



(12) 发明专利



(10) 授权公告号 CN 112142858 B

(45) 授权公告日 2024. 08. 27

(21) 申请号 202011069672.X

(22) 申请日 2015.10.15

(65) 同一申请的已公布的文献号
申请公布号 CN 112142858 A

(43) 申请公布日 2020.12.29

(30) 优先权数据
62/064,989 2014.10.16 US

(62) 分案原申请数据
201580055840.0 2015.10.15

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(51) Int.Cl.

C07K 19/00 (2006.01)

C12N 15/62 (2006.01)

C12N 1/21 (2006.01)

C12N 5/10 (2006.01)

A01N 63/23 (2020.01)

A01P 7/04 (2006.01)

C12R 1/01 (2006.01)

C12R 1/41 (2006.01)

C12R 1/07 (2006.01)

C12R 1/08 (2006.01)

C12R 1/185 (2006.01)

C12R 1/38 (2006.01)

C12R 1/22 (2006.01)

C12R 1/18 (2006.01)

(56) 对比文件

CN 102821596 A, 2012.12.12

CN 103596436 A, 2014.02.19

审查员 杨莽嘉

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(54) 发明名称

对鳞翅目害虫具有毒性或抑制性的嵌合杀
昆虫蛋白质

(57) 摘要

公开了编码新型嵌合杀昆虫蛋白质的核苷
酸序列,所述嵌合杀昆虫蛋白质表现出鳞翅目抑
制活性。具体实施方案提供了含有编码一种或多
种所述嵌合杀昆虫蛋白质的重组核酸分子的组
合物以及经过转化的植物、植物部分和种子。

1. 一种嵌合杀昆虫蛋白质,其氨基酸序列如SEQ ID NO:21所示。
2. 一种编码嵌合杀昆虫蛋白质的多核苷酸,其中所述多核苷酸可操作地连接至异源启动子,并且所述嵌合杀昆虫蛋白质的氨基酸序列如SEQ ID NO:21所示。
3. 一种编码嵌合杀昆虫蛋白质的多核苷酸,其编码氨基酸序列如SEQ ID NO:21所示的嵌合杀昆虫蛋白质。
4. 一种宿主细胞,其包含编码嵌合杀昆虫蛋白质的多核苷酸,其中所述嵌合杀昆虫蛋白质的氨基酸序列如SEQ ID NO:21所示或其中所述多核苷酸序列如SEQ ID NO:20所示,其中所述宿主细胞是选自由细菌宿主细胞和不可再生的植物宿主细胞组成的群组。
5. 如权利要求4所述的宿主细胞,其中所述细菌宿主细胞是选自由以下各项组成的群组:土壤杆菌、根瘤菌、芽孢杆菌、短芽孢杆菌、埃希氏杆菌、假单胞菌、克雷伯氏杆菌和欧文氏菌。
6. 如权利要求4所述的宿主细胞,其中所述宿主细胞是源自选自由单子叶植物和双子叶植物组成的植物群组的植物的不可再生的植物宿主细胞。
7. 一种昆虫抑制性组合物,其包含氨基酸序列如SEQ ID NO:21所示的嵌合杀昆虫蛋白质。
8. 如权利要求7所述的昆虫抑制性组合物,其还包含与所述嵌合杀昆虫蛋白质不同的至少一种昆虫抑制剂。
9. 如权利要求8所述的昆虫抑制性组合物,其中所述至少一种昆虫抑制剂是选自由以下各项组成的群组:昆虫抑制性蛋白质、昆虫抑制性dsRNA分子和昆虫抑制性化学物质。
10. 如权利要求8所述的昆虫抑制性组合物,其中所述至少一种昆虫抑制剂对鳞翅目、鞘翅目、半翅目、同翅目或缨翅目的一个或多个害虫物种表现出活性。
11. 一种防治鳞翅目害虫的方法,所述方法包括使所述鳞翅目害虫与抑制量的如权利要求1所述的嵌合杀昆虫蛋白质接触。
12. 一种不可再生的转基因植物细胞,其包含嵌合杀昆虫蛋白质,其中:
所述嵌合杀昆虫蛋白质的氨基酸序列如SEQ ID NO:21所示。
13. 一种防治鳞翅目害虫的方法,其包括使所述害虫暴露于如权利要求12所述的不可再生的转基因植物细胞,其中所述不可再生的植物细胞表达鳞翅目抑制量的所述嵌合杀昆虫蛋白质。
14. 一种来源于如权利要求12所述的不可再生的植物细胞的商品产品,其中所述产品包含可检测量的所述嵌合杀昆虫蛋白质。
15. 如权利要求14所述的商品产品,其中所述产品是选自由以下各项组成的群组:油、膳食、动物饲料、面粉、薄片、糠、棉绒和外壳。
16. 一种编码如权利要求1所述的嵌合杀昆虫蛋白质的重组多核苷酸分子,其包含编码嵌合杀昆虫蛋白质的多核苷酸,其中所述嵌合杀昆虫蛋白质的氨基酸序列如SEQ ID NO:21所示或其中所述多核苷酸序列如SEQ ID NO:20所示;和编码与所述嵌合杀昆虫蛋白质不同的昆虫抑制剂的多核苷酸序列。
17. 一种重组核酸分子,其包含可操作地连接至编码嵌合杀昆虫蛋白质的多核苷酸片段的异源启动子,其中:
所述嵌合杀昆虫蛋白质的氨基酸序列如SEQ ID NO:21所示。

对鳞翅目害虫具有毒性或抑制性的嵌合杀昆虫蛋白质

[0001] 本申请是申请日为2015年10月15日、申请号为201580055840.0、发明名称为“对鳞翅目害虫具有毒性或抑制性的新型嵌合杀昆虫蛋白质”的发明专利申请的分案申请。

[0002] 对相关申请的引用

[0003] 本申请要求2014年10月16日提交的美国临时申请No.62/064,989的权益,该申请以全文引用的方式并入本文中。

[0004] 序列表的并入

[0005] 序列表的计算机可读形式与本申请一起通过电子提交方式提交并且以全文引用的方式并入本申请。序列表包含在2016年8月23日创建的文件中,具有文件名P34230W000_Seq_PCT_Art34.txt,并且大小是898,229字节(如在MS-Windows®操作系统中所测量)。

发明领域

[0006] 本发明总体上涉及昆虫抑制性蛋白质领域。在本申请中公开了对农作物和种子的农业相关害虫表现出昆虫抑制性活性的一类新型的嵌合杀昆虫蛋白质。具体来说,所公开的类别的蛋白质对鳞翅目昆虫害虫表现出了杀昆虫活性。提供了含有编码所公开的毒素蛋白质中的一种或多种的重组核酸分子的植物、植物部分和种子。

发明背景

[0007] 提高重要农业植物,尤其包括玉米、大豆、甘蔗、水稻、小麦、蔬菜和棉花的作物产量已经变得越来越重要。除了日益增长的人口对于提供食物、衣物和能量的农产品的需要日益增长以外,预计气候相关的影响和来自于日益增长的人口将土地用于除农业耕作以外的用途的压力也会减少可耕种耕地的量。这些因素已经导致了关于粮食保障的严峻预测,特别是在植物生物技术和农业技术不存在重大改进的情况下。鉴于这些压力,技术、农业技术和害虫治理的环境可持续改进是在有限量的可耕种耕地上使作物增产的重要工具。

[0008] 昆虫,特别是鳞翅目内的昆虫,被认为是破坏农田作物,从而降低受侵扰区域中的作物产量的主要原因。会对农业造成负面影响的鳞翅目害虫物种包括但不限于草地粘虫(草地贪夜蛾)、甜菜粘虫(甜菜夜蛾)、披肩粘虫(蓓带夜蛾)、黑切根虫(球菜夜蛾)、甘蓝尺蠖(粉纹夜蛾)、大豆尺蠖(大豆夜蛾)、黎豆毛虫(黎豆夜蛾)、绿叶虫(苜蓿绿夜蛾)、烟草芽虫(烟芽夜蛾)、颗粒切根虫(黄地老虎)、粘虫(一星粘虫)、西方切根虫(灰地老虎)、欧洲玉米钻心虫(欧洲玉米螟)、脐橙虫(脐橙螟)、玉米根结网虫(玉米根网螟)、草地结网虫(水稻切叶野螟)、向日葵蛾(向日葵螟)、小玉米茎钻心虫(南美玉米苗斑螟)、苹果蛾(苹果蠹蛾)、葡萄果蛾(葡萄小食心虫)、东方果蛾(梨小食心虫)、向日葵芽小卷蛾(向日葵芽卷叶蛾)、钻背蛾(小菜蛾)、粉红棉铃虫(棉红铃虫)、粉红钻茎虫(大螟)、吉普赛蛾(舞毒蛾)、棉叶虫(棉叶波纹夜蛾)、果树卷叶虫(果树黄卷蛾)、欧洲卷叶虫(玫瑰黄卷蛾)、亚洲水稻钻心虫或水稻钻茎虫(二化螟)、水稻卷叶虫(稻纵卷叶螟)、玉米根结网虫(玉米根网螟)、蓝草结网虫(早熟禾草螟)、西南玉米钻心虫(西南玉米螟)、蔗螟(小蔗螟)、多刺棉铃虫(埃及金刚钻)、斑点棉铃虫(翠纹金刚钻)、古棉铃虫(棉铃实夜蛾)、玉米穗虫、大豆荚虫或棉铃虫(玉米穗

夜蛾)、草地结网虫(水稻切叶野螟)、欧洲葡萄蛾(欧洲葡萄缀穗蛾)、柑桔潜叶虫(柑桔潜叶蛾)、大白蝴蝶(大菜粉蛾)、菜青虫或小白蝴蝶(小菜粉蛾)、烟草切根虫或茶蚕(斜纹夜蛾)和番茄潜叶虫(番茄潜叶蛾)。

[0009] 在历史上,农业依赖于密集施用作为害虫防治剂的合成化学杀昆虫剂。除了出现抗性问题的以外,对环境和人类健康的忧虑也刺激了生物杀虫剂的研究和开发。这种研究工作导致逐渐发现和使用多种昆虫病原微生物物种,包括细菌。

[0010] 当发现了昆虫病原细菌,尤其是属于芽孢杆菌属的细菌,并且将其开发为生物害虫防治剂时,生物防治模式发生了变化。细菌苏云金芽孢杆菌(Bt)的菌株已经被用作杀昆虫蛋白质的来源,因为人们发现Bt菌株会对特定的昆虫显示出高毒性。已知Bt菌株会在孢子形成开始时和在稳定生长期期间产生位于伴孢结晶包涵体内的 δ 内毒素(例如Cry蛋白),而且还已知其能产生分泌型杀昆虫蛋白质。在被易感昆虫摄取后, δ 内毒素以及分泌型毒素在中肠皮膜细胞表面发挥其作用,从而破坏细胞膜,导致细胞破坏和死亡。除Bt以外的细菌物种中也鉴别出了编码杀昆虫蛋白质的基因,所述细菌物种包括其他芽孢杆菌和多种其他细菌物种,诸如侧孢短芽孢杆菌(*Brevibacillus laterosporus*)、球形赖氨酸芽孢杆菌(*Lysinibacillus sphaericus*) (“Ls”先前被称为球形芽孢杆菌(*Bacillus sphaericus*))和日本甲虫芽孢杆菌(*Paenibacillus popilliae*)。

[0011] 结晶型和分泌型可溶性杀昆虫蛋白质毒素对其宿主具有高度特异性,并且已经得以在世界范围内被接受作为化学杀昆虫剂的替代物。举例来说,杀昆虫毒素蛋白质已经被用于多种农业应用中,以防止重要农业植物遭到昆虫侵扰,减少对化学杀虫剂施用的需要和增加产量。通过机械方法诸如将含有多种细菌菌株的微生物制剂分散至植物表面上的喷雾和通过使用旨在产生表达杀昆虫毒素蛋白质的转基因植物和种子的基因转化技术将杀昆虫毒素蛋白质用来防治作物植物的农业相关害虫。

