ile Cys Leu Pro
545 550

<210> 18
<211> 570
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 用接头代替B HA的切割位点的氨基酸序列
<400> 18

Asp Arg Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His Val Val
1 5 10 15
Lys Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Val Ile Pro Leu
20 25 30
Thr Thr Thr Pro Thr Lys Ser His Phe Ala Asn Leu Lys Gly Thr Glu
35 40 45
Thr Arg Gly Lys Leu Cys Pro Lys Cys Leu Asn Cys Thr Asp Leu Asp
50 55 60
Val Ala Leu Gly Arg Pro Lys Cys Thr Gly Lys Ile Pro Ser Ala Arg
65 70 75 80
Val Ser Ile Leu His Glu Val Arg Pro Val Thr Ser Gly Cys Phe Pro
85 90 95
Ile Met His Asp Arg Thr Lys Ile Arg Gln Leu Pro Asn Leu Leu Arg
100 105 110

Gly Tyr Glu His Ile Arg Leu Ser Thr His Asn Val Ile Asn Ala Glu
115 120 125
Asn Ala Pro Gly Gly Pro Tyr Lys Ile Gly Thr Ser Gly Ser Cys Pro
130 135 140
Asn Ile Thr Asn Gly Asn Gly Phe Phe Ala Thr Met Ala Trp Ala Val
145 150 155 160
Pro Lys Asn Asp Lys Asn Lys Thr Ala Thr Asn Pro Leu Thr Ile Glu
165 170 175
Val Pro Tyr Ile Cys Thr Glu Gly Glu Asp Gln Ile Thr Val Trp Gly
180 185 190
Phe His Ser Asp Asn Glu Thr Gln Met Ala Lys Leu Tyr Gly Asp Ser
195 200 205
Lys Pro Gln Lys Phe Thr Ser Ser Ala Asn Gly Val Thr Thr His Tyr
210 215 220
Val Ser Gln Ile Gly Gly Phe Pro Asn Gln Thr Glu Asp Gly Gly Leu
225 230 235 240
Pro Gln Ser Gly Arg Ile Val Val Asp Tyr Met Val Gln Lys Ser Gly
245 250 255
Lys Thr Gly Thr Ile Thr Tyr Gln Arg Gly Ile Leu Leu Pro Gln Lys
260 265 270
Val Trp Cys Ala Ser Gly Arg Ser Lys Val Ile Lys Gly Ser Leu Pro
275 280 285
Leu Ile Gly Glu Ala Asp Cys Leu His Glu Lys Tyr Gly Gly Leu Asn
290 295 300
Lys Ser Lys Pro Tyr Tyr Thr Gly Glu His Ala Lys Ala Ile Gly Asn
305 310 315 320
Cys Pro Ile Trp Val Lys Thr Pro Leu Lys Leu Ala Asn Gly Thr Lys
325 330 335
Tyr Arg Gly Ser Ser Ser Gly Ser Ser Ser Gly Gly Phe Phe Gly Ala
340 345 350
Ile Ala Gly Phe Leu Glu Gly Gly Trp Glu Gly Met Ile Ala Gly Trp
355 360 365
His Gly Tyr Thr Ser His Gly Ala His Gly Val Ala Val Ala Ala Asp
370 375 380
Leu Lys Ser Thr Gln Glu Ala Ile Asn Lys Ile Thr Lys Asn Leu Asn
385 390 395 400
Ser Leu Ser Glu Leu Glu Val Lys Asn Leu Gln Arg Leu Ser Gly Ala
405 410 415
Met Asp Glu Leu His Asn Glu Ile Leu Glu Leu Asp Glu Lys Val Asp

420 425 430

Asp Leu Arg Ala Asp Thr Ile Ser Ser Gln Ile Glu Leu Ala Val Leu
435 440 445

Leu Ser Asn Glu Gly Ile Ile Asn Ser Glu Asp Glu His Leu Leu Ala
450 455 460

Leu Glu Arg Lys Leu Lys Lys Met Leu Gly Pro Ser Ala Val Glu Ile
465 470 475 480

Gly Asn Gly Cys Phe Glu Thr Lys His Lys Cys Asn Gln Thr Cys Leu
485 490 495

Asp Arg Ile Ala Ala Gly Thr Phe Asp Ala Gly Glu Phe Ser Leu Pro
500 505 510

Thr Phe Asp Ser Leu Asn Ile Thr Ala Ala Ser Leu Asn Asp Asp Gly
515 520 525

Leu Asp Asn His Thr Ile Leu Leu Tyr Tyr Ser Thr Ala Ala Ser Ser
530 535 540

Leu Ala Val Thr Leu Met Ile Ala Ile Phe Val Val Tyr Met Val Ser
545 550 555 560

Arg Asp Asn Val Ser Cys Ser Ile Cys Leu
565 570

<210> 19

<211> 550

<212> PRT

<213> 流感H3 A/珀斯/16/2009(Influenza H3 A/Perth/16/2009)

<400> 19

Gln Lys Leu Pro Gly Asn Asp Asn Ser Thr Ala Thr Leu Cys Leu Gly
1 5 10 15

His His Ala Val Pro Asn Gly Thr Ile Val Lys Thr Ile Thr Asn Asp
20 25 30

Gln Ile Glu Val Thr Asn Ala Thr Glu Leu Val Gln Ser Ser Ser Thr
35 40 45

Gly Glu Ile Cys Asp Ser Pro His Gln Ile Leu Asp Gly Lys Asn Cys
50 55 60

Thr Leu Ile Asp Ala Leu Leu Gly Asp Pro Gln Cys Asp Gly Phe Gln
65 70 75 80

Asn Lys Lys Trp Asp Leu Phe Val Glu Arg Ser Lys Ala Tyr Ser Asn
85 90 95

Cys Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Ser Leu Arg Ser Leu Val
100 105 110

Ala Ser Ser Gly Thr Leu Glu Phe Asn Asn Glu Ser Phe Asn Trp Thr

115 120 125

Gly Val Thr Gln Asn Gly Thr Ser Ser Ala Cys Ile Arg Arg Ser Lys

130 135 140

Asn Ser Phe Phe Ser Arg Leu Asn Trp Leu Thr His Leu Asn Phe Lys

145 150 155 160

Tyr Pro Ala Leu Asn Val Thr Met Pro Asn Asn Glu Gln Phe Asp Lys

165 170 175

Leu Tyr Ile Trp Gly Val His His Pro Gly Thr Asp Lys Asp Gln Ile

180 185 190

Phe Leu Tyr Ala Gln Ala Ser Gly Arg Ile Thr Val Ser Thr Lys Arg

195 200 205

Ser Gln Gln Thr Val Ser Pro Asn Ile Gly Ser Arg Pro Arg Val Arg

210 215 220

Asn Ile Pro Ser Arg Ile Ser Ile Tyr Trp Thr Ile Val Lys Pro Gly

225 230 235 240

Asp Ile Leu Leu Ile Asn Ser Thr Gly Asn Leu Ile Ala Pro Arg Gly

245 250 255

Tyr Phe Lys Ile Arg Ser Gly Lys Ser Ser Ile Met Arg Ser Asp Ala

260 265 270

Pro Ile Gly Lys Cys Asn Ser Glu Cys Ile Thr Pro Asn Gly Ser Ile

275 280 285

Pro Asn Asp Lys Pro Phe Gln Asn Val Asn Arg Ile Thr Tyr Gly Ala

290 295 300

Cys Pro Arg Tyr Val Lys Gln Asn Thr Leu Lys Leu Ala Thr Gly Met

305 310 315 320

Arg Asn Val Pro Glu Lys Gln Thr Arg Gly Ile Phe Gly Ala Ile Ala

325 330 335

Gly Phe Ile Glu Asn Gly Trp Glu Gly Met Val Asp Gly Trp Tyr Gly

340 345 350

Phe Arg His Gln Asn Ser Glu Gly Arg Gly Gln Ala Ala Asp Leu Lys

355 360 365

Ser Thr Gln Ala Ala Ile Asp Gln Ile Asn Gly Lys Leu Asn Arg Leu

370 375 380

Ile Gly Lys Thr Asn Glu Lys Phe His Gln Ile Glu Lys Glu Phe Ser

385 390 395 400

Glu Val Glu Gly Arg Ile Gln Asp Leu Glu Lys Tyr Val Glu Asp Thr

405 410 415

Lys Ile Asp Leu Trp Ser Tyr Asn Ala Glu Leu Leu Val Ala Leu Glu

420 425 430

Asn Gln His Thr Ile Asp Leu Thr Asp Ser Glu Met Asn Lys Leu Phe
435 440 445
Glu Lys Thr Lys Lys Gln Leu Arg Glu Asn Ala Glu Asp Met Gly Asn
450 455 460
Gly Cys Phe Lys Ile Tyr His Lys Cys Asp Asn Ala Cys Ile Gly Ser
465 470 475 480
Ile Arg Asn Gly Thr Tyr Asp His Asp Val Tyr Arg Asp Glu Ala Leu
485 490 495
Asn Asn Arg Phe Gln Ile Lys Gly Val Glu Leu Lys Ser Gly Tyr Lys
500 505 510
Asp Trp Ile Leu Trp Ile Ser Phe Ala Ile Ser Cys Phe Leu Leu Cys
515 520 525
Val Ala Leu Leu Gly Phe Ile Met Trp Ala Cys Gln Lys Gly Asn Ile
530 535 540
Arg Cys Asn Ile Cys Ile
545 550

<210> 20
<211> 540
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> δ-蛋白水解环H3(具有接头GS)的氨基酸序列
<400> 20
Gln Lys Leu Pro Gly Asn Asp Asn Ser Thr Ala Thr Leu Cys Leu Gly
1 5 10 15
His His Ala Val Pro Asn Gly Thr Ile Val Lys Thr Ile Thr Asn Asp
20 25 30
Gln Ile Glu Val Thr Asn Ala Thr Glu Leu Val Gln Ser Ser Ser Thr
35 40 45
Gly Glu Ile Cys Asp Ser Pro His Gln Ile Leu Asp Gly Lys Asn Cys
50 55 60
Thr Leu Ile Asp Ala Leu Leu Gly Asp Pro Gln Cys Asp Gly Phe Gln
65 70 75 80
Asn Lys Lys Trp Asp Leu Phe Val Glu Arg Ser Lys Ala Tyr Ser Asn
85 90 95
Cys Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Ser Leu Arg Ser Leu Val
100 105 110

Ala Ser Ser Gly Thr Leu Glu Phe Asn Asn Glu Ser Phe Asn Trp Thr
115 120 125
Gly Val Thr Gln Asn Gly Thr Ser Ser Ala Cys Ile Arg Arg Ser Lys
130 135 140
Asn Ser Phe Phe Ser Arg Leu Asn Trp Leu Thr His Leu Asn Phe Lys
145 150 155 160
Tyr Pro Ala Leu Asn Val Thr Met Pro Asn Asn Glu Gln Phe Asp Lys
165 170 175
Leu Tyr Ile Trp Gly Val His His Pro Gly Thr Asp Lys Asp Gln Ile
180 185 190
Phe Leu Tyr Ala Gln Ala Ser Gly Arg Ile Thr Val Ser Thr Lys Arg
195 200 205
Ser Gln Gln Thr Val Ser Pro Asn Ile Gly Ser Arg Pro Arg Val Arg
210 215 220
Asn Ile Pro Ser Arg Ile Ser Ile Tyr Trp Thr Ile Val Lys Pro Gly
225 230 235 240
Asp Ile Leu Leu Ile Asn Ser Thr Gly Asn Leu Ile Ala Pro Arg Gly
245 250 255
Tyr Phe Lys Ile Arg Ser Gly Lys Ser Ser Ile Met Arg Ser Asp Ala
260 265 270
Pro Ile Gly Lys Cys Asn Ser Glu Cys Ile Thr Pro Asn Gly Ser Ile
275 280 285
Pro Asn Asp Lys Pro Phe Gln Asn Val Asn Arg Ile Thr Tyr Gly Ala
290 295 300
Cys Pro Arg Tyr Val Lys Gln Asn Thr Leu Lys Leu Ala Thr Gly Met
305 310 315 320
Gly Ser Gly Ala Ile Ala Gly Phe Ile Glu Asn Gly Trp Glu Gly Met
325 330 335
Val Asp Gly Trp Tyr Gly Phe Arg His Gln Asn Ser Glu Gly Arg Gly
340 345 350
Gln Ala Ala Asp Leu Lys Ser Thr Gln Ala Ala Ile Asp Gln Ile Asn
355 360 365
Gly Lys Leu Asn Arg Leu Ile Gly Lys Thr Asn Glu Lys Phe His Gln
370 375 380
Ile Glu Lys Glu Phe Ser Glu Val Glu Gly Arg Ile Gln Asp Leu Glu
385 390 395 400
Lys Tyr Val Glu Asp Thr Lys Ile Asp Leu Trp Ser Tyr Asn Ala Glu
405 410 415
Leu Leu Val Ala Leu Glu Asn Gln His Thr Ile Asp Leu Thr Asp Ser

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

<210> 21
<211> 550
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 用接头代替H3的切割位点的氨基酸序列
<400> 21
Gln Lys Leu Pro Gly Asn Asp Asn Ser Thr Ala Thr Leu Cys Leu Gly
1 5 10 15
His His Ala Val Pro Asn Gly Thr Ile Val Lys Thr Ile Thr Asn Asp
20 25 30
Gln Ile Glu Val Thr Asn Ala Thr Glu Leu Val Gln Ser Ser Ser Thr
35 40 45
Gly Glu Ile Cys Asp Ser Pro His Gln Ile Leu Asp Gly Lys Asn Cys
50 55 60
Thr Leu Ile Asp Ala Leu Leu Gly Asp Pro Gln Cys Asp Gly Phe Gln
65 70 75 80
Asn Lys Lys Trp Asp Leu Phe Val Glu Arg Ser Lys Ala Tyr Ser Asn
85 90 95
Cys Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Ser Leu Arg Ser Leu Val
100 105 110
Ala Ser Ser Gly Thr Leu Glu Phe Asn Asn Glu Ser Phe Asn Trp Thr

115 120 125

Gly Val Thr Gln Asn Gly Thr Ser Ser Ala Cys Ile Arg Arg Ser Lys

130 135 140

Asn Ser Phe Phe Ser Arg Leu Asn Trp Leu Thr His Leu Asn Phe Lys

145 150 155 160

Tyr Pro Ala Leu Asn Val Thr Met Pro Asn Asn Glu Gln Phe Asp Lys

165 170 175

Leu Tyr Ile Trp Gly Val His His Pro Gly Thr Asp Lys Asp Gln Ile

180 185 190

Phe Leu Tyr Ala Gln Ala Ser Gly Arg Ile Thr Val Ser Thr Lys Arg

195 200 205

Ser Gln Gln Thr Val Ser Pro Asn Ile Gly Ser Arg Pro Arg Val Arg

210 215 220

Asn Ile Pro Ser Arg Ile Ser Ile Tyr Trp Thr Ile Val Lys Pro Gly

225 230 235 240

Asp Ile Leu Leu Ile Asn Ser Thr Gly Asn Leu Ile Ala Pro Arg Gly

245 250 255

Tyr Phe Lys Ile Arg Ser Gly Lys Ser Ser Ile Met Arg Ser Asp Ala

260 265 270

Pro Ile Gly Lys Cys Asn Ser Glu Cys Ile Thr Pro Asn Gly Ser Ile

275 280 285

Pro Asn Asp Lys Pro Phe Gln Asn Val Asn Arg Ile Thr Tyr Gly Ala

290 295 300

Cys Pro Arg Tyr Val Lys Gln Asn Thr Leu Lys Leu Ala Thr Gly Met

305 310 315 320

Gly Ser Ser Gly Ser Ser Gly Ser Ser Gly Ile Phe Gly Ala Ile Ala

325 330 335

Gly Phe Ile Glu Asn Gly Trp Glu Gly Met Val Asp Gly Trp Tyr Gly

340 345 350

Phe Arg His Gln Asn Ser Glu Gly Arg Gly Gln Ala Ala Asp Leu Lys

355 360 365

Ser Thr Gln Ala Ala Ile Asp Gln Ile Asn Gly Lys Leu Asn Arg Leu

370 375 380

Ile Gly Lys Thr Asn Glu Lys Phe His Gln Ile Glu Lys Glu Phe Ser

385 390 395 400

Glu Val Glu Gly Arg Ile Gln Asp Leu Glu Lys Tyr Val Glu Asp Thr

405 410 415

Lys Ile Asp Leu Trp Ser Tyr Asn Ala Glu Leu Leu Val Ala Leu Glu

420 425 430

Asn Gln His Thr Ile Asp Leu Thr Asp Ser Glu Met Asn Lys Leu Phe
435 440 445
Glu Lys Thr Lys Lys Gln Leu Arg Glu Asn Ala Glu Asp Met Gly Asn
450 455 460
Gly Cys Phe Lys Ile Tyr His Lys Cys Asp Asn Ala Cys Ile Gly Ser
465 470 475 480
Ile Arg Asn Gly Thr Tyr Asp His Asp Val Tyr Arg Asp Glu Ala Leu
485 490 495
Asn Asn Arg Phe Gln Ile Lys Gly Val Glu Leu Lys Ser Gly Tyr Lys
500 505 510
Asp Trp Ile Leu Trp Ile Ser Phe Ala Ile Ser Cys Phe Leu Leu Cys
515 520 525
Val Ala Leu Leu Gly Phe Ile Met Trp Ala Cys Gln Lys Gly Asn Ile
530 535 540
Arg Cys Asn Ile Cys Ile
545 550

<210> 22
<211> 31
<212> PRT
<213> 流感H1新喀里多尼亚(Influenza H1 New Cal)
<400> 22
Ile Pro Ser Ile Gln Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe
1 5 10 15
Ile Glu Gly Gly Trp Thr Gly Met Val Asp Gly Trp Tyr Gly Tyr
20 25 30

<210> 23
<211> 31
<212> PRT
<213> 流感H1布里斯班(Influenza H1 Brisbane)
<400> 23
Ile Pro Ser Ile Gln Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe
1 5 10 15
Ile Glu Gly Gly Trp Thr Gly Met Val Asp Gly Trp Tyr Gly Tyr
20 25 30

<210> 24
<211> 31
<212> PRT
<213> 流感H1所罗门群岛(Influenza H1 Sol Islands)
<400> 24

Ile Pro Ser Ile Gln Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe
1 5 10 15
Ile Glu Gly Gly Trp Thr Gly Met Val Asp Gly Trp Tyr Gly Tyr
20 25 30

<210> 25
<211> 31
<212> PRT
<213> 流感H2A新加坡(Influenza H2A Singapore)
<400> 25
Val Pro Gln Ile Glu Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe
1 5 10 15
Ile Glu Gly Gly Trp Gln Gly Met Val Asp Gly Trp Tyr Gly Tyr
20 25 30

<210> 26
<211> 31
<212> PRT
<213> 流感H3A布里斯班(Influenza H3A Brisbane)
<400> 26
Val Pro Glu Lys Gln Thr Arg Gly Ile Phe Gly Ala Ile Ala Gly Phe
1 5 10 15
Ile Glu Asn Gly Trp Glu Gly Met Val Asp Gly Trp Tyr Gly Phe
20 25 30

<210> 27
<211> 31
<212> PRT
<213> 流感H3A WCN(Influenza H3A WCN)
<400> 27
Val Pro Glu Lys Gln Thr Arg Gly Ile Phe Gly Ala Ile Ala Gly Phe
1 5 10 15
Ile Glu Asn Gly Trp Glu Gly Met Val Asp Gly Trp Tyr Gly Phe
20 25 30

<210> 28
<211> 34
<212> PRT
<213> 流感H5安徽(Influenza H5 Anhui)
<400> 28
Ser Pro Leu Arg Glu Arg Arg Arg Lys Arg Gly Leu Phe Gly Ala Ile
1 5 10 15
Ala Gly Phe Ile Glu Gly Gly Trp Gln Gly Met Val Asp Gly Trp Tyr

20 25 30

Gly Tyr

<210> 29

<211> 35

<212> PRT

<213> 流感H5印度尼西亚(Influenza H5 Indo)

<400> 29

Ser Pro Gln Arg Glu Ser Arg Arg Lys Lys Arg Gly Leu Phe Gly Ala
1 5 10 15
Ile Ala Gly Phe Ile Glu Gly Gly Trp Gln Gly Met Val Asp Gly Trp
20 25 30
Tyr Gly Tyr
35

<210> 30

<211> 35

<212> PRT

<213> 流感H5越南(Influenza H5 Vietnam)

<400> 30

Ser Pro Gln Arg Glu Arg Arg Arg Lys Lys Arg Gly Leu Phe Gly Ala
1 5 10 15
Ile Ala Gly Phe Ile Glu Gly Gly Trp Gln Gly Met Val Asp Gly Trp
20 25 30
Tyr Gly Tyr
35

<210> 31

<211> 6861

<212> DNA

<213> 人工序列(Artificial Sequence)

<220>

<221>

<222>

<223> 构建体1194

<400> 31

tggcaggata tattgtggtg taaacaaatt gacgcttaga caacttaata acacattgcg 60
gacgttttta atgtactgaa ttaacgccga atcccgggct ggtatattta tatgttgtca 120
aataactcaa aaaccataaa agtttaagtt agcaagtgtg tacattttta cttgaacaaa 180
aatattcacc tactactgtt ataaatcatt attaaacatt agagtaaaga aatatggatg 240
ataagaacaa gagtagtgat attttgacaa caattttgtt gcaacatttg agaaaatttt 300
gttgttctct cttttcattg gtcaaaaaca atagagagag aaaaaggaag agggagaata 360

aaaacataat gtgagtatga gagagaaagt tgtacaaaag ttgtaccaaa atagttgtac 420
aaatatcatt gaggaatttg acaaaagcta cacaaataag ggttaattgc tgtaaataaa 480
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aagaataaat tatttttaaa attaaaagtt gagtcatttg attaaacatg tgattattta 600
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gtatatttac taaaaaatct aagccacgta ggaggataac aggatccccg taggaggata 840
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ctgtggcaca tctacattat ctaaatcaca cattcttcca cacatctgag ccacacaaaa 960
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gtggttaata tagctcaaat atatggtcaa gttcaataga ttaataatgg aaatatcagt 1920
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attaaataac gtgtacttgt cctattcttg tcggtgtggt cttgggaaaa gaaagcttgc 3540
tggaggctgc tgttcagccc catacattac ttgttacgat tctgctgact ttcggcgggt 3600
gcaatatctc tacttctgct tgacgaggta ttgttgcctg tacttctttc ttcttcttct 3660
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<213> 流感H7 Eq布拉格(Influenza H7 Eq Prague)
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<210> 41
<211> 577
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>

<223> PDISP/HA 流感B/布里斯班/60/2008(Influenza B/Brisbane/60/2008)(缺失的蛋白水解环)的氨基酸序列

<400> 41

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

Arg Gly Ile Leu Leu Pro Gln Lys Val Trp Cys Ala Ser Gly Arg Ser
290 295 300
Lys Val Ile Lys Gly Ser Leu Pro Leu Ile Gly Glu Ala Asp Cys Leu
305 310 315 320
His Glu Lys Tyr Gly Gly Leu Asn Lys Ser Lys Pro Tyr Tyr Thr Gly
325 330 335
Glu His Ala Lys Ala Ile Gly Asn Cys Pro Ile Trp Val Lys Thr Pro
340 345 350
Leu Lys Leu Ala Asn Gly Thr Lys Tyr Arg Pro Pro Gly Gly Gly Trp
355 360 365
Glu Gly Met Ile Ala Gly Trp His Gly Tyr Thr Ser His Gly Ala His
370 375 380
Gly Val Ala Val Ala Ala Asp Leu Lys Ser Thr Gln Glu Ala Ile Asn
385 390 395 400
Lys Ile Thr Lys Asn Leu Asn Ser Leu Ser Glu Leu Glu Val Lys Asn
405 410 415
Leu Gln Arg Leu Ser Gly Ala Met Asp Glu Leu His Asn Glu Ile Leu
420 425 430
Glu Leu Asp Glu Lys Val Asp Asp Leu Arg Ala Asp Thr Ile Ser Ser
435 440 445
Gln Ile Glu Leu Ala Val Leu Leu Ser Asn Glu Gly Ile Ile Asn Ser
450 455 460
Glu Asp Glu His Leu Leu Ala Leu Glu Arg Lys Leu Lys Lys Met Leu
465 470 475 480
Gly Pro Ser Ala Val Glu Ile Gly Asn Gly Cys Phe Glu Thr Lys His
485 490 495
Lys Cys Asn Gln Thr Cys Leu Asp Arg Ile Ala Ala Gly Thr Phe Asp
500 505 510
Ala Gly Glu Phe Ser Leu Pro Thr Phe Asp Ser Leu Asn Ile Thr Ala
515 520 525
Ala Ser Leu Asn Asp Asp Gly Leu Asp Asn His Thr Ile Leu Leu Tyr
530 535 540
Tyr Ser Thr Ala Ala Ser Ser Leu Ala Val Thr Leu Met Ile Ala Ile
545 550 555 560
Phe Val Val Tyr Met Val Ser Arg Asp Asn Val Ser Cys Ser Ile Cys
565 570 575
Leu

<210> 42

<211> 1707

<212> DNA

<213> 流感H5 A/印度尼西亚/5/2005(H5N1)(Influenza H5 A/Indonesia/5/2005 (H5N1))

<400> 42

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actgttacac atgcccaaga catactggaa aagacacaca acgggaagct ctgcgatcta 180
gatggagtga agcctctaat tttaagagat tgtagtgtag ctggatggct cctcgggaac 240
ccaatgtgtg acgaattcat caatgtaccg gaatggtctt acatagtgga gaaggccaat 300
ccaaccaatg acctctgtta cccagggagt ttcaacgact atgaagaact gaaacaccta 360
ttgagcagaa taaaccattt tgagaaaatt caaatcatcc ccaaaagttc ttggtccgat 420
catgaagcct catcaggagt tagctcagca tgtccatacc tgggaagtcc ctccttttt 480
agaaatgtgg tatggcttat caaaaagaac agtacatacc caacaataaa gaaaagctac 540
aataatacca accaagagga tcttttggta ctgtggggaa ttcaccatcc taatgatgcg 600
gcagagcaga caaggctata tcaaaaccca accacctata tttccattgg gacatcaaca 660
ctaaaccaga gattggtacc aaaaatagct actagatcca aagtaaacgg gcaaagtgga 720
aggatggagt tcttctggac aattttaaaa cctaatgatg caatcaactt cgagagtaat 780
ggaaatttca ttgctccaga atatgcatac aaaattgtca agaaagggga ctcagcaatt 840
atgaaaagtg aattggaata tggtaactgc aacaccaagt gtcaaactcc aatgggggcg 900
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ggaacgtaca actatccgca gtattcagaa gaagcaagat taaaaagaga ggaaataagt 1560
ggggtaaaat tggaatcaat aggaacttac caaatactgt caatttattc aacagtggcg 1620
agttccctag cactggcaat catgatggct ggtctatctt tatggatgtg ctccaatgga 1680
tcgttacaat gcagaatttg catttaa 1707

<210> 43

<211> 1734

<212> DNA

<213> 人工序列(Artificial Sequence)

<220>

<221>

<222>

<223> PDISP/HA 流感B/布里斯班/60/2008(Influenza B/Brisbane/60/2008)(缺失的蛋白水解环)的核苷酸序列

<400> 43

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cagatcttcg ccgatcgaat ctgcactgga ataacatcgt caaactcacc acatgtcgtc 120
aaaactgcta ctcaagggga ggtcaatgtg actggtgtaa taccactgac aacaacaccc 180
accaaatctc attttgcaaa tctcaaagga acagaaacca gggggaaact atgcccaaaa 240
tgcctcaact gcacagatct ggacgtagcc ttgggcagac caaaatgcac ggggaaaata 300
ccctcggcaa gagtttcaat actccatgaa gtcagacctg ttacatctgg gtgctttcct 360
ataatgcacg acagaacaaa aattagacag ctgcctaacc ttctccgagg atacgaacat 420
atcaggttat caacccataa cgttatcaat gcagaaaatg caccaggagg accctacaaa 480
attggaacct cagggtcttg ccctaacatt accaatggaa acggattttt cgcaacaatg 540
gcttgggccg tcccaaaaaa cgacaaaaac aaaacagcaa caaatccatt aacaatagaa 600
gtaccataca tttgtacaga aggagaagac caaattaccg tttgggggtt ccactctgac 660
aacgagaccc aaatggcaaa gctctatggg gactcaaagc cccagaagtt cacctcatct 720
gccaacggag tgaccacaca ttacgtttca cagattggtg gcttcccaaa tcaaacagaa 780
gacggaggac taccacaaag tggtagaatt gttgttgatt acatggtgca aaaatctggg 840
aaaacaggaa caattaccta tcaaaggggt attttattgc ctcaaaaggt gtggtgcgca 900
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ataataaaca gtgaagatga acatctcttg gcgcttgaaa gaaagctgaa gaaaatgctg 1440
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acctgtctcg acagaatagc tgctggtacc tttgatgcag gagaattttc tctccccacc 1560
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atactgcttt actactcaac tgctgcctcc agtttggctg taacactgat gatagctatc 1680
tttgttgttt atatggtctc cagagacaat gtttcttgct ccatctgtct ataa 1734

<210> 44

<211> 19

<212> PRT

<213> 人工序列(Artificial Sequence)

<220>

<221>

<222>
<223> H5/Indo切割位点的天然序列
<400> 44
Thr Gly Leu Arg Asn Ser Pro Gln Arg Glu Ser Arg Arg Lys Lys Arg
1 5 10 15
Gly Leu Phe
<210> 45
<211> 15
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> H5/印度尼西亚 修饰的切割位点(TETR)
<400> 45
Thr Gly Leu Arg Asn Ser Pro Gln Thr Glu Thr Arg Gly Leu Phe
1 5 10 15
<210> 46
<211> 15
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> H5/印度尼西亚 修饰的切割位点(TETQ)
<400> 46
Thr Gly Leu Arg Asn Ser Pro Gln Thr Glu Thr Gln Gly Leu Phe
1 5 10 15
<210> 47
<211> 11
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> H1切割位点
<400> 47
Asn Ile Pro Ser Ile Gln Ser Arg Gly Leu Phe
1 5 10

<210> 48
<211> 11
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> H3切割位点
<400> 48
Asn Val Pro Glu Lys Gln Thr Arg Gly Ile Phe
1 5 10
<210> 49
<211> 46
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 构建体1462 IF-HAB110.S1+3c
<400> 49
Ala Ala Ala Thr Thr Thr Gly Thr Cys Gly Gly Gly Cys Cys Cys Ala
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Thr Gly Ala Ala Gly Gly Cys Ala Ala Thr Ala Ala Thr Thr Gly Thr
20 25 30
Ala Cys Thr Ala Cys Thr Cys Ala Thr Gly Gly Thr Ala Gly
35 40 45
<210> 50
<211> 52
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 构建体1462号 IF-HAB110.s1-4r
<400> 50
actaaagaaa ataggccttt atagacagat ggagcatgaa acgttgtctc tg 52
<210> 51
<211> 1755
<212> DNA

<213> 流感HA B威斯康星州(Influenza HA B wisconsin)

<400> 51

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gggataacat cttcaaactc acctcatgtg gtcaaaacag ctactcaagg ggaggtcaat 120
gtgactggcg tgataccact gacaacaaca ccaacaaaat cttattttgc aaatctcaaa 180
ggaacaagga ccagagggaa actatgcccg gactgtctca actgtacaga tctggatgtg 240
gccttgggca ggccaatgtg tgtggggacc acaccttctg ctaaagcttc aatactccac 300
gaggtcagac ctgttacatc cgggtgcttt cctataatgc acgacagaac aaaaatcagg 360
caactaccca atcttctcag aggatatgaa aatatcaggt tatcaaccca aaacgttatc 420
gatgcagaaa aagcaccagg aggaccctac agacttggaa cctcaggatc ttgccctaac 480
gctaccagta aaatcggatt ttttgcaaca atggcttggg ctgtcccaaa ggacaactac 540
aaaaatgcaa cgaacccact aacagtagaa gtaccataca tttgtacaga aggggaagac 600
caaattactg tttgggggtt ccattcagat aacaaaaccc aaatgaagag cctctatgga 660
gactcaaatc ctcaaaagtt cacctcatct gctaatggag taaccacaca ttatgtttct 720
cagattggcg acttcccaga tcaaacagaa gacggaggac taccacaaag cggcagaatt 780
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agtacacaag aagctataaa taagataaca aaaaatctca attctttgag tgagctagaa 1260
gtaaagaacc ttcaaagact aagtggtgcc atggatgaac tccacaacga aatactcgag 1320
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agaaaactaa agaaaatgct gggtccctct gctgtagaca taggaaacgg atgcttcgaa 1500
accaaacaca aatgcaacca gacctgctta gacaggatag ctgctggcac ctttaatgca 1560
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gatggattgg ataaccatac tatactgctc tattactcaa ctgctgcttc tagtttggct 1680
gtaacattaa tgctagctat ttttattgtt tatatggtct ccagagacaa cgtttcatgc 1740
tccatctgtc tataa 1755

<210> 52

<211> 6745

<212> DNA

<213> 人工序列(Artificial Sequence)

<220>

<221>

<222>

<223> 构建体193

<400> 52

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aataactcaa aaaccataaa agtttaagtt agcaagtgtg tacattttta cttgaacaaa 180
aatattcacc tactactgtt ataaatcatt attaaacatt agagtaaaga aatatggatg 240
ataagaacaa gagtagtgat attttgacaa caattttgtt gcaacatttg agaaaatttt 300
gttgttctct cttttcattg gtcaaaaaca atagagagag aaaaaggaag agggagaata 360
aaaacataat gtgagtatga gagagaaagt tgtacaaaag ttgtaccaaa atagttgtac 420
aaatatcatt gaggaatttg acaaaagcta cacaaataag ggttaattgc tgtaaataaa 480
taaggatgac gcattagaga gatgtaccat tagagaattt ttggcaagtc attaaaaaga 540
aagaataaat tatttttaaa attaaaagtt gagtcatttg attaaacatg tgattattta 600
atgaattgat gaaagagttg gattaaagtt gtattagtaa ttagaatttg gtgtcaaatt 660
taatttgaca tttgatcttt tcctatatat tgccccatag agtcagttaa ctcatttta 720
tatttcatag atcaaataag agaaataacg gtatattaat ccctccaaaa aaaaaaaacg 780
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acatccaatc caaccaatca caacaatcct gatgagataa cccactttaa gcccacgcat 900
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cgcgttggga attactagcg cgtgtcgaga cgcgttgttg ttgtgactcc gaggggttgc 2160
ctcaaactct atcttataac cggcgtggag gcatggaggc aggggtattt tggtcatttt 2220

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ataaaagcat atacgatgtg atggtatttg gtcgacaagc ttgcatgccg gtcaacatgg 2460
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Ser Asp Asn Lys Thr Gln Met Lys Ser Leu Tyr Gly Asp Ser Asn Pro
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485 490 495
Gly Cys Phe Glu Thr Lys His Lys Cys Asn Gln Thr Cys Leu Asp Arg
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<211> 567

<212> PRT
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50 55 60
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145 150 155 160
Ala Thr Ser Lys Ile Gly Phe Phe Ala Thr Met Ala Trp Ala Val Pro
165 170 175
Lys Asp Asn Tyr Lys Asn Ala Thr Asn Pro Leu Thr Val Glu Val Pro
180 185 190
Tyr Ile Cys Thr Glu Gly Glu Asp Gln Ile Thr Val Trp Gly Phe His
195 200 205
Ser Asp Asn Lys Thr Gln Met Lys Ser Leu Tyr Gly Asp Ser Asn Pro
210 215 220
Gln Lys Phe Thr Ser Ser Ala Asn Gly Val Thr Thr His Tyr Val Ser
225 230 235 240
Gln Ile Gly Asp Phe Pro Asp Gln Thr Glu Asp Gly Gly Leu Pro Gln
245 250 255

Ser Gly Arg Ile Val Val Asp Tyr Met Met Gln Lys Pro Gly Lys Thr
260 265 270
Gly Thr Ile Val Tyr Gln Arg Gly Val Leu Leu Pro Gln Lys Val Trp
275 280 285
Cys Ala Ser Gly Arg Ser Lys Val Ile Lys Gly Ser Leu Pro Leu Ile
290 295 300
Gly Glu Ala Asp Cys Leu His Glu Lys Tyr Gly Gly Leu Asn Lys Ser
305 310 315 320
Lys Pro Tyr Tyr Thr Gly Glu His Ala Lys Ala Ile Gly Asn Cys Pro
325 330 335
Ile Trp Val Lys Thr Pro Leu Lys Leu Ala Asn Gly Thr Lys Tyr Arg
340 345 350
Pro Pro Gly Gly Gly Trp Glu Gly Met Ile Ala Gly Trp His Gly Tyr
355 360 365
Thr Ser His Gly Ala His Gly Val Ala Val Ala Ala Asp Leu Lys Ser
370 375 380
Thr Gln Glu Ala Ile Asn Lys Ile Thr Lys Asn Leu Asn Ser Leu Ser
385 390 395 400
Glu Leu Glu Val Lys Asn Leu Gln Arg Leu Ser Gly Ala Met Asp Glu
405 410 415
Leu His Asn Glu Ile Leu Glu Leu Asp Glu Lys Val Asp Asp Leu Arg
420 425 430
Ala Asp Thr Ile Ser Ser Gln Ile Glu Leu Ala Val Leu Leu Ser Asn
435 440 445
Glu Gly Ile Ile Asn Ser Glu Asp Glu His Leu Leu Ala Leu Glu Arg
450 455 460
Lys Leu Lys Lys Met Leu Gly Pro Ser Ala Val Asp Ile Gly Asn Gly
465 470 475 480
Cys Phe Glu Thr Lys His Lys Cys Asn Gln Thr Cys Leu Asp Arg Ile
485 490 495
Ala Ala Gly Thr Phe Asn Ala Gly Glu Phe Ser Leu Pro Thr Phe Asp
500 505 510
Ser Leu Asn Ile Thr Ala Ala Ser Leu Asn Asp Asp Gly Leu Asp Asn
515 520 525
His Thr Ile Leu Leu Tyr Tyr Ser Thr Ala Ala Ser Ser Leu Ala Val
530 535 540
Thr Leu Met Leu Ala Ile Phe Ile Val Tyr Met Val Ser Arg Asp Asn
545 550 555 560
Val Ser Cys Ser Ile Cys Leu

565

<210> 59
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<400> 59
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<210> 60
<211> 19
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
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<222>
<223> H5/印度尼西亚 天然的切割位点
<400> 60
Thr Gly Leu Arg Asn Ser Pro Gln Arg Glu Ser Arg Arg Lys Lys Arg
1 5 10 15
Gly Leu Phe
<210> 61
<211> 15
<212> PRT
<213> 人工序列(Artificial Sequence)
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<223> H5/印度尼西亚 修饰的切割位点
<400> 61
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1 5 10 15
<210> 62
<211> 15
<212> PRT
<213> 人工序列(Artificial Sequence)

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<223> H5/印度尼西亚 修饰的切割位点
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1 5 10 15
<210> 63
<211> 11
<212> PRT
<213> 人工序列(Artificial Sequence)
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<221>
<222>
<223> H1/布里斯班 修饰的切割位点
<400> 63
Asn Ile Pro Ser Ile Gln Ser Gln Gly Leu Phe
1 5 10
<210> 64
<211> 11
<212> PRT
<213> 人工序列(Artificial Sequence)
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<222>
<223> H3/布里斯班 修饰的切割位点
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1 5 10
<210> 65
<211> 11
<212> PRT
<213> 人工序列(Artificial Sequence)
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<222>
<223> B/佛罗里达、B/布里斯班 修饰的切割位点
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1 5 10

<210> 66

<211> 19

<212> PRT

<213> 人工序列(Artificial Sequence)

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<223> A/H3/HA0共有序列

<400> 66

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Phe Ile Glu

<210> 67

<211> 19

<212> PRT

<213> 人工序列(Artificial Sequence)

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<223> A/H1/HA0共有序列

<400> 67

Asn Ile Pro Ser Ile Gln Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly
1 5 10 15

Phe Ile Glu

<210> 68

<211> 19

<212> PRT

<213> 人工序列(Artificial Sequence)

<220>

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<222>

<223> B/HA0共有序列

<400> 68

Pro Ala Lys Leu Leu Lys Glu Arg Gly Phe Phe Gly Ala Ile Ala Gly
1 5 10 15

Phe Leu Glu

<210> 69

<211> 16

<212> PRT
<213> 人工序列(Artificial Sequence)
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<223> H5安徽蛋白水解环缺失
<400> 69
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1 5 10 15
<210> 70
<211> 16
<212> PRT
<213> 人工序列(Artificial Sequence)
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<222>
<223> H5印度尼西亚蛋白水解环缺失
<400> 70
Ser Pro Gln Gly Gly Trp Gln Gly Met Val Asp Gly Trp Tyr Gly Tyr
1 5 10 15
<210> 71
<211> 16
<212> PRT
<213> 人工序列(Artificial Sequence)
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<223> H5越南蛋白水解环缺失
<400> 71
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1 5 10 15
<210> 72
<211> 25
<212> PRT
<213> 人工序列(Artificial Sequence)
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<221>
<222>
<223> B佛罗里达蛋白水解环缺失

<400> 72
Leu Lys Leu Ala Asn Gly Thr Lys Tyr Arg Pro Pro Gly Gly Trp Glu
1 5 10 15
Gly Met Ile Ala Gly Trp His Gly Tyr
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<210> 73
<211> 25
<212> PRT
<213> 人工序列(Artificial Sequence)
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<221>
<222>
<223> B马来西亚蛋白水解环缺失
<400> 73
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Gly Met Ile Ala Gly Trp His Gly Tyr
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<210> 74
<211> 45
<212> DNA
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<400> 74
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<210> 75
<211> 45
<212> DNA
<213> 人工序列(Artificial Sequence)
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<223> 引物MutCleavage-H5(Indo).c
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<210> 76

<211> 3435
<212> DNA
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<223> 盒676
<400> 76

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tacaaatgcc atcattgcga taaaggaaag gccatcgttg aagatgcctc tgccgacagt 240
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tccaaaaata tcaaagatac agtctcagaa gaccaaaggg caattgagac ttttcaacaa 420
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tccactgacg taagggatga cgcacaatcc cactatcctt cgcaagaccc ttcctctata 720
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gcgatgatta tcatataatt tctgttgaat tacgttaagc atgtaataat taacatgtaa 3300
tgcatgacgt tatttatgag atgggttttt atgattagag tcccgcaatt atacatttaa 3360
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<210> 77
<211> 564
<212> PRT
<213> 人工序列(Artificial Sequence)
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<400> 77

Met Glu Lys Ile Val Leu Leu Leu Ala Ile Val Ser Leu Val Lys Ser
1 5 10 15
Asp Gln Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Glu Gln Val

20 25 30

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

Pro Gln Thr Glu Thr Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile
340 345 350
Glu Gly Gly Trp Gln Gly Met Val Asp Gly Trp Tyr Gly Tyr His His
355 360 365
Ser Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Lys Glu Ser Thr Gln
370 375 380
Lys Ala Ile Asp Gly Val Thr Asn Lys Val Asn Ser Ile Ile Asp Lys
385 390 395 400
Met Asn Thr Gln Phe Glu Ala Val Gly Arg Glu Phe Asn Asn Leu Glu
405 410 415
Arg Arg Ile Glu Asn Leu Asn Lys Lys Met Glu Asp Gly Phe Leu Asp
420 425 430
Val Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Met Glu Asn Glu Arg
435 440 445
Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Asp Lys Val
450 455 460
Arg Leu Gln Leu Arg Asp Asn Ala Lys Glu Leu Gly Asn Gly Cys Phe
465 470 475 480
Glu Phe Tyr His Lys Cys Asp Asn Glu Cys Met Glu Ser Ile Arg Asn
485 490 495
Gly Thr Tyr Asn Tyr Pro Gln Tyr Ser Glu Glu Ala Arg Leu Lys Arg
500 505 510
Glu Glu Ile Ser Gly Val Lys Leu Glu Ser Ile Gly Thr Tyr Gln Ile
515 520 525
Leu Ser Ile Tyr Ser Thr Val Ala Ser Ser Leu Ala Leu Ala Ile Met
530 535 540
Met Ala Gly Leu Ser Leu Trp Met Cys Ser Asn Gly Ser Leu Gln Cys
545 550 555 560
Arg Ile Cys Ile

<210> 78
<211> 48
<212> DNA
<213> 人工序列(Artificial Sequence)
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<222>
<223> 引物H5I505_TETQ.r
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<210> 79
<211> 48
<212> DNA
<213> 人工序列(Artificial Sequence)
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<223> 引物H5I505_TETQ.c
<400> 79
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<210> 80
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<212> DNA
<213> 人工序列(Artificial Sequence)
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<400> 80
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ttccattgcc cagctatctg tcactttatt gtgaagatag tggaaaagga aggtggctcc 180
tacaaatgcc atcattgcga taaaggaaag gccatcgttg aagatgcctc tgccgacagt 240
ggtcccaaag atggaccccc acccacgagg agcatcgtgg aaaaagaaga cgttccaacc 300
acgtcttcaa agcaagtgga ttgatgtgat aacatggtgg agcacgacac acttgtctac 360
tccaaaaata tcaaagatac agtctcagaa gaccaaaggg caattgagac ttttcaacaa 420
agggtaatat ccggaaacct cctcggattc cattgcccag ctatctgtca ctttattgtg 480
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tccactgacg taagggatga cgcacaatcc cactatcctt cgcaagaccc ttcctctata 720
taaggaagtt catttcattt ggagaggtat taaaatctta ataggttttg ataaaagcga 780
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tgcatgacgt tatttatgag atgggttttt atgattagag tcccgcaatt atacatttaa 3360
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tctatgttac tagat 3435

<210> 81

<211> 564
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
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<223> 流感A/印度尼西亚/5/2005(H5N1)TETQ切割位点突变的氨基酸序列
<400> 81

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

245 250 255
Phe Glu Ser Asn Gly Asn Phe Ile Ala Pro Glu Tyr Ala Tyr Lys Ile
260 265 270
Val Lys Lys Gly Asp Ser Ala Ile Met Lys Ser Glu Leu Glu Tyr Gly
275 280 285
Asn Cys Asn Thr Lys Cys Gln Thr Pro Met Gly Ala Ile Asn Ser Ser
290 295 300
Met Pro Phe His Asn Ile His Pro Leu Thr Ile Gly Glu Cys Pro Lys
305 310 315 320
Tyr Val Lys Ser Asn Arg Leu Val Leu Ala Thr Gly Leu Arg Asn Ser
325 330 335
Pro Gln Thr Glu Thr Gln Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile
340 345 350
Glu Gly Gly Trp Gln Gly Met Val Asp Gly Trp Tyr Gly Tyr His His
355 360 365
Ser Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Lys Glu Ser Thr Gln
370 375 380
Lys Ala Ile Asp Gly Val Thr Asn Lys Val Asn Ser Ile Ile Asp Lys
385 390 395 400
Met Asn Thr Gln Phe Glu Ala Val Gly Arg Glu Phe Asn Asn Leu Glu
405 410 415
Arg Arg Ile Glu Asn Leu Asn Lys Lys Met Glu Asp Gly Phe Leu Asp
420 425 430
Val Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Met Glu Asn Glu Arg
435 440 445
Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Asp Lys Val
450 455 460
Arg Leu Gln Leu Arg Asp Asn Ala Lys Glu Leu Gly Asn Gly Cys Phe
465 470 475 480
Glu Phe Tyr His Lys Cys Asp Asn Glu Cys Met Glu Ser Ile Arg Asn
485 490 495
Gly Thr Tyr Asn Tyr Pro Gln Tyr Ser Glu Glu Ala Arg Leu Lys Arg
500 505 510
Glu Glu Ile Ser Gly Val Lys Leu Glu Ser Ile Gly Thr Tyr Gln Ile
515 520 525
Leu Ser Ile Tyr Ser Thr Val Ala Ser Ser Leu Ala Leu Ala Ile Met
530 535 540
Met Ala Gly Leu Ser Leu Trp Met Cys Ser Asn Gly Ser Leu Gln Cys
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<210> 82

<211> 50

<212> DNA

<213> 人工序列(Artificial Sequence)

<220>

<221>

<222>

<223> 引物H5I505(PrL-).r

<400> 82

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<210> 83

<211> 52

<212> DNA

<213> 人工序列(Artificial Sequence)

<220>

<221>

<222>

<223> 引物H5I505(PrL-).c

<400> 83

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<210> 84

<211> 3390

<212> DNA

<213> 人工序列(Artificial Sequence)

<220>

<221>

<222>

<223> 盒928

<400> 84

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ttccattgcc cagctatctg tcactttatt gtgaagatag tggaaaagga aggtggctcc 180

tacaaatgcc atcattgcga taaaggaaag gccatcgttg aagatgcctc tgccgacagt 240

ggtcccaaag atggaccccc acccacgagg agcatcgtgg aaaaagaaga cgttccaacc 300

acgtcttcaa agcaagtgga ttgatgtgat aacatggtgg agcacgacac acttgtctac 360

tccaaaaata tcaaagatac agtctcagaa gaccaaaggg caattgagac ttttcaacaa 420

agggtaatat ccggaaacct cctcggattc cattgcccag ctatctgtca ctttattgtg 480

aagatagtgg aaaaggaagg tggctcctac aaatgccatc attgcgataa aggaaaggcc 540

atcgttgaag atgcctctgc cgacagtggt cccaaagatg gacccccacc cacgaggagc 600
atcgtggaaa aagaagacgt tccaaccacg tcttcaaagc aagtggattg atgtgatatc 660
tccactgacg taagggatga cgcacaatcc cactatcctt cgcaagaccc ttcctctata 720
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caatggatcg ttacaatgca gaatttgcat ttaaaggcct attttcttta gtttgaattt 2940
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tattttatgt aatttaattt ctttgtgagc tcctgtttag caggtcgtcc cttcagcaag 3060
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caagcttatc gacctgcaga tcgttcaaac atttggcaat aaagtttctt aagattgaat 3180
cctgttgccg gtcttgcgat gattatcata taatttctgt tgaattacgt taagcatgta 3240
ataattaaca tgtaatgcat gacgttattt atgagatggg tttttatgat tagagtcccg 3300
caattataca tttaatacgc gatagaaaac aaaatatagc gcgcaaacta ggataaatta 3360
tcgcgcgcgg tgtcatctat gttactagat 3390

<210> 85
<211> 549
<212> PRT
<213> 人工序列(Artificial Sequence)
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<221>
<222>
<223> 流感A/印度尼西亚/5/2005(H5N1)的具有缺失的蛋白水解环的氨基酸序列
<400> 85

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

165 170 175
Lys Lys Ser Tyr Asn Asn Thr Asn Gln Glu Asp Leu Leu Val Leu Trp
180 185 190
Gly Ile His His Pro Asn Asp Ala Ala Glu Gln Thr Arg Leu Tyr Gln
195 200 205
Asn Pro Thr Thr Tyr Ile Ser Ile Gly Thr Ser Thr Leu Asn Gln Arg
210 215 220
Leu Val Pro Lys Ile Ala Thr Arg Ser Lys Val Asn Gly Gln Ser Gly
225 230 235 240
Arg Met Glu Phe Phe Trp Thr Ile Leu Lys Pro Asn Asp Ala Ile Asn
245 250 255
Phe Glu Ser Asn Gly Asn Phe Ile Ala Pro Glu Tyr Ala Tyr Lys Ile
260 265 270
Val Lys Lys Gly Asp Ser Ala Ile Met Lys Ser Glu Leu Glu Tyr Gly
275 280 285
Asn Cys Asn Thr Lys Cys Gln Thr Pro Met Gly Ala Ile Asn Ser Ser
290 295 300
Met Pro Phe His Asn Ile His Pro Leu Thr Ile Gly Glu Cys Pro Lys
305 310 315 320
Tyr Val Lys Ser Asn Arg Leu Val Leu Ala Thr Gly Leu Arg Asn Ser
325 330 335
Pro Gly Gly Gly Trp Gln Gly Met Val Asp Gly Trp Tyr Gly Tyr His
340 345 350
His Ser Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Lys Glu Ser Thr
355 360 365
Gln Lys Ala Ile Asp Gly Val Thr Asn Lys Val Asn Ser Ile Ile Asp
370 375 380
Lys Met Asn Thr Gln Phe Glu Ala Val Gly Arg Glu Phe Asn Asn Leu
385 390 395 400
Glu Arg Arg Ile Glu Asn Leu Asn Lys Lys Met Glu Asp Gly Phe Leu
405 410 415
Asp Val Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Met Glu Asn Glu
420 425 430
Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Asp Lys
435 440 445
Val Arg Leu Gln Leu Arg Asp Asn Ala Lys Glu Leu Gly Asn Gly Cys
450 455 460
Phe Glu Phe Tyr His Lys Cys Asp Asn Glu Cys Met Glu Ser Ile Arg
465 470 475 480

Asn Gly Thr Tyr Asn Tyr Pro Gln Tyr Ser Glu Glu Ala Arg Leu Lys
485 490 495
Arg Glu Glu Ile Ser Gly Val Lys Leu Glu Ser Ile Gly Thr Tyr Gln
500 505 510
Ile Leu Ser Ile Tyr Ser Thr Val Ala Ser Ser Leu Ala Leu Ala Ile
515 520 525
Met Met Ala Gly Leu Ser Leu Trp Met Cys Ser Asn Gly Ser Leu Gln
530 535 540
Cys Arg Ile Cys Ile
545

<210> 86
<211> 40
<212> DNA
<213> 人工序列(Artificial Sequence)
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<221>
<222>
<223> 构建体1029 IF-S2+S4-B Bris.c
<400> 86
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<210> 87
<211> 43
<212> DNA
<213> 人工序列(Artificial Sequence)
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<221>
<222>
<223> 构建体1029 IF-S1a4-B Bris.r
<400> 87
actaaagaaa ataggccttt atagacagat ggagcaagaa aca 43

<210> 88
<211> 1758
<212> DNA
<213> 人工序列(Artificial Sequence)
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<223> 合成的HA B布里斯班基因
<400> 88

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gtgactggtg taataccact gacaacaaca cccaccaaat ctcattttgc aaatctcaaa 180
ggaacagaaa ccaggggggaa actatgccca aaatgcctca actgcacaga tctggacgta 240
gccttgggca gaccaaaatg cacggggaaa ataccctcgg caagagtttc aatactccat 300
gaagtcagac ctgttacatc tgggtgcttt cctataatgc acgacagaac aaaaattaga 360
cagctgccta accttctccg aggatacgaa catatcaggt tatcaaccca taacgttatc 420
aatgcagaaa atgcaccagg aggaccctac aaaattggaa cctcagggtc ttgccctaac 480
attaccaatg gaaacggatt tttcgcaaca atggcttggg ccgtcccaaa aaacgacaaa 540
aacaaaacag caacaaatcc attaacaata gaagtaccat acatttgtac agaaggagaa 600
gaccaaatta ccgtttgggg gttccactct gacaacgaga cccaaatggc aaagctctat 660
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tccttgcctt taattggaga agcagattgc ctccacgaaa aatacggtgg attaaacaaa 960
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<210> 89
<211> 3525
<212> DNA
<213> 人工序列(Artificial Sequence)
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<221>
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<223> 盒1029
<400> 89

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aactaggata aattatcgcg cgcggtgtca tctatgttac tagat 3525

<210> 90
<211> 594
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 来自流感B/布里斯班/60/2008(Influenza B/Brisbane/60/2008)的PDISP/HA的氨基酸序列

<400> 90

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

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

Trp Glu Gly Met Ile Ala Gly Trp His Gly Tyr Thr Ser His Gly Ala
385 390 395 400
His Gly Val Ala Val Ala Ala Asp Leu Lys Ser Thr Gln Glu Ala Ile
405 410 415
Asn Lys Ile Thr Lys Asn Leu Asn Ser Leu Ser Glu Leu Glu Val Lys
420 425 430
Asn Leu Gln Arg Leu Ser Gly Ala Met Asp Glu Leu His Asn Glu Ile
435 440 445
Leu Glu Leu Asp Glu Lys Val Asp Asp Leu Arg Ala Asp Thr Ile Ser
450 455 460
Ser Gln Ile Glu Leu Ala Val Leu Leu Ser Asn Glu Gly Ile Ile Asn
465 470 475 480
Ser Glu Asp Glu His Leu Leu Ala Leu Glu Arg Lys Leu Lys Lys Met
485 490 495
Leu Gly Pro Ser Ala Val Glu Ile Gly Asn Gly Cys Phe Glu Thr Lys
500 505 510
His Lys Cys Asn Gln Thr Cys Leu Asp Arg Ile Ala Ala Gly Thr Phe
515 520 525
Asp Ala Gly Glu Phe Ser Leu Pro Thr Phe Asp Ser Leu Asn Ile Thr
530 535 540
Ala Ala Ser Leu Asn Asp Asp Gly Leu Asp Asn His Thr Ile Leu Leu
545 550 555 560
Tyr Tyr Ser Thr Ala Ala Ser Ser Leu Ala Val Thr Leu Met Ile Ala
565 570 575
Ile Phe Val Val Tyr Met Val Ser Arg Asp Asn Val Ser Cys Ser Ile
580 585 590
Cys Leu

<210> 91
<211> 1734
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> PDISP/HA B布里斯班(PrL-)的核苷酸序列
<400> 91
atggcgaaaa acgttgcgat tttcggctta ttgttttctc ttcttgtgtt ggttccttct 60
cagatcttcg ccgatcgaat ctgcactgga ataacatcgt caaactcacc acatgtcgtc 120
aaaactgcta ctcaagggga ggtcaatgtg actggtgtaa taccactgac aacaacaccc 180

accaaatctc attttgcaaa tctcaaagga acagaaacca gggggaaact atgcccaaaa 240
tgcctcaact gcacagatct ggacgtagcc ttgggcagac caaaatgcac ggggaaaata 300
ccctcggcaa gagtttcaat actccatgaa gtcagacctg ttacatctgg gtgctttcct 360
ataatgcacg acagaacaaa aattagacag ctgcctaacc ttctccgagg atacgaacat 420
atcaggttat caacccataa cgttatcaat gcagaaaatg caccaggagg accctacaaa 480
attggaacct cagggtcttg ccctaacatt accaatggaa acggattttt cgcaacaatg 540
gcttgggccg tcccaaaaaa cgacaaaaac aaaacagcaa caaatccatt aacaatagaa 600
gtaccataca tttgtacaga aggagaagac caaattaccg tttgggggtt ccactctgac 660
aacgagaccc aaatggcaaa gctctatggg gactcaaagc cccagaagtt cacctcatct 720
gccaacggag tgaccacaca ttacgtttca cagattggtg gcttcccaaa tcaaacagaa 780
gacggaggac taccacaaag tggtagaatt gttgttgatt acatggtgca aaaatctggg 840
aaaacaggaa caattaccta tcaaaggggt attttattgc ctcaaaaggt gtggtgcgca 900
agtggcagga gcaaggtaat aaaaggatcc ttgcctttaa ttggagaagc agattgcctc 960
cacgaaaaat acggtggatt aaacaaaagc aagccttact acacagggga acatgcaaag 1020
gccataggaa attgcccaat atgggtgaaa acacccttga agctggccaa tggaaccaaa 1080
tatagacctc ctggtggagg atgggaagga atgattgcag gttggcacgg atacacatcc 1140
catggggcac atggagtagc ggtggcagca gaccttaaga gcactcaaga ggccataaac 1200
aagataacaa aaaatctcaa ctctttgagt gagctggaag taaagaatct tcaaagacta 1260
agcggtgcca tggatgaact ccacaacgaa atactagaac tagatgagaa agtggatgat 1320
ctcagagctg atacaataag ctcacaaata gaactcgcag tcctgctttc caatgaagga 1380
ataataaaca gtgaagatga acatctcttg gcgcttgaaa gaaagctgaa gaaaatgctg 1440
ggcccctctg ctgtagagat agggaatgga tgctttgaaa ccaaacacaa gtgcaaccag 1500
acctgtctcg acagaatagc tgctggtacc tttgatgcag gagaattttc tctccccacc 1560
tttgattcac tgaatattac tgctgcatct ttaaatgacg atggattgga taatcatact 1620
atactgcttt actactcaac tgctgcctcc agtttggctg taacactgat gatagctatc 1680
tttgttgttt atatggtctc cagagacaat gtttcttgct ccatctgtct ataa 1734

<210> 92
<211> 577
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> PDISP/HA B布里斯班(PrL-)的氨基酸序列
<400> 92

Met Ala Lys Asn Val Ala Ile Phe Gly Leu Leu Phe Ser Leu Leu Val
1 5 10 15
Leu Val Pro Ser Gln Ile Phe Ala Asp Arg Ile Cys Thr Gly Ile Thr
20 25 30

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

340 345 350
Leu Lys Leu Ala Asn Gly Thr Lys Tyr Arg Pro Pro Gly Gly Gly Trp
355 360 365
Glu Gly Met Ile Ala Gly Trp His Gly Tyr Thr Ser His Gly Ala His
370 375 380
Gly Val Ala Val Ala Ala Asp Leu Lys Ser Thr Gln Glu Ala Ile Asn
385 390 395 400
Lys Ile Thr Lys Asn Leu Asn Ser Leu Ser Glu Leu Glu Val Lys Asn
405 410 415
Leu Gln Arg Leu Ser Gly Ala Met Asp Glu Leu His Asn Glu Ile Leu
420 425 430
Glu Leu Asp Glu Lys Val Asp Asp Leu Arg Ala Asp Thr Ile Ser Ser
435 440 445
Gln Ile Glu Leu Ala Val Leu Leu Ser Asn Glu Gly Ile Ile Asn Ser
450 455 460
Glu Asp Glu His Leu Leu Ala Leu Glu Arg Lys Leu Lys Lys Met Leu
465 470 475 480
Gly Pro Ser Ala Val Glu Ile Gly Asn Gly Cys Phe Glu Thr Lys His
485 490 495
Lys Cys Asn Gln Thr Cys Leu Asp Arg Ile Ala Ala Gly Thr Phe Asp
500 505 510
Ala Gly Glu Phe Ser Leu Pro Thr Phe Asp Ser Leu Asn Ile Thr Ala
515 520 525
Ala Ser Leu Asn Asp Asp Gly Leu Asp Asn His Thr Ile Leu Leu Tyr
530 535 540
Tyr Ser Thr Ala Ala Ser Ser Leu Ala Val Thr Leu Met Ile Ala Ile
545 550 555 560
Phe Val Val Tyr Met Val Ser Arg Asp Asn Val Ser Cys Ser Ile Cys
565 570 575
Leu

<210> 93
<211> 160
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 表达增强子CPMVX
<400> 93

tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc 60
ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgcgtgagc 120
gatcttcaac gttgtcagat cgtgcttcgg caccagtaca 160
<210> 94
<211> 29
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 跨结构域膜的共有的氨基酸序列
<220>
<221>
<222>
<221> SITE
<222> (3)..(3)
<223> Xaa可为任何天然存在的氨基酸
<220>
<221>
<222>
<221> SITE
<222> (15)..(15)
<223> Xaa可为任何天然存在的氨基酸
<220>
<221>
<222>
<221> SITE
<222> (17)..(18)
<223> Xaa可为任何天然存在的氨基酸
<220>
<221>
<222>
<221> SITE
<222> (23)..(23)
<223> Xaa可为任何天然存在的氨基酸
<220>
<221>
<222>
<221> SITE

<222> (25)..(25)
<223> Xaa可为任何天然存在的氨基酸
<400> 94
Ile Leu Xaa Ile Tyr Tyr Ser Thr Val Ala Ile Ser Ser Leu Xaa Leu
1 5 10 15
Xaa Xaa Met Leu Ala Gly Xaa Ser Xaa Trp Met Cys Ser
20 25
<210> 95
<211> 1734
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT的核苷酸序列
<400> 95
atggcgaaaa acgttgcgat tttcggctta ttgttttctc ttcttgtgtt ggttccttct 60
cagatcttcg ccgatcgaat ctgcactgga ataacatcgt caaactcacc acatgtcgtc 120
aaaactgcta ctcaagggga ggtcaatgtg actggtgtaa taccactgac aacaacaccc 180
accaaatctc attttgcaaa tctcaaagga acagaaacca gggggaaact atgcccaaaa 240
tgcctcaact gcacagatct ggacgtagcc ttgggcagac caaaatgcac ggggaaaata 300
ccctcggcaa gagtttcaat actccatgaa gtcagacctg ttacatctgg gtgctttcct 360
ataatgcacg acagaacaaa aattagacag ctgcctaacc ttctccgagg atacgaacat 420
atcaggttat caacccataa cgttatcaat gcagaaaatg caccaggagg accctacaaa 480
attggaacct cagggtcttg ccctaacatt accaatggaa acggattttt cgcaacaatg 540
gcttgggccg tcccaaaaaa cgacaaaaac aaaacagcaa caaatccatt aacaatagaa 600
gtaccataca tttgtacaga aggagaagac caaattaccg tttgggggtt ccactctgac 660
aacgagaccc aaatggcaaa gctctatggg gactcaaagc cccagaagtt cacctcatct 720
gccaacggag tgaccacaca ttacgtttca cagattggtg gcttcccaaa tcaaacagaa 780
gacggaggac taccacaaag tggtagaatt gttgttgatt acatggtgca aaaatctggg 840
aaaacaggaa caattaccta tcaaaggggt attttattgc ctcaaaaggt gtggtgcgca 900
agtggcagga gcaaggtaat aaaaggatcc ttgcctttaa ttggagaagc agattgcctc 960
cacgaaaaat acggtggatt aaacaaaagc aagccttact acacagggga acatgcaaag 1020
gccataggaa attgcccaat atgggtgaaa acacccttga agctggccaa tggaaccaaa 1080
tatagacctc ctggtggagg atgggaagga atgattgcag gttggcacgg atacacatcc 1140
catggggcac atggagtagc ggtggcagca gaccttaaga gcactcaaga ggccataaac 1200
aagataacaa aaaatctcaa ctctttgagt gagctggaag taaagaatct tcaaagacta 1260
agcggtgcca tggatgaact ccacaacgaa atactagaac tagatgagaa agtggatgat 1320
ctcagagctg atacaataag ctcacaaata gaactcgcag tcctgctttc caatgaagga 1380

ataataaaca gtgaagatga acatctcttg gcgcttgaaa gaaagctgaa gaaaatgctg 1440
ggcccctctg ctgtagagat agggaatgga tgctttgaaa ccaaacacaa gtgcaaccag 1500
acctgtctcg acagaatagc tgctggtacc tttgatgcag gagaattttc tctccccacc 1560
tttgattcac tgaatattac tgctgcatct ttaaatgacg atggattgga taattaccag 1620
attttggcga tctattcaac tgtcgccagt tcattggtac tggtagtctc cctgggggca 1680
atcagtttct ggatgtgctc taatgggtct ctacagtgta gaatatgtat ttaa 1734

<210> 96
<211> 577
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT的氨基酸序列
<400> 96

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

Ala Thr Asn Pro Leu Thr Ile Glu Val Pro Tyr Ile Cys Thr Glu Gly
195 200 205
Glu Asp Gln Ile Thr Val Trp Gly Phe His Ser Asp Asn Glu Thr Gln
210 215 220
Met Ala Lys Leu Tyr Gly Asp Ser Lys Pro Gln Lys Phe Thr Ser Ser
225 230 235 240
Ala Asn Gly Val Thr Thr His Tyr Val Ser Gln Ile Gly Gly Phe Pro
245 250 255
Asn Gln Thr Glu Asp Gly Gly Leu Pro Gln Ser Gly Arg Ile Val Val
260 265 270
Asp Tyr Met Val Gln Lys Ser Gly Lys Thr Gly Thr Ile Thr Tyr Gln
275 280 285
Arg Gly Ile Leu Leu Pro Gln Lys Val Trp Cys Ala Ser Gly Arg Ser
290 295 300
Lys Val Ile Lys Gly Ser Leu Pro Leu Ile Gly Glu Ala Asp Cys Leu
305 310 315 320
His Glu Lys Tyr Gly Gly Leu Asn Lys Ser Lys Pro Tyr Tyr Thr Gly
325 330 335
Glu His Ala Lys Ala Ile Gly Asn Cys Pro Ile Trp Val Lys Thr Pro
340 345 350
Leu Lys Leu Ala Asn Gly Thr Lys Tyr Arg Pro Pro Gly Gly Gly Trp
355 360 365
Glu Gly Met Ile Ala Gly Trp His Gly Tyr Thr Ser His Gly Ala His
370 375 380
Gly Val Ala Val Ala Ala Asp Leu Lys Ser Thr Gln Glu Ala Ile Asn
385 390 395 400
Lys Ile Thr Lys Asn Leu Asn Ser Leu Ser Glu Leu Glu Val Lys Asn
405 410 415
Leu Gln Arg Leu Ser Gly Ala Met Asp Glu Leu His Asn Glu Ile Leu
420 425 430
Glu Leu Asp Glu Lys Val Asp Asp Leu Arg Ala Asp Thr Ile Ser Ser
435 440 445
Gln Ile Glu Leu Ala Val Leu Leu Ser Asn Glu Gly Ile Ile Asn Ser
450 455 460
Glu Asp Glu His Leu Leu Ala Leu Glu Arg Lys Leu Lys Lys Met Leu
465 470 475 480
Gly Pro Ser Ala Val Glu Ile Gly Asn Gly Cys Phe Glu Thr Lys His
485 490 495
Lys Cys Asn Gln Thr Cys Leu Asp Arg Ile Ala Ala Gly Thr Phe Asp

500 505 510

Ala Gly Glu Phe Ser Leu Pro Thr Phe Asp Ser Leu Asn Ile Thr Ala

515 520 525

Ala Ser Leu Asn Asp Asp Gly Leu Asp Asn Tyr Gln Ile Leu Ala Ile

530 535 540

Tyr Ser Thr Val Ala Ser Ser Leu Val Leu Val Val Ser Leu Gly Ala

545 550 555 560

Ile Ser Phe Trp Met Cys Ser Asn Gly Ser Leu Gln Cys Arg Ile Cys

565 570 575

Ile

<210> 97

<211> 1731

<212> DNA

<213> 人工序列(Artificial Sequence)

<220>

<221>

<222>

<223> PDISP/HA B马萨诸塞州(PrL-)的核苷酸序列

<400> 97

atggcgaaaa acgttgcgat tttcggctta ttgttttctc ttcttgtgtt ggttccttct 60
cagatcttcg ccgatcgaat ctgcactggg ataacatctt caaactcacc tcatgtggtc 120
aaaacagcta ctcaagggga ggtcaatgtg actggtgtga taccactaac aacaacacca 180
acaaaatctt attttgcaaa tctcaaagga acaaagacca gagggaaact atgcccagac 240
tgtctcaact gtacagatct ggatgtggcc ctgggcaggc caatgtgtgt gggaactaca 300
ccttctgcga aagcttcaat acttcacgaa gtcagacctg ttacatccgg gtgcttccct 360
ataatgcacg acagaacaaa aatcaggcaa ctagccaatc ttctcagagg atatgaaaat 420
atcaggttat caacccaaaa cgttatcgat gcagaaaagg caccaggagg accctacaga 480
cttggaacct caggatcttg ccctaacgct accagtaaaa gcggattttt cgcaacaatg 540
gcttgggctg tcccaaagga caacaacaaa aatgcaacga acccattaac agtagaagta 600
ccatacattt gtgcagaagg ggaagaccaa attactgttt gggggttcca ttcagataac 660
aaaacccaaa tgaagaacct ctatggagac tcaaatcctc aaaagttcac ctcatctgct 720
aatggagtaa ccacacatta tgtttctcag attggcggct tcccagatca aacagaagac 780
ggaggactac cacaaagcgg cagaattgtc gttgattaca tgatgcaaaa acctgggaaa 840
acaggaacaa ttgtctatca aagaggtgtt ttgttgcctc aaaaggtgtg gtgcgcgagt 900
ggcaggagca aagtaataaa agggtccttg cctttaattg gtgaagcaga ttgccttcat 960
gaaaaatacg gtggattaaa caaaagcaag ccttactaca caggagaaca tgcaaaagcc 1020
ataggaaatt gcccaatatg ggtgaaaaca cctttgaagc ttgccaatgg aaccaaatat 1080
agacctcctg gtggaggatg ggaaggaatg attgcaggtt ggcacggata cacatctcac 1140
ggagcacatg gagtggcagt tgctgcagac cttaagagca cacaagaagc tataaacaag 1200

ataacaaaaa atctcaactc tttgagtgag ctagaagtaa agaatcttca aaggctaagt 1260
ggtgccatgg atgaactcca caacgaaata ctcgagctgg atgagaaagt ggatgacctc 1320
agagctgaca ctataagttc acaaatagaa cttgcagtct tgctttccaa cgaaggaata 1380
ataaacagtg aagacgagca tctattggca cttgagagaa aactaaagaa aatgctgggt 1440
ccctctgctg tagacatagg aaatggatgc ttcgaaacca aacacaaatg caaccagacc 1500
tgcttagaca ggatagctgc tggcaccttt aatgcaggag agttttctct ccccactttt 1560
gattcattga acattactgc tgcatcttta aatgatgatg gattggataa ccatactata 1620
ctgctctatt actcaactgc tgcttctagt ttggctgtaa cattgatgct agctattttt 1680
attgtttata tggtctccag agacaacgtt tcatgctcca tctgtctata a 1731

<210> 98
<211> 576
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> PDISP/HA B马萨诸塞州(PrL-)的氨基酸序列
<400> 98

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

165 170 175
Phe Ala Thr Met Ala Trp Ala Val Pro Lys Asp Asn Asn Lys Asn Ala
180 185 190
Thr Asn Pro Leu Thr Val Glu Val Pro Tyr Ile Cys Ala Glu Gly Glu
195 200 205
Asp Gln Ile Thr Val Trp Gly Phe His Ser Asp Asn Lys Thr Gln Met
210 215 220
Lys Asn Leu Tyr Gly Asp Ser Asn Pro Gln Lys Phe Thr Ser Ser Ala
225 230 235 240
Asn Gly Val Thr Thr His Tyr Val Ser Gln Ile Gly Gly Phe Pro Asp
245 250 255
Gln Thr Glu Asp Gly Gly Leu Pro Gln Ser Gly Arg Ile Val Val Asp
260 265 270
Tyr Met Met Gln Lys Pro Gly Lys Thr Gly Thr Ile Val Tyr Gln Arg
275 280 285
Gly Val Leu Leu Pro Gln Lys Val Trp Cys Ala Ser Gly Arg Ser Lys
290 295 300
Val Ile Lys Gly Ser Leu Pro Leu Ile Gly Glu Ala Asp Cys Leu His
305 310 315 320
Glu Lys Tyr Gly Gly Leu Asn Lys Ser Lys Pro Tyr Tyr Thr Gly Glu
325 330 335
His Ala Lys Ala Ile Gly Asn Cys Pro Ile Trp Val Lys Thr Pro Leu
340 345 350
Lys Leu Ala Asn Gly Thr Lys Tyr Arg Pro Pro Gly Gly Gly Trp Glu
355 360 365
Gly Met Ile Ala Gly Trp His Gly Tyr Thr Ser His Gly Ala His Gly
370 375 380
Val Ala Val Ala Ala Asp Leu Lys Ser Thr Gln Glu Ala Ile Asn Lys
385 390 395 400
Ile Thr Lys Asn Leu Asn Ser Leu Ser Glu Leu Glu Val Lys Asn Leu
405 410 415
Gln Arg Leu Ser Gly Ala Met Asp Glu Leu His Asn Glu Ile Leu Glu
420 425 430
Leu Asp Glu Lys Val Asp Asp Leu Arg Ala Asp Thr Ile Ser Ser Gln
435 440 445
Ile Glu Leu Ala Val Leu Leu Ser Asn Glu Gly Ile Ile Asn Ser Glu
450 455 460
Asp Glu His Leu Leu Ala Leu Glu Arg Lys Leu Lys Lys Met Leu Gly
465 470 475 480

Pro Ser Ala Val Asp Ile Gly Asn Gly Cys Phe Glu Thr Lys His Lys
485 490 495
Cys Asn Gln Thr Cys Leu Asp Arg Ile Ala Ala Gly Thr Phe Asn Ala
500 505 510
Gly Glu Phe Ser Leu Pro Thr Phe Asp Ser Leu Asn Ile Thr Ala Ala
515 520 525
Ser Leu Asn Asp Asp Gly Leu Asp Asn His Thr Ile Leu Leu Tyr Tyr
530 535 540
Ser Thr Ala Ala Ser Ser Leu Ala Val Thr Leu Met Leu Ala Ile Phe
545 550 555 560
Ile Val Tyr Met Val Ser Arg Asp Asn Val Ser Cys Ser Ile Cys Leu
565 570 575

<210> 99
<211> 1731
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> PDISP/HA B马萨诸塞州(PrL-)+H1加利福尼亚TMCT的核苷酸序列
<400> 99

atggcgaaaa acgttgcgat tttcggctta ttgttttctc ttcttgtgtt ggttccttct 60
cagatcttcg ccgatcgaat ctgcactggg ataacatctt caaactcacc tcatgtggtc 120
aaaacagcta ctcaagggga ggtcaatgtg actggtgtga taccactaac aacaacacca 180
acaaaatctt attttgcaaa tctcaaagga acaaagacca gagggaaact atgcccagac 240
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ccttctgcga aagcttcaat acttcacgaa gtcagacctg ttacatccgg gtgcttccct 360
ataatgcacg acagaacaaa aatcaggcaa ctagccaatc ttctcagagg atatgaaaat 420
atcaggttat caacccaaaa cgttatcgat gcagaaaagg caccaggagg accctacaga 480
cttggaacct caggatcttg ccctaacgct accagtaaaa gcggattttt cgcaacaatg 540
gcttgggctg tcccaaagga caacaacaaa aatgcaacga acccattaac agtagaagta 600
ccatacattt gtgcagaagg ggaagaccaa attactgttt gggggttcca ttcagataac 660
aaaacccaaa tgaagaacct ctatggagac tcaaatcctc aaaagttcac ctcatctgct 720
aatggagtaa ccacacatta tgtttctcag attggcggct tcccagatca aacagaagac 780
ggaggactac cacaaagcgg cagaattgtc gttgattaca tgatgcaaaa acctgggaaa 840
acaggaacaa ttgtctatca aagaggtgtt ttgttgcctc aaaaggtgtg gtgcgcgagt 900
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gaaaaatacg gtggattaaa caaaagcaag ccttactaca caggagaaca tgcaaaagcc 1020
ataggaaatt gcccaatatg ggtgaaaaca cctttgaagc ttgccaatgg aaccaaatat 1080

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ataacaaaaa atctcaactc tttgagtgag ctagaagtaa agaatcttca aaggctaagt 1260
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agagctgaca ctataagttc acaaatagaa cttgcagtct tgctttccaa cgaaggaata 1380
ataaacagtg aagacgagca tctattggca cttgagagaa aactaaagaa aatgctgggt 1440
ccctctgctg tagacatagg aaatggatgc ttcgaaacca aacacaaatg caaccagacc 1500
tgcttagaca ggatagctgc tggcaccttt aatgcaggag agttttctct ccccactttt 1560
gattcattga acattactgc tgcatcttta aatgatgatg gattggataa ctaccagatt 1620
ttggcgatct attcaactgt cgccagttca ttggtactgg tagtctccct gggggcaatc 1680
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<210> 100
<211> 576
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> PDISP/HA B马萨诸塞州(PrL-)+H1加利福尼亚TMCT的氨基酸序列
<400> 100

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

145 150 155 160
Leu Gly Thr Ser Gly Ser Cys Pro Asn Ala Thr Ser Lys Ser Gly Phe
165 170 175
Phe Ala Thr Met Ala Trp Ala Val Pro Lys Asp Asn Asn Lys Asn Ala
180 185 190
Thr Asn Pro Leu Thr Val Glu Val Pro Tyr Ile Cys Ala Glu Gly Glu
195 200 205
Asp Gln Ile Thr Val Trp Gly Phe His Ser Asp Asn Lys Thr Gln Met
210 215 220
Lys Asn Leu Tyr Gly Asp Ser Asn Pro Gln Lys Phe Thr Ser Ser Ala
225 230 235 240
Asn Gly Val Thr Thr His Tyr Val Ser Gln Ile Gly Gly Phe Pro Asp
245 250 255
Gln Thr Glu Asp Gly Gly Leu Pro Gln Ser Gly Arg Ile Val Val Asp
260 265 270
Tyr Met Met Gln Lys Pro Gly Lys Thr Gly Thr Ile Val Tyr Gln Arg
275 280 285
Gly Val Leu Leu Pro Gln Lys Val Trp Cys Ala Ser Gly Arg Ser Lys
290 295 300
Val Ile Lys Gly Ser Leu Pro Leu Ile Gly Glu Ala Asp Cys Leu His
305 310 315 320
Glu Lys Tyr Gly Gly Leu Asn Lys Ser Lys Pro Tyr Tyr Thr Gly Glu
325 330 335
His Ala Lys Ala Ile Gly Asn Cys Pro Ile Trp Val Lys Thr Pro Leu
340 345 350
Lys Leu Ala Asn Gly Thr Lys Tyr Arg Pro Pro Gly Gly Gly Trp Glu
355 360 365
Gly Met Ile Ala Gly Trp His Gly Tyr Thr Ser His Gly Ala His Gly
370 375 380
Val Ala Val Ala Ala Asp Leu Lys Ser Thr Gln Glu Ala Ile Asn Lys
385 390 395 400
Ile Thr Lys Asn Leu Asn Ser Leu Ser Glu Leu Glu Val Lys Asn Leu
405 410 415
Gln Arg Leu Ser Gly Ala Met Asp Glu Leu His Asn Glu Ile Leu Glu
420 425 430
Leu Asp Glu Lys Val Asp Asp Leu Arg Ala Asp Thr Ile Ser Ser Gln
435 440 445
Ile Glu Leu Ala Val Leu Leu Ser Asn Glu Gly Ile Ile Asn Ser Glu
450 455 460

Asp Glu His Leu Leu Ala Leu Glu Arg Lys Leu Lys Lys Met Leu Gly
465 470 475 480
Pro Ser Ala Val Asp Ile Gly Asn Gly Cys Phe Glu Thr Lys His Lys
485 490 495
Cys Asn Gln Thr Cys Leu Asp Arg Ile Ala Ala Gly Thr Phe Asn Ala
500 505 510
Gly Glu Phe Ser Leu Pro Thr Phe Asp Ser Leu Asn Ile Thr Ala Ala
515 520 525
Ser Leu Asn Asp Asp Gly Leu Asp Asn Tyr Gln Ile Leu Ala Ile Tyr
530 535 540
Ser Thr Val Ala Ser Ser Leu Val Leu Val Val Ser Leu Gly Ala Ile
545 550 555 560
Ser Phe Trp Met Cys Ser Asn Gly Ser Leu Gln Cys Arg Ile Cys Ile
565 570 575