[0012] 表达杀昆虫蛋白质的转基因植物的使用已经在全世界得到了采用。举例来说,在2012年,有2610万公顷种植了表达Bt毒素的转基因作物(James,C.,Global Status of Commercialized Biotech/GM Crops:2012.ISAAA Brief第44期)。转基因昆虫防护作物的全球使用和被用于这些作物中的杀昆虫蛋白质的有限数目已经对能赋予针对目前所利用的杀昆虫蛋白质的抗性的现有昆虫等位基因产生了选择压力。

[0013] 目标害虫中发展对杀昆虫蛋白质的抗性引起持续需要发现和开发可用于控制昆虫对表达杀昆虫蛋白质的转基因作物的抗性增加的杀昆虫蛋白质新形式。具有提高的效力并且对更宽范围的易感昆虫物种表现出控制的新杀昆虫蛋白质将减少可能发展抗性等位基因的存活昆虫的数目。另外,在一种植物中使用两种或更多种对同一昆虫害虫有毒并且呈现出不同的作用模式的转基因杀昆虫蛋白质能降低任何单一目标昆虫物种中出现抗性的概率。

[0014] 因此,极度需要鉴别出具有改善的杀昆虫性质,诸如与农业耕作中目前使用的毒素相比对更宽范围的目标昆虫害虫物种和不同的作用模式的功效有所增加的其他杀昆虫蛋白质。为了满足这个需要,本发明公开了对主要目标鳞翅目害虫物种表现出活性的新型Cry1嵌合杀昆虫蛋白质。

[0015] 本领域中已知Cry1晶体蛋白质家族成员会对鳞翅目害虫表现出生物活性。Cry1晶体蛋白质的前体形式由两个近似相等尺寸的片段组成。所述前体蛋白质的羧基末端部分,

称为原毒素片段,能使晶体形成稳定并且不表现杀昆虫活性。所述前体蛋白质的氨基末端部分包含Cry1蛋白的毒素片段,并且基于Cry1家族成员内的保守或基本保守序列的比对,可以进一步再分成三个结构域,即结构域I、结构域II和结构域III。结构域I包含活性毒素片段的约前三分之一,而且已经被证明对通道形成是不可或缺的。结构域II和结构域III都牵涉受体结合和昆虫物种特异性,这取决于所研究的昆虫和杀昆虫蛋白质。

[0016] 由对本领域中已知的众多天然杀昆虫蛋白质的结构域结构进行拣选来任意地产生具有增强的嵌合蛋白质的可能性微乎其微。这是蛋白质结构、寡聚和释放杀昆虫蛋白质片段所需的激活(包括对嵌合前体进行正确蛋白水解处理,如果以此种形式表达的话)的复杂特性的结果。只有通过小心地选择各亲本蛋白质内的原毒素和具体靶标来产生嵌合结构才可能构建出与得到嵌合体的亲本蛋白质相比表现出有所改善的杀昆虫活性的功能嵌合杀昆虫毒素。本领域中已知,重新组装原毒素与任何两种或更多种彼此不同的毒素的毒素结构域I、结构域II和结构域III往往会构建出表现出错误晶体形成或完全缺乏针对优选目标昆虫害虫物种的任何可检测杀昆虫活性的蛋白质。只有通过试错法才能设计出有效的杀昆虫嵌合体,而且即使那样,熟练的技术人员最后也不一定能得到与可能得到嵌合体组成原毒素或毒素结构域的任何单个亲本毒素蛋白质相比表现出等效或改善的杀昆虫活性的嵌合体。举例来说,文献报告了由两种或更多种晶体蛋白质前体构建或组装嵌合蛋白质的众多实例。参见例如Jacqueline S.Knight等人,“A Strategy for Shuffling Numerous *Bacillus thuringiensis* Crystal Protein Domains.”*J.Economic Entomology*,97(6)(2004):1805-1813;Bosch等人(美国专利No.6,204,246);Malvar和Gilmer(美国专利No.6,017,534)。在这些实例中的每一个中,所得嵌合体中有许多与得到嵌合体组分的前体蛋白质相比不能表现出等效或有所改善的杀昆虫或晶体形成性质。

发明概述

[0017] 提供了编码对鳞翅类植物害虫具有毒性的嵌合杀昆虫蛋白质的重组核酸分子。每一种嵌合杀昆虫蛋白质都可以单独使用或与彼此以及与其他杀昆虫蛋白质和昆虫抑制剂组合于制剂和活体中;从而提供农业系统中目前使用的杀昆虫蛋白质和杀昆虫化学物质的替代物。

[0018] 在某些实施方案中,本文中公开了一种嵌合杀昆虫蛋白质,其包含如SEQ ID NO: 21、10、28、7、4、13、16、19、23、25、30、33、36、39、41、43、45、47、50、53、55、57、59、61、63、65、67、69、71、73、75、77、79、81、83、85、87、89、91、93、95、97、99、101、103、105、107、109或111中的任意者中所示出的氨基酸序列。这种嵌合杀昆虫蛋白质对鳞翅目昆虫物种表现出抑制活性,所述鳞翅目昆虫物种诸如但不限于黎豆夜蛾、小蔗螟、南美玉米苗斑螟、玉米穗夜蛾、烟芽夜蛾、大豆夜蛾、考斯夜蛾、亚热带粘虫、草地贪夜蛾、甜菜夜蛾、棉铃实夜蛾、斜纹夜蛾、棉红铃虫、西南玉米螟、翠纹金刚钻、南美棉铃虫和薄荷灰夜蛾。

[0019] 在另一个实施方案中,公开了一种编码嵌合杀昆虫蛋白质的多核苷酸,其中所述多核苷酸可操作地连接至异源启动子,并且所述嵌合杀昆虫蛋白质包含如SEQ ID NO:21、10、28、7、4、13、16、19、23、25、30、33、36、39、41、43、45、47、50、53、55、57、59、61、63、65、67、69、71、73、75、77、79、81、83、85、87、89、91、93、95、97、99、101、103、105、107、109或111中的任意者中所示出的氨基酸序列。还设想了一种编码嵌合杀昆虫蛋白质的多核苷酸,其中所

述多核苷酸包含的核苷酸序列任选地：在严格条件下与如SEQ ID NO:1、2、3、5、6、8、9、11、12、14、15、17、18、20、22、24、26、27、29、31、32、34、35、37、38、40、42、44、46、48、49、51、52、54、56、58、60、62、64、66、68、70、72、74、76、78、80、82、84、86、88、90、92、94、96、98、100、102、104、106、108、110、112、113、114、115、116、117、118、119、120、121、122、123、124、125、126、127、128、129或130中的任意者中所示出的多核苷酸序列的反向互补序列杂交；或编码包含如SEQ ID NO:21、10、28、7、4、13、16、19、23、25、30、33、36、39、41、43、45、47、50、53、55、57、59、61、63、65、67、69、71、73、75、77、79、81、83、85、87、89、91、93、95、97、99、101、103、105、107、109或111中的任意者中所示出的氨基酸序列的嵌合杀昆虫蛋白质。

[0020] 在其他实施方案中，本文中公开了一种宿主细胞，其包含SEQ ID NO:1、2、3、5、6、8、9、11、12、14、15、17、18、20、22、24、26、27、29、31、32、34、35、37、38、40、42、44、46、48、49、51、52、54、56、58、60、62、64、66、68、70、72、74、76、78、80、82、84、86、88、90、92、94、96、98、100、102、104、106、108、110、112、113、114、115、116、117、118、119、120、121、122、123、124、125、126、127、128、129或130中的任意者中所示出的多核苷酸，其中所述宿主细胞是选自由细菌宿主细胞或植物宿主细胞组成的群组。所设想的细菌宿主包括土壤杆菌、根瘤菌、芽孢杆菌、短芽孢杆菌、埃希氏杆菌、假单胞菌、克雷伯氏杆菌和欧文氏菌；并且其中所述芽孢杆菌属是蜡样芽孢杆菌或苏云金芽孢杆菌，所述短芽孢杆菌是侧孢短芽孢杆菌，且所述埃希氏杆菌是大肠埃希氏杆菌。所设想的植物细胞包括单子叶植物和双子叶植物。

[0021] 本文中所公开的其他实施方案包括昆虫抑制性组合物，其包含有包含如SEQ ID NO:21、10、28、7、4、13、16、19、23、25、30、33、36、39、41、43、45、47、50、53、55、57、59、61、63、65、67、69、71、73、75、77、79、81、83、85、87、89、91、93、95、97、99、101、103、105、107、109或111中的任意者中所示出的氨基酸序列的嵌合杀昆虫蛋白质。在某些实施方案中，所述昆虫抑制性组合物还包含与所述嵌合杀昆虫蛋白质不同的至少一种昆虫抑制剂。所设想的与所述嵌合杀昆虫蛋白质不同的昆虫抑制剂包括昆虫抑制性蛋白质、昆虫抑制性dsRNA分子和昆虫抑制性化学物质。这些与所述嵌合杀昆虫蛋白质不同的昆虫抑制剂可以对鳞翅目、鞘翅目、半翅目、同翅目或缨翅目的一种或多种害虫物种表现出活性。

[0022] 在又一个实施方案中，本文中公开了一种种子，其包含昆虫抑制有效量的以下各物：包含如SEQ ID NO:21、10、28、7、4、13、16、19、23、25、30、33、36、39、41、43、45、47、50、53、55、57、59、61、63、65、67、69、71、73、75、77、79、81、83、85、87、89、91、93、95、97、99、101、103、105、107、109或111中的任意者中所示出的氨基酸序列的嵌合杀昆虫蛋白质；或SEQ ID NO:1、2、3、5、6、8、9、11、12、14、15、17、18、20、22、24、26、27、29、31、32、34、35、37、38、40、42、44、46、48、49、51、52、54、56、58、60、62、64、66、68、70、72、74、76、78、80、82、84、86、88、90、92、94、96、98、100、102、104、106、108、110、112、113、114、115、116、117、118、119、120、121、122、123、124、125、126、127、128、129或130中的任意者中所示出的多核苷酸。

[0023] 还设想了一种防治鳞翅目害虫的方法，所述方法包括使所述鳞翅目害虫与抑制量的本发明嵌合杀昆虫蛋白质接触。

[0024] 在另一个实施方案中，本文中公开了一种转基因植物细胞、植物或植物部分，其包含嵌合杀昆虫蛋白质，其中：所述嵌合杀昆虫蛋白质包含如SEQ ID NO:21、10、28、7、4、13、16、19、23、25、30、33、36、39、41、43、45、47、50、53、55、57、59、61、63、65、67、69、71、73、75、77、79、81、83、85、87、89、91、93、95、97、99、101、103、105、107、109或111中的任意者中所示