<210> 101
<211> 1704
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> HA B威斯康星州(PrL-)的核苷酸序列
<400> 101
atgaaggcaa taattgtact actcatggta gtaacatcca atgcagatcg aatctgcact 60
gggataacat cttcaaactc acctcatgtg gtcaaaacag ctactcaagg ggaggtcaat 120
gtgactggcg tgataccact gacaacaaca ccaacaaaat cttattttgc aaatctcaaa 180
ggaacaagga ccagagggaa actatgcccg gactgtctca actgtacaga tctggatgtg 240
gccttgggca ggccaatgtg tgtggggacc acaccttctg ctaaagcttc aatactccac 300
gaggtcagac ctgttacatc cgggtgcttt cctataatgc acgacagaac aaaaatcagg 360
caactaccca atcttctcag aggatatgaa aatatcaggt tatcaaccca aaacgttatc 420
gatgcagaaa aagcaccagg aggaccctac agacttggaa cctcaggatc ttgccctaac 480
gctaccagta aaatcggatt ttttgcaaca atggcttggg ctgtcccaaa ggacaactac 540
aaaaatgcaa cgaacccact aacagtagaa gtaccataca tttgtacaga aggggaagac 600
caaattactg tttgggggtt ccattcagat aacaaaaccc aaatgaagag cctctatgga 660
gactcaaatc ctcaaaagtt cacctcatct gctaatggag taaccacaca ttatgtttct 720
cagattggcg acttcccaga tcaaacagaa gacggaggac taccacaaag cggcagaatt 780
gttgttgatt acatgatgca aaaacctggg aaaacaggaa caattgtcta tcaaagaggt 840
gttttgttgc ctcaaaaggt gtggtgcgcg agtggcagga gcaaagtaat aaaagggtca 900
ttgcctttaa ttggtgaagc agattgcctt catgaaaaat acggtggatt aaacaaaagc 960

aagccttact acacaggaga acatgcaaaa gccataggaa attgcccaat atgggtaaaa 1020
acacctttga agcttgccaa tggaaccaaa tatagacctc ctggtggagg atgggaagga 1080
atgattgcag gttggcacgg atacacatct cacggagcac atggagtggc agtggcggca 1140
gaccttaaga gtacacaaga agctataaat aagataacaa aaaatctcaa ttctttgagt 1200
gagctagaag taaagaacct tcaaagacta agtggtgcca tggatgaact ccacaacgaa 1260
atactcgagc tggatgagaa agtggatgat ctcagagctg acactataag ctcacaaata 1320
gaacttgcag tcttgctttc caacgaagga ataataaaca gtgaagacga gcatctattg 1380
gcacttgaga gaaaactaaa gaaaatgctg ggtccctctg ctgtagacat aggaaacgga 1440
tgcttcgaaa ccaaacacaa atgcaaccag acctgcttag acaggatagc tgctggcacc 1500
tttaatgcag gagaattttc tctccccact tttgattcat tgaacattac tgctgcatct 1560
ttaaatgatg atggattgga taaccatact atactgctct attactcaac tgctgcttct 1620
agtttggctg taacattaat gctagctatt tttattgttt atatggtctc cagagacaac 1680
gtttcatgct ccatctgtct ataa 1704

<210> 102
<211> 567
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> HA B威斯康星州(PrL-)的氨基酸序列
<400> 102

Met Lys Ala Ile Ile Val Leu Leu Met Val Val Thr Ser Asn Ala Asp
1 5 10 15
Arg Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His Val Val Lys
20 25 30
Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Val Ile Pro Leu Thr
35 40 45
Thr Thr Pro Thr Lys Ser Tyr Phe Ala Asn Leu Lys Gly Thr Arg Thr
50 55 60
Arg Gly Lys Leu Cys Pro Asp Cys Leu Asn Cys Thr Asp Leu Asp Val
65 70 75 80
Ala Leu Gly Arg Pro Met Cys Val Gly Thr Thr Pro Ser Ala Lys Ala
85 90 95
Ser Ile Leu His Glu Val Arg Pro Val Thr Ser Gly Cys Phe Pro Ile
100 105 110
Met His Asp Arg Thr Lys Ile Arg Gln Leu Pro Asn Leu Leu Arg Gly
115 120 125
Tyr Glu Asn Ile Arg Leu Ser Thr Gln Asn Val Ile Asp Ala Glu Lys

130 135 140
Ala Pro Gly Gly Pro Tyr Arg Leu Gly Thr Ser Gly Ser Cys Pro Asn
145 150 155 160
Ala Thr Ser Lys Ile Gly Phe Phe Ala Thr Met Ala Trp Ala Val Pro
165 170 175
Lys Asp Asn Tyr Lys Asn Ala Thr Asn Pro Leu Thr Val Glu Val Pro
180 185 190
Tyr Ile Cys Thr Glu Gly Glu Asp Gln Ile Thr Val Trp Gly Phe His
195 200 205
Ser Asp Asn Lys Thr Gln Met Lys Ser Leu Tyr Gly Asp Ser Asn Pro
210 215 220
Gln Lys Phe Thr Ser Ser Ala Asn Gly Val Thr Thr His Tyr Val Ser
225 230 235 240
Gln Ile Gly Asp Phe Pro Asp Gln Thr Glu Asp Gly Gly Leu Pro Gln
245 250 255
Ser Gly Arg Ile Val Val Asp Tyr Met Met Gln Lys Pro Gly Lys Thr
260 265 270
Gly Thr Ile Val Tyr Gln Arg Gly Val Leu Leu Pro Gln Lys Val Trp
275 280 285
Cys Ala Ser Gly Arg Ser Lys Val Ile Lys Gly Ser Leu Pro Leu Ile
290 295 300
Gly Glu Ala Asp Cys Leu His Glu Lys Tyr Gly Gly Leu Asn Lys Ser
305 310 315 320
Lys Pro Tyr Tyr Thr Gly Glu His Ala Lys Ala Ile Gly Asn Cys Pro
325 330 335
Ile Trp Val Lys Thr Pro Leu Lys Leu Ala Asn Gly Thr Lys Tyr Arg
340 345 350
Pro Pro Gly Gly Gly Trp Glu Gly Met Ile Ala Gly Trp His Gly Tyr
355 360 365
Thr Ser His Gly Ala His Gly Val Ala Val Ala Ala Asp Leu Lys Ser
370 375 380
Thr Gln Glu Ala Ile Asn Lys Ile Thr Lys Asn Leu Asn Ser Leu Ser
385 390 395 400
Glu Leu Glu Val Lys Asn Leu Gln Arg Leu Ser Gly Ala Met Asp Glu
405 410 415
Leu His Asn Glu Ile Leu Glu Leu Asp Glu Lys Val Asp Asp Leu Arg
420 425 430
Ala Asp Thr Ile Ser Ser Gln Ile Glu Leu Ala Val Leu Leu Ser Asn
435 440 445

Glu Gly Ile Ile Asn Ser Glu Asp Glu His Leu Leu Ala Leu Glu Arg
450 455 460
Lys Leu Lys Lys Met Leu Gly Pro Ser Ala Val Asp Ile Gly Asn Gly
465 470 475 480
Cys Phe Glu Thr Lys His Lys Cys Asn Gln Thr Cys Leu Asp Arg Ile
485 490 495
Ala Ala Gly Thr Phe Asn Ala Gly Glu Phe Ser Leu Pro Thr Phe Asp
500 505 510
Ser Leu Asn Ile Thr Ala Ala Ser Leu Asn Asp Asp Gly Leu Asp Asn
515 520 525
His Thr Ile Leu Leu Tyr Tyr Ser Thr Ala Ala Ser Ser Leu Ala Val
530 535 540
Thr Leu Met Leu Ala Ile Phe Ile Val Tyr Met Val Ser Arg Asp Asn
545 550 555 560
Val Ser Cys Ser Ile Cys Leu
565

<210> 103
<211> 1704
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> HA B威斯康星州(PrL-)+H1加利福尼亚TMCT的核苷酸序列
<400> 103
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gggataacat cttcaaactc acctcatgtg gtcaaaacag ctactcaagg ggaggtcaat 120
gtgactggcg tgataccact gacaacaaca ccaacaaaat cttattttgc aaatctcaaa 180
ggaacaagga ccagagggaa actatgcccg gactgtctca actgtacaga tctggatgtg 240
gccttgggca ggccaatgtg tgtggggacc acaccttctg ctaaagcttc aatactccac 300
gaggtcagac ctgttacatc cgggtgcttt cctataatgc acgacagaac aaaaatcagg 360
caactaccca atcttctcag aggatatgaa aatatcaggt tatcaaccca aaacgttatc 420
gatgcagaaa aagcaccagg aggaccctac agacttggaa cctcaggatc ttgccctaac 480
gctaccagta aaatcggatt ttttgcaaca atggcttggg ctgtcccaaa ggacaactac 540
aaaaatgcaa cgaacccact aacagtagaa gtaccataca tttgtacaga aggggaagac 600
caaattactg tttgggggtt ccattcagat aacaaaaccc aaatgaagag cctctatgga 660
gactcaaatc ctcaaaagtt cacctcatct gctaatggag taaccacaca ttatgtttct 720
cagattggcg acttcccaga tcaaacagaa gacggaggac taccacaaag cggcagaatt 780
gttgttgatt acatgatgca aaaacctggg aaaacaggaa caattgtcta tcaaagaggt 840

gttttgttgc ctcaaaaggt gtggtgcgcg agtggcagga gcaaagtaat aaaagggtca 900
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aagccttact acacaggaga acatgcaaaa gccataggaa attgcccaat atgggtaaaa 1020
acacctttga agcttgccaa tggaaccaaa tatagacctc ctggtggagg atgggaagga 1080
atgattgcag gttggcacgg atacacatct cacggagcac atggagtggc agtggcggca 1140
gaccttaaga gtacacaaga agctataaat aagataacaa aaaatctcaa ttctttgagt 1200
gagctagaag taaagaacct tcaaagacta agtggtgcca tggatgaact ccacaacgaa 1260
atactcgagc tggatgagaa agtggatgat ctcagagctg acactataag ctcacaaata 1320
gaacttgcag tcttgctttc caacgaagga ataataaaca gtgaagacga gcatctattg 1380
gcacttgaga gaaaactaaa gaaaatgctg ggtccctctg ctgtagacat aggaaacgga 1440
tgcttcgaaa ccaaacacaa atgcaaccag acctgcttag acaggatagc tgctggcacc 1500
tttaatgcag gagaattttc tctccccact tttgattcat tgaacattac tgctgcatct 1560
ttaaatgatg atggattgga taactaccag attttggcga tctattcaac tgtcgccagt 1620
tcattggtac tggtagtctc cctgggggca atcagtttct ggatgtgctc taatgggtct 1680
ctacagtgta gaatatgtat ttaa 1704

<210> 104
<211> 567
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> HA B威斯康星州(PrL-)+H1加利福尼亚TMCT的氨基酸序列
<400> 104

Met Lys Ala Ile Ile Val Leu Leu Met Val Val Thr Ser Asn Ala Asp
1 5 10 15
Arg Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His Val Val Lys
20 25 30
Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Val Ile Pro Leu Thr
35 40 45
Thr Thr Pro Thr Lys Ser Tyr Phe Ala Asn Leu Lys Gly Thr Arg Thr
50 55 60
Arg Gly Lys Leu Cys Pro Asp Cys Leu Asn Cys Thr Asp Leu Asp Val
65 70 75 80
Ala Leu Gly Arg Pro Met Cys Val Gly Thr Thr Pro Ser Ala Lys Ala
85 90 95
Ser Ile Leu His Glu Val Arg Pro Val Thr Ser Gly Cys Phe Pro Ile
100 105 110
Met His Asp Arg Thr Lys Ile Arg Gln Leu Pro Asn Leu Leu Arg Gly

115 120 125

Tyr Glu Asn Ile Arg Leu Ser Thr Gln Asn Val Ile Asp Ala Glu Lys
130 135 140

Ala Pro Gly Gly Pro Tyr Arg Leu Gly Thr Ser Gly Ser Cys Pro Asn
145 150 155 160

Ala Thr Ser Lys Ile Gly Phe Phe Ala Thr Met Ala Trp Ala Val Pro
165 170 175

Lys Asp Asn Tyr Lys Asn Ala Thr Asn Pro Leu Thr Val Glu Val Pro
180 185 190

Tyr Ile Cys Thr Glu Gly Glu Asp Gln Ile Thr Val Trp Gly Phe His
195 200 205

Ser Asp Asn Lys Thr Gln Met Lys Ser Leu Tyr Gly Asp Ser Asn Pro
210 215 220

Gln Lys Phe Thr Ser Ser Ala Asn Gly Val Thr Thr His Tyr Val Ser
225 230 235 240

Gln Ile Gly Asp Phe Pro Asp Gln Thr Glu Asp Gly Gly Leu Pro Gln
245 250 255

Ser Gly Arg Ile Val Val Asp Tyr Met Met Gln Lys Pro Gly Lys Thr
260 265 270

Gly Thr Ile Val Tyr Gln Arg Gly Val Leu Leu Pro Gln Lys Val Trp
275 280 285

Cys Ala Ser Gly Arg Ser Lys Val Ile Lys Gly Ser Leu Pro Leu Ile
290 295 300

Gly Glu Ala Asp Cys Leu His Glu Lys Tyr Gly Gly Leu Asn Lys Ser
305 310 315 320

Lys Pro Tyr Tyr Thr Gly Glu His Ala Lys Ala Ile Gly Asn Cys Pro
325 330 335

Ile Trp Val Lys Thr Pro Leu Lys Leu Ala Asn Gly Thr Lys Tyr Arg
340 345 350

Pro Pro Gly Gly Gly Trp Glu Gly Met Ile Ala Gly Trp His Gly Tyr
355 360 365

Thr Ser His Gly Ala His Gly Val Ala Val Ala Ala Asp Leu Lys Ser
370 375 380

Thr Gln Glu Ala Ile Asn Lys Ile Thr Lys Asn Leu Asn Ser Leu Ser
385 390 395 400

Glu Leu Glu Val Lys Asn Leu Gln Arg Leu Ser Gly Ala Met Asp Glu
405 410 415

Leu His Asn Glu Ile Leu Glu Leu Asp Glu Lys Val Asp Asp Leu Arg
420 425 430

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

<210> 105
<211> 1734
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT的核苷酸序列
<400> 105
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cagatcttcg ccgatcgaat ctgcactgga ataacatcgt caaactcacc acatgtcgtc 120
aaaactgcta ctcaagggga ggtcaatgtg actggtgtaa taccactgac aacaacaccc 180
accaaatctc attttgcaaa tctcaaagga acagaaacca gggggaaact atgcccaaaa 240
tgcctcaact gcacagatct ggacgtagcc ttgggcagac caaaatgcac ggggaaaata 300
ccctcggcaa gagtttcaat actccatgaa gtcagacctg ttacatctgg gtgctttcct 360
ataatgcacg acagaacaaa aattagacag ctgcctaacc ttctccgagg atacgaacat 420
atcaggttat caacccataa cgttatcaat gcagaaaatg caccaggagg accctacaaa 480
attggaacct cagggtcttg ccctaacatt accaatggaa acggattttt cgcaacaatg 540
gcttgggccg tcccaaaaaa cgacaaaaac aaaacagcaa caaatccatt aacaatagaa 600
gtaccataca tttgtacaga aggagaagac caaattaccg tttgggggtt ccactctgac 660
aacgagaccc aaatggcaaa gctctatggg gactcaaagc cccagaagtt cacctcatct 720

gccaacggag tgaccacaca ttacgtttca cagattggtg gcttcccaaa tcaaacagaa 780
gacggaggac taccacaaag tggtagaatt gttgttgatt acatggtgca aaaatctggg 840
aaaacaggaa caattaccta tcaaaggggt attttattgc ctcaaaaggt gtggtgcgca 900
agtggcagga gcaaggtaat aaaaggatcc ttgcctttaa ttggagaagc agattgcctc 960
cacgaaaaat acggtggatt aaacaaaagc aagccttact acacagggga acatgcaaag 1020
gccataggaa attgcccaat atgggtgaaa acacccttga agctggccaa tggaaccaaa 1080
tatagacctc ctggtggagg atgggaagga atgattgcag gttggcacgg atacacatcc 1140
catggggcac atggagtagc ggtggcagca gaccttaaga gcactcaaga ggccataaac 1200
aagataacaa aaaatctcaa ctctttgagt gagctggaag taaagaatct tcaaagacta 1260
agcggtgcca tggatgaact ccacaacgaa atactagaac tagatgagaa agtggatgat 1320
ctcagagctg atacaataag ctcacaaata gaactcgcag tcctgctttc caatgaagga 1380
ataataaaca gtgaagatga acatctcttg gcgcttgaaa gaaagctgaa gaaaatgctg 1440
ggcccctctg ctgtagagat agggaatgga tgctttgaaa ccaaacacaa gtgcaaccag 1500
acctgtctcg acagaatagc tgctggtacc tttgatgcag gagaattttc tctccccacc 1560
tttgattcac tgaatattac tgctgcatct ttaaatgacg atggattgga taattaccag 1620
attttggcga tctattcaac tgtcgccagt tcattggtac tggtagtctc cctgggggca 1680
atcagtttct ggatgtgctc taatgggtct ctacagtgta gaatatgtat ttaa 1734

<210> 106
<211> 577
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT的氨基酸序列
<400> 106

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

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

Leu Gln Arg Leu Ser Gly Ala Met Asp Glu Leu His Asn Glu Ile Leu
420 425 430
Glu Leu Asp Glu Lys Val Asp Asp Leu Arg Ala Asp Thr Ile Ser Ser
435 440 445
Gln Ile Glu Leu Ala Val Leu Leu Ser Asn Glu Gly Ile Ile Asn Ser
450 455 460
Glu Asp Glu His Leu Leu Ala Leu Glu Arg Lys Leu Lys Lys Met Leu
465 470 475 480
Gly Pro Ser Ala Val Glu Ile Gly Asn Gly Cys Phe Glu Thr Lys His
485 490 495
Lys Cys Asn Gln Thr Cys Leu Asp Arg Ile Ala Ala Gly Thr Phe Asp
500 505 510
Ala Gly Glu Phe Ser Leu Pro Thr Phe Asp Ser Leu Asn Ile Thr Ala
515 520 525
Ala Ser Leu Asn Asp Asp Gly Leu Asp Asn Tyr Gln Ile Leu Ala Ile
530 535 540
Tyr Ser Thr Val Ala Ser Ser Leu Val Leu Val Val Ser Leu Gly Ala
545 550 555 560
Ile Ser Phe Trp Met Cys Ser Asn Gly Ser Leu Gln Cys Arg Ile Cys
565 570 575
Ile

<210> 107
<211> 1731
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> PDISP/HA B马萨诸塞州(PrL-)的核苷酸序列
<400> 107

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cagatcttcg ccgatcgaat ctgcactggg ataacatctt caaactcacc tcatgtggtc 120
aaaacagcta ctcaagggga ggtcaatgtg actggtgtga taccactaac aacaacacca 180
acaaaatctt attttgcaaa tctcaaagga acaaagacca gagggaaact atgcccagac 240
tgtctcaact gtacagatct ggatgtggcc ctgggcaggc caatgtgtgt gggaactaca 300
ccttctgcga aagcttcaat acttcacgaa gtcagacctg ttacatccgg gtgcttccct 360
ataatgcacg acagaacaaa aatcaggcaa ctagccaatc ttctcagagg atatgaaaat 420
atcaggttat caacccaaaa cgttatcgat gcagaaaagg caccaggagg accctacaga 480
cttggaacct caggatcttg ccctaacgct accagtaaaa gcggattttt cgcaacaatg 540

gcttgggctg tcccaaagga caacaacaaa aatgcaacga acccattaac agtagaagta 600
ccatacattt gtgcagaagg ggaagaccaa attactgttt gggggttcca ttcagataac 660
aaaacccaaa tgaagaacct ctatggagac tcaaatcctc aaaagttcac ctcatctgct 720
aatggagtaa ccacacatta tgtttctcag attggcggct tcccagatca aacagaagac 780
ggaggactac cacaaagcgg cagaattgtc gttgattaca tgatgcaaaa acctgggaaa 840
acaggaacaa ttgtctatca aagaggtgtt ttgttgcctc aaaaggtgtg gtgcgcgagt 900
ggcaggagca aagtaataaa agggtccttg cctttaattg gtgaagcaga ttgccttcat 960
gaaaaatacg gtggattaaa caaaagcaag ccttactaca caggagaaca tgcaaaagcc 1020
ataggaaatt gcccaatatg ggtgaaaaca cctttgaagc ttgccaatgg aaccaaatat 1080
agacctcctg gtggaggatg ggaaggaatg attgcaggtt ggcacggata cacatctcac 1140
ggagcacatg gagtggcagt tgctgcagac cttaagagca cacaagaagc tataaacaag 1200
ataacaaaaa atctcaactc tttgagtgag ctagaagtaa agaatcttca aaggctaagt 1260
ggtgccatgg atgaactcca caacgaaata ctcgagctgg atgagaaagt ggatgacctc 1320
agagctgaca ctataagttc acaaatagaa cttgcagtct tgctttccaa cgaaggaata 1380
ataaacagtg aagacgagca tctattggca cttgagagaa aactaaagaa aatgctgggt 1440
ccctctgctg tagacatagg aaatggatgc ttcgaaacca aacacaaatg caaccagacc 1500
tgcttagaca ggatagctgc tggcaccttt aatgcaggag agttttctct ccccactttt 1560
gattcattga acattactgc tgcatcttta aatgatgatg gattggataa ccatactata 1620
ctgctctatt actcaactgc tgcttctagt ttggctgtaa cattgatgct agctattttt 1680
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<210> 108
<211> 576
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> PDISP/HA B马萨诸塞州(PrL-)的氨基酸序列
<400> 108

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

Cys Leu Asn Cys Thr Asp Leu Asp Val Ala Leu Gly Arg Pro Met Cys
85 90 95
Val Gly Thr Thr Pro Ser Ala Lys Ala Ser Ile Leu His Glu Val Arg
100 105 110
Pro Val Thr Ser Gly Cys Phe Pro Ile Met His Asp Arg Thr Lys Ile
115 120 125
Arg Gln Leu Ala Asn Leu Leu Arg Gly Tyr Glu Asn Ile Arg Leu Ser
130 135 140
Thr Gln Asn Val Ile Asp Ala Glu Lys Ala Pro Gly Gly Pro Tyr Arg
145 150 155 160
Leu Gly Thr Ser Gly Ser Cys Pro Asn Ala Thr Ser Lys Ser Gly Phe
165 170 175
Phe Ala Thr Met Ala Trp Ala Val Pro Lys Asp Asn Asn Lys Asn Ala
180 185 190
Thr Asn Pro Leu Thr Val Glu Val Pro Tyr Ile Cys Ala Glu Gly Glu
195 200 205
Asp Gln Ile Thr Val Trp Gly Phe His Ser Asp Asn Lys Thr Gln Met
210 215 220
Lys Asn Leu Tyr Gly Asp Ser Asn Pro Gln Lys Phe Thr Ser Ser Ala
225 230 235 240
Asn Gly Val Thr Thr His Tyr Val Ser Gln Ile Gly Gly Phe Pro Asp
245 250 255
Gln Thr Glu Asp Gly Gly Leu Pro Gln Ser Gly Arg Ile Val Val Asp
260 265 270
Tyr Met Met Gln Lys Pro Gly Lys Thr Gly Thr Ile Val Tyr Gln Arg
275 280 285
Gly Val Leu Leu Pro Gln Lys Val Trp Cys Ala Ser Gly Arg Ser Lys
290 295 300
Val Ile Lys Gly Ser Leu Pro Leu Ile Gly Glu Ala Asp Cys Leu His
305 310 315 320
Glu Lys Tyr Gly Gly Leu Asn Lys Ser Lys Pro Tyr Tyr Thr Gly Glu
325 330 335
His Ala Lys Ala Ile Gly Asn Cys Pro Ile Trp Val Lys Thr Pro Leu
340 345 350
Lys Leu Ala Asn Gly Thr Lys Tyr Arg Pro Pro Gly Gly Gly Trp Glu
355 360 365
Gly Met Ile Ala Gly Trp His Gly Tyr Thr Ser His Gly Ala His Gly
370 375 380
Val Ala Val Ala Ala Asp Leu Lys Ser Thr Gln Glu Ala Ile Asn Lys

385 390 395 400
Ile Thr Lys Asn Leu Asn Ser Leu Ser Glu Leu Glu Val Lys Asn Leu
405 410 415
Gln Arg Leu Ser Gly Ala Met Asp Glu Leu His Asn Glu Ile Leu Glu
420 425 430
Leu Asp Glu Lys Val Asp Asp Leu Arg Ala Asp Thr Ile Ser Ser Gln
435 440 445
Ile Glu Leu Ala Val Leu Leu Ser Asn Glu Gly Ile Ile Asn Ser Glu
450 455 460
Asp Glu His Leu Leu Ala Leu Glu Arg Lys Leu Lys Lys Met Leu Gly
465 470 475 480
Pro Ser Ala Val Asp Ile Gly Asn Gly Cys Phe Glu Thr Lys His Lys
485 490 495
Cys Asn Gln Thr Cys Leu Asp Arg Ile Ala Ala Gly Thr Phe Asn Ala
500 505 510
Gly Glu Phe Ser Leu Pro Thr Phe Asp Ser Leu Asn Ile Thr Ala Ala
515 520 525
Ser Leu Asn Asp Asp Gly Leu Asp Asn His Thr Ile Leu Leu Tyr Tyr
530 535 540
Ser Thr Ala Ala Ser Ser Leu Ala Val Thr Leu Met Leu Ala Ile Phe
545 550 555 560
Ile Val Tyr Met Val Ser Arg Asp Asn Val Ser Cys Ser Ile Cys Leu
565 570 575

<210> 109
<211> 1731
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> PDISP/HA B马萨诸塞州(PrL-)+H1加利福尼亚TMCT的核苷酸序列
<400> 109
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cagatcttcg ccgatcgaat ctgcactggg ataacatctt caaactcacc tcatgtggtc 120
aaaacagcta ctcaagggga ggtcaatgtg actggtgtga taccactaac aacaacacca 180
acaaaatctt attttgcaaa tctcaaagga acaaagacca gagggaaact atgcccagac 240
tgtctcaact gtacagatct ggatgtggcc ctgggcaggc caatgtgtgt gggaactaca 300
ccttctgcga aagcttcaat acttcacgaa gtcagacctg ttacatccgg gtgcttccct 360
ataatgcacg acagaacaaa aatcaggcaa ctagccaatc ttctcagagg atatgaaaat 420

atcaggttat caacccaaaa cgttatcgat gcagaaaagg caccaggagg accctacaga 480
cttggaacct caggatcttg ccctaacgct accagtaaaa gcggattttt cgcaacaatg 540
gcttgggctg tcccaaagga caacaacaaa aatgcaacga acccattaac agtagaagta 600
ccatacattt gtgcagaagg ggaagaccaa attactgttt gggggttcca ttcagataac 660
aaaacccaaa tgaagaacct ctatggagac tcaaatcctc aaaagttcac ctcatctgct 720
aatggagtaa ccacacatta tgtttctcag attggcggct tcccagatca aacagaagac 780
ggaggactac cacaaagcgg cagaattgtc gttgattaca tgatgcaaaa acctgggaaa 840
acaggaacaa ttgtctatca aagaggtgtt ttgttgcctc aaaaggtgtg gtgcgcgagt 900
ggcaggagca aagtaataaa agggtccttg cctttaattg gtgaagcaga ttgccttcat 960
gaaaaatacg gtggattaaa caaaagcaag ccttactaca caggagaaca tgcaaaagcc 1020
ataggaaatt gcccaatatg ggtgaaaaca cctttgaagc ttgccaatgg aaccaaatat 1080
agacctcctg gtggaggatg ggaaggaatg attgcaggtt ggcacggata cacatctcac 1140
ggagcacatg gagtggcagt tgctgcagac cttaagagca cacaagaagc tataaacaag 1200
ataacaaaaa atctcaactc tttgagtgag ctagaagtaa agaatcttca aaggctaagt 1260
ggtgccatgg atgaactcca caacgaaata ctcgagctgg atgagaaagt ggatgacctc 1320
agagctgaca ctataagttc acaaatagaa cttgcagtct tgctttccaa cgaaggaata 1380
ataaacagtg aagacgagca tctattggca cttgagagaa aactaaagaa aatgctgggt 1440
ccctctgctg tagacatagg aaatggatgc ttcgaaacca aacacaaatg caaccagacc 1500
tgcttagaca ggatagctgc tggcaccttt aatgcaggag agttttctct ccccactttt 1560
gattcattga acattactgc tgcatcttta aatgatgatg gattggataa ctaccagatt 1620
ttggcgatct attcaactgt cgccagttca ttggtactgg tagtctccct gggggcaatc 1680
agtttctgga tgtgctctaa tgggtctcta cagtgtagaa tatgtattta a 1731

<210> 110
<211> 576
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> PDISP/HA B马萨诸塞州(PrL-)+H1加利福尼亚TMCT的氨基酸序列
<400> 110

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

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

370 375 380
Val Ala Val Ala Ala Asp Leu Lys Ser Thr Gln Glu Ala Ile Asn Lys
385 390 395 400
Ile Thr Lys Asn Leu Asn Ser Leu Ser Glu Leu Glu Val Lys Asn Leu
405 410 415
Gln Arg Leu Ser Gly Ala Met Asp Glu Leu His Asn Glu Ile Leu Glu
420 425 430
Leu Asp Glu Lys Val Asp Asp Leu Arg Ala Asp Thr Ile Ser Ser Gln
435 440 445
Ile Glu Leu Ala Val Leu Leu Ser Asn Glu Gly Ile Ile Asn Ser Glu
450 455 460
Asp Glu His Leu Leu Ala Leu Glu Arg Lys Leu Lys Lys Met Leu Gly
465 470 475 480
Pro Ser Ala Val Asp Ile Gly Asn Gly Cys Phe Glu Thr Lys His Lys
485 490 495
Cys Asn Gln Thr Cys Leu Asp Arg Ile Ala Ala Gly Thr Phe Asn Ala
500 505 510
Gly Glu Phe Ser Leu Pro Thr Phe Asp Ser Leu Asn Ile Thr Ala Ala
515 520 525
Ser Leu Asn Asp Asp Gly Leu Asp Asn Tyr Gln Ile Leu Ala Ile Tyr
530 535 540
Ser Thr Val Ala Ser Ser Leu Val Leu Val Val Ser Leu Gly Ala Ile
545 550 555 560
Ser Phe Trp Met Cys Ser Asn Gly Ser Leu Gln Cys Arg Ile Cys Ile
565 570 575

<210> 111
<211> 1704
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> HA B威斯康星州(PrL-)的核苷酸序列
<400> 111
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gggataacat cttcaaactc acctcatgtg gtcaaaacag ctactcaagg ggaggtcaat 120
gtgactggcg tgataccact gacaacaaca ccaacaaaat cttattttgc aaatctcaaa 180
ggaacaagga ccagagggaa actatgcccg gactgtctca actgtacaga tctggatgtg 240
gccttgggca ggccaatgtg tgtggggacc acaccttctg ctaaagcttc aatactccac 300

gaggtcagac ctgttacatc cgggtgcttt cctataatgc acgacagaac aaaaatcagg 360
caactaccca atcttctcag aggatatgaa aatatcaggt tatcaaccca aaacgttatc 420
gatgcagaaa aagcaccagg aggaccctac agacttggaa cctcaggatc ttgccctaac 480
gctaccagta aaatcggatt ttttgcaaca atggcttggg ctgtcccaaa ggacaactac 540
aaaaatgcaa cgaacccact aacagtagaa gtaccataca tttgtacaga aggggaagac 600
caaattactg tttgggggtt ccattcagat aacaaaaccc aaatgaagag cctctatgga 660
gactcaaatc ctcaaaagtt cacctcatct gctaatggag taaccacaca ttatgtttct 720
cagattggcg acttcccaga tcaaacagaa gacggaggac taccacaaag cggcagaatt 780
gttgttgatt acatgatgca aaaacctggg aaaacaggaa caattgtcta tcaaagaggt 840
gttttgttgc ctcaaaaggt gtggtgcgcg agtggcagga gcaaagtaat aaaagggtca 900
ttgcctttaa ttggtgaagc agattgcctt catgaaaaat acggtggatt aaacaaaagc 960
aagccttact acacaggaga acatgcaaaa gccataggaa attgcccaat atgggtaaaa 1020
acacctttga agcttgccaa tggaaccaaa tatagacctc ctggtggagg atgggaagga 1080
atgattgcag gttggcacgg atacacatct cacggagcac atggagtggc agtggcggca 1140
gaccttaaga gtacacaaga agctataaat aagataacaa aaaatctcaa ttctttgagt 1200
gagctagaag taaagaacct tcaaagacta agtggtgcca tggatgaact ccacaacgaa 1260
atactcgagc tggatgagaa agtggatgat ctcagagctg acactataag ctcacaaata 1320
gaacttgcag tcttgctttc caacgaagga ataataaaca gtgaagacga gcatctattg 1380
gcacttgaga gaaaactaaa gaaaatgctg ggtccctctg ctgtagacat aggaaacgga 1440
tgcttcgaaa ccaaacacaa atgcaaccag acctgcttag acaggatagc tgctggcacc 1500
tttaatgcag gagaattttc tctccccact tttgattcat tgaacattac tgctgcatct 1560
ttaaatgatg atggattgga taaccatact atactgctct attactcaac tgctgcttct 1620
agtttggctg taacattaat gctagctatt tttattgttt atatggtctc cagagacaac 1680
gtttcatgct ccatctgtct ataa 1704

<210> 112
<211> 567
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> HA B威斯康星州(PrL-)的氨基酸序列
<400> 112

Met Lys Ala Ile Ile Val Leu Leu Met Val Val Thr Ser Asn Ala Asp
1 5 10 15
Arg Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His Val Val Lys
20 25 30
Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Val Ile Pro Leu Thr
35 40 45

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

355 360 365
Thr Ser His Gly Ala His Gly Val Ala Val Ala Ala Asp Leu Lys Ser
370 375 380
Thr Gln Glu Ala Ile Asn Lys Ile Thr Lys Asn Leu Asn Ser Leu Ser
385 390 395 400
Glu Leu Glu Val Lys Asn Leu Gln Arg Leu Ser Gly Ala Met Asp Glu
405 410 415
Leu His Asn Glu Ile Leu Glu Leu Asp Glu Lys Val Asp Asp Leu Arg
420 425 430
Ala Asp Thr Ile Ser Ser Gln Ile Glu Leu Ala Val Leu Leu Ser Asn
435 440 445
Glu Gly Ile Ile Asn Ser Glu Asp Glu His Leu Leu Ala Leu Glu Arg
450 455 460
Lys Leu Lys Lys Met Leu Gly Pro Ser Ala Val Asp Ile Gly Asn Gly
465 470 475 480
Cys Phe Glu Thr Lys His Lys Cys Asn Gln Thr Cys Leu Asp Arg Ile
485 490 495
Ala Ala Gly Thr Phe Asn Ala Gly Glu Phe Ser Leu Pro Thr Phe Asp
500 505 510
Ser Leu Asn Ile Thr Ala Ala Ser Leu Asn Asp Asp Gly Leu Asp Asn
515 520 525
His Thr Ile Leu Leu Tyr Tyr Ser Thr Ala Ala Ser Ser Leu Ala Val
530 535 540
Thr Leu Met Leu Ala Ile Phe Ile Val Tyr Met Val Ser Arg Asp Asn
545 550 555 560
Val Ser Cys Ser Ile Cys Leu
565

<210> 113
<211> 1704
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> HA B威斯康星州(PrL-)+H1加利福尼亚TMCT的核苷酸序列
<400> 113
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gggataacat cttcaaactc acctcatgtg gtcaaaacag ctactcaagg ggaggtcaat 120
gtgactggcg tgataccact gacaacaaca ccaacaaaat cttattttgc aaatctcaaa 180

ggaacaagga ccagagggaa actatgcccg gactgtctca actgtacaga tctggatgtg 240
gccttgggca ggccaatgtg tgtggggacc acaccttctg ctaaagcttc aatactccac 300
gaggtcagac ctgttacatc cgggtgcttt cctataatgc acgacagaac aaaaatcagg 360
caactaccca atcttctcag aggatatgaa aatatcaggt tatcaaccca aaacgttatc 420
gatgcagaaa aagcaccagg aggaccctac agacttggaa cctcaggatc ttgccctaac 480
gctaccagta aaatcggatt ttttgcaaca atggcttggg ctgtcccaaa ggacaactac 540
aaaaatgcaa cgaacccact aacagtagaa gtaccataca tttgtacaga aggggaagac 600
caaattactg tttgggggtt ccattcagat aacaaaaccc aaatgaagag cctctatgga 660
gactcaaatc ctcaaaagtt cacctcatct gctaatggag taaccacaca ttatgtttct 720
cagattggcg acttcccaga tcaaacagaa gacggaggac taccacaaag cggcagaatt 780
gttgttgatt acatgatgca aaaacctggg aaaacaggaa caattgtcta tcaaagaggt 840
gttttgttgc ctcaaaaggt gtggtgcgcg agtggcagga gcaaagtaat aaaagggtca 900
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aagccttact acacaggaga acatgcaaaa gccataggaa attgcccaat atgggtaaaa 1020
acacctttga agcttgccaa tggaaccaaa tatagacctc ctggtggagg atgggaagga 1080
atgattgcag gttggcacgg atacacatct cacggagcac atggagtggc agtggcggca 1140
gaccttaaga gtacacaaga agctataaat aagataacaa aaaatctcaa ttctttgagt 1200
gagctagaag taaagaacct tcaaagacta agtggtgcca tggatgaact ccacaacgaa 1260
atactcgagc tggatgagaa agtggatgat ctcagagctg acactataag ctcacaaata 1320
gaacttgcag tcttgctttc caacgaagga ataataaaca gtgaagacga gcatctattg 1380
gcacttgaga gaaaactaaa gaaaatgctg ggtccctctg ctgtagacat aggaaacgga 1440
tgcttcgaaa ccaaacacaa atgcaaccag acctgcttag acaggatagc tgctggcacc 1500
tttaatgcag gagaattttc tctccccact tttgattcat tgaacattac tgctgcatct 1560
ttaaatgatg atggattgga taactaccag attttggcga tctattcaac tgtcgccagt 1620
tcattggtac tggtagtctc cctgggggca atcagtttct ggatgtgctc taatgggtct 1680
ctacagtgta gaatatgtat ttaa 1704

<210> 114
<211> 567
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> HA B威斯康星州(PrL-)+H1加利福尼亚TMCT的氨基酸序列
<400> 114

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

340 345 350
Pro Pro Gly Gly Gly Trp Glu Gly Met Ile Ala Gly Trp His Gly Tyr
355 360 365
Thr Ser His Gly Ala His Gly Val Ala Val Ala Ala Asp Leu Lys Ser
370 375 380
Thr Gln Glu Ala Ile Asn Lys Ile Thr Lys Asn Leu Asn Ser Leu Ser
385 390 395 400
Glu Leu Glu Val Lys Asn Leu Gln Arg Leu Ser Gly Ala Met Asp Glu
405 410 415
Leu His Asn Glu Ile Leu Glu Leu Asp Glu Lys Val Asp Asp Leu Arg
420 425 430
Ala Asp Thr Ile Ser Ser Gln Ile Glu Leu Ala Val Leu Leu Ser Asn
435 440 445
Glu Gly Ile Ile Asn Ser Glu Asp Glu His Leu Leu Ala Leu Glu Arg
450 455 460
Lys Leu Lys Lys Met Leu Gly Pro Ser Ala Val Asp Ile Gly Asn Gly
465 470 475 480
Cys Phe Glu Thr Lys His Lys Cys Asn Gln Thr Cys Leu Asp Arg Ile
485 490 495
Ala Ala Gly Thr Phe Asn Ala Gly Glu Phe Ser Leu Pro Thr Phe Asp
500 505 510
Ser Leu Asn Ile Thr Ala Ala Ser Leu Asn Asp Asp Gly Leu Asp Asn
515 520 525
Tyr Gln Ile Leu Ala Ile Tyr Ser Thr Val Ala Ser Ser Leu Val Leu
530 535 540
Val Val Ser Leu Gly Ala Ile Ser Phe Trp Met Cys Ser Asn Gly Ser
545 550 555 560
Leu Gln Cys Arg Ile Cys Ile
565

<210> 115
<211> 1707
<212> DNA
<213> 流感H5印度尼西亚(Influenza H5 Indo)
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gatggagtga agcctctaat tttaagagat tgtagtgtag ctggatggct cctcgggaac 240
ccaatgtgtg acgaattcat caatgtaccg gaatggtctt acatagtgga gaaggccaat 300

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ttgagcagaa taaaccattt tgagaaaatt caaatcatcc ccaaaagttc ttggtccgat 420
catgaagcct catcaggagt tagctcagca tgtccatacc tgggaagtcc ctccttttt 480
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aataatacca accaagagga tcttttggta ctgtggggaa ttcaccatcc taatgatgcg 600
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<210> 116
<211> 568
<212> PRT
<213> 流感H5印度尼西亚(Influenza H5 Indo)
<400> 116

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Asp Gln Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Glu Gln Val
20 25 30
Asp Thr Ile Met Glu Lys Asn Val Thr Val Thr His Ala Gln Asp Ile
35 40 45
Leu Glu Lys Thr His Asn Gly Lys Leu Cys Asp Leu Asp Gly Val Lys
50 55 60
Pro Leu Ile Leu Arg Asp Cys Ser Val Ala Gly Trp Leu Leu Gly Asn
65 70 75 80

Pro Met Cys Asp Glu Phe Ile Asn Val Pro Glu Trp Ser Tyr Ile Val
85 90 95
Glu Lys Ala Asn Pro Thr Asn Asp Leu Cys Tyr Pro Gly Ser Phe Asn
100 105 110
Asp Tyr Glu Glu Leu Lys His Leu Leu Ser Arg Ile Asn His Phe Glu
115 120 125
Lys Ile Gln Ile Ile Pro Lys Ser Ser Trp Ser Asp His Glu Ala Ser
130 135 140
Ser Gly Val Ser Ser Ala Cys Pro Tyr Leu Gly Ser Pro Ser Phe Phe
145 150 155 160
Arg Asn Val Val Trp Leu Ile Lys Lys Asn Ser Thr Tyr Pro Thr Ile
165 170 175
Lys Lys Ser Tyr Asn Asn Thr Asn Gln Glu Asp Leu Leu Val Leu Trp
180 185 190
Gly Ile His His Pro Asn Asp Ala Ala Glu Gln Thr Arg Leu Tyr Gln
195 200 205
Asn Pro Thr Thr Tyr Ile Ser Ile Gly Thr Ser Thr Leu Asn Gln Arg
210 215 220
Leu Val Pro Lys Ile Ala Thr Arg Ser Lys Val Asn Gly Gln Ser Gly
225 230 235 240
Arg Met Glu Phe Phe Trp Thr Ile Leu Lys Pro Asn Asp Ala Ile Asn
245 250 255
Phe Glu Ser Asn Gly Asn Phe Ile Ala Pro Glu Tyr Ala Tyr Lys Ile
260 265 270
Val Lys Lys Gly Asp Ser Ala Ile Met Lys Ser Glu Leu Glu Tyr Gly
275 280 285
Asn Cys Asn Thr Lys Cys Gln Thr Pro Met Gly Ala Ile Asn Ser Ser
290 295 300
Met Pro Phe His Asn Ile His Pro Leu Thr Ile Gly Glu Cys Pro Lys
305 310 315 320
Tyr Val Lys Ser Asn Arg Leu Val Leu Ala Thr Gly Leu Arg Asn Ser
325 330 335
Pro Gln Arg Glu Ser Arg Arg Lys Lys Arg Gly Leu Phe Gly Ala Ile
340 345 350
Ala Gly Phe Ile Glu Gly Gly Trp Gln Gly Met Val Asp Gly Trp Tyr
355 360 365
Gly Tyr His His Ser Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Lys
370 375 380
Glu Ser Thr Gln Lys Ala Ile Asp Gly Val Thr Asn Lys Val Asn Ser

385 390 395 400
Ile Ile Asp Lys Met Asn Thr Gln Phe Glu Ala Val Gly Arg Glu Phe
405 410 415
Asn Asn Leu Glu Arg Arg Ile Glu Asn Leu Asn Lys Lys Met Glu Asp
420 425 430
Gly Phe Leu Asp Val Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Met
435 440 445
Glu Asn Glu Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu
450 455 460
Tyr Asp Lys Val Arg Leu Gln Leu Arg Asp Asn Ala Lys Glu Leu Gly
465 470 475 480
Asn Gly Cys Phe Glu Phe Tyr His Lys Cys Asp Asn Glu Cys Met Glu
485 490 495
Ser Ile Arg Asn Gly Thr Tyr Asn Tyr Pro Gln Tyr Ser Glu Glu Ala
500 505 510
Arg Leu Lys Arg Glu Glu Ile Ser Gly Val Lys Leu Glu Ser Ile Gly
515 520 525
Thr Tyr Gln Ile Leu Ser Ile Tyr Ser Thr Val Ala Ser Ser Leu Ala
530 535 540
Leu Ala Ile Met Met Ala Gly Leu Ser Leu Trp Met Cys Ser Asn Gly
545 550 555 560
Ser Leu Gln Cys Arg Ile Cys Ile
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<210> 117
<211> 52
<212> DNA
<213> 人工序列(Artificial Sequence)
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<223> 构建体1800 IF**(SacII)-PDI.s1+4c
<400> 117
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<210> 118
<211> 51
<212> DNA
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<223> 构建体1800 IF-H3V36111.s1-4r

<400> 118

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<210> 119

<211> 1725

<212> DNA

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<223> PDISP/H3维多利亚的核苷酸序列

<400> 119

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caccatgcag taccaaacgg aacgatagtg aaaacaatca cgaatgacca aattgaagtt 180
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cagatccttg atggagaaaa ctgcacacta atagatgctc tattgggaga ccctcagtgt 300
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aacaaccggt tccagatcaa gggagttgag ctgaagtcag ggtacaaaga ttggatccta 1620
tggatttcct ttgccatatc atgtttttg ctttgtgttg ctttgttggg gttcatcatg 1680
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<210> 120
<211> 4644
<212> DNA
<213> 人工序列(Artificial Sequence)
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<221>
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<223> 构建体2171
<400> 120

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aataactcaa aaaccataaa agtttaagtt agcaagtgtg tacattttta cttgaacaaa 180
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aaacctaaga gaaaagagcg ttta 4644

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<211> 3129
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
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<223> 构建体1800
<400> 121

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Gln Ile Leu Asp Gly Glu Asn Cys Thr Leu Ile Asp Ala Leu Leu Gly
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Asp Pro Gln Cys Asp Gly Phe Gln Asn Lys Lys Trp Asp Leu Phe Val
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Glu Arg Ser Lys Ala Tyr Ser Asn Cys Tyr Pro Tyr Asp Val Pro Asp
115 120 125
Tyr Ala Ser Leu Arg Ser Leu Val Ala Ser Ser Gly Thr Leu Glu Phe
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Pro Asn Asn Glu Gln Phe Asp Lys Leu Tyr Ile Trp Gly Val His His
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Tyr Trp Thr Ile Val Lys Pro Gly Asp Ile Leu Leu Ile Asn Ser Thr
260 265 270

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<210> 129

<211> 3471

<212> DNA

<213> 人工序列(Artificial Sequence)

<220>

<221>

<222>

<223> 表达盒2220号

<400> 129

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<210> 130
<211> 571
<212> PRT
<213> 人工序列(Artificial Sequence)
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<221>
<222>
<223> PDISP/H2新加坡的氨基酸序列
<400> 130

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Ala Asn Asn Ser Thr Glu Lys Val Asp Thr Ile Leu Glu Arg Asn Val
35 40 45
Thr Val Thr His Ala Lys Asp Ile Leu Glu Lys Thr His Asn Gly Lys
50 55 60
Leu Cys Lys Leu Asn Gly Ile Pro Pro Leu Glu Leu Gly Asp Cys Ser
65 70 75 80
Ile Ala Gly Trp Leu Leu Gly Asn Pro Glu Cys Asp Arg Leu Leu Ser

85 90 95

Val Pro Glu Trp Ser Tyr Ile Met Glu Lys Glu Asn Pro Arg Asp Gly
100 105 110

Leu Cys Tyr Pro Gly Ser Phe Asn Asp Tyr Glu Glu Leu Lys His Leu
115 120 125

Leu Ser Ser Val Lys His Phe Glu Lys Val Lys Ile Leu Pro Lys Asp
130 135 140

Arg Trp Thr Gln His Thr Thr Thr Gly Gly Ser Arg Ala Cys Ala Val
145 150 155 160

Ser Gly Asn Pro Ser Phe Phe Arg Asn Met Val Trp Leu Thr Lys Lys
165 170 175

Glu Ser Asn Tyr Pro Val Ala Lys Gly Ser Tyr Asn Asn Thr Ser Gly
180 185 190

Glu Gln Met Leu Ile Ile Trp Gly Val His His Pro Asn Asp Glu Thr
195 200 205

Glu Gln Arg Thr Leu Tyr Gln Asn Val Gly Thr Tyr Val Ser Val Gly
210 215 220

Thr Ser Thr Leu Asn Lys Arg Ser Thr Pro Asp Ile Ala Thr Arg Pro
225 230 235 240

Lys Val Asn Gly Leu Gly Ser Arg Met Glu Phe Ser Trp Thr Leu Leu
245 250 255

Asp Met Trp Asp Thr Ile Asn Phe Glu Ser Thr Gly Asn Leu Ile Ala
260 265 270

Pro Glu Tyr Gly Phe Lys Ile Ser Lys Arg Gly Ser Ser Gly Ile Met
275 280 285

Lys Thr Glu Gly Thr Leu Glu Asn Cys Glu Thr Lys Cys Gln Thr Pro
290 295 300

Leu Gly Ala Ile Asn Thr Thr Leu Pro Phe His Asn Val His Pro Leu
305 310 315 320

Thr Ile Gly Glu Cys Pro Lys Tyr Val Lys Ser Glu Lys Leu Val Leu
325 330 335

Ala Thr Gly Leu Arg Asn Val Pro Gln Ile Glu Ser Arg Gly Leu Phe
340 345 350

Gly Ala Ile Ala Gly Phe Ile Glu Gly Gly Trp Gln Gly Met Val Asp
355 360 365

Gly Trp Tyr Gly Tyr His His Ser Asn Asp Gln Gly Ser Gly Tyr Ala
370 375 380

Ala Asp Lys Glu Ser Thr Gln Lys Ala Phe Asp Gly Ile Thr Asn Lys
385 390 395 400

Val Asn Ser Val Ile Glu Lys Met Asn Thr Gln Phe Glu Ala Val Gly
405 410 415
Lys Glu Phe Ser Asn Leu Glu Arg Arg Leu Glu Asn Leu Asn Lys Lys
420 425 430
Met Glu Asp Gly Phe Leu Asp Val Trp Thr Tyr Asn Ala Glu Leu Leu
435 440 445
Val Leu Met Glu Asn Glu Arg Thr Leu Asp Phe His Asp Ser Asn Val
450 455 460
Lys Asn Leu Tyr Asp Lys Val Arg Met Gln Leu Arg Asp Asn Val Lys
465 470 475 480
Glu Leu Gly Asn Gly Cys Phe Glu Phe Tyr His Lys Cys Asp Asp Glu
485 490 495
Cys Met Asn Ser Val Lys Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Glu
500 505 510
Glu Glu Ser Lys Leu Asn Arg Asn Glu Ile Lys Gly Val Lys Leu Ser
515 520 525
Ser Met Gly Val Tyr Gln Ile Leu Ala Ile Tyr Ala Thr Val Ala Gly
530 535 540
Ser Leu Ser Leu Ala Ile Met Met Ala Gly Ile Ser Phe Trp Met Cys
545 550 555 560
Ser Asn Gly Ser Leu Gln Cys Arg Ile Cys Ile
565 570

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<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> H2S157(Prl-).r
<400> 131
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<210> 132
<211> 50
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
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<223> H2S157(Prl-).c
<400> 132
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<210> 133
<211> 3426
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
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<223> 盒2221
<400> 133
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ctagat 3426

<210> 134
<211> 556
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 具有缺失的蛋白水解环的PDISP/H2新加坡的氨基酸序列

<400> 134

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

Leu Gly Ala Ile Asn Thr Thr Leu Pro Phe His Asn Val His Pro Leu
305 310 315 320
Thr Ile Gly Glu Cys Pro Lys Tyr Val Lys Ser Glu Lys Leu Val Leu
325 330 335
Ala Thr Gly Leu Arg Asn Val Pro Gly Gly Gly Trp Gln Gly Met Val
340 345 350
Asp Gly Trp Tyr Gly Tyr His His Ser Asn Asp Gln Gly Ser Gly Tyr
355 360 365
Ala Ala Asp Lys Glu Ser Thr Gln Lys Ala Phe Asp Gly Ile Thr Asn
370 375 380
Lys Val Asn Ser Val Ile Glu Lys Met Asn Thr Gln Phe Glu Ala Val
385 390 395 400
Gly Lys Glu Phe Ser Asn Leu Glu Arg Arg Leu Glu Asn Leu Asn Lys
405 410 415
Lys Met Glu Asp Gly Phe Leu Asp Val Trp Thr Tyr Asn Ala Glu Leu
420 425 430
Leu Val Leu Met Glu Asn Glu Arg Thr Leu Asp Phe His Asp Ser Asn
435 440 445
Val Lys Asn Leu Tyr Asp Lys Val Arg Met Gln Leu Arg Asp Asn Val
450 455 460
Lys Glu Leu Gly Asn Gly Cys Phe Glu Phe Tyr His Lys Cys Asp Asp
465 470 475 480
Glu Cys Met Asn Ser Val Lys Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr
485 490 495
Glu Glu Glu Ser Lys Leu Asn Arg Asn Glu Ile Lys Gly Val Lys Leu
500 505 510
Ser Ser Met Gly Val Tyr Gln Ile Leu Ala Ile Tyr Ala Thr Val Ala
515 520 525
Gly Ser Leu Ser Leu Ala Ile Met Met Ala Gly Ile Ser Phe Trp Met
530 535 540
Cys Ser Asn Gly Ser Leu Gln Cys Arg Ile Cys Ile
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<210> 135
<211> 3120
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>

<223> 盒2222

<400> 135

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<210> 136
<211> 3075
<212> DNA
<213> 人工序列(Artificial Sequence)
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<400> 136

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tacaaatgcc atcattgcga taaaggaaag gccatcgttg aagatgcctc tgccgacagt 240
ggtcccaaag atggaccccc acccacgagg agcatcgtgg aaaaagaaga cgttccaacc 300
acgtcttcaa agcaagtgga ttgatgtgat aacatggtgg agcacgacac acttgtctac 360
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<210> 137

<211> 1725

<212> DNA
<213> 人工序列(Artificial Sequence)
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<221>
<222>
<223> PDISP/H3珀斯的核苷酸序列
<400> 137
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caccatgcag taccaaacgg aacgatagtg aaaacaatca cgaatgacca aattgaagtt 180
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cagatccttg atggaaaaaa ctgcacacta atagatgctc tattgggaga ccctcagtgt 300
gatggcttcc aaaataagaa atgggacctt tttgttgaac gcagcaaagc ctacagcaac 360
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acactggagt ttaacaatga aagcttcaat tggactggag tcactcaaaa cggaacaagc 480
tctgcttgca taaggagatc taaaaacagt ttctttagta gattgaattg gttgacccac 540
ttaaacttca aatacccagc attgaacgtg actatgccaa acaatgaaca atttgacaaa 600
ttgtacattt ggggggttca ccacccgggt acggacaaag accaaatctt cctgtatgct 660
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atcggatcta gacccagagt aaggaatatc cctagcagaa taagcatcta ttggacaata 780
gtaaaaccgg gagacatact tttgattaac agcacaggga atctaattgc tcctaggggt 840
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<210> 138
<211> 50
<212> DNA

<213> 人工序列(Artificial Sequence)
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<223> IF**-H3P1609.S1-6r
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<210> 139
<211> 574
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> PDISP/H3珀斯的氨基酸序列
<400> 139

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

180 185 190

Pro Asn Asn Glu Gln Phe Asp Lys Leu Tyr Ile Trp Gly Val His His

195 200 205

Pro Gly Thr Asp Lys Asp Gln Ile Phe Leu Tyr Ala Gln Ala Ser Gly

210 215 220

Arg Ile Thr Val Ser Thr Lys Arg Ser Gln Gln Thr Val Ser Pro Asn

225 230 235 240

Ile Gly Ser Arg Pro Arg Val Arg Asn Ile Pro Ser Arg Ile Ser Ile

245 250 255

Tyr Trp Thr Ile Val Lys Pro Gly Asp Ile Leu Leu Ile Asn Ser Thr

260 265 270

Gly Asn Leu Ile Ala Pro Arg Gly Tyr Phe Lys Ile Arg Ser Gly Lys

275 280 285

Ser Ser Ile Met Arg Ser Asp Ala Pro Ile Gly Lys Cys Asn Ser Glu

290 295 300

Cys Ile Thr Pro Asn Gly Ser Ile Pro Asn Asp Lys Pro Phe Gln Asn

305 310 315 320

Val Asn Arg Ile Thr Tyr Gly Ala Cys Pro Arg Tyr Val Lys Gln Asn

325 330 335

Thr Leu Lys Leu Ala Thr Gly Met Arg Asn Val Pro Glu Lys Gln Thr

340 345 350

Arg Gly Ile Phe Gly Ala Ile Ala Gly Phe Ile Glu Asn Gly Trp Glu

355 360 365

Gly Met Val Asp Gly Trp Tyr Gly Phe Arg His Gln Asn Ser Glu Gly

370 375 380

Arg Gly Gln Ala Ala Asp Leu Lys Ser Thr Gln Ala Ala Ile Asp Gln

385 390 395 400

Ile Asn Gly Lys Leu Asn Arg Leu Ile Gly Lys Thr Asn Glu Lys Phe

405 410 415

His Gln Ile Glu Lys Glu Phe Ser Glu Val Glu Gly Arg Ile Gln Asp

420 425 430

Leu Glu Lys Tyr Val Glu Asp Thr Lys Ile Asp Leu Trp Ser Tyr Asn

435 440 445

Ala Glu Leu Leu Val Ala Leu Glu Asn Gln His Thr Ile Asp Leu Thr

450 455 460

Asp Ser Glu Met Asn Lys Leu Phe Glu Lys Thr Lys Lys Gln Leu Arg

465 470 475 480

Glu Asn Ala Glu Asp Met Gly Asn Gly Cys Phe Lys Ile Tyr His Lys

485 490 495

Cys Asp Asn Ala Cys Ile Gly Ser Ile Arg Asn Gly Thr Tyr Asp His
500 505 510
Asp Val Tyr Arg Asp Glu Ala Leu Asn Asn Arg Phe Gln Ile Lys Gly
515 520 525
Val Glu Leu Lys Ser Gly Tyr Lys Asp Trp Ile Leu Trp Ile Ser Phe
530 535 540
Ala Ile Ser Cys Phe Leu Leu Cys Val Ala Leu Leu Gly Phe Ile Met
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<210> 140
<211> 1680
<212> DNA
<213> 人工序列(Artificial Sequence)
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<222>
<223> 具有缺失的蛋白水解环的PDISP/H3珀斯的核苷酸序列
<400> 140

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caccatgcag taccaaacgg aacgatagtg aaaacaatca cgaatgacca aattgaagtt 180
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gatggcttcc aaaataagaa atgggacctt tttgttgaac gcagcaaagc ctacagcaac 360
tgttaccctt atgatgtgcc ggattatgcc tcccttaggt cactagttgc ctcatccggc 420
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ttaaacttca aatacccagc attgaacgtg actatgccaa acaatgaaca atttgacaaa 600
ttgtacattt gggggttca ccacccgggt acggacaaag accaaatctt cctgtatgct 660
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gtaaaaccgg gagacatact tttgattaac agcacaggga atctaattgc tcctaggggt 840
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<210> 141
<211> 48
<212> DNA
<213> 人工序列(Artificial Sequence)
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<400> 141
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<210> 142
<211> 48
<212> DNA
<213> 人工序列(Artificial Sequence)
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<221>
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<223> H3P1609(Prl-)#2.c
<400> 142
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<210> 143
<211> 559
<212> PRT
<213> 人工序列(Artificial Sequence)
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<221>
<222>
<223> 具有缺失的蛋白水解环的PDISP/H3珀斯的氨基酸序列
<400> 143

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

325 330 335
Thr Leu Lys Leu Ala Thr Gly Met Arg Asn Val Pro Gly Gly Gly Trp
340 345 350
Glu Gly Met Val Asp Gly Trp Tyr Gly Phe Arg His Gln Asn Ser Glu
355 360 365
Gly Arg Gly Gln Ala Ala Asp Leu Lys Ser Thr Gln Ala Ala Ile Asp
370 375 380
Gln Ile Asn Gly Lys Leu Asn Arg Leu Ile Gly Lys Thr Asn Glu Lys
385 390 395 400
Phe His Gln Ile Glu Lys Glu Phe Ser Glu Val Glu Gly Arg Ile Gln
405 410 415
Asp Leu Glu Lys Tyr Val Glu Asp Thr Lys Ile Asp Leu Trp Ser Tyr
420 425 430
Asn Ala Glu Leu Leu Val Ala Leu Glu Asn Gln His Thr Ile Asp Leu
435 440 445
Thr Asp Ser Glu Met Asn Lys Leu Phe Glu Lys Thr Lys Lys Gln Leu
450 455 460
Arg Glu Asn Ala Glu Asp Met Gly Asn Gly Cys Phe Lys Ile Tyr His
465 470 475 480
Lys Cys Asp Asn Ala Cys Ile Gly Ser Ile Arg Asn Gly Thr Tyr Asp
485 490 495
His Asp Val Tyr Arg Asp Glu Ala Leu Asn Asn Arg Phe Gln Ile Lys
500 505 510
Gly Val Glu Leu Lys Ser Gly Tyr Lys Asp Trp Ile Leu Trp Ile Ser
515 520 525
Phe Ala Ile Ser Cys Phe Leu Leu Cys Val Ala Leu Leu Gly Phe Ile
530 535 540
Met Trp Ala Cys Gln Lys Gly Asn Ile Arg Cys Asn Ile Cys Ile
545 550 555

<210> 144
<211> 1680
<212> DNA
<213> 人工序列(Artificial Sequence)
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<223> 具有缺失的蛋白水解环的PDISP/H3维多利亚的核苷酸序列
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caccatgcag taccaaacgg aacgatagtg aaaacaatca cgaatgacca aattgaagtt 180
actaatgcta ctgagctggt tcagaattcc tcaataggtg aaatatgcga cagtcctcat 240
cagatccttg atggagaaaa ctgcacacta atagatgctc tattgggaga ccctcagtgt 300
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tctgcttgca taaggagatc taataatagt ttctttagta gattaaattg gttgacccac 540
ttaaacttca aatacccagc attgaacgtg actatgccaa acaatgaaca atttgacaaa 600
ttgtacattt ggggggttca ccacccgggt acggacaagg accaaatctt cctgtatgct 660
caatcatcag gaagaatcac agtatctacc aaaagaagcc aacaagctgt aatcccgaat 720
atcggatcta gacccagaat aaggaatatc cctagcagaa taagcatcta ttggacaata 780
gtaaaaccgg gagacatact tttgattaac agcacaggga atctaattgc tcctaggggt 840
tacttcaaaa tacgaagtgg gaaaagctca ataatgagat cagatgcacc cattggcaaa 900
tgcaattctg aatgcatcac tccaaatgga agcattccca atgacaaacc attccaaaat 960
gtaaacagga tcacatacgg ggcctgtccc agatatgtta agcaaagcac tctgaaattg 1020
gcaacaggaa tgcgaaatgt accaggcgga ggttgggagg gaatggtgga tggttggtac 1080
ggtttcaggc atcaaaattc tgagggaaga ggacaagcag cagatctcaa aagcactcaa 1140
gcagcaatcg atcaaatcaa tgggaagctg aatcgattga tcgggaaaac caacgagaaa 1200
ttccatcaga ttgaaaaaga attctcagaa gtcgaaggga gaattcagga ccttgagaaa 1260
tatgttgagg acactaaaat agatctctgg tcatacaacg cggagcttct tgttgccctg 1320
gagaaccaac atacaattga tctaactgac tcagaaatga acaaactgtt tgaaaaaaca 1380
aagaagcaac taagggaaaa tgctgaggat atgggcaatg gttgtttcaa aatataccac 1440
aaatgtgaca atgcctgcat aggatcaatc agaaatggaa cttatgacca cgatgtatac 1500
agagatgaag cattaaacaa ccggttccag atcaagggag ttgagctgaa gtcagggtac 1560
aaagattgga tcctatggat ttcctttgcc atatcatgtt ttttgctttg tgttgctttg 1620
ttggggttca tcatgtgggc ctgccaaaag ggcaacatta ggtgcaacat ttgcatttga 1680

<210> 145
<211> 48
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> H3V36111(Prl-).r
<400> 145
ccctcccaac ctccgcctgg tacatttcgc attcctgttg ccaatttc 48

<210> 146
<211> 48

<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> H3V36111(Prl-).c
<400> 146
aatgtaccag gcggaggttg ggagggaatg gtggatggtt ggtacggt 48
<210> 147
<211> 559
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 具有缺失的蛋白水解环的PDISP/H3维多利亚的氨基酸序列
<400> 147

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

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

485 490 495
His Asp Val Tyr Arg Asp Glu Ala Leu Asn Asn Arg Phe Gln Ile Lys
500 505 510
Gly Val Glu Leu Lys Ser Gly Tyr Lys Asp Trp Ile Leu Trp Ile Ser
515 520 525
Phe Ala Ile Ser Cys Phe Leu Leu Cys Val Ala Leu Leu Gly Phe Ile
530 535 540
Met Trp Ala Cys Gln Lys Gly Asn Ile Arg Cys Asn Ile Cys Ile
545 550 555

<210> 148
<211> 1701
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> PDISP/H7杭州的核苷酸序列
<400> 148

atggcgaaaa acgttgcgat tttcggctta ttgttttctc ttcttgtgtt ggttccttct 60
cagatcttcg cggacaaaat ctgcctcgga catcatgccg tgtcaaacgg aaccaaagta 120
aacacattaa ctgaaagagg agtggaagtc gtcaatgcaa ctgaaacagt ggaacgaaca 180
aacatcccca ggatctgctc aaaagggaaa aggacagttg acctcggtca atgtggactc 240
ctggggacaa tcactggacc acctcaatgt gaccaattcc tagaattttc agccgattta 300
attattgaga ggcgagaagg aagtgatgtc tgttatcctg ggaaattcgt gaatgaagaa 360
gctctgaggc aaattctcag agaatcaggc ggaattgaca aggaagcaat gggattcaca 420
tacagtggaa taagaactaa tggagcaacc agtgcatgta ggagatcagg atcttcattc 480
tatgcagaaa tgaaatggct cctgtcaaac acagataatg ctgcattccc gcagatgact 540
aagtcatata aaaatacaag aaaaagccca gctctaatag tatgggggat ccatcattcc 600
gtatcaactg cagagcaaac caagctatat gggagtggaa acaaactggt gacagttggg 660
agttctaatt atcaacaatc ttttgtaccg agtccaggag cgagaccaca agttaatggt 720
atatctggaa gaattgactt tcattggcta atgctaaatc ccaatgatac agtcactttc 780
agtttcaatg gggctttcat agctccagac cgtgcaagct tcctgagagg aaaatctatg 840
ggaatccaga gtggagtaca ggttgatgcc aattgtgaag gggactgcta tcatagtgga 900
gggacaataa taagtaactt gccatttcag aacatagata gcagggcagt tggaaaatgt 960
ccgagatatg ttaagcaaag gagtctgctg ctagcaacag ggatgaagaa tgttcctgag 1020
attccaaagg gaagaggcct atttggtgct atagcgggtt tcattgaaaa tggatgggaa 1080
ggcctaattg atggttggta tggtttcaga caccagaatg cacagggaga gggaactgct 1140
gcagattaca aaagcactca atcggcaatt gatcaaataa caggaaaatt aaaccggctt 1200
atagaaaaaa ccaaccaaca atttgagttg atcgacaatg aattcaatga ggtagagaag 1260

caaatcggta atgtgataaa ttggaccaga gattctataa cagaagtgtg gtcatacaat 1320
gctgaactct tggtagcaat ggagaaccag catacaattg atctggctga ttcagaaatg 1380
gacaaactgt acgaacgagt gaaaagacag ctgagagaga atgctgaaga agatggcact 1440
ggttgctttg aaatatttca caagtgtgat gatgactgta tggccagtat tagaaataac 1500
acctatgatc acagcaaata cagggaagag gcaatgcaaa atagaataca gattgaccca 1560
gtcaaactaa gcagcggcta caaagatgtg atactttggt ttagcttcgg ggcatcatgt 1620
ttcatacttc tagccattgt aatgggcctt gtcttcatat gtgtaaagaa tggaaacatg 1680
cggtgcacta tttgtatata a 1701

<210> 149
<211> 50
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> IF*-H7H113.s1-6r
<400> 149
actaaagaaa ataggccttt atatacaaat agtgcaccgc atgtttccat 50

<210> 150
<211> 566
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> PDISP/H7杭州的氨基酸序列
<400> 150

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

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

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

<210> 151
<211> 1650
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 具有缺失的蛋白水解环的PDISP/H7杭州的核苷酸序列
<400> 151
atggcgaaaa acgttgcgat tttcggctta ttgttttctc ttcttgtgtt ggttccttct 60
cagatcttcg cggacaaaat ctgcctcgga catcatgccg tgtcaaacgg aaccaaagta 120
aacacattaa ctgaaagagg agtggaagtc gtcaatgcaa ctgaaacagt ggaacgaaca 180
aacatcccca ggatctgctc aaaagggaaa aggacagttg acctcggtca atgtggactc 240
ctggggacaa tcactggacc acctcaatgt gaccaattcc tagaattttc agccgattta 300
attattgaga ggcgagaagg aagtgatgtc tgttatcctg ggaaattcgt gaatgaagaa 360
gctctgaggc aaattctcag agaatcaggc ggaattgaca aggaagcaat gggattcaca 420
tacagtggaa taagaactaa tggagcaacc agtgcatgta ggagatcagg atcttcattc 480
tatgcagaaa tgaaatggct cctgtcaaac acagataatg ctgcattccc gcagatgact 540

aagtcatata aaaatacaag aaaaagccca gctctaatag tatgggggat ccatcattcc 600
gtatcaactg cagagcaaac caagctatat gggagtggaa acaaactggt gacagttggg 660
agttctaatt atcaacaatc ttttgtaccg agtccaggag cgagaccaca agttaatggt 720
atatctggaa gaattgactt tcattggcta atgctaaatc ccaatgatac agtcactttc 780
agtttcaatg gggctttcat agctccagac cgtgcaagct tcctgagagg aaaatctatg 840
ggaatccaga gtggagtaca ggttgatgcc aattgtgaag gggactgcta tcatagtgga 900
gggacaataa taagtaactt gccatttcag aacatagata gcagggcagt tggaaaatgt 960
ccgagatatg ttaagcaaag gagtctgctg ctagcaacag ggatgaagaa tgttcctggc 1020
ggatgggaag gcctaattga tggttggtat ggtttcagac accagaatgc acagggagag 1080
ggaactgctg cagattacaa aagcactcaa tcggcaattg atcaaataac aggaaaatta 1140
aaccggctta tagaaaaaac caaccaacaa tttgagttga tcgacaatga attcaatgag 1200
gtagagaagc aaatcggtaa tgtgataaat tggaccagag attctataac agaagtgtgg 1260
tcatacaatg ctgaactctt ggtagcaatg gagaaccagc atacaattga tctggctgat 1320
tcagaaatgg acaaactgta cgaacgagtg aaaagacagc tgagagagaa tgctgaagaa 1380
gatggcactg gttgctttga aatatttcac aagtgtgatg atgactgtat ggccagtatt 1440
agaaataaca cctatgatca cagcaaatac agggaagagg caatgcaaaa tagaatacag 1500
attgacccag tcaaactaag cagcggctac aaagatgtga tactttggtt tagcttcggg 1560
gcatcatgtt tcatacttct agccattgta atgggccttg tcttcatatg tgtaaagaat 1620
ggaaacatgc ggtgcactat ttgtatataa 1650

<210> 152
<211> 49
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> H7H113(PrL-).r
<400> 152
ccttcccatc cgccaggaac attcttcatc cctgttgcta gcagcagac 49

<210> 153
<211> 48
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> H7H113(PrL-).c
<400> 153
agaatgttcc tggcggatgg gaaggcctaa ttgatggttg gtatggtt 48

<210> 154
<211> 549
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 具有缺失的蛋白水解环的PDISP/H7杭州的的氨基酸序列
<400> 154

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

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

545

<210> 155

<211> 1701

<212> DNA

<213> 人工序列(Artificial Sequence)

<220>

<221>

<222>

<223> PDISP/H9杭州的核苷酸序列

<400> 155

atggcgaaaa acgttgcgat tttcggctta ttgttttctc ttcttgtgtt ggttccttct 60
cagatcttcg cggataaaat ctgcatcggc caccagtcaa caaactccac agaaactgtg 120
gacacgctaa cagaaaccaa tgttcctgtg acacatgcca aagaattgct ccacacagag 180
cataatggaa tgctgtgtgc aacaagcctg ggacatcccc tcattctaga cacatgcact 240
attgaaggac tagtctatgg caacccttct tgtgacctgc tgttgggagg aagagaatgg 300
tcctacatcg tcgaaagatc atcagctgta aatggaacgt gttaccctgg gaatgtagaa 360
aacctagagg aactcaggac acttttagt tccgctagtt cctaccaaag aatccaaatc 420
ttcccagaca caacctggaa tgtgacttac actggaacaa gcagagcatg ttcaggttca 480
ttctacagga gtatgagatg gctgactcaa aagagcggtt tttaccctgt tcaagacgcc 540
caatacacaa ataacagggg aaagagcatt cttttcgtgt ggggcataca tcacccaccc 600
acctataccg agcaaacaaa tttgtacata agaaacgaca caacaacaag cgtgacaaca 660
gaagatttga ataggacctt caaaccagtg atagggccaa ggccccttgt caatggtctg 720
cagggaagaa ttgattatta ttggtcggta ctaaaaccag gccaaacatt gcgagtacga 780
tccaatggga atctaattgc tccatggtat ggacacgttc tttcaggagg gagccatgga 840
agaatcctga agactgattt aaaaggtggt aattgtgtag tgcaatgtca gactgaaaaa 900
ggtggcttaa acagtacatt gccattccac aatatcagta aatatgcatt tggaacctgc 960
cccaaatatg taagagttaa tagtctcaaa ctggcagtcg gtctgaggaa cgtgcctgct 1020
agatcaagta gaggactatt tggagccata gctggattca tagaaggagg ttggccagga 1080
ctagtcgctg gctggtatgg tttccagcat tcaaatgatc aaggggttgg tatggctgca 1140
gatagggatt caactcaaaa ggcaattgat aaaataacat ccaaggtgaa taatatagtc 1200
gacaagatga acaagcaata tgaaataatt gatcatgaat ttagtgaggt tgaaactaga 1260
ctcaatatga tcaataataa gattgatgac caaatacaag acgtatgggc atataatgca 1320
gaattgctag tactacttga aaatcaaaaa acactcgatg agcatgatgc gaacgtgaac 1380
aatctatata acaaggtgaa gagggcactg ggctccaatg ctatggaaga tgggaaaggc 1440
tgtttcgagc tataccataa atgtgatgat cagtgcatgg aaacaattcg gaacgggacc 1500
tataatagga gaaagtatag agaggaatca agactagaaa ggcagaaaat agagggggtt 1560
aagctggaat ctgagggaac ttacaaaatc ctcaccattt attcgactgt cgcctcatct 1620
cttgtgcttg caatggggtt tgctgccttc ctgttctggg ccatgtccaa tggatcttgc 1680
agatgcaaca tttgtatata a 1701

<210> 156
<211> 50
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> IF**-H9HK107399.S1-6r
<400> 156
actaaagaaa ataggccttt atatacaaat gttgcatctg caagatccat 50
<210> 157
<211> 566
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> PDISP/H9杭州的氨基酸序列
<400> 157

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

Phe Tyr Arg Ser Met Arg Trp Leu Thr Gln Lys Ser Gly Phe Tyr Pro
165 170 175
Val Gln Asp Ala Gln Tyr Thr Asn Asn Arg Gly Lys Ser Ile Leu Phe
180 185 190
Val Trp Gly Ile His His Pro Pro Thr Tyr Thr Glu Gln Thr Asn Leu
195 200 205
Tyr Ile Arg Asn Asp Thr Thr Thr Ser Val Thr Thr Glu Asp Leu Asn
210 215 220
Arg Thr Phe Lys Pro Val Ile Gly Pro Arg Pro Leu Val Asn Gly Leu
225 230 235 240
Gln Gly Arg Ile Asp Tyr Tyr Trp Ser Val Leu Lys Pro Gly Gln Thr
245 250 255
Leu Arg Val Arg Ser Asn Gly Asn Leu Ile Ala Pro Trp Tyr Gly His
260 265 270
Val Leu Ser Gly Gly Ser His Gly Arg Ile Leu Lys Thr Asp Leu Lys
275 280 285
Gly Gly Asn Cys Val Val Gln Cys Gln Thr Glu Lys Gly Gly Leu Asn
290 295 300
Ser Thr Leu Pro Phe His Asn Ile Ser Lys Tyr Ala Phe Gly Thr Cys
305 310 315 320
Pro Lys Tyr Val Arg Val Asn Ser Leu Lys Leu Ala Val Gly Leu Arg
325 330 335
Asn Val Pro Ala Arg Ser Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly
340 345 350
Phe Ile Glu Gly Gly Trp Pro Gly Leu Val Ala Gly Trp Tyr Gly Phe
355 360 365
Gln His Ser Asn Asp Gln Gly Val Gly Met Ala Ala Asp Arg Asp Ser
370 375 380
Thr Gln Lys Ala Ile Asp Lys Ile Thr Ser Lys Val Asn Asn Ile Val
385 390 395 400
Asp Lys Met Asn Lys Gln Tyr Glu Ile Ile Asp His Glu Phe Ser Glu
405 410 415
Val Glu Thr Arg Leu Asn Met Ile Asn Asn Lys Ile Asp Asp Gln Ile
420 425 430
Gln Asp Val Trp Ala Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn
435 440 445
Gln Lys Thr Leu Asp Glu His Asp Ala Asn Val Asn Asn Leu Tyr Asn
450 455 460
Lys Val Lys Arg Ala Leu Gly Ser Asn Ala Met Glu Asp Gly Lys Gly

465 470 475 480
Cys Phe Glu Leu Tyr His Lys Cys Asp Asp Gln Cys Met Glu Thr Ile
485 490 495
Arg Asn Gly Thr Tyr Asn Arg Arg Lys Tyr Arg Glu Glu Ser Arg Leu
500 505 510
Glu Arg Gln Lys Ile Glu Gly Val Lys Leu Glu Ser Glu Gly Thr Tyr
515 520 525
Lys Ile Leu Thr Ile Tyr Ser Thr Val Ala Ser Ser Leu Val Leu Ala
530 535 540
Met Gly Phe Ala Ala Phe Leu Phe Trp Ala Met Ser Asn Gly Ser Cys
545 550 555 560
Arg Cys Asn Ile Cys Ile
565

<210> 158
<211> 1656
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 具有缺失的蛋白水解环的PDISP/H9杭州的核苷酸序列
<400> 158
atggcgaaaa acgttgcgat tttcggctta ttgttttctc ttcttgtgtt ggttccttct 60
cagatcttcg cggataaaat ctgcatcggc caccagtcaa caaactccac agaaactgtg 120
gacacgctaa cagaaaccaa tgttcctgtg acacatgcca aagaattgct ccacacagag 180
cataatggaa tgctgtgtgc aacaagcctg ggacatcccc tcattctaga cacatgcact 240
attgaaggac tagtctatgg caacccttct tgtgacctgc tgttgggagg aagagaatgg 300
tcctacatcg tcgaaagatc atcagctgta aatggaacgt gttaccctgg gaatgtagaa 360
aacctagagg aactcaggac acttttagt tccgctagtt cctaccaaag aatccaaatc 420
ttcccagaca caacctggaa tgtgacttac actggaacaa gcagagcatg ttcaggttca 480
ttctacagga gtatgagatg gctgactcaa aagagcggtt tttaccctgt tcaagacgcc 540
caatacacaa ataacagggg aaagagcatt cttttcgtgt ggggcataca tcacccaccc 600
acctataccg agcaaacaaa tttgtacata agaaacgaca caacaacaag cgtgacaaca 660
gaagatttga ataggacctt caaaccagtg atagggccaa ggccccttgt caatggtctg 720
cagggaagaa ttgattatta ttggtcggta ctaaaaccag gccaaacatt gcgagtacga 780
tccaatggga atctaattgc tccatggtat ggacacgttc tttcaggagg gagccatgga 840
agaatcctga agactgattt aaaaggtggt aattgtgtag tgcaatgtca gactgaaaaa 900
ggtggcttaa acagtacatt gccattccac aatatcagta aatatgcatt tggaacctgc 960
cccaaatatg taagagttaa tagtctcaaa ctggcagtcg gtctgaggaa cgtgcctggc 1020

ggaggttggc caggactagt cgctggctgg tatggtttcc agcattcaaa tgatcaaggg 1080
gttggtatgg ctgcagatag ggattcaact caaaaggcaa ttgataaaat aacatccaag 1140
gtgaataata tagtcgacaa gatgaacaag caatatgaaa taattgatca tgaatttagt 1200
gaggttgaaa ctagactcaa tatgatcaat aataagattg atgaccaaat acaagacgta 1260
tgggcatata atgcagaatt gctagtacta cttgaaaatc aaaaaacact cgatgagcat 1320
gatgcgaacg tgaacaatct atataacaag gtgaagaggg cactgggctc caatgctatg 1380
gaagatggga aaggctgttt cgagctatac cataaatgtg atgatcagtg catggaaaca 1440
attcggaacg ggacctataa taggagaaag tatagagagg aatcaagact agaaaggcag 1500
aaaatagagg gggttaagct ggaatctgag ggaacttaca aaatcctcac catttattcg 1560
actgtcgcct catctcttgt gcttgcaatg gggtttgctg ccttcctgtt ctgggccatg 1620
tccaatggat cttgcagatg caacatttgt atataa 1656