出的任意氨基酸序列;或所述嵌合杀昆虫蛋白质包含如下蛋白质:与SEQ ID NO:21、10具有至少94%同一性;与SEQ ID NO:28具有至少93%同一性;与SEQ ID NO:7具有至少87%同一性;与SEQ ID NO:4具有至少90%同一性;与SEQ ID NO:13具有至少91%同一性;与SEQ ID NO:16具有至少64%同一性;与SEQ ID NO:19具有至少66%同一性;与SEQ ID NO:23具有至少86%同一性;与SEQ ID NO:25具有至少91%同一性;与SEQ ID NO:30具有至少94%同一性;与SEQ ID NO:33具有至少91%同一性;与SEQ ID NO:36具有至少64%同一性;与SEQ ID NO:39具有至少66%同一性;与SEQ ID NO:41具有至少94%同一性;与SEQ ID NO:43具有至少84%同一性;与SEQ ID NO:45具有至少93%同一性;与SEQ ID NO:47具有至少94%同一性;与SEQ ID NO:50具有至少91%同一性;或与SEQ ID NO:53具有至少93%同一性;或与SEQ ID NO:85、93、105具有至少87%同一性;或与SEQ ID NO:55、57、59、61、63、65、67、69、71、73、75、77、79具有至少85%同一性;或与SEQ ID NO:91、87、89具有至少88%同一性;或与SEQ ID NO:107、111具有至少89%同一性;或与SEQ ID NO:97具有至少90%同一性;与SEQ ID NO:109具有至少91%同一性;或与SEQ ID NO:83具有至少93%同一性;或与SEQ ID NO:91或103具有至少94%同一性;或与SEQ ID NO:95、101具有至少95%同一性;或与SEQ ID NO:99具有至少98%同一性。还设想了防治鳞翅目害虫的方法,其包括使所述害虫暴露于这种转基因植物细胞、植物或植物部分,其中所述植物细胞、植物或植物部分表达鳞翅目抑制量的所述嵌合杀昆虫蛋白质。

[0025] 在本文中的其他实施方案中,提供了来源于所述植物细胞、植物或植物部分的商品产品,其中所述产品包含可检测量的所述嵌合杀昆虫蛋白质。所设想的商品产品包括植物生物质、油、膳食、动物饲料、面粉、薄片、糠、棉绒、外壳和经过处理的种子。

[0026] 本文中所公开的又另一种方法是一种生产包含嵌合杀昆虫蛋白质的种子的方法,所述方法包括:种植至少一个包含嵌合杀昆虫蛋白质的种子;从所述种子长出植物;和从所述植物收获种子,其中所述收获的种子包含所述嵌合杀昆虫蛋白质。

[0027] 本文中还设想了编码嵌合杀昆虫蛋白质的重组多核苷酸分子,其包含选自由以下各项组成的群组的核苷酸序列:1、2、3、5、6、8、9、11、12、14、15、17、18、20、22、24、26、27、29、31、32、34、35、37、38、40、42、44、46、48、49、51、52、54、56、58、60、62、64、66、68、70、72、74、76、78、80、82、84、86、88、90、92、94、96、98、100、102、104、106、108、110、112、113、114、115、116、117、118、119、120、121、122、123、124、125、126、127、128、129或130;和任选地,编码与所述嵌合杀昆虫蛋白质不同的昆虫抑制剂的多核苷酸序列。

[0028] 本文中所设想的另一种重组核酸分子包含可操作地连接至编码嵌合杀昆虫蛋白质的多核苷酸片段的异源启动子,其中:所述嵌合杀昆虫蛋白质包含如SEQ ID NO:21、10、28、7、4、13、16、19、23、25、30、33、36、39、41、43、45、47、50、53、55、57、59、61、63、65、67、69、71、73、75、77、79、81、83、85、87、89、91、93、95、97、99、101、103、105、107、109或111中的任意者中所示出的任意氨基酸序列;或所述嵌合杀昆虫蛋白质包含如下蛋白质:与SEQ ID NO:21、10具有至少94%同一性;与SEQ ID NO:28具有至少93%同一性;与SEQ ID NO:7具有至少87%同一性;与SEQ ID NO:4具有至少90%同一性;与SEQ ID NO:13具有至少91%同一性;与SEQ ID NO:16具有至少64%同一性;与SEQ ID NO:19具有至少66%同一性;与SEQ ID NO:23具有至少86%同一性;与SEQ ID NO:25具有至少91%同一性;与SEQ ID NO:30具有至少94%同一性;与SEQ ID NO:33具有至少91%同一性;与SEQ ID NO:36具有至少64%同一

性;与SEQ ID NO:39具有至少66%同一性;与SEQ ID NO:41具有至少94%同一性;与SEQ ID NO:43具有至少84%同一性;与SEQ ID NO:45具有至少93%同一性;与SEQ ID NO:47具有至少94%同一性;与SEQ ID NO:50具有至少91%同一性;或与SEQ ID NO:53具有至少93%同一性;或与SEQ ID NO:85、93、105具有87%同一性;或与SEQ ID NO:55、57、59、61、63、65、67、69、71、73、75、77、79具有至少85%同一性;或与SEQ ID NO:91、87、89具有至少88%同一性;或与SEQ ID NO:107、111具有至少89%同一性;或与SEQ ID NO:97具有至少90%同一性;或与SEQ ID NO:109具有至少91%同一性;或与SEQ ID NO:83具有至少93%同一性;或与SEQ ID NO:91、103具有至少94%同一性;或与SEQ ID NO:95、101具有至少95%同一性;或与SEQ ID NO:99具有至少98%同一性;或者所述多核苷酸片段与具有如SEQ ID NO:1、2、3、5、6、8、9、11、12、14、15、17、18、20、22、24、26、27、29、31、32、34、35、37、38、40、42、44、46、48、49、51、52、54、56、58、60、62、64、66、68、70、72、74、76、78、80、82、84、86、88、90、92、94、96、98、100、102、104、106、108、110、112、113、114、115、116、117、118、119、120、121、122、123、124、125、126、127、128、129或130中的任意者中所示出的核苷酸序列的多核苷酸杂交。

[0029] 本发明的其他实施方案、特征和优点将从以下详细描述、实施例和权利要求书中显而易见。

[0030] 序列简述

[0031] SEQ ID NO:1是用于在细菌细胞中表达的编码TIC1100的重组DNA序列。

[0032] SEQ ID NO:2是用于在植物细胞中表达的编码TIC1100的合成DNA序列。

[0033] SEQ ID NO:3是用于在植物细胞中表达的编码TIC1100的合成DNA序列。

[0034] SEQ ID NO:4是TIC1100的氨基酸序列。

[0035] SEQ ID NO:5是用于在细菌细胞中表达的编码TIC860的重组DNA序列。

[0036] SEQ ID NO:6是用于在植物细胞中表达的编码TIC860的合成DNA序列。

[0037] SEQ ID NO:7是TIC860的氨基酸序列。

[0038] SEQ ID NO:8是用于在细菌细胞中表达的编码TIC867的重组DNA序列。

[0039] SEQ ID NO:9是用于在植物细胞中表达的编码TIC867的合成DNA序列。

[0040] SEQ ID NO:10是TIC867的氨基酸序列。

[0041] SEQ ID NO:11是用于在细菌细胞中表达的编码TIC867_20的重组DNA序列。

[0042] SEQ ID NO:12是用于在植物细胞中表达的编码TIC867_20的合成DNA序列。

[0043] SEQ ID NO:13是TIC867_20的氨基酸序列。

[0044] SEQ ID NO:14是用于在细菌细胞中表达的编码TIC867_21的重组DNA序列。

[0045] SEQ ID NO:15是用于在植物细胞中表达的编码TIC867_21的合成DNA序列。

[0046] SEQ ID NO:16是TIC867_21的氨基酸序列。

[0047] SEQ ID NO:17是用于在细菌细胞中表达的编码TIC867_22的重组DNA序列。

[0048] SEQ ID NO:18是用于在植物细胞中表达的编码TIC867_22的合成DNA序列。

[0049] SEQ ID NO:19是TIC867_22的氨基酸序列。

[0050] SEQ ID NO:20是用于在植物细胞中表达的编码TIC867_23的合成DNA序列。

[0051] SEQ ID NO:21是TIC867_23的氨基酸序列。

[0052] SEQ ID NO:22是用于在植物细胞中表达的编码TIC867_24的合成DNA序列。

[0053] SEQ ID NO:23是TIC867_24的氨基酸序列。

- [0054] SEQ ID NO:24是用于在植物细胞中表达的编码TIC867_24的合成DNA序列。
- [0055] SEQ ID NO:25是TIC867_25的氨基酸序列。
- [0056] SEQ ID NO:26是用于在细菌细胞中表达的编码TIC868的重组DNA序列。
- [0057] SEQ ID NO:27是用于在植物细胞中表达的编码TIC868的合成DNA序列。
- [0058] SEQ ID NO:28是TIC868的氨基酸序列。
- [0059] SEQ ID NO:29是用于在植物细胞中表达的编码TIC868_9的合成DNA序列。
- [0060] SEQ ID NO:30是TIC868_9的氨基酸序列。
- [0061] SEQ ID NO:31是用于在细菌细胞中表达的编码TIC868_10的重组DNA序列。
- [0062] SEQ ID NO:32是用于在植物细胞中表达的编码TIC868变异体TIC868_10的合成DNA序列。
- [0063] SEQ ID NO:33是TIC868_10的氨基酸序列。
- [0064] SEQ ID NO:34是用于在细菌细胞中表达的编码TIC868_11的重组DNA序列。
- [0065] SEQ ID NO:35是用于在植物细胞中表达的编码TIC868_11的合成DNA序列。
- [0066] SEQ ID NO:36是TIC868_11的氨基酸序列。
- [0067] SEQ ID NO:37是用于在细菌细胞中表达的编码TIC868_12的重组DNA序列。
- [0068] SEQ ID NO:38是用于在植物细胞中表达的编码TIC868_12的合成DNA序列。
- [0069] SEQ ID NO:39是TIC868_12的氨基酸序列。
- [0070] SEQ ID NO:40是用于在植物细胞中表达的编码TIC868_13的合成DNA序列。
- [0071] SEQ ID NO:41是TIC868_13的氨基酸序列。
- [0072] SEQ ID NO:42是用于在植物细胞中表达的编码TIC868_14的合成DNA序列。
- [0073] SEQ ID NO:43是TIC868_14的氨基酸序列。
- [0074] SEQ ID NO:44是用于在植物细胞中表达的编码TIC868_15的合成DNA序列。
- [0075] SEQ ID NO:45是TIC868_15的氨基酸序列。
- [0076] SEQ ID NO:46是用于在植物细胞中表达的编码TIC868_29的合成DNA序列。
- [0077] SEQ ID NO:47是TIC868_29的氨基酸序列。
- [0078] SEQ ID NO:48是用于在细菌细胞中表达的编码TIC869的重组DNA序列。
- [0079] SEQ ID NO:49是用于在植物细胞中表达的编码TIC869的合成DNA序列。
- [0080] SEQ ID NO:50是TIC869的氨基酸序列。
- [0081] SEQ ID NO:51是用于在细菌细胞中表达的编码TIC836的重组DNA序列。
- [0082] SEQ ID NO:52是用于在植物细胞中表达的编码TIC836的合成DNA序列。
- [0083] SEQ ID NO:53是TIC836的氨基酸序列。
- [0084] SEQ ID NO:54是编码嵌合TIC713氨基酸序列的DNA序列。
- [0085] SEQ ID NO:55是从SEQ ID NO:54中所示出的开放阅读框翻译而来的TIC713氨基酸序列。
- [0086] SEQ ID NO:56是编码嵌合TIC843氨基酸序列的DNA序列。
- [0087] SEQ ID NO:57是从SEQ ID NO:56中所示出的开放阅读框翻译而来的TIC843氨基酸序列。
- [0088] SEQ ID NO:58是编码嵌合TIC862氨基酸序列的DNA序列。
- [0089] SEQ ID NO:59是从SEQ ID NO:58中所示出的开放阅读框翻译而来的TIC862氨基

酸序列。

[0090] SEQ ID NO:60是编码嵌合TIC1099氨基酸序列的DNA序列。

[0091] SEQ ID NO:61是从SEQ ID NO:60中所示出的开放阅读框翻译而来的TIC1099氨基酸序列。

[0092] SEQ ID NO:62是编码嵌合TIC1099-T507E氨基酸序列的DNA序列。

[0093] SEQ ID NO:63是从SEQ ID NO:62中所示出的开放阅读框翻译而来的TIC1099-T507E氨基酸序列。

[0094] SEQ ID NO:64是编码嵌合TIC1099-R522K氨基酸序列的DNA序列。

[0095] SEQ ID NO:65是从SEQ ID NO:64中所示出的开放阅读框翻译而来的TIC1099-R522K氨基酸序列。

[0096] SEQ ID NO:66是编码嵌合TIC1099-K490S氨基酸序列的DNA序列。

[0097] SEQ ID NO:67是从SEQ ID NO:66中所示出的开放阅读框翻译而来的TIC1099-K490S氨基酸序列。

[0098] SEQ ID NO:68是编码嵌合TIC1099-T562R氨基酸序列的DNA序列。

[0099] SEQ ID NO:69是从SEQ ID NO:68中所示出的开放阅读框翻译而来的TIC1099-T562R氨基酸序列。

[0100] SEQ ID NO:70是编码嵌合TIC1099-S553R氨基酸序列的DNA序列。

[0101] SEQ ID NO:71是从SEQ ID NO:70中所示出的开放阅读框翻译而来的TIC1099-S553R氨基酸序列。

[0102] SEQ ID NO:72是编码嵌合TIC1099-G498D氨基酸序列的DNA序列。

[0103] SEQ ID NO:73是从SEQ ID NO:72中所示出的开放阅读框翻译而来的TIC1099-G498D氨基酸序列。

[0104] SEQ ID NO:74是编码嵌合TIC1099-K490A氨基酸序列的DNA序列。

[0105] SEQ ID NO:75是从SEQ ID NO:74中所示出的开放阅读框翻译而来的TIC1099-K490A氨基酸序列。

[0106] SEQ ID NO:76是编码嵌合TIC1099-E564A氨基酸序列的DNA序列。

[0107] SEQ ID NO:77是从SEQ ID NO:76中所示出的开放阅读框翻译而来的TIC1099-E564A氨基酸序列。

[0108] SEQ ID NO:78是编码嵌合TIC1103氨基酸序列的DNA序列。

[0109] SEQ ID NO:79是从SEQ ID NO:78中所示出的开放阅读框翻译而来的TIC1103氨基酸序列。

[0110] SEQ ID NO:80是编码嵌合TIC1101氨基酸序列的DNA序列。

[0111] SEQ ID NO:81是从SEQ ID NO:80中所示出的开放阅读框翻译而来的TIC1101氨基酸序列。

[0112] SEQ ID NO:82是编码嵌合TIC845氨基酸序列的DNA序列。

[0113] SEQ ID NO:83是从SEQ ID NO:82中所示出的开放阅读框翻译而来的TIC845氨基酸序列。

[0114] SEQ ID NO:84是编码嵌合TIC846氨基酸序列的DNA序列。

[0115] SEQ ID NO:85是从SEQ ID NO:84中所示出的开放阅读框翻译而来的TIC846氨基

酸序列。

[0116] SEQ ID NO:86是编码嵌合TIC858氨基酸序列的DNA序列。

[0117] SEQ ID NO:87是从SEQ ID NO:86中所示出的开放阅读框翻译而来的TIC858氨基酸序列。

[0118] SEQ ID NO:88是编码嵌合TIC865氨基酸序列的DNA序列。

[0119] SEQ ID NO:89是从SEQ ID NO:88中所示出的开放阅读框翻译而来的TIC865氨基酸序列。

[0120] SEQ ID NO:90是编码嵌合TIC866氨基酸序列的DNA序列。

[0121] SEQ ID NO:91是从SEQ ID NO:90中所示出的开放阅读框翻译而来的TIC866氨基酸序列。

[0122] SEQ ID NO:92是编码嵌合TIC838氨基酸序列的DNA序列。

[0123] SEQ ID NO:93是从SEQ ID NO:92中所示出的开放阅读框翻译而来的TIC838氨基酸序列。

[0124] SEQ ID NO:94是编码嵌合TIC839氨基酸序列的DNA序列。

[0125] SEQ ID NO:95是从SEQ ID NO:94中所示出的开放阅读框翻译而来的TIC839氨基酸序列。

[0126] SEQ ID NO:96是编码嵌合TIC841氨基酸序列的DNA序列。

[0127] SEQ ID NO:97是从SEQ ID NO:96中所示出的开放阅读框翻译而来的TIC841氨基酸序列。

[0128] SEQ ID NO:98是编码嵌合TIC842氨基酸序列的DNA序列。

[0129] SEQ ID NO:99是从SEQ ID NO:98中所示出的开放阅读框翻译而来的TIC842氨基酸序列。

[0130] SEQ ID NO:100是编码嵌合TIC850氨基酸序列的DNA序列。

[0131] SEQ ID NO:101是从SEQ ID NO:100中所示出的开放阅读框翻译而来的TIC850氨基酸序列。

[0132] SEQ ID NO:102是编码嵌合TIC859氨基酸序列的DNA序列。

[0133] SEQ ID NO:103是从SEQ ID NO:102中所示出的开放阅读框翻译而来的TIC859氨基酸序列。

[0134] SEQ ID NO:104是编码嵌合TIC861氨基酸序列的DNA序列。

[0135] SEQ ID NO:105是从SEQ ID NO:104中所示出的开放阅读框翻译而来的TIC861氨基酸序列。

[0136] SEQ ID NO:106是编码嵌合TIC848氨基酸序列的DNA序列。

[0137] SEQ ID NO:107是从SEQ ID NO:106中所示出的开放阅读框翻译而来的TIC848氨基酸序列。

[0138] SEQ ID NO:108是编码嵌合TIC849氨基酸序列的DNA序列。

[0139] SEQ ID NO:109是从SEQ ID NO:108中所示出的开放阅读框翻译而来的TIC849氨基酸序列。

[0140] SEQ ID NO:110是编码嵌合TIC847氨基酸序列的DNA序列。

[0141] SEQ ID NO:111是从SEQ ID NO:110中所示出的开放阅读框翻译而来的TIC847氨

基酸序列。

- [0142] SEQ ID NO:112是用于在植物细胞中表达的编码TIC713的合成DNA序列。
- [0143] SEQ ID NO:113是用于在植物细胞中表达的编码TIC713的合成DNA序列。
- [0144] SEQ ID NO:114是用于在植物细胞中表达的编码TIC843的合成DNA序列。
- [0145] SEQ ID NO:115是用于在植物细胞中表达的编码TIC862的合成DNA序列。
- [0146] SEQ ID NO:116是用于在植物细胞中表达的编码TIC1099的合成DNA序列。
- [0147] SEQ ID NO:117是用于在植物细胞中表达的编码TIC1103的合成DNA序列。
- [0148] SEQ ID NO:118是用于在植物细胞中表达的编码TIC845的合成DNA序列。
- [0149] SEQ ID NO:119是用于在植物细胞中表达的编码TIC846的合成DNA序列。
- [0150] SEQ ID NO:120是用于在植物细胞中表达的编码TIC858的合成DNA序列。
- [0151] SEQ ID NO:121是用于在植物细胞中表达的编码TIC866的合成DNA序列。
- [0152] SEQ ID NO:122是用于在植物细胞中表达的编码TIC838的合成DNA序列。
- [0153] SEQ ID NO:123是用于在植物细胞中表达的编码TIC841的合成DNA序列。
- [0154] SEQ ID NO:124是用于在植物细胞中表达的编码TIC842的合成DNA序列。
- [0155] SEQ ID NO:125是用于在植物细胞中表达的编码TIC850的合成DNA序列。
- [0156] SEQ ID NO:126是用于在植物细胞中表达的编码TIC859的合成DNA序列。
- [0157] SEQ ID NO:127是用于在植物细胞中表达的编码TIC861的合成DNA序列。
- [0158] SEQ ID NO:128是用于在植物细胞中表达的编码TIC848的合成DNA序列。
- [0159] SEQ ID NO:129是用于在植物细胞中表达的编码TIC849的合成DNA序列。
- [0160] SEQ ID NO:130是用于在植物细胞中表达的编码TIC847的合成DNA序列。

发明详述

[0161] 农业害虫防治领域中的问题的特征是需要对目标害虫有效果、对目标害虫物种表现出广谱毒性、能够在植物中表达而不会引起不希望的农艺学问题并且与市面上用于植物的当前毒素相比可提供替代作用模式的新杀昆虫蛋白质。本文中公开了新型嵌合杀昆虫蛋白质,并且解决了这些需要中的每一个,特别是对抗较宽范围的鳞翅目昆虫害虫。

[0162] 为了避免发展或规避昆虫对目前使用的杀昆虫蛋白质的抗性,需要具有不同的作用模式(MOA)以及广谱性和功效的新杀昆虫蛋白质用于鳞翅目防治。解决这种需要的一种方式是从不同的生物学来源,优选地从细菌、真菌或植物中发现新的杀昆虫蛋白质。另一种方法是在表现出结构相似性的各种Bt蛋白质之间互换片段以产生具有昆虫抑制性质的新嵌合Bt蛋白质。本领域中已知由对本领域中已知的众多天然杀昆虫晶体蛋白质的结构域结构进行再挑选来产生具有增强的嵌合蛋白质的可能性微乎其微。参见例如Jacqueline S.Knight等人,“A Strategy for Shuffling Numerous *Bacillus thuringiensis* Crystal Protein Domains.”*J.Economic Entomology*,97(6) (2004):1805-1813。

[0163] 本文中公开了编码新型嵌合杀昆虫蛋白质的重组核酸分子序列。这些杀昆虫蛋白质解决了本领域中持续需要工程改造其他毒性杀昆虫蛋白质以具有改善的杀昆虫性质(诸如对较宽范围的目标昆虫害虫物种的功效有所增加)和不同的作用模式。这组蛋白质的成员,包括本文中所公开的示例性蛋白质,对鳞翅目昆虫害虫物种表现出杀昆虫活性。

[0164] 术语“片段(segment/fragment)”在本申请中用于描述比描述所公开的嵌合杀昆

虫蛋白质的完整氨基酸或核酸序列短连续氨基酸或核酸序列。本申请中还公开了表现出昆虫抑制活性的片段,如果将所述片段与所述嵌合杀昆虫蛋白质的相应部分比对,那么将得到在所述片段与所述嵌合杀昆虫蛋白质的相应部分之间存在从约65%至约100%的任何分数百分比的氨基酸序列同一性。

[0165] 在本申请中提及术语“活性的”或“活性”、“杀虫活性”或“杀虫的”,或者“杀昆虫活性”、“昆虫抑制性”或“杀昆虫的”是指毒性剂,诸如杀昆虫蛋白质,在抑制(抑制生长、摄食、繁殖力或存活力)、遏制(遏制生长、摄食、繁殖力或存活力)、控制(控制害虫侵扰、控制含有有效量的杀昆虫蛋白质的特定作物上的害虫摄食活动)或杀死(引起发病、死亡或降低繁殖力)害虫方面的功效。这些术语意图包括对害虫提供杀虫有效量的杀昆虫蛋白质的结果,其中使所述害虫暴露于所述杀昆虫蛋白质将导致发病、死亡、繁殖力降低或发育迟缓。这些术语还包括由于在植物中或在植物上提供杀虫有效量的杀昆虫蛋白质而将害虫从植物、植物组织、植物部分、种子、植物细胞或从植物可能生长的特定地理位置中驱离。一般来说,杀虫活性是指杀昆虫蛋白质在抑制特定目标害虫(包括但不限于鳞翅目昆虫)的生长、发育、存活力、摄食行为、交配行为、繁殖力方面有效或对由以这种蛋白质、蛋白质片段、蛋白质片段或多核苷酸为食的昆虫引起的不利作用产生任何可测量的减少的能力。所述杀昆虫蛋白质可以由植物产生,或可以被施用至所述植物或施用至所述植物所处位置内的环境。术语“生物活性”、“有效的”、“有效果的”或其变化形式也是本申请中可互换用于描述本发明的嵌合杀昆虫蛋白质对目标昆虫害虫的作用的术语。