<210> 159
<211> 48
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> H9HK107399(Prl-).r
<400> 159
gtcctggcca acctccgcca ggcacgttcc tcagaccgac tgccagtt 48

<210> 160
<211> 48
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> H9HK107399(Prl-).c
<400> 160
ggaacgtgcc tggcggaggt tggccaggac tagtcgctgg ctggtatg 48

<210> 161
<211> 551
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 具有缺失的蛋白水解环的PDISP/H9杭州的氨基酸序列

<400> 161

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

Ser Thr Leu Pro Phe His Asn Ile Ser Lys Tyr Ala Phe Gly Thr Cys
305 310 315 320
Pro Lys Tyr Val Arg Val Asn Ser Leu Lys Leu Ala Val Gly Leu Arg
325 330 335
Asn Val Pro Gly Gly Gly Trp Pro Gly Leu Val Ala Gly Trp Tyr Gly
340 345 350
Phe Gln His Ser Asn Asp Gln Gly Val Gly Met Ala Ala Asp Arg Asp
355 360 365
Ser Thr Gln Lys Ala Ile Asp Lys Ile Thr Ser Lys Val Asn Asn Ile
370 375 380
Val Asp Lys Met Asn Lys Gln Tyr Glu Ile Ile Asp His Glu Phe Ser
385 390 395 400
Glu Val Glu Thr Arg Leu Asn Met Ile Asn Asn Lys Ile Asp Asp Gln
405 410 415
Ile Gln Asp Val Trp Ala Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu
420 425 430
Asn Gln Lys Thr Leu Asp Glu His Asp Ala Asn Val Asn Asn Leu Tyr
435 440 445
Asn Lys Val Lys Arg Ala Leu Gly Ser Asn Ala Met Glu Asp Gly Lys
450 455 460
Gly Cys Phe Glu Leu Tyr His Lys Cys Asp Asp Gln Cys Met Glu Thr
465 470 475 480
Ile Arg Asn Gly Thr Tyr Asn Arg Arg Lys Tyr Arg Glu Glu Ser Arg
485 490 495
Leu Glu Arg Gln Lys Ile Glu Gly Val Lys Leu Glu Ser Glu Gly Thr
500 505 510
Tyr Lys Ile Leu Thr Ile Tyr Ser Thr Val Ala Ser Ser Leu Val Leu
515 520 525
Ala Met Gly Phe Ala Ala Phe Leu Phe Trp Ala Met Ser Asn Gly Ser
530 535 540
Cys Arg Cys Asn Ile Cys Ile
545 550

<210> 162
<211> 1785
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>

<223> PDISP/HA B马来西亚的核苷酸序列

<400> 162

atggcgaaaa acgttgcgat tttcggctta ttgttttctc ttcttgtgtt ggttccttct 60
cagatcttcg cggatcgaat ctgcactggg ataacatcgt caaactcacc acatgttgtc 120
aaaactgcta ctcaagggga ggtcaatgtg actggtgtaa taccactgac aacaacaccc 180
accaaatctc attttgcaaa tctcaaagga acagaaacca gagggaaact atgcccaaaa 240
tgcctcaact gcacagatct ggacgtggcc ttgggcagac caaaatgcac ggggaacata 300
ccctcggcaa gagtttcaat actccatgaa gtcagacctg ttacatctgg gtgctttcct 360
ataatgcacg acagaacaaa aattagacag ctgcctaaac ttctcagagg atacgaacat 420
atcaggttat caactcataa cgttatcaat gcagaaaatg caccaggagg accctacaaa 480
attggaacct cagggtcttg ccctaacgtt accaatggaa acggattttt cgcaacaatg 540
gcttgggccg tcccaaaaaa cgacaacaac aaaacagcaa caaattcatt aacaatagaa 600
gtaccataca tttgtacaga aggagaagac caaattaccg tttgggggtt ccactctgat 660
aacgaaaccc aaatggcaaa gctctatggg gactcaaagc cccagaagtt cacctcatct 720
gccaacggag tgaccacaca ttacgtttca cagattggtg gcttcccaaa tcaaacagaa 780
gacggaggac taccacaaag cggtagaatt gttgttgatt acatggtgca aaaatctggg 840
aaaacaggaa caattaccta tcaaagaggt attttattgc ctcaaaaagt gtggtgcgca 900
agtggcagga gcaaggtaat aaaaggatcg ttgcctttaa ttggagaagc agattgcctc 960
cacgaaaaat acggtggatt aaacaaaagc aagccttact acacagggga acatgcaaag 1020
gccataggaa attgcccaat atgggtgaaa acacccttga agctggccaa tggaaccaaa 1080
tatagacctc ctgcaaaact attaaaggaa aggggtttct tcggagctat tgctggtttc 1140
ttagaaggag gatgggaagg aatgattgca ggttggcacg gatacacatc ccatggggca 1200
catggagtag cggtggcagc agaccttaag agcactcaag aggccataaa caagataaca 1260
aaaaatctca actctttgag tgagctggaa gtaaagaatc ttcaaagact aagcggtgcc 1320
atggatgaac tccacaacga aatactagaa ctagacgaga aagtggatga tctcagagct 1380
gatacaataa gctcacaaat agaactcgca gtcctgcttt ccaatgaagg aataataaac 1440
agtgaagatg agcatctctt ggcgcttgaa agaaagctga agaaaatgct gggcccctct 1500
gctgtagaga tagggaatgg atgctttgaa accaaacaca agtgcaacca gacctgtctc 1560
gacagaatag ctgctggtac ctttgatgca ggagaatttt ctctccccac ttttgattca 1620
ctgaatatta ctgctgcatc tttaaatgac gatggattgg ataatcatac tatactgctt 1680
tactactcaa ctgctgcctc cagtttggct gtaacattga tgatagctat ctttgttgtt 1740
tatatggtct ccagagacaa tgtttcttgc tccatctgtc tataa 1785

<210> 163

<211> 50

<212> DNA

<213> 人工序列(Artificial Sequence)

<220>

<221>

<222>

<223> IF**-HBM250604.S1-6r
<400> 163
actaaagaaa ataggccttt atagacagat ggagcaagaa acattgtctc 50
<210> 164
<211> 594
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> PDISP/HA B马来西亚的氨基酸序列
<400> 164

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

210 215 220
Met Ala Lys Leu Tyr Gly Asp Ser Lys Pro Gln Lys Phe Thr Ser Ser
225 230 235 240
Ala Asn Gly Val Thr Thr His Tyr Val Ser Gln Ile Gly Gly Phe Pro
245 250 255
Asn Gln Thr Glu Asp Gly Gly Leu Pro Gln Ser Gly Arg Ile Val Val
260 265 270
Asp Tyr Met Val Gln Lys Ser Gly Lys Thr Gly Thr Ile Thr Tyr Gln
275 280 285
Arg Gly Ile Leu Leu Pro Gln Lys Val Trp Cys Ala Ser Gly Arg Ser
290 295 300
Lys Val Ile Lys Gly Ser Leu Pro Leu Ile Gly Glu Ala Asp Cys Leu
305 310 315 320
His Glu Lys Tyr Gly Gly Leu Asn Lys Ser Lys Pro Tyr Tyr Thr Gly
325 330 335
Glu His Ala Lys Ala Ile Gly Asn Cys Pro Ile Trp Val Lys Thr Pro
340 345 350
Leu Lys Leu Ala Asn Gly Thr Lys Tyr Arg Pro Pro Ala Lys Leu Leu
355 360 365
Lys Glu Arg Gly Phe Phe Gly Ala Ile Ala Gly Phe Leu Glu Gly Gly
370 375 380
Trp Glu Gly Met Ile Ala Gly Trp His Gly Tyr Thr Ser His Gly Ala
385 390 395 400
His Gly Val Ala Val Ala Ala Asp Leu Lys Ser Thr Gln Glu Ala Ile
405 410 415
Asn Lys Ile Thr Lys Asn Leu Asn Ser Leu Ser Glu Leu Glu Val Lys
420 425 430
Asn Leu Gln Arg Leu Ser Gly Ala Met Asp Glu Leu His Asn Glu Ile
435 440 445
Leu Glu Leu Asp Glu Lys Val Asp Asp Leu Arg Ala Asp Thr Ile Ser
450 455 460
Ser Gln Ile Glu Leu Ala Val Leu Leu Ser Asn Glu Gly Ile Ile Asn
465 470 475 480
Ser Glu Asp Glu His Leu Leu Ala Leu Glu Arg Lys Leu Lys Lys Met
485 490 495
Leu Gly Pro Ser Ala Val Glu Ile Gly Asn Gly Cys Phe Glu Thr Lys
500 505 510
His Lys Cys Asn Gln Thr Cys Leu Asp Arg Ile Ala Ala Gly Thr Phe
515 520 525

Asp Ala Gly Glu Phe Ser Leu Pro Thr Phe Asp Ser Leu Asn Ile Thr
530 535 540
Ala Ala Ser Leu Asn Asp Asp Gly Leu Asp Asn His Thr Ile Leu Leu
545 550 555 560
Tyr Tyr Ser Thr Ala Ala Ser Ser Leu Ala Val Thr Leu Met Ile Ala
565 570 575
Ile Phe Val Val Tyr Met Val Ser Arg Asp Asn Val Ser Cys Ser Ile
580 585 590
Cys Leu

<210> 165
<211> 1734
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 具有缺失的蛋白水解环的PDISP/HA B马来西亚的核苷酸序列
<400> 165

atggcgaaaa acgttgcgat tttcggctta ttgttttctc ttcttgtgtt ggttccttct 60
cagatcttcg cggatcgaat ctgcactggg ataacatcgt caaactcacc acatgttgtc 120
aaaactgcta ctcaagggga ggtcaatgtg actggtgtaa taccactgac aacaacaccc 180
accaaatctc attttgcaaa tctcaaagga acagaaacca gagggaaact atgcccaaaa 240
tgcctcaact gcacagatct ggacgtggcc ttgggcagac caaaatgcac ggggaacata 300
ccctcggcaa gagtttcaat actccatgaa gtcagacctg ttacatctgg gtgctttcct 360
ataatgcacg acagaacaaa aattagacag ctgcctaaac ttctcagagg atacgaacat 420
atcaggttat caactcataa cgttatcaat gcagaaaatg caccaggagg accctacaaa 480
attggaacct cagggtcttg ccctaacgtt accaatggaa acggattttt cgcaacaatg 540
gcttgggccg tcccaaaaaa cgacaacaac aaaacagcaa caaattcatt aacaatagaa 600
gtaccataca tttgtacaga aggagaagac caaattaccg tttgggggtt ccactctgat 660
aacgaaaccc aaatggcaaa gctctatggg gactcaaagc cccagaagtt cacctcatct 720
gccaacggag tgaccacaca ttacgtttca cagattggtg gcttcccaaa tcaaacagaa 780
gacggaggac taccacaaag cggtagaatt gttgttgatt acatggtgca aaaatctggg 840
aaaacaggaa caattaccta tcaaagaggt attttattgc ctcaaaaagt gtggtgcgca 900
agtggcagga gcaaggtaat aaaaggatcg ttgcctttaa ttggagaagc agattgcctc 960
cacgaaaaat acggtggatt aaacaaaagc aagccttact acacagggga acatgcaaag 1020
gccataggaa attgcccaat atgggtgaaa acacccttga agctggccaa tggaaccaaa 1080
tatagacctc ctggtggagg atgggaagga atgattgcag gttggcacgg atacacatcc 1140
catggggcac atggagtagc ggtggcagca gaccttaaga gcactcaaga ggccataaac 1200
aagataacaa aaaatctcaa ctctttgagt gagctggaag taaagaatct tcaaagacta 1260

agcggtgcca tggatgaact ccacaacgaa atactagaac tagacgagaa agtggatgat 1320
ctcagagctg atacaataag ctcacaaata gaactcgcag tcctgctttc caatgaagga 1380
ataataaaca gtgaagatga gcatctcttg gcgcttgaaa gaaagctgaa gaaaatgctg 1440
ggcccctctg ctgtagagat agggaatgga tgctttgaaa ccaaacacaa gtgcaaccag 1500
acctgtctcg acagaatagc tgctggtacc tttgatgcag gagaattttc tctccccact 1560
tttgattcac tgaatattac tgctgcatct ttaaatgacg atggattgga taatcatact 1620
atactgcttt actactcaac tgctgcctcc agtttggctg taacattgat gatagctatc 1680
tttgttgttt atatggtctc cagagacaat gtttcttgct ccatctgtct ataa 1734

<210> 166
<211> 48
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> HBM250604(PrL-).r
<400> 166
cattccttcc catcctccac caggaggtct atatttggtt ccattggc 48

<210> 167
<211> 48
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> HBM250604(PrL-).c
<400> 167
agacctcctg gtggaggatg ggaaggaatg attgcaggtt ggcacgga 48

<210> 168
<211> 577
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 具有缺失的蛋白水解环的PDISP/HA B马来西亚的氨基酸序列
<400> 168
Met Ala Lys Asn Val Ala Ile Phe Gly Leu Leu Phe Ser Leu Leu Val
1 5 10 15

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

325 330 335
Glu His Ala Lys Ala Ile Gly Asn Cys Pro Ile Trp Val Lys Thr Pro
340 345 350
Leu Lys Leu Ala Asn Gly Thr Lys Tyr Arg Pro Pro Gly Gly Gly Trp
355 360 365
Glu Gly Met Ile Ala Gly Trp His Gly Tyr Thr Ser His Gly Ala His
370 375 380
Gly Val Ala Val Ala Ala Asp Leu Lys Ser Thr Gln Glu Ala Ile Asn
385 390 395 400
Lys Ile Thr Lys Asn Leu Asn Ser Leu Ser Glu Leu Glu Val Lys Asn
405 410 415
Leu Gln Arg Leu Ser Gly Ala Met Asp Glu Leu His Asn Glu Ile Leu
420 425 430
Glu Leu Asp Glu Lys Val Asp Asp Leu Arg Ala Asp Thr Ile Ser Ser
435 440 445
Gln Ile Glu Leu Ala Val Leu Leu Ser Asn Glu Gly Ile Ile Asn Ser
450 455 460
Glu Asp Glu His Leu Leu Ala Leu Glu Arg Lys Leu Lys Lys Met Leu
465 470 475 480
Gly Pro Ser Ala Val Glu Ile Gly Asn Gly Cys Phe Glu Thr Lys His
485 490 495
Lys Cys Asn Gln Thr Cys Leu Asp Arg Ile Ala Ala Gly Thr Phe Asp
500 505 510
Ala Gly Glu Phe Ser Leu Pro Thr Phe Asp Ser Leu Asn Ile Thr Ala
515 520 525
Ala Ser Leu Asn Asp Asp Gly Leu Asp Asn His Thr Ile Leu Leu Tyr
530 535 540
Tyr Ser Thr Ala Ala Ser Ser Leu Ala Val Thr Leu Met Ile Ala Ile
545 550 555 560
Phe Val Val Tyr Met Val Ser Arg Asp Asn Val Ser Cys Ser Ile Cys
565 570 575
Leu

<210> 169
<211> 1782
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>

<223> PDISP/HA B马萨诸塞州的核苷酸序列

<400> 169

atggcgaaaa acgttgcgat tttcggctta ttgttttctc ttcttgtgtt ggttccttct 60
cagatcttcg ccgatcgaat ctgcactggg ataacatctt caaactcacc tcatgtggtc 120
aaaacagcta ctcaagggga ggtcaatgtg actggtgtga taccactaac aacaacacca 180
acaaaatctt attttgcaaa tctcaaagga acaaagacca gagggaaact atgcccagac 240
tgtctcaact gtacagatct ggatgtggcc ctgggcaggc caatgtgtgt gggaactaca 300
ccttctgcga aagcttcaat acttcacgaa gtcagacctg ttacatccgg gtgcttccct 360
ataatgcacg acagaacaaa aatcaggcaa ctagccaatc ttctcagagg atatgaaaat 420
atcaggttat caacccaaaa cgttatcgat gcagaaaagg caccaggagg accctacaga 480
cttggaacct caggatcttg ccctaacgct accagtaaaa gcggattttt cgcaacaatg 540
gcttgggctg tcccaaagga caacaacaaa aatgcaacga acccattaac agtagaagta 600
ccatacattt gtgcagaagg ggaagaccaa attactgttt gggggttcca ttcagataac 660
aaaacccaaa tgaagaacct ctatggagac tcaaatcctc aaaagttcac ctcatctgct 720
aatggagtaa ccacacatta tgtttctcag attggcggct tcccagatca aacagaagac 780
ggaggactac cacaaagcgg cagaattgtc gttgattaca tgatgcaaaa acctgggaaa 840
acaggaacaa ttgtctatca aagaggtgtt ttgttgcctc aaaaggtgtg gtgcgcgagt 900
ggcaggagca aagtaataaa agggtccttg cctttaattg gtgaagcaga ttgccttcat 960
gaaaaatacg gtggattaaa caaaagcaag ccttactaca caggagaaca tgcaaaagcc 1020
ataggaaatt gcccaatatg ggtgaaaaca cctttgaagc ttgccaatgg aaccaaatat 1080
agacctcctg caaaactatt aaaggaaagg ggtttcttcg gagctattgc tggtttccta 1140
gaaggaggat gggaaggaat gattgcaggt tggcacggat acacatctca cggagcacat 1200
ggagtggcag ttgctgcaga ccttaagagc acacaagaag ctataaacaa gataacaaaa 1260
aatctcaact ctttgagtga gctagaagta aagaatcttc aaaggctaag tggtgccatg 1320
gatgaactcc acaacgaaat actcgagctg gatgagaaag tggatgacct cagagctgac 1380
actataagtt cacaaataga acttgcagtc ttgctttcca acgaaggaat aataaacagt 1440
gaagacgagc atctattggc acttgagaga aaactaaaga aaatgctggg tccctctgct 1500
gtagacatag gaaatggatg cttcgaaacc aaacacaaat gcaaccagac ctgcttagac 1560
aggatagctg ctggcacctt taatgcagga gagttttctc tccccacttt tgattcattg 1620
aacattactg ctgcatcttt aaatgatgat ggattggata accatactat actgctctat 1680
tactcaactg ctgcttctag tttggctgta acattgatgc tagctatttt tattgtttat 1740
atggtctcca gagacaacgt ttcatgctcc atctgtctat aa 1782

<210> 170

<211> 593

<212> PRT

<213> 人工序列(Artificial Sequence)

<220>

<221>

<222>

<223> PDISP/HA B马萨诸塞州的氨基酸序列

<400> 170

Met Ala Lys Asn Val Ala Ile Phe Gly Leu Leu Phe Ser Leu Leu Val
1 5 10 15
Leu Val Pro Ser Gln Ile Phe Ala Asp Arg Ile Cys Thr Gly Ile Thr
20 25 30
Ser Ser Asn Ser Pro His Val Val Lys Thr Ala Thr Gln Gly Glu Val
35 40 45
Asn Val Thr Gly Val Ile Pro Leu Thr Thr Thr Pro Thr Lys Ser Tyr
50 55 60
Phe Ala Asn Leu Lys Gly Thr Lys Thr Arg Gly Lys Leu Cys Pro Asp
65 70 75 80
Cys Leu Asn Cys Thr Asp Leu Asp Val Ala Leu Gly Arg Pro Met Cys
85 90 95
Val Gly Thr Thr Pro Ser Ala Lys Ala Ser Ile Leu His Glu Val Arg
100 105 110
Pro Val Thr Ser Gly Cys Phe Pro Ile Met His Asp Arg Thr Lys Ile
115 120 125
Arg Gln Leu Ala Asn Leu Leu Arg Gly Tyr Glu Asn Ile Arg Leu Ser
130 135 140
Thr Gln Asn Val Ile Asp Ala Glu Lys Ala Pro Gly Gly Pro Tyr Arg
145 150 155 160
Leu Gly Thr Ser Gly Ser Cys Pro Asn Ala Thr Ser Lys Ser Gly Phe
165 170 175
Phe Ala Thr Met Ala Trp Ala Val Pro Lys Asp Asn Asn Lys Asn Ala
180 185 190
Thr Asn Pro Leu Thr Val Glu Val Pro Tyr Ile Cys Ala Glu Gly Glu
195 200 205
Asp Gln Ile Thr Val Trp Gly Phe His Ser Asp Asn Lys Thr Gln Met
210 215 220
Lys Asn Leu Tyr Gly Asp Ser Asn Pro Gln Lys Phe Thr Ser Ser Ala
225 230 235 240
Asn Gly Val Thr Thr His Tyr Val Ser Gln Ile Gly Gly Phe Pro Asp
245 250 255
Gln Thr Glu Asp Gly Gly Leu Pro Gln Ser Gly Arg Ile Val Val Asp
260 265 270
Tyr Met Met Gln Lys Pro Gly Lys Thr Gly Thr Ile Val Tyr Gln Arg
275 280 285
Gly Val Leu Leu Pro Gln Lys Val Trp Cys Ala Ser Gly Arg Ser Lys

290 295 300

Val Ile Lys Gly Ser Leu Pro Leu Ile Gly Glu Ala Asp Cys Leu His
305 310 315 320

Glu Lys Tyr Gly Gly Leu Asn Lys Ser Lys Pro Tyr Tyr Thr Gly Glu
325 330 335

His Ala Lys Ala Ile Gly Asn Cys Pro Ile Trp Val Lys Thr Pro Leu
340 345 350

Lys Leu Ala Asn Gly Thr Lys Tyr Arg Pro Pro Ala Lys Leu Leu Lys
355 360 365

Glu Arg Gly Phe Phe Gly Ala Ile Ala Gly Phe Leu Glu Gly Gly Trp
370 375 380

Glu Gly Met Ile Ala Gly Trp His Gly Tyr Thr Ser His Gly Ala His
385 390 395 400

Gly Val Ala Val Ala Ala Asp Leu Lys Ser Thr Gln Glu Ala Ile Asn
405 410 415

Lys Ile Thr Lys Asn Leu Asn Ser Leu Ser Glu Leu Glu Val Lys Asn
420 425 430

Leu Gln Arg Leu Ser Gly Ala Met Asp Glu Leu His Asn Glu Ile Leu
435 440 445

Glu Leu Asp Glu Lys Val Asp Asp Leu Arg Ala Asp Thr Ile Ser Ser
450 455 460

Gln Ile Glu Leu Ala Val Leu Leu Ser Asn Glu Gly Ile Ile Asn Ser
465 470 475 480

Glu Asp Glu His Leu Leu Ala Leu Glu Arg Lys Leu Lys Lys Met Leu
485 490 495

Gly Pro Ser Ala Val Asp Ile Gly Asn Gly Cys Phe Glu Thr Lys His
500 505 510

Lys Cys Asn Gln Thr Cys Leu Asp Arg Ile Ala Ala Gly Thr Phe Asn
515 520 525

Ala Gly Glu Phe Ser Leu Pro Thr Phe Asp Ser Leu Asn Ile Thr Ala
530 535 540

Ala Ser Leu Asn Asp Asp Gly Leu Asp Asn His Thr Ile Leu Leu Tyr
545 550 555 560

Tyr Ser Thr Ala Ala Ser Ser Leu Ala Val Thr Leu Met Leu Ala Ile
565 570 575

Phe Ile Val Tyr Met Val Ser Arg Asp Asn Val Ser Cys Ser Ile Cys
580 585 590

Leu

<210> 171

<211> 63
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 马铃薯糖蛋白A信号肽的核苷酸序列
<400> 171
atggcaacta ctaaaacttt tttaatttta ttttttatga tattagcaac tactagttca 60
aca 63
<210> 172
<211> 23
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 马铃薯糖蛋白A信号肽的氨基酸序列
<400> 172
Met Ala Thr Thr Lys Thr Phe Leu Ile Leu Phe Phe Met Ile Leu Ala
1 5 10 15
Thr Thr Ser Ser Thr Cys Ala
20
<210> 173
<211> 517
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> CMPV HT序列
<400> 173
tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc 60
ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgcgtgagc 120
gatcttcaac gttgtcagat cgtgcttcgg caccagtaca acgttttctt tcactgaagc 180
gaaatcaaag atctctttgt ggacacgtag tgcggcgcca ttaaataacg tgtacttgtc 240
ctattcttgt cggtgtggtc ttgggaaaag aaagcttgct ggaggctgct gttcagcccc 300
atacattact tgttacgatt ctgctgactt tcggcgggtg caatatctct acttctgctt 360
gacgaggtat tgttgcctgt acttctttct tcttcttctt gctgattggt tctataagaa 420

atctagtatt ttctttgaaa cagagttttc ccgtggtttt cgaacttgga gaaagattgt 480
taagcttctg tatattctgc ccaaatttgt cgggccc 517

<210> 174

<400> 174

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<210> 175

<400> 175

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<210> 176

<400> 176

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<210> 177

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<210> 178

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<210> 183

<400> 183

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<210> 184

<400> 184

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<210> 185

<400> 185

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<210> 186

<400> 186
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<210> 187
<211> 528
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 具有植物kozak共有序列的CPMV HT+
<400> 187
tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc 60
ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgcgtgagc 120
gatcttcaac gttgtcagat cgtgcttcgg caccagtaca acgttttctt tcactgaagc 180
gaaatcaaag atctctttgt ggacacgtag tgcggcgcca ttaaataacg tgtacttgtc 240
ctattcttgt cggtgtggtc ttgggaaaag aaagcttgct ggaggctgct gttcagcccc 300
atacattact tgttacgatt ctgctgactt tcggcgggtg caatatctct acttctgctt 360
gacgaggtat tgttgcctgt acttctttct tcttcttctt gctgattggt tctataagaa 420
atctagtatt ttctttgaaa cagagttttc ccgtggtttt cgaacttgga gaaagattgt 480
taagcttctg tatattctgc ccaaatttgt tcgggcccaa taccgcgg 528
<210> 188
<211> 511
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> CPMV HT+ 511的核苷酸序列
<400> 188
tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc 60
ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgcgtgagc 120
gatcttcaac gttgtcagat cgtgcttcgg caccagtaca acgttttctt tcactgaagc 180
gaaatcaaag atctctttgt ggacacgtag tgcggcgcca ttaaataacg tgtacttgtc 240
ctattcttgt cggtgtggtc ttgggaaaag aaagcttgct ggaggctgct gttcagcccc 300
atacattact tgttacgatt ctgctgactt tcggcgggtg caatatctct acttctgctt 360
gacgaggtat tgttgcctgt acttctttct tcttcttctt gctgattggt tctataagaa 420
atctagtatt ttctttgaaa cagagttttc ccgtggtttt cgaacttgga gaaagattgt 480
taagcttctg tatattctgc ccaaatttga a 511
<210> 189

<211> 534
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> CPMV HT+ [WT115]的核苷酸序列
<400> 189
tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc 60
ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgcatgagc 120
gatcttcaac gttgtcagat cgtgcttcgg caccagtaca acgttttctt tcactgaagc 180
gaaatcaaag atctctttgt ggacacgtag tgcggcgcca ttaaataacg tgtacttgtc 240
ctattcttgt cggtgtggtc ttgggaaaag aaagcttgct ggaggctgct gttcagcccc 300
atacattact tgttacgatt ctgctgactt tcggcgggtg caatatctct acttctgctt 360
gacgaggtat tgttgcctgt acttctttct tcttcttctt gctgattggt tctataagaa 420
atctagtatt ttctttgaaa cagagttttc ccgtggtttt cgaacttgga gaaagattgt 480
taagcttctg tatattctgc ccaaatttgt tcgggcccaa taccgcggag aaaa 534
<210> 190
<211> 56
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> HBF406(PrL-).r(构建体2102和2104)
<400> 190
tccttcccat cctccaccag gaggtctata tttggttcca ttggcgagct tcaaag 56
<210> 191
<211> 52
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> HBF406(PrL-).c(构建体2102和2104)
<400> 191
atatagacct cctggtggag gatgggaagg aatgattgca ggctggcacg ga 52
<210> 192
<211> 50

<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> IF*-HBF406.s1-6r(构建体2102和2104)
<400> 192
actaaagaaa ataggccttt atagacagat ggagcatgaa acgttgtctc 50
<210> 193
<211> 1731
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 具有缺失的蛋白水解环的PDISP/HA B佛罗里达(构建体2102和2104)
的核苷酸序列
<400> 193
atggcgaaaa acgttgcgat tttcggctta ttgttttctc ttcttgtgtt ggttccttct 60
cagatcttcg cggatcgaat ctgcactgga ataacatctt caaactcacc tcatgtggtc 120
aaaacagcca ctcaagggga ggtcaatgtg actggtgtga taccactaac aacaacacca 180
acaaaatctt attttgcaaa tctcaaagga acaaggacca gagggaaact atgcccagac 240
tgtctcaact gcacagatct ggatgtggct ttgggcagac caatgtgtgt ggggaccaca 300
ccttcggcga aggcttcaat actccacgaa gtcaaacctg ttacatccgg gtgctttcct 360
ataatgcacg acagaacaaa aatcaggcaa ctacccaatc ttctcagagg atatgaaaat 420
atcaggctat caacccaaaa cgtcatcgat gcggaaaagg caccaggagg accctacaga 480
cttggaacct caggatcttg ccctaacgct accagtaaga gcggattttt cgcaacaatg 540
gcttgggctg tcccaaagga caacaacaaa aatgcaacga acccactaac agtagaagta 600
ccatacattt gtacagaagg ggaagaccaa atcactgttt gggggttcca ttcagataac 660
aaaacccaaa tgaagaacct ctatggagac tcaaatcctc aaaagttcac ctcatctgct 720
aatggagtaa ccacacacta tgtttctcag attggcagct tcccagatca aacagaagac 780
ggaggactac cacaaagcgg caggattgtt gttgattaca tgatgcaaaa acctgggaaa 840
acaggaacaa ttgtctacca aagaggtgtt ttgttgcctc aaaaggtgtg gtgcgcgagt 900
ggcaggagca aagtaataaa agggtccttg cctttaattg gtgaagcaga ttgccttcat 960
gaaaaatacg gtggattaaa caaaagcaag ccttactaca caggagaaca tgcaaaagcc 1020
ataggaaatt gcccaatatg ggtgaaaaca cctttgaagc tcgccaatgg aaccaaatat 1080
agacctcctg gtggaggatg ggaaggaatg attgcaggct ggcacggata cacatctcac 1140
ggagcacatg gagtggcagt ggcggcggac cttaagagta cgcaagaagc tataaacaag 1200
ataacaaaaa atctcaattc tttgagtgag ctagaagtaa agaatcttca aagactaagt 1260

ggtgccatgg atgaactcca caacgaaata ctcgagctgg atgagaaagt ggatgatctc 1320
agagctgaca ctataagctc gcaaatagaa cttgcagtct tgctttccaa cgaaggaata 1380
ataaacagtg aagatgagca tctattggca cttgagagaa aactaaagaa aatgctgggt 1440
ccctctgctg tagagatagg aaatggatgc ttcgaaacca aacacaagtg caaccagacc 1500
tgcttagaca ggatagctgc tggcaccttt aatgcaggag aattttctct ccccactttt 1560
gattcactga acattactgc tgcatcttta aatgatgatg gattggataa ccatactata 1620
ctgctctatt actcaactgc tgcttctagt ttggctgtaa cattgatgct agctattttt 1680
attgtttata tggtctccag agacaacgtt tcatgctcca tctgtctata a 1731

<210> 194
<211> 576
<212> PRT
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 具有缺失的蛋白水解环的PDISP/HA B佛罗里达(构建体2102和2104)的氨基酸序列

<400> 194

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

165 170 175
Phe Ala Thr Met Ala Trp Ala Val Pro Lys Asp Asn Asn Lys Asn Ala
180 185 190
Thr Asn Pro Leu Thr Val Glu Val Pro Tyr Ile Cys Thr Glu Gly Glu
195 200 205
Asp Gln Ile Thr Val Trp Gly Phe His Ser Asp Asn Lys Thr Gln Met
210 215 220
Lys Asn Leu Tyr Gly Asp Ser Asn Pro Gln Lys Phe Thr Ser Ser Ala
225 230 235 240
Asn Gly Val Thr Thr His Tyr Val Ser Gln Ile Gly Ser Phe Pro Asp
245 250 255
Gln Thr Glu Asp Gly Gly Leu Pro Gln Ser Gly Arg Ile Val Val Asp
260 265 270
Tyr Met Met Gln Lys Pro Gly Lys Thr Gly Thr Ile Val Tyr Gln Arg
275 280 285
Gly Val Leu Leu Pro Gln Lys Val Trp Cys Ala Ser Gly Arg Ser Lys
290 295 300
Val Ile Lys Gly Ser Leu Pro Leu Ile Gly Glu Ala Asp Cys Leu His
305 310 315 320
Glu Lys Tyr Gly Gly Leu Asn Lys Ser Lys Pro Tyr Tyr Thr Gly Glu
325 330 335
His Ala Lys Ala Ile Gly Asn Cys Pro Ile Trp Val Lys Thr Pro Leu
340 345 350
Lys Leu Ala Asn Gly Thr Lys Tyr Arg Pro Pro Gly Gly Gly Trp Glu
355 360 365
Gly Met Ile Ala Gly Trp His Gly Tyr Thr Ser His Gly Ala His Gly
370 375 380
Val Ala Val Ala Ala Asp Leu Lys Ser Thr Gln Glu Ala Ile Asn Lys
385 390 395 400
Ile Thr Lys Asn Leu Asn Ser Leu Ser Glu Leu Glu Val Lys Asn Leu
405 410 415
Gln Arg Leu Ser Gly Ala Met Asp Glu Leu His Asn Glu Ile Leu Glu
420 425 430
Leu Asp Glu Lys Val Asp Asp Leu Arg Ala Asp Thr Ile Ser Ser Gln
435 440 445
Ile Glu Leu Ala Val Leu Leu Ser Asn Glu Gly Ile Ile Asn Ser Glu
450 455 460
Asp Glu His Leu Leu Ala Leu Glu Arg Lys Leu Lys Lys Met Leu Gly
465 470 475 480

Pro Ser Ala Val Glu Ile Gly Asn Gly Cys Phe Glu Thr Lys His Lys
485 490 495
Cys Asn Gln Thr Cys Leu Asp Arg Ile Ala Ala Gly Thr Phe Asn Ala
500 505 510
Gly Glu Phe Ser Leu Pro Thr Phe Asp Ser Leu Asn Ile Thr Ala Ala
515 520 525
Ser Leu Asn Asp Asp Gly Leu Asp Asn His Thr Ile Leu Leu Tyr Tyr
530 535 540
Ser Thr Ala Ala Ser Ser Leu Ala Val Thr Leu Met Leu Ala Ile Phe
545 550 555 560
Ile Val Tyr Met Val Ser Arg Asp Asn Val Ser Cys Ser Ile Cys Leu
565 570 575

<210> 195
<211> 3486
<212> DNA
<213> 人工序列(Artificial Sequence)
<220>
<221>
<222>
<223> 盒2102
<400> 195

gtcaacatgg tggagcacga cacacttgtc tactccaaaa atatcaaaga tacagtctca 60
gaagaccaaa gggcaattga gacttttcaa caaagggtaa tatccggaaa cctcctcgga 120
ttccattgcc cagctatctg tcactttatt gtgaagatag tggaaaagga aggtggctcc 180
tacaaatgcc atcattgcga taaaggaaag gccatcgttg aagatgcctc tgccgacagt 240
ggtcccaaag atggaccccc acccacgagg agcatcgtgg aaaaagaaga cgttccaacc 300
acgtcttcaa agcaagtgga ttgatgtgat aacatggtgg agcacgacac acttgtctac 360
tccaaaaata tcaaagatac agtctcagaa gaccaaaggg caattgagac ttttcaacaa 420
agggtaatat ccggaaacct cctcggattc cattgcccag ctatctgtca ctttattgtg 480
aagatagtgg aaaaggaagg tggctcctac aaatgccatc attgcgataa aggaaaggcc 540
atcgttgaag atgcctctgc cgacagtggt cccaaagatg gacccccacc cacgaggagc 600
atcgtggaaa aagaagacgt tccaaccacg tcttcaaagc aagtggattg atgtgatatc 660
tccactgacg taagggatga cgcacaatcc cactatcctt cgcaagaccc ttcctctata 720
taaggaagtt catttcattt ggagaggtat taaaatctta ataggttttg ataaaagcga 780
acgtggggaa acccgaacca aaccttcttc taaactctct ctcatctctc ttaaagcaaa 840
cttctctctt gtctttcttg cgtgagcgat cttcaacgtt gtcagatcgt gcttcggcac 900
cagtacaacg ttttctttca ctgaagcgaa atcaaagatc tctttgtgga cacgtagtgc 960
ggcgccatta ataacgtgt acttgtccta ttcttgtcgg tgtggtcttg ggaaaagaaa 1020
gcttgctgga ggctgctgtt cagccccata cattacttgt tacgattctg ctgactttcg 1080

gcgggtgcaa tatctctact tctgcttgac gaggtattgt tgcctgtact tctttcttct 1140
tcttcttgct gattggttct ataagaaatc tagtattttc tttgaaacag agttttcccg 1200
tggttttcga acttggagaa agattgttaa gcttctgtat attctgccca aatttgtcgg 1260
gcccaatacc gcggagaaaa tggcgaaaaa cgttgcgatt ttcggcttat tgttttctct 1320
tcttgtgttg gttccttctc agatcttcgc ggatcgaatc tgcactggaa taacatcttc 1380
aaactcacct catgtggtca aaacagccac tcaaggggag gtcaatgtga ctggtgtgat 1440
accactaaca acaacaccaa caaaatctta ttttgcaaat ctcaaaggaa caaggaccag 1500
agggaaacta tgcccagact gtctcaactg cacagatctg gatgtggctt tgggcagacc 1560
aatgtgtgtg gggaccacac cttcggcgaa ggcttcaata ctccacgaag tcaaacctgt 1620
tacatccggg tgctttccta taatgcacga cagaacaaaa atcaggcaac tacccaatct 1680
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<210> 198
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<223> 具有缺失的蛋白水解环的PDISP/HA B佛罗里达+H1加利福尼亚TMCT (构建体2106和2108)的核苷酸序列

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<210> 199

<211> 576

<212> PRT

<213> 人工序列(Artificial Sequence)

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<221>

<222>

<223> 具有缺失的蛋白水解环的PDISP/HA B佛罗里达+H1加利福尼亚TMCT(构建体2106和2108)的氨基酸序列

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

275 280 285

Gly Val Leu Leu Pro Gln Lys Val Trp Cys Ala Ser Gly Arg Ser Lys

290 295 300

Val Ile Lys Gly Ser Leu Pro Leu Ile Gly Glu Ala Asp Cys Leu His

305 310 315 320

Glu Lys Tyr Gly Gly Leu Asn Lys Ser Lys Pro Tyr Tyr Thr Gly Glu

325 330 335

His Ala Lys Ala Ile Gly Asn Cys Pro Ile Trp Val Lys Thr Pro Leu

340 345 350

Lys Leu Ala Asn Gly Thr Lys Tyr Arg Pro Pro Gly Gly Gly Trp Glu

355 360 365

Gly Met Ile Ala Gly Trp His Gly Tyr Thr Ser His Gly Ala His Gly

370 375 380

Val Ala Val Ala Ala Asp Leu Lys Ser Thr Gln Glu Ala Ile Asn Lys

385 390 395 400

Ile Thr Lys Asn Leu Asn Ser Leu Ser Glu Leu Glu Val Lys Asn Leu

405 410 415

Gln Arg Leu Ser Gly Ala Met Asp Glu Leu His Asn Glu Ile Leu Glu

420 425 430

Leu Asp Glu Lys Val Asp Asp Leu Arg Ala Asp Thr Ile Ser Ser Gln

435 440 445

Ile Glu Leu Ala Val Leu Leu Ser Asn Glu Gly Ile Ile Asn Ser Glu

450 455 460

Asp Glu His Leu Leu Ala Leu Glu Arg Lys Leu Lys Lys Met Leu Gly

465 470 475 480

Pro Ser Ala Val Glu Ile Gly Asn Gly Cys Phe Glu Thr Lys His Lys

485 490 495

Cys Asn Gln Thr Cys Leu Asp Arg Ile Ala Ala Gly Thr Phe Asn Ala

500 505 510

Gly Glu Phe Ser Leu Pro Thr Phe Asp Ser Leu Asn Ile Thr Ala Ala

515 520 525

Ser Leu Asn Asp Asp Gly Leu Asp Asn Tyr Gln Ile Leu Ala Ile Tyr

530 535 540

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Ser Phe Trp Met Cys Ser Asn Gly Ser Leu Gln Cys Arg Ile Cys Ile

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图 1A

IF-H5A-I-05.s1+3c SEQ ID NO: 2

AAATTTGTCGGGCCCATGGAGAAAATAGTGCTTCTTCTTGC

图 1B

IF-H5dTm.r SEQ ID NO: 3

ACTAAAGAAAATAGGCCTTTAAATGCAAATTCTGCATTGTAACGATCCAT

图 1C

构建体1191的示意图。在示意图上注释用于质粒线性化的SacII和StuI限制性酶切位点

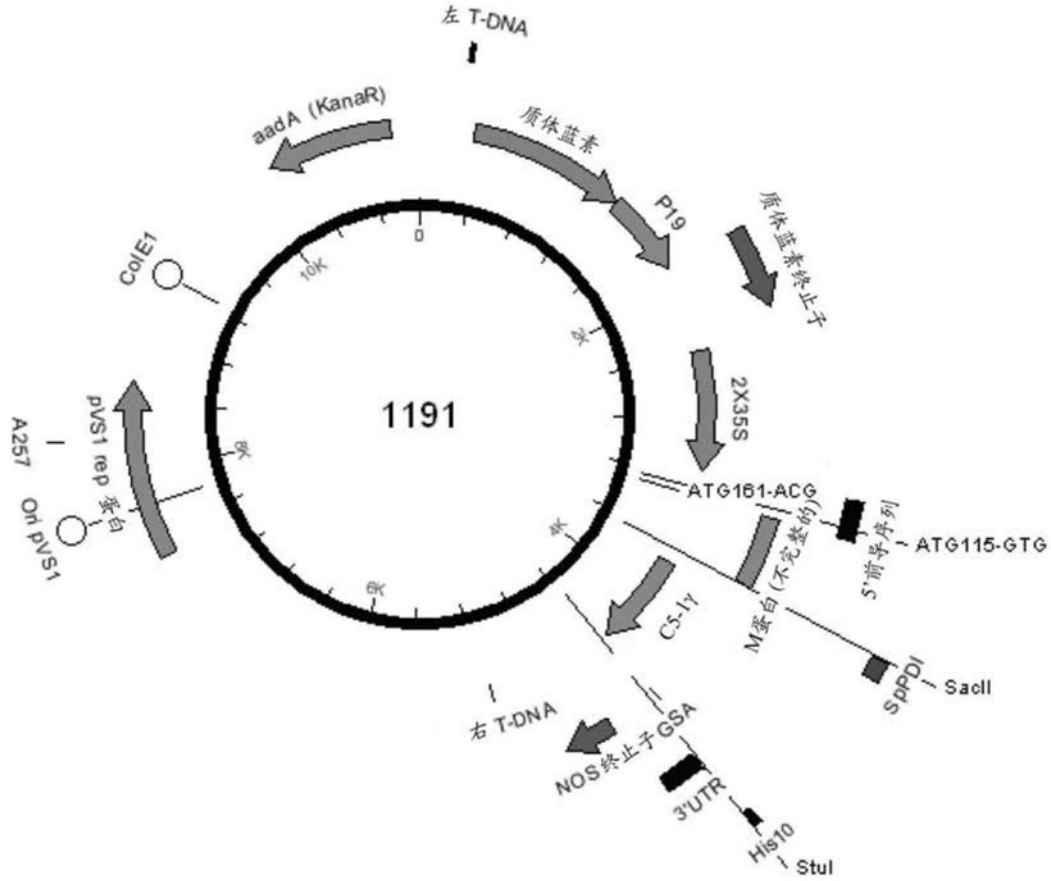


图 1D

从左至右t-DNA边界的构建体1191(加下划线)。具有质体蓝素-P19-质体蓝素沉默抑制子表达盒的2X35S/CPMV-HT/NOS(SEQ ID NO: 4)

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图 1D(续)

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图 1E

从2X35S启动子至NOS终止子的表达盒489号。来自流感A/印度尼西亚/5/2005(H5N1)的H5加下划线(SEQ ID NO: 5)

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图 1E(续)

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GGCGAGTTCCCTAGCACTGGCAATCATGATGGCTGGTCTATCTTTATGGATGTGCTCCAATGGA
TCGTTACAATGCAGAATTTGCATTTAAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCG
GTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTT
AATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAAT
TTTATTAAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCAGATCG
TTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATC
ATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTA
TGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAAT
ATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGAT

图 1F

来自流感A/印度尼西亚/5/2005(H5N1)的H5的氨基酸序列(SEQ ID NO: 6)

MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCDLDGVK
PLILRDCSVAGWLLGNPMCDEFINVPEWSYIVEKANPTNDLCYPGSFNDYEELKHLLSRINHFE
KIQIIPKSSWSDHEASSGVSSACPYLGSPSFFRNVVWLIKKNSTYPTIKKSYNNTNQEDLLVLW
GIHHPNDAAEQTRLYQNPTTYISIGTSTLNQRLVPKIATRSKVNGQSGRMEFFWTILKPNDAIN
FESNGNFIAPEYAYKIVKKGDSAIMKSELEYGNCNTKCQTPMGAINSSMPFHNIHPLTIGECPK
YVKSNRLVLATGLRNSPQRESRRKKRGLFGAIAGFIEGGWQGMVDGWYGYHHSNEQGSGYAADK
ESTQKAIDGVTNKVNSIIDKMNTQFEAVGREFNNLERRIENLNKKMEDGFLDVWTYNAELLVLM
ENERTLDFHDSNVKNLYDKVRLQLRDNAKELGNGCFEFYHKCDNECMESIRNGTYNYPQYSEEA
RLKREEISGVKLESIGTYQILSIYSTVASSLALAIMMAGLSLWMCSNGSLQCRICI

图 1G

编码来自流感A/印度尼西亚/5/2005(H5N1)的H5的核苷酸序列(SEQ ID NO: 42)

ATGGAGAAAATAGTGCTTCTTCTTGCAATAGTCAGTCTTGTTAAAAGTGATCAGATTTGCATTG
GTTACCATGCAAACAATTCAACAGAGCAGGTTGACACAATCATGGAAAAGAACGTTACTGTTAC
ACATGCCCAAGACATACTGGAAAAGACACACAACGGGAAGCTCTGCGATCTAGATGGAGTGAAG
CCTCTAATTTTAAGAGATTGTAGTGTAGCTGGATGGCTCCTCGGGAACCCAATGTGTGACGAAT
TCATCAATGTACCGGAATGGTCTTACATAGTGGAGAAGGCCAATCCAACCAATGACCTCTGTTA
CCCAGGGAGTTTCAACGACTATGAAGAACTGAAACACCTATTGAGCAGAATAAACCATTTTGAG
AAAATTCAAATCATCCCCAAAAGTTCTTGGTCCGATCATGAAGCCTCATCAGGAGTTAGCTCAG
CATGTCCATACCTGGGAAGTCCCTCCTTTTTTAGAAATGTGGTATGGCTTATCAAAAAGAACAG
TACATACCCAACAATAAAGAAAAGCTACAATAATACCAACCAAGAGGATCTTTTGGTACTGTGG
GGAATTCACCATCCTAATGATGCGGCAGAGCAGACAAGGCTATATCAAAACCCAACCACCTATA
TTTCCATTGGGACATCAACACTAAACCAGAGATTGGTACCAAAAATAGCTACTAGATCCAAAGT
AAACGGGCAAAGTGGAAGGATGGAGTTCTTCTGGACAATTTTAAAACCTAATGATGCAATCAAC
TTCGAGAGTAATGGAAATTTCATTGCTCCAGAATATGCATACAAAATTGTCAAGAAAGGGGACT
CAGCAATTATGAAAAGTGAATTGGAATATGGTAACTGCAACACCAAGTGTCAAACTCCAATGGG
GGCGATAAACTCTAGTATGCCATTCCACAACATACACCCTCTCACCATCGGGGAATGCCCCAAA
TATGTGAAATCAAACAGATTAGTCCTTGCAACAGGGCTCAGAAATAGCCCTCAAAGAGAGAGCA
GAAGAAAAAAGAGAGGACTATTTGGAGCTATAGCAGGTTTTATAGAGGGAGGATGGCAGGGAAT
GGTAGATGGTTGGTATGGGTACCACCATAGCAATGAGCAGGGGAGTGGGTACGCTGCAGACAAA
GAATCCACTCAAAAGGCAATAGATGGAGTCACCAATAAGGTCAACTCAATCATTGACAAAATGA
ACACTCAGTTTGAGGCCGTTGGAAGGGAATTTAATAACTTAGAAAGGAGAATAGAGAATTTAAA
CAAGAAGATGGAAGACGGGTTTCTAGATGTCTGGACTTATAATGCCGAACTTCTGGTTCTCATG
GAAAATGAGAGAACTCTAGACTTTCATGACTCAAATGTTAAGAACCTCTACGACAAGGTCCGAC
TACAGCTTAGGGATAATGCAAAGGAGCTGGGTAACGGTTGTTTCGAGTTCTATCACAAATGTGA
TAATGAATGTATGGAAAGTATAAGAAACGGAACGTACAACTATCCGCAGTATTCAGAAGAAGCA
AGATTAAAAAGAGAGGAAATAAGTGGGGTAAAATTGGAATCAATAGGAACTTACCAAATACTGT
CAATTTATTCAACAGTGGCGAGTTCCCTAGCACTGGCAATCATGATGGCTGGTCTATCTTTATG
GATGTGCTCCAATGGATCGTTACAATGCAGAATTTGCATTTAA

图1:A-2X35S/CPMV-HT/H5印度尼西亚/NOS(构建体489号)

图 2A

IF-S1-M1+M2ANC.c (SEQ ID NO:7)

AAATTTGTCGGGCCCATGAGTCTTCTAACCGAGGTCGAAACG

图 2B

IF-S1-4-M2ANC.r (SEQ ID NO: 8)

ACTAAAGAAAATAGGCCTTTACTCCAGCTCTATGCTGACAAAA

图 2C

合成的M2基因(对应于连接到来自Genbank登录号DQ508860的nt 715-982的nt 1-26)
(SEQ ID NO: 9)

ATGAGTCTTCTAACCGAGGTCGAAACGCCTATCAGAAACGAATGGGGGTGCAGATGCAACGATT
CAAGTGATCCTCTTGTTGTTGCCGCAAGTATAATTGGGATTGTGCACCTGATATTGTGGATTAT
TGATCGCCTTTTTTCCAAAAGCATTTATCGTATCTTTAAACACGGTTTAAAAAGAGGGCCTTCT
ACGGAAGGAGTACCAGAGTCTATGAGGGAAGAATATCGAGAGGAACAGCAGAATGCTGTGGATG
CTGACGATGGTCATTTTGTCAGCATAGAGCTGGAGTAA

图 2D

从2X35S启动子至NOS终止子的表达盒1261号。来自流感A/新喀里多尼亚/20/1999(H1N1)
的M2加下划线(SEQ ID NO: 10)

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAG
ACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTG
CCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCAT
CATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGAC
CCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGA
TTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTC
TCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGAT
TCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAA
ATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAA
GATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGC
AAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCA

图 2D(续)

AGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTT
TGATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTT
AAAGCAAACTTCTCTCTTGTCTTTCTTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCG
GCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTTTGTGGACACGTAGTGC
GGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGTGGTCTTGGGAAAAGAAAGCTT
GCTGGAGGCTGCTGTTCAGCCCCATACATTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGC
AATATCTCTACTTCTGCTTGACGAGGTATTGTTGCCTGTACTTCTTTCTTCTTCTTCTTGCTGA
TTGGTTCTATAAGAAATCTAGTATTTTCTTTGAAACAGAGTTTTCCCGTGGTTTTCGAACTTGG
AGAAAGATTGTTAAGCTTCTGTATATTCTGCCCAAATTTGTCGGGCCCATGAGTCTTCTAACCG
AGGTCGAAACGCCTATCAGAAACGAATGGGGGTGCAGATGCAACGATTCAAGTGATCCTCTTGT
TGTTGCCGCAAGTATAATTGGGATTGTGCACCTGATATTGTGGATTATTGATCGCCTTTTTTCC
AAAAGCATTTATCGTATCTTTAAACACGGTTTAAAAAGAGGGCCTTCTACGGAAGGAGTACCAG
AGTCTATGAGGGAAGAATATCGAGAGGAACAGCAGAATGCTGTGGATGCTGACGATGGTCATTT
TGTCAGCATAGAGCTGGAGTAAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTG
CATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTT
CTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTTAT
TAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCAGATCGTTCAA
ACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATA
ATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGA
TGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGC
GCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGAT

图 2E

来自流感A/新喀里多尼亚/20/1999(H1N1)的M2的氨基酸序列(SEQ ID NO:11)

MSLLTEVETPIRNEWGCRCNDSSDPLVVAASIIGIVHLILWIIDRLFSKSIYRIFKHGLKRGPS
TEGVPESMREEYREEQQNAVDADDGHFVSIELE

图2:B-2X35S/CPMV HT/M2新喀里多尼亚/NOS(构建体1261号)

图 3A

合成的M2基因(对应于连接到来自Genebank登录号EF467824的nt 740-1007的nt 26-51)
(SEQ ID NO: 12)

ATGAGTCTTCTAACCGAGGTCGAAACGCCTATCAGAAACGAATGGGGGTGCAGATGCAACGGTT
CAAGTGATCCTCTCACTATTGCCGCAAATATCATTGGGATCTTGCACTTGACATTGTGGATTCT
TGATCGTCTTTTTTTCAAATGCATTTACCGTCGCTTTAAATACGGACTGAAAGGAGGGCCTTCT
ACGGAAGGAGTGCCAAAGTCTATGAGGGAAGAATATCGAAAGGAACAGCAGAGTGCTGTGGATG
CTGACGATGGTCATTTTGTCAGCATAGAGCTGGAGTAA

图 3B

从2X35S启动子至NOS终止子的表达盒859号。来自流感A/波多黎各/8/1934(H1N1)的M2
加下划线(SEQ ID NO: 13)

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAG
ACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTG
CCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCAT
CATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGAC
CCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGA
TTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTC
TCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGAT
TCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAA
ATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAA
GATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGC
AAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCA
AGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTT
TGATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTT
AAAGCAAACTTCTCTCTTGTCTTTCTTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCG
GCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTTTGTGGACACGTAGTGC
GGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGTGGTCTTGGGAAAAGAAAGCTT
GCTGGAGGCTGCTGTTCAGCCCCATACATTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGC
AATATCTCTACTTCTGCTTGACGAGGTATTGTTGCCTGTACTTCTTTCTTCTTCTTCTTGCTGA
TTGGTTCTATAAGAAATCTAGTATTTTCTTTGAAACAGAGTTTTCCCGTGGTTTTCGAACTTGG
AGAAAGATTGTTAAGCTTCTGTATATTCTGCCCAAATTTGTCGGGCCCATGAGTCTTCTAACCG
AGGTCGAAACGCCTATCAGAAACGAATGGGGGTGCAGATGCAACGGTTCAAGTGATCCTCTCAC
TATTGCCGCAAATATCATTGGGATCTTGCACTTGACATTGTGGATTCTTGATCGTCTTTTTTTC
AAATGCATTTACCGTCGCTTTAAATACGGACTGAAAGGAGGGCCTTCTACGGAAGGAGTGCCAA
AGTCTATGAGGGAAGAATATCGAAAGGAACAGCAGAGTGCTGTGGATGCTGACGATGGTCATTT
TGTCAGCATAGAGCTGGAGTAAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTG
CATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTT

图 3B(续)

CTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTTAT
TAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCAGATCGTTCAA
ACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATA
ATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGA
TGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGC
GCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGAT

图 3C

来自流感A/波多黎各/8/1934(H1N1)的M2的氨基酸序列(SEQ ID NO:14)

MSLLTEVETPIRNEWGCRCNGSSDPLTIAANIIGILHLTLWILDRLFFKCIYRRFKYGLKGGPS
TEGVPKSMREEYRKEQQSAVDADDGHFVSIELE

图3:C-2X35S/CPMV-HT/M2波多黎各/NOS(构建体859号)

图 4A

构建体1194的示意图。示意图上注释用于质粒线性化的SacII和StuI限制性酶切位点

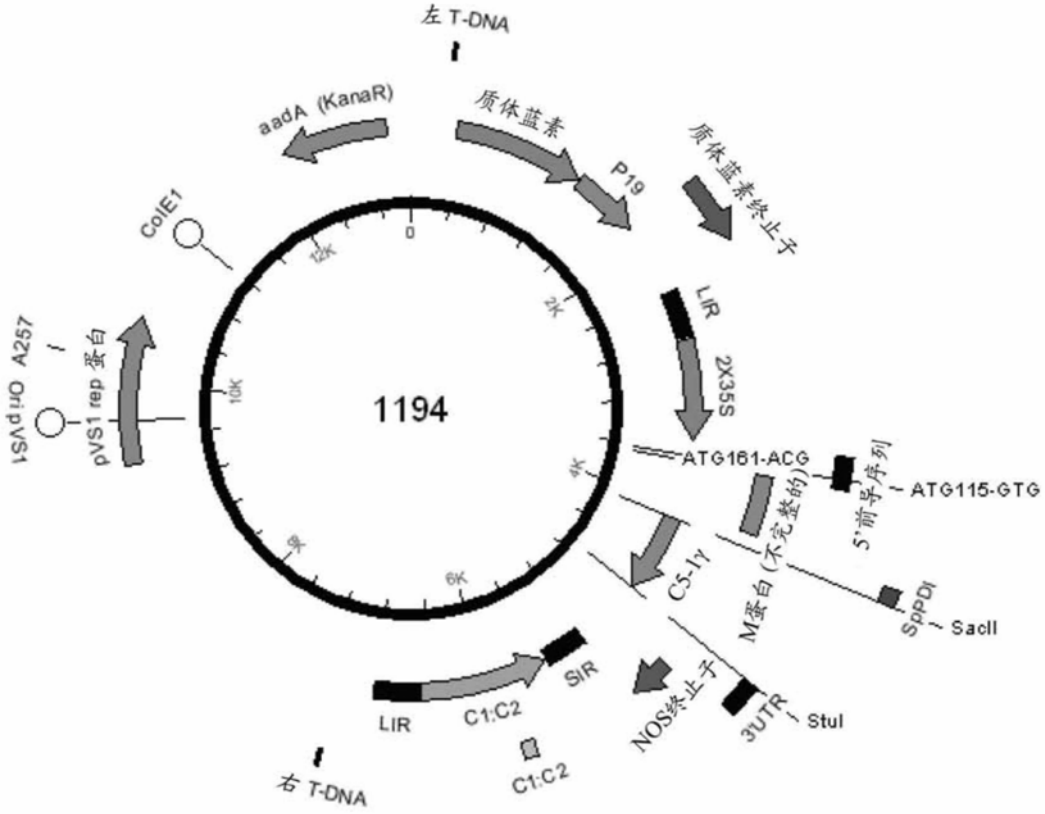


图 4B

从左至右t-DNA边界的构建体1194(加下划线)。引入BeYDV+复制酶扩增系统中的具有质体蓝素-P19-质体蓝素沉默抑制子表达盒的2X35S/CPMV-HT/PDISP/NOS(SEQ ID NO: 31)

TGGCAGGATATATTGTGGTGTAAACAAATTGACGCTTAGACAACTTAATAACACATTGCGGACG
TTTTTAATGTACTGAATTAACGCCGAATCCCGGGCTGGTATATTTATATGTTGTCAAATAACTC
AAAAACCATAAAAGTTTAAGTTAGCAAGTGTGTACATTTTTACTTGAACAAAAATATTCACCTA
CTACTGTTATAAATCATTATTAAACATTAGAGTAAAGAAATATGGATGATAAGAACAAGAGTAG
TGATATTTTGACAACAATTTTGTTGCAACATTTGAGAAAATTTTGTTGTTCTCTCTTTTCATTG
GTCAAAAACAATAGAGAGAGAAAAAGGAAGAGGGAGAATAAAAACATAATGTGAGTATGAGAGA
GAAAGTTGTACAAAAGTTGTACCAAAATAGTTGTACAAATATCATTGAGGAATTTGACAAAAGC

图 4B(续)

TACACAAATAAGGGTTAATTGCTGTAAATAAATAAGGATGACGCATTAGAGAGATGTACCATTA
GAGAATTTTTGGCAAGTCATTAAAAAGAAAGAATAAATTATTTTTAAAATTAAAAGTTGAGTCA
TTTGATTAAACATGTGATTATTTAATGAATTGATGAAAGAGTTGGATTAAAGTTGTATTAGTAA
TTAGAATTTGGTGTCAAATTTAATTTGACATTTGATCTTTTCCTATATATTGCCCCATAGAGTC
AGTTAACTCATTTTTATATTTCATAGATCAAATAAGAGAAATAACGGTATATTAATCCCTCCAA
AAAAAAAAAACGGTATATTTACTAAAAAATCTAAGCCACGTAGGAGGATAACAGGATCCCCGTA
GGAGGATAACATCCAATCCAACCAATCACAACAATCCTGATGAGATAACCCACTTTAAGCCCAC
GCATCTGTGGCACATCTACATTATCTAAATCACACATTCTTCCACACATCTGAGCCACACAAAA
ACCAATCCACATCTTTATCACCCATTCTATAAAAAATCACACTTTGTGAGTCTACACTTTGATT
CCCTTCAAACACATACAAAGAGAAGAGACTAATTAATTAATTAATCATCTTGAGAGAAAATGGA
ACGAGCTATACAAGGAAACGACGCTAGGGAACAAGCTAACAGTGAACGTTGGGATGGAGGATCA
GGAGGTACCACTTCTCCCTTCAAACTTCCTGACGAAAGTCCGAGTTGGACTGAGTGGCGGCTAC
ATAACGATGAGACGAATTCGAATCAAGATAATCCCCTTGGTTTCAAGGAAAGCTGGGGTTTCGG
GAAAGTTGTATTTAAGAGATATCTCAGATACGACAGGACGGAAGCTTCACTGCACAGAGTCCTT
GGATCTTGGACGGGAGATTCGGTTAACTATGCAGCATCTCGATTTTTCGGTTTCGACCAGATCG
GATGTACCTATAGTATTCGGTTTCGAGGAGTTAGTATCACCGTTTCTGGAGGGTCGCGAACTCT
TCAGCATCTCTGTGAGATGGCAATTCGGTCTAAGCAAGAACTGCTACAGCTTGCCCCAATCGAA
GTGGAAAGTAATGTATCAAGAGGATGCCCTGAAGGTACTCAAACCTTCGAAAAAGAAAGCGAGT
AAGTTAAAATGCTTCTTCGTCTCCTATTTATAATATGGTTTGTTATTGTTAATTTTGTTCTTGT
AGAAGAGCTTAATTAATCGTTGTTGTTATGAAATACTATTTGTATGAGATGAACTGGTGTAATG
TAATTCATTTACATAAGTGGAGTCAGAATCAGAATGTTTCCTCCATAACTAACTAGACATGAAG
ACCTGCCGCGTACAATTGTCTTATATTTGAACAACTAAAATTGAACATCTTTTGCCACAACTTT
ATAAGTGGTTAATATAGCTCAAATATATGGTCAAGTTCAATAGATTAATAATGGAAATATCAGT
TATCGAAATTCATTAACAATCAACTTAACGTTATTAACTACTAATTTTATATCATCCCCTTTGA
TAAATGATAGTACACCAATTAGGAAGGAGCATGCTCGCCTAGGAGATTGTCGTTTCCCGCCTTC
AGTTTGCAAGCTGCTCTAGCCGTGTAGCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGGAAT
TACTAGCGCGTGTCGACACGCGTGGCGCGCCCTAGCAGAAGGCATGTTGTTGTGACTCCGAGGG
GTTGCCTCAAACTCTATCTTATAACCGGCGTGGAGGCATGGAGGCAAGGGCATTTTGGTAATTT
AAGTAGTTAGTGGAAAATGACGTCATTTACTTAAAGACGAAGTCTTGCGACAAGGGGGGCCCAC
GCCGAATTTTAATATTACCGGCGTGGCCCCACCTTATCGCGAGTGCTTTAGCACGAGCGGTCCA
GATTTAAAGTAGAAAAGTTCCCGCCCACTAGGGTTAAAGGTGTTCACACTATAAAAGCATATAC
GATGTGATGGTATTTGATAAAGCGTATATTGTATCAGGTATTTCCGTCGGATACGAATTATTCG
TACAAGCTTCTTAAGCCGGTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCA
AAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAA
CCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGT
GGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACA
GTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCAC
GTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAA
AATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATAT
CCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAA
GGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCT
GCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTC
CAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACA
ATCCCACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTAT
TAAAATCTTAATAGGTTTTGATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAA
CTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTCTTGCGTGAGCGATCTTCAACG
TTGTCAGATCGTGCTTCGGCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTC

图 4B(续)

TTTGTGGACACGTAGTGCGGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGTGGT
CTTGGGAAAAGAAAGCTTGCTGGAGGCTGCTGTTCAGCCCCATACATTACTTGTTACGATTCTG
CTGACTTTCGGCGGGTGCAATATCTCTACTTCTGCTTGACGAGGTATTGTTGCCTGTACTTCTT
TCTTCTTCTTCTTGCTGATTGGTTCTATAAGAAATCTAGTATTTTCTTTGAAACAGAGTTTTCC
CGTGGTTTTCGAACTTGGAGAAAGATTGTTAAGCTTCTGTATATTCTGCCCAAATTTGTCGGGC
CCATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCA
GATCTTCGCCGCGGCTCCTCAGCCAAAACGACACCCCCATCTGTCTATCCACTGGCCCCTGGAT
CTGCTGCCCAAACTAACTCCATGGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCC
AGTGACAGTGACCTGGAACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTG
CAGTCTGACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGA
CCGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCCAG
GGATTGTGGTTGTAAGCCTTGCATATGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCC
CCAAAGCCCAAGGATGTGCTCACCATTACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACA
TCAGCAAGGATGATCCCGAGGTCCAGTTCAGCTGGTTTGTAGATGATGTGGAGGTGCACACAGC
TCAGACGCAACCCCGGGAGGAGCAGTTCAACAGCACTTTCCGCTCAGTCAGTGAACTTCCCATC
ATGCACCAGGACTGGCTCAATGGCAAGGAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATT
CGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAAT
TTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTA
ATTTTATTAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCAGAT
CGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTA
TCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATT
TATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAA
ATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGATCTCTAGA
GTCTCAAGCTTGGCGCGGGGTACCGAGCTCGAATTCCGAGTGTACTTCAAGTCAGTTGGAAATC
AATAAAATGATTATTTTATGAATATATTTCATTGTGCAAGTAGATAGAAATTACATATGTTACA
TAACACACGAAATAAACAAAAAAACACAATCCAAAACAAACACCCCAAACAAAATAACACTATA
TATATCCTCGTATGAGGAGAGGCACGTTCAGTGACTCGACGATTCCCGAGCAAAAAAAGTCTCC
CCGTCACACATATAGTGGGTGACGCAATTATCTTCAAAGTAATCCTTCTGTTGACTTGTCATTG
ATAACATCCAGTCTTCGTCAGGATTGCAAAGAATTATAGAAGGGATCCCACCTTTTATTTTCTT
CTTTTTTCCATATTTAGGGTTGACAGTGAAATCAGACTGGCAACCTATTAATTGCTTCCACAAT
GGGACGAACTTGAAGGGGATGTCGTCGATGATATTATAGGTGGCGTGTTCATCGTAGTTGGTGA
AGTCGATGGTCCCGTTCCAGTAGTTGTGTCGCCCGAGACTTCTAGCCCAGGTGGTCTTTCCGGT
ACGAGTTGGTCCGCAGATGTAGAGGCTGGGGTGTCTGACCCCAGTCCTTCCCTCATCCTGGTTA
GATCGGCCATCCACTCAAGGTCAGATTGTGCTTGATCGTAGGAGACAGGATGTATGAAAGTGTA
GGCATCGATGCTTACATGATATAGGTGCGTCTCTCTCCAGTTGTGCAGATCTTCGTGGCAGCGG
AGATCTGATTCTGTGAAGGGCGACACGTACTGCTCAGGTTGTGGAGGAAATAATTTGTTGGCTG
AATATTCCAGCCATTGAAGCTTTGTTGCCCATTCATGAGGGAATTCTTCTTTGATCATGTCAAG
ATACTCCTCCTTAGACGTTGCAGTCTGGATAATAGTTCGCCATCGTGCGTCAGATTTGCGAGGA
GAGACCTTATGATCTCGGAAATCTCCTCTGGTTTTAATATCTCCGTCCTTTGATATGTAATCAA
GGACTTGTTTAGAGTTTCTAGCTGGCTGGATATTAGGGTGATTTCCTTCAAAATCGAAAAAAGA
AGGATCCCTAATACAAGGTTTTTTATCAAGCTGGATAAGAGCATGATAGTGGGTAGTGCCATCT
TGATGAAGCTCAGAAGCAACACCAAGGAAGAAAATAAGAAAAGGTGTGAGTTTCTCCCAGAGAA
ACTGGAATAAATCATCTCTTTGAGATGAGCACTTGGGGTAGGTAAGGAAAACATATTTAGATTG
GAGTCTGAAGTTCTTGCTAGCAGAAGGCATGTTGTTGTGACTCCGAGGGGTTGCCTCAAACTCT
ATCTTATAACCGGCGTGGAGGCATGGAGGCAAGGGCATTTTGGTAATTTAAGTAGTTAGTGGAA
AATGACGTCATTTACTTAAAGACGAAGTCTTGCGACAAGGGGGGCCCACGCCGAATTTTAATAT
TACCGGCGTGGCCCCACCTTATCGCGAGTGCTTTAGCACGAGCGGTCCAGATTTAAAGTAGAAA

图 4B(续)

AGTTCCCGCCCACTAGGGTTAAAGGTGTTCACACTATAAAAGCATATACGATGTGATGGTATTT
GATGGAGCGTATATTGTATCAGGTATTTCCGTCGGATACGAATTATTCGTACGGCCGGCCACTA
GTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATC
GCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCC
TTCCCAACAGTTGCGCAGCCTGAATGGCGAATGCTAGAGCAGCTTGAGCTTGGATCAGATTGTC
GTTTCCCGCCTTCAGTTTAAACTATCAGTGTTTGACAGGATATATTGGCGGGTAAACCTAAGAG
AAAAGAGCGTTTA

图 4C

从BeYDV左LIR至BeYDV右LIR的表达盒1008号。来自流感B/布里斯班/60/2008的PDISP/HA加下划线(SEQ ID NO: 32)

CTAGCAGAAGGCATGTTGTTGTGACTCCGAGGGGTTGCCTCAAACTCTATCTTATAACCGGCGT
GGAGGCATGGAGGCAAGGGCATTTTGGTAATTTAAGTAGTTAGTGGAAAATGACGTCATTTACT
TAAAGACGAAGTCTTGCGACAAGGGGGGCCCACGCCGAATTTTAATATTACCGGCGTGGCCCCA
CCTTATCGCGAGTGCTTTAGCACGAGCGGTCCAGATTTAAAGTAGAAAAGTTCCCGCCCACTAG
GGTTAAAGGTGTTCACACTATAAAAGCATATACGATGTGATGGTATTTGATAAAGCGTATATTG
TATCAGGTATTTCCGTCGGATACGAATTATTCGTACAAGCTTCTTAAGCCGGTCAACATGGTGG
AGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAAT
TGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGT
CACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAG
GAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAG
GAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAAC
ATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAA
GGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGC
TATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGC
GATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCAC
CCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATG
TGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCCTTCCTCT
ATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTTGATAAAAGCGAA
CGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCT
CTCTTGTCTTTCTTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAAC
GTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTTTGTGGACACGTAGTGCGGCGCCATTAAAT
AACGTGTACTTGTCCTATTCTTGTCGGTGTGGTCTTGGGAAAAGAAAGCTTGCTGGAGGCTGCT
GTTCAGCCCCATACATTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGCAATATCTCTACTT
CTGCTTGACGAGGTATTGTTGCCTGTACTTCTTTCTTCTTCTTCTTGCTGATTGGTTCTATAAG
AAATCTAGTATTTTCTTTGAAACAGAGTTTTCCCGTGGTTTTCGAACTTGGAGAAAGATTGTTA
AGCTTCTGTATATTCTGCCCAAATTTGTCGGGCCCATGGCGAAAAACGTTGCGATTTTCGGCTT
ATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCCGATCGAATCTGCACTGGAATA
ACATCGTCAAACTCACCACATGTCGTCAAAACTGCTACTCAAGGGGAGGTCAATGTGACTGGTG
TAATACCACTGACAACAACACCCACCAAATCTCATTTTGCAAATCTCAAAGGAACAGAAACCAG
GGGGAAACTATGCCCAAAATGCCTCAACTGCACAGATCTGGACGTAGCCTTGGGCAGACCAAAA
TGCACGGGGAAAATACCCTCGGCAAGAGTTTCAATACTCCATGAAGTCAGACCTGTTACATCTG
GGTGCTTTCCTATAATGCACGACAGAACAAAAATTAGACAGCTGCCTAACCTTCTCCGAGGATA
CGAACATATCAGGTTATCAACCCATAACGTTATCAATGCAGAAAATGCACCAGGAGGACCCTAC

图 4C(续)

AAAATTGGAACCTCAGGGTCTTGCCCTAACATTACCAATGGAAACGGATTTTTCGCAACAATGG
CTTGGGCCGTCCCAAAAAACGACAAAAACAAAACAGCAACAAATCCATTAACAATAGAAGTACC
ATACATTTGTACAGAAGGAGAAGACCAAATTACCGTTTGGGGGTTCCACTCTGACAACGAGACC
CAAATGGCAAAGCTCTATGGGGACTCAAAGCCCCAGAAGTTCACCTCATCTGCCAACGGAGTGA
CCACACATTACGTTTCACAGATTGGTGGCTTCCCAAATCAAACAGAAGACGGAGGACTACCACA
AAGTGGTAGAATTGTTGTTGATTACATGGTGCAAAAATCTGGGAAAACAGGAACAATTACCTAT
CAAAGGGGTATTTTATTGCCTCAAAAGGTGTGGTGCGCAAGTGGCAGGAGCAAGGTAATAAAAG
GATCCTTGCCTTTAATTGGAGAAGCAGATTGCCTCCACGAAAAATACGGTGGATTAAACAAAAG
CAAGCCTTACTACACAGGGGAACATGCAAAGGCCATAGGAAATTGCCCAATATGGGTGAAAACA
CCCTTGAAGCTGGCCAATGGAACCAAATATAGACCTCCTGCAAAACTATTAAAGGAAAGGGGTT
TCTTCGGAGCTATTGCTGGTTTCTTAGAAGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGG
ATACACATCCCATGGGGCACATGGAGTAGCGGTGGCAGCAGACCTTAAGAGCACTCAAGAGGCC
ATAAACAAGATAACAAAAAATCTCAACTCTTTGAGTGAGCTGGAAGTAAAGAATCTTCAAAGAC
TAAGCGGTGCCATGGATGAACTCCACAACGAAATACTAGAACTAGATGAGAAAGTGGATGATCT
CAGAGCTGATACAATAAGCTCACAAATAGAACTCGCAGTCCTGCTTTCCAATGAAGGAATAATA
AACAGTGAAGATGAACATCTCTTGGCGCTTGAAAGAAAGCTGAAGAAAATGCTGGGCCCCTCTG
CTGTAGAGATAGGGAATGGATGCTTTGAAACCAAACACAAGTGCAACCAGACCTGTCTCGACAG
AATAGCTGCTGGTACCTTTGATGCAGGAGAATTTTCTCTCCCCACCTTTGATTCACTGAATATT
ACTGCTGCATCTTTAAATGACGATGGATTGGATAATCATACTATACTGCTTTACTACTCAACTG
CTGCCTCCAGTTTGGCTGTAACACTGATGATAGCTATCTTTGTTGTTTATATGGTCTCCAGAGA
CAATGTTTCTTGCTCCATCTGTCTATAAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTC
GGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATT
TAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAA
TTTTATTAAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCAGATC
GTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTAT
CATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTT
ATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAA
TATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGATCTCTAGAG
TCTCAAGCTTGGCGCGGGGTACCGAGCTCGAATTCCGAGTGTACTTCAAGTCAGTTGGAAATCA
ATAAAATGATTATTTTATGAATATATTTCATTGTGCAAGTAGATAGAAATTACATATGTTACAT
AACACACGAAATAAACAAAAAAACACAATCCAAAACAAACACCCCAAACAAAATAACACTATAT
ATATCCTCGTATGAGGAGAGGCACGTTCAGTGACTCGACGATTCCCGAGCAAAAAAAGTCTCCC
CGTCACACATATAGTGGGTGACGCAATTATCTTCAAAGTAATCCTTCTGTTGACTTGTCATTGA
TAACATCCAGTCTTCGTCAGGATTGCAAAGAATTATAGAAGGGATCCCACCTTTTATTTTCTTC
TTTTTTCCATATTTAGGGTTGACAGTGAAATCAGACTGGCAACCTATTAATTGCTTCCACAATG
GGACGAACTTGAAGGGGATGTCGTCGATGATATTATAGGTGGCGTGTTCATCGTAGTTGGTGAA
GTCGATGGTCCCGTTCCAGTAGTTGTGTCGCCCGAGACTTCTAGCCCAGGTGGTCTTTCCGGTA
CGAGTTGGTCCGCAGATGTAGAGGCTGGGGTGTCTGACCCCAGTCCTTCCCTCATCCTGGTTAG
ATCGGCCATCCACTCAAGGTCAGATTGTGCTTGATCGTAGGAGACAGGATGTATGAAAGTGTAG
GCATCGATGCTTACATGATATAGGTGCGTCTCTCTCCAGTTGTGCAGATCTTCGTGGCAGCGGA
GATCTGATTCTGTGAAGGGCGACACGTACTGCTCAGGTTGTGGAGGAAATAATTTGTTGGCTGA
ATATTCCAGCCATTGAAGCTTTGTTGCCCATTCATGAGGGAATTCTTCTTTGATCATGTCAAGA
TACTCCTCCTTAGACGTTGCAGTCTGGATAATAGTTCGCCATCGTGCGTCAGATTTGCGAGGAG
AGACCTTATGATCTCGGAAATCTCCTCTGGTTTTAATATCTCCGTCCTTTGATATGTAATCAAG
GACTTGTTTAGAGTTTCTAGCTGGCTGGATATTAGGGTGATTTCCTTCAAAATCGAAAAAAGAA
GGATCCCTAATACAAGGTTTTTTATCAAGCTGGATAAGAGCATGATAGTGGGTAGTGCCATCTT
GATGAAGCTCAGAAGCAACACCAAGGAAGAAAATAAGAAAAGGTGTGAGTTTCTCCCAGAGAAA

图 4C(续)

CTGGAATAAATCATCTCTTTGAGATGAGCACTTGGGGTAGGTAAGGAAAACATATTTAGATTGG
AGTCTGAAGTTCTTGCTAGCAGAAGGCATGTTGTTGTGACTCCGAGGGGTTGCCTCAAACTCTA
TCTTATAACCGGCGTGGAGGCATGGAGGCAAGGGCATTTTGGTAATTTAAGTAGTTAGTGGAAA
ATGACGTCATTTACTTAAAGACGAAGTCTTGCGACAAGGGGGGCCCACGCCGAATTTTAATATT
ACCGGCGTGGCCCCACCTTATCGCGAGTGCTTTAGCACGAGCGGTCCAGATTTAAAGTAGAAAA
GTTCCCGCCCACTAGGGTTAAAGGTGTTCACACTATAAAAGCATATACGATGTGATGGTATTTG
ATGGAGCGTATATTGTATCAGGTATTTCCGTCGGATACGAATTATTCGTAC

图4:引入BeYDV+复制酶扩增系统中的G-2X35S/CPMV-HT/PDISP/HA B布里斯班/NOS(构建体1008号)

图 5A

1039+1059.r (SEQ ID NO: 38)

CTTCCCATCCTCCACCAGGAGGTCTATATTTGGTTCCATTGGCCAGCTTCAA

图 5B

1039+1059.c (SEQ ID NO: 39)

CAAATATAGACCTCCTGGTGGAGGATGGGAAGGAATGATTGCAGGTTGGCAC

图 5C

从BeYDV左LIR至BeYDV右LIR的表达盒1059号。来自流感B/布里斯班/60/2008的具有缺失的蛋白水解环的PDISP/HA加下划线(SEQ ID NO: 40)

CTAGCAGAAGGCATGTTGTTGTGACTCCGAGGGGTTGCCTCAAACTCTATCTTATAACCGGCGT
GGAGGCATGGAGGCAAGGGCATTTTGGTAATTTAAGTAGTTAGTGGAAAATGACGTCATTTACT
TAAAGACGAAGTCTTGCGACAAGGGGGGCCCACGCCGAATTTTAATATTACCGGCGTGGCCCCA
CCTTATCGCGAGTGCTTTAGCACGAGCGGTCCAGATTTAAAGTAGAAAAGTTCCCGCCCACTAG
GGTTAAAGGTGTTCACACTATAAAAGCATATACGATGTGATGGTATTTGATAAAGCGTATATTG
TATCAGGTATTTCCGTCGGATACGAATTATTCGTACAAGCTTCTTAAGCCGGTCAACATGGTGG
AGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAAT
TGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGT
CACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAG
GAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAG
GAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAAC

图 5C(续)

ATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAA
GGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGC
TATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGC
GATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCAC
CCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATG
TGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCCTTCCTCT
ATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTTGATAAAAGCGAA
CGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCT
CTCTTGTCTTTCTTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAAC
GTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTTTGTGGACACGTAGTGCGGCGCCATTAAAT
AACGTGTACTTGTCCTATTCTTGTCGGTGTGGTCTTGGGAAAAGAAAGCTTGCTGGAGGCTGCT
GTTCAGCCCCATACATTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGCAATATCTCTACTT
CTGCTTGACGAGGTATTGTTGCCTGTACTTCTTTCTTCTTCTTCTTGCTGATTGGTTCTATAAG
AAATCTAGTATTTTCTTTGAAACAGAGTTTTCCCGTGGTTTTCGAACTTGGAGAAAGATTGTTA
AGCTTCTGTATATTCTGCCCAAATTTGTCGGGCCCATGGCGAAAAACGTTGCGATTTTCGGCTT
ATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCCGATCGAATCTGCACTGGAATA
ACATCGTCAAACTCACCACATGTCGTCAAAACTGCTACTCAAGGGGAGGTCAATGTGACTGGTG
TAATACCACTGACAACAACACCCACCAAATCTCATTTTGCAAATCTCAAAGGAACAGAAACCAG
GGGGAAACTATGCCCAAAATGCCTCAACTGCACAGATCTGGACGTAGCCTTGGGCAGACCAAAA
TGCACGGGGAAAATACCCTCGGCAAGAGTTTCAATACTCCATGAAGTCAGACCTGTTACATCTG
GGTGCTTTCCTATAATGCACGACAGAACAAAAATTAGACAGCTGCCTAACCTTCTCCGAGGATA
CGAACATATCAGGTTATCAACCCATAACGTTATCAATGCAGAAAATGCACCAGGAGGACCCTAC
AAAATTGGAACCTCAGGGTCTTGCCCTAACATTACCAATGGAAACGGATTTTTCGCAACAATGG
CTTGGGCCGTCCCAAAAAACGACAAAAACAAAACAGCAACAAATCCATTAACAATAGAAGTACC
ATACATTTGTACAGAAGGAGAAGACCAAATTACCGTTTGGGGGTTCCACTCTGACAACGAGACC
CAAATGGCAAAGCTCTATGGGGACTCAAAGCCCCAGAAGTTCACCTCATCTGCCAACGGAGTGA
CCACACATTACGTTTCACAGATTGGTGGCTTCCCAAATCAAACAGAAGACGGAGGACTACCACA
AAGTGGTAGAATTGTTGTTGATTACATGGTGCAAAAATCTGGGAAAACAGGAACAATTACCTAT
CAAAGGGGTATTTTATTGCCTCAAAAGGTGTGGTGCGCAAGTGGCAGGAGCAAGGTAATAAAAG
GATCCTTGCCTTTAATTGGAGAAGCAGATTGCCTCCACGAAAAATACGGTGGATTAAACAAAAG
CAAGCCTTACTACACAGGGGAACATGCAAAGGCCATAGGAAATTGCCCAATATGGGTGAAAACA
CCCTTGAAGCTGGCCAATGGAACCAAATATAGACCTCCTGGTGGAGGATGGGAAGGAATGATTG
CAGGTTGGCACGGATACACATCCCATGGGGCACATGGAGTAGCGGTGGCAGCAGACCTTAAGAG
CACTCAAGAGGCCATAAACAAGATAACAAAAAATCTCAACTCTTTGAGTGAGCTGGAAGTAAAG
AATCTTCAAAGACTAAGCGGTGCCATGGATGAACTCCACAACGAAATACTAGAACTAGATGAGA
AAGTGGATGATCTCAGAGCTGATACAATAAGCTCACAAATAGAACTCGCAGTCCTGCTTTCCAA
TGAAGGAATAATAAACAGTGAAGATGAACATCTCTTGGCGCTTGAAAGAAAGCTGAAGAAAATG
CTGGGCCCCTCTGCTGTAGAGATAGGGAATGGATGCTTTGAAACCAAACACAAGTGCAACCAGA
CCTGTCTCGACAGAATAGCTGCTGGTACCTTTGATGCAGGAGAATTTTCTCTCCCCACCTTTGA
TTCACTGAATATTACTGCTGCATCTTTAAATGACGATGGATTGGATAATCATACTATACTGCTT
TACTACTCAACTGCTGCCTCCAGTTTGGCTGTAACACTGATGATAGCTATCTTTGTTGTTTATA
TGGTCTCCAGAGACAATGTTTCTTGCTCCATCTGTCTATAAAGGCCTATTTTCTTTAGTTTGAA
TTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTT
ATTTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACAC
AAAAAGATTTTAATTTTATTAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTAT
CGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTC
TTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATG

图 5C (续)

CATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCG
ATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTAC
TAGATCTCTAGAGTCTCAAGCTTGGCGCGGGGTACCGAGCTCGAATTCCGAGTGTACTTCAAGT
CAGTTGGAAATCAATAAAATGATTATTTTATGAATATATTTCATTGTGCAAGTAGATAGAAATT
ACATATGTTACATAACACACGAAATAAACAAAAAAACACAATCCAAAACAAACACCCCAAACAA
AATAACACTATATATATCCTCGTATGAGGAGAGGCACGTTCAGTGACTCGACGATTCCCGAGCA
AAAAAAGTCTCCCCGTCACACATATAGTGGGTGACGCAATTATCTTCAAAGTAATCCTTCTGTT
GACTTGTCATTGATAACATCCAGTCTTCGTCAGGATTGCAAAGAATTATAGAAGGGATCCCACC
TTTTATTTTCTTCTTTTTTCCATATTTAGGGTTGACAGTGAAATCAGACTGGCAACCTATTAAT
TGCTTCCACAATGGGACGAACTTGAAGGGGATGTCGTCGATGATATTATAGGTGGCGTGTTCAT
CGTAGTTGGTGAAGTCGATGGTCCCGTTCCAGTAGTTGTGTCGCCCGAGACTTCTAGCCCAGGT
GGTCTTTCCGGTACGAGTTGGTCCGCAGATGTAGAGGCTGGGGTGTCTGACCCCAGTCCTTCCC
TCATCCTGGTTAGATCGGCCATCCACTCAAGGTCAGATTGTGCTTGATCGTAGGAGACAGGATG
TATGAAAGTGTAGGCATCGATGCTTACATGATATAGGTGCGTCTCTCTCCAGTTGTGCAGATCT
TCGTGGCAGCGGAGATCTGATTCTGTGAAGGGCGACACGTACTGCTCAGGTTGTGGAGGAAATA
ATTTGTTGGCTGAATATTCCAGCCATTGAAGCTTTGTTGCCCATTCATGAGGGAATTCTTCTTT
GATCATGTCAAGATACTCCTCCTTAGACGTTGCAGTCTGGATAATAGTTCGCCATCGTGCGTCA
GATTTGCGAGGAGAGACCTTATGATCTCGGAAATCTCCTCTGGTTTTAATATCTCCGTCCTTTG
ATATGTAATCAAGGACTTGTTTAGAGTTTCTAGCTGGCTGGATATTAGGGTGATTTCCTTCAAA
ATCGAAAAAAGAAGGATCCCTAATACAAGGTTTTTTATCAAGCTGGATAAGAGCATGATAGTGG
GTAGTGCCATCTTGATGAAGCTCAGAAGCAACACCAAGGAAGAAAATAAGAAAAGGTGTGAGTT
TCTCCCAGAGAAACTGGAATAAATCATCTCTTTGAGATGAGCACTTGGGGTAGGTAAGGAAAAC
ATATTTAGATTGGAGTCTGAAGTTCTTGCTAGCAGAAGGCATGTTGTTGTGACTCCGAGGGGTT
GCCTCAAACTCTATCTTATAACCGGCGTGGAGGCATGGAGGCAAGGGCATTTTGGTAATTTAAG
TAGTTAGTGGAAAATGACGTCATTTACTTAAAGACGAAGTCTTGCGACAAGGGGGGCCCACGCC
GAATTTTAATATTACCGGCGTGGCCCCACCTTATCGCGAGTGCTTTAGCACGAGCGGTCCAGAT
TTAAAGTAGAAAAGTTCCCGCCCACTAGGGTTAAAGGTGTTCACACTATAAAAGCATATACGAT
GTGATGGTATTTGATGGAGCGTATATTGTATCAGGTATTTCCGTCGGATACGAATTATTCGTAC

图 5D

来自流感B/布里斯班/60/2008的具有缺失的蛋白水解环的PDISP/HA的氨基酸序列
(SEQ ID NO: 41)

MAKNVAIFGLLFSLLVLVPSQIFADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSH
FANLKGTETRGKLCPKCLNCTDLDVALGRPKCTGKIPSARVSILHEVRPVTSGCFPIMHDRTKI
RQLPNLLRGYEHIRLSTHNVINAENAPGGPYKIGTSGSCPNITNGNGFFATMAWAVPKNDKNKT
ATNPLTIEVPYICTEGEDQITVWGFHSDNETQMAKLYGDSKPQKFTSSANGVTTHYVSQIGGFP
NQTEDGGLPQSGRIVVDYMVQKSGKTGTITYQRGILLPQKVWCASGRSKVIKGSLPLIGEADCL
HEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPGGGWEGMIAGWHGYTSHGAH
GVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISS
QIELAVLLSNEGIINSEDEHLLALERKLKKMLGPSAVEIGNGCFETKHKCNQTCLDRIAAGTFD
AGEFSLPTFDSLNITAASLNDDGLDNHTILLYYSTAASSLAVTLMIAIFVVYMVSRDNVSCSIC
L

图 5E

来自流感B/布里斯班/60/2008的具有缺失的蛋白水解环的PDISP/HA的核苷酸序列
(SEQ ID NO: 43)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGA
TCTTCGCCGATCGAATCTGCACTGGAATAACATCGTCAAACTCACCACATGTCGTCAAAACTGC
TACTCAAGGGGAGGTCAATGTGACTGGTGTAATACCACTGACAACAACACCCACCAAATCTCAT
TTTGCAAATCTCAAAGGAACAGAAACCAGGGGGAAACTATGCCCAAAATGCCTCAACTGCACAG
ATCTGGACGTAGCCTTGGGCAGACCAAAATGCACGGGGAAAATACCCTCGGCAAGAGTTTCAAT
ACTCCATGAAGTCAGACCTGTTACATCTGGGTGCTTTCCTATAATGCACGACAGAACAAAAATT
AGACAGCTGCCTAACCTTCTCCGAGGATACGAACATATCAGGTTATCAACCCATAACGTTATCA
ATGCAGAAAATGCACCAGGAGGACCCTACAAAATTGGAACCTCAGGGTCTTGCCCTAACATTAC
CAATGGAAACGGATTTTTCGCAACAATGGCTTGGGCCGTCCCAAAAAACGACAAAAACAAAACA
GCAACAAATCCATTAACAATAGAAGTACCATACATTTGTACAGAAGGAGAAGACCAAATTACCG
TTTGGGGGTTCCACTCTGACAACGAGACCCAAATGGCAAAGCTCTATGGGGACTCAAAGCCCCA
GAAGTTCACCTCATCTGCCAACGGAGTGACCACACATTACGTTTCACAGATTGGTGGCTTCCCA
AATCAAACAGAAGACGGAGGACTACCACAAAGTGGTAGAATTGTTGTTGATTACATGGTGCAAA
AATCTGGGAAAACAGGAACAATTACCTATCAAAGGGGTATTTTATTGCCTCAAAAGGTGTGGTG
CGCAAGTGGCAGGAGCAAGGTAATAAAAGGATCCTTGCCTTTAATTGGAGAAGCAGATTGCCTC
CACGAAAAATACGGTGGATTAAACAAAAGCAAGCCTTACTACACAGGGGAACATGCAAAGGCCA
TAGGAAATTGCCCAATATGGGTGAAAACACCCTTGAAGCTGGCCAATGGAACCAAATATAGACC
TCCTGGTGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGATACACATCCCATGGGGCACAT
GGAGTAGCGGTGGCAGCAGACCTTAAGAGCACTCAAGAGGCCATAAACAAGATAACAAAAAATC
TCAACTCTTTGAGTGAGCTGGAAGTAAAGAATCTTCAAAGACTAAGCGGTGCCATGGATGAACT
CCACAACGAAATACTAGAACTAGATGAGAAAGTGGATGATCTCAGAGCTGATACAATAAGCTCA
CAAATAGAACTCGCAGTCCTGCTTTCCAATGAAGGAATAATAAACAGTGAAGATGAACATCTCT
TGGCGCTTGAAAGAAAGCTGAAGAAAATGCTGGGCCCCTCTGCTGTAGAGATAGGGAATGGATG
CTTTGAAACCAAACACAAGTGCAACCAGACCTGTCTCGACAGAATAGCTGCTGGTACCTTTGAT
GCAGGAGAATTTTCTCTCCCCACCTTTGATTCACTGAATATTACTGCTGCATCTTTAAATGACG
ATGGATTGGATAATCATACTATACTGCTTTACTACTCAACTGCTGCCTCCAGTTTGGCTGTAAC
ACTGATGATAGCTATCTTTGTTGTTTATATGGTCTCCAGAGACAATGTTTCTTGCTCCATCTGT
CTATAA

图5:引入BeYDV+复制酶扩增系统中的具有缺失的蛋白水解环的I-2X35S/CPMV-HT/PDISP/HA B布里斯班(构建体1059号)

图 6A, SEQ ID NO: 49

IF-HAB110.S1+3c

AAATTTGTCGGGCCCATGAAGGCAATAATTGTACTACTCATGGTAG

图 6B, SEQ ID NO: 50

IF-HAB110.s1-4r

ACTAAAGAAAATAGGCCTTTATAGACAGATGGAGCATGAAACGTTGTCTCTG

图 6C, SEQ ID NO: 51

合成的HA B威斯康星州(Genbank登录号JN993010)

ATGAAGGCAATAATTGTACTACTCATGGTAGTAACATCCAATGCAGATCGAATCTGCACTGGGA
TAACATCTTCAAACTCACCTCATGTGGTCAAAACAGCTACTCAAGGGGAGGTCAATGTGACTGG
CGTGATACCACTGACAACAACACCAACAAAATCTTATTTTGCAAATCTCAAAGGAACAAGGACC
AGAGGGAAACTATGCCCGGACTGTCTCAACTGTACAGATCTGGATGTGGCCTTGGGCAGGCCAA
TGTGTGTGGGGACCACACCTTCTGCTAAAGCTTCAATACTCCACGAGGTCAGACCTGTTACATC
CGGGTGCTTTCCTATAATGCACGACAGAACAAAAATCAGGCAACTACCCAATCTTCTCAGAGGA
TATGAAAATATCAGGTTATCAACCCAAAACGTTATCGATGCAGAAAAAGCACCAGGAGGACCCT
ACAGACTTGGAACCTCAGGATCTTGCCCTAACGCTACCAGTAAAATCGGATTTTTTGCAACAAT
GGCTTGGGCTGTCCCAAAGGACAACTACAAAAATGCAACGAACCCACTAACAGTAGAAGTACCA
TACATTTGTACAGAAGGGGAAGACCAAATTACTGTTTGGGGGTTCCATTCAGATAACAAAACCC
AAATGAAGAGCCTCTATGGAGACTCAAATCCTCAAAAGTTCACCTCATCTGCTAATGGAGTAAC
CACACATTATGTTTCTCAGATTGGCGACTTCCCAGATCAAACAGAAGACGGAGGACTACCACAA
AGCGGCAGAATTGTTGTTGATTACATGATGCAAAAACCTGGGAAAACAGGAACAATTGTCTATC
AAAGAGGTGTTTTGTTGCCTCAAAAGGTGTGGTGCGCGAGTGGCAGGAGCAAAGTAATAAAAGG
GTCATTGCCTTTAATTGGTGAAGCAGATTGCCTTCATGAAAAATACGGTGGATTAAACAAAAGC
AAGCCTTACTACACAGGAGAACATGCAAAAGCCATAGGAAATTGCCCAATATGGGTAAAAACAC
CTTTGAAGCTTGCCAATGGAACCAAATATAGACCTCCTGCAAAACTATTGAAGGAAAGGGGTTT
CTTCGGAGCTATTGCTGGTTTCCTAGAAGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGA
TACACATCTCACGGAGCACATGGAGTGGCAGTGGCGGCAGACCTTAAGAGTACACAAGAAGCTA
TAAATAAGATAACAAAAAATCTCAATTCTTTGAGTGAGCTAGAAGTAAAGAACCTTCAAAGACT
AAGTGGTGCCATGGATGAACTCCACAACGAAATACTCGAGCTGGATGAGAAAGTGGATGATCTC
AGAGCTGACACTATAAGCTCACAAATAGAACTTGCAGTCTTGCTTTCCAACGAAGGAATAATAA
ACAGTGAAGACGAGCATCTATTGGCACTTGAGAGAAAACTAAAGAAAATGCTGGGTCCCTCTGC
TGTAGACATAGGAAACGGATGCTTCGAAACCAAACACAAATGCAACCAGACCTGCTTAGACAGG
ATAGCTGCTGGCACCTTTAATGCAGGAGAATTTTCTCTCCCCACTTTTGATTCATTGAACATTA
CTGCTGCATCTTTAAATGATGATGGATTGGATAACCATACTATACTGCTCTATTACTCAACTGC
TGCTTCTAGTTTGGCTGTAACATTAATGCTAGCTATTTTTATTGTTTATATGGTCTCCAGAGAC
AACGTTTCATGCTCCATCTGTCTATAA

图 6D

构建体193的示意图。示意图上注释用于质粒线性化的SacII和StuI限制性酶切位点

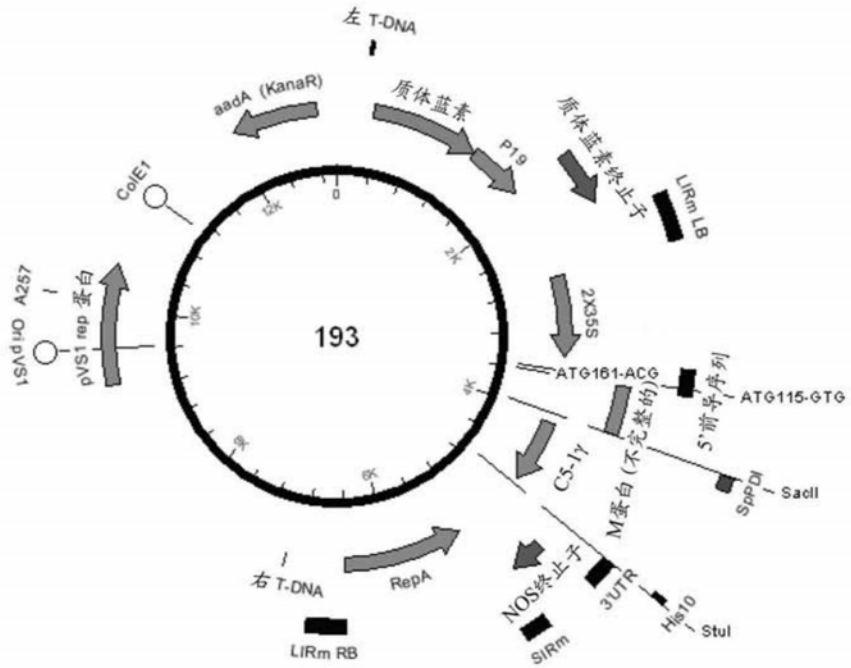


图 6E, SEQ ID NO: 52

从左至右t-DNA边界的构建体194(加下划线)。引入BeYDV+复制酶扩增系统中的具有质体蓝素-P19-质体蓝素沉默抑制子表达盒的2X35S/CPMV-HT/NOS

TGGCAGGATATATTGTGGTGTAAACAAATTGACGCTTAGACAACTTAATAACACATTGCGGACG
TTTTTAATGTACTGAATTAACGCCGAATCCCGGGCTGGTATATTTATATGTTGTCAAATAACTC
AAAAACCATAAAAGTTTAAGTTAGCAAGTGTGTACATTTTTACTTGAACAAAAATATTCACCTA
CTACTGTTATAAATCATTATTAAACATTAGAGTAAAGAAATATGGATGATAAGAACAAGAGTAG
TGATATTTTGACAACAATTTTGTTGCAACATTTGAGAAAATTTTGTTGTTCTCTCTTTTCATTG
GTCAAAAACAATAGAGAGAGAAAAAGGAAGAGGGAGAATAAAAACATAATGTGAGTATGAGAGA
GAAAGTTGTACAAAAGTTGTACCAAAATAGTTGTACAAATATCATTGAGGAATTTGACAAAAGC
TACACAAATAAGGGTTAATTGCTGTAAATAAATAAGGATGACGCATTAGAGAGATGTACCATTA
GAGAATTTTTGGCAAGTCATTAAAAAGAAAGAATAAATTATTTTTAAAATTAAAAGTTGAGTCA
TTTGATTAAACATGTGATTATTTAATGAATTGATGAAAGAGTTGGATTAAAGTTGTATTAGTAA
TTAGAATTTGGTGTCAAATTTAATTTGACATTTGATCTTTTCCTATATATTGCCCCATAGAGTC
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AAAAAAAAAACGGTATATTTACTAAAAAATCTAAGCCACGTAGGAGGATAACAGGATCCCCGTA
GGAGGATAACATCCAATCCAACCAATCACAACAATCCTGATGAGATAACCCACTTTAAGCCCAC
GCATCTGTGGCACATCTACATTATCTAAATCACACATTCTTCCACACATCTGAGCCACACAAAA
ACCAATCCACATCTTTATCACCCATTCTATAAAAAATCACACTTTGTGAGTCTACACTTTGATT

图 6E(续)

CCCTTCAAACACATACAAAGAGAAGAGACTAATTAATTAATTAATCATCTTGAGAGAAAATGGA
ACGAGCTATACAAGGAAACGACGCTAGGGAACAAGCTAACAGTGAACGTTGGGATGGAGGATCA
GGAGGTACCACTTCTCCCTTCAAACTTCCTGACGAAAGTCCGAGTTGGACTGAGTGGCGGCTAC
ATAACGATGAGACGAATTCGAATCAAGATAATCCCCTTGGTTTCAAGGAAAGCTGGGGTTTCGG
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GGATCTTGGACGGGAGATTCGGTTAACTATGCAGCATCTCGATTTTTCGGTTTCGACCAGATCG
GATGTACCTATAGTATTCGGTTTCGAGGAGTTAGTATCACCGTTTCTGGAGGGTCGCGAACTCT
TCAGCATCTCTGTGAGATGGCAATTCGGTCTAAGCAAGAACTGCTACAGCTTGCCCCAATCGAA
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AAGTTAAAATGCTTCTTCGTCTCCTATTTATAATATGGTTTGTTATTGTTAATTTTGTTCTTGT
AGAAGAGCTTAATTAATCGTTGTTGTTATGAAATACTATTTGTATGAGATGAACTGGTGTAATG
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ACCTGCCGCGTACAATTGTCTTATATTTGAACAACTAAAATTGAACATCTTTTGCCACAACTTT
ATAAGTGGTTAATATAGCTCAAATATATGGTCAAGTTCAATAGATTAATAATGGAAATATCAGT
TATCGAAATTCATTAACAATCAACTTAACGTTATTAACTACTAATTTTATATCATCCCCTTTGA
TAAATGATAGTACACCAATTAGGAAGGAGCATGCTCGCCTAGGAGATTGTCGTTTCCCGCCTTC
AGTTTGCAAGCTGCTCTAGCCGTGTAGCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGGAAT
TACTAGCGCGTGTCGAGACGCGTTGTTGTTGTGACTCCGAGGGGTTGCCTCAAACTCTATCTTA
TAACCGGCGTGGAGGCATGGAGGCAGGGGTATTTTGGTCATTTTAATAGATAGTGGAAAATGAC
GTGGAATTTACTTAAAGACGAAGTCTTTGCGACAAGGGGGGGCCCACGCCGAATTTAATATTAC
CGGCGTGGCCCCCCCTTATCGCGAGTGCTTTAGCACGAGCGGTCCAGATTTAAAGTAGAAAATT
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GTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAA
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TTGTCAGATCGTGCTTCGGCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTC
TTTGTGGACACGTAGTGCGGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGTGGT
CTTGGGAAAAGAAAGCTTGCTGGAGGCTGCTGTTCAGCCCCATACATTACTTGTTACGATTCTG
CTGACTTTCGGCGGGTGCAATATCTCTACTTCTGCTTGACGAGGTATTGTTGCCTGTACTTCTT
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CTGGATCTGCTGCCCAAACTAACTCCATGGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCCC
TGAGCCAGTGACAGTGACCTGGAACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCT
GTCCTGCAGTCTGACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCA

图 6E(续)

GCGAGACCGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGT
GCCCAGGGATTGTGGTTGTAAGCCTTGCATATGTACAGTCCCAGAAGTATCATCTGTCTTCATC
TTCCCCCCAAAGCCCAAGGATGTGCTCACCATTACTCTGACTCCTAAGGTCACGTGTGTTGTGG
TAGACATCAGCAAGGATGATCCCGAGGTCCAGTTCAGCTGGTTTGTAGATGATGTGGAGGTGCA
CACAGCTCAGACGCAACCCCGGGAGGAGCAGTTCAACAGCACTTTCCGCTCAGTCAGTGAACTT
CCCATCATGCACCAGGACTGGCTCAATGGCAAGGAGCGATCGCTCACCATCACCATCACCATCA
CCATCACCATTAAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTAT
GTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTTCTTTGTGAG
CTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAAA
AAAAAAAAAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCAGATCGTTCAAACATTTGGC
AATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTT
GAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTT
ATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACT
AGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGATCTCTAGAGTCTCAAGCTTGGCGC
GCCATAAAATGATTATTTTATGAATATATTTCATTGTGCAAGTAGATAGAAATTACATATGTTA
CATAACACACGAAATAAACAAAAAAAGACAATCCAAAAACAAACACCCCAAAAAAAATAATCAC
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CTCCCCGTCACACATATAGTGGGTGACGCAATTATCTTTAAAGTAATCCTTCTGTTGACTTGTC
ATTGATAACATCCAGTCTTCGTCAGGATTGCAAAGAATTATAGAAGGGATCCCACCTTTTATTT
TCTTCTTTTTTCCATATTTAGGGTTGACAGTGAAATCAGACTGGCAACCTATTAATTGCTTCCA
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CGGTACGAGTTGGTCCGCAGATGTAGAGGCTGGGGTGTCGGATTCCATTCCTTCCATTGTCCTG
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CAAGATATTCCTCCTTAGACGTTGCAGTCTGGATAATAGTTCTCCATCGTGCGTCAGATTTGCG
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TCAAGGACTTGTTTAGAGTTTCTAGCTGGCTGGATATTAGGGTGATTTCCTTCAAAATCGAAAA
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GGTATTTGACTAGTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTA
CCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCG
CACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGCTAGAGCAGCTTGAGCTTG
GATCAGATTGTCGTTTCCCGCCTTCAGTTTAAACTATCAGTGTTTGACAGGATATATTGGCGGG
TAAACCTAAGAGAAAAGAGCGTTTA

图 6F, SEQ ID NO: 53

从2X35S启动子至NOS终止子的表达盒1462号。来自流感B/威斯康星州/1/2010的HA加下划线

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAG
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CATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGAC
CCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGA
TTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTC
TCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGAT
TCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAA
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GATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGC
AAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCA
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TGATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTT
AAAGCAAACTTCTCTCTTGTCTTTCTTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCG
GCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTTTGTGGACACGTAGTGC
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GCTGGAGGCTGCTGTTCAGCCCCATACATTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGC
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CAGATTGGCGACTTCCCAGATCAAACAGAAGACGGAGGACTACCACAAAGCGGCAGAATTGTTG
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GCCTCAAAAGGTGTGGTGCGCGAGTGGCAGGAGCAAAGTAATAAAAGGGTCATTGCCTTTAATT
GGTGAAGCAGATTGCCTTCATGAAAAATACGGTGGATTAAACAAAAGCAAGCCTTACTACACAG
GAGAACATGCAAAAGCCATAGGAAATTGCCCAATATGGGTAAAAACACCTTTGAAGCTTGCCAA
TGGAACCAAATATAGACCTCCTGCAAAACTATTGAAGGAAAGGGGTTTCTTCGGAGCTATTGCT
GGTTTCCTAGAAGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGATACACATCTCACGGAG
CACATGGAGTGGCAGTGGCGGCAGACCTTAAGAGTACACAAGAAGCTATAAATAAGATAACAAA
AAATCTCAATTCTTTGAGTGAGCTAGAAGTAAAGAACCTTCAAAGACTAAGTGGTGCCATGGAT
GAACTCCACAACGAAATACTCGAGCTGGATGAGAAAGTGGATGATCTCAGAGCTGACACTATAA
GCTCACAAATAGAACTTGCAGTCTTGCTTTCCAACGAAGGAATAATAAACAGTGAAGACGAGCA
TCTATTGGCACTTGAGAGAAAACTAAAGAAAATGCTGGGTCCCTCTGCTGTAGACATAGGAAAC

图 6F(续)

GGATGCTTCGAAACCAAACACAAATGCAACCAGACCTGCTTAGACAGGATAGCTGCTGGCACCT
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TGATGATGGATTGGATAACCATACTATACTGCTCTATTACTCAACTGCTGCTTCTAGTTTGGCT
GTAACATTAATGCTAGCTATTTTTATTGTTTATATGGTCTCCAGAGACAACGTTTCATGCTCCA
TCTGTCTATAAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTATGT
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TAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGA
ATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTAT
GATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAG
GATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGAT

图 6G, SEQ ID NO: 54

来自流感B/威斯康星州/1/2010的HA的氨基酸序列

MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSYFANLKGTRT
RGKLCPDCLNCTDLDVALGRPMCVGTTPSAKASILHEVRPVTSGCFPIMHDRTKIRQLPNLLRG
YENIRLSTQNVIDAEKAPGGPYRLGTSGSCPNATSKIGFFATMAWAVPKDNYKNATNPLTVEVP
YICTEGEDQITVWGFHSDNKTQMKSLYGDSNPQKFTSSANGVTTHYVSQIGDFPDQTEDGGLPQ
SGRIVVDYMMQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKGSLPLIGEADCLHEKYGGLNKS
KPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPAKLLKERGFFGAIAGFLEGGWEGMIAGWHG
YTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDL
RADTISSQIELAVLLSNEGIINSEDEHLLALERKLKKMLGPSAVDIGNGCFETKHKCNQTCLDR
IAAGTFNAGEFSLPTFDSLNITAASLNDDGLDNHTILLYYSTAASSLAVTLMLAIFIVYMVSRD
NVSCSICL*

图 6H

构建体1462号的示意图

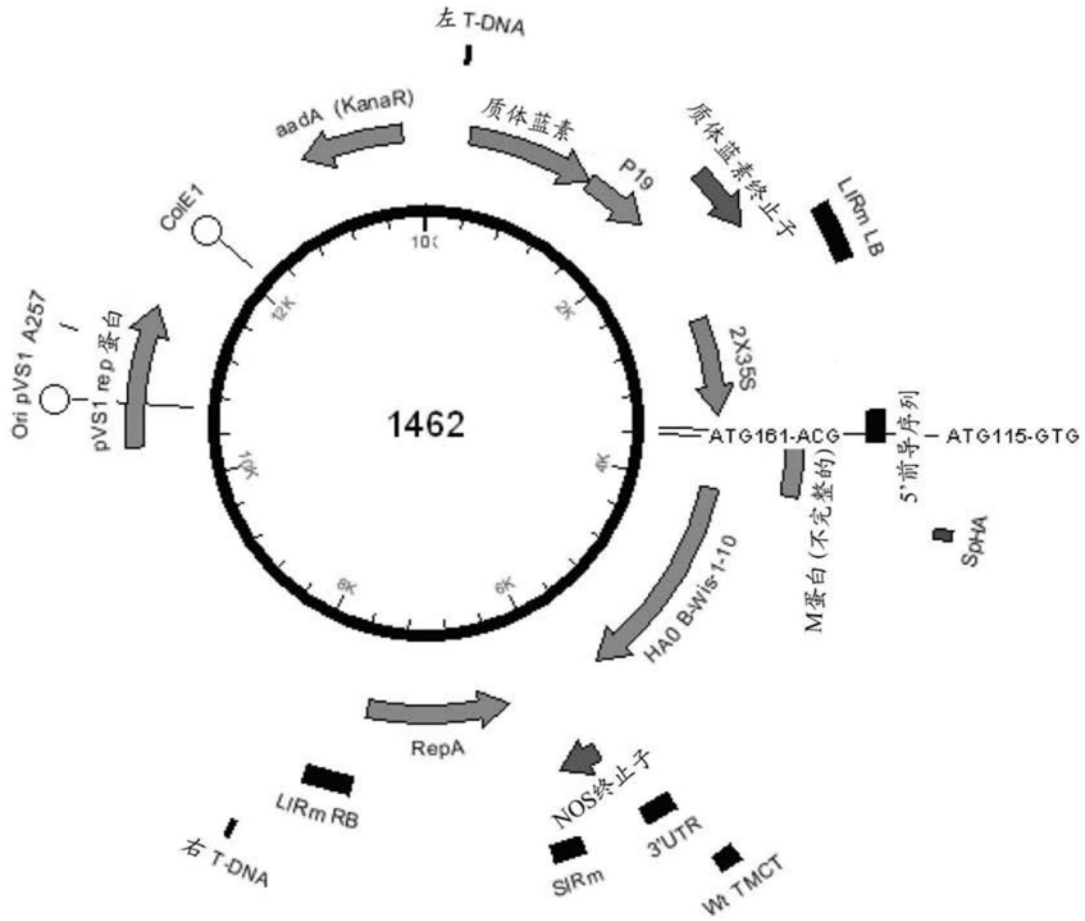


图6:引入BeYDV+复制酶扩增系统中的B-2X35S/CPMV-HT/HA B威斯康星州/NOS(构建体1462)

图 7A, SEQ ID NO: 55

HAB110(PrL-).r

TCCTTCCCATCCTCCACCAGGAGGTCTATATTTGGTTCCATTGGCAAGCTTCAAAG

图 7B, SEQ ID NO: 56

HAB110(PrL-).c

ATATAGACCTCCTGGTGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGA

图 7C, SEQ ID NO: 57

从2X35S启动子至NOS终止子的表达盒1467号。来自流感B/威斯康星州/1/2010的具有缺失的蛋白水解环的HA加下划线

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAG
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CCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCAT
CATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGAC
CCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGA
TTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTC
TCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGAT
TCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAA
ATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAA
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TATCAACCCAAAACGTTATCGATGCAGAAAAAGCACCAGGAGGACCCTACAGACTTGGAACCTC
AGGATCTTGCCCTAACGCTACCAGTAAAATCGGATTTTTTGCAACAATGGCTTGGGCTGTCCCA
AAGGACAACTACAAAAATGCAACGAACCCACTAACAGTAGAAGTACCATACATTTGTACAGAAG
GGGAAGACCAAATTACTGTTTGGGGGTTCCATTCAGATAACAAAACCCAAATGAAGAGCCTCTA

图 7C(续)

TGGAGACTCAAATCCTCAAAAGTTCACCTCATCTGCTAATGGAGTAACCACACATTATGTTTCT
CAGATTGGCGACTTCCCAGATCAAACAGAAGACGGAGGACTACCACAAAGCGGCAGAATTGTTG
TTGATTACATGATGCAAAAACCTGGGAAAACAGGAACAATTGTCTATCAAAGAGGTGTTTTGTT
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GAGAACATGCAAAAGCCATAGGAAATTGCCCAATATGGGTAAAAACACCTTTGAAGCTTGCCAA
TGGAACCAAATATAGACCTCCTGGTGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGATAC
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ATAAGATAACAAAAAATCTCAATTCTTTGAGTGAGCTAGAAGTAAAGAACCTTCAAAGACTAAG
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GCTGACACTATAAGCTCACAAATAGAACTTGCAGTCTTGCTTTCCAACGAAGGAATAATAAACA
GTGAAGACGAGCATCTATTGGCACTTGAGAGAAAACTAAAGAAAATGCTGGGTCCCTCTGCTGT
AGACATAGGAAACGGATGCTTCGAAACCAAACACAAATGCAACCAGACCTGCTTAGACAGGATA
GCTGCTGGCACCTTTAATGCAGGAGAATTTTCTCTCCCCACTTTTGATTCATTGAACATTACTG
CTGCATCTTTAAATGATGATGGATTGGATAACCATACTATACTGCTCTATTACTCAACTGCTGC
TTCTAGTTTGGCTGTAACATTAATGCTAGCTATTTTTATTGTTTATATGGTCTCCAGAGACAAC
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TAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGA
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图 7D, SEQ ID NO: 58

来自流感B/威斯康星州/1/2010的具有缺失的蛋白水解环的HA的氨基酸序列

MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSYFANLKGTRT
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YENIRLSTQNVIDAEKAPGGPYRLGTSGSCPNATSKIGFFATMAWAVPKDNYKNATNPLTVEVP
YICTEGEDQITVWGFHSDNKTQMKSLYGDSNPQKFTSSANGVTTHYVSQIGDFPDQTEDGGLPQ
SGRIVVDYMMQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKGSLPLIGEADCLHEKYGGLNKS
KPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPGGGWEGMIAGWHGYTSHGAHGVAVAADLKS
TQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELAVLLSN
EGIINSEDEHLLALERKLKKMLGPSAVDIGNGCFETKHKCNQTCLDRIAAGTFNAGEFSLPTFD
SLNITAASLNDDGLDNHTILLYYSTAASSLAVTLMLAIFIVYMVSRDNVSCSICL*

图 7E

构建体1467号的示意图

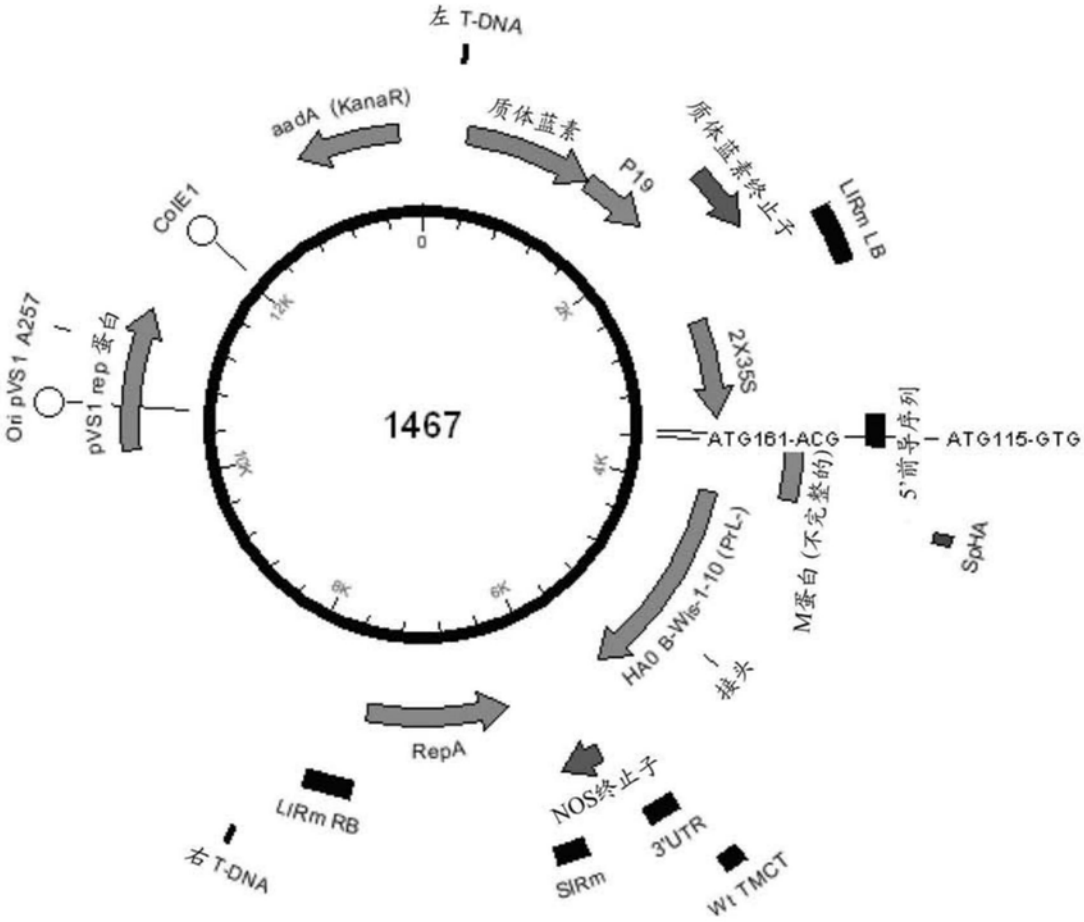


图7:引入BeYDV+复制酶扩增系统中的具有缺失的蛋白水解环的C-2X35S/CPMV-HT/HA B威斯康星州(构建体1467号)

图 8A(SEQ ID NO: 15)

从2X35S启动子至NOS终止子的表达盒1039号。来自流感B/布里斯班/60/2008的具有缺失的蛋白水解环的PDISP/HA加下划线

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAG
ACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTG
CCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCAT
CATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGAC
CCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGA
TTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTC
TCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGAT
TCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAA
ATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAA
GATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGC
AAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCA
AGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTT
TGATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTT
AAAGCAAACTTCTCTCTTGTCTTTCTTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCG
GCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTTTGTGGACACGTAGTGC
GGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGTGGTCTTGGGAAAAGAAAGCTT
GCTGGAGGCTGCTGTTCAGCCCCATACATTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGC
AATATCTCTACTTCTGCTTGACGAGGTATTGTTGCCTGTACTTCTTTCTTCTTCTTCTTGCTGA
TTGGTTCTATAAGAAATCTAGTATTTTCTTTGAAACAGAGTTTTCCCGTGGTTTTCGAACTTGG
AGAAAGATTGTTAAGCTTCTGTATATTCTGCCCAAATTTGTCGGGCCCATGGCGAAAAACGTTG
CGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCCGATCGAAT
CTGCACTGGAATAACATCGTCAAACTCACCACATGTCGTCAAAACTGCTACTCAAGGGGAGGTC
AATGTGACTGGTGTAATACCACTGACAACAACACCCACCAAATCTCATTTTGCAAATCTCAAAG
GAACAGAAACCAGGGGGAAACTATGCCCAAAATGCCTCAACTGCACAGATCTGGACGTAGCCTT
GGGCAGACCAAAATGCACGGGGAAAATACCCTCGGCAAGAGTTTCAATACTCCATGAAGTCAGA
CCTGTTACATCTGGGTGCTTTCCTATAATGCACGACAGAACAAAAATTAGACAGCTGCCTAACC
TTCTCCGAGGATACGAACATATCAGGTTATCAACCCATAACGTTATCAATGCAGAAAATGCACC
AGGAGGACCCTACAAAATTGGAACCTCAGGGTCTTGCCCTAACATTACCAATGGAAACGGATTT
TTCGCAACAATGGCTTGGGCCGTCCCAAAAAACGACAAAAACAAAACAGCAACAAATCCATTAA
CAATAGAAGTACCATACATTTGTACAGAAGGAGAAGACCAAATTACCGTTTGGGGGTTCCACTC
TGACAACGAGACCCAAATGGCAAAGCTCTATGGGGACTCAAAGCCCCAGAAGTTCACCTCATCT
GCCAACGGAGTGACCACACATTACGTTTCACAGATTGGTGGCTTCCCAAATCAAACAGAAGACG
GAGGACTACCACAAAGTGGTAGAATTGTTGTTGATTACATGGTGCAAAAATCTGGGAAAACAGG
AACAATTACCTATCAAAGGGGTATTTTATTGCCTCAAAAGGTGTGGTGCGCAAGTGGCAGGAGC
AAGGTAATAAAAGGATCCTTGCCTTTAATTGGAGAAGCAGATTGCCTCCACGAAAAATACGGTG
GATTAAACAAAAGCAAGCCTTACTACACAGGGGAACATGCAAAGGCCATAGGAAATTGCCCAAT
ATGGGTGAAAACACCCTTGAAGCTGGCCAATGGAACCAAATATAGACCTCCTGGTGGAGGATGG
GAAGGAATGATTGCAGGTTGGCACGGATACACATCCCATGGGGCACATGGAGTAGCGGTGGCAG
CAGACCTTAAGAGCACTCAAGAGGCCATAAACAAGATAACAAAAAATCTCAACTCTTTGAGTGA

图 8A(续)

GCTGGAAGTAAAGAATCTTCAAAGACTAAGCGGTGCCATGGATGAACTCCACAACGAAATACTA
GAACTAGATGAGAAAGTGGATGATCTCAGAGCTGATACAATAAGCTCACAAATAGAACTCGCAG
TCCTGCTTTCCAATGAAGGAATAATAAACAGTGAAGATGAACATCTCTTGGCGCTTGAAAGAAA
GCTGAAGAAAATGCTGGGCCCCTCTGCTGTAGAGATAGGGAATGGATGCTTTGAAACCAAACAC
AAGTGCAACCAGACCTGTCTCGACAGAATAGCTGCTGGTACCTTTGATGCAGGAGAATTTTCTC
TCCCCACCTTTGATTCACTGAATATTACTGCTGCATCTTTAAATGACGATGGATTGGATAATCA
TACTATACTGCTTTACTACTCAACTGCTGCCTCCAGTTTGGCTGTAACACTGATGATAGCTATC
TTTGTTGTTTATATGGTCTCCAGAGACAATGTTTCTTGCTCCATCTGTCTATAAAGGCCTATTT
TCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGC
TCAGAGTGTGTTTATTTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCT
TCAGCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAAAAAAAAAAAAAGACCGGGAATTCG
ATATCAAGCTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAAT
CCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAA
TTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATA
CATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTG
TCATCTATGTTACTAGAT

图 8B

构建体1039号的示意图

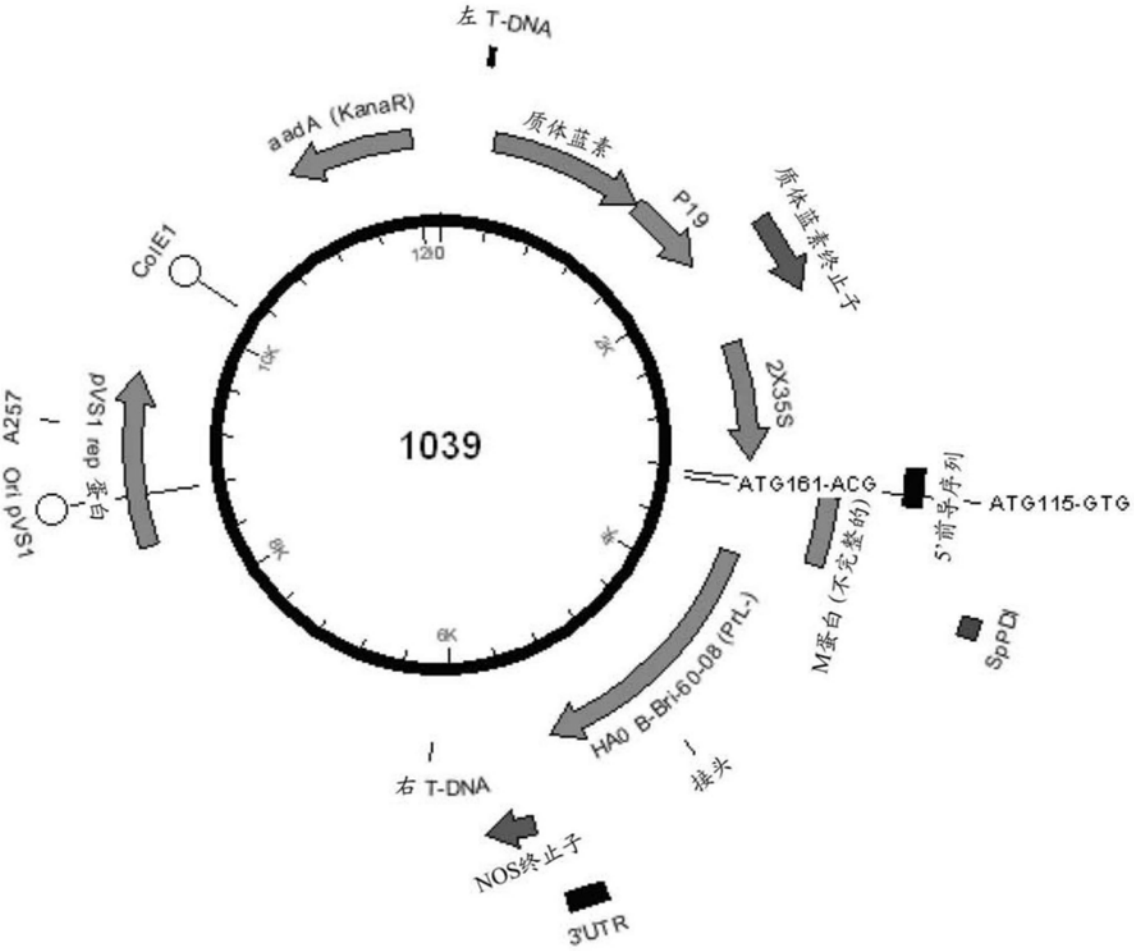


图8:A-2X35S/CPMV-HT/具有缺失的蛋白水解环的PDISP/HA B/NOS(构建体1039号)

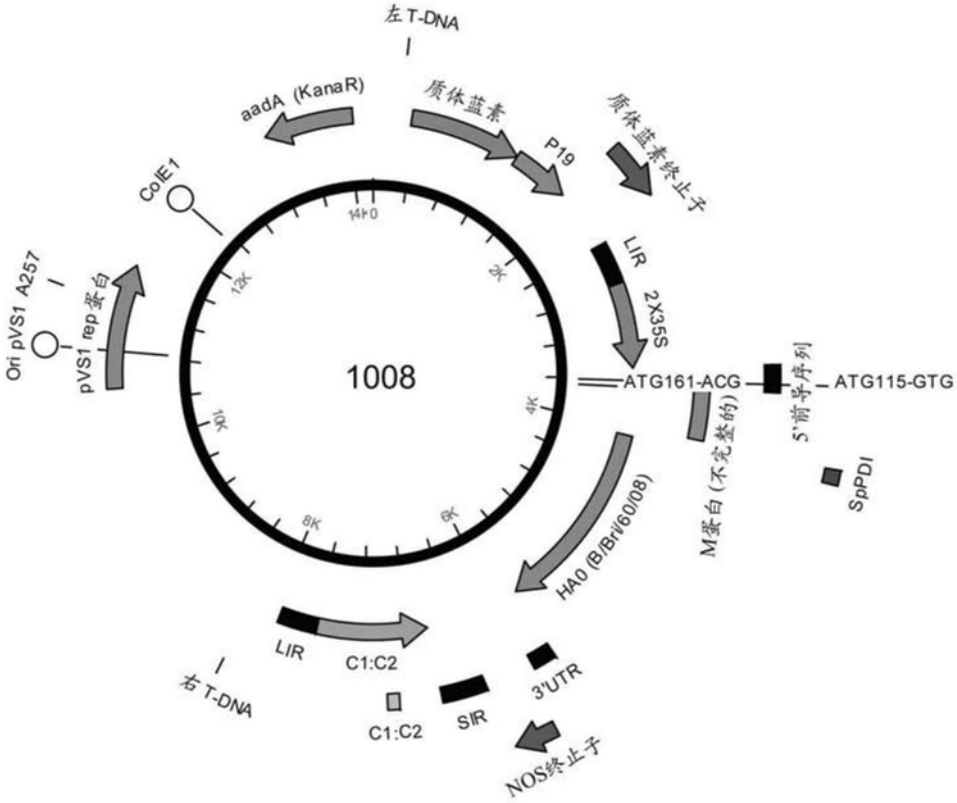

左T-DNA
aadA (KanaR)
质体蓝素
P19
质体蓝素终止子
CoIE1
LIR
2X35S
Ori pVS1 A257
pVS1 rep蛋白
1008
ATG161-ACG
ATG115-GTG
5'前导序列
M蛋白(不完整的)
SpPDI
HA0 (B/Bri/60/08)
LIR
C1:C2
右T-DNA
C1:C2
SIR
3'UTR
NOS终止子

图9

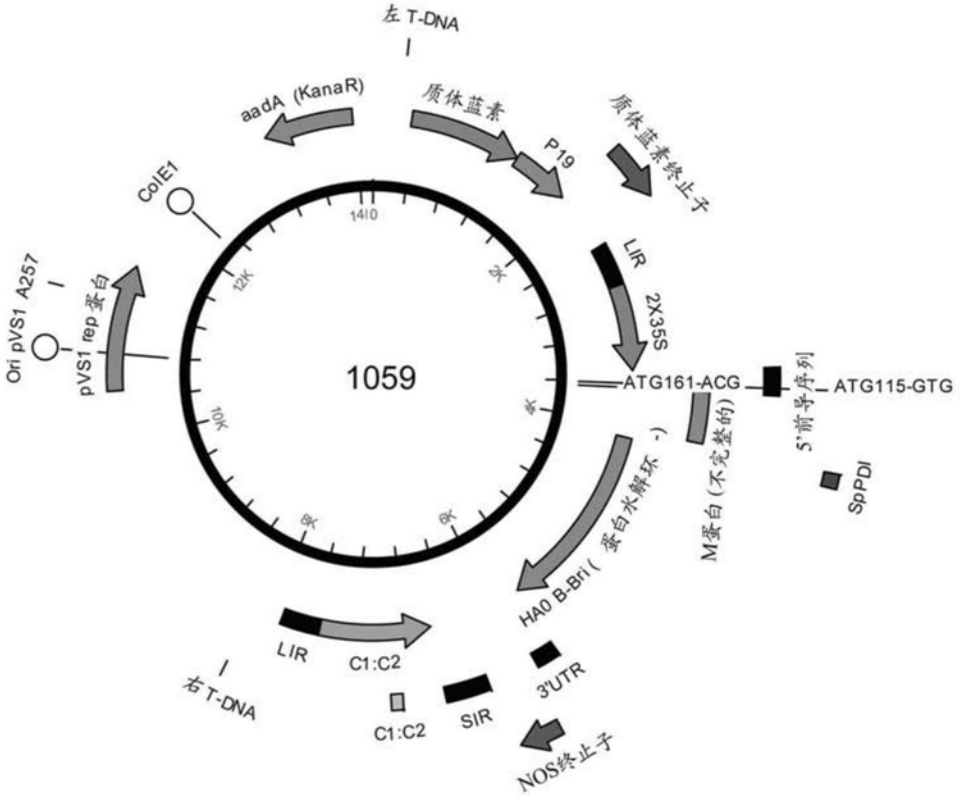

左 T-DNA
aadA (KanaR)
质体蓝素
P19
质体蓝素终止子
ColE1
LIR
2X35S
Ori pVS1
A257
pVS1 rep 蛋白
1059
ATG161-ACG
ATG115-GTG
5'前导序列
M蛋白(不完整的)
SpPDI
HA0 B-Bri(蛋白水解环 -)
LIR
C1:C2
右 T-DNA
C1:C2
SIR
3'UTR
NOS终止子

图10

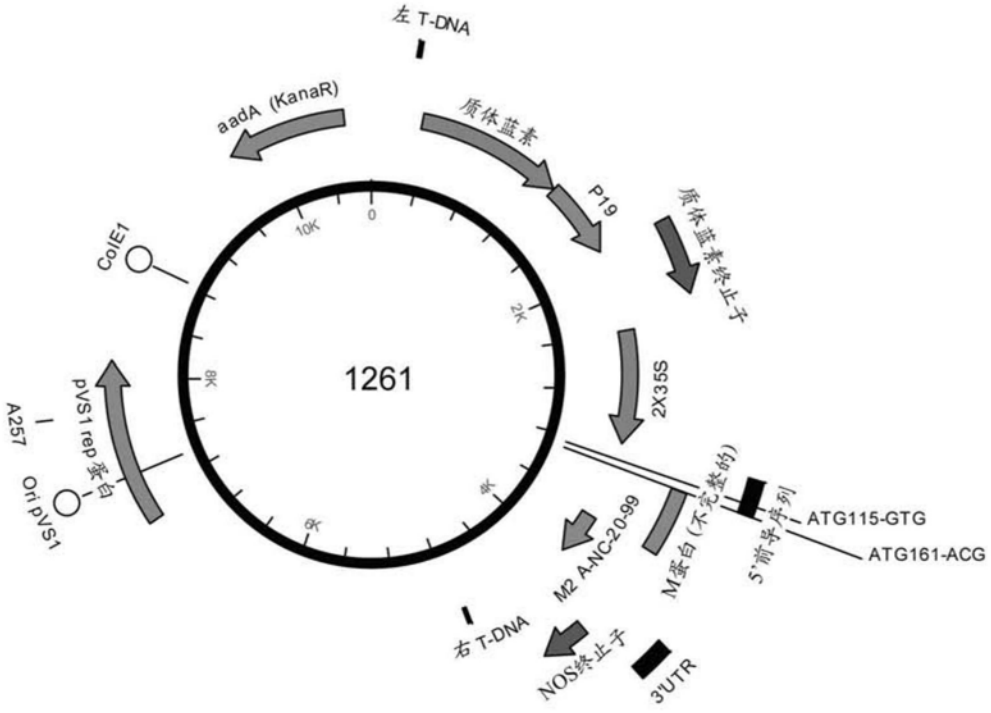

左 T-DNA
aadA (KanaR)
质体蓝素
P19
质体蓝素终止子
ColE1
2X35S
pVS1 rep 蛋白
A257
Ori pVS1
1261
M2 A-NC-20-99
M蛋白(不完整的)
5'前导序列
ATG115-GTG
ATG161-ACG
右 T-DNA
NOS终止子
3'UTR

图11

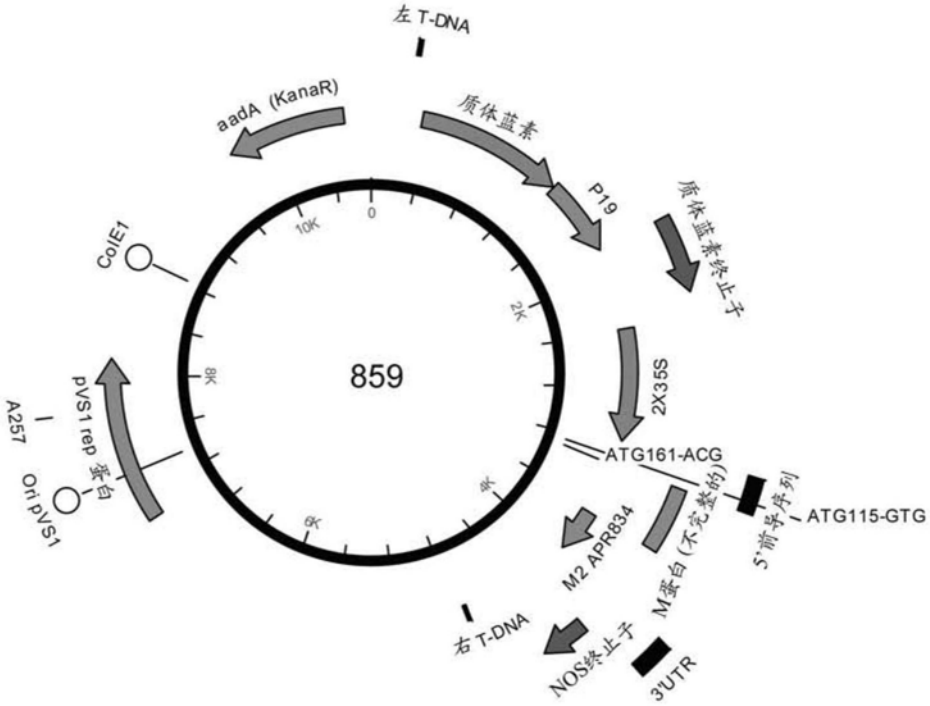

左 T-DNA
aadA (KanaR)
质体蓝素
P19
质体蓝素终止子
2X35S
ATG161-ACG
M蛋白(不完整的)
5'前导序列
ATG115-GTG
M2 APR834
右 T-DNA
NOS终止子
3'UTR
ColE1
pVS1 rep 蛋白
Ori pVS1
A257
859
0
2K
4K
6K
8K
10K

图12

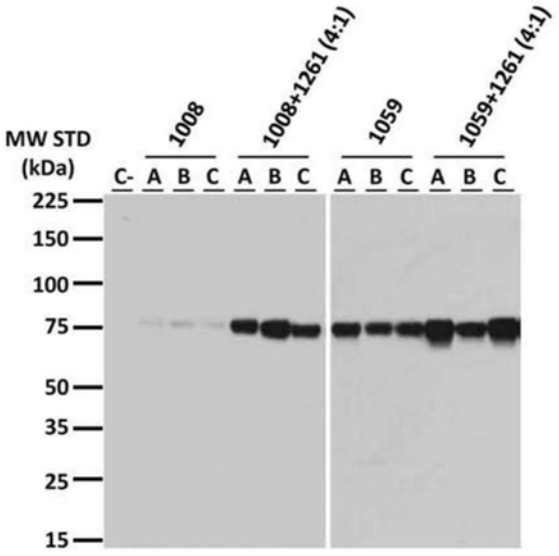

MW STD
(kDa)
C-
1008
1008+1261 (4:1)
1059
1059+1261 (4:1)
A B C A B C A B C A B C
225
150
100
75
50
35
25
15

图13A

构建体号	血凝能力 (HA单位/ mg蛋白)[a]
1008	533
1008 + 1261 (4:1)	3200
1059	4267
1059 + 1261 (4:1)	34133

[a] 用于在200 μl最终体积中进行阳性血凝反应所需总蛋白的最小量的倒数

图13B

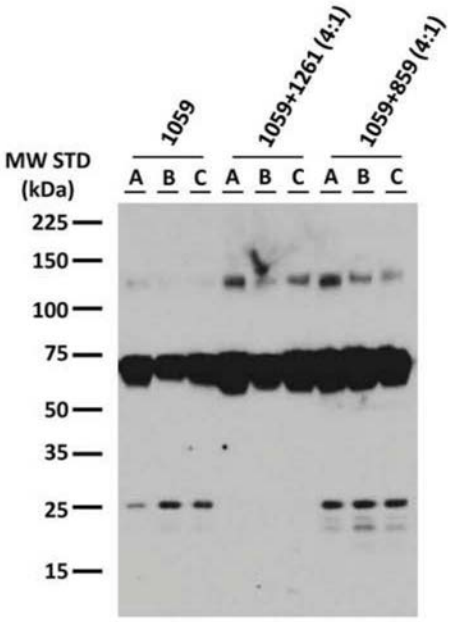


图14

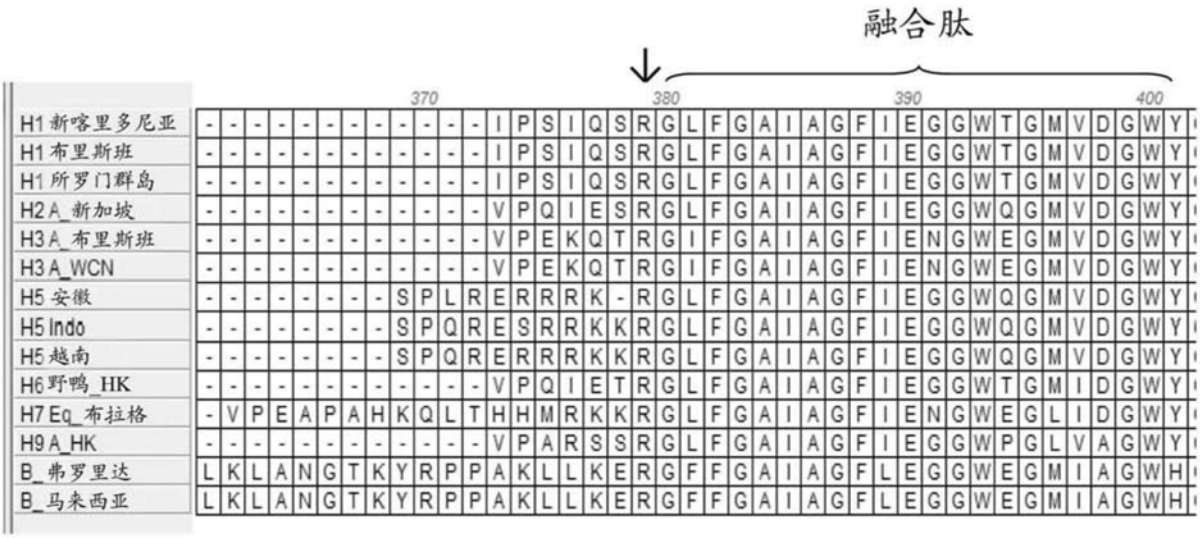

融合肽
370
380
390
400
H1 新喀里多尼亚 ---------IPSIQSRGLFGAIAGFIEGGWTGMVDGWY
H1 布里斯班 ---------IPSIQSRGLFGAIAGFIEGGWTGMVDGWY
H1 所罗门群岛 ---------IPSIQSRGLFGAIAGFIEGGWTGMVDGWY
H2 A_新加坡 ---------VPQIESRGLFGAIAGFIEGGWQGMVDGWY
H3 A_布里斯班 ---------VPEKQTRGIFGAIAGFIENGWEGMVDGWY
H3 A_WCN ---------VPEKQTRGIFGAIAGFIENGWEGMVDGWY
H5 安徽 --------SPLRERRRK-RGLFGAIAGFIEGGWQGMVDGWY
H5 Indo --------SPQRESRRKKRGLFGAIAGFIEGGWQGMVDGWY
H5 越南 --------SPQRERRRKKRGLFGAIAGFIEGGWQGMVDGWY
H6 野鸭_HK ---------VPQIETRGLFGAIAGFIEGGWTGMIDGWY
H7 Eq_布拉格 -VPEAPAHKQLTHHMRKKRGLFGAIAGFIENGWEGLIDGWY
H9 A_HK ---------VPARSSRGLFGAIAGFIEGGWPGLVAGWY
B_弗罗里达 LKLANGTKYRPPAKLLKERGFFGAIAGFLEGGWEGMIAGWH
B_马来西亚 LKLANGTKYRPPAKLLKERGFFGAIAGFLEGGWEGMIAGWH

图15

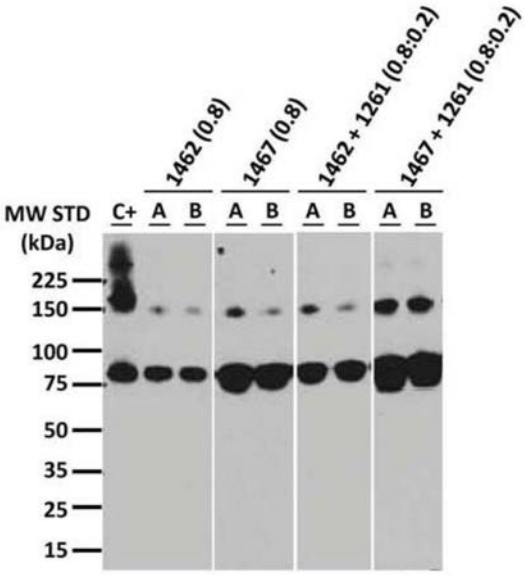

1462 (0.8)
1467 (0.8)
1462 + 1261 (0.8:0.2)
1467 + 1261 (0.8:0.2)
MW STD
(kDa)
C+
A
B
A
B
A
B
A
B
225
150
100
75
50
35
25
15

图16A

构建号	血凝能力(HA滴度)[a]
1462 (0.8)	136 ± 48
1467 (0.8)	2660 ± 384
1462 + 1261 (0.8:0.2)	941 ± 192
1467 + 1261 (0.8:0.2)	4344 ± 1536

[a] 能够使红血细胞凝集的最高稀释度的倒数

图16B

构建体号	调控元件	BeYDV	蛋白水解环	M2共表达	血凝能力(HA单位/ml)
1008	CPMV-HT	是	存在	否	1216
1008 + 1261	CPMV-HT	是	存在	是	5696
1039	CPMV-HT	否	去除	否	141
1039 + 1261	CPMV-HT	否	去除	是	2944
1059	CPMV-HT	是	去除	否	10197
1059 + 1261	CPMV-HT	是	去除	是	43008

图17A

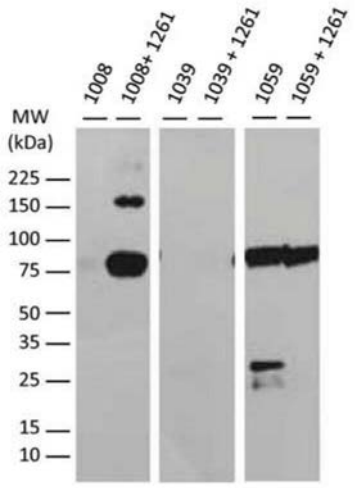


图17B

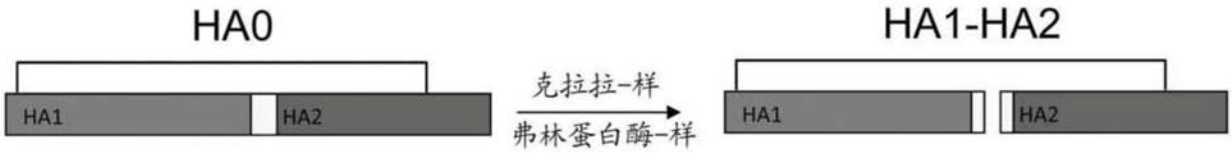


图18A

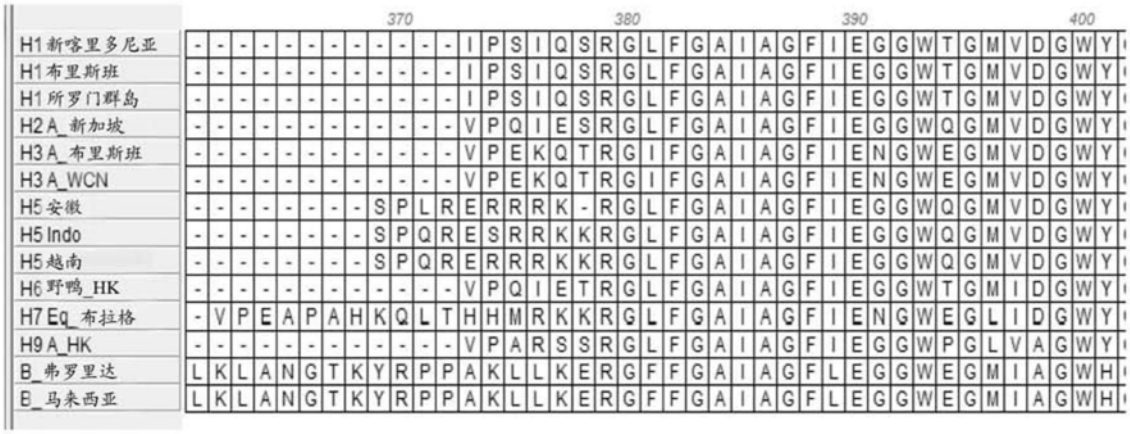


图18B

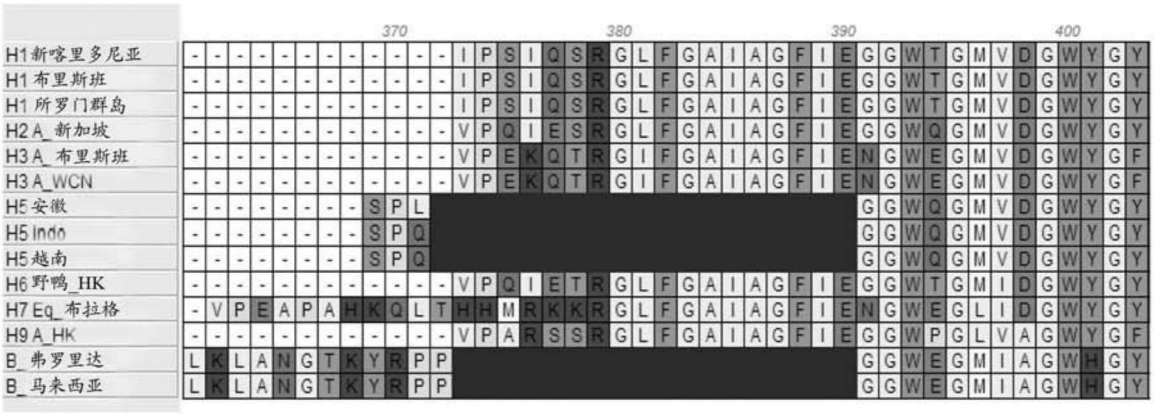
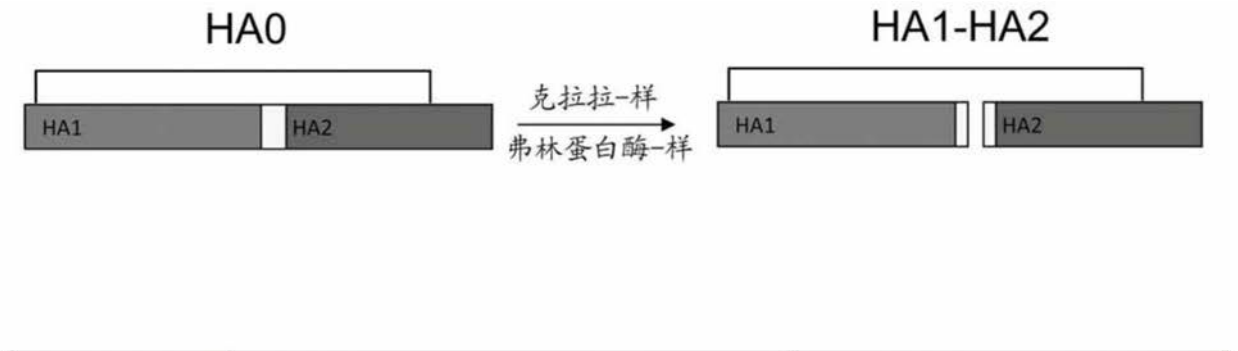


图18C



株	天然序列	前体切割位点的废除
H5/Indo	TGLRNSPQ**RESRRKKR**↓GLF	TGLRNSPQTETR↓GLF TGLRNSPQTETQGLF

图19

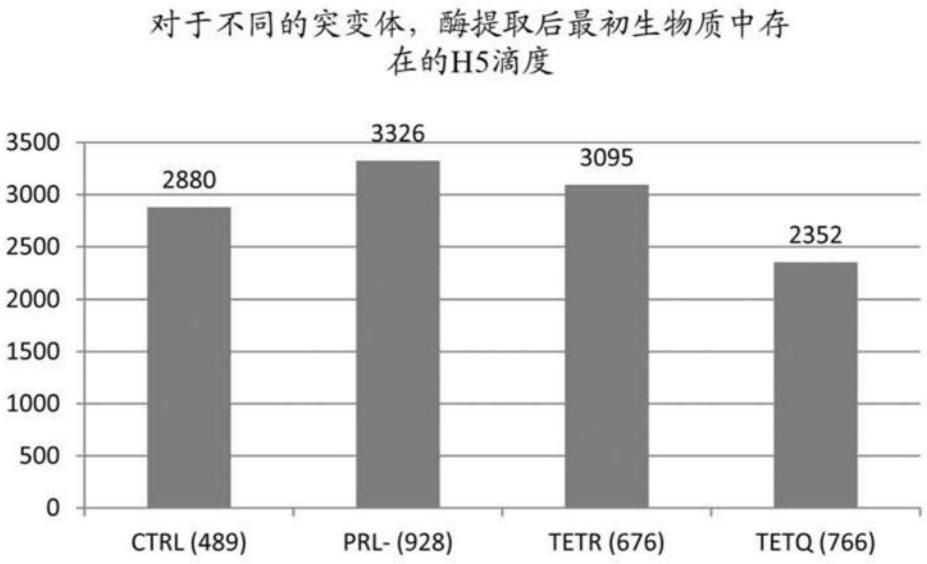

对于不同的突变体，酶提取后最初生物质中存在的H5滴度
3500
3000
2500
2000
1500
1000
500
0
2880
3326
3095
2352
CTRL (489)
PRL- (928)
TETR (676)
TETQ (766)

图20

图 21A

天然的B/布里斯班/60/2008的一级序列(SEQ ID NO: 16)

DRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKGTETRGKLCPKCLNCTDLD
VALGRPKCTGKIPSARVSILHEVRPVTSGCFPIMHDRTKIRQLPNLLRGYEHIRLSTHNVINAE
NAPGGPYKIGTSGSCPNITNGNGFFATMAWAVPKNDKNKTATNPLTIEVPYICTEGEDQITVWG
FHSDNETQMAKLYGDSKPQKFTSSANGVTTHYVSQIGGFPNQTEDGGLPQSGRIVVDYMVQKSG
KTGTITYQRGILLPQKVWCASGRSKVIKGSLPLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGN
CPIWVKTPLKLANGTKYRPPAKLLKERGFFGAIAGFLEGGWEGMIAGWHGYTSHGAHGVAVAAD
LKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELAVL
LSNEGIINSEDEHLLALERKLKKMLGPSAVEIGNGCFETKHKCNQTCLDRIAAGTFDAGEFSLP
TFDSLNITAASLNDDGLDNHTILLYYSTAASSLAVTLMIAIFVVYMVSRDNVSCSICL

图 21B

B型HA的Δ蛋白水解环(SEQ ID NO:17)

DRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKGTETRGKLCPKCLNCTDLD
VALGRPKCTGKIPSARVSILHEVRPVTSGCFPIMHDRTKIRQLPNLLRGYEHIRLSTHNVINAE
NAPGGPYKIGTSGSCPNITNGNGFFATMAWAVPKNDKNKTATNPLTIEVPYICTEGEDQITVWG
FHSDNETQMAKLYGDSKPQKFTSSANGVTTHYVSQIGGFPNQTEDGGLPQSGRIVVDYMVQKSG
KTGTITYQRGILLPQKVWCASGRSKVIKGSLPLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGN
CPIWVKTPLKLANGTKYRPP-*GG*-
GWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNE
ILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKLKKMLGPSAVEIGNGCFET
KHKCNQTCLDRIAAGTFDAGEFSLPTFDSLNITAASLNDDGLDNHTILLYYSTAASSLAVTLMI
AIFVVYMVSRDNVSCSICL

图 21C

通过接头代替B型HA的切割位点(SEQ ID NO:18)

DRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKGTETRGKLCPKCLNCTDLD
VALGRPKCTGKIPSARVSILHEVRPVTSGCFPIMHDRTKIRQLPNLLRGYEHIRLSTHNVINAE
NAPGGPYKIGTSGSCPNITNGNGFFATMAWAVPKNDKNKTATNPLTIEVPYICTEGEDQITVWG
FHSDNETQMAKLYGDSKPQKFTSSANGVTTHYVSQIGGFPNQTEDGGLPQSGRIVVDYMVQKSG
KTGTITYQRGILLPQKVWCASGRSKVIKGSLPLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGN
CPIWVKTPLKLANGTKYR *-GSSSGSSSG-*
GFFGAIAGFLEGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQ
RLSGAMDELHNEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKLKKMLGP
SAVEIGNGCFETKHKCNQTCLDRIAAGTFDAGEFSLPTFDSLNITAASLNDDGLDNHTILLYYS
TAASSLAVTLMIAIFVVYMVSRDNVSCSICL

图 21D

H3的缺失/代替方法

天然的H3 A/珀斯/16/2009的一级序列(SEQ ID NO:19)

QKLPGNDNSTATLCLGHHAVPNGTIVKTITNDQIEVTNATELVQSSSTGEICDSPHQILDGKNC
TLIDALLGDPQCDGFQNKKWDLFVERSKAYSNCYPYDVPDYASLRSLVASSGTLEFNNESFNWT
GVTQNGTSSACIRRSKNSFFSRLNWLTHLNFKYPALNVTMPNNEQFDKLYIWGVHHPGTDKDQI
FLYAQASGRITVSTKRSQQTVSPNIGSRPRVRNIPSRISIYWTIVKPGDILLINSTGNLIAPRG
YFKIRSGKSSIMRSDAPIGKCNSECITPNGSIPNDKPFQNVNRITYGACPRYVKQNTLKLATGM
RNVPEKQTRGIFGAIAGFIENGWEGMVDGWYGFRHQNSEGRGQAADLKSTQAAIDQINGKLNRL
IGKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNKLF
EKTKKQLRENAEDMGNGCFKIYHKCDNACIGSIRNGTYDHDVYRDEALNNRFQIKGVELKSGYK
DWILWISFAISCFLLCVALLGFIMWACQKGNIRCNICI

图 21E

Δ蛋白水解环H3(BeYDV, 1096)(SEQ ID NO:20)

QKLPGNDNSTATLCLGHHAVPNGTIVKTITNDQIEVTNATELVQSSSTGEICDSPHQILDGKNC
TLIDALLGDPQCDGFQNKKWDLFVERSKAYSNCYPYDVPDYASLRSLVASSGTLEFNNESFNWT
GVTQNGTSSACIRRSKNSFFSRLNWLTHLNFKYPALNVTMPNNEQFDKLYIWGVHHPGTDKDQI
FLYAQASGRITVSTKRSQQTVSPNIGSRPRVRNIPSRISIYWTIVKPGDILLINSTGNLIAPRG
YFKIRSGKSSIMRSDAPIGKCNSECITPNGSIPNDKPFQNVNRITYGACPRYVKQNTLKLATGM
-GS-
GAIAGFIENGWEGMVDGWYGFRHQNSEGRGQAADLKSTQAAIDQINGKLNRLIGKTNEKFHQIE
KEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNKLFEKTKKQLRENAE
DMGNGCFKIYHKCDNACIGSIRNGTYDHDVYRDEALNNRFQIKGVELKSGYKDWILWISFAISC
FLLCVALLGFIMWACQKGNIRCNICI

图 21F

通过接头代替H3的切割位点(BeYDV, 1097)(SEQ ID NO:21)

QKLPGNDNSTATLCLGHHAVPNGTIVKTITNDQIEVTNATELVQSSSTGEICDSPHQILDGKNC
TLIDALLGDPQCDGFQNKKWDLFVERSKAYSNCYPYDVPDYASLRSLVASSGTLEFNNESFNWT
GVTQNGTSSACIRRSKNSFFSRLNWLTHLNFKYPALNVTMPNNEQFDKLYIWGVHHPGTDKDQI
FLYAQASGRITVSTKRSQQTVSPNIGSRPRVRNIPSRISIYWTIVKPGDILLINSTGNLIAPRG
YFKIRSGKSSIMRSDAPIGKCNSECITPNGSIPNDKPFQNVNRITYGACPRYVKQNTLKLATGM
-GSSGSSGSS-
GIFGAIAGFIENGWEGMVDGWYGFRHQNSEGRGQAADLKSTQAAIDQINGKLNRLIGKTNEKFH
QIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNKLFEKTKKQLRE
NAEDMGNGCFKIYHKCDNACIGSIRNGTYDHDVYRDEALNNRFQIKGVELKSGYKDWILWISFA
ISCFLLCVALLGFIMWACQKGNIRCNICI

图21:B型HA的缺失/代替方法

泳道#	样本号	描述
1	496-59-001A	H5/Indo CTRL (# 489)
2	496-59-001B	H5/Indo CTRL (# 489)
3	496-59-002A	H5/Indo PRL- (# 928)
4	496-59-002B	H5/Indo PRL- (# 928)
5	496-59-003A	H5/Indo TETR (# 676)
6	496-59-003B	H5/Indo TETR (# 676)
7	496-59-004A	H5/Indo TETQ (# 766)
8	496-59-004B	H5/Indo TETQ (# 766)
9	MW	
10	C+	H5 25 ng