[0166] 当提供于目标害虫的食物中时,杀虫有效量的毒性剂在所述毒性剂接触所述害虫时表现出杀虫活性。毒性剂可以是杀昆虫蛋白质或本领域中已知的一种或多种化学试剂。杀昆虫化学试剂和杀昆虫蛋白质试剂可以单独使用或与彼此组合使用。化学试剂包括但不限于靶向目标害虫中的特定基因以便遏制的dsRNA分子、有机氯化物、有机磷酸酯、氨基甲酸酯、拟除虫菊酯、新烟碱和莱恩碱。杀昆虫蛋白质试剂包括本申请中示出的嵌合杀昆虫蛋白质,以及其他蛋白质毒性剂,包括靶向鳞翅目害虫物种的那些,以及用于防治其他植物害虫的蛋白质毒素,诸如本领域中可用于防治鞘翅目、缨翅目、半翅目和同翅目物种的Cry蛋白质。

[0167] 意图提及害虫、特别是作物植物的害虫意指作物植物的昆虫害虫,特别是通过所公开的嵌合杀昆虫蛋白质加以防治的那些鳞翅目昆虫害虫。然而,当靶向这些害虫的毒性剂与所述嵌合杀昆虫蛋白质或同所述嵌合杀昆虫蛋白质具有65%至约100%同一性的蛋白质共处或共存时,提及害虫还可以包括植物的鞘翅目、半翅目和同翅目昆虫害虫以及线虫和真菌。

[0168] 本文中所公开的嵌合杀昆虫蛋白质对鳞翅目昆虫物种的昆虫害虫(包括成虫、蛹、幼虫和初孵幼虫)以及半翅目昆虫物种(包括成虫和若虫)表现出杀昆虫活性。鳞翅目昆虫包括但不限于夜蛾科的粘虫、切根虫、尺蠖和夜蛾,例如草地粘虫(草地贪夜蛾)、甜菜粘虫(甜菜夜蛾)、披肩粘虫(蓓带夜蛾)、黑切根虫(球菜夜蛾)、甘蓝尺蠖(粉纹夜蛾)、大豆尺蠖(大豆夜蛾)、黎豆毛虫(黎豆夜蛾)、绿啃叶虫(苜蓿绿夜蛾)、烟草芽虫(烟芽夜蛾)、颗粒切根虫(黄地老虎)、粘虫(一星粘虫)、西方切根虫(灰地老虎);螟蛾科的钻心虫、鞘蛾、结网虫、球果虫、甘蓝虫和雕叶虫,例如,欧洲玉米钻心虫(欧洲玉米螟)、脐橙虫(脐橙螟)、玉米根结网虫(玉米根网螟)、草地结网虫(水稻切叶野螟)、向日葵蛾(向日葵螟)、小玉米茎钻心

虫(南美玉米苗斑螟);卷蛾科的卷叶虫、芽虫、种虫和果虫,例如,苹果蛾(苹果蠹蛾)、葡萄果实蛾(葡萄小食心虫)、东方果蛾(梨小食心虫)、向日葵芽小卷蛾(向日葵芽卷叶蛾);和许多其他经济上重要的鳞翅目昆虫,例如钻背蛾(小菜蛾)、粉红棉铃虫(棉红铃虫)和吉普赛蛾(舞毒蛾)。其他鳞翅目昆虫害虫包括例如棉叶波纹夜蛾(棉叶虫)、果树黄卷蛾(果树卷叶虫)、玫瑰黄卷蛾(欧洲卷叶虫)和其他黄卷蛾属、二化螟(亚洲水稻钻心虫或水稻钻茎虫)、稻纵卷叶螟(水稻卷叶虫)、玉米根网螟(玉米根结网虫)、早熟禾草螟(蓝草结网虫)、西南玉米螟(西南玉米钻心虫)、小蔗螟(蔗螟)、埃及金刚钻(多刺棉铃虫)、翠纹金刚钻(斑点棉铃虫)、棉铃实夜蛾(美洲棉铃虫)、玉米穗夜蛾(玉米穗虫或棉铃虫)、烟芽夜蛾(烟草芽虫)、水稻切叶野螟(草地结网虫)、欧洲葡萄缀穗蛾(欧洲葡萄蛾)、柑桔潜叶蛾(柑桔潜叶虫)、大菜粉蛾(大白蝴蝶)、小菜粉蛾(菜青虫或小白蝴蝶)、小菜蛾(钻背蛾)、甜菜夜蛾(甜菜粘虫)、斜纹夜蛾(烟草切根虫、茶蚕)和番茄潜叶蛾(番茄潜叶虫)。

[0169] 本申请中提及“分离的DNA分子”或者等效术语或短语意图意指所述DNA分子是单独或与其他组合物组合存在但不在其天然环境内的DNA分子。举例来说,只要生物体基因组DNA内天然存在的核酸元件,诸如编码序列、内含子序列、非翻译前导序列、启动子序列、转录终止序列等在所述生物体基因组内且在天然存在其的基因组内位置上,所述元件就不被认为是“分离的”。然而,只要这些元件中的每一个和这些元件的子部分不是在所述生物体基因组内且在天然存在其的基因组内位置上,所述元件在本公开的范围就将是“分离的”。类似地,只要编码杀昆虫蛋白质或所述蛋白质的任何天然存在的杀昆虫变体的核苷酸序列不在从中天然存在编码所述蛋白质的序列的细菌DNA内,所述核苷酸序列就将是分离的核苷酸序列。出于本公开的目的,编码天然存在的杀昆虫蛋白质的氨基酸序列的合成核苷酸序列将被认为是分离的。出于本公开的目的,任何转基因核苷酸序列,即插入植物或细菌细胞基因组中或存在于染色体外载体中的DNA核苷酸序列将被认为是分离的核苷酸序列,无论其存在于质粒内或用于转化所述细胞的类似结构内、所述植物或细菌的基因组内,还是以可检测的量存在于来源于所述植物或细菌的组织、子代、生物样品或商品产品中。

[0170] 如实施例中进一步描述,通过嵌合工作,由已知杀昆虫毒素(在本文中被成为“亲本蛋白质”的原毒素和毒素结构域构建了约八百四十四(844)个编码嵌合杀昆虫蛋白质的核苷酸序列,并且加以表达且在生物测定中测试鳞翅目活性。与从中得到其毒素组分的亲本蛋白质相比,所构建的嵌合杀昆虫蛋白质中少数表现出提高的鳞翅目活性或增大的鳞翅目谱。

[0171] 这些具有提高的鳞翅目活性或增大的鳞翅目谱的新型嵌合杀昆虫蛋白质是由以下杀昆虫亲本蛋白质原毒素和毒素结构域构建:Cry1Ah(结构域I)、Cry1Bb1(结构域I和II)、Cry1Be2(结构域I和II)、Cry1Ja1(结构域I和II)、Cry1Fa1(结构域I和II)、Cry1Ac(结构域II和结构域原毒素)、Cry1Ca(结构域III和结构域原毒素)、Cry1Ka(结构域III和结构域原毒素)、Cry1Jx(结构域III)、Cry1Ab(结构域III)、Cry1Ab3(原毒素)、Cry1Da1(原毒素)、Cry4(原毒素)、Cry9(原毒素)、Cry1Be(原毒素),和结构域Cry1Ka(原毒素)。

[0172] 具体来说,本发明的具有提高的鳞翅目活性或增大的鳞翅目谱的新型嵌合杀昆虫蛋白质包含以下原毒素与结构域组合:TIC1100/SEQ ID NO:4(结构域I-Cry1Ah、结构域II-Cry1Ac、结构域III-Cry1Ca、原毒素-Cry1Ac)、TIC860/SEQ ID NO:7(结构域I-Cry1Bb1、结构域II-Cry1BB1、结构域III-Cry1Ca、原毒素-Cry1Ac)、TIC867/SEQ ID NO:10(结构域I-

Cry1Be2、结构域II-Cry1Be2、结构域III-Cry1Ka、原毒素-Cry1Ab3)、TIC868/SEQ ID NO:28 (结构域I-Cry1Be2、结构域II-Cry1Be2和结构域III-Cry1Ca、原毒素-Cry1Ab3)、TIC869/SEQ ID NO:50 (结构域I-Cry1Ja1、结构域II-Cry1Ja1、结构域III-Cry1Jx、原毒素-Cry1Ab3) 和TIC836/SEQ ID NO:53 (结构域I-Cry1Fa1、结构域II-Cry1Fa1、结构域III-Cry1Ab、原毒素-Cry1Ac)。

[0173] 对于嵌合杀昆虫蛋白质TIC867和TIC868,还构建了引入氨基酸取代或替代原毒素结构域的变体。具体来说,TIC867和TIC868的这些变体包含以下氨基酸取代或替代原毒素结构域:TIC867_20/SEQ ID NO:13(替代原毒素结构域Cry1Da1)、TIC867_21/SEQ ID NO:16(替代原毒素结构域Cry4)、TIC867_22/SEQ ID NO:19(替代原毒素结构域Cry9)、TIC867_23/SEQ ID NO:21(替代原毒素结构域Cry1Be)、TIC867_24/SEQ ID NO:23(替代原毒素结构域Cry1Ka)、TIC867_25/SEQ ID NO:25(替代原毒素结构域Cry1Ka)、TIC868_9/SEQ ID NO:30(氨基酸修饰N240S_Y343Q_N349T)、TIC868_10/SEQ ID NO:33(替代原毒素结构域Cry1Da1)、TIC868_11/SEQ ID NO:36(替代原毒素结构域Cry4)、TIC868_12/SEQ ID NO:39(替代原毒素结构域Cry 9),TIC868_13/SEQ ID NO:41(替代原毒素结构域Cry1Be)、TIC868_14/SEQ ID NO:43(替代原毒素结构域Cry1Ka)、TIC868_15/SEQ ID NO:45(替代原毒素结构域Cry1Ca)和TIC868_29/SEQ ID NO:47(氨基酸修饰Q136Y_Y343Q_N349T)。

[0174] 如实施例中所显示,这些TIC867和TIC868变体中的每一种都能改变亲本嵌合杀昆虫蛋白质的鳞翅目活性和/或减小鳞翅目活性谱,因此表明替代原毒素结构域和氨基酸取代对嵌合杀昆虫蛋白质TIC867和TIC868的杀昆虫活性和谱具有直接后果。

[0175] 所述嵌合杀昆虫蛋白质中有许多种对多个鳞翅目昆虫害虫物种显示了杀昆虫活性。具体来说,本申请中所公开的新型嵌合杀昆虫蛋白质对一种或多种以下鳞翅目昆虫害虫表现出活性:黎豆毛虫(VBC,黎豆夜蛾)、蔗螟(SCB,小蔗螟)、小玉米茎钻心虫(LSCB,南美玉米苗斑螟)、玉米穗虫(CEW,玉米穗夜蛾)、大豆荚虫(SPW,玉米穗夜蛾)、棉铃虫(CBW,玉米穗夜蛾)、烟草芽虫(TBW,烟芽夜蛾)、大豆尺蠖(SBL,大豆夜蛾)、黑粘虫(BLAW,考斯夜蛾)、南方粘虫(SAW,亚热带粘虫)、草地粘虫(FAW,草地贪夜蛾)、甜菜粘虫(BAW,甜菜夜蛾)、古棉铃虫(OBW,棉铃实夜蛾)、东方叶虫(OLW,斜纹夜蛾)、粉红棉铃虫(PBW,棉红铃虫)、西南玉米钻心虫(SWCB,西南玉米螟)、斑点棉铃虫(SBW,翠纹金刚钻)、美洲棉铃虫(SABW,南美棉铃虫)和向日葵尺蠖(SFL,薄荷灰夜蛾)。因此,本申请中所描述的示例性蛋白质因常见功能而相关并且对鳞翅目昆虫物种的昆虫害虫,包括成虫、幼虫和蛹,表现出杀昆虫活性。