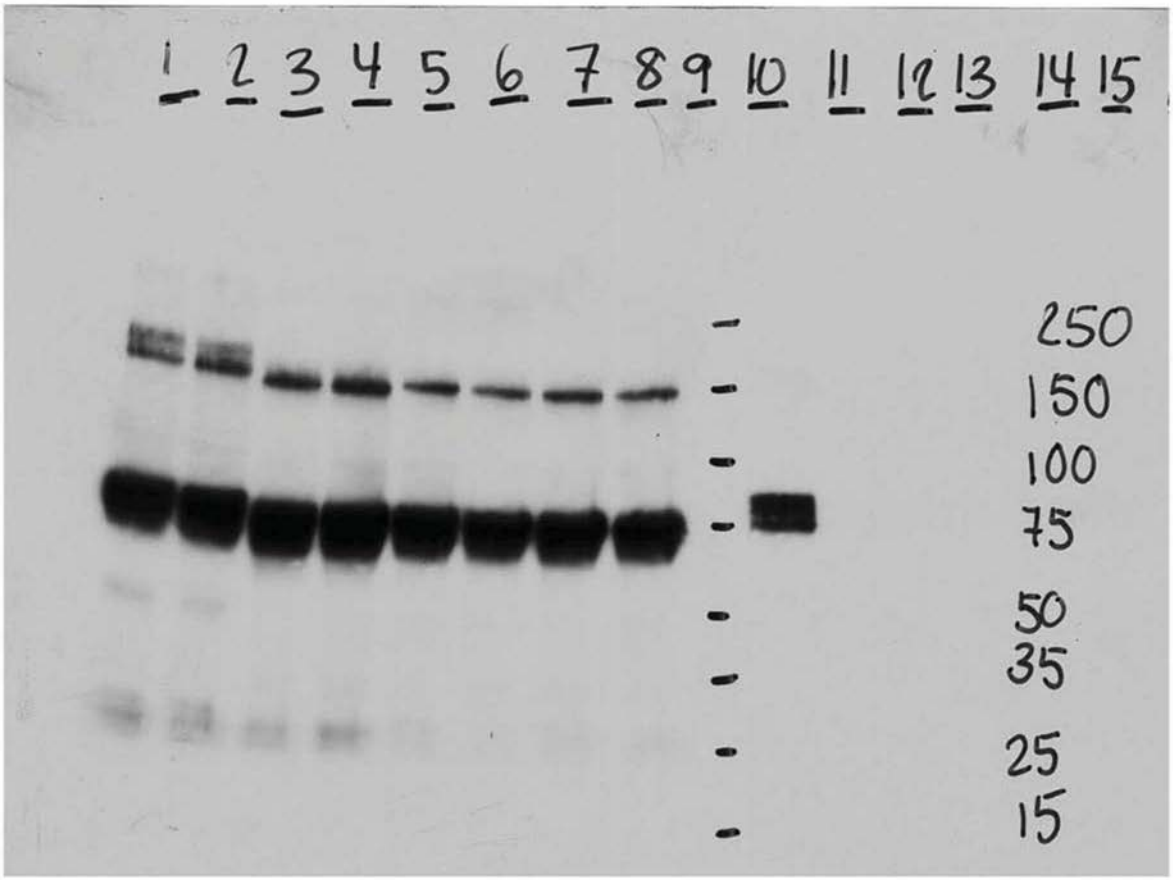


图22

图 23A, SEQ ID NO : 7 4 MutCleavage-H5(Indo).r

TAGTCCTCTTGTCTCTGTTTGAGGGCTATTTCTGAGCCCTGTTGC

图 23B, SEQ ID NO : 75 MutCleavage-H5(Indo).c

TAGCCCTCAAACAGAGACAAGAGGACTATTTGGAGCTATAGCAGG

图 23C, SEQ ID NO : 76

从2X35S启动子至NOS终止子的表达盒676号。来自流感A/印度尼西亚/5/2005(H5N1)的H5的TETR切割位点突变加下划线

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTC
AGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCG
GATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGC
TCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGA
CAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTC
CAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGGAGCACGACACACTT
GTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTT
TCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACT
TTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAA
GGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACC
CACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATT
GATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGAC
CCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTT
TTGATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATC
TCTCTTAAAGCAAACTTCTCTCTTGTCTTTCTTGCGTGAGCGATCTTCAACGTTGTCAG
ATCGTGCTTCGGCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTT
TGTGGACACGTAGTGCGGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGT
GGTCTTGGGAAAAGAAAGCTTGCTGGAGGCTGCTGTTCAGCCCCATACATTACTTGTTA
CGATTCTGCTGACTTTCGGCGGGTGCAATATCTCTACTTCTGCTTGACGAGGTATTGTT
GCCTGTACTTCTTTCTTCTTCTTCTTGCTGATTGGTTCTATAAGAAATCTAGTATTTTC
TTTGAAACAGAGTTTTCCCGTGGTTTTCGAACTTGGAGAAAGATTGTTAAGCTTCTGTA
TATTCTGCCCAAATTTGTCGGGCCCATGGAGAAAATAGTGCTTCTTCTTGCAATAGTCA
GTCTTGTTAAAAGTGATCAGATTTGCATTGGTTACCATGCAAACAATTCAACAGAGCAG

图 23C(续)

GTTGACACAATCATGGAAAAGAACGTTACTGTTACACATGCCCAAGACATACTGGAAAA
GACACACAACGGGAAGCTCTGCGATCTAGATGGAGTGAAGCCTCTAATTTTAAGAGATT
GTAGTGTAGCTGGATGGCTCCTCGGGAACCCAATGTGTGACGAATTCATCAATGTACCG
GAATGGTCTTACATAGTGGAGAAGGCCAATCCAACCAATGACCTCTGTTACCCAGGGAG
TTTCAACGACTATGAAGAACTGAAACACCTATTGAGCAGAATAAACCATTTTGAGAAAA
TTCAAATCATCCCCAAAAGTTCTTGGTCCGATCATGAAGCCTCATCAGGAGTTAGCTCA
GCATGTCCATACCTGGGAAGTCCCTCCTTTTTTAGAAATGTGGTATGGCTTATCAAAAA
GAACAGTACATACCCAACAATAAAGAAAAGCTACAATAATACCAACCAAGAGGATCTTT
TGGTACTGTGGGGAATTCACCATCCTAATGATGCGGCAGAGCAGACAAGGCTATATCAA
AACCCAACCACCTATATTTCCATTGGGACATCAACACTAAACCAGAGATTGGTACCAAA
AATAGCTACTAGATCCAAAGTAAACGGGCAAAGTGGAAGGATGGAGTTCTTCTGGACAA
TTTTAAAACCTAATGATGCAATCAACTTCGAGAGTAATGGAAATTTCATTGCTCCAGAA
TATGCATACAAAATTGTCAAGAAAGGGGACTCAGCAATTATGAAAAGTGAATTGGAATA
TGGTAACTGCAACACCAAGTGTCAAACTCCAATGGGGGCGATAAACTCTAGTATGCCAT
TCCACAACATACACCCTCTCACCATCGGGGAATGCCCCAAATATGTGAAATCAAACAGA
TTAGTCCTTGCAACAGGGCTCAGAAATAGCCCTCAAACAGAGACAAGAGGACTATTTGG
AGCTATAGCAGGTTTTATAGAGGGAGGATGGCAGGGAATGGTAGATGGTTGGTATGGGT
ACCACCATAGCAATGAGCAGGGGAGTGGGTACGCTGCAGACAAAGAATCCACTCAAAAG
GCAATAGATGGAGTCACCAATAAGGTCAACTCAATCATTGACAAAATGAACACTCAGTT
TGAGGCCGTTGGAAGGGAATTTAATAACTTAGAAAGGAGAATAGAGAATTTAAACAAGA
AGATGGAAGACGGGTTTCTAGATGTCTGGACTTATAATGCCGAACTTCTGGTTCTCATG
GAAAATGAGAGAACTCTAGACTTTCATGACTCAAATGTTAAGAACCTCTACGACAAGGT
CCGACTACAGCTTAGGGATAATGCAAAGGAGCTGGGTAACGGTTGTTTCGAGTTCTATC
ACAAATGTGATAATGAATGTATGGAAAGTATAAGAAACGGAACGTACAACTATCCGCAG
TATTCAGAAGAAGCAAGATTAAAAAGAGAGGAAATAAGTGGGGTAAAATTGGAATCAAT
AGGAACTTACCAAATACTGTCAATTTATTCAACAGTGGCGAGTTCCCTAGCACTGGCAA
TCATGATGGCTGGTCTATCTTTATGGATGTGCTCCAATGGATCGTTACAATGCAGAATT
TGCATTTAAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTA
TGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTTCTT
TGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTT
ATTAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCAGAT
CGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGAT
GATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCA
TGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATAC
GCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATC
TATGTTACTAGAT

图 23D, SEQ ID NO : 77

来自流感A/印度尼西亚/5/2005的H5的TETR切割位点的氨基酸序列

MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCD
LDGVKPLILRDCSVAGWLLGNPMCDEFINVPEWSYIVEKANPTNDLCYPGSFNDYEELK
HLLSRINHFEKIQIIPKSSWSDHEASSGVSSACPYLGSPSFFRNVVWLIKKNSTYPTIK
KSYNNTNQEDLLVLWGIHHPNDAAEQTRLYQNPTTYISIGTSTLNQRLVPKIATRSKVN
GQSGRMEFFWTILKPNDAINFESNGNFIAPEYAYKIVKKGDSAIMKSELEYGNCNTKCQ
TPMGAINSSMPFHNIHPLTIGECPKYVKSNRLVLATGLRNSPQTETRGLFGAIAGFIEG
GWQGMVDGWYGYHHSNEQGSGYAADKESTQKAIDGVTNKVNSIIDKMNTQFEAVGREFN
NLERRIENLNKKMEDGFLDVWTYNAELLVLMENERTLDFHDSNVKNLYDKVRLQLRDNA
KELGNGCFEFYHKCDNECMESIRNGTYNYPQYSEEARLKREEISGVKLESIGTYQILSI
YSTVASSLALAIMMAGLSLWMCSNGSLQCRICI

图 23E

构建体676号的示意图

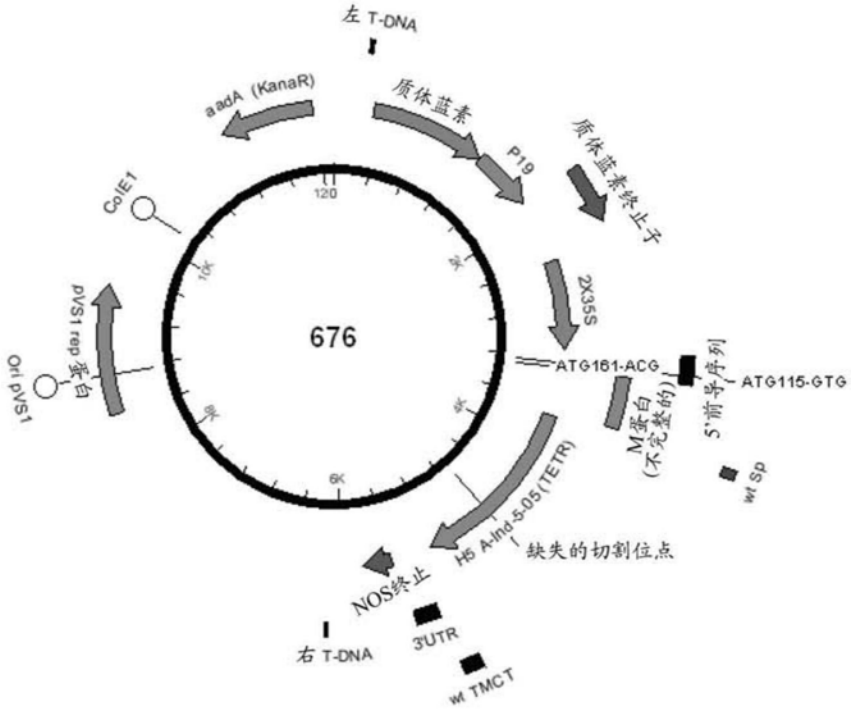


图23:来自A/印度尼西亚/5/2005的具有TETR切割位点突变的A-2X35S/CPMV-HT/H5(构建体676号)

图 24A, SEQ ID NO : 78 H5I505_TETQ.r

TCCAAATAGTCCTTGTGTCTCTGTTTGAGGGCTATTTCTGAGCCCTGT

图 24B, SEQ ID NO : 79 H5I505_TETQ.c

AAATAGCCCTCAAACAGAGACACAAGGACTATTTGGAGCTATAGCAGG

图 24C, SEQ ID NO : 80

从2X35S启动子至NOS终止子的表达盒766号。来自流感A/印度尼西亚/5/2005(H5N1)的H5的TETQ切割位点突变加下划线

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTC
AGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCG
GATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGC
TCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGA
CAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTC
CAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGGAGCACGACACACTT
GTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTT
TCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACT
TTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAA
GGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACC
CACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATT
GATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGAC
CCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTT
TTGATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATC
TCTCTTAAAGCAAACTTCTCTCTTGTCTTTCTTGCGTGAGCGATCTTCAACGTTGTCAG
ATCGTGCTTCGGCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTT
TGTGGACACGTAGTGCGGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGT
GGTCTTGGGAAAAGAAAGCTTGCTGGAGGCTGCTGTTCAGCCCCATACATTACTTGTTA
CGATTCTGCTGACTTTCGGCGGGTGCAATATCTCTACTTCTGCTTGACGAGGTATTGTT
GCCTGTACTTCTTTCTTCTTCTTCTTGCTGATTGGTTCTATAAGAAATCTAGTATTTTC
TTTGAAACAGAGTTTTCCCGTGGTTTTCGAACTTGGAGAAAGATTGTTAAGCTTCTGTA
TATTCTGCCCAAATTTGTCGGGCCCATGGAGAAAATAGTGCTTCTTCTTGCAATAGTCA
GTCTTGTTAAAAGTGATCAGATTTGCATTGGTTACCATGCAAACAATTCAACAGAGCAG

图 24C（续）

GTTGACACAATCATGGAAAAGAACGTTACTGTTACACATGCCCAAGACATACTGGAAAA
GACACACAACGGGAAGCTCTGCGATCTAGATGGAGTGAAGCCTCTAATTTTAAGAGATT
GTAGTGTAGCTGGATGGCTCCTCGGGAACCCAATGTGTGACGAATTCATCAATGTACCG
GAATGGTCTTACATAGTGGAGAAGGCCAATCCAACCAATGACCTCTGTTACCCAGGGAG
TTTCAACGACTATGAAGAACTGAAACACCTATTGAGCAGAATAAACCATTTTGAGAAAA
TTCAAATCATCCCCAAAAGTTCTTGGTCCGATCATGAAGCCTCATCAGGAGTTAGCTCA
GCATGTCCATACCTGGGAAGTCCCTCCTTTTTTAGAAATGTGGTATGGCTTATCAAAAA
GAACAGTACATACCCAACAATAAAGAAAAGCTACAATAATACCAACCAAGAGGATCTTT
TGGTACTGTGGGGAATTCACCATCCTAATGATGCGGCAGAGCAGACAAGGCTATATCAA
AACCCAACCACCTATATTTCCATTGGGACATCAACACTAAACCAGAGATTGGTACCAAA
AATAGCTACTAGATCCAAAGTAAACGGGCAAAGTGGAAGGATGGAGTTCTTCTGGACAA
TTTTAAAACCTAATGATGCAATCAACTTCGAGAGTAATGGAAATTTCATTGCTCCAGAA
TATGCATACAAAATTGTCAAGAAAGGGGACTCAGCAATTATGAAAAGTGAATTGGAATA
TGGTAACTGCAACACCAAGTGTCAAACTCCAATGGGGGCGATAAACTCTAGTATGCCAT
TCCACAACATACACCCTCTCACCATCGGGGAATGCCCCAAATATGTGAAATCAAACAGA
TTAGTCCTTGCAACAGGGCTCAGAAATAGCCCTCAAACAGAGACACAAGGACTATTTGG
AGCTATAGCAGGTTTTATAGAGGGAGGATGGCAGGGAATGGTAGATGGTTGGTATGGGT
ACCACCATAGCAATGAGCAGGGGAGTGGGTACGCTGCAGACAAAGAATCCACTCAAAAG
GCAATAGATGGAGTCACCAATAAGGTCAACTCAATCATTGACAAAATGAACACTCAGTT
TGAGGCCGTTGGAAGGGAATTTAATAACTTAGAAAGGAGAATAGAGAATTTAAACAAGA
AGATGGAAGACGGGTTTCTAGATGTCTGGACTTATAATGCCGAACTTCTGGTTCTCATG
GAAAATGAGAGAACTCTAGACTTTCATGACTCAAATGTTAAGAACCTCTACGACAAGGT
CCGACTACAGCTTAGGGATAATGCAAAGGAGCTGGGTAACGGTTGTTTCGAGTTCTATC
ACAAATGTGATAATGAATGTATGGAAAGTATAAGAAACGGAACGTACAACTATCCGCAG
TATTCAGAAGAAGCAAGATTAAAAAGAGAGGAAATAAGTGGGGTAAAATTGGAATCAAT
AGGAACTTACCAAATACTGTCAATTTATTCAACAGTGGCGAGTTCCCTAGCACTGGCAA
TCATGATGGCTGGTCTATCTTTATGGATGTGCTCCAATGGATCGTTACAATGCAGAATT
TGCATTTAAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTA
TGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTTCTT
TGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTT
ATTAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCAGAT
CGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGAT
GATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCA
TGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATAC
GCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATC
TATGTTACTAGAT

图 24D, SEQ ID NO : 81

来自流感A/印度尼西亚/5/2005(H5N1)的H5的TETQ切割位点突变的氨基酸序列

MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCD
LDGVKPLILRDCSVAGWLLGNPMCDEFINVPEWSYIVEKANPTNDLCYPGSFNDYEELK
HLLSRINHFEKIQIIPKSSWSDHEASSGVSSACPYLGSPSFFRNVVWLIKKNSTYPTIK
KSYNNTNQEDLLVLWGIHHPNDAAEQTRLYQNPTTYISIGTSTLNQRLVPKIATRSKVN
GQSGRMEFFWTILKPNDAINFESNGNFIAPEYAYKIVKKGDSAIMKSELEYGNCNTKCQ
TPMGAINSSMPFHNIHPLTIGECPKYVKSNRLVLATGLRNSPQTETQGLFGAIAGFIEG
GWQGMVDGWYGYHHSNEQGSGYAADKESTQKAIDGVTNKVNSIIDKMNTQFEAVGREFN
NLERRIENLNKKMEDGFLDVWTYNAELLVLMENERTLDFHDSNVKNLYDKVRLQLRDNA
KELGNGCFEFYHKCDNECMESIRNGTYNYPQYSEEARLKREEISGVKLESIGTYQILSI
YSTVASSLALAIMMAGLSLWMCSNGSLQCRICI

图 24E

构建体766号的示意图

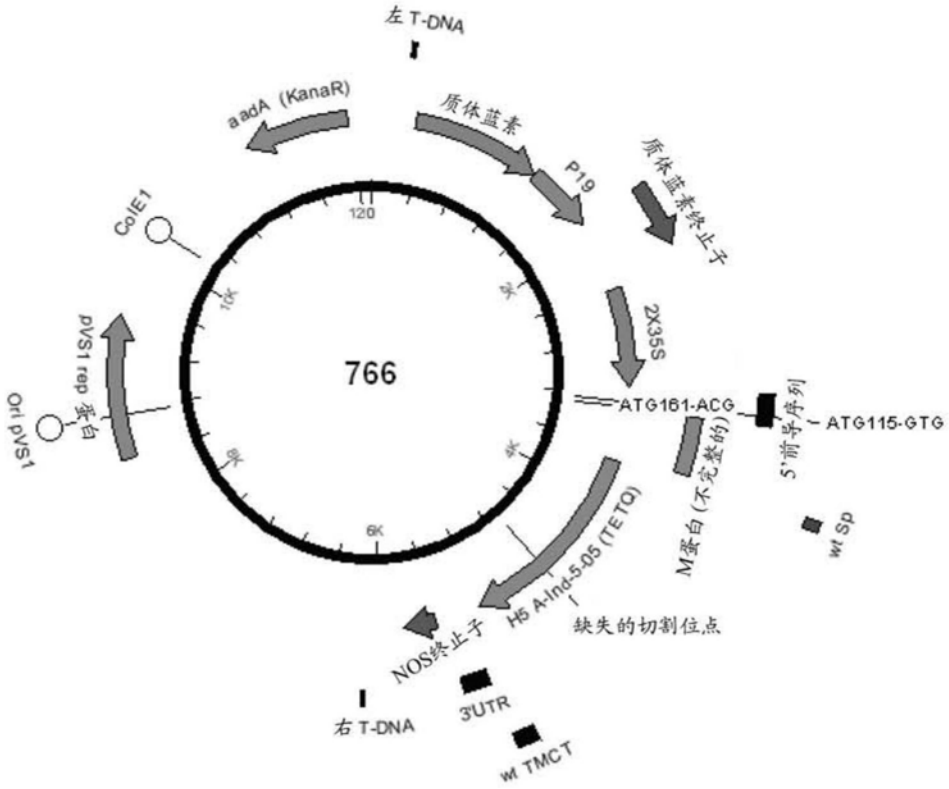


图24:来自A/印度尼西亚/5/2005的具有TETQ切割位点突变的B-2X35S/CPMV-HT/H5(构建体766号)

图 25A, SEQ ID NO : 82 H5I505(PrL-).r

CTGCCATCCTCCGCCAGGGCTATTTCTGAGCCCTGTTGCAAGGACTAATC

图 25B, SEQ ID NO : 83 H5I505(PrL-).c

GAAATAGCCCTGGCGGAGGATGGCAGGGAATGGTAGATGGTTGGTATGGGTA

图 25C, SEQ ID NO : 84

从2X35S启动子至NOS终止子的表达盒928号。来自流感A/印度尼西亚/5/2005(H5N1)的具有缺失的蛋白水解环的H5加下划线

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTC
AGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCG
GATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGC
TCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGA
CAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTC
CAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGGAGCACGACACACTT
GTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTT
TCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACT
TTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAA
GGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACC
CACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATT
GATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGAC
CCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTT
TTGATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATC
TCTCTTAAAGCAAACTTCTCTCTTGTCTTTCTTGCGTGAGCGATCTTCAACGTTGTCAG
ATCGTGCTTCGGCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTT
TGTGGACACGTAGTGCGGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGT
GGTCTTGGGAAAAGAAAGCTTGCTGGAGGCTGCTGTTCAGCCCCATACATTACTTGTTA
CGATTCTGCTGACTTTCGGCGGGTGCAATATCTCTACTTCTGCTTGACGAGGTATTGTT
GCCTGTACTTCTTTCTTCTTCTTCTTGCTGATTGGTTCTATAAGAAATCTAGTATTTTC
TTTGAAACAGAGTTTTCCCGTGGTTTTCGAACTTGGAGAAAGATTGTTAAGCTTCTGTA
TATTCTGCCCAAATTTGTCGGGCCCATGGAGAAAATAGTGCTTCTTCTTGCAATAGTCA
GTCTTGTTAAAAGTGATCAGATTTGCATTGGTTACCATGCAAACAATTCAACAGAGCAG

图 25C（续）

GTTGACACAATCATGGAAAAGAACGTTACTGTTACACATGCCCAAGACATACTGGAAAA
GACACACAACGGGAAGCTCTGCGATCTAGATGGAGTGAAGCCTCTAATTTTAAGAGATT
GTAGTGTAGCTGGATGGCTCCTCGGGAACCCAATGTGTGACGAATTCATCAATGTACCG
GAATGGTCTTACATAGTGGAGAAGGCCAATCCAACCAATGACCTCTGTTACCCAGGGAG
TTTCAACGACTATGAAGAACTGAAACACCTATTGAGCAGAATAAACCATTTTGAGAAAA
TTCAAATCATCCCCAAAAGTTCTTGGTCCGATCATGAAGCCTCATCAGGAGTTAGCTCA
GCATGTCCATACCTGGGAAGTCCCTCCTTTTTTAGAAATGTGGTATGGCTTATCAAAAA
GAACAGTACATACCCAACAATAAAGAAAAGCTACAATAATACCAACCAAGAGGATCTTT
TGGTACTGTGGGGAATTCACCATCCTAATGATGCGGCAGAGCAGACAAGGCTATATCAA
AACCCAACCACCTATATTTCCATTGGGACATCAACACTAAACCAGAGATTGGTACCAAA
AATAGCTACTAGATCCAAAGTAAACGGGCAAAGTGGAAGGATGGAGTTCTTCTGGACAA
TTTTAAAACCTAATGATGCAATCAACTTCGAGAGTAATGGAAATTTCATTGCTCCAGAA
TATGCATACAAAATTGTCAAGAAAGGGGACTCAGCAATTATGAAAAGTGAATTGGAATA
TGGTAACTGCAACACCAAGTGTCAAACTCCAATGGGGGCGATAAACTCTAGTATGCCAT
TCCACAACATACACCCTCTCACCATCGGGGAATGCCCCAAATATGTGAAATCAAACAGA
TTAGTCCTTGCAACAGGGCTCAGAAATAGCCCTGGCGGAGGATGGCAGGGAATGGTAGA
TGGTTGGTATGGGTACCACCATAGCAATGAGCAGGGGAGTGGGTACGCTGCAGACAAAG
AATCCACTCAAAAGGCAATAGATGGAGTCACCAATAAGGTCAACTCAATCATTGACAAA
ATGAACACTCAGTTTGAGGCCGTTGGAAGGGAATTTAATAACTTAGAAAGGAGAATAGA
GAATTTAAACAAGAAGATGGAAGACGGGTTTCTAGATGTCTGGACTTATAATGCCGAAC
TTCTGGTTCTCATGGAAAATGAGAGAACTCTAGACTTTCATGACTCAAATGTTAAGAAC
CTCTACGACAAGGTCCGACTACAGCTTAGGGATAATGCAAAGGAGCTGGGTAACGGTTG
TTTCGAGTTCTATCACAAATGTGATAATGAATGTATGGAAAGTATAAGAAACGGAACGT
ACAACTATCCGCAGTATTCAGAAGAAGCAAGATTAAAAAGAGAGGAAATAAGTGGGGTA
AAATTGGAATCAATAGGAACTTACCAAATACTGTCAATTTATTCAACAGTGGCGAGTTC
CCTAGCACTGGCAATCATGATGGCTGGTCTATCTTTATGGATGTGCTCCAATGGATCGT
TACAATGCAGAATTTGCATTTAAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTC
GGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATG
TAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAA
AAGATTTTAATTTTATTAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTT
ATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTT
GCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAAT
TAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAAT
TATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCG
CGCGCGGTGTCATCTATGTTACTAGAT

图 25D, SEQ ID NO : 85

来自流感A/印度尼西亚/5/2005(H5N1)的具有缺失的蛋白水解环的H5的氨基酸序列

MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCD
LDGVKPLILRDCSVAGWLLGNPMCDEFINVPEWSYIVEKANPTNDLCYPGSFNDYEELK
HLLSRINHFEKIQIIPKSSWSDHEASSGVSSACPYLGSPSFFRNVVWLIKKNSTYPTIK
KSYNNTNQEDLLVLWGIHHPNDAAEQTRLYQNPTTYISIGTSTLNQRLVPKIATRSKVN
GQSGRMEFFWTILKPNDAINFESNGNFIAPEYAYKIVKKGDSAIMKSELEYGNCNTKCQ
TPMGAINSSMPFHNIHPLTIGECPKYVKSNRLVLATGLRNSPGGGWQGMVDGWYGYHHS
NEQGSGYAADKESTQKAIDGVTNKVNSIIDKMNTQFEAVGREFNNLERRIENLNKKMED
GFLDVWTYNAELLVLMENERTLDFHDSNVKNLYDKVRLQLRDNAKELGNGCFEFYHKCD
NECMESIRNGTYNYPQYSEEARLKREEISGVKLESIGTYQILSIYSTVASSLALAIMMA
GLSLWMCSNGSLQCRICI

图 25E

构建体928号的示意图

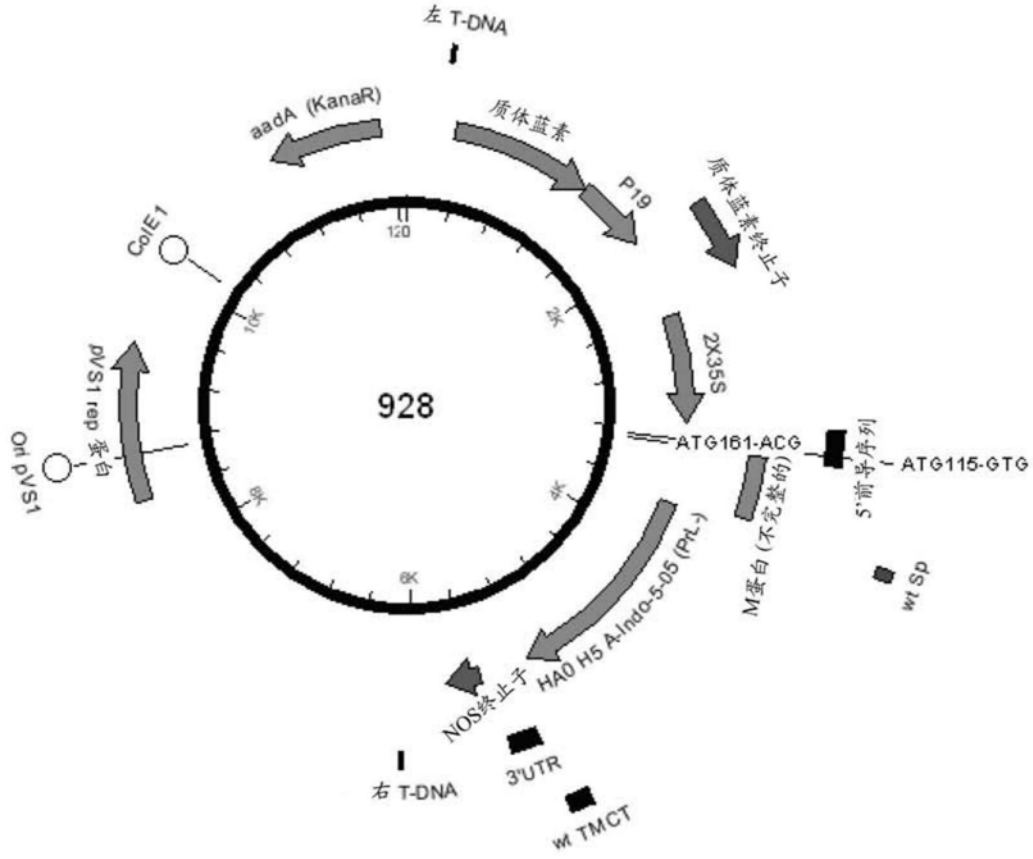


图25:来自A/印度尼西亚/5/2005的具有缺失的蛋白水解环的C-2X35S/CPMV-HT/H5(构建体928号)

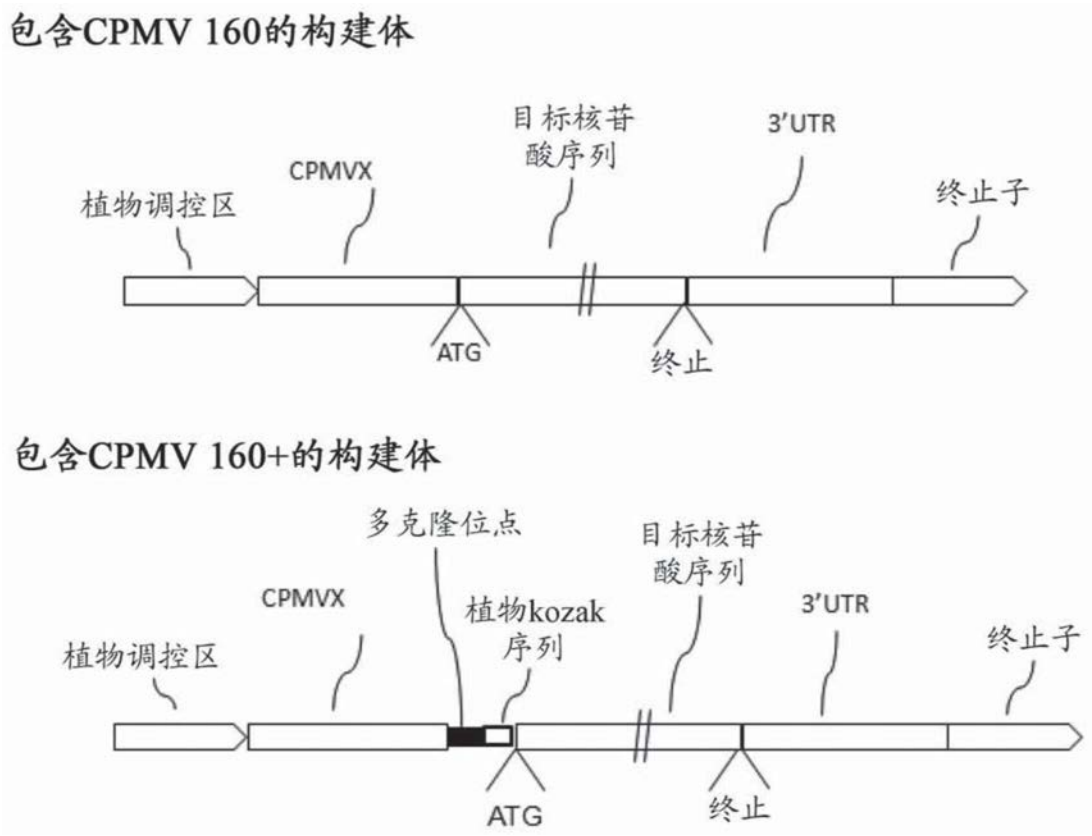

包含CPMV 160的构建体
植物调控区
CPMVX
目标核苷酸序列
3'UTR
终止子
ATG
终止
包含CPMV 160+的构建体
多克隆位点
植物kozak序列
CPMVX
目标核苷酸序列
3'UTR
植物调控区
终止子
ATG
终止

图26A

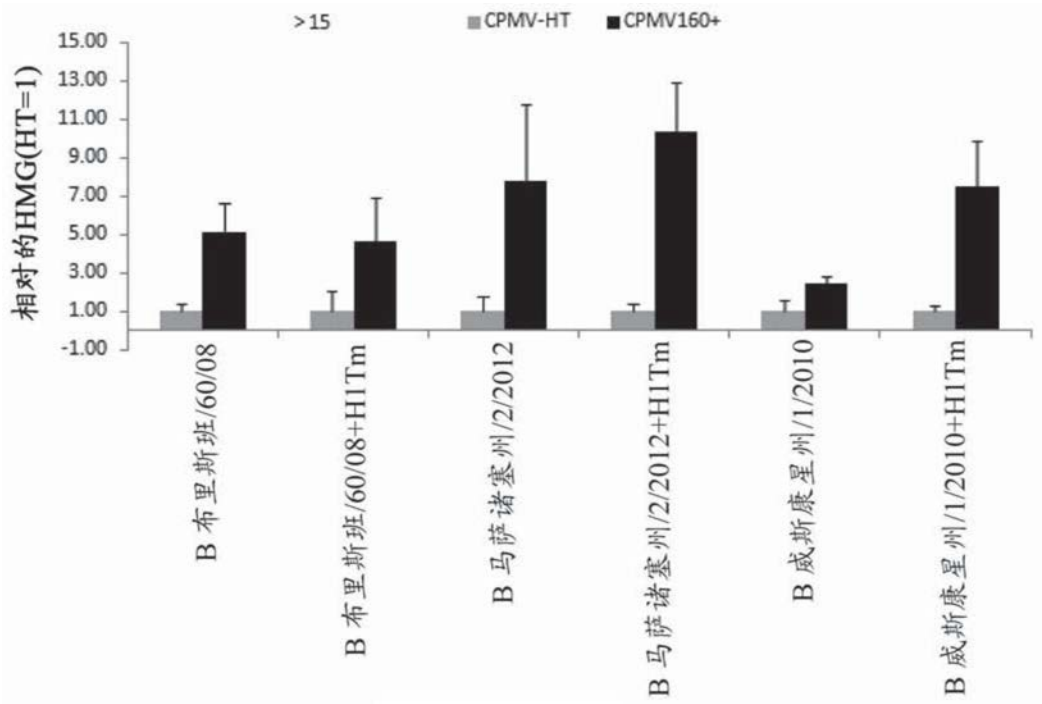

>15
CPMV-HT
CPMV160+
相对的HMG(HT=1)
15.00
13.00
11.00
9.00
7.00
5.00
3.00
1.00
-1.00
B 布里斯班/60/08
B 布里斯班/60/08+H1Tm
B 马萨诸塞州/2/2012
B 马萨诸塞州/2/2012+H1Tm
B 威斯康星州/1/2010
B 威斯康星州/1/2010+H1Tm

图26B

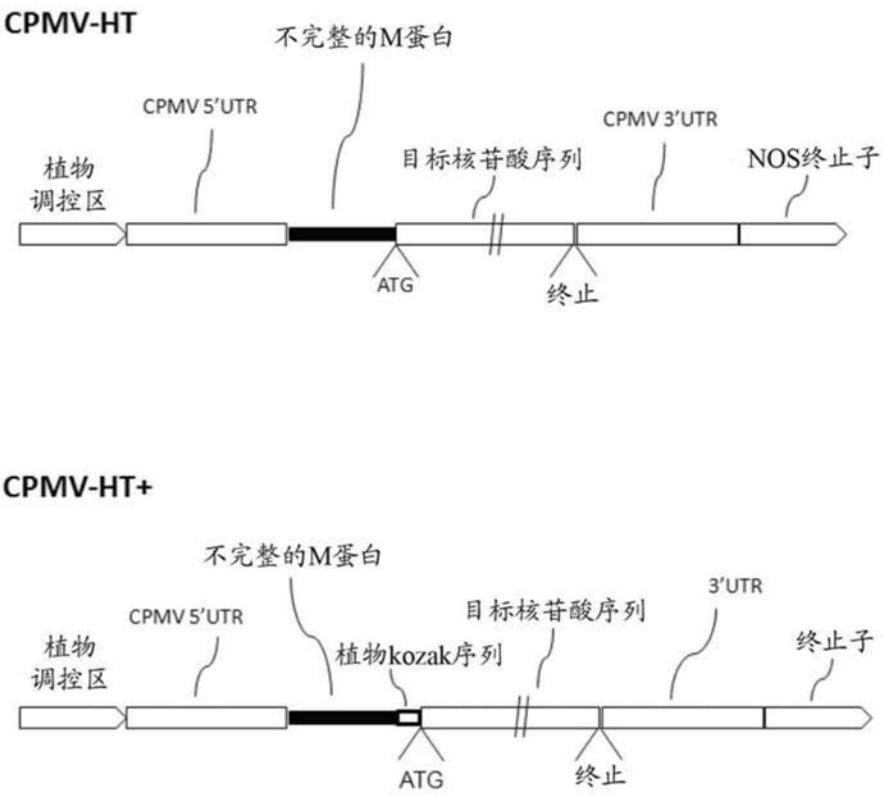

CPMV-HT
不完整的M蛋白
CPMV 5'UTR
CPMV 3'UTR
植物
调控区
目标核苷酸序列
NOS终止子
ATG
终止
CPMV-HT+
不完整的M蛋白
3'UTR
CPMV 5'UTR
目标核苷酸序列
植物
调控区
植物kozak序列
终止子
ATG
终止

图27A

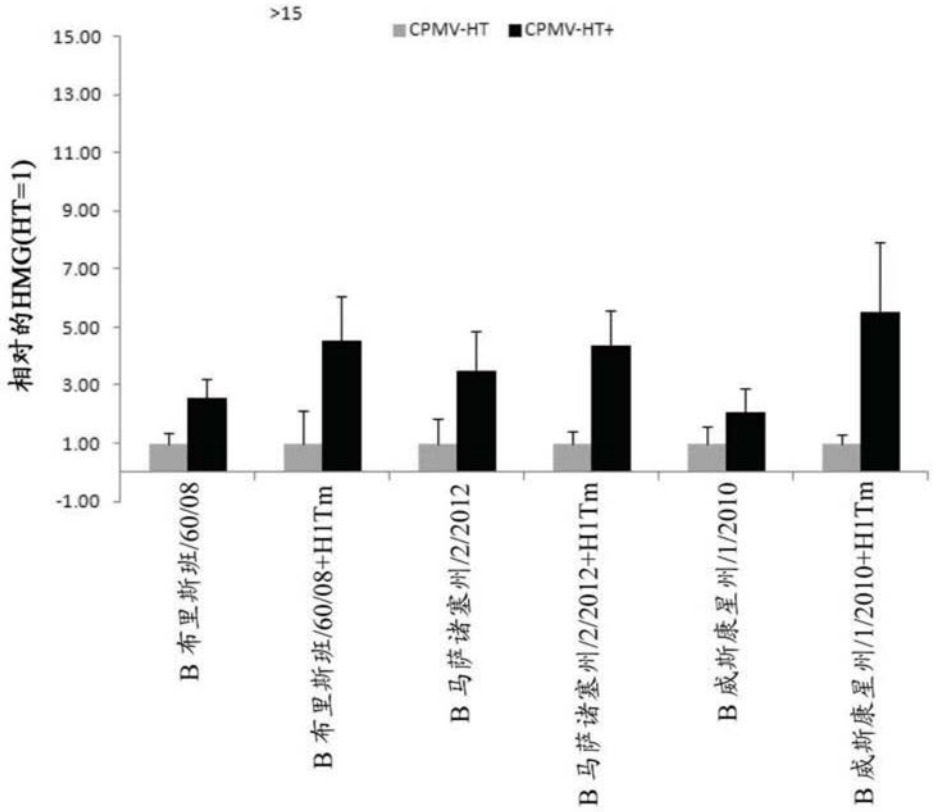

>15
CPMV-HT
CPMV-HT+
15.00
13.00
11.00
9.00
7.00
5.00
3.00
1.00
-1.00
相对的HMG(HT=1)
B 布里斯班/60/08
B 布里斯班/60/08+H1Tm
B 马萨诸塞州/2/2012
B 马萨诸塞州/2/2012+H1Tm
B 威斯康星州/1/2010
B 威斯康星州/1/2010+H1Tm

图27B

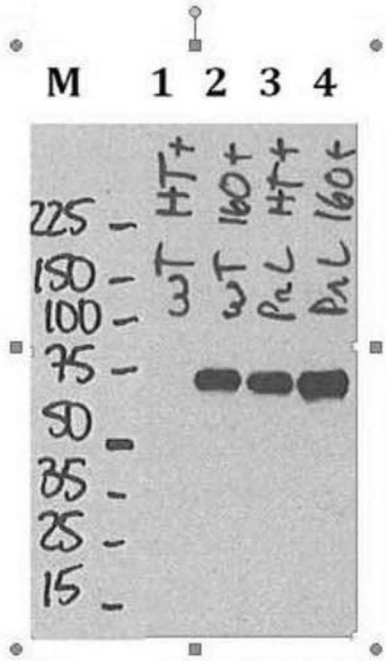

M
1
2
3
4
225
150
100
75
50
35
25
15
WT HT+
WT 160+
PnL HT+
PnL 160+

图28A

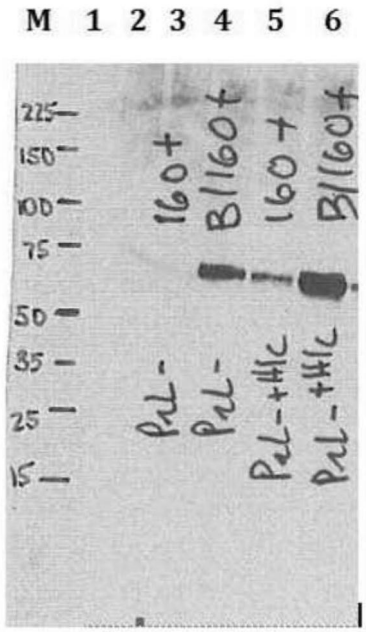

M 1 2 3 4 5 6
225
150
100
75
50
35
25
15
160+
B1160+
160+
B1160+
PrL-
PrL-
PrL-+HC
PrL-+HC

图28B

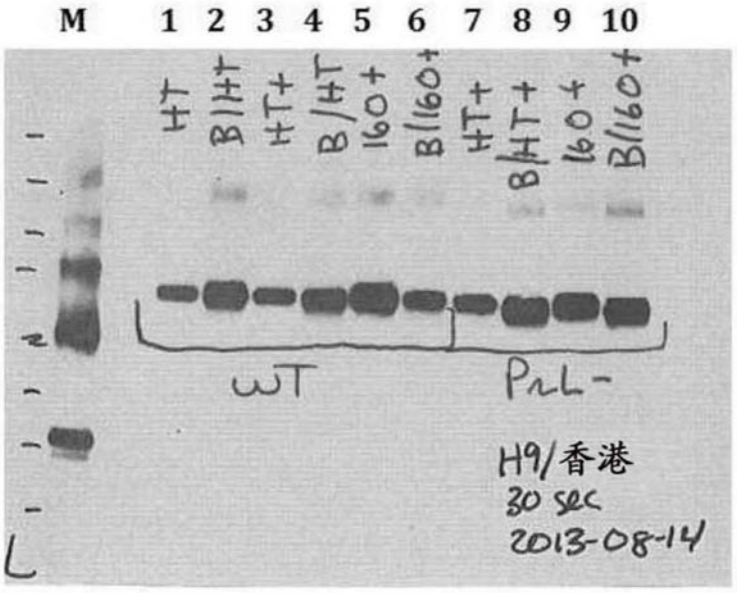

M 1 2 3 4 5 6 7 8 9 10
HT
B1HT+
HT+
B/HT
160+
B1160+
HT+
B/HT+
160+
B1160+
WT
PrL-
H9/香港
30 sec
2013-08-14

图28C

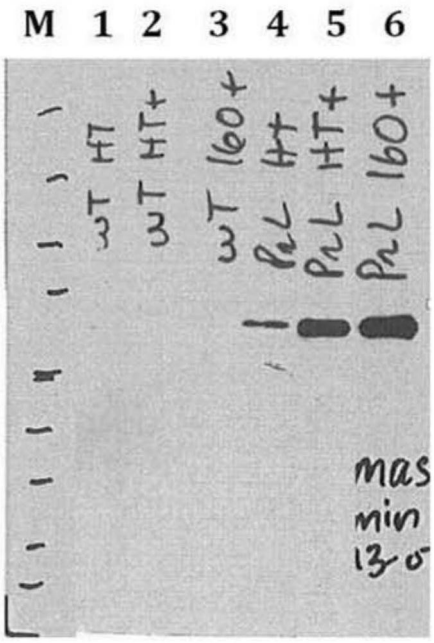

M 1 2 3 4 5 6
3T HT
3T HT+
3T 160+
PrL HT
PrL HT+
PrL 160+
mas
min
130

图28D

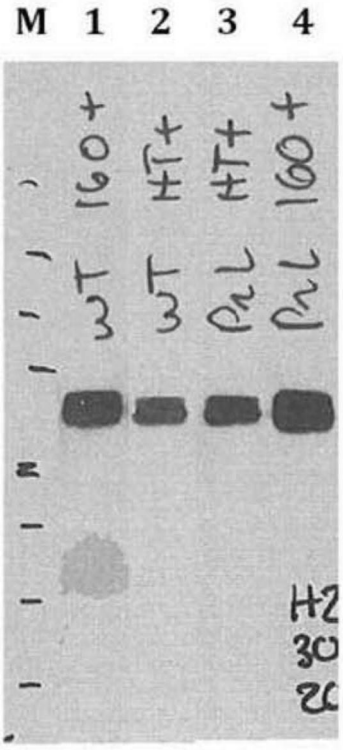

M 1 2 3 4
3T 160+
3T HT+
PrL HT
PrL 160+
H2
30
2

图28E

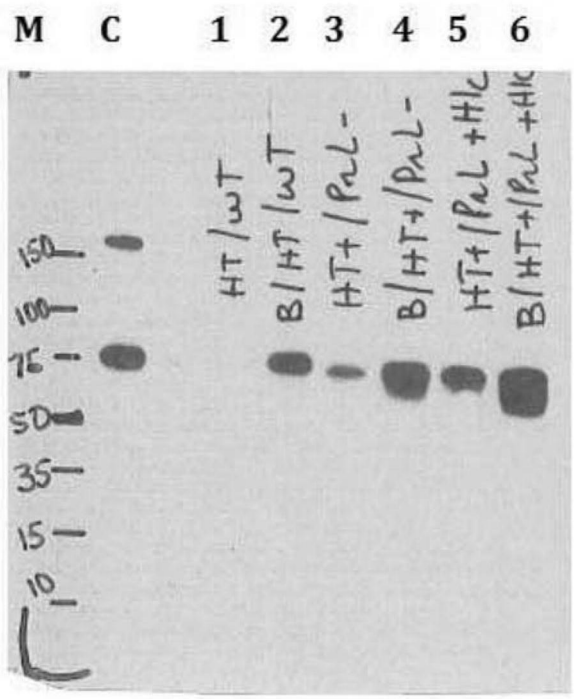

M
C
1
2
3
4
5
6
150
100
75
50
35
15
10
HT/wT
B/HT/wT
HT+/PrL-
B/HT+/PrL-
HT+/PrL+Hc
B/HT+/PrL+Hc

图28F

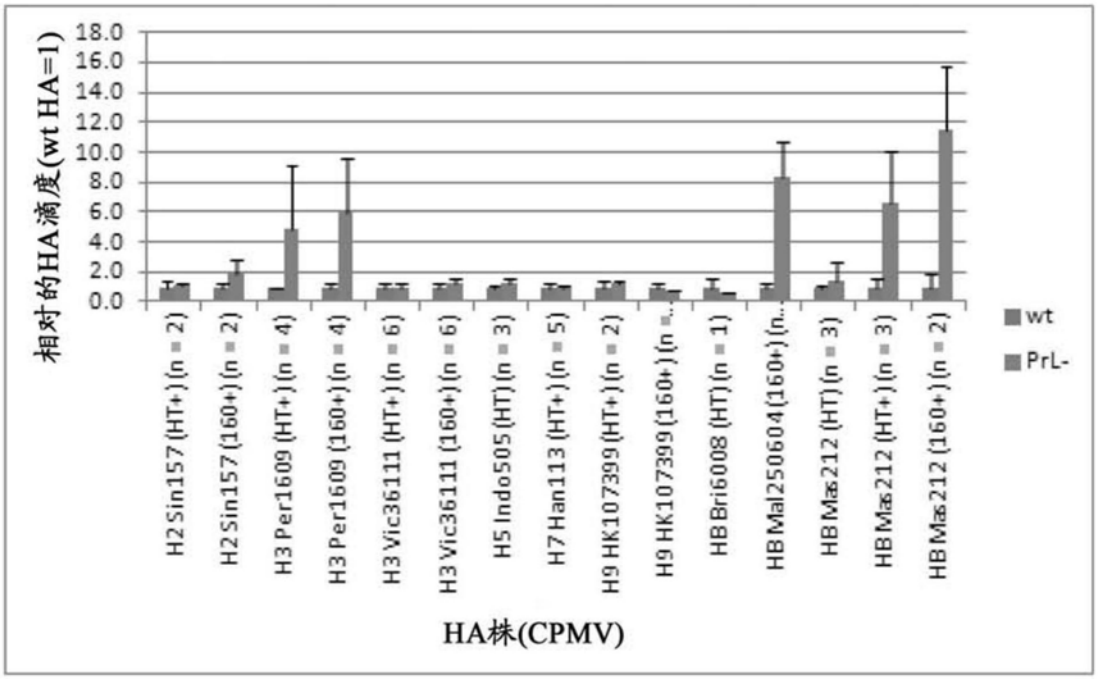

相对的HA滴度(wt HA=1)
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
H2 Sin157 (HT+) (n = 2)
H2 Sin157 (160+) (n = 2)
H3 Per1609 (HT+) (n = 4)
H3 Per1609 (160+) (n = 4)
H3 Vic36111 (HT+) (n = 6)
H3 Vic36111 (160+) (n = 6)
H5 Indo505 (HT) (n = 3)
H7 Han113 (HT+) (n = 5)
H9 HK107399 (HT+) (n = 2)
H9 HK107399 (160+) (n =...
HB Bri6008 (HT) (n = 1)
HB Mal2506O4 (160+) (n...
HB Mas212 (HT) (n = 3)
HB Mas212 (HT+) (n = 3)
HB Mas212 (160+) (n = 2)
HA株(CPMV)
wt
PrL-

图29A

图 30A

IF-S2+S4-B Bris.c (SEQ ID NO: 86)

TCTCAGATCTTCGCCGATCGAATCTGCACTGGAATAACAT

图 30B

IF-S2+S4-B Bris.c (SEQ ID NO: 87)

ACTAAAGAAAATAGGCCTTTATAGACAGATGGAGCAAGAAACA

图 30C

合成的HA B布里斯班基因 (对应于来自Genbank登录号FJ766840的nt 34-1791)
(SEQ ID NO: 88)

ATGAAGGCAATAATTGTACTACTCATGGTAGTAACATCCAATGCAGATCGAATCTGCACTGGAA
TAACATCGTCAAACTCACCACATGTCGTCAAAACTGCTACTCAAGGGGAGGTCAATGTGACTGG
TGTAATACCACTGACAACAACACCCACCAAATCTCATTTTGCAAATCTCAAAGGAACAGAAACC
AGGGGGAAACTATGCCCAAAATGCCTCAACTGCACAGATCTGGACGTAGCCTTGGGCAGACCAA
AATGCACGGGGAAAATACCCTCGGCAAGAGTTTCAATACTCCATGAAGTCAGACCTGTTACATC
TGGGTGCTTTCCTATAATGCACGACAGAACAAAAATTAGACAGCTGCCTAACCTTCTCCGAGGA
TACGAACATATCAGGTTATCAACCCATAACGTTATCAATGCAGAAAATGCACCAGGAGGACCCT
ACAAAATTGGAACCTCAGGGTCTTGCCCTAACATTACCAATGGAAACGGATTTTTCGCAACAAT
GGCTTGGGCCGTCCCAAAAAACGACAAAAACAAAACAGCAACAAATCCATTAACAATAGAAGTA
CCATACATTTGTACAGAAGGAGAAGACCAAATTACCGTTTGGGGGTTCCACTCTGACAACGAGA
CCCAAATGGCAAAGCTCTATGGGGACTCAAAGCCCCAGAAGTTCACCTCATCTGCCAACGGAGT
GACCACACATTACGTTTCACAGATTGGTGGCTTCCCAAATCAAACAGAAGACGGAGGACTACCA
CAAAGTGGTAGAATTGTTGTTGATTACATGGTGCAAAAATCTGGGAAAACAGGAACAATTACCT
ATCAAAGGGGTATTTTATTGCCTCAAAAGGTGTGGTGCGCAAGTGGCAGGAGCAAGGTAATAAA
AGGATCCTTGCCTTTAATTGGAGAAGCAGATTGCCTCCACGAAAAATACGGTGGATTAAACAAA
AGCAAGCCTTACTACACAGGGGAACATGCAAAGGCCATAGGAAATTGCCCAATATGGGTGAAAA
CACCCTTGAAGCTGGCCAATGGAACCAAATATAGACCTCCTGCAAAACTATTAAAGGAAAGGGG
TTTCTTCGGAGCTATTGCTGGTTTCTTAGAAGGAGGATGGGAAGGAATGATTGCAGGTTGGCAC
GGATACACATCCCATGGGGCACATGGAGTAGCGGTGGCAGCAGACCTTAAGAGCACTCAAGAGG
CCATAAACAAGATAACAAAAAATCTCAACTCTTTGAGTGAGCTGGAAGTAAAGAATCTTCAAAG
ACTAAGCGGTGCCATGGATGAACTCCACAACGAAATACTAGAACTAGATGAGAAAGTGGATGAT
CTCAGAGCTGATACAATAAGCTCACAAATAGAACTCGCAGTCCTGCTTTCCAATGAAGGAATAA
TAAACAGTGAAGATGAACATCTCTTGGCGCTTGAAAGAAAGCTGAAGAAAATGCTGGGCCCCTC
TGCTGTAGAGATAGGGAATGGATGCTTTGAAACCAAACACAAGTGCAACCAGACCTGTCTCGAC
AGAATAGCTGCTGGTACCTTTGATGCAGGAGAATTTTCTCTCCCCACCTTTGATTCACTGAATA

图 30C（续）

TTACTGCTGCATCTTTAAATGACGATGGATTGGATAATCATACTATACTGCTTTACTACTCAAC
TGCTGCCTCCAGTTTGGCTGTAACACTGATGATAGCTATCTTTGTTGTTTATATGGTCTCCAGA
GACAATGTTTCTTGCTCCATCTGTCTATAA

图 30D

从2X35S启动子至NOS终止子的表达盒1029。来自流感B/布里斯班/60/2008的PDISP/HA加下划线(SEQ ID NO: 89)

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAG
ACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTG
CCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCAT
CATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGAC
CCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGA
TTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTC
TCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGAT
TCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAA
ATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAA
GATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGC
AAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCA
AGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTT
TGATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTT
AAAGCAAACTTCTCTCTTGTCTTTCTTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCG
GCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTTTGTGGACACGTAGTGC
GGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGTGGTCTTGGGAAAAGAAAGCTT
GCTGGAGGCTGCTGTTCAGCCCCATACATTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGC
AATATCTCTACTTCTGCTTGACGAGGTATTGTTGCCTGTACTTCTTTCTTCTTCTTCTTGCTGA
TTGGTTCTATAAGAAATCTAGTATTTTCTTTGAAACAGAGTTTTCCCGTGGTTTTCGAACTTGG
AGAAAGATTGTTAAGCTTCTGTATATTCTGCCCAAATTTGTCGGGCCCATGGCGAAAAACGTTG
CGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCCGATCGAAT
CTGCACTGGAATAACATCGTCAAACTCACCACATGTCGTCAAAACTGCTACTCAAGGGGAGGTC
AATGTGACTGGTGTAATACCACTGACAACAACACCCACCAAATCTCATTTTGCAAATCTCAAAG
GAACAGAAACCAGGGGGAAACTATGCCCAAAATGCCTCAACTGCACAGATCTGGACGTAGCCTT
GGGCAGACCAAAATGCACGGGGAAAATACCCTCGGCAAGAGTTTCAATACTCCATGAAGTCAGA
CCTGTTACATCTGGGTGCTTTCCTATAATGCACGACAGAACAAAAATTAGACAGCTGCCTAACC
TTCTCCGAGGATACGAACATATCAGGTTATCAACCCATAACGTTATCAATGCAGAAAATGCACC
AGGAGGACCCTACAAAATTGGAACCTCAGGGTCTTGCCCTAACATTACCAATGGAAACGGATTT
TTCGCAACAATGGCTTGGGCCGTCCCAAAAAACGACAAAAACAAAACAGCAACAAATCCATTAA
CAATAGAAGTACCATACATTTGTACAGAAGGAGAAGACCAAATTACCGTTTGGGGGTTCCACTC
TGACAACGAGACCCAAATGGCAAAGCTCTATGGGGACTCAAAGCCCCAGAAGTTCACCTCATCT
GCCAACGGAGTGACCACACATTACGTTTCACAGATTGGTGGCTTCCCAAATCAAACAGAAGACG
GAGGACTACCACAAAGTGGTAGAATTGTTGTTGATTACATGGTGCAAAAATCTGGGAAAACAGG
AACAATTACCTATCAAAGGGGTATTTTATTGCCTCAAAAGGTGTGGTGCGCAAGTGGCAGGAGC
AAGGTAATAAAAGGATCCTTGCCTTTAATTGGAGAAGCAGATTGCCTCCACGAAAAATACGGTG
GATTAAACAAAAGCAAGCCTTACTACACAGGGGAACATGCAAAGGCCATAGGAAATTGCCCAAT
ATGGGTGAAAACACCCTTGAAGCTGGCCAATGGAACCAAATATAGACCTCCTGCAAAACTATTA
AAGGAAAGGGGTTTCTTCGGAGCTATTGCTGGTTTCTTAGAAGGAGGATGGGAAGGAATGATTG

图 30D（续）

CAGGTTGGCACGGATACACATCCCATGGGGCACATGGAGTAGCGGTGGCAGCAGACCTTAAGAG
CACTCAAGAGGCCATAAACAAGATAACAAAAAATCTCAACTCTTTGAGTGAGCTGGAAGTAAAG
AATCTTCAAAGACTAAGCGGTGCCATGGATGAACTCCACAACGAAATACTAGAACTAGATGAGA
AAGTGGATGATCTCAGAGCTGATACAATAAGCTCACAAATAGAACTCGCAGTCCTGCTTTCCAA
TGAAGGAATAATAAACAGTGAAGATGAACATCTCTTGGCGCTTGAAAGAAAGCTGAAGAAAATG
CTGGGCCCCTCTGCTGTAGAGATAGGGAATGGATGCTTTGAAACCAAACACAAGTGCAACCAGA
CCTGTCTCGACAGAATAGCTGCTGGTACCTTTGATGCAGGAGAATTTTCTCTCCCCACCTTTGA
TTCACTGAATATTACTGCTGCATCTTTAAATGACGATGGATTGGATAATCATACTATACTGCTT
TACTACTCAACTGCTGCCTCCAGTTTGGCTGTAACACTGATGATAGCTATCTTTGTTGTTTATA
TGGTCTCCAGAGACAATGTTTCTTGCTCCATCTGTCTATAAAGGCCTATTTTCTTTAGTTTGAA
TTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTT
ATTTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACAC
AAAAAGATTTTAATTTTATTAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTAT
CGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTC
TTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATG
CATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCG
ATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTAC
TAGAT

图 30E

来自流感B/布里斯班/60/2008的PDISP/HA的氨基酸序列(SEQ ID NO: 90)

MAKNVAIFGLLFSLLVLVPSQIFADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSH
FANLKGTETRGKLCPKCLNCTDLDVALGRPKCTGKIPSARVSILHEVRPVTSGCFPIMHDRTKI
RQLPNLLRGYEHIRLSTHNVINAENAPGGPYKIGTSGSCPNITNGNGFFATMAWAVPKNDKNKT
ATNPLTIEVPYICTEGEDQITVWGFHSDNETQMAKLYGDSKPQKFTSSANGVTTHYVSQIGGFP
NQTEDGGLPQSGRIVVDYMVQKSGKTGTITYQRGILLPQKVWCASGRSKVIKGSLPLIGEADCL
HEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPAKLLKERGFFGAIAGFLEGG
WEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEI
LELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKLKKMLGPSAVEIGNGCFETK
HKCNQTCLDRIAAGTFDAGEFSLPTFDSLNITAASLNDDGLDNHTILLYYSTAASSLAVTLMIA
IFVVYMVSRDNVSCSICL

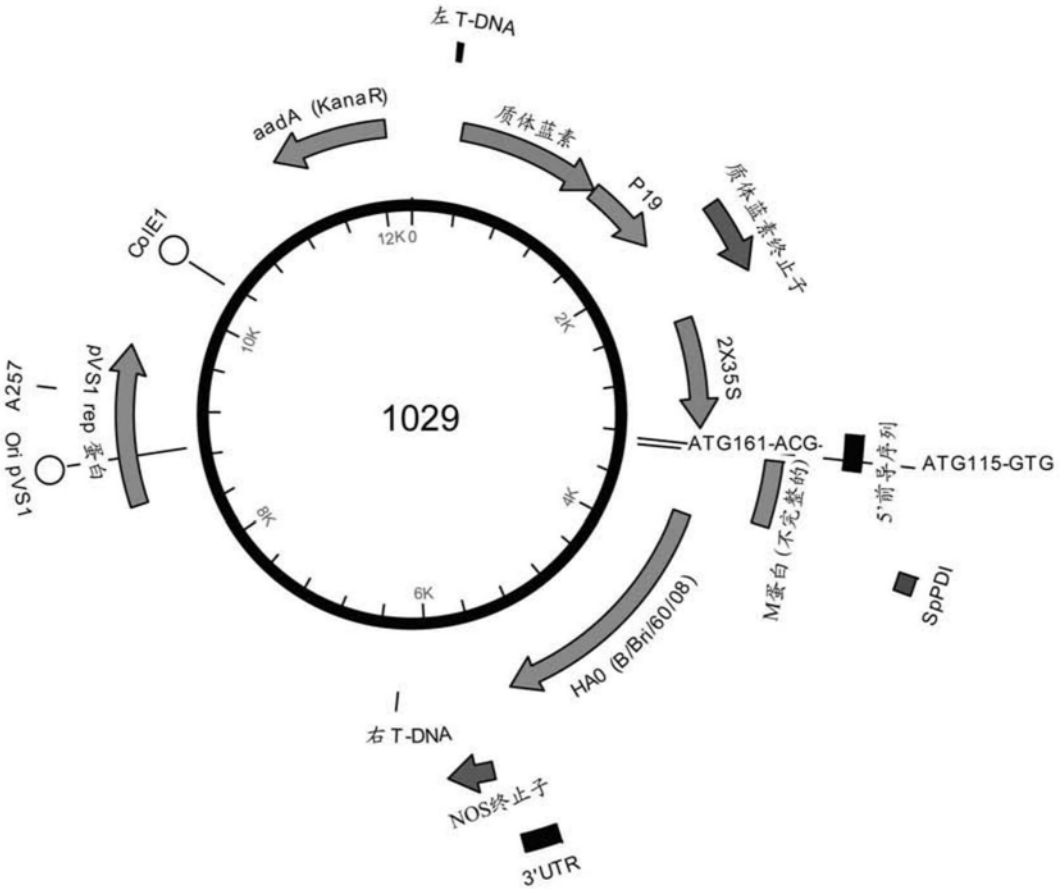


图 30F

图30:F-2X35S/CPMV-HT/PDISP/HA B布里斯班/NOS(构建体1029号)

图 31A，SEQ ID NO：91

PDISP/HA B布里斯班(PrL-)的核苷酸序列

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGA
TCTTCGCCGATCGAATCTGCACTGGAATAACATCGTCAAACTCACCACATGTCGTCAAAACTGC
TACTCAAGGGGAGGTCAATGTGACTGGTGTAATACCACTGACAACAACACCCACCAAATCTCAT
TTTGCAAATCTCAAAGGAACAGAAACCAGGGGGAAACTATGCCCAAAATGCCTCAACTGCACAG
ATCTGGACGTAGCCTTGGGCAGACCAAAATGCACGGGGAAAATACCCTCGGCAAGAGTTTCAAT
ACTCCATGAAGTCAGACCTGTTACATCTGGGTGCTTTCCTATAATGCACGACAGAACAAAAATT
AGACAGCTGCCTAACCTTCTCCGAGGATACGAACATATCAGGTTATCAACCCATAACGTTATCA
ATGCAGAAAATGCACCAGGAGGACCCTACAAAATTGGAACCTCAGGGTCTTGCCCTAACATTAC
CAATGGAAACGGATTTTTCGCAACAATGGCTTGGGCCGTCCCAAAAAACGACAAAAACAAAACA
GCAACAAATCCATTAACAATAGAAGTACCATACATTTGTACAGAAGGAGAAGACCAAATTACCG
TTTGGGGGTTCCACTCTGACAACGAGACCCAAATGGCAAAGCTCTATGGGGACTCAAAGCCCCA
GAAGTTCACCTCATCTGCCAACGGAGTGACCACACATTACGTTTCACAGATTGGTGGCTTCCCA
AATCAAACAGAAGACGGAGGACTACCACAAAGTGGTAGAATTGTTGTTGATTACATGGTGCAAA
AATCTGGGAAAACAGGAACAATTACCTATCAAAGGGGTATTTTATTGCCTCAAAAGGTGTGGTG
CGCAAGTGGCAGGAGCAAGGTAATAAAAGGATCCTTGCCTTTAATTGGAGAAGCAGATTGCCTC
CACGAAAAATACGGTGGATTAAACAAAAGCAAGCCTTACTACACAGGGGAACATGCAAAGGCCA
TAGGAAATTGCCCAATATGGGTGAAAACACCCTTGAAGCTGGCCAATGGAACCAAATATAGACC
TCCTGGTGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGATACACATCCCATGGGGCACAT
GGAGTAGCGGTGGCAGCAGACCTTAAGAGCACTCAAGAGGCCATAAACAAGATAACAAAAAATC
TCAACTCTTTGAGTGAGCTGGAAGTAAAGAATCTTCAAAGACTAAGCGGTGCCATGGATGAACT
CCACAACGAAATACTAGAACTAGATGAGAAAGTGGATGATCTCAGAGCTGATACAATAAGCTCA
CAAATAGAACTCGCAGTCCTGCTTTCCAATGAAGGAATAATAAACAGTGAAGATGAACATCTCT
TGGCGCTTGAAAGAAAGCTGAAGAAAATGCTGGGCCCCTCTGCTGTAGAGATAGGGAATGGATG
CTTTGAAACCAAACACAAGTGCAACCAGACCTGTCTCGACAGAATAGCTGCTGGTACCTTTGAT
GCAGGAGAATTTTCTCTCCCCACCTTTGATTCACTGAATATTACTGCTGCATCTTTAAATGACG
ATGGATTGGATAATCATACTATACTGCTTTACTACTCAACTGCTGCCTCCAGTTTGGCTGTAAC
ACTGATGATAGCTATCTTTGTTGTTTATATGGTCTCCAGAGACAATGTTTCTTGCTCCATCTGT
CTATAA

图 31B，SEQ ID NO：92

PDISP/HA B布里斯班(PrL-)的氨基酸序列

MAKNVAIFGLLFSLLVLVPSQIFADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSH
FANLKGTETRGKLCPKCLNCTDLDVALGRPKCTGKIPSARVSILHEVRPVTSGCFPIMHDRTKI
RQLPNLLRGYEHIRLSTHNVINAENAPGGPYKIGTSGSCPNITNGNGFFATMAWAVPKNDKNKT
ATNPLTIEVPYICTEGEDQITVWGFHSDNETQMAKLYGDSKPQKFTSSANGVTTHYVSQIGGFP
NQTEDGGLPQSGRIVVDYMVQKSGKTGTITYQRGILLPQKVWCASGRSKVIKGSLPLIGEADCL

图 31B（续）

HEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPGGGWEGMIAGWHGYTSHGAH
GVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISS
QIELAVLLSNEGIINSEDEHLLALERKLKKMLGPSAVEIGNGCFETKHKCNQTCLDRIAAGTFD
AGEFSLPTFDSLNITAASLNDDGLDNHTILLYYSTAASSLAVTLMIAIFVVYMVSRDNVSCSIC
L*

图 31C

构建体1829号(2X35S/CPMV HT*)的示意图

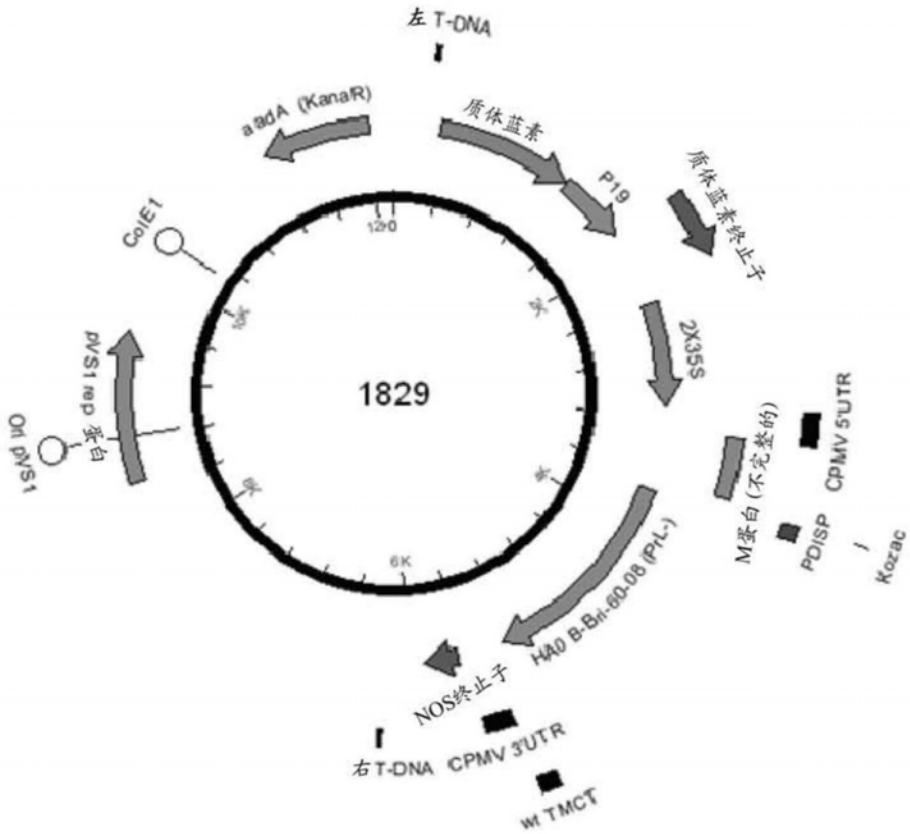


图31:用于PDISP/HA B布里斯班(PrL-)的K-2X35S/CPMV HT(构建体1039号)和HT*(构建体1829号)

图 32A

构建体1937号(2X35S/CPMV HT*(-Mprot))的示意图



图32:用于PDISP/HA B布里斯班(PrL-)的2X35S/CPMV HT(构建体1039号)和HT*(-Mprot)(构建体1937号)

图 33A, SEQ ID NO : 95

PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT的核苷酸序列

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCCGATCGA
ATCTGCACTGGAATAACATCGTCAAACTCACCACATGTCGTCAAAACTGCTACTCAAGGGGAGGTCAATGTGACTG
GTGTAATACCACTGACAACAACACCCACCAAATCTCATTTTGCAAATCTCAAAGGAACAGAAACCAGGGGGAAAC
TATGCCCAAAATGCCTCAACTGCACAGATCTGGACGTAGCCTTGGGCAGACCAAAATGCACGGGGAAAATACCCT
CGGCAAGAGTTTCAATACTCCATGAAGTCAGACCTGTTACATCTGGGTGCTTTCCTATAATGCACGACAGAACAAA
AATTAGACAGCTGCCTAACCTTCTCCGAGGATACGAACATATCAGGTTATCAACCCATAACGTTATCAATGCAGAA
AATGCACCAGGAGGACCCTACAAAATTGGAACCTCAGGGTCTTGCCCTAACATTACCAATGGAAACGGATTTTTCG
CAACAATGGCTTGGGCCGTCCCAAAAAACGACAAAAACAAAACAGCAACAAATCCATTAACAATAGAAGTACCA
TACATTTGTACAGAAGGAGAAGACCAAATTACCGTTTGGGGGTTCCACTCTGACAACGAGACCCAAATGGCAAAG
CTCTATGGGGACTCAAAGCCCCAGAAGTTCACCTCATCTGCCAACGGAGTGACCACACATTACGTTTCACAGATTG
GTGGCTTCCCAAATCAAACAGAAGACGGAGGACTACCACAAAGTGGTAGAATTGTTGTTGATTACATGGTGCAAA
AATCTGGGAAAACAGGAACAATTACCTATCAAAGGGGTATTTTATTGCCTCAAAAGGTGTGGTGCGCAAGTGGCA
GGAGCAAGGTAATAAAAGGATCCTTGCCTTTAATTGGAGAAGCAGATTGCCTCCACGAAAAATACGGTGGATTAA
ACAAAAGCAAGCCTTACTACACAGGGGAACATGCAAAGGCCATAGGAAATTGCCCAATATGGGTGAAAACACCCT
TGAAGCTGGCCAATGGAACCAAATATAGACCTCCTGGTGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGAT
ACACATCCCATGGGGCACATGGAGTAGCGGTGGCAGCAGACCTTAAGAGCACTCAAGAGGCCATAAACAAGATA
ACAAAAAATCTCAACTCTTTGAGTGAGCTGGAAGTAAAGAATCTTCAAAGACTAAGCGGTGCCATGGATGAACTC
CACAACGAAATACTAGAACTAGATGAGAAAGTGGATGATCTCAGAGCTGATACAATAAGCTCACAAATAGAACTC
GCAGTCCTGCTTTCCAATGAAGGAATAATAAACAGTGAAGATGAACATCTCTTGGCGCTTGAAAGAAAGCTGAAG
AAAATGCTGGGCCCCTCTGCTGTAGAGATAGGGAATGGATGCTTTGAAACCAAACACAAGTGCAACCAGACCTGT
CTCGACAGAATAGCTGCTGGTACCTTTGATGCAGGAGAATTTTCTCTCCCCACCTTTGATTCACTGAATATTACTGC
TGCATCTTTAAATGACGATGGATTGGATAATTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTCATTGGTAC
TGGTAGTCTCCCTGGGGGCAATCAGTTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

图 33B, SEQ ID NO : 96

PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT的氨基酸序列

MAKNVAIFGLLFSLLVLVPSQIFADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKGTETRGKLCPKC
LNCTDLDVALGRPKCTGKIPSARVSILHEVRPVTSGCFPIMHDRTKIRQLPNLLRGYEHIRLSTHNVINAENAPGGPYKIG
TSGSCPNITNGNGFFATMAWAVPKNDKNKTATNPLTIEVPYICTEGEDQITVWGFHSDNETQMAKLYGDSKPQKFTSSA
NGVTTHYVSQIGGFPNQTEDGGLPQSGRIVVDYMVQKSGKTGTITYQRGILLPQKVWCASGRSKVIKGSLPLIGEADCL
HEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPGGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQ
EAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKLKK
MLGPSAVEIGNGCFETKHKCNQTCLDRIAAGTFDAGEFSLPTFDSLNITAASLNDDGLDNYQILAIYSTVASSLVLVVSL
GAISFWMCSNGSLQCRICI*

图 33C

构建体1067号(2X35S/CPMV HT)的示意图

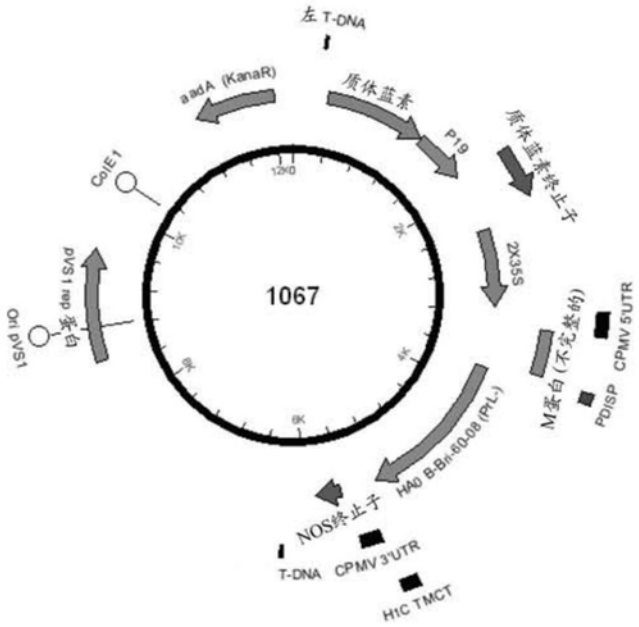


图 33D

构建体1977号(2X35S/CPMV HT*(-Mprot))的示意图

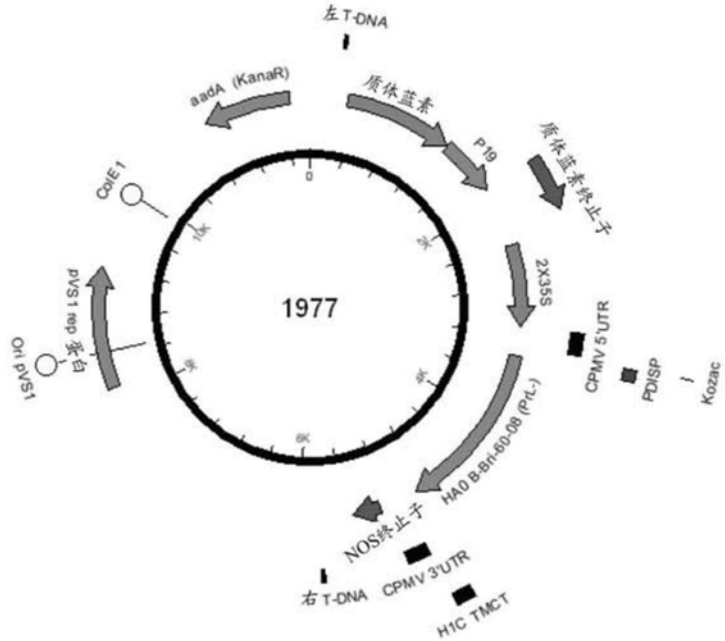


图33:用于PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT的2X35S/CPMV HT(构建体1067号)和HT*(-Mprot)(构建体1977号)

图 34A SEQ ID NO: 97

PDISP/HA B马萨诸塞州(PrL-)的核苷酸序列

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCCGATCG
AATCTGCACTGGGATAACATCTTCAAACTCACCTCATGTGGTCAAAACAGCTACTCAAGGGGAGGTCAATGTGACTG
GTGTGATACCACTAACAACAACACCAACAAAATCTTATTTTGCAAATCTCAAAGGAACAAAGACCAGAGGGAAACTA
TGCCCAGACTGTCTCAACTGTACAGATCTGGATGTGGCCCTGGGCAGGCCAATGTGTGTGGGAACTACACCTTCTGC
GAAAGCTTCAATACTTCACGAAGTCAGACCTGTTACATCCGGGTGCTTCCCTATAATGCACGACAGAACAAAAATCA
GGCAACTAGCCAATCTTCTCAGAGGATATGAAAATATCAGGTTATCAACCCAAAACGTTATCGATGCAGAAAAGGCA
CCAGGAGGACCCTACAGACTTGGAACCTCAGGATCTTGCCCTAACGCTACCAGTAAAAGCGGATTTTTCGCAACAAT
GGCTTGGGCTGTCCCAAAGGACAACAACAAAAATGCAACGAACCCATTAACAGTAGAAGTACCATACATTTGTGCAG
AAGGGGAAGACCAAATTACTGTTTGGGGGTTCCATTCAGATAACAAAACCCAAATGAAGAACCTCTATGGAGACTCA
AATCCTCAAAAGTTCACCTCATCTGCTAATGGAGTAACCACACATTATGTTTCTCAGATTGGCGGCTTCCCAGATCA
AACAGAAGACGGAGGACTACCACAAAGCGGCAGAATTGTCGTTGATTACATGATGCAAAAACCTGGGAAAACAGGAA
CAATTGTCTATCAAAGAGGTGTTTTGTTGCCTCAAAAGGTGTGGTGCGCGAGTGGCAGGAGCAAAGTAATAAAAGGG
TCCTTGCCTTTAATTGGTGAAGCAGATTGCCTTCATGAAAAATACGGTGGATTAAACAAAAGCAAGCCTTACTACAC
AGGAGAACATGCAAAAGCCATAGGAAATTGCCCAATATGGGTGAAAACACCTTTGAAGCTTGCCAATGGAACCAAAT
ATAGACCTCCTGGTGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGATACACATCTCACGGAGCACATGGAGTG
GCAGTTGCTGCAGACCTTAAGAGCACACAAGAAGCTATAAACAAGATAACAAAAAATCTCAACTCTTTGAGTGAGCT
AGAAGTAAAGAATCTTCAAAGGCTAAGTGGTGCCATGGATGAACTCCACAACGAAATACTCGAGCTGGATGAGAAAG
TGGATGACCTCAGAGCTGACACTATAAGTTCACAAATAGAACTTGCAGTCTTGCTTTCCAACGAAGGAATAATAAAC
AGTGAAGACGAGCATCTATTGGCACTTGAGAGAAAACTAAAGAAAATGCTGGGTCCCTCTGCTGTAGACATAGGAAA
TGGATGCTTCGAAACCAAACACAAATGCAACCAGACCTGCTTAGACAGGATAGCTGCTGGCACCTTTAATGCAGGAG
AGTTTTCTCTCCCCACTTTTGATTCATTGAACATTACTGCTGCATCTTTAAATGATGATGGATTGGATAACCATACT
ATACTGCTCTATTACTCAACTGCTGCTTCTAGTTTGGCTGTAACATTGATGCTAGCTATTTTTATTGTTTATATGGT
CTCCAGAGACAACGTTTCATGCTCCATCTGTCTATAA

图 34B SEQ ID NO: 98

PDISP/HA B马萨诸塞州(PrL-)的氨基酸序列

MAKNVAIFGLLFSLLVLVPSQIFADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSYFANLKGTKTRGKL
CPDCLNCTDLDVALGRPMCVGTTPSAKASILHEVRPVTSGCFPIMHDRTKIRQLANLLRGYENIRLSTQNVIDAEKA
PGGPYRLGTSGSCPNATSKSGFFATMAWAVPKDNNKNATNPLTVEVPYICAEGEDQITVWGFHSDNKTQMKNLYGDS
NPQKFTSSANGVTTHYVSQIGGFPDQTEDGGLPQSGRIVVDYMMQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKG
SLPLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPGGGWEGMIAGWHGYTSHGAHGV
AVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELAVLLSNEGIIN
SEDEHLLALERKLKKMLGPSAVDIGNGCFETKHKCNQTCLDRIAAGTFNAGEFSLPTFDSLNITAASLNDDGLDNHT
ILLYYSTAASSLAVTLMLAIFIVYMVSRDNVSCSICL*

图 34C

构建体2072号(2X35S/CPMV HT)的示意图

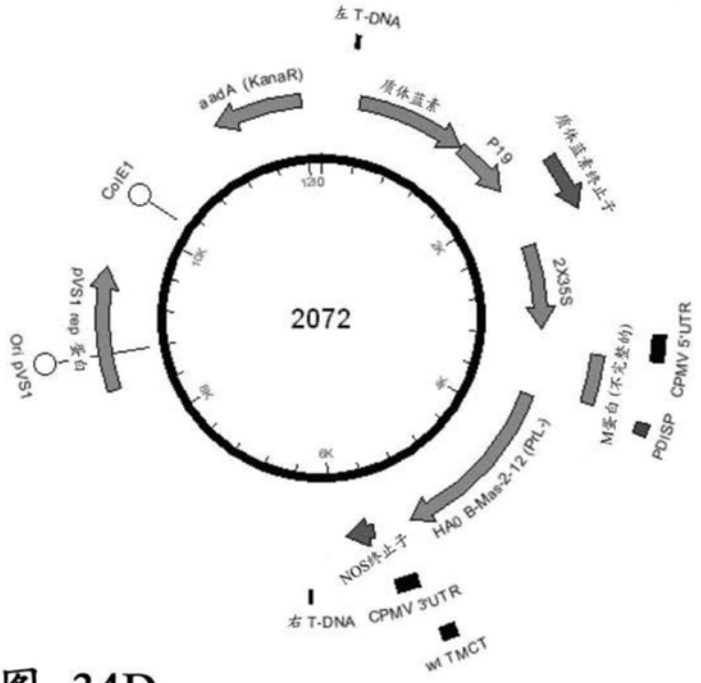


图 34D

构建体2050号(2X35S/CPMV HT*(-Mprot))的示意图

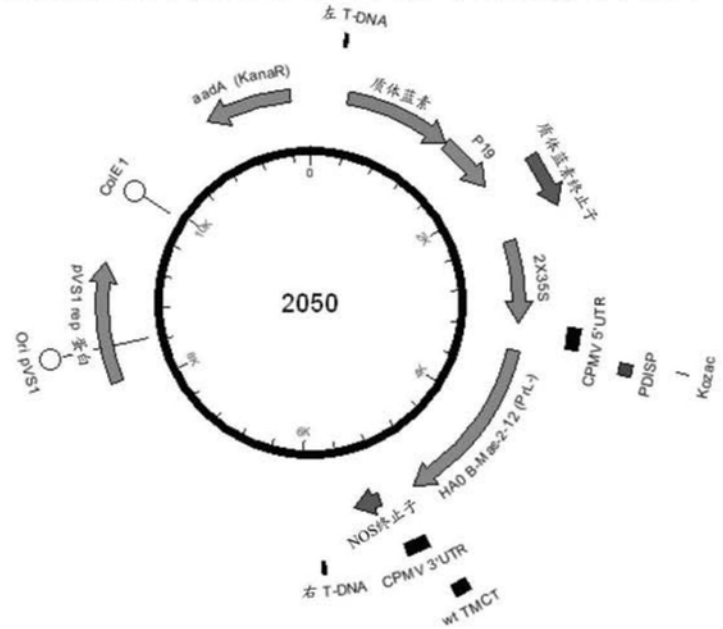


图34:用于PDISP/HA B马萨诸塞州(PrL-)的2X35S/CPMV HT(构建体2072号)和HT*(-Mprot)(构建体2050号)

图 35A SEQ ID NO : 99

PDISP/HA B马萨诸塞州(PrL-)+H1加利福尼亚TMCT的核苷酸序列

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCCGATCG
AATCTGCACTGGGATAACATCTTCAAACTCACCTCATGTGGTCAAAACAGCTACTCAAGGGGAGGTCAATGTGACTG
GTGTGATACCACTAACAACAACACCAACAAAATCTTATTTTGCAAATCTCAAAGGAACAAAGACCAGAGGGAAACTA
TGCCCAGACTGTCTCAACTGTACAGATCTGGATGTGGCCCTGGGCAGGCCAATGTGTGTGGGAACTACACCTTCTGC
GAAAGCTTCAATACTTCACGAAGTCAGACCTGTTACATCCGGGTGCTTCCCTATAATGCACGACAGAACAAAAATCA
GGCAACTAGCCAATCTTCTCAGAGGATATGAAAATATCAGGTTATCAACCCAAAACGTTATCGATGCAGAAAAGGCA
CCAGGAGGACCCTACAGACTTGGAACCTCAGGATCTTGCCCTAACGCTACCAGTAAAAGCGGATTTTTCGCAACAAT
GGCTTGGGCTGTCCCAAAGGACAACAACAAAAATGCAACGAACCCATTAACAGTAGAAGTACCATACATTTGTGCAG
AAGGGGAAGACCAAATTACTGTTTGGGGGTTCCATTCAGATAACAAAACCCAAATGAAGAACCTCTATGGAGACTCA
AATCCTCAAAAGTTCACCTCATCTGCTAATGGAGTAACCACACATTATGTTTCTCAGATTGGCGGCTTCCCAGATCA
AACAGAAGACGGAGGACTACCACAAAGCGGCAGAATTGTCGTTGATTACATGATGCAAAAACCTGGGAAAACAGGAA
CAATTGTCTATCAAAGAGGTGTTTTGTTGCCTCAAAAGGTGTGGTGCGCGAGTGGCAGGAGCAAAGTAATAAAAGGG
TCCTTGCCTTTAATTGGTGAAGCAGATTGCCTTCATGAAAAATACGGTGGATTAAACAAAAGCAAGCCTTACTACAC
AGGAGAACATGCAAAAGCCATAGGAAATTGCCCAATATGGGTGAAAACACCTTTGAAGCTTGCCAATGGAACCAAAT
ATAGACCTCCTGGTGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGATACACATCTCACGGAGCACATGGAGTG
GCAGTTGCTGCAGACCTTAAGAGCACACAAGAAGCTATAAACAAGATAACAAAAAATCTCAACTCTTTGAGTGAGCT
AGAAGTAAAGAATCTTCAAAGGCTAAGTGGTGCCATGGATGAACTCCACAACGAAATACTCGAGCTGGATGAGAAAG
TGGATGACCTCAGAGCTGACACTATAAGTTCACAAATAGAACTTGCAGTCTTGCTTTCCAACGAAGGAATAATAAAC
AGTGAAGACGAGCATCTATTGGCACTTGAGAGAAAACTAAAGAAAATGCTGGGTCCCTCTGCTGTAGACATAGGAAA
TGGATGCTTCGAAACCAAACACAAATGCAACCAGACCTGCTTAGACAGGATAGCTGCTGGCACCTTTAATGCAGGAG
AGTTTTCTCTCCCCACTTTTGATTCATTGAACATTACTGCTGCATCTTTAAATGATGATGGATTGGATAACTACCAG
ATTTTGGCGATCTATTCAACTGTCGCCAGTTCATTGGTACTGGTAGTCTCCCTGGGGGCAATCAGTTTCTGGATGTG
CTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

图 35B SEQ ID NO : 100

PDISP/HA B马萨诸塞州(PrL-)+H1加利福尼亚TMCT的氨基酸序列

MAKNVAIFGLLFSLLVLVPSQIFADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSYFANLKGTKTRGKL
CPDCLNCTDLDVALGRPMCVGTTPSAKASILHEVRPVTSGCFPIMHDRTKIRQLANLLRGYENIRLSTQNVIDAEKA
PGGPYRLGTSGSCPNATSKSGFFATMAWAVPKDNNKNATNPLTVEVPYICAEGEDQITVWGFHSDNKTQMKNLYGDS
NPQKFTSSANGVTTHYVSQIGGFPDQTEDGGLPQSGRIVVDYMMQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKG
SLPLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPGGGWEGMIAGWHGYTSHGAHGV
AVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELAVLLSNEGIIN
SEDEHLLALERKLKKMLGPSAVDIGNGCFETKHKCNQTCLDRIAAGTFNAGEFSLPTFDSLNITAASLNDDGLDNYQ
ILAIYSTVASSLVLVVSLGAISFWMCSNGSLQCRICI*

图 35C

构建体2074号(2X35S/CPMV HT)的示意图

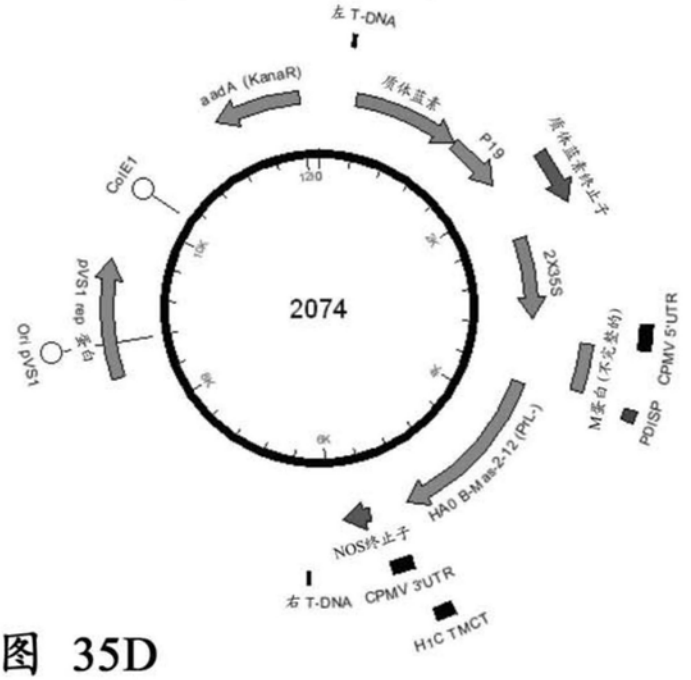


图 35D

构建体2060号(2X35S/CPMV HT*(-Mprot))的示意图

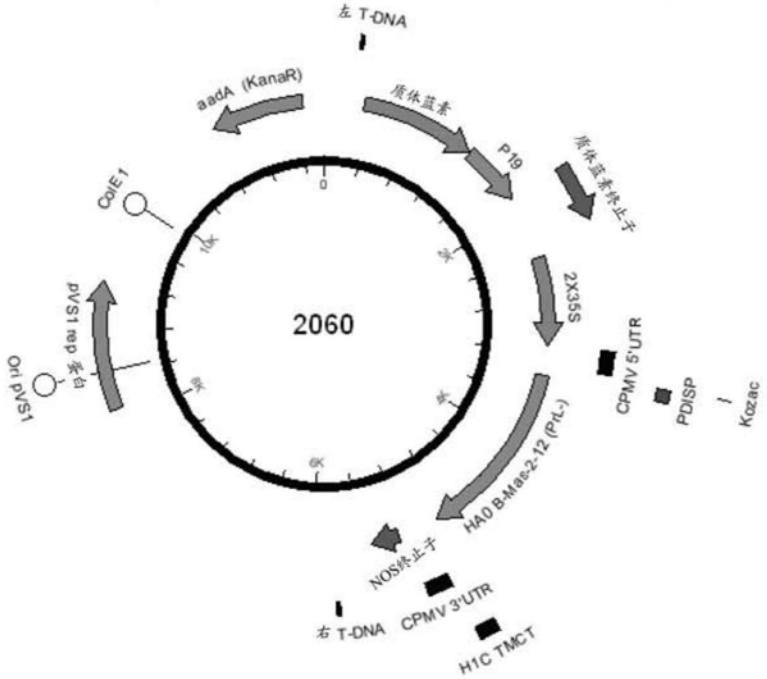


图35:用于PDISP/HA B马萨诸塞州(PrL-)+H1加利福尼亚TMCT的2X35S/CPMV HT(构建体2074号)和HT*(-Mprot)(构建体2060号)

图 36A, SEQ ID NO : 101

HA B威斯康星州(PrL-)的核苷酸序列

ATGAAGGCAATAATTGTACTACTCATGGTAGTAACATCCAATGCAGATCGAATCTGCACTGGGATAACATCTTCAAA
CTCACCTCATGTGGTCAAAACAGCTACTCAAGGGGAGGTCAATGTGACTGGCGTGATACCACTGACAACAACACCAA
CAAAATCTTATTTTGCAAATCTCAAAGGAACAAGGACCAGAGGGAAACTATGCCCGGACTGTCTCAACTGTACAGAT
CTGGATGTGGCCTTGGGCAGGCCAATGTGTGTGGGGACCACACCTTCTGCTAAAGCTTCAATACTCCACGAGGTCAG
ACCTGTTACATCCGGGTGCTTTCCTATAATGCACGACAGAACAAAAATCAGGCAACTACCCAATCTTCTCAGAGGAT
ATGAAAATATCAGGTTATCAACCCAAAACGTTATCGATGCAGAAAAAGCACCAGGAGGACCCTACAGACTTGGAACC
TCAGGATCTTGCCCTAACGCTACCAGTAAAATCGGATTTTTTGCAACAATGGCTTGGGCTGTCCCAAAGGACAACTA
CAAAAATGCAACGAACCCACTAACAGTAGAAGTACCATACATTTGTACAGAAGGGGAAGACCAAATTACTGTTTGGG
GGTTCCATTCAGATAACAAAACCCAAATGAAGAGCCTCTATGGAGACTCAAATCCTCAAAAGTTCACCTCATCTGCT
AATGGAGTAACCACACATTATGTTTCTCAGATTGGCGACTTCCCAGATCAAACAGAAGACGGAGGACTACCACAAAG
CGGCAGAATTGTTGTTGATTACATGATGCAAAAACCTGGGAAAACAGGAACAATTGTCTATCAAAGAGGTGTTTTGT
TGCCTCAAAAGGTGTGGTGCGCGAGTGGCAGGAGCAAAGTAATAAAAGGGTCATTGCCTTTAATTGGTGAAGCAGAT
TGCCTTCATGAAAAATACGGTGGATTAAACAAAAGCAAGCCTTACTACACAGGAGAACATGCAAAAGCCATAGGAAA
TTGCCCAATATGGGTAAAAACACCTTTGAAGCTTGCCAATGGAACCAAATATAGACCTCCTGGTGGAGGATGGGAAG
GAATGATTGCAGGTTGGCACGGATACACATCTCACGGAGCACATGGAGTGGCAGTGGCGGCAGACCTTAAGAGTACA
CAAGAAGCTATAAATAAGATAACAAAAAATCTCAATTCTTTGAGTGAGCTAGAAGTAAAGAACCTTCAAAGACTAAG
TGGTGCCATGGATGAACTCCACAACGAAATACTCGAGCTGGATGAGAAAGTGGATGATCTCAGAGCTGACACTATAA
GCTCACAAATAGAACTTGCAGTCTTGCTTTCCAACGAAGGAATAATAAACAGTGAAGACGAGCATCTATTGGCACTT
GAGAGAAAACTAAAGAAAATGCTGGGTCCCTCTGCTGTAGACATAGGAAACGGATGCTTCGAAACCAAACACAAATG
CAACCAGACCTGCTTAGACAGGATAGCTGCTGGCACCTTTAATGCAGGAGAATTTTCTCTCCCCACTTTTGATTCAT
TGAACATTACTGCTGCATCTTTAAATGATGATGGATTGGATAACCATACTATACTGCTCTATTACTCAACTGCTGCT
TCTAGTTTGGCTGTAACATTAATGCTAGCTATTTTTATTGTTTATATGGTCTCCAGAGACAACGTTTCATGCTCCAT
CTGTCTATAA

图 36B, SEQ ID NO : 102

HA B威斯康星州(PrL-)的氨基酸序列

MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSYFANLKGTRTRGKLCPDCLNCTD
LDVALGRPMCVGTTPSAKASILHEVRPVTSGCFPIMHDRTKIRQLPNLLRGYENIRLSTQNVIDAEKAPGGPYRLGT
SGSCPNATSKIGFFATMAWAVPKDNYKNATNPLTVEVPYICTEGEDQITVWGFHSDNKTQMKSLYGDSNPQKFTSSA
NGVTTHYVSQIGDFPDQTEDGGLPQSGRIVVDYMMQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKGSLPLIGEAD
CLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPGGGWEGMIAGWHGYTSHGAHGVAVAADLKST
QEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLAL
ERKLKKMLGPSAVDIGNGCFETKHKCNQTCLDRIAAGTFNAGEFSLPTFDSLNITAASLNDDGLDNHTILLYYSTAA
SSLAVTLMLAIFIVYMVSRDNVSCSICL*

图 36C

构建体1445号(2X35S/CPMV HT)的示意图

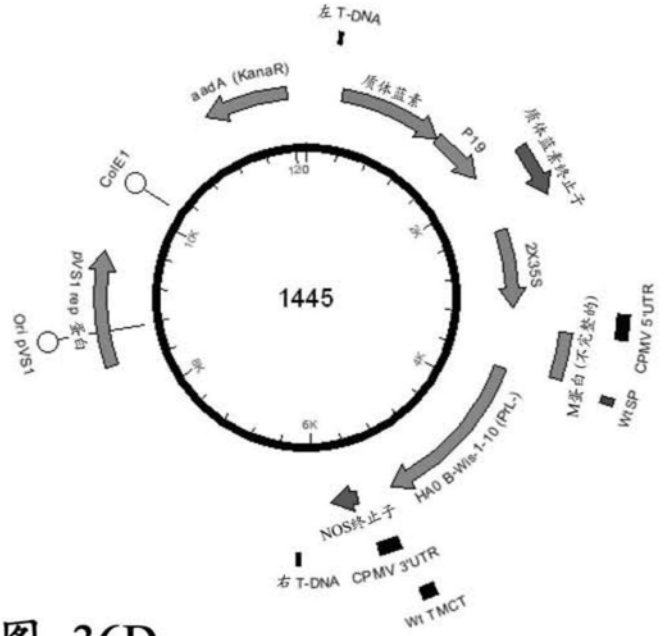


图 36D

构建体1820号(2X35S/CPMV HT*(-Mprot))的示意图

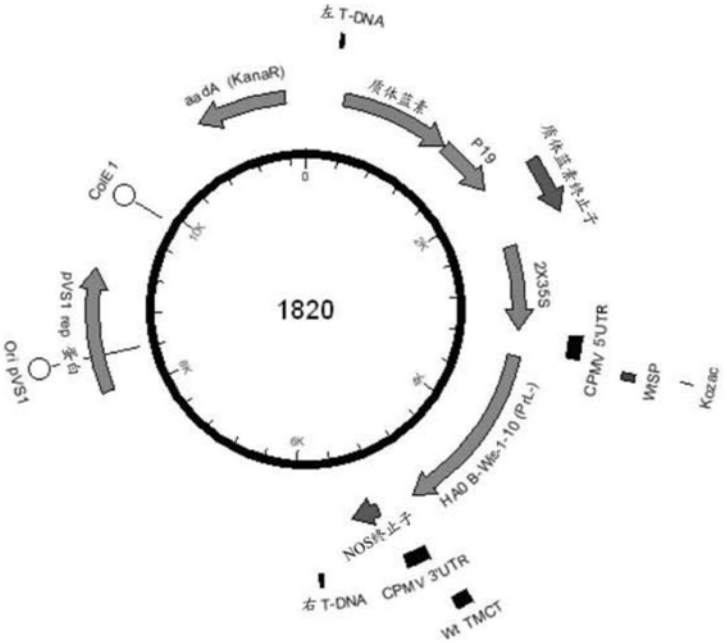


图 36E

构建体1975号(2X35S/CPMV HT*(f15'UTR))的示意图

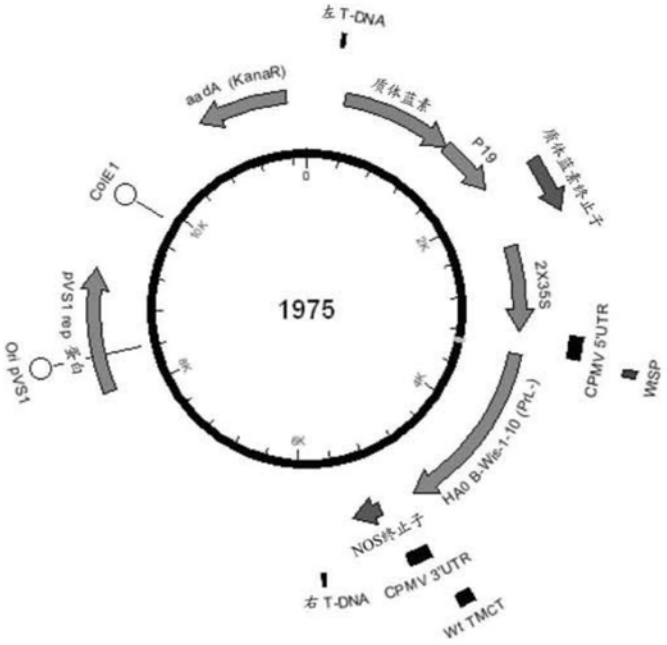


图36:用于HA B威斯康星州(PrL-)的2X35S/CPMV HT(构建体1445号)、HT*(-Mprot)(构建体1820号)和HT(f15'UTR)(构建体1975号)

图 37A, SEQ ID NO : 103

HA B威斯康星州(PrL-)+H1加利福尼亚TMCT的核苷酸序列

ATGAAGGCAATAATTGTACTACTCATGGTAGTAACATCCAATGCAGATCGAATCTGCACTGGGATAACATCTTCAAA
CTCACCTCATGTGGTCAAAACAGCTACTCAAGGGGAGGTCAATGTGACTGGCGTGATACCACTGACAACAACACCAA
CAAAATCTTATTTTGCAAATCTCAAAGGAACAAGGACCAGAGGGAAACTATGCCCGGACTGTCTCAACTGTACAGAT
CTGGATGTGGCCTTGGGCAGGCCAATGTGTGTGGGGACCACACCTTCTGCTAAAGCTTCAATACTCCACGAGGTCAG
ACCTGTTACATCCGGGTGCTTTCCTATAATGCACGACAGAACAAAAATCAGGCAACTACCCAATCTTCTCAGAGGAT
ATGAAAATATCAGGTTATCAACCCAAAACGTTATCGATGCAGAAAAAGCACCAGGAGGACCCTACAGACTTGGAACC
TCAGGATCTTGCCCTAACGCTACCAGTAAAATCGGATTTTTTGCAACAATGGCTTGGGCTGTCCCAAAGGACAACTA
CAAAAATGCAACGAACCCACTAACAGTAGAAGTACCATACATTTGTACAGAAGGGGAAGACCAAATTACTGTTTGGG
GGTTCCATTCAGATAACAAAACCCAAATGAAGAGCCTCTATGGAGACTCAAATCCTCAAAAGTTCACCTCATCTGCT
AATGGAGTAACCACACATTATGTTTCTCAGATTGGCGACTTCCCAGATCAAACAGAAGACGGAGGACTACCACAAAG
CGGCAGAATTGTTGTTGATTACATGATGCAAAAACCTGGGAAAACAGGAACAATTGTCTATCAAAGAGGTGTTTTGT
TGCCTCAAAAGGTGTGGTGCGCGAGTGGCAGGAGCAAAGTAATAAAAGGGTCATTGCCTTTAATTGGTGAAGCAGAT
TGCCTTCATGAAAAATACGGTGGATTAAACAAAAGCAAGCCTTACTACACAGGAGAACATGCAAAAGCCATAGGAAA
TTGCCCAATATGGGTAAAAACACCTTTGAAGCTTGCCAATGGAACCAAATATAGACCTCCTGGTGGAGGATGGGAAG
GAATGATTGCAGGTTGGCACGGATACACATCTCACGGAGCACATGGAGTGGCAGTGGCGGCAGACCTTAAGAGTACA
CAAGAAGCTATAAATAAGATAACAAAAAATCTCAATTCTTTGAGTGAGCTAGAAGTAAAGAACCTTCAAAGACTAAG
TGGTGCCATGGATGAACTCCACAACGAAATACTCGAGCTGGATGAGAAAGTGGATGATCTCAGAGCTGACACTATAA
GCTCACAAATAGAACTTGCAGTCTTGCTTTCCAACGAAGGAATAATAAACAGTGAAGACGAGCATCTATTGGCACTT
GAGAGAAAACTAAAGAAAATGCTGGGTCCCTCTGCTGTAGACATAGGAAACGGATGCTTCGAAACCAAACACAAATG
CAACCAGACCTGCTTAGACAGGATAGCTGCTGGCACCTTTAATGCAGGAGAATTTTCTCTCCCCACTTTTGATTCAT
TGAACATTACTGCTGCATCTTTAAATGATGATGGATTGGATAACTACCAGATTTTGGCGATCTATTCAACTGTCGCC
AGTTCATTGGTACTGGTAGTCTCCCTGGGGGCAATCAGTTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAAT
ATGTATTTAA

图 37B, SEQ ID NO : 104

HA B威斯康星州(PrL-)+H1加利福尼亚TMCT的氨基酸序列

MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSYFANLKGTRTRGKLCPDCLNCTD
LDVALGRPMCVGTTPSAKASILHEVRPVTSGCFPIMHDRTKIRQLPNLLRGYENIRLSTQNVIDAEKAPGGPYRLGT
SGSCPNATSKIGFFATMAWAVPKDNYKNATNPLTVEVPYICTEGEDQITVWGFHSDNKTQMKSLYGDSNPQKFTSSA
NGVTTHYVSQIGDFPDQTEDGGLPQSGRIVVDYMMQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKGSLPLIGEAD
CLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPGGGWEGMIAGWHGYTSHGAHGVAVAADLKST
QEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLAL
ERKLKKMLGPSAVDIGNGCFETKHKCNQTCLDRIAAGTFNAGEFSLPTFDSLNITAASLNDDGLDNYQILAIYSTVA
SSLVLVVSLGAISFWMCSNGSLQCRICI*

图 37C

构建体1454号(2X35S/CPMV HT)的示意图

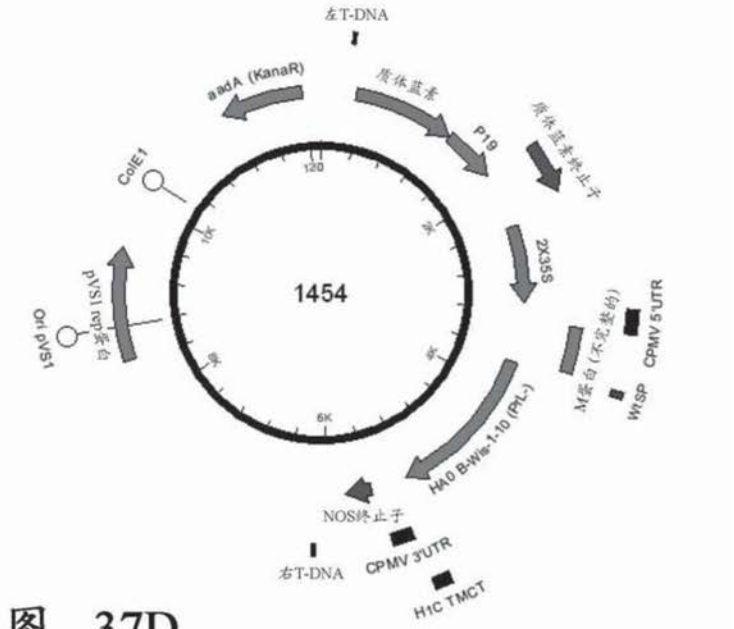


图 37D

构建体1893号(2X35S/CPMV HT*(-Mprot))的示意图



图37:用于HA B威斯康星州(PrL-)+H1加利福尼亚TMCT的2X35S/CPMV HT(构建体1454号)和HT*(-Mprot)(构建体1893号)

图 38A, SEQ ID NO : 105

PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT的核苷酸序列

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCCGATCGA
ATCTGCACTGGAATAACATCGTCAAACTCACCACATGTCGTCAAAACTGCTACTCAAGGGGAGGTCAATGTGACTG
GTGTAATACCACTGACAACAACACCCACCAAATCTCATTTTGCAAATCTCAAAGGAACAGAAACCAGGGGGAAAC
TATGCCCAAAATGCCTCAACTGCACAGATCTGGACGTAGCCTTGGGCAGACCAAAATGCACGGGGAAAATACCCT
CGGCAAGAGTTTCAATACTCCATGAAGTCAGACCTGTTACATCTGGGTGCTTTCCTATAATGCACGACAGAACAAA
AATTAGACAGCTGCCTAACCTTCTCCGAGGATACGAACATATCAGGTTATCAACCCATAACGTTATCAATGCAGAA
AATGCACCAGGAGGACCCTACAAAATTGGAACCTCAGGGTCTTGCCCTAACATTACCAATGGAAACGGATTTTTCG
CAACAATGGCTTGGGCCGTCCCAAAAAACGACAAAAACAAAACAGCAACAAATCCATTAACAATAGAAGTACCA
TACATTTGTACAGAAGGAGAAGACCAAATTACCGTTTGGGGGTTCCACTCTGACAACGAGACCCAAATGGCAAAG
CTCTATGGGGACTCAAAGCCCCAGAAGTTCACCTCATCTGCCAACGGAGTGACCACACATTACGTTTCACAGATTG
GTGGCTTCCCAAATCAAACAGAAGACGGAGGACTACCACAAAGTGGTAGAATTGTTGTTGATTACATGGTGCAAA
AATCTGGGAAAACAGGAACAATTACCTATCAAAGGGGTATTTTATTGCCTCAAAAGGTGTGGTGCGCAAGTGGCA
GGAGCAAGGTAATAAAAGGATCCTTGCCTTTAATTGGAGAAGCAGATTGCCTCCACGAAAAATACGGTGGATTAA
ACAAAAGCAAGCCTTACTACACAGGGGAACATGCAAAGGCCATAGGAAATTGCCCAATATGGGTGAAAACACCCT
TGAAGCTGGCCAATGGAACCAAATATAGACCTCCTGGTGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGAT
ACACATCCCATGGGGCACATGGAGTAGCGGTGGCAGCAGACCTTAAGAGCACTCAAGAGGCCATAAACAAGATA
ACAAAAAATCTCAACTCTTTGAGTGAGCTGGAAGTAAAGAATCTTCAAAGACTAAGCGGTGCCATGGATGAACTC
CACAACGAAATACTAGAACTAGATGAGAAAGTGGATGATCTCAGAGCTGATACAATAAGCTCACAAATAGAACTC
GCAGTCCTGCTTTCCAATGAAGGAATAATAAACAGTGAAGATGAACATCTCTTGGCGCTTGAAAGAAAGCTGAAG
AAAATGCTGGGCCCCTCTGCTGTAGAGATAGGGAATGGATGCTTTGAAACCAAACACAAGTGCAACCAGACCTGT
CTCGACAGAATAGCTGCTGGTACCTTTGATGCAGGAGAATTTTCTCTCCCCACCTTTGATTCACTGAATATTACTGC
TGCATCTTTAAATGACGATGGATTGGATAATTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTCATTGGTAC
TGGTAGTCTCCCTGGGGGCAATCAGTTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

图 38B, SEQ ID NO : 106

PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT的氨基酸序列

MAKNVAIFGLLFSLLVLVPSQIFADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKGTETRGKLCPKC
LNCTDLDVALGRPKCTGKIPSARVSILHEVRPVTSGCFPIMHDRTKIRQLPNLLRGYEHIRLSTHNVINAENAPGGPYKIG
TSGSCPNITNGNGFFATMAWAVPKNDKNKTATNPLTIEVPYICTEGEDQITVWGFHSDNETQMAKLYGDSKPQKFTSSA
NGVTTHYVSQIGGFPNQTEDGGLPQSGRIVVDYMVQKSGKTGTITYQRGILLPQKVWCASGRSKVIKGSLPLIGEADCL
HEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPGGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQ
EAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKLKK
MLGPSAVEIGNGCFETKHKCNQTCLDRIAAGTFDAGEFSLPTFDSLNITAASLNDDGLDNYQILAIYSTVASSLVLVVSL
GAISFWMCSNGSLQCRICI*

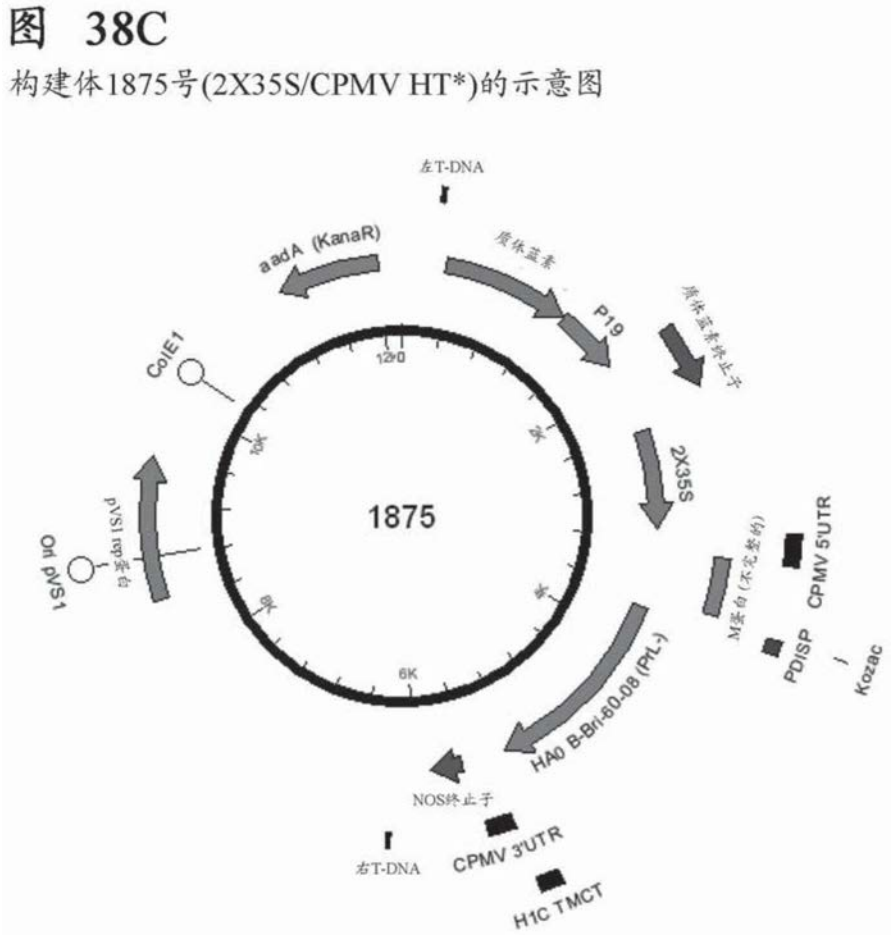


图38:用于PDISP/HA B布里斯班(PrL-)+H1加利福尼亚TMCT的L-2X35S/CPMV HT(构建体1067号)和HT*(构建体1875号)

图 39A, SEQ ID NO : 107

PDISP/HA B马萨诸塞州(PrL-)的核苷酸序列

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCCGATCGA
ATCTGCACTGGGATAACATCTTCAAACTCACCTCATGTGGTCAAAACAGCTACTCAAGGGGAGGTCAATGTGACTG
GTGTGATACCACTAACAACAACACCAACAAAATCTTATTTTGCAAATCTCAAAGGAACAAAGACCAGAGGGAAAC
TATGCCCAGACTGTCTCAACTGTACAGATCTGGATGTGGCCCTGGGCAGGCCAATGTGTGTGGGAACTACACCTTC
TGCGAAAGCTTCAATACTTCACGAAGTCAGACCTGTTACATCCGGGTGCTTCCCTATAATGCACGACAGAACAAAA
ATCAGGCAACTAGCCAATCTTCTCAGAGGATATGAAAATATCAGGTTATCAACCCAAAACGTTATCGATGCAGAA
AAGGCACCAGGAGGACCCTACAGACTTGGAACCTCAGGATCTTGCCCTAACGCTACCAGTAAAAGCGGATTTTTC
GCAACAATGGCTTGGGCTGTCCCAAAGGACAACAACAAAAATGCAACGAACCCATTAACAGTAGAAGTACCATAC
ATTTGTGCAGAAGGGGAAGACCAAATTACTGTTTGGGGGTTCCATTCAGATAACAAAACCCAAATGAAGAACCTC
TATGGAGACTCAAATCCTCAAAAGTTCACCTCATCTGCTAATGGAGTAACCACACATTATGTTTCTCAGATTGGCG
GCTTCCCAGATCAAACAGAAGACGGAGGACTACCACAAAGCGGCAGAATTGTCGTTGATTACATGATGCAAAAAC
CTGGGAAAACAGGAACAATTGTCTATCAAAGAGGTGTTTTGTTGCCTCAAAAGGTGTGGTGCGCGAGTGGCAGGA
GCAAAGTAATAAAAGGGTCCTTGCCTTTAATTGGTGAAGCAGATTGCCTTCATGAAAAATACGGTGGATTAAACA
AAAGCAAGCCTTACTACACAGGAGAACATGCAAAAGCCATAGGAAATTGCCCAATATGGGTGAAAACACCTTTGA
AGCTTGCCAATGGAACCAAATATAGACCTCCTGGTGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGATACA
CATCTCACGGAGCACATGGAGTGGCAGTTGCTGCAGACCTTAAGAGCACACAAGAAGCTATAAACAAGATAACAA
AAAATCTCAACTCTTTGAGTGAGCTAGAAGTAAAGAATCTTCAAAGGCTAAGTGGTGCCATGGATGAACTCCACA
ACGAAATACTCGAGCTGGATGAGAAAGTGGATGACCTCAGAGCTGACACTATAAGTTCACAAATAGAACTTGCAG
TCTTGCTTTCCAACGAAGGAATAATAAACAGTGAAGACGAGCATCTATTGGCACTTGAGAGAAAACTAAAGAAAA
TGCTGGGTCCCTCTGCTGTAGACATAGGAAATGGATGCTTCGAAACCAAACACAAATGCAACCAGACCTGCTTAG
ACAGGATAGCTGCTGGCACCTTTAATGCAGGAGAGTTTTCTCTCCCCACTTTTGATTCATTGAACATTACTGCTGCA
TCTTTAAATGATGATGGATTGGATAACCATACTATACTGCTCTATTACTCAACTGCTGCTTCTAGTTTGGCTGTAAC
ATTGATGCTAGCTATTTTTATTGTTTATATGGTCTCCAGAGACAACGTTTCATGCTCCATCTGTCTATAA

图 39B, SEQ ID NO :108

PDISP/HA B马萨诸塞州(PrL-)的氨基酸序列

MAKNVAIFGLLFSLLVLVPSQIFADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSYFANLKGTKTRGKLCPDC
LNCTDLDVALGRPMCVGTTPSAKASILHEVRPVTSGCFPIMHDRTKIRQLANLLRGYENIRLSTQNVIDAEKAPGGPYRL
GTSGSCPNATSKSGFFATMAWAVPKDNNKNATNPLTVEVPYICAEGEDQITVWGFHSDNKTQMKNLYGDSNPQKFTSS
ANGVTTHYVSQIGGFPDQTEDGGLPQSGRIVVDYMMQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKGSLPLIGEAD
CLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPGGGWEGMIAGWHGYTSHGAHGVAVAADLKS
TQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKL
KKMLGPSAVDIGNGCFETKHKCNQTCLDRIAAGTFNAGEFSLPTFDSLNITAASLNDDGLDNHTILLYYSTAASSLAVTL
MLAIFIVYMVSRDNVSCSICL*

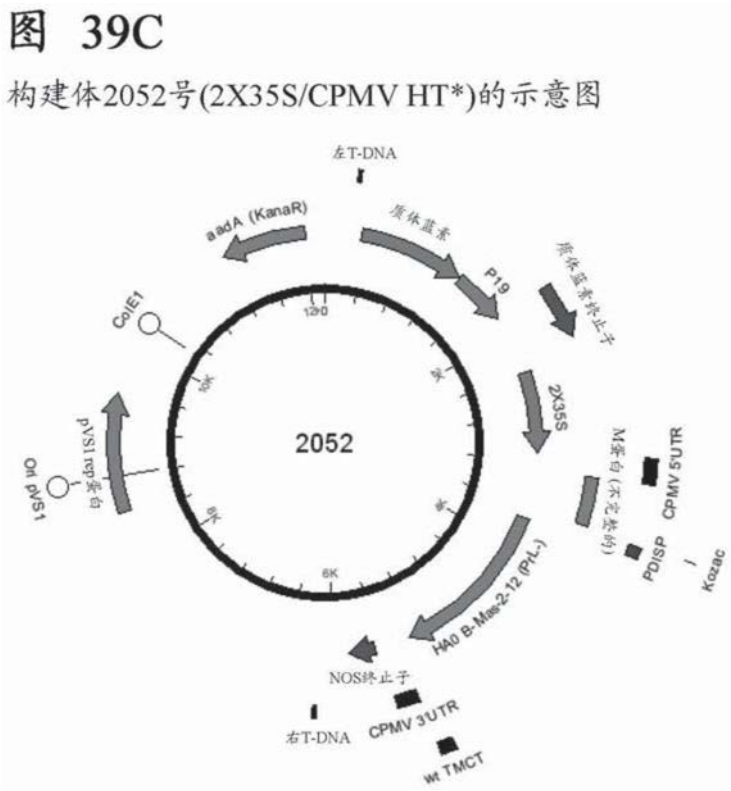


图39:用于PDISP/HA B马萨诸塞州(PrL-)的M-2X35S/CPMV HT(构建体2072号)和HT*(构建体2052号)

图 40A, SEQ ID NO : 109

PDISP/HA B马萨诸塞州(PrL-)+H1加利福尼亚TMCT的核苷酸序列

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCCGATCGA
ATCTGCACTGGGATAACATCTTCAAACTCACCTCATGTGGTCAAAACAGCTACTCAAGGGGAGGTCAATGTGACTG
GTGTGATACCACTAACAACAACACCAACAAAATCTTATTTTGCAAATCTCAAAGGAACAAAGACCAGAGGGAAAC
TATGCCCAGACTGTCTCAACTGTACAGATCTGGATGTGGCCCTGGGCAGGCCAATGTGTGTGGGAACTACACCTTC
TGCGAAAGCTTCAATACTTCACGAAGTCAGACCTGTTACATCCGGGTGCTTCCCTATAATGCACGACAGAACAAAA
ATCAGGCAACTAGCCAATCTTCTCAGAGGATATGAAAATATCAGGTTATCAACCCAAAACGTTATCGATGCAGAA
AAGGCACCAGGAGGACCCTACAGACTTGGAACCTCAGGATCTTGCCCTAACGCTACCAGTAAAAGCGGATTTTTC
GCAACAATGGCTTGGGCTGTCCCAAAGGACAACAACAAAAATGCAACGAACCCATTAACAGTAGAAGTACCATAC
ATTTGTGCAGAAGGGGAAGACCAAATTACTGTTTGGGGGTTCCATTCAGATAACAAAACCCAAATGAAGAACCTC
TATGGAGACTCAAATCCTCAAAAGTTCACCTCATCTGCTAATGGAGTAACCACACATTATGTTTCTCAGATTGGCG
GCTTCCCAGATCAAACAGAAGACGGAGGACTACCACAAAGCGGCAGAATTGTCGTTGATTACATGATGCAAAAAC
CTGGGAAAACAGGAACAATTGTCTATCAAAGAGGTGTTTTGTTGCCTCAAAAGGTGTGGTGCGCGAGTGGCAGGA
GCAAAGTAATAAAAGGGTCCTTGCCTTTAATTGGTGAAGCAGATTGCCTTCATGAAAAATACGGTGGATTAAACA
AAAGCAAGCCTTACTACACAGGAGAACATGCAAAAGCCATAGGAAATTGCCCAATATGGGTGAAAACACCTTTGA
AGCTTGCCAATGGAACCAAATATAGACCTCCTGGTGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGATACA
CATCTCACGGAGCACATGGAGTGGCAGTTGCTGCAGACCTTAAGAGCACACAAGAAGCTATAAACAAGATAACAA
AAAATCTCAACTCTTTGAGTGAGCTAGAAGTAAAGAATCTTCAAAGGCTAAGTGGTGCCATGGATGAACTCCACA
ACGAAATACTCGAGCTGGATGAGAAAGTGGATGACCTCAGAGCTGACACTATAAGTTCACAAATAGAACTTGCAG
TCTTGCTTTCCAACGAAGGAATAATAAACAGTGAAGACGAGCATCTATTGGCACTTGAGAGAAAACTAAAGAAAA
TGCTGGGTCCCTCTGCTGTAGACATAGGAAATGGATGCTTCGAAACCAAACACAAATGCAACCAGACCTGCTTAG
ACAGGATAGCTGCTGGCACCTTTAATGCAGGAGAGTTTTCTCTCCCCACTTTTGATTCATTGAACATTACTGCTGCA
TCTTTAAATGATGATGGATTGGATAACTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTCATTGGTACTGGT
AGTCTCCCTGGGGGCAATCAGTTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

图 40B, SEQ ID NO : 110

PDISP/HA B马萨诸塞州(PrL-)+H1加利福尼亚TMCT的氨基酸序列

MAKNVAIFGLLFSLLVLVPSQIFADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSYFANLKGTKTRGKLCPDC
LNCTDLDVALGRPMCVGTTPSAKASILHEVRPVTSGCFPIMHDRTKIRQLANLLRGYENIRLSTQNVIDAEKAPGGPYRL
GTSGSCPNATSKSGFFATMAWAVPKDNNKNATNPLTVEVPYICAEGEDQITVWGFHSDNKTQMKNLYGDSNPQKFTSS
ANGVTTHYVSQIGGFPDQTEDGGLPQSGRIVVDYMMQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKGSLPLIGEAD
CLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPGGGWEGMIAGWHGYTSHGAHGVAVAADLKS
TQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKL
KKMLGPSAVDIGNGCFETKHKCNQTCLDRIAAGTFNAGEFSLPTFDSLNITAASLNDDGLDNYQILAIYSTVASSLVLVV
SLGAISFWMCSNGSLQCRICI*

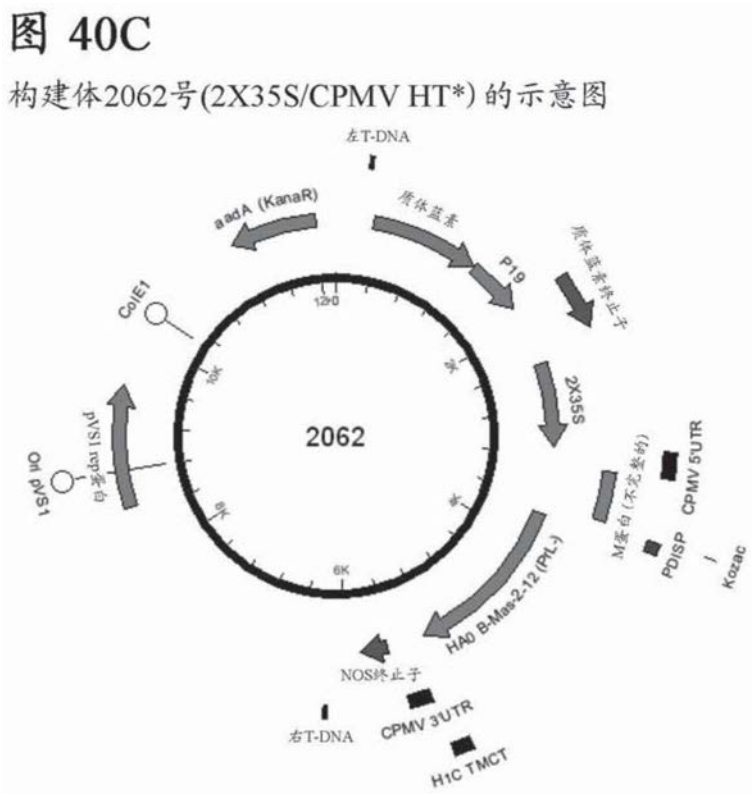


图40:用于PDISP/HA B马萨诸塞州(PrL-)+H1加利福尼亚TMCT的N-2X35S/CPMV HT(构建体2074号)和HT*(构建体2062号)

图 41A, SEQ ID NO :111

HA B威斯康星州(PrL-)的核苷酸序列

ATGAAGGCAATAATTGTACTACTCATGGTAGTAACATCCAATGCAGATCGAATCTGCACTGGGATAACATCTTCAA
ACTCACCTCATGTGGTCAAAACAGCTACTCAAGGGGAGGTCAATGTGACTGGCGTGATACCACTGACAACAACAC
CAACAAAATCTTATTTTGCAAATCTCAAAGGAACAAGGACCAGAGGGAAACTATGCCCGGACTGTCTCAACTGTA
CAGATCTGGATGTGGCCTTGGGCAGGCCAATGTGTGTGGGGACCACACCTTCTGCTAAAGCTTCAATACTCCACGA
GGTCAGACCTGTTACATCCGGGTGCTTTCCTATAATGCACGACAGAACAAAAATCAGGCAACTACCCAATCTTCTC
AGAGGATATGAAAATATCAGGTTATCAACCCAAAACGTTATCGATGCAGAAAAAGCACCAGGAGGACCCTACAGA
CTTGGAACCTCAGGATCTTGCCCTAACGCTACCAGTAAAATCGGATTTTTTGCAACAATGGCTTGGGCTGTCCCAA
AGGACAACTACAAAAATGCAACGAACCCACTAACAGTAGAAGTACCATACATTTGTACAGAAGGGGAAGACCAA
ATTACTGTTTGGGGGTTCCATTCAGATAACAAAACCCAAATGAAGAGCCTCTATGGAGACTCAAATCCTCAAAAGT
TCACCTCATCTGCTAATGGAGTAACCACACATTATGTTTCTCAGATTGGCGACTTCCCAGATCAAACAGAAGACGG
AGGACTACCACAAAGCGGCAGAATTGTTGTTGATTACATGATGCAAAAACCTGGGAAAACAGGAACAATTGTCTA
TCAAAGAGGTGTTTTGTTGCCTCAAAAGGTGTGGTGCGCGAGTGGCAGGAGCAAAGTAATAAAAGGGTCATTGCC
TTTAATTGGTGAAGCAGATTGCCTTCATGAAAAATACGGTGGATTAAACAAAAGCAAGCCTTACTACACAGGAGA
ACATGCAAAAGCCATAGGAAATTGCCCAATATGGGTAAAAACACCTTTGAAGCTTGCCAATGGAACCAAATATAG
ACCTCCTGGTGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGATACACATCTCACGGAGCACATGGAGTGGC
AGTGGCGGCAGACCTTAAGAGTACACAAGAAGCTATAAATAAGATAACAAAAAATCTCAATTCTTTGAGTGAGCT
AGAAGTAAAGAACCTTCAAAGACTAAGTGGTGCCATGGATGAACTCCACAACGAAATACTCGAGCTGGATGAGAA
AGTGGATGATCTCAGAGCTGACACTATAAGCTCACAAATAGAACTTGCAGTCTTGCTTTCCAACGAAGGAATAATA
AACAGTGAAGACGAGCATCTATTGGCACTTGAGAGAAAACTAAAGAAAATGCTGGGTCCCTCTGCTGTAGACATA
GGAAACGGATGCTTCGAAACCAAACACAAATGCAACCAGACCTGCTTAGACAGGATAGCTGCTGGCACCTTTAAT
GCAGGAGAATTTTCTCTCCCCACTTTTGATTCATTGAACATTACTGCTGCATCTTTAAATGATGATGGATTGGATAA
CCATACTATACTGCTCTATTACTCAACTGCTGCTTCTAGTTTGGCTGTAACATTAATGCTAGCTATTTTTATTGTTTA
TATGGTCTCCAGAGACAACGTTTCATGCTCCATCTGTCTATAA

图 41B, SEQ ID NO : 112

HA B威斯康星州(PrL-)的氨基酸序列

MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSYFANLKGTRTRGKLCPDCLNCTDLDV
ALGRPMCVGTTPSAKASILHEVRPVTSGCFPIMHDRTKIRQLPNLLRGYENIRLSTQNVIDAEKAPGGPYRLGTSGSCPN
ATSKIGFFATMAWAVPKDNYKNATNPLTVEVPYICTEGEDQITVWGFHSDNKTQMKSLYGDSNPQKFTSSANGVTTHY
VSQIGDFPDQTEDGGLPQSGRIVVDYMMQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKGSLPLIGEADCLHEKYGG
LNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPGGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKIT
KNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKLKKMLGPSA
VDIGNGCFETKHKCNQTCLDRIAAGTFNAGEFSLPTFDSLNITAASLNDDGLDNHTILLYYSTAASSLAVTLMLAIFIVY
MVSRDNVSCSICL*

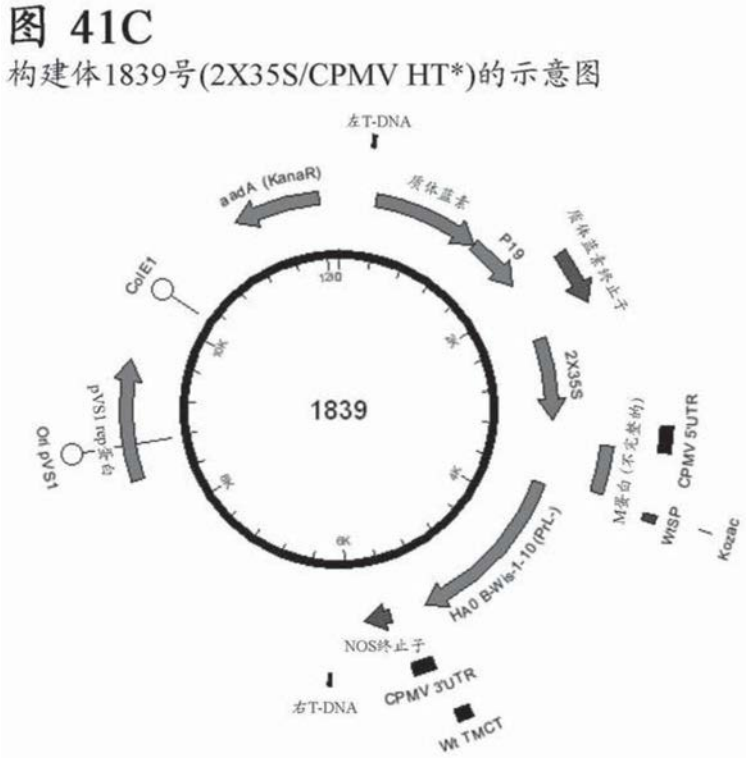


图41:用于HA B威斯康星州(PrL-)的O-2X35S/CPMV HT(构建体1445号)、HT*(构建体1839号)、HT*(-Mprot)(构建体1820号)和HT(f15'UTR)(构建体1975号)

图 42A, SEQ ID NO : 113

HA B威斯康星州(PrL-)+H1加利福尼亚TMCT的核苷酸序列

ATGAAGGCAATAATTGTACTACTCATGGTAGTAACATCCAATGCAGATCGAATCTGCACTGGGATAACATCTTCAA
ACTCACCTCATGTGGTCAAAACAGCTACTCAAGGGGAGGTCAATGTGACTGGCGTGATACCACTGACAACAACAC
CAACAAAATCTTATTTTGCAAATCTCAAAGGAACAAGGACCAGAGGGAAACTATGCCCGGACTGTCTCAACTGTA
CAGATCTGGATGTGGCCTTGGGCAGGCCAATGTGTGTGGGGACCACACCTTCTGCTAAAGCTTCAATACTCCACGA
GGTCAGACCTGTTACATCCGGGTGCTTTCCTATAATGCACGACAGAACAAAAATCAGGCAACTACCCAATCTTCTC
AGAGGATATGAAAATATCAGGTTATCAACCCAAAACGTTATCGATGCAGAAAAAGCACCAGGAGGACCCTACAGA
CTTGGAACCTCAGGATCTTGCCCTAACGCTACCAGTAAAATCGGATTTTTTGCAACAATGGCTTGGGCTGTCCCAA
AGGACAACTACAAAAATGCAACGAACCCACTAACAGTAGAAGTACCATACATTTGTACAGAAGGGGAAGACCAA
ATTACTGTTTGGGGGTTCCATTCAGATAACAAAACCCAAATGAAGAGCCTCTATGGAGACTCAAATCCTCAAAAGT
TCACCTCATCTGCTAATGGAGTAACCACACATTATGTTTCTCAGATTGGCGACTTCCCAGATCAAACAGAAGACGG
AGGACTACCACAAAGCGGCAGAATTGTTGTTGATTACATGATGCAAAAACCTGGGAAAACAGGAACAATTGTCTA
TCAAAGAGGTGTTTTGTTGCCTCAAAAGGTGTGGTGCGCGAGTGGCAGGAGCAAAGTAATAAAAGGGTCATTGCC
TTTAATTGGTGAAGCAGATTGCCTTCATGAAAAATACGGTGGATTAAACAAAAGCAAGCCTTACTACACAGGAGA
ACATGCAAAAGCCATAGGAAATTGCCCAATATGGGTAAAAACACCTTTGAAGCTTGCCAATGGAACCAAATATAG
ACCTCCTGGTGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGATACACATCTCACGGAGCACATGGAGTGGC
AGTGGCGGCAGACCTTAAGAGTACACAAGAAGCTATAAATAAGATAACAAAAAATCTCAATTCTTTGAGTGAGCT
AGAAGTAAAGAACCTTCAAAGACTAAGTGGTGCCATGGATGAACTCCACAACGAAATACTCGAGCTGGATGAGAA
AGTGGATGATCTCAGAGCTGACACTATAAGCTCACAAATAGAACTTGCAGTCTTGCTTTCCAACGAAGGAATAATA
AACAGTGAAGACGAGCATCTATTGGCACTTGAGAGAAAACTAAAGAAAATGCTGGGTCCCTCTGCTGTAGACATA
GGAAACGGATGCTTCGAAACCAAACACAAATGCAACCAGACCTGCTTAGACAGGATAGCTGCTGGCACCTTTAAT
GCAGGAGAATTTTCTCTCCCCACTTTTGATTCATTGAACATTACTGCTGCATCTTTAAATGATGATGGATTGGATAA
CTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTCATTGGTACTGGTAGTCTCCCTGGGGGCAATCAGTTTCT
GGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

图 42B, SEQ ID NO : 114

HA B威斯康星州(PrL-)+H1加利福尼亚TMCT的氨基酸序列

MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSYFANLKGTRTRGKLCPDCLNCTDLDV
ALGRPMCVGTTPSAKASILHEVRPVTSGCFPIMHDRTKIRQLPNLLRGYENIRLSTQNVIDAEKAPGGPYRLGTSGSCPN
ATSKIGFFATMAWAVPKDNYKNATNPLTVEVPYICTEGEDQITVWGFHSDNKTQMKSLYGDSNPQKFTSSANGVTTHY
VSQIGDFPDQTEDGGLPQSGRIVVDYMMQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKGSLPLIGEADCLHEKYGG
LNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPGGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKIT
KNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKLKKMLGPSA
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MCSNGSLQCRICI*

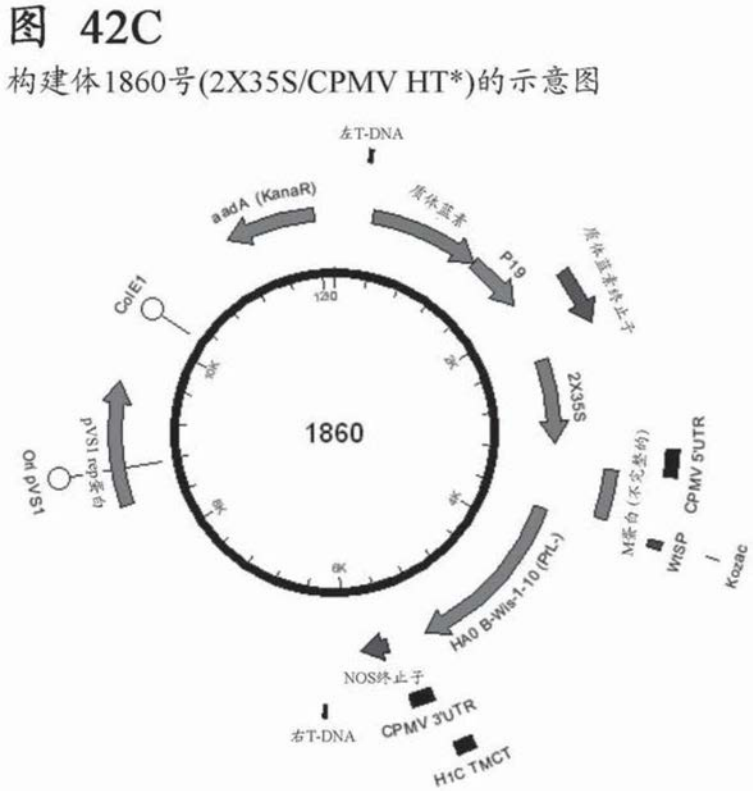


图42:用于HA B威斯康星州(PrL-)+H1加利福尼亚TMCT的P-2X35S/CPMV HT(构建体1454号)和HT*(构建体1860号)

图 43A

天然的H5印度尼西亚的核苷酸序列(SEQ ID NO: 115)

ATGGAGAAAATAGTGCTTCTTCTTGCAATAGTCAGTCTTGTTAAAAGTGATCAGATTTGCATTGGTTACCATGCAAA
CAATTCAACAGAGCAGGTTGACACAATCATGGAAAAGAACGTTACTGTTACACATGCCCAAGACATACTGGAAAAGA
CACACAACGGGAAGCTCTGCGATCTAGATGGAGTGAAGCCTCTAATTTTAAGAGATTGTAGTGTAGCTGGATGGCTC
CTCGGGAACCCAATGTGTGACGAATTCATCAATGTACCGGAATGGTCTTACATAGTGGAGAAGGCCAATCCAACCAA
TGACCTCTGTTACCCAGGGAGTTTCAACGACTATGAAGAACTGAAACACCTATTGAGCAGAATAAACCATTTTGAGA
AAATTCAAATCATCCCCAAAAGTTCTTGGTCCGATCATGAAGCCTCATCAGGAGTTAGCTCAGCATGTCCATACCTG
GGAAGTCCCTCCTTTTTTAGAAATGTGGTATGGCTTATCAAAAAGAACAGTACATACCCAACAATAAAGAAAAGCTA
CAATAATACCAACCAAGAGGATCTTTTGGTACTGTGGGGAATTCACCATCCTAATGATGCGGCAGAGCAGACAAGGC
TATATCAAAACCCAACCACCTATATTTCCATTGGGACATCAACACTAAACCAGAGATTGGTACCAAAAATAGCTACT
AGATCCAAAGTAAACGGGCAAAGTGGAAGGATGGAGTTCTTCTGGACAATTTTAAAACCTAATGATGCAATCAACTT
CGAGAGTAATGGAAATTTCATTGCTCCAGAATATGCATACAAAATTGTCAAGAAAGGGGACTCAGCAATTATGAAAA
GTGAATTGGAATATGGTAACTGCAACACCAAGTGTCAAACTCCAATGGGGGCGATAAACTCTAGTATGCCATTCCAC
AACATACACCCTCTCACCATCGGGGAATGCCCCAAATATGTGAAATCAAACAGATTAGTCCTTGCAACAGGGCTCAG
AAATAGCCCTCAAAGAGAGAGCAGAAGAAAAAAGAGAGGACTATTTGGAGCTATAGCAGGTTTTATAGAGGGAGGAT
GGCAGGGAATGGTAGATGGTTGGTATGGGTACCACCATAGCAATGAGCAGGGGAGTGGGTACGCTGCAGACAAAGAA
TCCACTCAAAAGGCAATAGATGGAGTCACCAATAAGGTCAACTCAATCATTGACAAAATGAACACTCAGTTTGAGGC
CGTTGGAAGGGAATTTAATAACTTAGAAAGGAGAATAGAGAATTTAAACAAGAAGATGGAAGACGGGTTTCTAGATG
TCTGGACTTATAATGCCGAACTTCTGGTTCTCATGGAAAATGAGAGAACTCTAGACTTTCATGACTCAAATGTTAAG
AACCTCTACGACAAGGTCCGACTACAGCTTAGGGATAATGCAAAGGAGCTGGGTAACGGTTGTTTCGAGTTCTATCA
CAAATGTGATAATGAATGTATGGAAAGTATAAGAAACGGAACGTACAACTATCCGCAGTATTCAGAAGAAGCAAGAT
TAAAAAGAGAGGAAATAAGTGGGGTAAAATTGGAATCAATAGGAACTTACCAAATACTGTCAATTTATTCAACAGTG
GCGAGTTCCCTAGCACTGGCAATCATGATGGCTGGTCTATCTTTATGGATGTGCTCCAATGGATCGTTACAATGCAG
AATTTGCATTTAA

图 43B

天然的H5印度尼西亚的氨基酸序列(SEQ ID NO: 116)

MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCDLDGVKPLILRDCSVAGWL
LGNPMCDEFINVPEWSYIVEKANPTNDLCYPGSFNDYEELKHLLSRINHFEKIQIIPKSSWSDHEASSGVSSACPYL
GSPSFFRNVVWLIKKNSTYPTIKKSYNNTNQEDLLVLWGIHHPNDAAEQTRLYQNPTTYISIGTSTLNQRLVPKIAT
RSKVNGQSGRMEFFWTILKPNDAINFESNGNFIAPEYAYKIVKKGDSAIMKSELEYGNCNTKCQTPMGAINSSMPFH
NIHPLTIGECPKYVKSNRLVLATGLRNSPQRESRRKKRGLFGAIAGFIEGGWQGMVDGWYGYHHSNEQGSGYAADKE
STQKAIDGVTNKVNSIIDKMNTQFEAVGREFNNLERRIENLNKKMEDGFLDVWTYNAELLVLMENERTLDFHDSNVK
NLYDKVRLQLRDNAKELGNGCFEFYHKCDNECMESIRNGTYNYPQYSEEARLKREEISGVKLESIGTYQILSIYSTV
ASSLALAIMMAGLSLWMCSNGSLQCRICI*

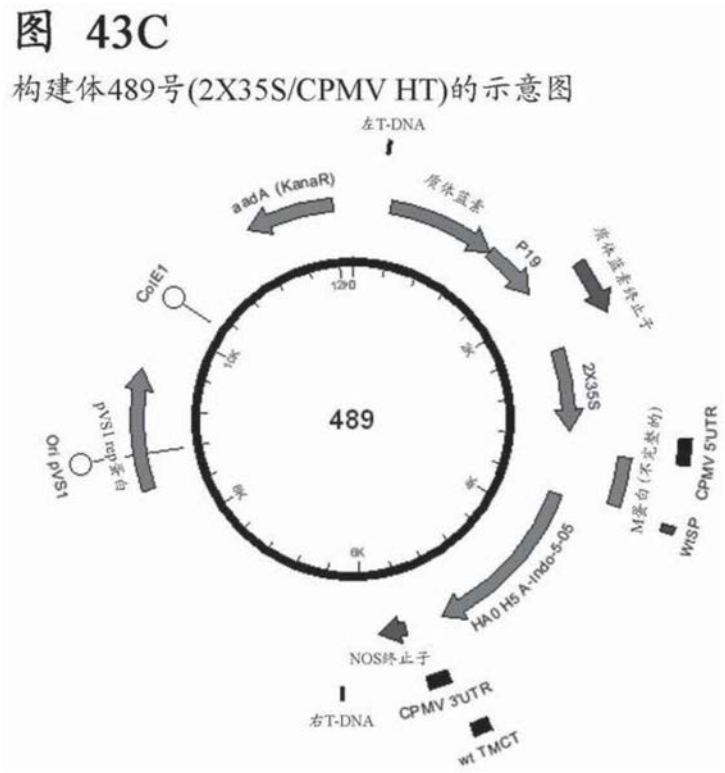


图43:用于H5印度尼西亚的2X35S/CPMV HT(构建体489号)、HT*(-Mprot)(构建体1880号)和HT(f15'UTR)(构建体1885号)

图 44A

IF**(SacII)-PDI.s1+4c

ACAGGGCCCAATACCGCGGAGAAAATGGCGAAAAACGTTGCGATTTTCGGCT (SEQ ID NO: 117)

图 44B

IF-H3V36111.s1-4r

ACTAAAGAAAATAGGCCTTCAAATGCAAATGTTGCACCTAATGTTGCCCTT (SEQ ID NO: 118)

图 44C

PDISP/H3维多利亚的核苷酸序列(SEQ ID NO: 119)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCCCAAAA
ACTTCCTGGAAATGACAACAGCACGGCAACGCTGTGCCTTGGGCACCATGCAGTACCAAACGGAACGATAGTGAAAA
CAATCACGAATGACCAAATTGAAGTTACTAATGCTACTGAGCTGGTTCAGAATTCCTCAATAGGTGAAATATGCGAC
AGTCCTCATCAGATCCTTGATGGAGAAAACTGCACACTAATAGATGCTCTATTGGGAGACCCTCAGTGTGATGGCTT
CCAAAATAAGAAATGGGACCTTTTTGTTGAACGAAGCAAAGCCTACAGCAACTGTTACCCTTATGATGTGCCGGATT
ATGCCTCCCTTAGGTCACTAGTTGCCTCATCCGGCACACTGGAGTTTAACAATGAAAGCTTCAATTGGACTGGAGTC
ACTCAAAACGGAACAAGTTCTGCTTGCATAAGGAGATCTAATAATAGTTTCTTTAGTAGATTAAATTGGTTGACCCA
CTTAAACTTCAAATACCCAGCATTGAACGTGACTATGCCAAACAATGAACAATTTGACAAATTGTACATTTGGGGGG
TTCACCACCCGGGTACGGACAAGGACCAAATCTTCCTGTATGCTCAATCATCAGGAAGAATCACAGTATCTACCAAA
AGAAGCCAACAAGCTGTAATCCCGAATATCGGATCTAGACCCAGAATAAGGAATATCCCTAGCAGAATAAGCATCTA
TTGGACAATAGTAAAACCGGGAGACATACTTTTGATTAACAGCACAGGGAATCTAATTGCTCCTAGGGGTTACTTCA
AAATACGAAGTGGGAAAAGCTCAATAATGAGATCAGATGCACCCATTGGCAAATGCAATTCTGAATGCATCACTCCA
AATGGAAGCATTCCCAATGACAAACCATTCCAAAATGTAAACAGGATCACATACGGGGCCTGTCCCAGATATGTTAA
GCAAAGCACTCTGAAATTGGCAACAGGAATGCGAAATGTACCAGAGAAACAAACTAGAGGCATATTTGGCGCAATAG
CGGGTTTCATAGAAAATGGTTGGGAGGGAATGGTGGATGGTTGGTACGGTTTCAGGCATCAAAATTCTGAGGGAAGA
GGACAAGCAGCAGATCTCAAAAGCACTCAAGCAGCAATCGATCAAATCAATGGGAAGCTGAATCGATTGATCGGGAA
AACCAACGAGAAATTCCATCAGATTGAAAAAGAATTCTCAGAAGTCGAAGGGAGAATTCAGGACCTTGAGAAATATG
TTGAGGACACTAAAATAGATCTCTGGTCATACAACGCGGAGCTTCTTGTTGCCCTGGAGAACCAACATACAATTGAT
CTAACTGACTCAGAAATGAACAAACTGTTTGAAAAAACAAAGAAGCAACTAAGGGAAAATGCTGAGGATATGGGCAA
TGGTTGTTTCAAAATATACCACAAATGTGACAATGCCTGCATAGGATCAATCAGAAATGGAACTTATGACCACGATG
TATACAGAGATGAAGCATTAAACAACCGGTTCCAGATCAAGGGAGTTGAGCTGAAGTCAGGGTACAAAGATTGGATC
CTATGGATTTCCTTTGCCATATCATGTTTTTTGCTTTGTGTTGCTTTGTTGGGGTTCATCATGTGGGCCTGCCAAAA
GGGCAACATTAGGTGCAACATTTGCATTTGA

图 44D

构建体2171的示意图。在示意图上注释用于质粒线性化的SacII和StuI的限制性酶切位点

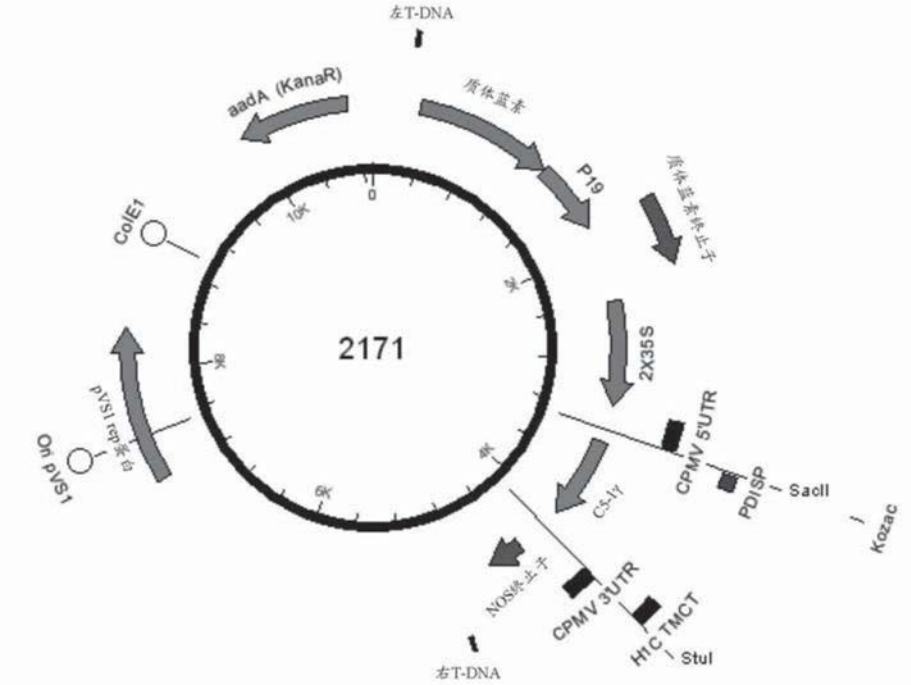


图 44E

从左至右t-DNA边界的构建体2171(加下划线)。具有质体蓝素-P19-质体蓝素沉默抑制子表达盒的2X35S/CPMV-HT*(-Mprot)/NOS(SEQ ID NO: 120)

TGGCAGGATATATTGTGGTGTAAACAAATTGACGCTTAGACAACTTAATAACACATTGCGGACGTTTTTAATGTACT
GAATTAACGCCGAATCCCGGGCTGGTATATTTATATGTTGTCAAATAACTCAAAAACCATAAAAGTTTAAGTTAGCA
AGTGTGTACATTTTTACTTGAACAAAAATATTCACCTACTACTGTTATAAATCATTATTAAACATTAGAGTAAAGAA
ATATGGATGATAAGAACAAGAGTAGTGATATTTTGACAACAATTTTGTTGCAACATTTGAGAAAATTTTGTTGTTCT
CTCTTTTCATTGGTCAAAAACAATAGAGAGAGAAAAAGGAAGAGGGAGAATAAAAACATAATGTGAGTATGAGAGAG
AAAGTTGTACAAAAGTTGTACCAAAATAGTTGTACAAATATCATTGAGGAATTTGACAAAAGCTACACAAATAAGGG
TTAATTGCTGTAAATAAATAAGGATGACGCATTAGAGAGATGTACCATTAGAGAATTTTTGGCAAGTCATTAAAAAG
AAAGAATAAATTATTTTTAAAATTAAAAGTTGAGTCATTTGATTAAACATGTGATTATTTAATGAATTGATGAAAGA
GTTGGATTAAAGTTGTATTAGTAATTAGAATTTGGTGTCAAATTTAATTTGACATTTGATCTTTTCCTATATATTGC
CCCATAGAGTCAGTTAACTCATTTTTATATTTCATAGATCAAATAAGAGAAATAACGGTATATTAATCCCTCCAAAA
AAAAAAAACGGTATATTTACTAAAAAATCTAAGCCACGTAGGAGGATAACAGGATCCCCGTAGGAGGATAACATCCA
ATCCAACCAATCACAACAATCCTGATGAGATAACCCACTTTAAGCCCACGCATCTGTGGCACATCTACATTATCTAA
ATCACACATTCTTCCACACATCTGAGCCACACAAAAACCAATCCACATCTTTATCACCCATTCTATAAAAAATCACA
CTTTGTGAGTCTACACTTTGATTCCCTTCAAACACATACAAAGAGAAGAGACTAATTAATTAATTAATCATCTTGAG
AGAAAATGGAACGAGCTATACAAGGAAACGACGCTAGGGAACAAGCTAACAGTGAACGTTGGGATGGAGGATCAGGA
GGTACCACTTCTCCCTTCAAACTTCCTGACGAAAGTCCGAGTTGGACTGAGTGGCGGCTACATAACGATGAGACGAA
TTCGAATCAAGATAATCCCCTTGGTTTCAAGGAAAGCTGGGGTTTCGGGAAAGTTGTATTTAAGAGATATCTCAGAT
ACGACAGGACGGAAGCTTCACTGCACAGAGTCCTTGGATCTTGGACGGGAGATTCGGTTAACTATGCAGCATCTCGA
TTTTTCGGTTTCGACCAGATCGGATGTACCTATAGTATTCGGTTTCGAGGAGTTAGTATCACCGTTTCTGGAGGGTC
GCGAACTCTTCAGCATCTCTGTGAGATGGCAATTCGGTCTAAGCAAGAACTGCTACAGCTTGCCCCAATCGAAGTGG
AAAGTAATGTATCAAGAGGATGCCCTGAAGGTACTCAAACCTTCGAAAAAGAAAGCGAGTAAGTTAAAATGCTTCTT
CGTCTCCTATTTATAATATGGTTTGTTATTGTTAATTTTGTTCTTGTAGAAGAGCTTAATTAATCGTTGTTGTTATG
AAATACTATTTGTATGAGATGAACTGGTGTAATGTAATTCATTTACATAAGTGGAGTCAGAATCAGAATGTTTCCTC
CATAACTAACTAGACATGAAGACCTGCCGCGTACAATTGTCTTATATTTGAACAACTAAAATTGAACATCTTTTGCC
ACAACTTTATAAGTGGTTAATATAGCTCAAATATATGGTCAAGTTCAATAGATTAATAATGGAAATATCAGTTATCG
AAATTCATTAACAATCAACTTAACGTTATTAACTACTAATTTTATATCATCCCCTTTGATAAATGATAGTACACCAA
TTAGGAAGGAGCATGCTCGCCTAGGAGATTGTCGTTTCCCGCCTTCAGTTTGCAAGCTGCTCTAGCCGTGTAGCCAA
TACGCAAACCGCCTCTCCCCGCGCGTTGGGAATTACTAGCGCGTGTCGACAAGCTTGCATGCCGGTCAACATGGTGG
AGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAA
CAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAA
GGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTC
CCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGAT
TGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAG
GGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTA
TTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGAT
GCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCAC
GTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAG
ACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTTGATAAAAGCGAACG
TGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTCTTG
CGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAGGGCCCAATACCGCGGAGAAAATGGCGAAA
AACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCGACGTCACTCCTCAG
CCAAAACGACACCCCCATCTGTCTATCCACTGGCCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACCCTGGGA
TGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACTCTGGATCCCTGTCCAGCGGTGTGCACAC
CTTCCCAGCTGTCCTGCAGTCTGACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCG
AGACCGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGTGGT
TGTAAGCCTTGCATATGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCCAAAGCCCAAGGATGTGCTCAC
CATTACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTCCAGTTCAGCTGGT
TTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAGTTCAACAGCACTTTCCGCTCAGTC
AGTGAACTTCCCATCATGCACCAGGACTGGCTCAATGGCAAGGAGACGTCCAGATTTTGGCGATCTATTCAACTGTC
GCCAGTTCATTGGTACTGGTAGTCTCCCTGGGGGCAATCAGTTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAG
AATATGTATTTAAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGG
TTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCA

图 44E(续)

GCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTATCGA
CCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCAT
ATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTA
TGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCG
CGCGCGGTGTCATCTATGTTACTAGATCTCTAGAGTCTCAAGCTTGGCGCGCCCACGTGACTAGTGGCACTGGCCGT
CGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
GCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGCTAGAGC
AGCTTGAGCTTGGATCAGATTGTCGTTTCCCGCCTTCAGTTTAAACTATCAGTGTTTGACAGGATATATTGGCGGGT
AAACCTAAGAGAAAAGAGCGTTTA

图 44F

从2X35S启动子至NOS终止子的表达盒1800号。PDISP/H3维多利亚核苷酸序列加下划线 (SEQ ID NO: 121)