[0176] 与所述嵌合杀昆虫蛋白质类似的蛋白质可以通过使用本领域中已知的基于计算机的算法与彼此比较来加以鉴别。举例来说,可以使用Clustal W比对,使用以下这些默认参数来分析蛋白质相对于嵌合杀昆虫蛋白质的氨基酸序列同一性:权重矩阵:blosum,空位开放罚分:10.0,空位延伸罚分:0.05,亲水性空位:0n,亲水性残基:GPSNDQERK,残基特异性空位罚分:0n(Thompson等人,(1994)Nucleic Acids Research,22:4673-4680)。通过100%×(氨基酸同一性/标的蛋白质的长度)的乘积来进一步计算氨基酸同一性百分比。还可利用本领域中的其他比对算法,得到与使用Clustal W比对获得的结果类似的结果,且涵盖在本申请中。

[0177] 意图本申请中公开一种表现出昆虫抑制活性的查询蛋白质,在比对此类查询蛋白质与SEQ ID NO:4、7、10、13、16、19、21、23、25、28、30、33、36、39、41、43、45、47、50、53、55、

57、59、61、63、65、67、69、71、73、75、77、79、81、83、85、87、89、91、93、95、97、99、101、103、105、107、109和111中所示出的标的嵌合杀昆虫蛋白质时,在所述查询蛋白质与所述标的蛋白质之间获得至少约64%、65%、66%、67%、68%、69%、70%、71%、72%、73%、74%、75%、76%、77%、78%、79%、80%、81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或约100%氨基酸序列同一性(或此范围中的任何分数百分比)。

[0178] 如本申请的实施例中进一步描述,设计编码所述嵌合杀昆虫蛋白质的合成序列或人工序列以供用于植物中。设计用于植物中的示例性合成核苷酸序列示出于SEQ ID NO:2和3(TIC1100)、SEQ ID NO:6(TIC860)、SEQ ID NO:9(TIC867)、SEQ ID NO:12(TIC867_20)、SEQ ID NO:15(TIC867_21)、SEQ ID NO:18(TIC867_22)、SEQ ID NO:20(TIC867_23)、SEQ ID NO:22(TIC867_24)、SEQ ID NO:24(TIC867_25)、SEQ ID NO:27(TIC868)、SEQ ID NO:29(TIC868_9)、SEQ ID NO:32(TIC868_10)、SEQ ID NO:35(TIC868_11)、SEQ ID NO:38(TIC868_12)、SEQ ID NO:40(TIC868_13)、SEQ ID NO:42(TIC868_14)、SEQ ID NO:44(TIC868_15)、SEQ ID NO:46(TIC868_29)、SEQ ID NO:49(TIC869)和SEQ ID NO:52(TIC836)、SEQ ID NO:112和113(TIC713)、SEQ ID NO:114(TIC843)、SEQ ID NO:115(TIC862)、SEQ ID NO:116(TIC1099)、SEQ ID NO:117(TIC1103)、SEQ ID NO:118(TIC845)、SEQ ID NO:119(TIC846)、SEQ ID NO:120(TIC858)、SEQ ID NO:121(TIC866)、SEQ ID NO:122(TIC838)、SEQ ID NO:123(TIC841)、SEQ ID NO:124(TIC842)、SEQ ID NO:125(TIC850)、SEQ ID NO:126(TIC859)、SEQ ID NO:127(TIC861)、SEQ ID NO:128(TIC848)、SEQ ID NO:129(TIC849)和SEQ ID NO:130(TIC847)中。

[0179] 为了在植物细胞中表达,所述嵌合杀昆虫蛋白质可以被表达以存在于细胞溶质中或靶向植物细胞的各种细胞器。举例来说,使蛋白质靶向叶绿体可能导致转基因植物中的表达蛋白质的水平增加,同时防止出现脱靶表型。靶向还可能导致转基因事件中的害虫抗性功效增加。靶标肽或转运肽是指导蛋白质转运至细胞中特定区域,包括细胞核、线粒体、内质网(ER)、叶绿体、质外体、过氧化物酶体和胞质膜的短(3至70个氨基酸长)肽链。在蛋白质被转运之后通过信号肽酶使一些靶标肽从所述蛋白质上裂解。为了靶向叶绿体,蛋白质含有大约40至50个氨基酸的转运肽。关于叶绿体转运肽的使用的描述,参见美国专利No.5,188,642和5,728,925。许多位于叶绿体的蛋白质是从细胞核基因表达为前体,并且通过叶绿体转运肽(CTP)靶向叶绿体。此类分离的叶绿体蛋白质的实例包括但不限于与核酮糖-1,5-双磷酸羧化酶、铁氧化还原蛋白、铁氧化还原蛋白氧化还原酶、捕光复合体蛋白I和蛋白II、硫氧化还原蛋白F、烯醇丙酮莽草酸磷酸合酶(EPSPS)和美国专利No.7,193,133中所描述的转运肽的小亚单元(SSU)缔合的那些。已经在活体内和活体外证明,可以通过使用与异源CTP的蛋白质融合物使非叶绿体蛋白质靶向叶绿体,而且CTP足以使蛋白质靶向叶绿体。并入合适的叶绿体转运肽,诸如阿拉伯芥EPSPS CTP(CTP2)(参见Klee等人,Mol.Gen.Genet.210:437-442,1987)或矮牵牛EPSPS CTP(CTP4)(参见della-Cioppa等人,Proc.Natl.Acad.Sci.USA 83:6873-6877,1986)已经证明异源EPSPS蛋白质序列靶向转基因植物中的叶绿体(参见美国专利No.5,627,061、5,633,435和5,312,910;以及EP 0218571、EP 189707、EP 508909和EP 924299)。为了使嵌合杀昆虫蛋白质靶向叶绿体,相对于已经设计的用于在植物细胞中进行最佳表达的编码嵌合杀昆虫蛋白质的合成编码序列,

将编码叶绿体转运肽的序列的放在5'处的可操作键联中和框架中。

[0180] 根据本领域中已知的转化方法和技术来构建含有这些合成或人工核苷酸序列的表达盒和载体并且将其引入玉米、棉花和大豆植物细胞中。使转化细胞再生出转化植物,观察到所述转化植物表达所述嵌合杀昆虫蛋白质。为了测试杀虫活性,在存在鳞翅目害虫幼虫的情况下使用获自转化植物的植物叶盘进行生物测定。设想了编码所述嵌合杀昆虫蛋白质的重组核酸分子组合物。举例来说,可以用重组DNA构建体表达所述嵌合杀昆虫蛋白质,其中编码嵌合杀昆虫蛋白质的具有ORF的多核苷酸分子可操作地连接至诸如启动子之类的基因表达元件和在所述构建体所意图的系统中表达所必需的任何其他调控元件。非限制性实例包括与合成嵌合杀昆虫蛋白质编码序列可操作地连接以便在植物中表达所述嵌合杀昆虫蛋白质的植物功能启动子或与嵌合杀昆虫蛋白质编码序列可操作地连接以便在Bt细菌或其他芽孢杆菌属中表达所述蛋白质的Bt功能启动子。其他元件可以可操作地连接至所述嵌合杀昆虫蛋白质编码序列,包括但不限于增强子、内含子、未翻译前导序列、编码蛋白质固定标签(HIS标签)、转位肽(即,质粒转运肽、信号肽)、针对翻译后修饰酶的多肽序列、核糖体结合位点和RNAi靶位点。

[0181] 本文中所提供的示例性重组多核苷酸分子包括但不限于与编码具有如SEQ ID NO:4(TIC1100)、7(TIC860)、10(TIC867)、13(TIC867_20)、16(TIC867_21)、19(TIC867_22)、21(TIC867_23)、23(TIC867_24)、25(TIC867_25)、28(TIC868)、30(TIC868_9)、33(TIC868_10)、36(TIC868_11)、39(TIC867_12)、41(TIC867_13)、43(TIC867_14)、45(TIC867_15)、47(TIC867_29)、50(TIC869)、53(TIC836)、55(TIC713)、57(TIC843)、59(TIC862)、61(TIC1099)、63(TIC1099-T507E)、65(TIC1099-R522K)、67(TIC1099-K490S)、69(TIC1099-T562R)、71(TIC1099-S533R)、73(TIC1099-G498D)、75(TIC1099-K490A)、77(TIC1099-E564A)、79(TIC1103)、81(TIC1101)、83(TIC845)、85(TIC846)、87(TIC858)、89(TIC865)、91(TIC866)、93(TIC838)、95(TIC839)、97(TIC841)、99(TIC842)、101(TIC850)、103(TIC859)、105(TIC861)、107(TIC848)、109(TIC849)和111(TIC847)中所示出的氨基酸序列的多肽或蛋白质的诸如SEQ ID NO:1、2、3、5、6、8、9、11、12、14、15、17、18、20、22、24、26、27、29、31、32、34、35、37、38、40、42、44、46、48、49、51、52、54、56、58、60、62、64、66、68、70、72、74、76、78、80、82、84、86、88、90、92、94、96、98、100、102、104、106、108、110、112、113、114、115、116、117、118、119、120、121、122、123、124、125、126、127、128、129和130之类的多核苷酸可操作地连接的异源启动子。异源启动子还可以可操作地连接至编码质粒靶向的嵌合杀昆虫蛋白质和未靶向的嵌合杀昆虫蛋白质的合成DNA编码序列。设想编码本文中所公开的嵌合杀昆虫蛋白质的重组核酸分子的密码子可以被同义密码子取代(在本领域中被称作沉默取代)。

[0182] 包含嵌合杀昆虫蛋白质编码序列的重组DNA分子或构建体可以进一步包含编码一种或多种毒性剂的DNA区域,所述DNA区域可以经配置以便与编码嵌合杀昆虫蛋白质、不同于嵌合杀昆虫蛋白质的蛋白质、昆虫抑制性dsRNA分子或辅助蛋白质的DNA序列伴随表达或共同表达。辅助蛋白质包括但不限于辅因子、酶、结合伴侣或功能在于辅助昆虫抑制剂的效果,例如通过辅助其表达、影响其在植物中的稳定性、优化寡聚自由能、加强其毒性和增加其活性谱的其他试剂。辅助蛋白质可以例如促进一种或多种昆虫抑制剂的吸收或增强毒性剂的毒性效果。

[0183] 可以组装重组DNA分子或构建体,使得所有蛋白质或dsRNA分子都可以从一种启动

子表达,或者每一种蛋白质或dsRNA分子都在单独启动子控制或其一些组合下。本发明的蛋白质可以由多基因表达系统表达,其中嵌合杀昆虫蛋白质由常见核苷酸片段表达,所述核苷酸片段还含有其他开放阅读框和启动子,取决于所选表达系统的类型。举例来说,细菌多基因表达系统可以利用单个启动子来驱动多个连接/串联开放阅读框从单个操纵子内表达(即,多顺反子表达)。在另一个实例中,植物多基因表达系统可以利用多个未连接的表达盒,每一个表达盒表达不同的蛋白质或其他毒性剂,诸如一或多个dsRNA分子。

[0184] 可以通过载体,例如质粒、杆状病毒、合成染色体、病毒体、粘粒、噬菌粒、噬菌体或病毒载体,将包含嵌合杀昆虫蛋白质编码序列的重组核酸分子或重组DNA构建体递送至宿主细胞。此类载体可用于实现嵌合杀昆虫蛋白质编码序列在宿主细胞中的稳定或暂时表达或者所编码的多肽的后续表达。包含嵌合杀昆虫蛋白质序列编码序列并且被引入宿主细胞中的外源重组多核苷酸或重组DNA构建体在本文中被称作“转基因”。