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTT
TCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAG
GTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCC
CCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGGAGCA
CGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATAT
CCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGC
CATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCAT
CGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAAT
CCCACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTTGATAA
AAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTCTTG
CGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAGGGCCCAATACCGCGGAGAAAATGGCGAAAAACGTTGCG
ATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCCCAAAAACTTCCTGGAAATGACAACAGCACGGC
AACGCTGTGCCTTGGGCACCATGCAGTACCAAACGGAACGATAGTGAAAACAATCACGAATGACCAAATTGAAGTTACTAATGCTA
CTGAGCTGGTTCAGAATTCCTCAATAGGTGAAATATGCGACAGTCCTCATCAGATCCTTGATGGAGAAAACTGCACACTAATAGAT
GCTCTATTGGGAGACCCTCAGTGTGATGGCTTCCAAAATAAGAAATGGGACCTTTTTGTTGAACGAAGCAAAGCCTACAGCAACTG
TTACCCTTATGATGTGCCGGATTATGCCTCCCTTAGGTCACTAGTTGCCTCATCCGGCACACTGGAGTTTAACAATGAAAGCTTCA
ATTGGACTGGAGTCACTCAAAACGGAACAAGTTCTGCTTGCATAAGGAGATCTAATAATAGTTTCTTTAGTAGATTAAATTGGTTG
ACCCACTTAAACTTCAAATACCCAGCATTGAACGTGACTATGCCAAACAATGAACAATTTGACAAATTGTACATTTGGGGGGTTCA
CCACCCGGGTACGGACAAGGACCAAATCTTCCTGTATGCTCAATCATCAGGAAGAATCACAGTATCTACCAAAAGAAGCCAACAAG
CTGTAATCCCGAATATCGGATCTAGACCCAGAATAAGGAATATCCCTAGCAGAATAAGCATCTATTGGACAATAGTAAAACCGGGA
GACATACTTTTGATTAACAGCACAGGGAATCTAATTGCTCCTAGGGGTTACTTCAAAATACGAAGTGGGAAAAGCTCAATAATGAG
ATCAGATGCACCCATTGGCAAATGCAATTCTGAATGCATCACTCCAAATGGAAGCATTCCCAATGACAAACCATTCCAAAATGTAA
ACAGGATCACATACGGGGCCTGTCCCAGATATGTTAAGCAAAGCACTCTGAAATTGGCAACAGGAATGCGAAATGTACCAGAGAAA
CAAACTAGAGGCATATTTGGCGCAATAGCGGGTTTCATAGAAAATGGTTGGGAGGGAATGGTGGATGGTTGGTACGGTTTCAGGCA
TCAAAATTCTGAGGGAAGAGGACAAGCAGCAGATCTCAAAAGCACTCAAGCAGCAATCGATCAAATCAATGGGAAGCTGAATCGAT
TGATCGGGAAAACCAACGAGAAATTCCATCAGATTGAAAAAGAATTCTCAGAAGTCGAAGGGAGAATTCAGGACCTTGAGAAATAT
GTTGAGGACACTAAAATAGATCTCTGGTCATACAACGCGGAGCTTCTTGTTGCCCTGGAGAACCAACATACAATTGATCTAACTGA
CTCAGAAATGAACAAACTGTTTGAAAAAACAAAGAAGCAACTAAGGGAAAATGCTGAGGATATGGGCAATGGTTGTTTCAAAATAT
ACCACAAATGTGACAATGCCTGCATAGGATCAATCAGAAATGGAACTTATGACCACGATGTATACAGAGATGAAGCATTAAACAAC
CGGTTCCAGATCAAGGGAGTTGAGCTGAAGTCAGGGTACAAAGATTGGATCCTATGGATTTCCTTTGCCATATCATGTTTTTTGCT
TTGTGTTGCTTTGTTGGGGTTCATCATGTGGGCCTGCCAAAAGGGCAACATTAGGTGCAACATTTGCATTTGAAGGCCTATTTTCT
TTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAAT
TTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAAAAAAAAA
AAAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGT
TGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTA
TGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAA
TTATCGCGCGCGGTGTCATCTATGTTACTAGAT

图 44G

PDISP/H3维多利亚的氨基酸序列(SEQ ID NO: 122)

MAKNVAIFGLLFSLLVLVPSQIFAQKLPGNDNSTATLCLGHHAVPNGTIVKTITNDQIEVTNATELVQNSSIGEICDSPHQILDGE
NCTLIDALLGDPQCDGFQNKKWDLFVERSKAYSNCYPYDVPDYASLRSLVASSGTLEFNNESFNWTGVTQNGTSSACIRRSNNSFF
SRLNWLTHLNFKYPALNVTMPNNEQFDKLYIWGVHHPGTDKDQIFLYAQSSGRITVSTKRSQQAVIPNIGSRPRIRNIPSRISIYW
TIVKPGDILLINSTGNLIAPRGYFKIRSGKSSIMRSDAPIGKCNSECITPNGSIPNDKPFQNVNRITYGACPRYVKQSTLKLATGM
RNVPEKQTRGIFGAIAGFIENGWEGMVDGWYGFRHQNSEGRGQAADLKSTQAAIDQINGKLNRLIGKTNEKFHQIEKEFSEVEGRI
QDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNKLFEKTKKQLRENAEDMGNGCFKIYHKCDNACIGSIRNGTYDHDVYR
DEALNNRFQIKGVELKSGYKDWILWISFAISCFLLCVALLGFIMWACQKGNIRCNICI*

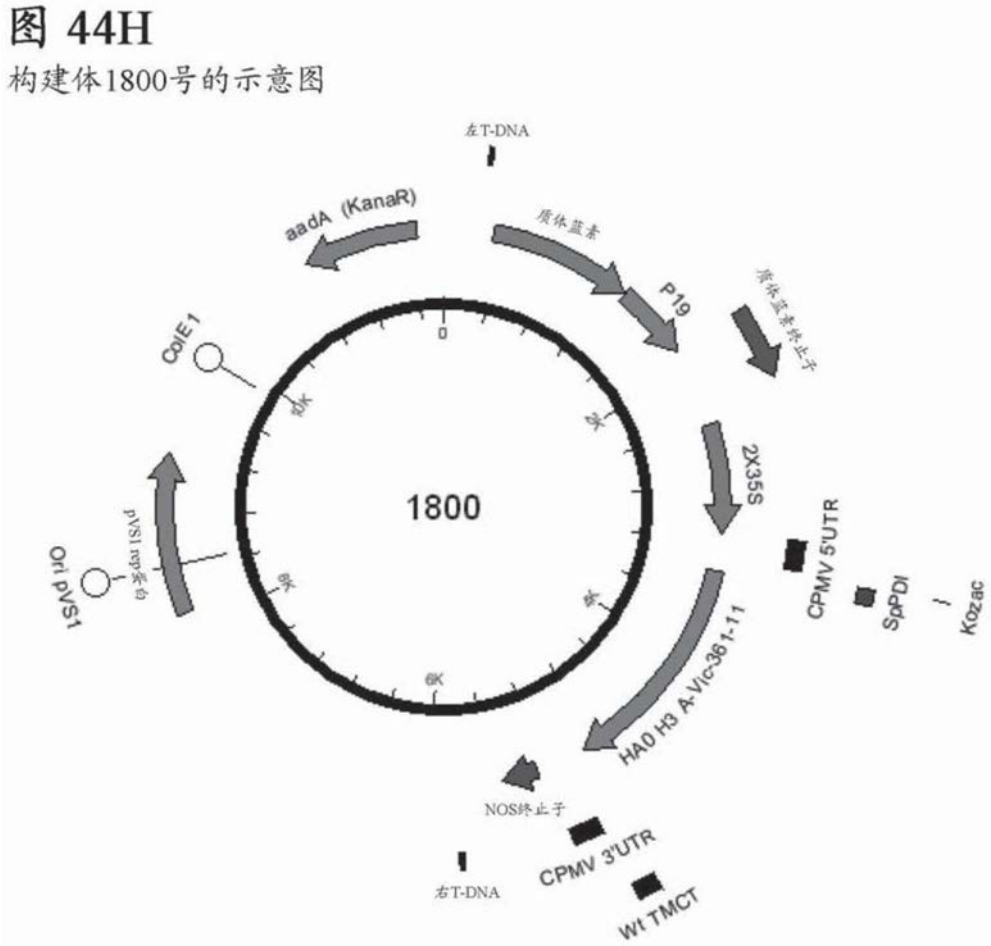


图44:2X35S/CPMV-HT*(-Mprot)/PDISP/H3维多利亚/NOS(构建体1800号)

图 45A

IF(SacII)-Kozac_PDI.c

GTCGGGCCCAATACCGCGGAGAAAATGGCGAAAAACGTTGCGATTTTC (SEQ ID NO.: 123)

图 45B

IF-H3V36111.s1-4r

ACTAAAGAAAATAGGCCTTCAAATGCAAATGTTGCACCTAATGTTGCCCTT (SEQ ID NO: 124)

图 45C

构建体2181的示意图。在示意图上注释用于质粒线性化的SacII和StuI的限制性酶切位点

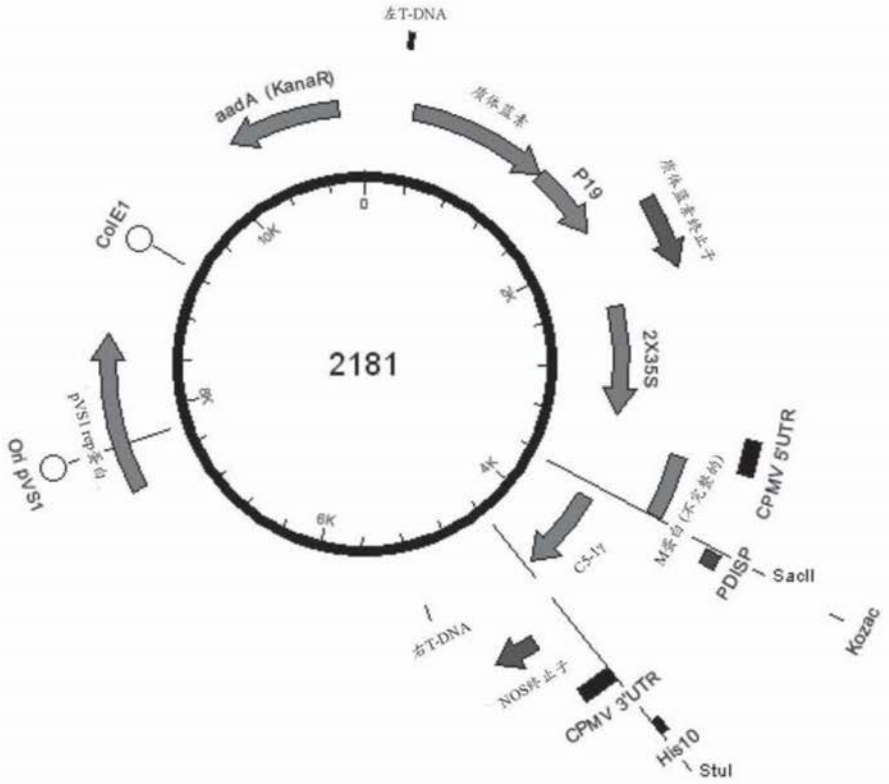


图 45D

从左至右t-DNA边界的构建体2181(加下划线)。具有质体蓝素-P19-质体蓝素沉默抑制子表达盒的2X35S/CPMV-HT*/NOS(SEQ ID NO: 125)

TGGCAGGATATATTGTGGTGTAAACAAATTGACGCTTAGACAACTTAATAACACATTGCGGACGTTTTTAATGTACTGAATTAACG
CCGAATCCCGGGCTGGTATATTTATATGTTGTCAAATAACTCAAAAACCATAAAAGTTTAAGTTAGCAAGTGTGTACATTTTTACT
TGAACAAAAATATTCACCTACTACTGTTATAAATCATTATTAAACATTAGAGTAAAGAAATATGGATGATAAGAACAAGAGTAGTG
ATATTTTGACAACAATTTTGTTGCAACATTTGAGAAAATTTTGTTGTTCTCTCTTTTCATTGGTCAAAAACAATAGAGAGAGAAAA
AGGAAGAGGGAGAATAAAAACATAATGTGAGTATGAGAGAGAAAGTTGTACAAAAGTTGTACCAAAATAGTTGTACAAATATCATT
GAGGAATTTGACAAAAGCTACACAAATAAGGGTTAATTGCTGTAAATAAATAAGGATGACGCATTAGAGAGATGTACCATTAGAGA
ATTTTTGGCAAGTCATTAAAAAGAAAGAATAAATTATTTTTAAAATTAAAAGTTGAGTCATTTGATTAAACATGTGATTATTTAAT
GAATTGATGAAAGAGTTGGATTAAAGTTGTATTAGTAATTAGAATTTGGTGTCAAATTTAATTTGACATTTGATCTTTTCCTATAT

图 45D(续)

ATTGCCCCATAGAGTCAGTTAACTCATTTTTATATTTCATAGATCAAATAAGAGAAATAACGGTATATTAATCCCTCCAAAAAAAA
AAAACGGTATATTTACTAAAAAATCTAAGCCACGTAGGAGGATAACAGGATCCCCGTAGGAGGATAACATCCAATCCAACCAATCA
CAACAATCCTGATGAGATAACCCACTTTAAGCCCACGCATCTGTGGCACATCTACATTATCTAAATCACACATTCTTCCACACATC
TGAGCCACACAAAAACCAATCCACATCTTTATCACCCATTCTATAAAAAATCACACTTTGTGAGTCTACACTTTGATTCCCTTCAA
ACACATACAAAGAGAAGAGACTAATTAATTAATTAATCATCTTGAGAGAAAATGGAACGAGCTATACAAGGAAACGACGCTAGGGA
ACAAGCTAACAGTGAACGTTGGGATGGAGGATCAGGAGGTACCACTTCTCCCTTCAAACTTCCTGACGAAAGTCCGAGTTGGACTG
AGTGGCGGCTACATAACGATGAGACGAATTCGAATCAAGATAATCCCCTTGGTTTCAAGGAAAGCTGGGGTTTCGGGAAAGTTGTA
TTTAAGAGATATCTCAGATACGACAGGACGGAAGCTTCACTGCACAGAGTCCTTGGATCTTGGACGGGAGATTCGGTTAACTATGC
AGCATCTCGATTTTTCGGTTTCGACCAGATCGGATGTACCTATAGTATTCGGTTTCGAGGAGTTAGTATCACCGTTTCTGGAGGGT
CGCGAACTCTTCAGCATCTCTGTGAGATGGCAATTCGGTCTAAGCAAGAACTGCTACAGCTTGCCCCAATCGAAGTGGAAAGTAAT
GTATCAAGAGGATGCCCTGAAGGTACTCAAACCTTCGAAAAAGAAAGCGAGTAAGTTAAAATGCTTCTTCGTCTCCTATTTATAAT
ATGGTTTGTTATTGTTAATTTTGTTCTTGTAGAAGAGCTTAATTAATCGTTGTTGTTATGAAATACTATTTGTATGAGATGAACTG
GTGTAATGTAATTCATTTACATAAGTGGAGTCAGAATCAGAATGTTTCCTCCATAACTAACTAGACATGAAGACCTGCCGCGTACA
ATTGTCTTATATTTGAACAACTAAAATTGAACATCTTTTGCCACAACTTTATAAGTGGTTAATATAGCTCAAATATATGGTCAAGT
TCAATAGATTAATAATGGAAATATCAGTTATCGAAATTCATTAACAATCAACTTAACGTTATTAACTACTAATTTTATATCATCCC
CTTTGATAAATGATAGTACACCAATTAGGAAGGAGCATGCTCGCCTAGGAGATTGTCGTTTCCCGCCTTCAGTTTGCAAGCTGCTC
TAGCCGTGTAGCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGGAATTACTAGCGCGTGTCGACAAGCTTGCATGCCGGTCAACA
TGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAA
AGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTC
CTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCA
CGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGGAGCACGACACA
CTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAA
CCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATT
GCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAA
AAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTA
TCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTTGATAAAAGCGAA
CGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTCTTGCGTGAGC
GATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTTTGTGGACAC
GTAGTGCGGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGTGGTCTTGGGAAAAGAAAGCTTGCTGGAGGCTGCTGT
TCAGCCCCATACATTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGCAATATCTCTACTTCTGCTTGACGAGGTATTGTTGCCT
GTACTTCTTTCTTCTTCTTCTTGCTGATTGGTTCTATAAGAAATCTAGTATTTTCTTTGAAACAGAGTTTTCCCGTGGTTTTCGAA
CTTGGAGAAAGATTGTTAAGCTTCTGTATATTCTGCCCAAATTTGTCGGGCCCAATACCGCGGAGAAAATGGCGAAAAACGTTGCG
ATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCGACGTCACTCCTCAGCCAAAACGACACCCCCAT
CTGTCTATCCACTGGCCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAG
CCAGTGACAGTGACCTGGAACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTACACTCT
GAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGACCGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGG
TGGACAAGAAAATTGTGCCCAGGGATTGTGGTTGTAAGCCTTGCATATGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCC
CCAAAGCCCAAGGATGTGCTCACCATTACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGT
CCAGTTCAGCTGGTTTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAGTTCAACAGCACTTTCCGCT
CAGTCAGTGAACTTCCCATCATGCACCAGGACTGGCTCAATGGCAAGGAGCGATCGCTCACCATCACCATCACCATCACCATCACC
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TATTAAAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTT
CTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGT
AATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAG
CGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGATCTCTAGAGTCTCAAGCTTGGCGCGCCCACGTGACT
AGTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTT
TCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGCTAGAGCAGC
TTGAGCTTGGATCAGATTGTCGTTTCCCGCCTTCAGTTTAAACTATCAGTGTTTGACAGGATATATTGGCGGGTAAACCTAAGAGA
AAAGAGCGTTTA

图 45E

从2X35S启动子至NOS终止子的表达盒1819号。PDISP/H3维多利亚核苷酸序列加下划线 (SEQ ID NO: 126)

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTT
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GTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCC
CCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGGAGCA
CGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATAT
CCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGC
CATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCAT
CGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAAT

图 45E(续)

CCCACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTTGATAA
AAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTCTTG
CGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTTTG
TGGACACGTAGTGCGGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGTGGTCTTGGGAAAAGAAAGCTTGCTGGAGG
CTGCTGTTCAGCCCCATACATTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGCAATATCTCTACTTCTGCTTGACGAGGTATT
GTTGCCTGTACTTCTTTCTTCTTCTTCTTGCTGATTGGTTCTATAAGAAATCTAGTATTTTCTTTGAAACAGAGTTTTCCCGTGGT
TTTCGAACTTGGAGAAAGATTGTTAAGCTTCTGTATATTCTGCCCAAATTTGTCGGGCCCAATACCGCGGAGAAAATGGCGAAAAA
CGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCCCAAAAACTTCCTGGAAATGACAACA
GCACGGCAACGCTGTGCCTTGGGCACCATGCAGTACCAAACGGAACGATAGTGAAAACAATCACGAATGACCAAATTGAAGTTACT
AATGCTACTGAGCTGGTTCAGAATTCCTCAATAGGTGAAATATGCGACAGTCCTCATCAGATCCTTGATGGAGAAAACTGCACACT
AATAGATGCTCTATTGGGAGACCCTCAGTGTGATGGCTTCCAAAATAAGAAATGGGACCTTTTTGTTGAACGAAGCAAAGCCTACA
GCAACTGTTACCCTTATGATGTGCCGGATTATGCCTCCCTTAGGTCACTAGTTGCCTCATCCGGCACACTGGAGTTTAACAATGAA
AGCTTCAATTGGACTGGAGTCACTCAAAACGGAACAAGTTCTGCTTGCATAAGGAGATCTAATAATAGTTTCTTTAGTAGATTAAA
TTGGTTGACCCACTTAAACTTCAAATACCCAGCATTGAACGTGACTATGCCAAACAATGAACAATTTGACAAATTGTACATTTGGG
GGGTTCACCACCCGGGTACGGACAAGGACCAAATCTTCCTGTATGCTCAATCATCAGGAAGAATCACAGTATCTACCAAAAGAAGC
CAACAAGCTGTAATCCCGAATATCGGATCTAGACCCAGAATAAGGAATATCCCTAGCAGAATAAGCATCTATTGGACAATAGTAAA
ACCGGGAGACATACTTTTGATTAACAGCACAGGGAATCTAATTGCTCCTAGGGGTTACTTCAAAATACGAAGTGGGAAAAGCTCAA
TAATGAGATCAGATGCACCCATTGGCAAATGCAATTCTGAATGCATCACTCCAAATGGAAGCATTCCCAATGACAAACCATTCCAA
AATGTAAACAGGATCACATACGGGGCCTGTCCCAGATATGTTAAGCAAAGCACTCTGAAATTGGCAACAGGAATGCGAAATGTACC
AGAGAAACAAACTAGAGGCATATTTGGCGCAATAGCGGGTTTCATAGAAAATGGTTGGGAGGGAATGGTGGATGGTTGGTACGGTT
TCAGGCATCAAAATTCTGAGGGAAGAGGACAAGCAGCAGATCTCAAAAGCACTCAAGCAGCAATCGATCAAATCAATGGGAAGCTG
AATCGATTGATCGGGAAAACCAACGAGAAATTCCATCAGATTGAAAAAGAATTCTCAGAAGTCGAAGGGAGAATTCAGGACCTTGA
GAAATATGTTGAGGACACTAAAATAGATCTCTGGTCATACAACGCGGAGCTTCTTGTTGCCCTGGAGAACCAACATACAATTGATC
TAACTGACTCAGAAATGAACAAACTGTTTGAAAAAACAAAGAAGCAACTAAGGGAAAATGCTGAGGATATGGGCAATGGTTGTTTC
AAAATATACCACAAATGTGACAATGCCTGCATAGGATCAATCAGAAATGGAACTTATGACCACGATGTATACAGAGATGAAGCATT
AAACAACCGGTTCCAGATCAAGGGAGTTGAGCTGAAGTCAGGGTACAAAGATTGGATCCTATGGATTTCCTTTGCCATATCATGTT
TTTTGCTTTGTGTTGCTTTGTTGGGGTTCATCATGTGGGCCTGCCAAAAGGGCAACATTAGGTGCAACATTTGCATTTGAAGGCCT
ATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTT
ATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAA
AAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGA
ATCCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACG
TTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTA
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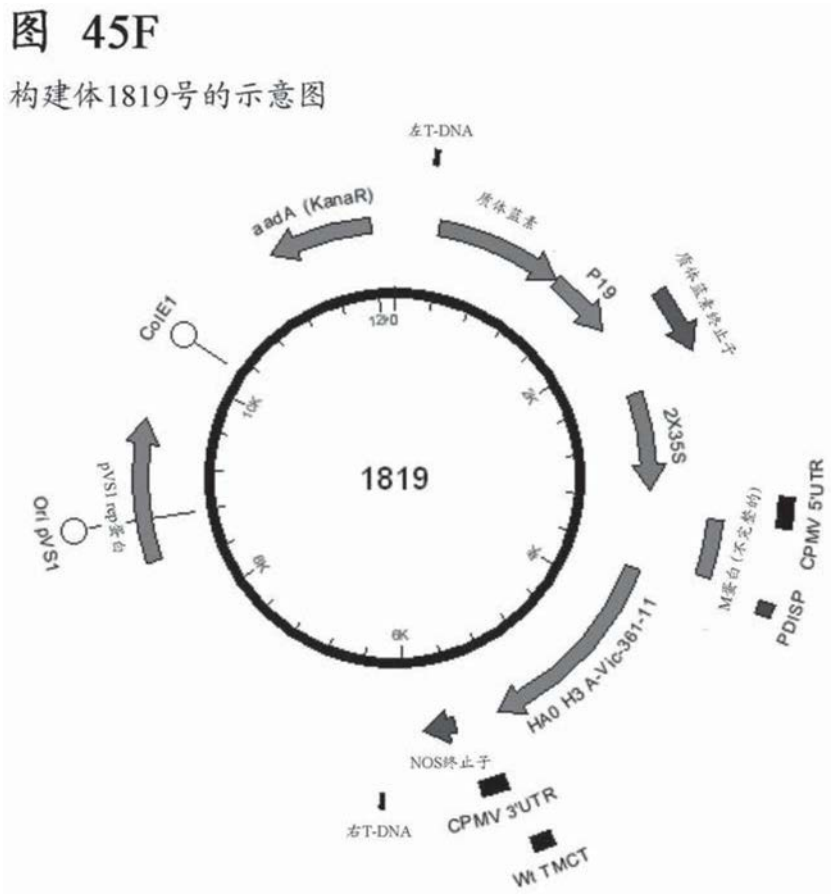


图45:2X3B-2X35S/CPMV-HT*/PDISP/H3维多利亚/NOS(构建体1819号)

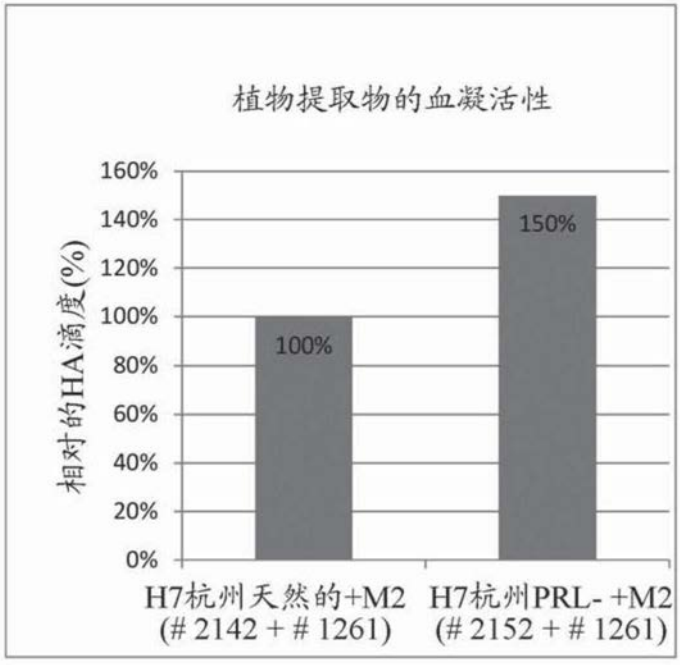


图46A

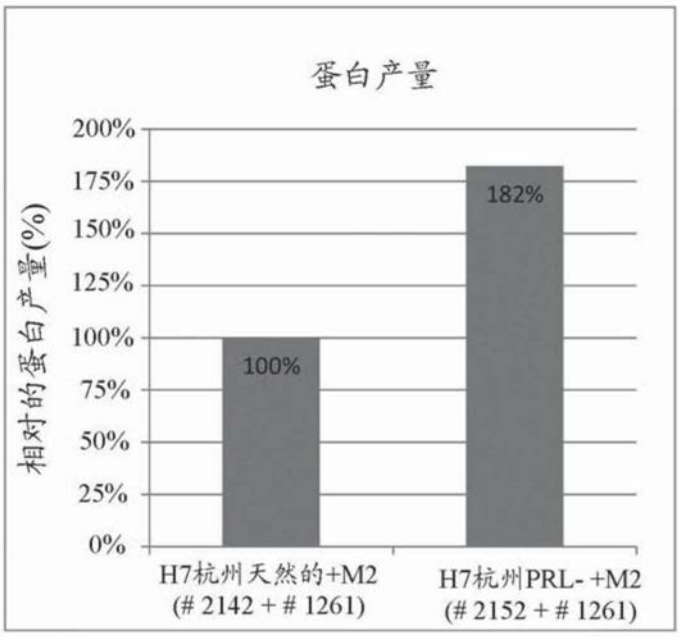

蛋白产量
相对的蛋白产量(%)
200%
175%
150%
125%
100%
75%
50%
25%
0%
100%
182%
H7杭州天然的+M2
(# 2142 + # 1261)
H7杭州PRL- +M2
(# 2152 + # 1261)

图46B

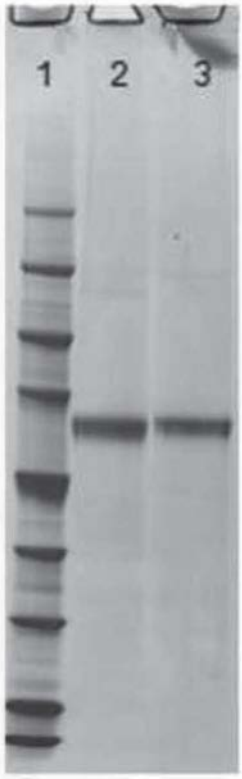

1
2
3

图46C

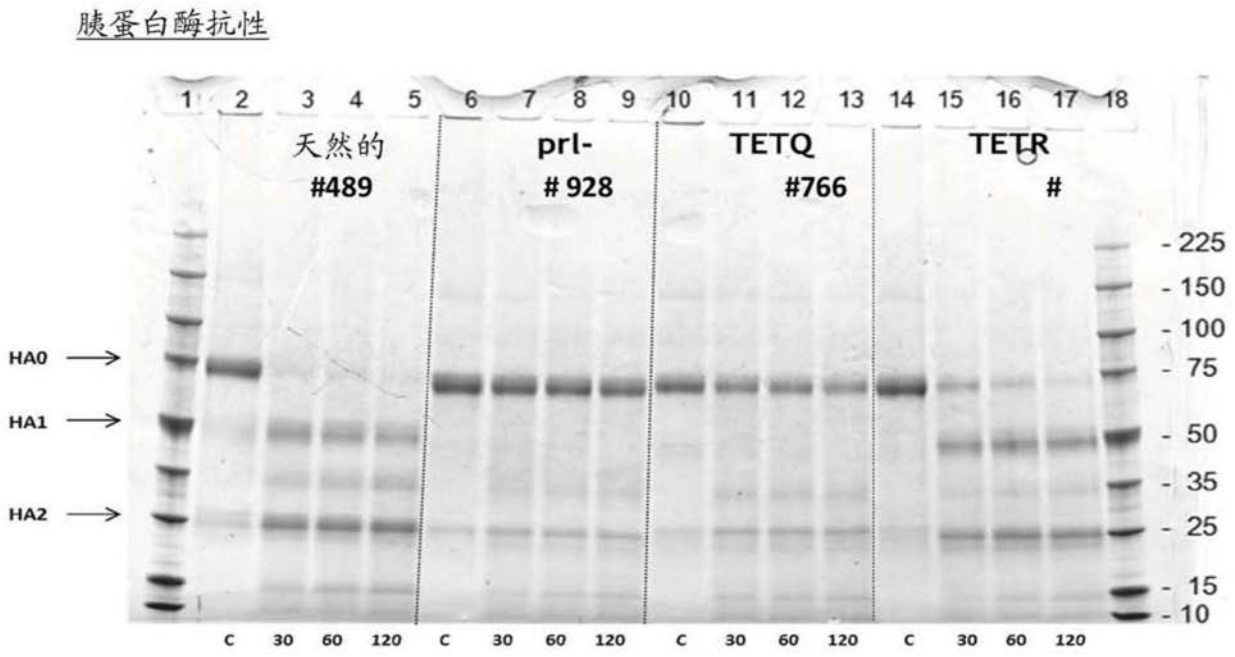

胰蛋白酶抗性
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
天然的
#489
prl-
928
TETQ
#766
TETR
#
HA0
HA1
HA2
225
150
100
75
50
35
25
15
10
C 30 60 120 C 30 60 120 C 30 60 120 C 30 60 120

图47A

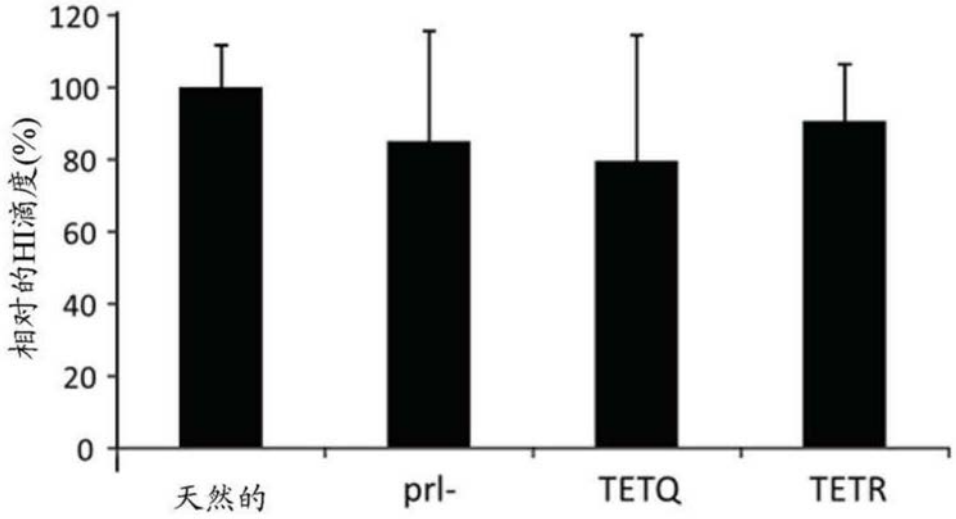

相对的HI滴度(%)
120
100
80
60
40
20
0
天然的
prl-
TETQ
TETR

图47B

图 48A, SEQ ID NO: 127

IF**-H2S157.s1-6r

ACTAAAGAAAATAGGCCTTCATATGCAGATCCTGCACTGCAGAGACCCGT

图 48B, SEQ ID NO: 128

PDISP/H2新加坡的核苷酸序列

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCGGACCAAA
TATGCATTGGATACCATGCCAATAATTCCACAGAGAAGGTCGACACAATTCTAGAGCGGAACGTCACTGTGACTCATGC
CAAGGACATTCTTGAGAAGACCCATAACGGAAAGTTATGCAAACTAAACGGAATCCCTCCACTTGAACTAGGGGACTGT
AGCATTGCCGGATGGCTCCTTGGAAATCCAGAATGTGATAGGCTTCTAAGTGTGCCAGAATGGTCCTATATAATGGAGA
AAGAAAACCCGAGAGACGGTTTGTGTTATCCAGGCAGCTTCAATGATTATGAAGAATTGAAACATCTCCTCAGCAGCGT
GAAACATTTCGAGAAAGTAAAGATTCTGCCCAAAGATAGATGGACACAGCATACAACAACTGGAGGTTCACGGGCCTGC
GCGGTGTCTGGTAATCCATCATTCTTCAGGAACATGGTCTGGCTGACAAAGAAAGAATCAAATTATCCGGTTGCCAAAG
GATCGTACAACAATACAAGCGGAGAACAAATGCTAATAATTTGGGGGGTGCACCATCCCAATGATGAGACAGAACAAAG
AACATTGTACCAGAATGTGGGAACCTATGTTTCCGTAGGCACATCAACATTGAACAAAAGGTCAACCCCAGACATAGCA
ACAAGGCCTAAAGTGAATGGACTAGGAAGTAGAATGGAGTTCTCTTGGACCCTATTGGATATGTGGGACACCATAAATT
TTGAGAGTACTGGTAATCTAATTGCACCAGAGTATGGATTCAAAATATCGAAAAGAGGTAGTTCAGGGATCATGAAAAC
AGAAGGAACACTTGAGAACTGTGAGACCAAATGCCAAACTCCTTTGGGAGCAATAAATACAACATTGCCTTTTCACAAT
GTCCACCCACTGACAATAGGTGAGTGCCCCAAATATGTAAAATCGGAGAAGTTGGTCTTAGCAACAGGACTAAGGAATG
TTCCCCAGATTGAATCAAGAGGATTGTTTGGGGCAATAGCTGGTTTTATAGAAGGAGGATGGCAAGGAATGGTTGATGG
TTGGTATGGATACCATCACAGCAATGACCAGGGATCAGGGTATGCAGCAGACAAAGAATCCACTCAAAAGGCATTTGAT
GGAATCACCAACAAGGTAAATTCTGTGATTGAAAAGATGAACACCCAATTTGAAGCTGTTGGGAAAGAGTTCAGTAACT
TAGAGAGAAGACTGGAGAACTTGAACAAAAAGATGGAAGACGGGTTTCTAGATGTGTGGACATACAATGCTGAGCTTCT
AGTTCTGATGGAAAATGAGAGGACACTTGACTTTCATGATTCTAATGTCAAGAATCTGTATGATAAAGTCAGAATGCAG
CTGAGAGACAACGTCAAAGAACTAGGAAATGGATGTTTTGAATTTTATCACAAATGTGATGATGAATGCATGAATAGTG
TGAAAAACGGGACGTATGATTATCCCAAGTATGAAGAAGAGTCTAAACTAAATAGAAATGAAATCAAAGGGGTAAAATT
GAGCAGCATGGGGGTTTATCAAATCCTTGCCATTTATGCTACAGTAGCAGGTTCTCTGTCACTGGCAATCATGATGGCT
GGGATCTCTTTCTGGATGTGCTCCAACGGGTCTCTGCAGTGCAGGATCTGCATATGA

图 48C, SEQ ID NO: 129

从2X35S启动子至NOS终止子的表达盒2220号。PDISP/H2新加坡核苷酸序列加下划线

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTG
AGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGAT
AGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGAC
AGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAG
TGGATTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCA
AAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTT
ATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATG
CCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTC
TTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCCT
TCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTTGATAAAAGCGAACGTGGGGAA
ACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTCTTGCGTGAGCGA
TCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTTTGTG
GACACGTAGTGCGGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGTGGTCTTGGGAAAAGAAAGCTTGCT
GGAGGCTGCTGTTCAGCCCCATACATTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGCAATATCTCTACTTCTGCT
TGACGAGGTATTGTTGCCTGTACTTCTTTCTTCTTCTTCTTGCTGATTGGTTCTATAAGAAATCTAGTATTTTCTTTGA

图 48C(续)

AACAGAGTTTTCCCGTGGTTTTCGAACTTGGAGAAAGATTGTTAAGCTTCTGTATATTCTGCCCAAATTTGTCGGGCCC
AATACCGCGGAGAAAATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGA
TCTTCGCGGACCAAATATGCATTGGATACCATGCCAATAATTCCACAGAGAAGGTCGACACAATTCTAGAGCGGAACGT
CACTGTGACTCATGCCAAGGACATTCTTGAGAAGACCCATAACGGAAAGTTATGCAAACTAAACGGAATCCCTCCACTT
GAACTAGGGGACTGTAGCATTGCCGGATGGCTCCTTGGAAATCCAGAATGTGATAGGCTTCTAAGTGTGCCAGAATGGT
CCTATATAATGGAGAAAGAAAACCCGAGAGACGGTTTGTGTTATCCAGGCAGCTTCAATGATTATGAAGAATTGAAACA
TCTCCTCAGCAGCGTGAAACATTTCGAGAAAGTAAAGATTCTGCCCAAAGATAGATGGACACAGCATACAACAACTGGA
GGTTCACGGGCCTGCGCGGTGTCTGGTAATCCATCATTCTTCAGGAACATGGTCTGGCTGACAAAGAAAGAATCAAATT
ATCCGGTTGCCAAAGGATCGTACAACAATACAAGCGGAGAACAAATGCTAATAATTTGGGGGGTGCACCATCCCAATGA
TGAGACAGAACAAAGAACATTGTACCAGAATGTGGGAACCTATGTTTCCGTAGGCACATCAACATTGAACAAAAGGTCA
ACCCCAGACATAGCAACAAGGCCTAAAGTGAATGGACTAGGAAGTAGAATGGAGTTCTCTTGGACCCTATTGGATATGT
GGGACACCATAAATTTTGAGAGTACTGGTAATCTAATTGCACCAGAGTATGGATTCAAAATATCGAAAAGAGGTAGTTC
AGGGATCATGAAAACAGAAGGAACACTTGAGAACTGTGAGACCAAATGCCAAACTCCTTTGGGAGCAATAAATACAACA
TTGCCTTTTCACAATGTCCACCCACTGACAATAGGTGAGTGCCCCAAATATGTAAAATCGGAGAAGTTGGTCTTAGCAA
CAGGACTAAGGAATGTTCCCCAGATTGAATCAAGAGGATTGTTTGGGGCAATAGCTGGTTTTATAGAAGGAGGATGGCA
AGGAATGGTTGATGGTTGGTATGGATACCATCACAGCAATGACCAGGGATCAGGGTATGCAGCAGACAAAGAATCCACT
CAAAAGGCATTTGATGGAATCACCAACAAGGTAAATTCTGTGATTGAAAAGATGAACACCCAATTTGAAGCTGTTGGGA
AAGAGTTCAGTAACTTAGAGAGAAGACTGGAGAACTTGAACAAAAAGATGGAAGACGGGTTTCTAGATGTGTGGACATA
CAATGCTGAGCTTCTAGTTCTGATGGAAAATGAGAGGACACTTGACTTTCATGATTCTAATGTCAAGAATCTGTATGAT
AAAGTCAGAATGCAGCTGAGAGACAACGTCAAAGAACTAGGAAATGGATGTTTTGAATTTTATCACAAATGTGATGATG
AATGCATGAATAGTGTGAAAAACGGGACGTATGATTATCCCAAGTATGAAGAAGAGTCTAAACTAAATAGAAATGAAAT
CAAAGGGGTAAAATTGAGCAGCATGGGGGTTTATCAAATCCTTGCCATTTATGCTACAGTAGCAGGTTCTCTGTCACTG
GCAATCATGATGGCTGGGATCTCTTTCTGGATGTGCTCCAACGGGTCTCTGCAGTGCAGGATCTGCATATGAAGGCCTA
TTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTT
TATTTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAAT
TTTATTAAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCAGATCGTTCAAACATTTGGCA
ATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCAT
GTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAAT
ACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGAT

图 48D, SEQ ID NO: 130

PDISP/H2新加坡的氨基酸序列

MAKNVAIFGLLFSLLVLVPSQIFADQICIGYHANNSTEKVDTILERNVTVTHAKDILEKTHNGKLCKLNGIPPLELGDC
SIAGWLLGNPECDRLLSVPEWSYIMEKENPRDGLCYPGSFNDYEELKHLLSSVKHFEKVKILPKDRWTQHTTTGGSRAC
AVSGNPSFFRNMVWLTKKESNYPVAKGSYNNTSGEQMLIIWGVHHPNDETEQRTLYQNVGTYVSVGTSTLNKRSTPDIA
TRPKVNGLGSRMEFSWTLLDMWDTINFESTGNLIAPEYGFKISKRGSSGIMKTEGTLENCETKCQTPLGAINTTLPFHN
VHPLTIGECPKYVKSEKLVLATGLRNVPQIESRGLFGAIAGFIEGGWQGMVDGWYGYHHSNDQGSGYAADKESTQKAFD
GITNKVNSVIEKMNTQFEAVGKEFSNLERRLENLNKKMEDGFLDVWTYNAELLVLMENERTLDFHDSNVKNLYDKVRMQ
LRDNVKELGNGCFEFYHKCDDECMNSVKNGTYDYPKYEEESKLNRNEIKGVKLSSMGVYQILAIYATVAGSLSLAIMMA
GISFWMCSNGSLQCRICI*

图 48E

构建体2220号的示意图

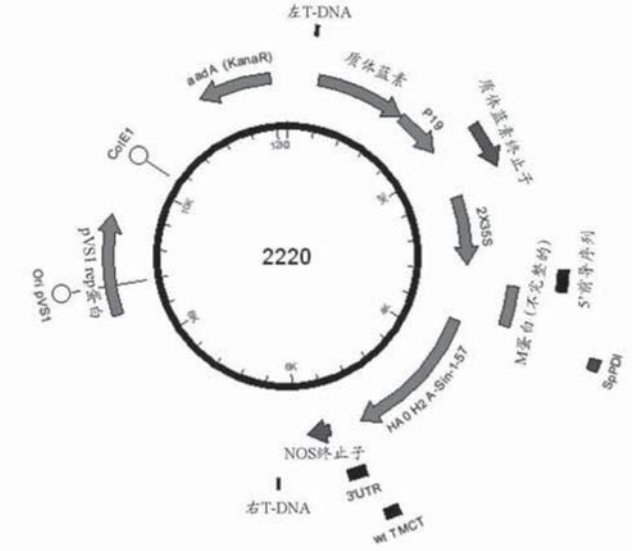


图48:2X35S/CPMV HT+/PDISP/H2新加坡/NOS(构建体2220号)

图 49A, SEQ ID NO: 131

H2S157(Prl-).r

TGCCATCCTCCGCCGGGAACATTCCTTAGTCCTGTTGCTAAGACCAAC

图 49B, SEQ ID NO: 132

H2S157(Prl-).c

AGGAATGTTCCCGGCGGAGGATGGCAAGGAATGGTTGATGGTTGGTATGG

图 49C, SEQ ID NO: 133

从2X35S启动子至NOS终止子的表达盒2221号。具有缺失的蛋白水解环的PDISP/H2新加坡的核苷酸序列加下划线

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTG
AGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGAT
AGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGAC
AGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAG
TGGATTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCA
AAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTT
ATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATG
CCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTC
TTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCCT
TCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTTGATAAAAGCGAACGTGGGGAA
ACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTCTTGCGTGAGCGA

图 49C(续)

TCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTTTGTG
GACACGTAGTGCGGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGTGGTCTTGGGAAAAGAAAGCTTGCT
GGAGGCTGCTGTTCAGCCCCATACATTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGCAATATCTCTACTTCTGCT
TGACGAGGTATTGTTGCCTGTACTTCTTTCTTCTTCTTCTTGCTGATTGGTTCTATAAGAAATCTAGTATTTTCTTTGA
AACAGAGTTTTCCCGTGGTTTTCGAACTTGGAGAAAGATTGTTAAGCTTCTGTATATTCTGCCCAAATTTGTCGGGCCC
AATACCGCGGAGAAAATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGA
TCTTCGCGGACCAAATATGCATTGGATACCATGCCAATAATTCCACAGAGAAGGTCGACACAATTCTAGAGCGGAACGT
CACTGTGACTCATGCCAAGGACATTCTTGAGAAGACCCATAACGGAAAGTTATGCAAACTAAACGGAATCCCTCCACTT
GAACTAGGGGACTGTAGCATTGCCGGATGGCTCCTTGGAAATCCAGAATGTGATAGGCTTCTAAGTGTGCCAGAATGGT
CCTATATAATGGAGAAAGAAAACCCGAGAGACGGTTTGTGTTATCCAGGCAGCTTCAATGATTATGAAGAATTGAAACA
TCTCCTCAGCAGCGTGAAACATTTCGAGAAAGTAAAGATTCTGCCCAAAGATAGATGGACACAGCATACAACAACTGGA
GGTTCACGGGCCTGCGCGGTGTCTGGTAATCCATCATTCTTCAGGAACATGGTCTGGCTGACAAAGAAAGAATCAAATT
ATCCGGTTGCCAAAGGATCGTACAACAATACAAGCGGAGAACAAATGCTAATAATTTGGGGGGTGCACCATCCCAATGA
TGAGACAGAACAAAGAACATTGTACCAGAATGTGGGAACCTATGTTTCCGTAGGCACATCAACATTGAACAAAAGGTCA
ACCCCAGACATAGCAACAAGGCCTAAAGTGAATGGACTAGGAAGTAGAATGGAGTTCTCTTGGACCCTATTGGATATGT
GGGACACCATAAATTTTGAGAGTACTGGTAATCTAATTGCACCAGAGTATGGATTCAAAATATCGAAAAGAGGTAGTTC
AGGGATCATGAAAACAGAAGGAACACTTGAGAACTGTGAGACCAAATGCCAAACTCCTTTGGGAGCAATAAATACAACA
TTGCCTTTTCACAATGTCCACCCACTGACAATAGGTGAGTGCCCCAAATATGTAAAATCGGAGAAGTTGGTCTTAGCAA
CAGGACTAAGGAATGTTCCCGGCGGAGGATGGCAAGGAATGGTTGATGGTTGGTATGGATACCATCACAGCAATGACCA
GGGATCAGGGTATGCAGCAGACAAAGAATCCACTCAAAAGGCATTTGATGGAATCACCAACAAGGTAAATTCTGTGATT
GAAAAGATGAACACCCAATTTGAAGCTGTTGGGAAAGAGTTCAGTAACTTAGAGAGAAGACTGGAGAACTTGAACAAAA
AGATGGAAGACGGGTTTCTAGATGTGTGGACATACAATGCTGAGCTTCTAGTTCTGATGGAAAATGAGAGGACACTTGA
CTTTCATGATTCTAATGTCAAGAATCTGTATGATAAAGTCAGAATGCAGCTGAGAGACAACGTCAAAGAACTAGGAAAT
GGATGTTTTGAATTTTATCACAAATGTGATGATGAATGCATGAATAGTGTGAAAAACGGGACGTATGATTATCCCAAGT
ATGAAGAAGAGTCTAAACTAAATAGAAATGAAATCAAAGGGGTAAAATTGAGCAGCATGGGGGTTTATCAAATCCTTGC
CATTTATGCTACAGTAGCAGGTTCTCTGTCACTGGCAATCATGATGGCTGGGATCTCTTTCTGGATGTGCTCCAACGGG
TCTCTGCAGTGCAGGATCTGCATATGAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTATG
TTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGT
CGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAG
CTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGAT
TATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTT
TTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTAT
CGCGCGCGGTGTCATCTATGTTACTAGAT

图 49D, SEQ ID NO: 134

具有缺失的蛋白水解环的PDISP/H2新加坡的氨基酸序列

MAKNVAIFGLLFSLLVLVPSQIFADQICIGYHANNSTEKVDTILERNVTVTHAKDILEKTHNGKLCKLNGIPPLELGDCSIAGWLLGNPECD
RLLSVPEWSYIMEKENPRDGLCYPGSFNDYEELKHLLSSVKHFEKVKILPKDRWTQHTTTGGSRACAVSGNPSFFRNMVWLTKKESNYP
VAKGSYNNTSGEQMLIIWGVHHPNDETEQRTLYQNVGTYVSVGTSTLNKRSTPDIATRPKVNGLGSRMEFSWTLLDMWDTINFESTG
NLIAPEYGFKISKRGSSGIMKTEGTLENCETKCQTPLGAINTTLPFHNVHPLTIGECPKYVKSEKLVLATGLRNVPGGGWQGMVDGWYG
YHHSNDQGSGYAADKESTQKAFDGITNKVNSVIEKMNTQFEAVGKEFSNLERRLENLNKKMEDGFLDVWTYNAELLVLMENERTLDF
HDSNVKNLYDKVRMQLRDNVKELGNGCFEFYHKCDDECMNSVKNGTYDYPKYEEESKLNRNEIKGVKLSSMGVYQILAIYATVAGSLSL
AIMMAGISFWMCSNGSLQCRICI*

图 49E

构建体2221号的示意图

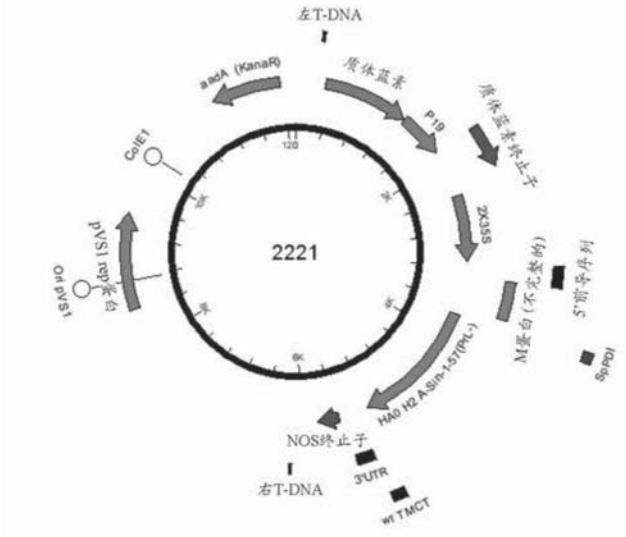


图49:2X35S/CPMV HT+/具有缺失的蛋白水解环的PDISP/H2新加坡/NOS(构建体2221号)

图 50A, SEQ ID NO: 135

从2X35S启动子至NOS终止子的表达盒2222号。PDISP/H2新加坡的核苷酸序列加下划线

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTG
AGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGAT
AGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGAC
AGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAG
TGGATTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCA
AAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTT
ATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATG
CCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTC
TTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCCT
TCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTTGATAAAAGCGAACGTGGGGAA
ACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTCTTGCGTGAGCGA
TCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAGGGCCCAATACCGCGGAGAAAATGGCGAAAAACGTTGCGAT
TTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCGGACCAAATATGCATTGGATACCATGCC
AATAATTCCACAGAGAAGGTCGACACAATTCTAGAGCGGAACGTCACTGTGACTCATGCCAAGGACATTCTTGAGAAGA
CCCATAACGGAAAGTTATGCAAACTAAACGGAATCCCTCCACTTGAACTAGGGGACTGTAGCATTGCCGGATGGCTCCT
TGGAAATCCAGAATGTGATAGGCTTCTAAGTGTGCCAGAATGGTCCTATATAATGGAGAAAGAAAACCCGAGAGACGGT
TTGTGTTATCCAGGCAGCTTCAATGATTATGAAGAATTGAAACATCTCCTCAGCAGCGTGAAACATTTCGAGAAAGTAA
AGATTCTGCCCAAAGATAGATGGACACAGCATACAACAACTGGAGGTTCACGGGCCTGCGCGGTGTCTGGTAATCCATC
ATTCTTCAGGAACATGGTCTGGCTGACAAAGAAAGAATCAAATTATCCGGTTGCCAAAGGATCGTACAACAATACAAGC
GGAGAACAAATGCTAATAATTTGGGGGGTGCACCATCCCAATGATGAGACAGAACAAAGAACATTGTACCAGAATGTGG
GAACCTATGTTTCCGTAGGCACATCAACATTGAACAAAAGGTCAACCCCAGACATAGCAACAAGGCCTAAAGTGAATGG
ACTAGGAAGTAGAATGGAGTTCTCTTGGACCCTATTGGATATGTGGGACACCATAAATTTTGAGAGTACTGGTAATCTA

图 50A(续)

ATTGCACCAGAGTATGGATTCAAAATATCGAAAAGAGGTAGTTCAGGGATCATGAAAACAGAAGGAACACTTGAGAACT
GTGAGACCAAATGCCAAACTCCTTTGGGAGCAATAAATACAACATTGCCTTTTCACAATGTCCACCCACTGACAATAGG
TGAGTGCCCCAAATATGTAAAATCGGAGAAGTTGGTCTTAGCAACAGGACTAAGGAATGTTCCCCAGATTGAATCAAGA
GGATTGTTTGGGGCAATAGCTGGTTTTATAGAAGGAGGATGGCAAGGAATGGTTGATGGTTGGTATGGATACCATCACA
GCAATGACCAGGGATCAGGGTATGCAGCAGACAAAGAATCCACTCAAAAGGCATTTGATGGAATCACCAACAAGGTAAA
TTCTGTGATTGAAAAGATGAACACCCAATTTGAAGCTGTTGGGAAAGAGTTCAGTAACTTAGAGAGAAGACTGGAGAAC
TTGAACAAAAAGATGGAAGACGGGTTTCTAGATGTGTGGACATACAATGCTGAGCTTCTAGTTCTGATGGAAAATGAGA
GGACACTTGACTTTCATGATTCTAATGTCAAGAATCTGTATGATAAAGTCAGAATGCAGCTGAGAGACAACGTCAAAGA
ACTAGGAAATGGATGTTTTGAATTTTATCACAAATGTGATGATGAATGCATGAATAGTGTGAAAAACGGGACGTATGAT
TATCCCAAGTATGAAGAAGAGTCTAAACTAAATAGAAATGAAATCAAAGGGGTAAAATTGAGCAGCATGGGGGTTTATC
AAATCCTTGCCATTTATGCTACAGTAGCAGGTTCTCTGTCACTGGCAATCATGATGGCTGGGATCTCTTTCTGGATGTG
CTCCAACGGGTCTCTGCAGTGCAGGATCTGCATATGAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTG
CATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTTCTTTGTGAGCTCCTG
TTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAAAAAAAAAAAAAGACCGGGAATT
CGATATCAAGCTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTC
TTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTAT
GAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAG
GATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGAT

图 50B, SEQ ID NO: 136

从2X35S启动子至NOS终止子的表达盒2223号。具有缺失的蛋白水解环的PDISP/H2新加坡的核苷酸序列加下划线

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTG
AGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGAT
AGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGAC
AGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAG
TGGATTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCA
AAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTT
ATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATG
CCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTC
TTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCCT
TCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTTGATAAAAGCGAACGTGGGGAA
ACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTCTTGCGTGAGCGA
TCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAGGGCCCAATACCGCGGAGAAAATGGCGAAAAACGTTGCGAT
TTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCGGACCAAATATGCATTGGATACCATGCC
AATAATTCCACAGAGAAGGTCGACACAATTCTAGAGCGGAACGTCACTGTGACTCATGCCAAGGACATTCTTGAGAAGA
CCCATAACGGAAAGTTATGCAAACTAAACGGAATCCCTCCACTTGAACTAGGGGACTGTAGCATTGCCGGATGGCTCCT
TGGAAATCCAGAATGTGATAGGCTTCTAAGTGTGCCAGAATGGTCCTATATAATGGAGAAAGAAAACCCGAGAGACGGT
TTGTGTTATCCAGGCAGCTTCAATGATTATGAAGAATTGAAACATCTCCTCAGCAGCGTGAAACATTTCGAGAAAGTAA
AGATTCTGCCCAAAGATAGATGGACACAGCATACAACAACTGGAGGTTCACGGGCCTGCGCGGTGTCTGGTAATCCATC
ATTCTTCAGGAACATGGTCTGGCTGACAAAGAAAGAATCAAATTATCCGGTTGCCAAAGGATCGTACAACAATACAAGC
GGAGAACAAATGCTAATAATTTGGGGGGTGCACCATCCCAATGATGAGACAGAACAAAGAACATTGTACCAGAATGTGG
GAACCTATGTTTCCGTAGGCACATCAACATTGAACAAAAGGTCAACCCCAGACATAGCAACAAGGCCTAAAGTGAATGG
ACTAGGAAGTAGAATGGAGTTCTCTTGGACCCTATTGGATATGTGGGACACCATAAATTTTGAGAGTACTGGTAATCTA
ATTGCACCAGAGTATGGATTCAAAATATCGAAAAGAGGTAGTTCAGGGATCATGAAAACAGAAGGAACACTTGAGAACT
GTGAGACCAAATGCCAAACTCCTTTGGGAGCAATAAATACAACATTGCCTTTTCACAATGTCCACCCACTGACAATAGG
TGAGTGCCCCAAATATGTAAAATCGGAGAAGTTGGTCTTAGCAACAGGACTAAGGAATGTTCCCGGCGGAGGATGGCAA
GGAATGGTTGATGGTTGGTATGGATACCATCACAGCAATGACCAGGGATCAGGGTATGCAGCAGACAAAGAATCCACTC
AAAAGGCATTTGATGGAATCACCAACAAGGTAAATTCTGTGATTGAAAAGATGAACACCCAATTTGAAGCTGTTGGGAA
AGAGTTCAGTAACTTAGAGAGAAGACTGGAGAACTTGAACAAAAAGATGGAAGACGGGTTTCTAGATGTGTGGACATAC
AATGCTGAGCTTCTAGTTCTGATGGAAAATGAGAGGACACTTGACTTTCATGATTCTAATGTCAAGAATCTGTATGATA
AAGTCAGAATGCAGCTGAGAGACAACGTCAAAGAACTAGGAAATGGATGTTTTGAATTTTATCACAAATGTGATGATGA
ATGCATGAATAGTGTGAAAAACGGGACGTATGATTATCCCAAGTATGAAGAAGAGTCTAAACTAAATAGAAATGAAATC

图 50B(续)

AAAGGGGTAAAATTGAGCAGCATGGGGGTTTATCAAATCCTTGCCATTTATGCTACAGTAGCAGGTTCTCTGTCACTGG
CAATCATGATGGCTGGGATCTCTTTCTGGATGTGCTCCAACGGGTCTCTGCAGTGCAGGATCTGCATATGAAGGCCTAT
TTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTT
ATTTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAATT
TTATTAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCAGATCGTTCAAACATTTGGCAA
TAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATG
TAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATA
CGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGAT

图 50C

构建体2222号的示意图

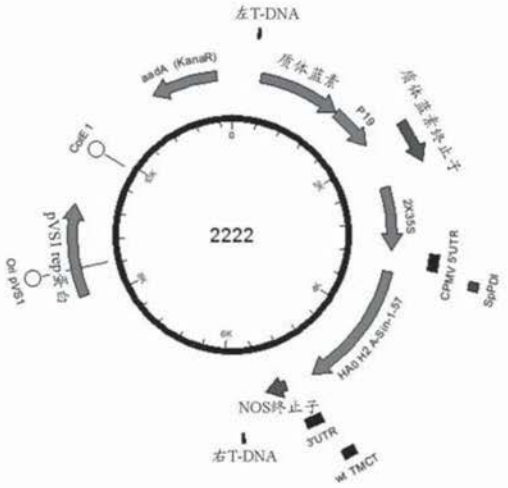


图 50D

构建体2223号的示意图

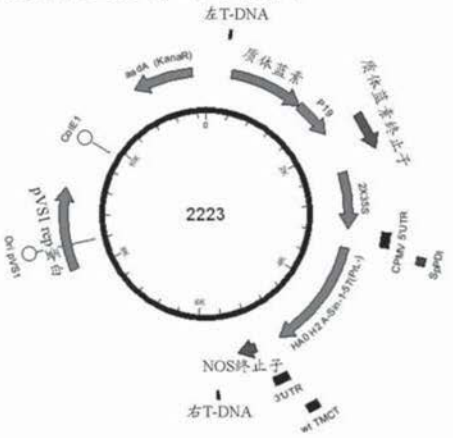


图50:2X35S/CPMV 160+/NOS表达系统中的PDISP/H2新加坡(构建体2222号)和具有缺失的蛋白水解环的PDISP/H2新加坡(构建体2223号)

图 51A, SEQ ID NO: 137

PDISP/H3珀斯的核苷酸序列

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCGCAAAAAC
TTCCTGGAAATGACAACAGCACGGCAACGCTGTGCCTTGGGCACCATGCAGTACCAAACGGAACGATAGTGAAAACAAT
CACGAATGACCAAATTGAAGTTACTAATGCTACTGAGCTGGTTCAGAGTTCCTCAACAGGTGAAATATGCGACAGTCCT
CATCAGATCCTTGATGGAAAAAACTGCACACTAATAGATGCTCTATTGGGAGACCCTCAGTGTGATGGCTTCCAAAATA
AGAAATGGGACCTTTTTGTTGAACGCAGCAAAGCCTACAGCAACTGTTACCCTTATGATGTGCCGGATTATGCCTCCCT
TAGGTCACTAGTTGCCTCATCCGGCACACTGGAGTTTAACAATGAAAGCTTCAATTGGACTGGAGTCACTCAAAACGGA
ACAAGCTCTGCTTGCATAAGGAGATCTAAAAACAGTTTCTTTAGTAGATTGAATTGGTTGACCCACTTAAACTTCAAAT
ACCCAGCATTGAACGTGACTATGCCAAACAATGAACAATTTGACAAATTGTACATTTGGGGGGTTCACCACCCGGGTAC
GGACAAAGACCAAATCTTCCTGTATGCTCAAGCATCAGGAAGAATCACAGTCTCTACCAAAAGAAGCCAACAAACCGTA
AGCCCGAATATCGGATCTAGACCCAGAGTAAGGAATATCCCTAGCAGAATAAGCATCTATTGGACAATAGTAAAACCGG
GAGACATACTTTTGATTAACAGCACAGGGAATCTAATTGCTCCTAGGGGTTACTTCAAAATACGAAGTGGGAAAAGCTC
AATAATGAGATCAGATGCACCCATTGGCAAATGCAATTCTGAATGCATCACTCCAAATGGAAGCATTCCCAATGACAAA
CCATTCCAAAATGTAAACAGGATCACATACGGGGCCTGTCCCAGATATGTTAAGCAAAACACTCTGAAATTGGCAACAG
GGATGCGAAATGTACCAGAGAAACAAACTAGAGGCATATTTGGCGCAATCGCGGGTTTCATAGAAAATGGTTGGGAGGG
AATGGTGGATGGTTGGTACGGTTTCAGGCATCAAAATTCTGAGGGAAGAGGACAAGCAGCAGATCTCAAAAGCACTCAA
GCAGCAATCGATCAAATCAATGGGAAGCTGAATAGATTGATCGGGAAAACCAACGAGAAATTCCATCAGATTGAAAAAG
AATTCTCAGAAGTCGAAGGGAGAATTCAGGACCTTGAGAAATATGTTGAGGACACTAAAATAGATCTCTGGTCATACAA
CGCGGAGCTTCTTGTTGCCCTGGAGAACCAACATACAATTGATCTAACTGACTCAGAAATGAACAAACTGTTTGAAAAA
ACAAAGAAGCAACTGAGGGAAAATGCTGAGGATATGGGCAATGGTTGTTTCAAAATATACCACAAATGTGACAATGCCT
GCATAGGATCAATCAGAAATGGAACTTATGACCACGATGTATACAGAGATGAAGCATTAAACAACCGGTTTCAGATCAA
GGGAGTTGAGCTGAAGTCAGGGTACAAAGATTGGATCCTATGGATTTCCTTTGCCATATCATGTTTTTTGCTTTGTGTT
GCTTTGTTGGGGTTCATCATGTGGGCCTGCCAAAAAGGCAACATTAGGTGCAACATTTGCATTTGA

图 51B, SEQ ID NO: 138

IF**-H3P1609.S1-6r

ACTAAAGAAAATAGGCCTTCAAATGCAAATGTTGCACCTAATGTTGCCTT

图 51C, SEQ ID NO: 139

PDISP/H3珀斯的氨基酸序列

MAKNVAIFGLLFSLLVLVPSQIFAQKLPGNDNSTATLCLGHHAVPNGTIVKTITNDQIEVTNATELVQSSSTGEICDSP
HQILDGKNCTLIDALLGDPQCDGFQNKKWDLFVERSKAYSNCYPYDVPDYASLRSLVASSGTLEFNNESFNWTGVTQNG
TSSACIRRSKNSFFSRLNWLTHLNFKYPALNVTMPNNEQFDKLYIWGVHHPGTDKDQIFLYAQASGRITVSTKRSQQTV
SPNIGSRPRVRNIPSRISIYWTIVKPGDILLINSTGNLIAPRGYFKIRSGKSSIMRSDAPIGKCNSECITPNGSIPNDK
PFQNVNRITYGACPRYVKQNTLKLATGMRNVPEKQTRGIFGAIAGFIENGWEGMVDGWYGFRHQNSEGRGQAADLKSTQ
AAIDQINGKLNRLIGKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNKLFEK
TKKQLRENAEDMGNGCFKIYHKCDNACIGSIRNGTYDHDVYRDEALNNRFQIKGVELKSGYKDWILWISFAISCFLLCV
ALLGFIMWACQKGNIRCNICI*

图 51D

构建体2019号的示意图

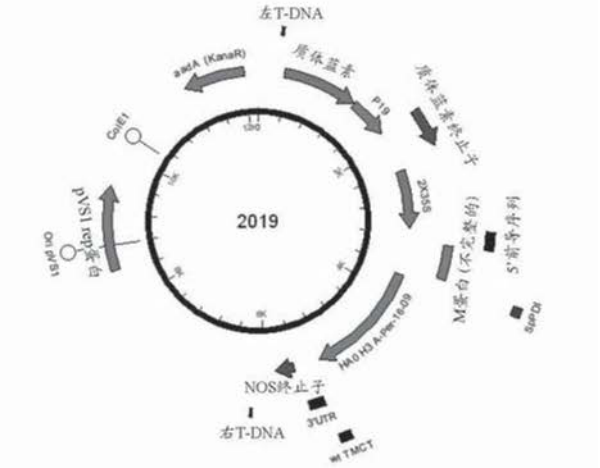


图 51E

构建体2139号的示意图

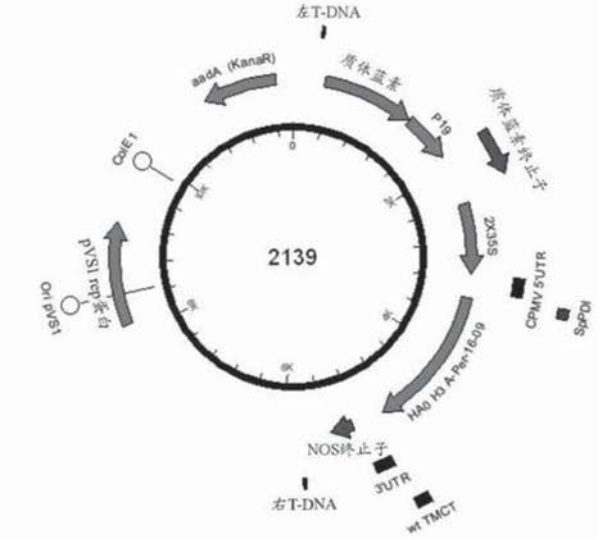


图51:用于PDISP/H3珀斯的2X35S/CPMV HT+(构建体2019号)和160+(构建体2139号)

图 52A, SEQ ID NO: 140

具有缺失的蛋白水解环的PDISP/H3珀斯的核苷酸序列

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCGCAAAAAC
TTCCTGGAAATGACAACAGCACGGCAACGCTGTGCCTTGGGCACCATGCAGTACCAAACGGAACGATAGTGAAAACAAT
CACGAATGACCAAATTGAAGTTACTAATGCTACTGAGCTGGTTCAGAGTTCCTCAACAGGTGAAATATGCGACAGTCCT
CATCAGATCCTTGATGGAAAAAACTGCACACTAATAGATGCTCTATTGGGAGACCCTCAGTGTGATGGCTTCCAAAATA
AGAAATGGGACCTTTTTGTTGAACGCAGCAAAGCCTACAGCAACTGTTACCCTTATGATGTGCCGGATTATGCCTCCCT
TAGGTCACTAGTTGCCTCATCCGGCACACTGGAGTTTAACAATGAAAGCTTCAATTGGACTGGAGTCACTCAAAACGGA
ACAAGCTCTGCTTGCATAAGGAGATCTAAAAACAGTTTCTTTAGTAGATTGAATTGGTTGACCCACTTAAACTTCAAAT
ACCCAGCATTGAACGTGACTATGCCAAACAATGAACAATTTGACAAATTGTACATTTGGGGGGTTCACCACCCGGGTAC
GGACAAAGACCAAATCTTCCTGTATGCTCAAGCATCAGGAAGAATCACAGTCTCTACCAAAAGAAGCCAACAAACCGTA
AGCCCGAATATCGGATCTAGACCCAGAGTAAGGAATATCCCTAGCAGAATAAGCATCTATTGGACAATAGTAAAACCGG
GAGACATACTTTTGATTAACAGCACAGGGAATCTAATTGCTCCTAGGGGTTACTTCAAAATACGAAGTGGGAAAAGCTC
AATAATGAGATCAGATGCACCCATTGGCAAATGCAATTCTGAATGCATCACTCCAAATGGAAGCATTCCCAATGACAAA
CCATTCCAAAATGTAAACAGGATCACATACGGGGCCTGTCCCAGATATGTTAAGCAAAACACTCTGAAATTGGCAACAG
GGATGCGAAATGTACCAGGCGGAGGTTGGGAGGGAATGGTGGATGGTTGGTACGGTTTCAGGCATCAAAATTCTGAGGG
AAGAGGACAAGCAGCAGATCTCAAAAGCACTCAAGCAGCAATCGATCAAATCAATGGGAAGCTGAATAGATTGATCGGG
AAAACCAACGAGAAATTCCATCAGATTGAAAAAGAATTCTCAGAAGTCGAAGGGAGAATTCAGGACCTTGAGAAATATG
TTGAGGACACTAAAATAGATCTCTGGTCATACAACGCGGAGCTTCTTGTTGCCCTGGAGAACCAACATACAATTGATCT
AACTGACTCAGAAATGAACAAACTGTTTGAAAAAACAAAGAAGCAACTGAGGGAAAATGCTGAGGATATGGGCAATGGT
TGTTTCAAAATATACCACAAATGTGACAATGCCTGCATAGGATCAATCAGAAATGGAACTTATGACCACGATGTATACA
GAGATGAAGCATTAAACAACCGGTTTCAGATCAAGGGAGTTGAGCTGAAGTCAGGGTACAAAGATTGGATCCTATGGAT
TTCCTTTGCCATATCATGTTTTTTGCTTTGTGTTGCTTTGTTGGGGTTCATCATGTGGGCCTGCCAAAAAGGCAACATT
AGGTGCAACATTTGCATTTGA

图 52B, SEQ ID NO: 141

H3P1609(Prl-)#2.r

CCCTCCCAACCTCCGCCTGGTACATTTCGCATCCCTGTTGCCAATTTC

图 52C, SEQ ID NO: 142

H3P1609(Prl-)#2.r

AATGTACCAGGCGGAGGTTGGGAGGGAATGGTGGATGGTTGGTACGGT

图 52D, SEQ ID NO: 143

具有缺失的蛋白水解环的PDISP/H3珀斯的氨基酸序列

MAKNVAIFGLLFSLLVLVPSQIFAQKLPGNDNSTATLCLGHHAVPNGTIVKTITNDQIEVTNATELVQSSSTGEICDSP
HQILDGKNCTLIDALLGDPQCDGFQNKKWDLFVERSKAYSNCYPYDVPDYASLRSLVASSGTLEFNNESFNWTGVTQNG
TSSACIRRSKNSFFSRLNWLTHLNFKYPALNVTMPNNEQFDKLYIWGVHHPGTDKDQIFLYAQASGRITVSTKRSQQTV
SPNIGSRPRVRNIPSRISIYWTIVKPGDILLINSTGNLIAPRGYFKIRSGKSSIMRSDAPIGKCNSECITPNGSIPNDK
PFQNVNRITYGACPRYVKQNTLKLATGMRNVPGGGWEGMVDGWYGFRHQNSEGRGQAADLKSTQAAIDQINGKLNRLIG
KTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNKLFEKTKKQLRENAEDMGNG
CFKIYHKCDNACIGSIRNGTYDHDVYRDEALNNRFQIKGVELKSGYKDWILWISFAISCFLLCVALLGFIMWACQKGNI
RCNICI*

图 52E

构建体2039号的示意图

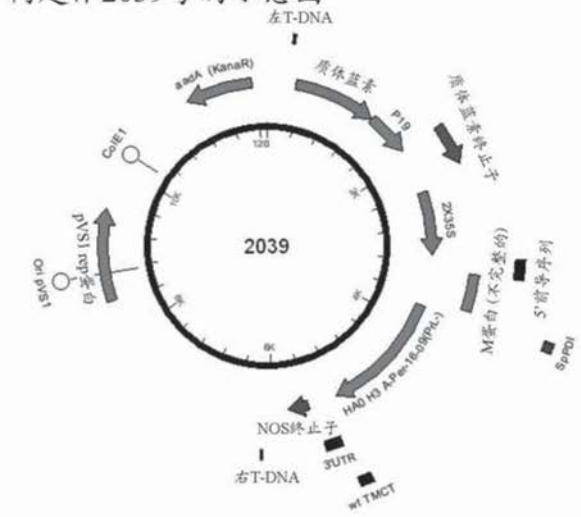


图 52F

构建体2159号的示意图

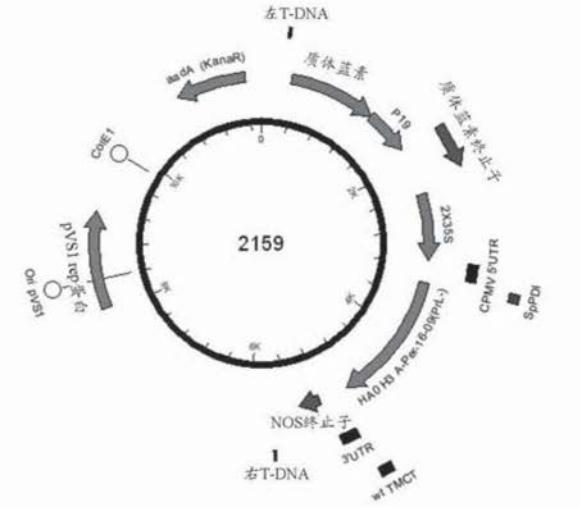


图52:用于具有缺失的蛋白水解环的PDISP/H3珀斯的2X35S/CPMV HT+(构建体2039号)和160+(构建体2159号)

图 53A, SEQ ID NO: 144

具有缺失的蛋白水解环的PDISP/H3维多利亚的核苷酸序列

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCGCAAAAACTTCCTGGAA
ATGACAACAGCACGGCAACGCTGTGCCTTGGGCACCATGCAGTACCAAACGGAACGATAGTGAAAACAATCACGAATGACCAAA
TTGAAGTTACTAATGCTACTGAGCTGGTTCAGAATTCCTCAATAGGTGAAATATGCGACAGTCCTCATCAGATCCTTGATGGAGAA
AACTGCACACTAATAGATGCTCTATTGGGAGACCCTCAGTGTGATGGCTTCCAAAATAAGAAATGGGACCTTTTTGTTGAACGAAG
CAAAGCCTACAGCAACTGTTACCCTTATGATGTGCCGGATTATGCCTCCCTTAGGTCACTAGTTGCCTCATCCGGCACACTGGAGTT
TAACAATGAAAGCTTCAATTGGACTGGAGTCACTCAAAACGGAACAAGTTCTGCTTGCATAAGGAGATCTAATAATAGTTTCTTTA
GTAGATTAAATTGGTTGACCCACTTAAACTTCAAATACCCAGCATTGAACGTGACTATGCCAAACAATGAACAATTTGACAAATTG
TACATTTGGGGGGTTCACCACCCGGGTACGGACAAGGACCAAATCTTCCTGTATGCTCAATCATCAGGAAGAATCACAGTATCTAC
CAAAAGAAGCCAACAAGCTGTAATCCCGAATATCGGATCTAGACCCAGAATAAGGAATATCCCTAGCAGAATAAGCATCTATTGG
ACAATAGTAAAACCGGGAGACATACTTTTGATTAACAGCACAGGGAATCTAATTGCTCCTAGGGGTTACTTCAAAATACGAAGTG
GGAAAAGCTCAATAATGAGATCAGATGCACCCATTGGCAAATGCAATTCTGAATGCATCACTCCAAATGGAAGCATTCCCAATGA
CAAACCATTCCAAAATGTAAACAGGATCACATACGGGGCCTGTCCCAGATATGTTAAGCAAAGCACTCTGAAATTGGCAACAGGA
ATGCGAAATGTACCAGGCGGAGGTTGGGAGGGAATGGTGGATGGTTGGTACGGTTTCAGGCATCAAAATTCTGAGGGAAGAGG
ACAAGCAGCAGATCTCAAAAGCACTCAAGCAGCAATCGATCAAATCAATGGGAAGCTGAATCGATTGATCGGGAAAACCAACGA
GAAATTCCATCAGATTGAAAAAGAATTCTCAGAAGTCGAAGGGAGAATTCAGGACCTTGAGAAATATGTTGAGGACACTAAAATA
GATCTCTGGTCATACAACGCGGAGCTTCTTGTTGCCCTGGAGAACCAACATACAATTGATCTAACTGACTCAGAAATGAACAAACT
GTTTGAAAAAACAAAGAAGCAACTAAGGGAAAATGCTGAGGATATGGGCAATGGTTGTTTCAAAATATACCACAAATGTGACAAT
GCCTGCATAGGATCAATCAGAAATGGAACTTATGACCACGATGTATACAGAGATGAAGCATTAAACAACCGGTTCCAGATCAAGG
GAGTTGAGCTGAAGTCAGGGTACAAAGATTGGATCCTATGGATTTCCTTTGCCATATCATGTTTTTTGCTTTGTGTTGCTTTGTTGG
GGTTCATCATGTGGGCCTGCCAAAAGGGCAACATTAGGTGCAACATTTGCATTTGA

图 53B, SEQ ID NO: 145

H3V36111(Prl-).r

CCCTCCCAACCTCCGCCTGGTACATTTCGCATTCCTGTTGCCAATTTC

图 53C, SEQ ID NO: 146

H3V36111(Prl-).c

AATGTACCAGGCGGAGGTTGGGAGGGAATGGTGGATGGTTGGTACGGT

图 53D, SEQ ID NO: 147

具有缺失的蛋白水解环的PDISP/H3维多利亚的氨基酸序列

MAKNVAIFGLLFSLLVLVPSQIFAQKLPGNDNSTATLCLGHHAVPNGTIVKTITNDQIEVTNATELVQNSSIGEICDSPHQILDGENCTLID
ALLGDPQCDGFQNKKWDLFVERSKAYSNCYPYDVPDYASLRSLVASSGTLEFNNESFNWTGVTQNGTSSACIRRSNNSFFSRLNWLTH
LNFKYPALNVTMPNNEQFDKLYIWGVHHPGTDKDQIFLYAQSSGRITVSTKRSQQAVIPNIGSRPRIRNIPSRISIYWTIVKPGDILLINST
GNLIAPRGYFKIRSGKSSIMRSDAPIGKCNSECITPNGSIPNDKPFQNVNRITYGACPRYVKQSTLKLATGMRNVPGGGWEGMVDGWY
GFRHQNSEGRGQAADLKSTQAAIDQINGKLNRLIGKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDS
EMNKLFEKTKKQLRENAEDMGNGCFKIYHKCDNACIGSIRNGTYDHDVYRDEALNNRFQIKGVELKSGYKDWILWISFAISCFLLCVALL
GFIMWACQKGNIRCNICI*

图 53E

构建体2230号的示意图

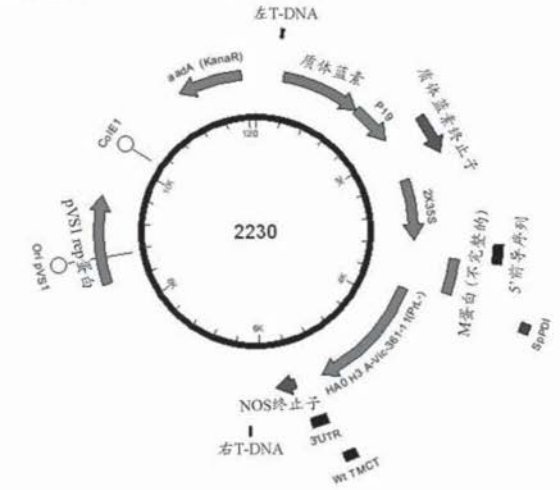


图 53F

构建体2250号的示意图

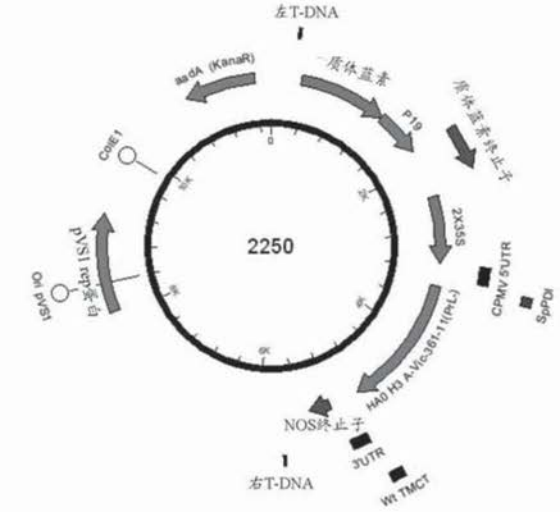


图53:用于具有缺失的蛋白水解环的PDISP/H3维多利亚的2X35S/CPMV HT(构建体2230号)和160+(构建体2250号)

图 54A, SEQ ID NO: 148

PDISP/H7杭州的核苷酸序列

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCGGACAAAATCTGCCTCG
GACATCATGCCGTGTCAAACGGAACCAAAGTAAACACATTAACTGAAAGAGGAGTGGAAGTCGTCAATGCAACTGAAACAGTGG
AACGAACAAACATCCCCAGGATCTGCTCAAAAGGGAAAAGGACAGTTGACCTCGGTCAATGTGGACTCCTGGGGACAATCACTG
GACCACCTCAATGTGACCAATTCCTAGAATTTTCAGCCGATTTAATTATTGAGAGGCGAGAAGGAAGTGATGTCTGTTATCCTGGG
AAATTCGTGAATGAAGAAGCTCTGAGGCAAATTCTCAGAGAATCAGGCGGAATTGACAAGGAAGCAATGGGATTCACATACAGT
GGAATAAGAACTAATGGAGCAACCAGTGCATGTAGGAGATCAGGATCTTCATTCTATGCAGAAATGAAATGGCTCCTGTCAAACA
CAGATAATGCTGCATTCCCGCAGATGACTAAGTCATATAAAAATACAAGAAAAAGCCCAGCTCTAATAGTATGGGGGATCCATCA
TTCCGTATCAACTGCAGAGCAAACCAAGCTATATGGGAGTGGAAACAAACTGGTGACAGTTGGGAGTTCTAATTATCAACAATCT
TTTGTACCGAGTCCAGGAGCGAGACCACAAGTTAATGGTATATCTGGAAGAATTGACTTTCATTGGCTAATGCTAAATCCCAATGA
TACAGTCACTTTCAGTTTCAATGGGGCTTTCATAGCTCCAGACCGTGCAAGCTTCCTGAGAGGAAAATCTATGGGAATCCAGAGTG
GAGTACAGGTTGATGCCAATTGTGAAGGGGACTGCTATCATAGTGGAGGGACAATAATAAGTAACTTGCCATTTCAGAACATAGA
TAGCAGGGCAGTTGGAAAATGTCCGAGATATGTTAAGCAAAGGAGTCTGCTGCTAGCAACAGGGATGAAGAATGTTCCTGAGAT
TCCAAAGGGAAGAGGCCTATTTGGTGCTATAGCGGGTTTCATTGAAAATGGATGGGAAGGCCTAATTGATGGTTGGTATGGTTTC
AGACACCAGAATGCACAGGGAGAGGGAACTGCTGCAGATTACAAAAGCACTCAATCGGCAATTGATCAAATAACAGGAAAATTA
AACCGGCTTATAGAAAAAACCAACCAACAATTTGAGTTGATCGACAATGAATTCAATGAGGTAGAGAAGCAAATCGGTAATGTGA
TAAATTGGACCAGAGATTCTATAACAGAAGTGTGGTCATACAATGCTGAACTCTTGGTAGCAATGGAGAACCAGCATACAATTGA
TCTGGCTGATTCAGAAATGGACAAACTGTACGAACGAGTGAAAAGACAGCTGAGAGAGAATGCTGAAGAAGATGGCACTGGTTG
CTTTGAAATATTTCACAAGTGTGATGATGACTGTATGGCCAGTATTAGAAATAACACCTATGATCACAGCAAATACAGGGAAGAG
GCAATGCAAAATAGAATACAGATTGACCCAGTCAAACTAAGCAGCGGCTACAAAGATGTGATACTTTGGTTTAGCTTCGGGGCAT
CATGTTTCATACTTCTAGCCATTGTAATGGGCCTTGTCTTCATATGTGTAAAGAATGGAAACATGCGGTGCACTATTTGTATATAA

图 54B, SEQ ID NO: 149

IF*-H7H113.s1-6r

ACTAAAGAAAATAGGCCTTTATATACAAATAGTGCACCGCATGTTTCCAT

图 54C, SEQ ID NO: 150

PDISP/H7杭州的氨基酸序列

MAKNVAIFGLLFSLLVLVPSQIFADKICLGHHAVSNGTKVNTLTERGVEVVNATETVERTNIPRICSKGKRTVDLGQCGLLGTITGPPQCD
QFLEFSADLIIERREGSDVCYPGKFVNEEALRQILRESGGIDKEAMGFTYSGIRTNGATSACRRSGSSFYAEMKWLLSNTDNAAFPQMTK
SYKNTRKSPALIVWGIHHSVSTAEQTKLYGSGNKLVTVGSSNYQQSFVPSPGARPQVNGISGRIDFHWLMLNPNDTVTFSFNGAFIAPD
RASFLRGKSMGIQSGVQVDANCEGDCYHSGGTIISNLPFQNIDSRAVGKCPRYVKQRSLLLATGMKNVPEIPKGRGLFGAIAGFIENGW
EGLIDGWYGFRHQNAQGEGTAADYKSTQSAIDQITGKLNRLIEKTNQQFELIDNEFNEVEKQIGNVINWTRDSITEVWSYNAELLVAME
NQHTIDLADSEMDKLYERVKRQLRENAEEDGTGCFEIFHKCDDDCMASIRNNTYDHSKYREEAMQNRIQIDPVKLSSGYKDVILWFSFG
ASCFILLAIVMGLVFICVKNGNMRCTICI*

图 54D

构建体2142号的示意图

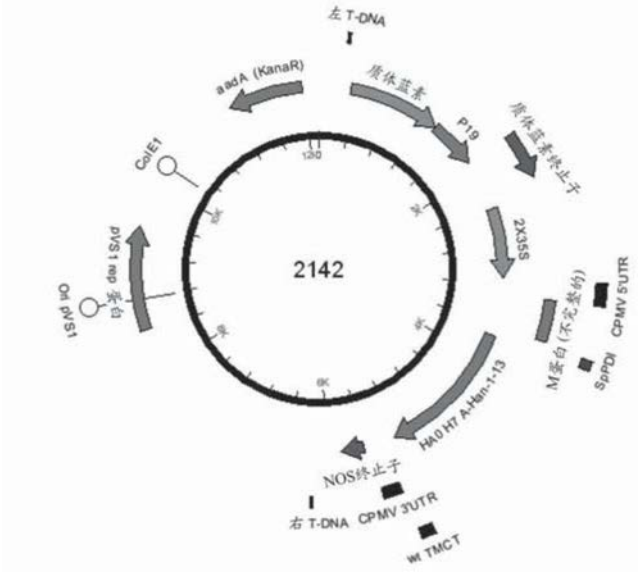


图54:2X35S/CPMV HT+/PDISP/H7杭州/NOS(构建体2142号)

图 55A, SEQ ID NO: 151

具有缺失的蛋白水解环的PDISP/H7杭州的核苷酸序列

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCGGACAAAATCTGCCTCG
GACATCATGCCGTGTCAAACGGAACCAAAGTAAACACATTAACTGAAAGAGGAGTGGAAGTCGTCAATGCAACTGAAACAGTGG
AACGAACAAACATCCCCAGGATCTGCTCAAAAGGGAAAAGGACAGTTGACCTCGGTCAATGTGGACTCCTGGGGACAATCACTG
GACCACCTCAATGTGACCAATTCCTAGAATTTTCAGCCGATTTAATTATTGAGAGGCGAGAAGGAAGTGATGTCTGTTATCCTGGG
AAATTCGTGAATGAAGAAGCTCTGAGGCAAATTCTCAGAGAATCAGGCGGAATTGACAAGGAAGCAATGGGATTCACATACAGT
GGAATAAGAACTAATGGAGCAACCAGTGCATGTAGGAGATCAGGATCTTCATTCTATGCAGAAATGAAATGGCTCCTGTCAAACA
CAGATAATGCTGCATTCCCGCAGATGACTAAGTCATATAAAAATACAAGAAAAAGCCCAGCTCTAATAGTATGGGGGATCCATCA
TTCCGTATCAACTGCAGAGCAAACCAAGCTATATGGGAGTGGAAACAAACTGGTGACAGTTGGGAGTTCTAATTATCAACAATCT
TTTGTACCGAGTCCAGGAGCGAGACCACAAGTTAATGGTATATCTGGAAGAATTGACTTTCATTGGCTAATGCTAAATCCCAATGA
TACAGTCACTTTCAGTTTCAATGGGGCTTTCATAGCTCCAGACCGTGCAAGCTTCCTGAGAGGAAAATCTATGGGAATCCAGAGTG
GAGTACAGGTTGATGCCAATTGTGAAGGGGACTGCTATCATAGTGGAGGGACAATAATAAGTAACTTGCCATTTCAGAACATAGA
TAGCAGGGCAGTTGGAAAATGTCCGAGATATGTTAAGCAAAGGAGTCTGCTGCTAGCAACAGGGATGAAGAATGTTCCTGGCGG
ATGGGAAGGCCTAATTGATGGTTGGTATGGTTTCAGACACCAGAATGCACAGGGAGAGGGAACTGCTGCAGATTACAAAAGCAC
TCAATCGGCAATTGATCAAATAACAGGAAAATTAAACCGGCTTATAGAAAAAACCAACCAACAATTTGAGTTGATCGACAATGAA
TTCAATGAGGTAGAGAAGCAAATCGGTAATGTGATAAATTGGACCAGAGATTCTATAACAGAAGTGTGGTCATACAATGCTGAAC
TCTTGGTAGCAATGGAGAACCAGCATACAATTGATCTGGCTGATTCAGAAATGGACAAACTGTACGAACGAGTGAAAAGACAGCT
GAGAGAGAATGCTGAAGAAGATGGCACTGGTTGCTTTGAAATATTTCACAAGTGTGATGATGACTGTATGGCCAGTATTAGAAAT
AACACCTATGATCACAGCAAATACAGGGAAGAGGCAATGCAAAATAGAATACAGATTGACCCAGTCAAACTAAGCAGCGGCTAC
AAAGATGTGATACTTTGGTTTAGCTTCGGGGCATCATGTTTCATACTTCTAGCCATTGTAATGGGCCTTGTCTTCATATGTGTAAAG
AATGGAAACATGCGGTGCACTATTTGTATATAA

图 55B, SEQ ID NO: 152

H7H113(PrL-).r

CCTTCCCATCCGCCAGGAACATTCTTCATCCCTGTTGCTAGCAGCAGAC

图 55C, SEQ ID NO: 153

H7H113(PrL-).c

AGAATGTTCCTGGCGGATGGGAAGGCCTAATTGATGGTTGGTATGGTT

图 55D, SEQ ID NO: 154

具有缺失的蛋白水解环的PDISP/H7杭州的氨基酸序列

MAKNVAIFGLLFSLLVLVPSQIFADKICLGHHAVSNGTKVNTLTERGVEVVNATETVERTNIPRICSKGKRTVDLGQCGLLGTITGPPQCD
QFLEFSADLIIERREGSDVCYPGKFVNEEALRQILRESGGIDKEAMGFTYSGIRTNGATSACRRSGSSFYAEMKWLLSNTDNAAFPQMTK
SYKNTRKSPALIVWGIHHSVSTAEQTKLYGSGNKLVTVGSSNYQQSFVPSPGARPQVNGISGRIDFHWLMLNPNDTVTFSFNGAFIAPD
RASFLRGKSMGIQSGVQVDANCEGDCYHSGGTIISNLPFQNIDSRAVGKCPRYVKQRSLLLATGMKNVPGGWEGLIDGWYGFRHQNA
QGEGTAADYKSTQSAIDQITGKLNRLIEKTNQQFELIDNEFNEVEKQIGNVINWTRDSITEVWSYNAELLVAMENQHTIDLADSEMDKL
YERVKRQLRENAEEDGTGCFEIFHKCDDDCMASIRNNTYDHSKYREEAMQNRIQIDPVKLSSGYKDVILWFSFGASCFILLAIVMGLVFIC
VKNGNMRCTICI*

图 55E

构建体2152号的示意图

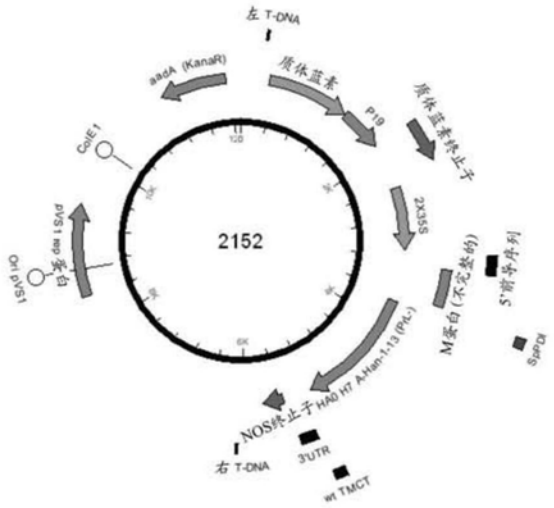


图55:2X35S/CPMV HT+/具有缺失的蛋白水解环的PDISP/H7杭州/NOS(构建体2152号)

图 56A, SEQ ID NO: 155

PDISP/H9香港的核苷酸序列

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCGGATAAAATCTGCATCG
GCCACCAGTCAACAAACTCCACAGAAACTGTGGACACGCTAACAGAAACCAATGTTCCTGTGACACATGCCAAAGAATTGCTCCAC
ACAGAGCATAATGGAATGCTGTGTGCAACAAGCCTGGGACATCCCCTCATTCTAGACACATGCACTATTGAAGGACTAGTCTATG
GCAACCCTTCTTGTGACCTGCTGTTGGGAGGAAGAGAATGGTCCTACATCGTCGAAAGATCATCAGCTGTAAATGGAACGTGTTA
CCCTGGGAATGTAGAAAACCTAGAGGAACTCAGGACACTTTTTAGTTCCGCTAGTTCCTACCAAAGAATCCAAATCTTCCCAGACA
CAACCTGGAATGTGACTTACACTGGAACAAGCAGAGCATGTTCAGGTTCATTCTACAGGAGTATGAGATGGCTGACTCAAAAGAG
CGGTTTTTACCCTGTTCAAGACGCCCAATACACAAATAACAGGGGAAAGAGCATTCTTTTCGTGTGGGGCATACATCACCCACCCA
CCTATACCGAGCAAACAAATTTGTACATAAGAAACGACACAACAACAAGCGTGACAACAGAAGATTTGAATAGGACCTTCAAACC
AGTGATAGGGCCAAGGCCCCTTGTCAATGGTCTGCAGGGAAGAATTGATTATTATTGGTCGGTACTAAAACCAGGCCAAACATTG
CGAGTACGATCCAATGGGAATCTAATTGCTCCATGGTATGGACACGTTCTTTCAGGAGGGAGCCATGGAAGAATCCTGAAGACTG
ATTTAAAAGGTGGTAATTGTGTAGTGCAATGTCAGACTGAAAAAGGTGGCTTAAACAGTACATTGCCATTCCACAATATCAGTAAA
TATGCATTTGGAACCTGCCCCAAATATGTAAGAGTTAATAGTCTCAAACTGGCAGTCGGTCTGAGGAACGTGCCTGCTAGATCAA
GTAGAGGACTATTTGGAGCCATAGCTGGATTCATAGAAGGAGGTTGGCCAGGACTAGTCGCTGGCTGGTATGGTTTCCAGCATTC
AAATGATCAAGGGGTTGGTATGGCTGCAGATAGGGATTCAACTCAAAAGGCAATTGATAAAATAACATCCAAGGTGAATAATATA
GTCGACAAGATGAACAAGCAATATGAAATAATTGATCATGAATTTAGTGAGGTTGAAACTAGACTCAATATGATCAATAATAAGA
TTGATGACCAAATACAAGACGTATGGGCATATAATGCAGAATTGCTAGTACTACTTGAAAATCAAAAAACACTCGATGAGCATGA
TGCGAACGTGAACAATCTATATAACAAGGTGAAGAGGGCACTGGGCTCCAATGCTATGGAAGATGGGAAAGGCTGTTTCGAGCT
ATACCATAAATGTGATGATCAGTGCATGGAAACAATTCGGAACGGGACCTATAATAGGAGAAAGTATAGAGAGGAATCAAGACT
AGAAAGGCAGAAAATAGAGGGGGTTAAGCTGGAATCTGAGGGAACTTACAAAATCCTCACCATTTATTCGACTGTCGCCTCATCT
CTTGTGCTTGCAATGGGGTTTGCTGCCTTCCTGTTCTGGGCCATGTCCAATGGATCTTGCAGATGCAACATTTGTATATAA

图 56B, SEQ ID NO: 156

IF**-H9HK107399.S1-6r

ACTAAAGAAAATAGGCCTTTATATACAAATGTTGCATCTGCAAGATCCAT

图 56C, SEQ ID NO: 157

PDISP/H9香港的氨基酸序列

MAKNVAIFGLLFSLLVLVPSQIFADKICIGHQSTNSTETVDTLTETNVPVTHAKELLHTEHNGMLCATSLGHPLILDTCTIEGLVYGNPSCD
LLLGGREWSYIVERSSAVNGTCYPGNVENLEELRTLFSSASSYQRIQIFPDTTWNVTYTGTSRACSGSFYRSMRWLTQKSGFYPVQDAQ
YTNNRGKSILFVWGIHHPPTYTEQTNLYIRNDTTTSVTTEDLNRTFKPVIGPRPLVNGLQGRIDYYWSVLKPGQTLRVRSNGNLIAPWYG
HVLSGGSHGRILKTDLKGGNCVVQCQTEKGGLNSTLPFHNISKYAFGTCPKYVRVNSLKLAVGLRNVPARSSRGLFGAIAGFIEGGWPGL
VAGWYGFQHSNDQGVGMAADRDSTQKAIDKITSKVNNIVDKMNKQYEIIDHEFSEVETRLNMINNKIDDQIQDVWAYNAELLVLLEN
QKTLDEHDANVNNLYNKVKRALGSNAMEDGKGCFELYHKCDDQCMETIRNGTYNRRKYREESRLERQKIEGVKLESEGTYKILTIYSTVA
SSLVLAMGFAAFLFWAMSNGSCRCNICI*

图 56D

构建体2224号的示意图

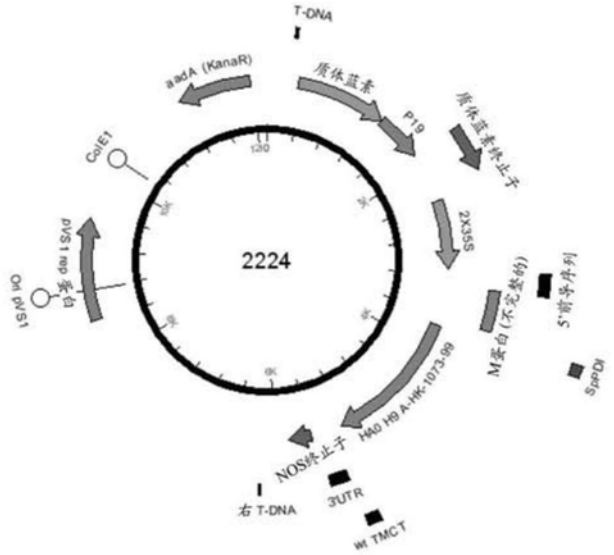


图 56E

构建体2226号的示意图



图56:用于PDISP/H9香港的2X35S/CPMV HT+(构建体2224号)和160+(构建体2226号)

图 57A, SEQ ID NO: 158

具有缺失的蛋白水解环的PDISP/H9香港的核苷酸序列

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCGGATAAAATCTGCATCG
GCCACCAGTCAACAAACTCCACAGAAACTGTGGACACGCTAACAGAAACCAATGTTCCTGTGACACATGCCAAAGAATTGCTCCAC
ACAGAGCATAATGGAATGCTGTGTGCAACAAGCCTGGGACATCCCCTCATTCTAGACACATGCACTATTGAAGGACTAGTCTATG
GCAACCCTTCTTGTGACCTGCTGTTGGGAGGAAGAGAATGGTCCTACATCGTCGAAAGATCATCAGCTGTAAATGGAACGTGTTA
CCCTGGGAATGTAGAAAACCTAGAGGAACTCAGGACACTTTTTAGTTCCGCTAGTTCCTACCAAAGAATCCAAATCTTCCCAGACA
CAACCTGGAATGTGACTTACACTGGAACAAGCAGAGCATGTTCAGGTTCATTCTACAGGAGTATGAGATGGCTGACTCAAAAGAG
CGGTTTTTACCCTGTTCAAGACGCCCAATACACAAATAACAGGGGAAAGAGCATTCTTTTCGTGTGGGGCATACATCACCCACCCA
CCTATACCGAGCAAACAAATTTGTACATAAGAAACGACACAACAACAAGCGTGACAACAGAAGATTTGAATAGGACCTTCAAACC
AGTGATAGGGCCAAGGCCCCTTGTCAATGGTCTGCAGGGAAGAATTGATTATTATTGGTCGGTACTAAAACCAGGCCAAACATTG
CGAGTACGATCCAATGGGAATCTAATTGCTCCATGGTATGGACACGTTCTTTCAGGAGGGAGCCATGGAAGAATCCTGAAGACTG
ATTTAAAAGGTGGTAATTGTGTAGTGCAATGTCAGACTGAAAAAGGTGGCTTAAACAGTACATTGCCATTCCACAATATCAGTAAA
TATGCATTTGGAACCTGCCCCAAATATGTAAGAGTTAATAGTCTCAAACTGGCAGTCGGTCTGAGGAACGTGCCTGGCGGAGGTT
GGCCAGGACTAGTCGCTGGCTGGTATGGTTTCCAGCATTCAAATGATCAAGGGGTTGGTATGGCTGCAGATAGGGATTCAACTCA
AAAGGCAATTGATAAAATAACATCCAAGGTGAATAATATAGTCGACAAGATGAACAAGCAATATGAAATAATTGATCATGAATTT
AGTGAGGTTGAAACTAGACTCAATATGATCAATAATAAGATTGATGACCAAATACAAGACGTATGGGCATATAATGCAGAATTGC
TAGTACTACTTGAAAATCAAAAAACACTCGATGAGCATGATGCGAACGTGAACAATCTATATAACAAGGTGAAGAGGGCACTGG
GCTCCAATGCTATGGAAGATGGGAAAGGCTGTTTCGAGCTATACCATAAATGTGATGATCAGTGCATGGAAACAATTCGGAACGG
GACCTATAATAGGAGAAAGTATAGAGAGGAATCAAGACTAGAAAGGCAGAAAATAGAGGGGGTTAAGCTGGAATCTGAGGGAA
CTTACAAAATCCTCACCATTTATTCGACTGTCGCCTCATCTCTTGTGCTTGCAATGGGGTTTGCTGCCTTCCTGTTCTGGGCCATGTC
CAATGGATCTTGCAGATGCAACATTTGTATATAA

图 57B, SEQ ID NO: 159

H9HK107399(Prl-).r

GTCCTGGCCAACCTCCGCCAGGCACGTTCCTCAGACCGACTGCCAGTT

图 57C, SEQ ID NO: 160

H9HK107399(Prl-).c

GGAACGTGCCTGGCGGAGGTTGGCCAGGACTAGTCGCTGGCTGGTATG

图 57D, SEQ ID NO: 161

具有缺失的蛋白水解环的PDISP/H9香港的氨基酸序列

MAKNVAIFGLLFSLLVLVPSQIFADKICIGHQSTNSTETVDTLTETNVPVTHAKELLHTEHNGMLCATSLGHPLILDTCTIEGLVYGNPSCD
LLLGGREWSYIVERSSAVNGTCYPGNVENLEELRTLFSSASSYQRIQIFPDTTWNVTYTGTSRACSGSFYRSMRWLTQKSGFYPVQDAQ
YTNNRGKSILFVWGIHHPPTYTEQTNLYIRNDTTTSVTTEDLNRTFKPVIGPRPLVNGLQGRIDYYWSVLKPGQTLRVRSNGNLIAPWYG
HVLSGGSHGRILKTDLKGGNCVVQCQTEKGGLNSTLPFHNISKYAFGTCPKYVRVNSLKLAVGLRNVPGGGWPGLVAGWYGFQHSND
QGVGMAADRDSTQKAIDKITSKVNNIVDKMNKQYEIIDHEFSEVETRLNMINNKIDDQIQDVWAYNAELLVLLENQKTLDEHDANVN
NLYNKVKRALGSNAMEDGKGCFELYHKCDDQCMETIRNGTYNRRKYREESRLERQKIEGVKLESEGTYKILTIYSTVASSLVLAMGFAAF
LFWAMSNGSCRCNICI*

图 57E

构建体2225号的示意图

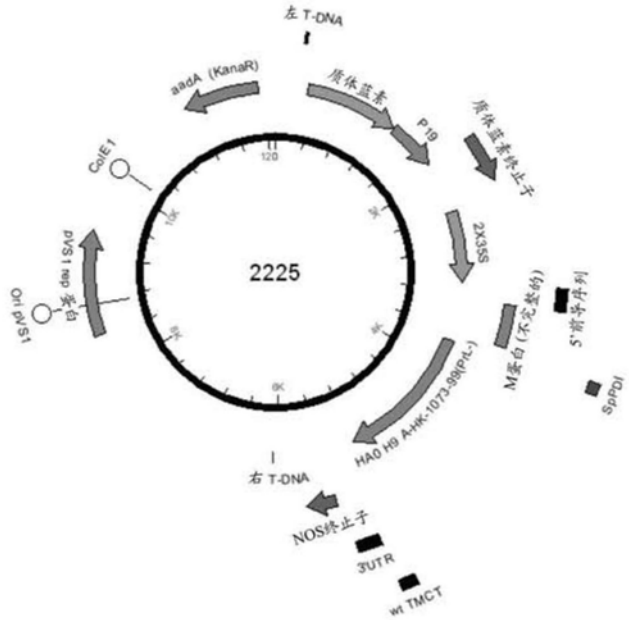


图 57F

构建体2227号的示意图

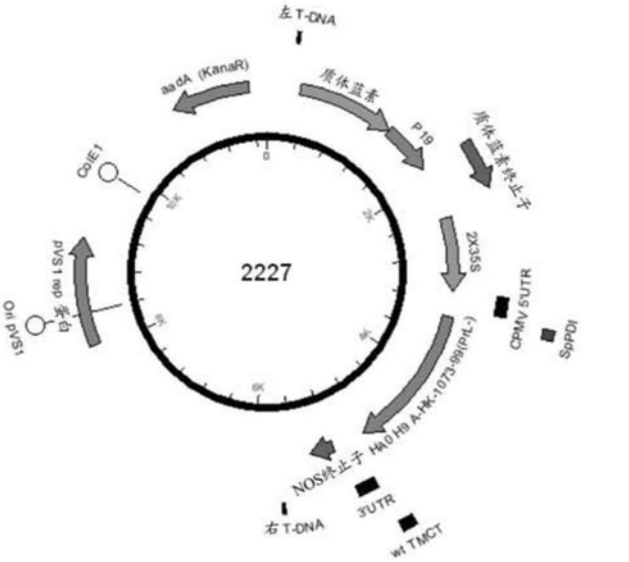


图57:用于具有缺失的蛋白水解环的PDISP/H9香港的2X35S/CPMV HT+(构建体2225号)和160+(构建体2227号)

图 58A, SEQ ID NO: 162

PDISP/HA B马来西亚的核苷酸序列

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCGGATCGAATCTGCACTG
GGATAACATCGTCAAACTCACCACATGTTGTCAAAACTGCTACTCAAGGGGAGGTCAATGTGACTGGTGTAATACCACTGACAAC
AACACCCACCAAATCTCATTTTGCAAATCTCAAAGGAACAGAAACCAGAGGGAAACTATGCCCAAAATGCCTCAACTGCACAGATC
TGGACGTGGCCTTGGGCAGACCAAAATGCACGGGGAACATACCCTCGGCAAGAGTTTCAATACTCCATGAAGTCAGACCTGTTAC
ATCTGGGTGCTTTCCTATAATGCACGACAGAACAAAAATTAGACAGCTGCCTAAACTTCTCAGAGGATACGAACATATCAGGTTAT
CAACTCATAACGTTATCAATGCAGAAAATGCACCAGGAGGACCCTACAAAATTGGAACCTCAGGGTCTTGCCCTAACGTTACCAAT
GGAAACGGATTTTTCGCAACAATGGCTTGGGCCGTCCCAAAAAACGACAACAACAAAACAGCAACAAATTCATTAACAATAGAAG
TACCATACATTTGTACAGAAGGAGAAGACCAAATTACCGTTTGGGGGTTCCACTCTGATAACGAAACCCAAATGGCAAAGCTCTAT
GGGGACTCAAAGCCCCAGAAGTTCACCTCATCTGCCAACGGAGTGACCACACATTACGTTTCACAGATTGGTGGCTTCCCAAATCA
AACAGAAGACGGAGGACTACCACAAAGCGGTAGAATTGTTGTTGATTACATGGTGCAAAAATCTGGGAAAACAGGAACAATTAC
CTATCAAAGAGGTATTTTATTGCCTCAAAAAGTGTGGTGCGCAAGTGGCAGGAGCAAGGTAATAAAAGGATCGTTGCCTTTAATT
GGAGAAGCAGATTGCCTCCACGAAAAATACGGTGGATTAAACAAAAGCAAGCCTTACTACACAGGGGAACATGCAAAGGCCATA
GGAAATTGCCCAATATGGGTGAAAACACCCTTGAAGCTGGCCAATGGAACCAAATATAGACCTCCTGCAAAACTATTAAAGGAAA
GGGGTTTCTTCGGAGCTATTGCTGGTTTCTTAGAAGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGATACACATCCCATG
GGGCACATGGAGTAGCGGTGGCAGCAGACCTTAAGAGCACTCAAGAGGCCATAAACAAGATAACAAAAAATCTCAACTCTTTGA
GTGAGCTGGAAGTAAAGAATCTTCAAAGACTAAGCGGTGCCATGGATGAACTCCACAACGAAATACTAGAACTAGACGAGAAAG
TGGATGATCTCAGAGCTGATACAATAAGCTCACAAATAGAACTCGCAGTCCTGCTTTCCAATGAAGGAATAATAAACAGTGAAGA
TGAGCATCTCTTGGCGCTTGAAAGAAAGCTGAAGAAAATGCTGGGCCCCTCTGCTGTAGAGATAGGGAATGGATGCTTTGAAACC
AAACACAAGTGCAACCAGACCTGTCTCGACAGAATAGCTGCTGGTACCTTTGATGCAGGAGAATTTTCTCTCCCCACTTTTGATTCA
CTGAATATTACTGCTGCATCTTTAAATGACGATGGATTGGATAATCATACTATACTGCTTTACTACTCAACTGCTGCCTCCAGTTTG
GCTGTAACATTGATGATAGCTATCTTTGTTGTTTATATGGTCTCCAGAGACAATGTTTCTTGCTCCATCTGTCTATAA

图 58B, SEQ ID NO: 163

IF**-HBM250604.S1-6r

ACTAAAGAAAATAGGCCTTTATAGACAGATGGAGCAAGAAACATTGTCTC

图 58C, SEQ ID NO: 164

PDISP/HA B马来西亚的氨基酸序列

MAKNVAIFGLLFSLLVLVPSQIFADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKGTETRGKLCPKCLNCTDLDVALG
RPKCTGNIPSARVSILHEVRPVTSGCFPIMHDRTKIRQLPKLLRGYEHIRLSTHNVINAENAPGGPYKIGTSGSCPNVTNGNGFFATMAW
AVPKNDNNKTATNSLTIEVPYICTEGEDQITVWGFHSDNETQMAKLYGDSKPQKFTSSANGVTTHYVSQIGGFPNQTEDGGLPQSGRI
VVDYMVQKSGKTGTITYQRGILLPQKVWCASGRSKVIKGSLPLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTK
YRPPAKLLKERGFFGAIAGFLEGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEIL
ELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKLKKMLGPSAVEIGNGCFETKHKCNQTCLDRIAAGTFDAGEFSLPTFDSLN
ITAASLNDDGLDNHTILLYYSTAASSLAVTLMIAIFVVYMVSRDNVSCSICL*

图 58D

构建体2013号的示意图

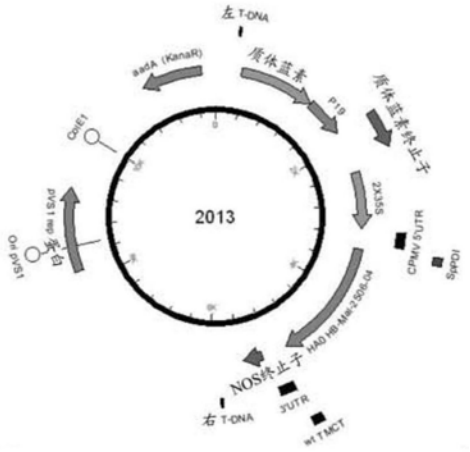


图58:2X35S/CPMV 160+/PDISP/HA B马来西亚/NOS(构建体2013号)

图 59A, SEQ ID NO: 165

具有缺失的蛋白水解环的PDISP/HA B马来西亚的核苷酸序列

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCGGATCGAA
TCTGCACTGGGATAACATCGTCAAACTCACCACATGTTGTCAAAACTGCTACTCAAGGGGAGGTCAATGTGACTGGTGT
AATACCACTGACAACAACACCCACCAAATCTCATTTTGCAAATCTCAAAGGAACAGAAACCAGAGGGAAACTATGCCCA
AAATGCCTCAACTGCACAGATCTGGACGTGGCCTTGGGCAGACCAAAATGCACGGGGAACATACCCTCGGCAAGAGTTT
CAATACTCCATGAAGTCAGACCTGTTACATCTGGGTGCTTTCCTATAATGCACGACAGAACAAAAATTAGACAGCTGCC
TAAACTTCTCAGAGGATACGAACATATCAGGTTATCAACTCATAACGTTATCAATGCAGAAAATGCACCAGGAGGACCC
TACAAAATTGGAACCTCAGGGTCTTGCCCTAACGTTACCAATGGAAACGGATTTTTCGCAACAATGGCTTGGGCCGTCC
CAAAAAACGACAACAACAAAACAGCAACAAATTCATTAACAATAGAAGTACCATACATTTGTACAGAAGGAGAAGACCA
AATTACCGTTTGGGGGTTCCACTCTGATAACGAAACCCAAATGGCAAAGCTCTATGGGGACTCAAAGCCCCAGAAGTTC
ACCTCATCTGCCAACGGAGTGACCACACATTACGTTTCACAGATTGGTGGCTTCCCAAATCAAACAGAAGACGGAGGAC
TACCACAAAGCGGTAGAATTGTTGTTGATTACATGGTGCAAAAATCTGGGAAAACAGGAACAATTACCTATCAAAGAGG
TATTTTATTGCCTCAAAAAGTGTGGTGCGCAAGTGGCAGGAGCAAGGTAATAAAAGGATCGTTGCCTTTAATTGGAGAA
GCAGATTGCCTCCACGAAAAATACGGTGGATTAAACAAAAGCAAGCCTTACTACACAGGGGAACATGCAAAGGCCATAG
GAAATTGCCCAATATGGGTGAAAACACCCTTGAAGCTGGCCAATGGAACCAAATATAGACCTCCTGGTGGAGGATGGGA
AGGAATGATTGCAGGTTGGCACGGATACACATCCCATGGGGCACATGGAGTAGCGGTGGCAGCAGACCTTAAGAGCACT
CAAGAGGCCATAAACAAGATAACAAAAAATCTCAACTCTTTGAGTGAGCTGGAAGTAAAGAATCTTCAAAGACTAAGCG
GTGCCATGGATGAACTCCACAACGAAATACTAGAACTAGACGAGAAAGTGGATGATCTCAGAGCTGATACAATAAGCTC
ACAAATAGAACTCGCAGTCCTGCTTTCCAATGAAGGAATAATAAACAGTGAAGATGAGCATCTCTTGGCGCTTGAAAGA
AAGCTGAAGAAAATGCTGGGCCCCTCTGCTGTAGAGATAGGGAATGGATGCTTTGAAACCAAACACAAGTGCAACCAGA
CCTGTCTCGACAGAATAGCTGCTGGTACCTTTGATGCAGGAGAATTTTCTCTCCCCACTTTTGATTCACTGAATATTAC
TGCTGCATCTTTAAATGACGATGGATTGGATAATCATACTATACTGCTTTACTACTCAACTGCTGCCTCCAGTTTGGCT
GTAACATTGATGATAGCTATCTTTGTTGTTTATATGGTCTCCAGAGACAATGTTTCTTGCTCCATCTGTCTATAA

图 59B, SEQ ID NO: 166

HBM250604(PrL-).r

CATTCCTTCCCATCCTCCACCAGGAGGTCTATATTTGGTTCCATTGGC

图 59C, SEQ ID NO: 167

HBM250604(PrL-).c

AGACCTCCTGGTGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGA

图 59D, SEQ ID NO: 168

具有缺失的蛋白水解环的PDISP/HA B马来西亚的氨基酸序列

MAKNVAIFGLLFSLLVLVPSQIFADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKGTETRGKLCPKCLNCTDLDVALG
RPKCTGNIPSARVSILHEVRPVTSGCFPIMHDRTKIRQLPKLLRGYEHIRLSTHNVINAENAPGGPYKIGTSGSCPNVTNGNGFFATMAW
AVPKNDNNKTATNSLTIEVPYICTEGEDQITVWGFHSDNETQMAKLYGDSKPQKFTSSANGVTTHYVSQIGGFPNQTEDGGLPQSGRI
VVDYMVQKSGKTGTITYQRGILLPQKVWCASGRSKVIKGSLPLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTK
YRPPGGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISS
QIELAVLLSNEGIINSEDEHLLALERKLKKMLGPSAVEIGNGCFETKHKCNQTCLDRIAAGTFDAGEFSLPTFDSLNITAASLNDDGLDNHT
ILLYYSTAASSLAVTLMIAIFVVYMVSRDNVSCSICL*

图 59E

构建体2014号的示意图

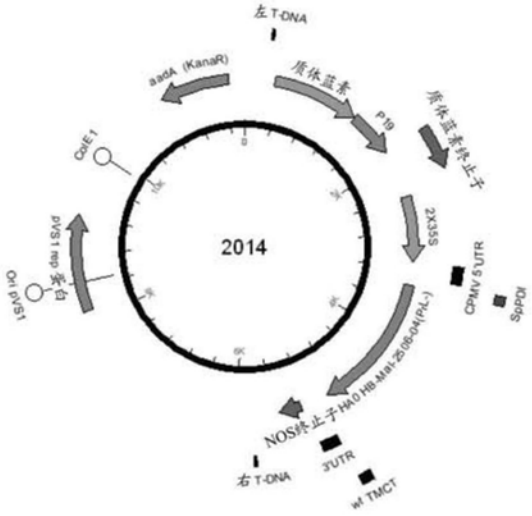


图59:2X35S/CPMV 160+/具有缺失的蛋白水解环的PDISP/HA B马来西亚/NOS(构建体2014号)

图 60A, SEQ ID NO: 169

PDISP/HA B马萨诸塞州的核苷酸序列

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCCGATCGAATCTGCACTG
GGATAACATCTTCAAACTCACCTCATGTGGTCAAAACAGCTACTCAAGGGGAGGTCAATGTGACTGGTGTGATACCACTAACAAC
AACACCAACAAAATCTTATTTTGCAAATCTCAAAGGAACAAAGACCAGAGGGAAACTATGCCCAGACTGTCTCAACTGTACAGATC
TGGATGTGGCCCTGGGCAGGCCAATGTGTGTGGGAACTACACCTTCTGCGAAAGCTTCAATACTTCACGAAGTCAGACCTGTTAC
ATCCGGGTGCTTCCCTATAATGCACGACAGAACAAAAATCAGGCAACTAGCCAATCTTCTCAGAGGATATGAAAATATCAGGTTAT

图 60A（续）

CAACCCAAAACGTTATCGATGCAGAAAAGGCACCAGGAGGACCCTACAGACTTGGAACCTCAGGATCTTGCCCTAACGCTACCAG
TAAAAGCGGATTTTTCGCAACAATGGCTTGGGCTGTCCCAAAGGACAACAACAAAAATGCAACGAACCCATTAACAGTAGAAGTA
CCATACATTTGTGCAGAAGGGGAAGACCAAATTACTGTTTGGGGGTTCCATTCAGATAACAAAACCCAAATGAAGAACCTCTATG
GAGACTCAAATCCTCAAAAGTTCACCTCATCTGCTAATGGAGTAACCACACATTATGTTTCTCAGATTGGCGGCTTCCCAGATCAAA
CAGAAGACGGAGGACTACCACAAAGCGGCAGAATTGTCGTTGATTACATGATGCAAAAACCTGGGAAAACAGGAACAATTGTCT
ATCAAAGAGGTGTTTTGTTGCCTCAAAAGGTGTGGTGCGCGAGTGGCAGGAGCAAAGTAATAAAAGGGTCCTTGCCTTTAATTGG
TGAAGCAGATTGCCTTCATGAAAAATACGGTGGATTAAACAAAAGCAAGCCTTACTACACAGGAGAACATGCAAAAGCCATAGG
AAATTGCCCAATATGGGTGAAAACACCTTTGAAGCTTGCCAATGGAACCAAATATAGACCTCCTGCAAAACTATTAAAGGAAAGG
GGTTTCTTCGGAGCTATTGCTGGTTTCCTAGAAGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGATACACATCTCACGGA
GCACATGGAGTGGCAGTTGCTGCAGACCTTAAGAGCACACAAGAAGCTATAAACAAGATAACAAAAAATCTCAACTCTTTGAGTG
AGCTAGAAGTAAAGAATCTTCAAAGGCTAAGTGGTGCCATGGATGAACTCCACAACGAAATACTCGAGCTGGATGAGAAAGTGG
ATGACCTCAGAGCTGACACTATAAGTTCACAAATAGAACTTGCAGTCTTGCTTTCCAACGAAGGAATAATAAACAGTGAAGACGA
GCATCTATTGGCACTTGAGAGAAAACTAAAGAAAATGCTGGGTCCCTCTGCTGTAGACATAGGAAATGGATGCTTCGAAACCAAA
CACAAATGCAACCAGACCTGCTTAGACAGGATAGCTGCTGGCACCTTTAATGCAGGAGAGTTTTCTCTCCCCACTTTTGATTCATTG
AACATTACTGCTGCATCTTTAAATGATGATGGATTGGATAACCATACTATACTGCTCTATTACTCAACTGCTGCTTCTAGTTTGGCTG
TAACATTGATGCTAGCTATTTTTATTGTTTATATGGTCTCCAGAGACAACGTTTCATGCTCCATCTGTCTATAA

图 60B, SEQ ID NO: 170

PDISP/HA B马萨诸塞州的氨基酸序列

MAKNVAIFGLLFSLLVLVPSQIFADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSYFANLKGTKTRGKLCPDCLNCTDLDVALG
RPMCVGTTPSAKASILHEVRPVTSGCFPIMHDRTKIRQLANLLRGYENIRLSTQNVIDAEKAPGGPYRLGTSGSCPNATSKSGFFATMA
WAVPKDNNKNATNPLTVEVPYICAEGEDQITVWGFHSDNKTQMKNLYGDSNPQKFTSSANGVTTHYVSQIGGFPDQTEDGGLPQSG
RIVVDYMMQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKGSLPLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLAN
GTKYRPPAKLLKERGFFGAIAGFLEGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELH
NEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKLKKMLGPSAVDIGNGCFETKHKCNQTCLDRIAAGTFNAGEFSLPTF
DSLNITAASLNDDGLDNHTILLYYSTAASSLAVTLMLAIFIVYMVSRDNVSCSICL*

图 60C

构建体2070号的示意图

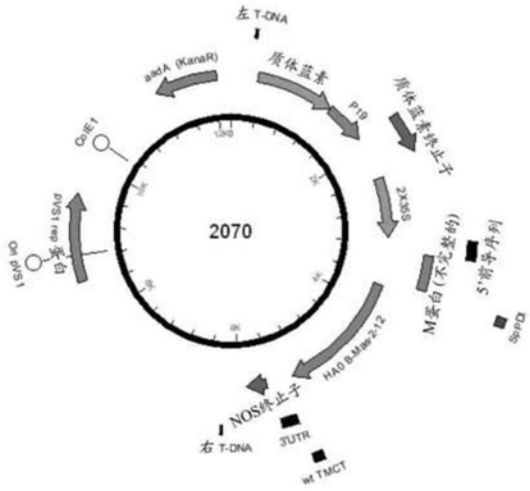


图 60D

构建体2080号的示意图

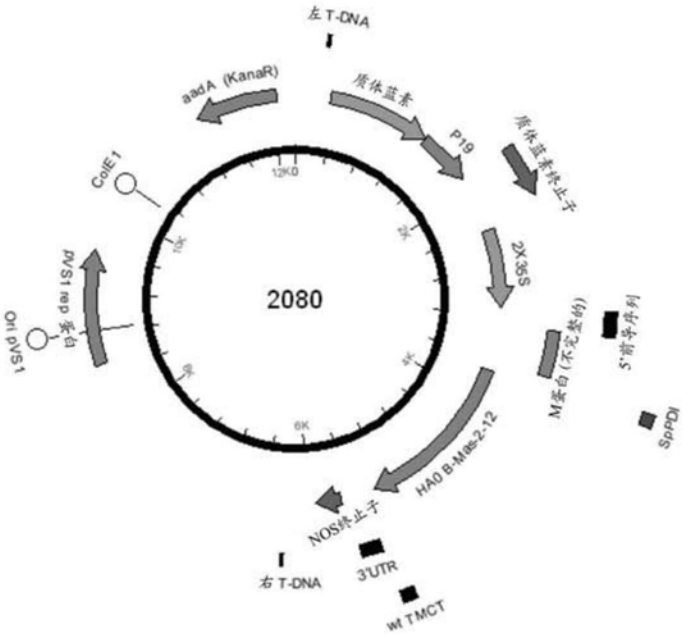


图 60E

构建体2090号的示意图

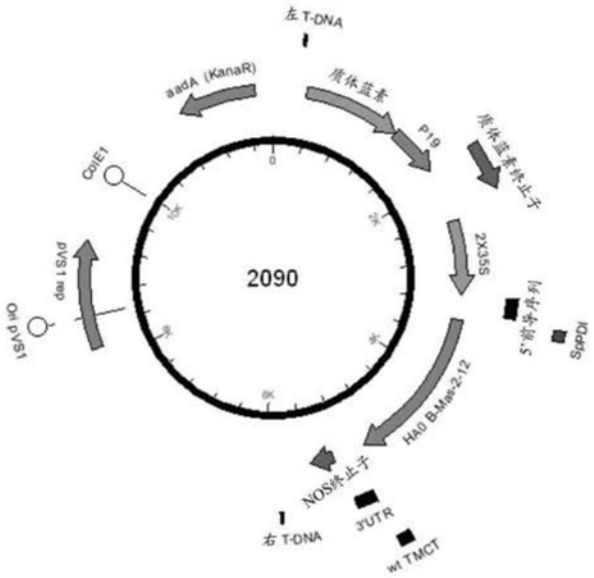


图60:用于PDISP/HA B马萨诸塞州的2X35S/CPMV HT(构建体2070号)、HT+(构建体2080号)和160+(-Mprot)(构建体2090号)

图 61A SEQ ID NO: 190

HBF406(PrL-).r (构建体2102和2104)

TCCTTCCCATCCTCCACCAGGAGGTCTATATTTGGTTCCATTGGCGAGCTTCAAAG

图 61B SEQ ID NO: 191

HBF406(PrL-).c (构建体2102和2104)

ATATAGACCTCCTGGTGGAGGATGGGAAGGAATGATTGCAGGCTGGCACGGA

图 61C SEQ ID NO: 192

IF*-HBF406.s1-6r (构建体2102和2104)

ACTAAAGAAAATAGGCCTTTATAGACAGATGGAGCATGAAACGTTGTCTC

图 61D SEQ ID NO: 193

具有缺失的蛋白水解环的PDISP/B佛罗里达的核苷酸序列(构建体2102和2104)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCGGATCGAA
TCTGCACTGGAATAACATCTTCAAACTCACCTCATGTGGTCAAAACAGCCACTCAAGGGGAGGTCAATGTGACTGGTGT
GATACCACTAACAACAACACCAACAAAATCTTATTTTGCAAATCTCAAAGGAACAAGGACCAGAGGGAAACTATGCCCA
GACTGTCTCAACTGCACAGATCTGGATGTGGCTTTGGGCAGACCAATGTGTGTGGGGACCACACCTTCGGCGAAGGCTT
CAATACTCCACGAAGTCAAACCTGTTACATCCGGGTGCTTTCCTATAATGCACGACAGAACAAAAATCAGGCAACTACC
CAATCTTCTCAGAGGATATGAAAATATCAGGCTATCAACCCAAAACGTCATCGATGCGGAAAAGGCACCAGGAGGACCC
TACAGACTTGGAACCTCAGGATCTTGCCCTAACGCTACCAGTAAGAGCGGATTTTTCGCAACAATGGCTTGGGCTGTCC
CAAAGGACAACAACAAAAATGCAACGAACCCACTAACAGTAGAAGTACCATACATTTGTACAGAAGGGGAAGACCAAAT
CACTGTTTGGGGGTTCCATTCAGATAACAAAACCCAAATGAAGAACCTCTATGGAGACTCAAATCCTCAAAAGTTCACC
TCATCTGCTAATGGAGTAACCACACACTATGTTTCTCAGATTGGCAGCTTCCCAGATCAAACAGAAGACGGAGGACTAC
CACAAAGCGGCAGGATTGTTGTTGATTACATGATGCAAAAACCTGGGAAAACAGGAACAATTGTCTACCAAAGAGGTGT
TTTGTTGCCTCAAAAGGTGTGGTGCGCGAGTGGCAGGAGCAAAGTAATAAAAGGGTCCTTGCCTTTAATTGGTGAAGCA
GATTGCCTTCATGAAAAATACGGTGGATTAAACAAAAGCAAGCCTTACTACACAGGAGAACATGCAAAAGCCATAGGAA
ATTGCCCAATATGGGTGAAAACACCTTTGAAGCTCGCCAATGGAACCAAATATAGACCTCCTGGTGGAGGATGGGAAGG
AATGATTGCAGGCTGGCACGGATACACATCTCACGGAGCACATGGAGTGGCAGTGGCGGCGGACCTTAAGAGTACGCAA
GAAGCTATAAACAAGATAACAAAAAATCTCAATTCTTTGAGTGAGCTAGAAGTAAAGAATCTTCAAAGACTAAGTGGTG
CCATGGATGAACTCCACAACGAAATACTCGAGCTGGATGAGAAAGTGGATGATCTCAGAGCTGACACTATAAGCTCGCA
AATAGAACTTGCAGTCTTGCTTTCCAACGAAGGAATAATAAACAGTGAAGATGAGCATCTATTGGCACTTGAGAGAAAA
CTAAAGAAAATGCTGGGTCCCTCTGCTGTAGAGATAGGAAATGGATGCTTCGAAACCAAACACAAGTGCAACCAGACCT
GCTTAGACAGGATAGCTGCTGGCACCTTTAATGCAGGAGAATTTTCTCTCCCCACTTTTGATTCACTGAACATTACTGC
TGCATCTTTAAATGATGATGGATTGGATAACCATACTATACTGCTCTATTACTCAACTGCTGCTTCTAGTTTGGCTGTA
ACATTGATGCTAGCTATTTTTATTGTTTATATGGTCTCCAGAGACAACGTTTCATGCTCCATCTGTCTATAA

图 61E SEQ ID NO: 194

具有缺失的蛋白水解环的PDISP/B佛罗里达的氨基酸序列(构建体2102和2104)

MAKNVAIFGLLFSLLVLVPSQIFADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSYFANLKGTRTRGKLCP
DCLNCTDLDVALGRPMCVGTTPSAKASILHEVKPVTSGCFPIMHDRTKIRQLPNLLRGYENIRLSTQNVIDAEKAPGGP
YRLGTSGSCPNATSKSGFFATMAWAVPKDNNKNATNPLTVEVPYICTEGEDQITVWGFHSDNKTQMKNLYGDSNPQKFT
SSANGVTTHYVSQIGSFPDQTEDGGLPQSGRIVVDYMMQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKGSLPLIGEA

图 61E （续）

DCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPGGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQ
EAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERK
LKKMLGPSAVEIGNGCFETKHKCNQTCLDRIAAGTFNAGEFSLPTFDSLNITAASLNDDGLDNHTILLYYSTAASSLAV
TLMLAIFIVYMVSRDNVSCSICL*

图 61F SEQ ID NO: 195

从2X35S启动子至NOS终止子的表达盒2102号。具有缺失的蛋白水解环的PDISP/ HA B佛罗里达的核苷酸序列加下划线

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTG
AGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGAT
AGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGAC
AGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAG
TGGATTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCA
AAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTT
ATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATG
CCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTC
TTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCCT
TCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTTGATAAAAGCGAACGTGGGGAA
ACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTCTTGCGTGAGCGA
TCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTTTGTG
GACACGTAGTGCGGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGTGGTCTTGGGAAAAGAAAGCTTGCT
GGAGGCTGCTGTTCAGCCCCATACATTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGCAATATCTCTACTTCTGCT
TGACGAGGTATTGTTGCCTGTACTTCTTTCTTCTTCTTCTTGCTGATTGGTTCTATAAGAAATCTAGTATTTTCTTTGA
AACAGAGTTTTCCCGTGGTTTTCGAACTTGGAGAAAGATTGTTAAGCTTCTGTATATTCTGCCCAAATTTGTCGGGCCC
AATACCGCGGAGAAAATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGA
TCTTCGCGGATCGAATCTGCACTGGAATAACATCTTCAAACTCACCTCATGTGGTCAAAACAGCCACTCAAGGGGAGGT
CAATGTGACTGGTGTGATACCACTAACAACAACACCAACAAAATCTTATTTTGCAAATCTCAAAGGAACAAGGACCAGA
GGGAAACTATGCCCAGACTGTCTCAACTGCACAGATCTGGATGTGGCTTTGGGCAGACCAATGTGTGTGGGGACCACAC
CTTCGGCGAAGGCTTCAATACTCCACGAAGTCAAACCTGTTACATCCGGGTGCTTTCCTATAATGCACGACAGAACAAA
AATCAGGCAACTACCCAATCTTCTCAGAGGATATGAAAATATCAGGCTATCAACCCAAAACGTCATCGATGCGGAAAAG
GCACCAGGAGGACCCTACAGACTTGGAACCTCAGGATCTTGCCCTAACGCTACCAGTAAGAGCGGATTTTTCGCAACAA
TGGCTTGGGCTGTCCCAAAGGACAACAACAAAAATGCAACGAACCCACTAACAGTAGAAGTACCATACATTTGTACAGA
AGGGGAAGACCAAATCACTGTTTGGGGGTTCCATTCAGATAACAAAACCCAAATGAAGAACCTCTATGGAGACTCAAAT
CCTCAAAAGTTCACCTCATCTGCTAATGGAGTAACCACACACTATGTTTCTCAGATTGGCAGCTTCCCAGATCAAACAG
AAGACGGAGGACTACCACAAAGCGGCAGGATTGTTGTTGATTACATGATGCAAAAACCTGGGAAAACAGGAACAATTGT
CTACCAAAGAGGTGTTTTGTTGCCTCAAAAGGTGTGGTGCGCGAGTGGCAGGAGCAAAGTAATAAAAGGGTCCTTGCCT
TTAATTGGTGAAGCAGATTGCCTTCATGAAAAATACGGTGGATTAAACAAAAGCAAGCCTTACTACACAGGAGAACATG
CAAAAGCCATAGGAAATTGCCCAATATGGGTGAAAACACCTTTGAAGCTCGCCAATGGAACCAAATATAGACCTCCTGG
TGGAGGATGGGAAGGAATGATTGCAGGCTGGCACGGATACACATCTCACGGAGCACATGGAGTGGCAGTGGCGGCGGAC
CTTAAGAGTACGCAAGAAGCTATAAACAAGATAACAAAAAATCTCAATTCTTTGAGTGAGCTAGAAGTAAAGAATCTTC
AAAGACTAAGTGGTGCCATGGATGAACTCCACAACGAAATACTCGAGCTGGATGAGAAAGTGGATGATCTCAGAGCTGA
CACTATAAGCTCGCAAATAGAACTTGCAGTCTTGCTTTCCAACGAAGGAATAATAAACAGTGAAGATGAGCATCTATTG
GCACTTGAGAGAAAACTAAAGAAAATGCTGGGTCCCTCTGCTGTAGAGATAGGAAATGGATGCTTCGAAACCAAACACA
AGTGCAACCAGACCTGCTTAGACAGGATAGCTGCTGGCACCTTTAATGCAGGAGAATTTTCTCTCCCCACTTTTGATTC
ACTGAACATTACTGCTGCATCTTTAAATGATGATGGATTGGATAACCATACTATACTGCTCTATTACTCAACTGCTGCT
TCTAGTTTGGCTGTAACATTGATGCTAGCTATTTTTATTGTTTATATGGTCTCCAGAGACAACGTTTCATGCTCCATCT
GTCTATAAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTG
TGCTCAGAGTGTGTTTATTTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACA
CAAAAAGATTTTAATTTTATTAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCAGATCG
TTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTG
AATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGC
AATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTAT
GTTACTAGAT

图 61G

构建体2102号的示意图

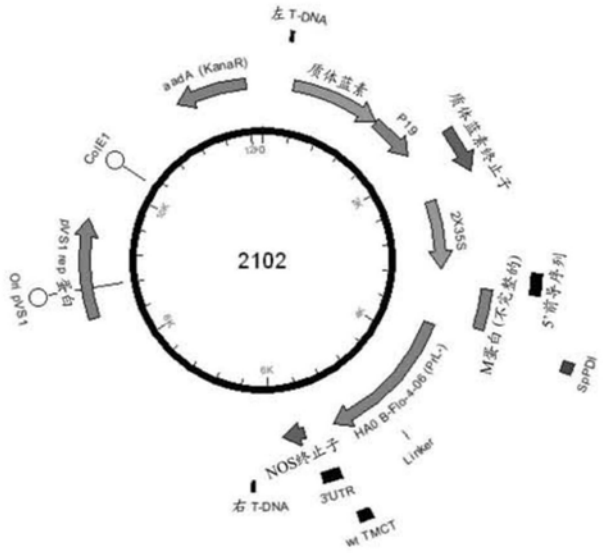


图 61H, SEQ ID NO: 196

从左至右LIR的表达盒2104号。具有缺失的蛋白水解环的PDISP/ HA B佛罗里达的核苷酸序列加下划线

TGTTGTTGTGACTCCGAGGGGTTGCCTCAAACTCTATCTTATAACCGGCGTGGAGGCATGGAGGCAGGGGTATTTTGGT
CATTTTAATAGATAGTGGAAAATGACGTGGAATTTACTTAAAGACGAAGTCTTTGCGACAAGGGGGGGCCCACGCCGAA
TTTAATATTACCGGCGTGGCCCCCCCTTATCGCGAGTGCTTTAGCACGAGCGGTCCAGATTTAAAGTAGAAAATTTCCC
GCCCACTAGGGTTAAAGGTGTTCACACTATAAAAGCATATACGATGTGATGGTATTTGGTCGACAAGCTTGCATGCCGG
TCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGA
GACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATA
GTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACA
GTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGT
GGATTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAA
AGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTA
TTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGC
CTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCT
TCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCCTT
CCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTTGATAAAAGCGAACGTGGGGAAA
CCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTCTTGCGTGAGCGAT
CTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTTTGTGG
ACACGTAGTGCGGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGTGGTCTTGGGAAAAGAAAGCTTGCTG
GAGGCTGCTGTTCAGCCCCATACATTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGCAATATCTCTACTTCTGCTT
GACGAGGTATTGTTGCCTGTACTTCTTTCTTCTTCTTCTTGCTGATTGGTTCTATAAGAAATCTAGTATTTTCTTTGAA
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ATACCGCGGAGAAAATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGAT
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AATGTGACTGGTGTGATACCACTAACAACAACACCAACAAAATCTTATTTTGCAAATCTCAAAGGAACAAGGACCAGAG
GGAAACTATGCCCAGACTGTCTCAACTGCACAGATCTGGATGTGGCTTTGGGCAGACCAATGTGTGTGGGGACCACACC
TTCGGCGAAGGCTTCAATACTCCACGAAGTCAAACCTGTTACATCCGGGTGCTTTCCTATAATGCACGACAGAACAAAA

图 61H（续）

ATCAGGCAACTACCCAATCTTCTCAGAGGATATGAAAATATCAGGCTATCAACCCAAAACGTCATCGATGCGGAAAAGG
CACCAGGAGGACCCTACAGACTTGGAACCTCAGGATCTTGCCCTAACGCTACCAGTAAGAGCGGATTTTTCGCAACAAT
GGCTTGGGCTGTCCCAAAGGACAACAACAAAAATGCAACGAACCCACTAACAGTAGAAGTACCATACATTTGTACAGAA
GGGGAAGACCAAATCACTGTTTGGGGGTTCCATTCAGATAACAAAACCCAAATGAAGAACCTCTATGGAGACTCAAATC
CTCAAAAGTTCACCTCATCTGCTAATGGAGTAACCACACACTATGTTTCTCAGATTGGCAGCTTCCCAGATCAAACAGA
AGACGGAGGACTACCACAAAGCGGCAGGATTGTTGTTGATTACATGATGCAAAAACCTGGGAAAACAGGAACAATTGTC
TACCAAAGAGGTGTTTTGTTGCCTCAAAAGGTGTGGTGCGCGAGTGGCAGGAGCAAAGTAATAAAAGGGTCCTTGCCTT
TAATTGGTGAAGCAGATTGCCTTCATGAAAAATACGGTGGATTAAACAAAAGCAAGCCTTACTACACAGGAGAACATGC
AAAAGCCATAGGAAATTGCCCAATATGGGTGAAAACACCTTTGAAGCTCGCCAATGGAACCAAATATAGACCTCCTGGT
GGAGGATGGGAAGGAATGATTGCAGGCTGGCACGGATACACATCTCACGGAGCACATGGAGTGGCAGTGGCGGCGGACC
TTAAGAGTACGCAAGAAGCTATAAACAAGATAACAAAAAATCTCAATTCTTTGAGTGAGCTAGAAGTAAAGAATCTTCA
AAGACTAAGTGGTGCCATGGATGAACTCCACAACGAAATACTCGAGCTGGATGAGAAAGTGGATGATCTCAGAGCTGAC
ACTATAAGCTCGCAAATAGAACTTGCAGTCTTGCTTTCCAACGAAGGAATAATAAACAGTGAAGATGAGCATCTATTGG
CACTTGAGAGAAAACTAAAGAAAATGCTGGGTCCCTCTGCTGTAGAGATAGGAAATGGATGCTTCGAAACCAAACACAA
GTGCAACCAGACCTGCTTAGACAGGATAGCTGCTGGCACCTTTAATGCAGGAGAATTTTCTCTCCCCACTTTTGATTCA
CTGAACATTACTGCTGCATCTTTAAATGATGATGGATTGGATAACCATACTATACTGCTCTATTACTCAACTGCTGCTT
CTAGTTTGGCTGTAACATTGATGCTAGCTATTTTTATTGTTTATATGGTCTCCAGAGACAACGTTTCATGCTCCATCTG
TCTATAAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGT
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AAAAAGATTTTAATTTTATTAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCAGATCGT
TCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGA
ATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCA
ATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATG
TTACTAGATCTCTAGAGTCTCAAGCTTGGCGCGCCATAAAATGATTATTTTATGAATATATTTCATTGTGCAAGTAGAT
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TGCAAAGAATTATAGAAGGGATCCCACCTTTTATTTTCTTCTTTTTTCCATATTTAGGGTTGACAGTGAAATCAGACTG
GCAACCTATTAATTGCTTCCACAATGGGACGAACTTGAAGGGGATGTCGTCGATGATATTATAGGTGGCGTGTTCATCG
TAGTTGGTGAAATCGATGGTACCGTTCCAATAGTTGTGTCGTCCGAGACTTCTAGCCCAGGTGGTCTTTCCGGTACGAG
TTGGTCCGCAGATGTAGAGGCTGGGGTGTCGGATTCCATTCCTTCCATTGTCCTGGTTAAATCGGCCATCCATTCAAGG
TCAGATTGAGCTTGTTGGTATGAGACAGGATGTATGTAAGTATAAGCGTCTATGCTTACATGGTATAGATGGGTTTCCC
TCCAGGAGTGTAGATCTTCGTGGCAGCGAAGATCTGATTCTGTGAAGGGCGACACATACGGTTCAGGTTGTGGAGGGAA
TAATTTGTTGGCTGAATATTCCAGCCATTGAAGTTTTGTTGCCCATTCATGAGGGAATTCTTCCTTGATCATGTCAAGA
TATTCCTCCTTAGACGTTGCAGTCTGGATAATAGTTCTCCATCGTGCGTCAGATTTGCGAGGAGAGACCTTATGATCTC
GGAAATCTCCTCTGGTTTTAATATCTCCGTCCTTTGATATGTAATCAAGGACTTGTTTAGAGTTTCTAGCTGGCTGGAT
ATTAGGGTGATTTCCTTCAAAATCGAAAAAAGAAGGATCCCTAATACAAGGTTTTTTATCAAGCTGGAGAAGAGCATGA
TAGTGGGTAGTGCCATCTTGATGAAGCTCAGAAGCAACACCAAGGAAGAAAATAAGAAAAGGTGTGAGTTTCTCCCAGA
GAAACTGGAATAAATCATCTCTTTGAGATGAGCACTTGGGATAGGTAAGGAAAACATATTTAGATTGGAGTCTGAAGTT
CTTACTAGCAGAAGGCATGTTGTTGTGACTCCGAGGGGTTGCCTCAAACTCTATCTTATAACCGGCGTGGAGGCATGGA
GGCAGGGGTATTTTGGTCATTTTAATAGATAGTGGAAAATGACGTGGAATTTACTTAAAGACGAAGTCTTTGCGACAAG
GGGGGGCCCACGCCGAATTTAATATTACCGGCGTGGCCCCCCCTTATCGCGAGTGCTTTAGCACGAGCGGTCCAGATTT
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图 61I

构建体2104号的示意图

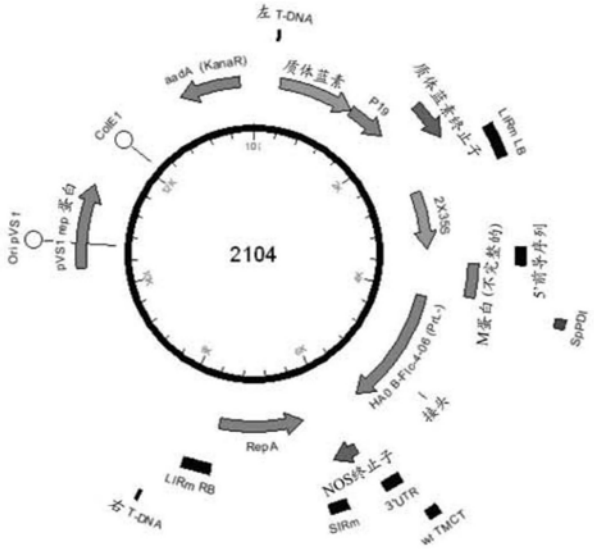


图61:2X35S/CPMV HT+/具有缺失的蛋白水解环的PDISP/B佛罗里达/NOS(构建体2102号)和2X35S/CPMV HT+/BeYDV/具有缺失的蛋白水解环PDISP/B佛罗里达/NOS(构建体2104号)

图 62A, SEQ ID NO: 197

IF-H1cTMCT.S1-4r (构建体2106和2108)

ACTAAAGAAAATAGGCCTTTAAATACATATTCTACACTGTAGAGAC

图 62B, SEQ ID NO: 198

具有缺失的蛋白水解环的PDISP/HA B佛罗里达+H1加利福尼亚TMCT的核苷酸序列(构建体2106和2108)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCGGATCGAA
TCTGCACTGGAATAACATCTTCAAACTCACCTCATGTGGTCAAAACAGCCACTCAAGGGGAGGTCAATGTGACTGGTGT
GATACCACTAACAACAACACCAACAAAATCTTATTTTGCAAATCTCAAAGGAACAAGGACCAGAGGGAAACTATGCCCA
GACTGTCTCAACTGCACAGATCTGGATGTGGCTTTGGGCAGACCAATGTGTGTGGGGACCACACCTTCGGCGAAGGCTT
CAATACTCCACGAAGTCAAACCTGTTACATCCGGGTGCTTTCCTATAATGCACGACAGAACAAAAATCAGGCAACTACC
CAATCTTCTCAGAGGATATGAAAATATCAGGCTATCAACCCAAAACGTCATCGATGCGGAAAAGGCACCAGGAGGACCC
TACAGACTTGGAACCTCAGGATCTTGCCCTAACGCTACCAGTAAGAGCGGATTTTTCGCAACAATGGCTTGGGCTGTCC
CAAAGGACAACAACAAAAATGCAACGAACCCACTAACAGTAGAAGTACCATACATTTGTACAGAAGGGGAAGACCAAAT
CACTGTTTGGGGGTTCCATTCAGATAACAAAACCCAAATGAAGAACCTCTATGGAGACTCAAATCCTCAAAAGTTCACC
TCATCTGCTAATGGAGTAACCACACACTATGTTTCTCAGATTGGCAGCTTCCCAGATCAAACAGAAGACGGAGGACTAC
CACAAAGCGGCAGGATTGTTGTTGATTACATGATGCAAAAACCTGGGAAAACAGGAACAATTGTCTACCAAAGAGGTGT
TTTGTTGCCTCAAAAGGTGTGGTGCGCGAGTGGCAGGAGCAAAGTAATAAAAGGGTCCTTGCCTTTAATTGGTGAAGCA
GATTGCCTTCATGAAAAATACGGTGGATTAAACAAAAGCAAGCCTTACTACACAGGAGAACATGCAAAAGCCATAGGAA
ATTGCCCAATATGGGTGAAAACACCTTTGAAGCTCGCCAATGGAACCAAATATAGACCTCCTGGTGGAGGATGGGAAGG
AATGATTGCAGGCTGGCACGGATACACATCTCACGGAGCACATGGAGTGGCAGTGGCGGCGGACCTTAAGAGTACGCAA

图 62B（续）

GAAGCTATAAACAAGATAACAAAAAATCTCAATTCTTTGAGTGAGCTAGAAGTAAAGAATCTTCAAAGACTAAGTGGTG
CCATGGATGAACTCCACAACGAAATACTCGAGCTGGATGAGAAAGTGGATGATCTCAGAGCTGACACTATAAGCTCGCA
AATAGAACTTGCAGTCTTGCTTTCCAACGAAGGAATAATAAACAGTGAAGATGAGCATCTATTGGCACTTGAGAGAAAA
CTAAAGAAAATGCTGGGTCCCTCTGCTGTAGAGATAGGAAATGGATGCTTCGAAACCAAACACAAGTGCAACCAGACCT
GCTTAGACAGGATAGCTGCTGGCACCTTTAATGCAGGAGAATTTTCTCTCCCCACTTTTGATTCACTGAACATTACTGC
TGCATCTTTAAATGATGATGGATTGGATAACTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTCATTGGTACTG
GTAGTCTCCCTGGGGGCAATCAGTTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

图 62C, SEQ ID NO: 199

具有缺失的蛋白水解环的PDISP/HA B佛罗里达+H1加利福尼亚TMCT的氨基酸序列
(构建体2106和2108)

MAKNVAIFGLLFSLLVLVPSQIFADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSYFANLKGTRTRGKLCP
DCLNCTDLDVALGRPMCVGTTPSAKASILHEVKPVTSGCFPIMHDRTKIRQLPNLLRGYENIRLSTQNVIDAEKAPGGP
YRLGTSGSCPNATSKSGFFATMAWAVPKDNNKNATNPLTVEVPYICTEGEDQITVWGFHSDNKTQMKNLYGDSNPQKFT
SSANGVTTHYVSQIGSFPDQTEDGGLPQSGRIVVDYMMQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKGSLPLIGEA
DCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPGGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQ
EAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERK
LKKMLGPSAVEIGNGCFETKHKCNQTCLDRIAAGTFNAGEFSLPTFDSLNITAASLNDDGLDNYQILAIYSTVASSLVL
VVSLGAISFWMCSNGSLQCRICI*

图 62D, SEQ ID NO: 200

从2X35S启动子至NOS终止子的表达盒2106号。具有缺失的蛋白水解环的PDISP/HA B佛罗里达+H1加利福尼亚TMCT的核苷酸序列加下划线

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTG
AGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGAT
AGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGAC
AGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAG
TGGATTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCA
AAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTT
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CCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTC
TTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCCT
TCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTTGATAAAAGCGAACGTGGGGAA
ACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTCTTGCGTGAGCGA
TCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTTTGTG
GACACGTAGTGCGGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGTGGTCTTGGGAAAAGAAAGCTTGCT
GGAGGCTGCTGTTCAGCCCCATACATTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGCAATATCTCTACTTCTGCT
TGACGAGGTATTGTTGCCTGTACTTCTTTCTTCTTCTTCTTGCTGATTGGTTCTATAAGAAATCTAGTATTTTCTTTGA
AACAGAGTTTTCCCGTGGTTTTCGAACTTGGAGAAAGATTGTTAAGCTTCTGTATATTCTGCCCAAATTTGTCGGGCCC
AATACCGCGGAGAAAATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGA
TCTTCGCGGATCGAATCTGCACTGGAATAACATCTTCAAACTCACCTCATGTGGTCAAAACAGCCACTCAAGGGGAGGT
CAATGTGACTGGTGTGATACCACTAACAACAACACCAACAAAATCTTATTTTGCAAATCTCAAAGGAACAAGGACCAGA
GGGAAACTATGCCCAGACTGTCTCAACTGCACAGATCTGGATGTGGCTTTGGGCAGACCAATGTGTGTGGGGACCACAC
CTTCGGCGAAGGCTTCAATACTCCACGAAGTCAAACCTGTTACATCCGGGTGCTTTCCTATAATGCACGACAGAACAAA
AATCAGGCAACTACCCAATCTTCTCAGAGGATATGAAAATATCAGGCTATCAACCCAAAACGTCATCGATGCGGAAAAG
GCACCAGGAGGACCCTACAGACTTGGAACCTCAGGATCTTGCCCTAACGCTACCAGTAAGAGCGGATTTTTCGCAACAA
TGGCTTGGGCTGTCCCAAAGGACAACAACAAAAATGCAACGAACCCACTAACAGTAGAAGTACCATACATTTGTACAGA
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CCTCAAAAGTTCACCTCATCTGCTAATGGAGTAACCACACACTATGTTTCTCAGATTGGCAGCTTCCCAGATCAAACAG
AAGACGGAGGACTACCACAAAGCGGCAGGATTGTTGTTGATTACATGATGCAAAAACCTGGGAAAACAGGAACAATTGT
CTACCAAAGAGGTGTTTTGTTGCCTCAAAAGGTGTGGTGCGCGAGTGGCAGGAGCAAAGTAATAAAAGGGTCCTTGCCT
TTAATTGGTGAAGCAGATTGCCTTCATGAAAAATACGGTGGATTAAACAAAAGCAAGCCTTACTACACAGGAGAACATG

图 62D（续）

CAAAAGCCATAGGAAATTGCCCAATATGGGTGAAAACACCTTTGAAGCTCGCCAATGGAACCAAATATAGACCTCCTGG
TGGAGGATGGGAAGGAATGATTGCAGGCTGGCACGGATACACATCTCACGGAGCACATGGAGTGGCAGTGGCGGCGGAC
CTTAAGAGTACGCAAGAAGCTATAAACAAGATAACAAAAAATCTCAATTCTTTGAGTGAGCTAGAAGTAAAGAATCTTC
AAAGACTAAGTGGTGCCATGGATGAACTCCACAACGAAATACTCGAGCTGGATGAGAAAGTGGATGATCTCAGAGCTGA
CACTATAAGCTCGCAAATAGAACTTGCAGTCTTGCTTTCCAACGAAGGAATAATAAACAGTGAAGATGAGCATCTATTG
GCACTTGAGAGAAAACTAAAGAAAATGCTGGGTCCCTCTGCTGTAGAGATAGGAAATGGATGCTTCGAAACCAAACACA
AGTGCAACCAGACCTGCTTAGACAGGATAGCTGCTGGCACCTTTAATGCAGGAGAATTTTCTCTCCCCACTTTTGATTC
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AGTTCATTGGTACTGGTAGTCTCCCTGGGGGCAATCAGTTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATAT
GTATTTAAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTG
TGCTCAGAGTGTGTTTATTTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACA
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TTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTG
AATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGC
AATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTAT
GTTACTAGAT

图 62E

构建体2106号的示意图

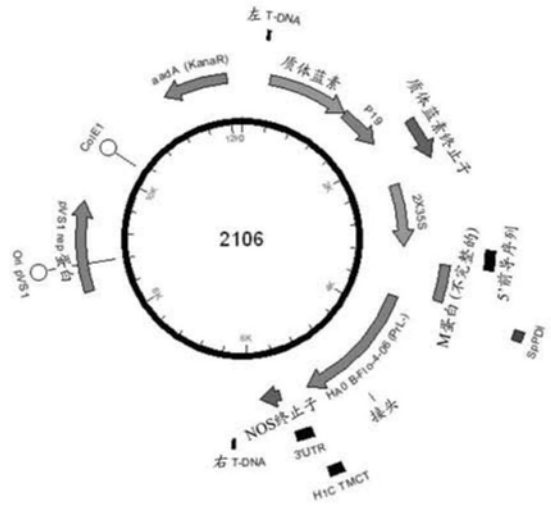


图 62F, SEQ ID NO: 201

从左至右LIR的表达盒2108号。具有缺失的蛋白水解环的PDISP/HA B佛罗里达+H1加利福尼亚TMCT的核苷酸序列加下划线

TGTTGTTGTGACTCCGAGGGGTTGCCTCAAACTCTATCTTATAACCGGCGTGGAGGCATGGAGGCAGGGGTATTTTGGT
CATTTTAATAGATAGTGGAAAATGACGTGGAATTTACTTAAAGACGAAGTCTTTGCGACAAGGGGGGGCCCACGCCGAA
TTTAATATTACCGGCGTGGCCCCCCCTTATCGCGAGTGCTTTAGCACGAGCGGTCCAGATTTAAAGTAGAAAATTTCCC
GCCCACTAGGGTTAAAGGTGTTCACACTATAAAAGCATATACGATGTGATGGTATTTGGTCGACAAGCTTGCATGCCGG
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GTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGT
GGATTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAA
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TTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGC
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CTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTTTGTGG
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GAGGCTGCTGTTCAGCCCCATACATTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGCAATATCTCTACTTCTGCTT
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ATACCGCGGAGAAAAATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGAT
CTTCGCGGATCGAATCTGCACTGGAATAACATCTTCAAACTCACCTCATGTGGTCAAAACAGCCACTCAAGGGGAGGTC
AATGTGACTGGTGTGATACCACTAACAACAACACCAACAAAATCTTATTTTGCAAATCTCAAAGGAACAAGGACCAGAG
GGAAACTATGCCCAGACTGTCTCAACTGCACAGATCTGGATGTGGCTTTGGGCAGACCAATGTGTGTGGGGACCACACC
TTCGGCGAAGGCTTCAATACTCCACGAAGTCAAACCTGTTACATCCGGGTGCTTTCCTATAATGCACGACAGAACAAAA
ATCAGGCAACTACCCAATCTTCTCAGAGGATATGAAAATATCAGGCTATCAACCCAAAACGTCATCGATGCGGAAAAGG
CACCAGGAGGACCCTACAGACTTGGAACCTCAGGATCTTGCCCTAACGCTACCAGTAAGAGCGGATTTTTCGCAACAAT
GGCTTGGGCTGTCCCAAAGGACAACAACAAAAATGCAACGAACCCACTAACAGTAGAAGTACCATACATTTGTACAGAA
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CTCAAAAGTTCACCTCATCTGCTAATGGAGTAACCACACACTATGTTTCTCAGATTGGCAGCTTCCCAGATCAAACAGA
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TAATTGGTGAAGCAGATTGCCTTCATGAAAAATACGGTGGATTAAACAAAAGCAAGCCTTACTACACAGGAGAACATGC
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GTGCAACCAGACCTGCTTAGACAGGATAGCTGCTGGCACCTTTAATGCAGGAGAATTTTCTCTCCCCACTTTTGATTCA
CTGAACATTACTGCTGCATCTTTAAATGATGATGGATTGGATAACTACCAGATTTTGGCGATCTATTCAACTGTCGCCA
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图 62F（续）

ACTTTAGATAAACTCGTATGAGGAGAGGCACGTTCAGTGACTCGACGATTCCCGAGCAAAAAAAGTCTCCCCGTCACAC
ATATAGTGGGTGACGCAATTATCTTTAAAGTAATCCTTCTGTTGACTTGTCATTGATAACATCCAGTCTTCGTCAGGAT
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图 62G

构建体2108号的示意图

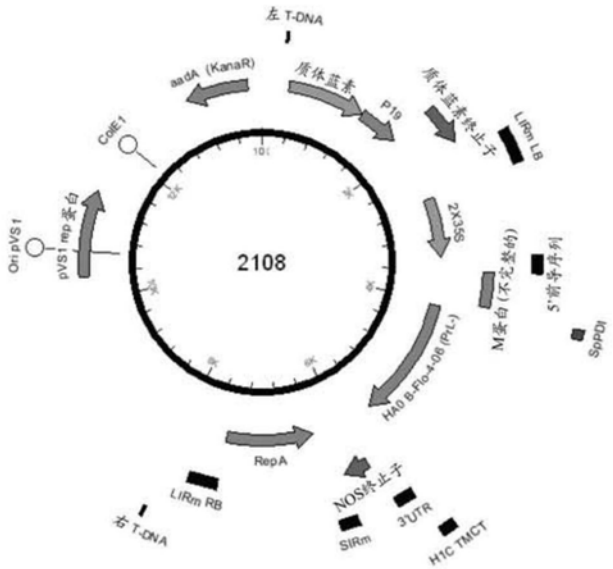


图62:2X35S/CPMV HT+/具有蛋白水解环缺失的PDISP/B佛罗里达+H1加利福尼亚TMCT/NOS(构建体2106号)和2X35S/CPMV HT+/BeYDV/具有蛋白水解环缺失的PDISP/B佛罗里达+H1加利福尼亚TMCT/NOS(构建体2108号)