[0185] 本文中提供了含有编码所述嵌合杀昆虫蛋白质中的任何一种或多种的多核苷酸的转基因细菌、转基因植物细胞、转基因植物和转基因植物部分。术语“细菌细胞”或“细菌”可以包括但不限于土壤杆菌、芽孢杆菌、埃希氏杆菌、沙门氏菌、假单胞菌或根瘤菌细胞。术语“植物细胞”或“植物”可以包括但不限于双子叶细胞或单子叶细胞。所设想的植物和植物细胞包括但不限于苜蓿、香蕉、大麦、豆、茎椰菜、甘蓝、芸苔、胡萝卜、木薯、蓖麻、花椰菜、芹菜、鹰嘴豆、大白菜、柑桔、椰子、咖啡、玉米、三叶草、棉花、葫芦、黄瓜、花旗松、茄子、桉树、亚麻、大蒜、葡萄、啤酒花、韭菜、莴苣、火炬松、小米、甜瓜、坚果、燕麦、橄榄树、洋葱、观赏植物、棕榈、牧草、豌豆、花生、胡椒、木豆、松树、马铃薯、白杨树、南瓜、辐射松、萝卜、油菜籽、水稻、根茎、黑麦、红花、灌木、高粱、南方松、大豆、菠菜、南瓜、草莓、糖用甜菜、甘蔗、向日葵、甜玉米、香枫、甜薯、柳枝稷、茶树、烟草、番茄、黑小麦、草坪草、西瓜和小麦植物细胞或植物。在某些实施方案中,提供了由转基因植物细胞再生的转基因植物和转基因植物部分。在某些实施方案中,所述转基因植物可以获自转基因种子、通过插枝、折断、研磨或其他方式使部分从植物脱离。在某些实施方案中,所述植物部分可以是种子、蒴、叶、花、茎、根或其任何部分,或者转基因植物部分的不可再生部分。如这种情况下所使用,转基因植物部分的“不可再生”部分是不能受到诱导而形成完整植物或不能受到诱导而形成能够进行有性繁殖和/或无性繁殖的完整植物的部分。在某些实施方案中,植物部分的不可再生部分是转基因种子、蒴、叶、花、茎或根的一部分。

[0186] 提供了制造包含鳞翅目抑制量的嵌合杀昆虫蛋白质的转基因植物的方法。此类植物可以通过将编码本申请中所提供的嵌合杀昆虫蛋白质的多核苷酸引入植物细胞中和选择表达昆虫或鳞翅目抑制量的嵌合杀昆虫蛋白质的来源于所述植物细胞的植物来制造。植物可以通过再生、种子、花粉或分生组织转化技术而来源于植物细胞。用于转化植物的方法在本领域中是已知的。举例来说,土壤杆菌介导的转化描述于美国专利申请公布2009/0138985A1(大豆)、2008/0280361A1(大豆)、2009/0142837A1(玉米)、2008/0282432(棉花)和2008/0256667(棉花)中。

[0187] 表达所述嵌合杀昆虫蛋白质的植物可以通过利用表达其他杀昆虫蛋白质和/或表达其他转基因特质(诸如其他昆虫防治特质)的转基因事件、除草剂耐受基因、赋予产量或应力耐受特质的基因等等进行育种来进行杂交,或可以将此类特质组合在单个载体中,从而将所述特质全部联系起来。

[0188] 本申请中还公开了经过加工的植物产品,其中所述经过加工的产品包含可检测量的嵌合杀昆虫蛋白质、其昆虫抑制性片段或其任何区别部分。在某些实施方案中,所述经过加工的产品是选自由以下各项组成的群组:植物部分、植物生物质、油、膳食、糖、动物饲料、面粉、薄片、糠、棉绒、外壳、经过加工的种子和种子。在某些实施方案中,所述经过加工的产品是不可再生的。所述植物产品可以包含来源于转基因植物或转基因植物部分的商品或其他商业产品,其中所述商品或其他产品可以通过检测编码或包含嵌合杀昆虫蛋白质的特色部分的核苷酸片段或者表达的RNA或蛋白质来通过商业进行追踪。

[0189] 本申请中还公开了用所述嵌合杀昆虫蛋白质来防治昆虫、特别是鳞翅目昆虫侵扰作物植物的方法。此类方法可以包括种植包含昆虫或鳞翅目抑制量的所述嵌合杀昆虫蛋白质的植物。在某些实施方案中,此类方法可以进一步包括以下操作中的任何一个或多个:(i) 向植物或能产生植物的种子施用包含或编码嵌合杀昆虫蛋白质的任何组合物;和(ii) 用编码嵌合杀昆虫蛋白质的多核苷酸对植物或能产生植物的植物细胞进行转化。一般来说,设想嵌合杀昆虫蛋白质可以提供于组合物中,提供于微生物中,或提供于转基因植物中,以赋予针对鳞翅目昆虫的昆虫抑制活性。

[0190] 在某些实施方案中,所述嵌合杀昆虫蛋白质是通过培养经过转化以便在适合表达的条件下表达嵌合杀昆虫蛋白质的重组芽孢杆菌或任何其他重组细菌细胞而制备的昆虫抑制性组合物的杀昆虫活性成分。此类组合物可以通过对表达/产生所述嵌合杀昆虫蛋白质的此类重组细胞的培养物进行干燥、冻干、均质化、提取、过滤、离心、沉降或浓缩来制备。此类方法可以获得芽孢杆菌或其他昆虫病原细菌细胞提取物、细胞悬浮液、细胞匀浆、细胞溶解产物、细胞上清液、细胞滤液或细胞球粒。通过获得如此产生的嵌合杀昆虫蛋白质,包括所述嵌合杀昆虫蛋白质的组合物可以包括细菌细胞、细菌孢子和伴孢包涵体,并且可以经过配制以用于各种用途,包括作为农业昆虫抑制性喷雾产品或作为食物生物测定中的昆虫抑制性制剂。

[0191] 上述化合物或制剂可以进一步包含农业上可接受的载体,诸如诱饵、粉末、粉尘、球粒、颗粒、喷雾、乳液、胶态悬浮液、水溶液、芽孢杆菌孢子或晶体制剂或种子处理。所述化合物或制剂还可以进一步包含经转化以表达一种或多种所述蛋白质的重组植物细胞、植物组织、种子或植物;或经转化以表达一种或多种所述蛋白质的细菌。取决于所述重组多肽固有的昆虫抑制或杀昆虫抑制水平和应用于植物或食物测定的化合物或制剂水平,所述化合物或制剂可以包括各种以重量计量的所述重组多肽,例如以重量计0.0001%至0.001%至0.01%至1%至99%的所述重组多肽。

[0192] 在一个实施方案中,为了降低抗性发展的可能性,包含嵌合杀昆虫蛋白质的昆虫抑制组合物或转基因植物可以进一步包含至少一种其他毒性剂,所述毒性剂对相同鳞翅目昆虫物种表现出昆虫抑制活性但不同于所述嵌合杀昆虫蛋白质。用于此类组合物的其他可能毒性剂包括昆虫抑制性蛋白质和昆虫抑制性dsRNA分子。使用此类核糖核苷酸序列来防治昆虫害虫的一个实例描述于Baum等人(美国专利公布2006/0021087A1)中。用于防治鳞翅目害虫的此类其他多肽可以选自由昆虫抑制性蛋白质组成的群组,诸如但不限于Cry1A(美国专利No.5,880,275)、Cry1Ab、Cry1Ac、Cry1A.105、Cry1Ae、Cry1B(美国专利公布No.10/525,318)、Cry1C(美国专利No.6,033,874)、Cry1D、Cry1E、Cry1F和Cry1A/F嵌合体(美国专利No.7,070,982、6,962,705和6,713,063)、Cry1G、Cry1H、Cry1I、Cry1J、Cry1K、Cry1L、

Cry2A、Cry2Ab(美国专利No.7,064,249)、Cry2Ae、Cry4B、Cry6、Cry7、Cry8、Cry9、Cry15、Cry43A、Cry43B、Cry51Aa1、ET66、TIC400、TIC800、TIC834、TIC1415、Vip3A、VIP3Ab、VIP3B、AXMI-001、AXMI-002、AXMI-030、AXMI-035和AXMI-045(美国专利公布2013-0117884A1)、AXMI-52、AXMI-58、AXMI-88、AXMI-97、AXMI-102、AXMI-112、AXMI-117、AXMI-100(美国专利公布2013-0310543A1)、AXMI-115、AXMI-113、AXMI-005(美国专利公布2013-0104259A1)、AXMI-134(美国专利公布2013-0167264A1)、AXMI-150(美国专利公布2010-0160231A1)、AXMI-184(美国专利公布2010-0004176A1)、AXMI-196、AXMI-204、AXMI-207、AXMI-209(美国专利公布2011-0030096A1)、AXMI-218、AXMI-220(美国专利公布2014-0245491A1)、AXMI-221z、AXMI-222z、AXMI-223z、AXMI-224z、AXMI-225z(美国专利公布2014-0196175A1)、AXMI-238(美国专利公布2014-0033363A1)、AXMI-270(美国专利公布2014-0223598A1)、AXMI-345(美国专利公布2014-0373195A1)、DIG-3(美国专利公布2013-0219570A1)、DIG-5(美国专利公布2010-0317569A1)、DIG-11(美国专利公布2010-0319093A1)、AfIP-1A及其衍生物(美国专利公布2014-0033361A1)、AfIP-1B及其衍生物(美国专利公布2014-0033361A1)、PIP-1APIP-1B(美国专利公布2014-0007292A1)、PSEEN3174(美国专利公布2014-0007292A1)、AECFG-592740(美国专利公布2014-0007292A1)、Pput_1063(美国专利公布2014-0007292A1)、Pput_1064(美国专利公布2014-0007292A1)、GS-135及其衍生物(美国专利公布2012-0233726A1)、GS153及其衍生物(美国专利公布2012-0192310A1)、GS154及其衍生物(美国专利公布2012-0192310A1)、GS155及其衍生物(美国专利公布2012-0192310A1)、如美国专利公布2012-0167259A1中所描述的SEQ ID NO:2及其衍生物、如美国专利公布2012-0047606A1中所描述的SEQ ID NO:2及其衍生物、如美国专利公布2011-0154536A1中所描述的SEQ ID NO:2及其衍生物、如美国专利公布2011-0112013A1中所描述的SEQ ID NO:2及其衍生物、如美国专利公布2010-0192256A1中所描述的SEQ ID NO:2和4及其衍生物、如美国专利公布2010-0077507A1中所描述的SEQ ID NO:2及其衍生物、如美国专利公布2010-0077508A1中所描述的SEQ ID NO:2及其衍生物、如美国专利公布2009-0313721A1中所描述的SEQ ID NO:2及其衍生物、如美国专利公布2010-0269221A1中所描述的SEQ ID NO:2或4及其衍生物、如美国专利No.7,772,465(B2)中所描述的SEQ ID NO:2及其衍生物、如W02014/008054A2中所描述的CF161_0085及其衍生物、如美国专利公布US2008-0172762A1、US2011-0055968A1和US2012-0117690A1中所描述的鳞翅目毒性蛋白质及其衍生物、如US7510878(B2)中所描述的SEQ ID NO:2及其衍生物、如美国专利No.7812129(B1)中所描述的SEQ ID NO:2及其衍生物;等等。

[0193] 在其他实施方案中,昆虫抑制组合物或转基因植物可以进一步包含对不受本发明的嵌合杀昆虫蛋白质抑制的昆虫害虫(诸如鞘翅目、半翅目和同翅目害虫)表现出昆虫抑制活性的至少一种其他毒性剂,以便扩大所获得的昆虫抑制的范围。

[0194] 用于防治鞘翅目害虫的此类其他毒性剂可以选自由昆虫抑制性蛋白质组成的群组,诸如但不限于Cry3Bb(美国专利No.6,501,009)、Cry1C变体、Cry3A变体、Cry3、Cry3B、Cry34/35、5307、AXMI134(美国专利公布2013-0167264A1)、AXMI-184(美国专利公布2010-0004176A1)、AXMI-205(美国专利公布2014-0298538A1)、axmi207(美国专利公布2013-0303440A1)、AXMI-218、AXMI-220(美国专利公布20140245491A1)、AXMI-221z、AXMI-223z(美国专利公布2014-0196175A1)、AXMI-279(美国专利公布2014-0223599A1)、AXMI-R1和其

变体(美国专利公布2010-0197592A1)、TIC407、TIC417、TIC431、TIC807、TIC853、TIC901、TIC1201、TIC3131、DIG-10(美国专利公布2010-0319092A1)、eHIP(美国专利申请公布No.2010/0017914)、IP3和其变体(美国专利公布2012-0210462A1)和 ω -Hexatoxin-Hv1a(美国专利申请公布US2014-0366227A1)。

[0195] 用于防治半翅目害虫的此类其他毒性剂可以选自由半翅目活性蛋白质组成的群组,诸如但不限于TIC1415(美国专利公布2013-0097735A1)、TIC807(美国专利No.8609936)、TIC834(美国专利公布2013-0269060A1)、AXMI-036(美国专利公布2010-0137216A1)和AXMI-171(美国专利公布2013-0055469A1)。用于防治鞘翅目、鳞翅目和半翅目昆虫害虫的其他多肽可以在由Neil Crickmore维护的苏云金芽孢杆菌毒素命名网站(www.btnomenclature.info)上找到。

[0196] 可以使用本领域技术人员已知的方法,诸如聚合酶链反应(PCR)、热扩增和杂交来鉴别嵌合杀昆虫蛋白质编码序列和与所述嵌合杀昆虫蛋白质具有相当大同一性百分比的序列。举例来说,所述嵌合杀昆虫蛋白质可用于产生特异性结合相关蛋白质的抗体,并且可用于筛选和发现密切相关的其他蛋白质。

[0197] 此外,编码所述嵌合杀昆虫蛋白质的核苷酸序列可以用作筛选用探针和引物,以便使用热循环或等温扩增和杂交方法来鉴别所述类别的其他成员。举例来说,来源于如SEQ ID NO:2中所示出的序列的寡核苷酸可用于确定来源于商品产品的脱氧核糖核酸样品中存在或不存在嵌合杀昆虫转基因。鉴于使用寡核苷酸的某些核酸检测方法的敏感性,预期来源于如SEQ ID NO:2中的任意者中所示出的序列的寡核苷酸可用于在来源于汇集来源的商品产品中检测相应嵌合杀昆虫蛋白质,其中仅一部分商品产品来源于含有SEQ ID NO:2中的任意者的转基因植物。

实施例

[0198] 鉴于上文,本领域技术人员应了解,以下公开实施方案仅代表本发明,本发明可以用多种形式体现。因此,本文中所公开的具体结构和功能细节不应理解为具有限制性。

[0199] 实施例1

[0200] 鳞翅目活性新型嵌合杀昆虫蛋白质编码序列的生成和克隆

[0201] 本实施例说明新型嵌合杀昆虫蛋白质的生成以及所述嵌合杀昆虫蛋白质的克隆和表达。

[0202] 由用于产生编码新型嵌合杀昆虫蛋白质的多核苷酸序列的已知Cry蛋白质基因构建了重组核酸序列。将所得多核苷酸序列克隆至苏云金芽孢杆菌(Bt)表达质粒载体中。在证实多核苷酸序列之后,将所述表达质粒转化至Bt中并且表达。测定所表达的新型嵌合蛋白质的制剂对各种鳞翅目害虫的活性。

[0203] 产生了许多编码嵌合杀昆虫蛋白质的多核苷酸序列并且在生物测定中加以测试。不是所有嵌合杀昆虫蛋白质都显示出活性。仅基于其在生物测定中显示的对特定鳞翅目的活性来选择一些嵌合杀昆虫蛋白质。还基于原始嵌合杀昆虫蛋白质TIC867和TIC868产生了引入氨基酸取代或替代原毒素结构域的氨基酸变体。本发明的嵌合杀昆虫蛋白质的组分(结构域I、II和III,以及原毒素)提供于表1中。还提供了TIC868变体中相对于原始TIC868蛋白质序列的氨基酸取代。

[0204] 表1.新型嵌合杀虫蛋白质和其组分。

[0205]

毒素	PRT SEQ ID NO:	结构域 1	结构域 2	结构 域3	原毒素	氨基酸修饰*
TIC1100	4	Cry1A h	Cry1A c	Cry1C a	Cry1A c	
TIC860	7	Cry1B b1	Cry1B b1	Cry1C a	Cry1A c	
TIC867	10	Cry1B e2	Cry1B e2	Cry1 Ka	Cry1A b3	
TIC867_ 20	13	Cry1B e2	Cry1B e2	Cry1 Ka	Cry1D a1	
TIC867_ 21	16	Cry1B e2	Cry1B e2	Cry1 Ka	Cry4	
TIC867_ 22	19	Cry1B e2	Cry1B e2	Cry1 Ka	Cry9	
TIC867_ 23	21	Cry1B e2	Cry1B e2	Cry1 Ka	Cry1B e	
TIC867_ 24	23	Cry1B e2	Cry1B e2	Cry1 Ka	Cry1K a	
TIC867_ 25	25	Cry1B e2	Cry1B e2	Cry1 Ka	Cry1C a	
TIC868	28	Cry1B e2	Cry1B e2	Cry1C a	Cry1A b3	
TIC868_ 9	30	Cry1B e2	Cry1B e2	Cry1C a	Cry1A b3	N240S_Y343Q_N3 49T
TIC868_ 10	33	Cry1B e2	Cry1B e2	Cry1C a	Cry1D a1	
TIC868_ 11	36	Cry1B e2	Cry1B e2	Cry1C a	Cry4	
TIC868_ 39	39	Cry1B	Cry1B	Cry1C	Cry9	

毒素	PRT SEQ ID NO:	结构域 1	结构域 2	结构域 3	原毒素	氨基酸修饰*
12		e2	e2	a		
TIC868_13	41	Cry1B e2	Cry1B e2	Cry1C a	Cry1B e	
TIC868_14	43	Cry1B e2	Cry1B e2	Cry1C a	Cry1K a	
TIC868_15	45	Cry1B e2	Cry1B e2	Cry1C a	Cry1C a	
TIC868_29	47	Cry1B e2	Cry1B e2	Cry1C a	Cry1A b3	Q136Y_Y343Q_N 349T
TIC869	50	Cry1Ja 1	Cry1Ja 1	Cry1J x	Cry1A b3	
TIC836	53	Cry1Fa 1	Cry1Fa 1	Cry1 Ab	Cry1A c	

[0207] *使用标准IUPAC氨基酸代码来标识氨基酸突变。参见IUPAC-IUB Joint Commission on Biochemical Nomenclature.Nomenclature and Symbolism for Amino Acids and Peptides.Eur.J.Biochem.138:9-37(1984)。第一个氨基酸序列缩写表示给定支架蛋白质中的原始氨基酸,数字表示所述氨基酸的位置,而第二个氨基酸序列缩写表示改进的变体蛋白质中放在该位置上的氨基酸。

[0208] 实施例2

[0209] 新型嵌合杀昆虫蛋白质对鳞翅目害虫显示出活性

[0210] 本实施例说明了对实施例1中所描述的嵌合杀昆虫蛋白质的测试和针对所述嵌合杀昆虫蛋白质观测到的鳞翅目活性。

[0211] 在Bt中表达编码嵌合杀昆虫蛋白质的多核苷酸序列。然后针对已知是玉米、甘蔗、大豆和棉花以及其他作物植物的害虫的多种鳞翅目昆虫来测定所表达的嵌合杀昆虫蛋白质。具体来说,测定杀昆虫蛋白质对黎豆毛虫(VBC,黎豆夜蛾)、蔗螟(SCB,小蔗螟)、小玉米茎钻心虫(LSCB,南美玉米苗斑螟)、玉米穗虫(CEW,玉米穗夜蛾)、烟草芽虫(TBW,烟芽夜蛾)、大豆尺蠖(SBL,大豆夜蛾)、黑粘虫(BLAW,考斯夜蛾)、南方粘虫(SAW,亚热带粘虫)、草地粘虫(FAW,草地贪夜蛾)、甜菜粘虫(BAW,甜菜夜蛾)、古棉铃虫(OBW,棉铃实夜蛾)、东方叶虫(OLW,斜纹夜蛾)、粉红棉铃虫(PBW,棉红铃虫)、黑切根虫(BCW,球菜夜蛾)、西南玉米钻心虫(SWCB,西南玉米螟)、斑点棉铃虫(SBW,翠纹金刚钻)和欧洲玉米钻心虫(ECB,欧洲玉米螟)的活性。玉米穗虫(CEW,玉米穗夜蛾)也称为大豆荚虫(SPW)和棉铃虫(CBW)。通过死亡率与发育迟缓评分以及MIC50评分的组合来测定活性。MIC50是指蜕变抑制浓度,其中将死幼虫和L1幼虫(未能蜕变至第二龄的幼虫)都考虑至所述评分中。表2示出了每一种嵌合杀昆虫蛋白质的活性。‘+’符号表示对特定昆虫害虫观测到的活性。

[0212]

表 2. 对所选鳞翅目的生物测定活性。

毒素	PRT SEQ ID NO:	昆虫																	S B W
		V B C	SCB	LS CB	CEW SPW CBW	BLAW	TBW	SBL	SAW	FAW	BAW	OBW	OLW	PBW	BCW	SWCB	EC B		
TIC1100	4	+	+			+			+		+		+	+					
TIC860	7	+	+	+		+		+	+	+	+		+	+		+			
TIC867	10	+	+			+		+		+	+	+	+			+			
TIC867_20	13																		
TIC867_21	16				+														
TIC867_22	19				+					+									
TIC868	28	+	+			+		+		+	+		+	+		+	+		
TIC868_10	33									+									
TIC868_11	36									+									
TIC868_12	39									+									
TIC869	50	+	+					+		+						+			
TIC836	53	+				+		+	+	+									

- [0213] 如以上表2中可见,大部分嵌合杀昆虫蛋白质对一种或多种鳞翅目害虫物种表现出活性。
- [0214] 实施例3
- [0215] 合成编码嵌合杀昆虫蛋白质的基因并且用于在植物中表达
- [0216] 本实施例说明合成编码嵌合杀昆虫蛋白质的多核苷酸以便在植物中表达。
- [0217] 构建合成编码序列以用于在植物中表达嵌合杀昆虫蛋白质。根据美国专利5,500,

365中大体描述的方法来设计并合成所述合成序列,从而避免某些有害的问题序列,诸如 ATTTA和富A/T植物聚腺苷酸化序列,同时保留嵌合杀昆虫蛋白质的氨基酸序列。表3中列出了用于在植物中表达的编码所述嵌合杀昆虫蛋白质的这些基因的核苷酸序列。

[0218] 表3.设计用于在植物中使用的编码嵌合杀昆虫蛋白质的多核苷酸序列。

[0219]

杀昆虫 蛋白质	DNA SEQ ID NO:	PRT SEQ ID NO:
TIC1100	2	4
TIC1100	3	4
TIC860	6	7
TIC867	9	10
TIC867_ 20	12	13
TIC867_ 21	15	16
TIC867_ 22	18	19
TIC867_ 23	20	21
TIC867_ 24	22	23

[0220]

杀昆虫 蛋白质	DNA SEQ ID NO:	PRT SEQ ID NO:
TIC867_ 25	24	25
TIC868	27	28
TIC868_ 9	29	30
TIC868_ 10	32	33
TIC868_ 11	35	36
TIC868_ 12	38	39
TIC868_ 13	40	41
TIC868_ 14	42	43
TIC868_ 15	44	45
TIC868_ 29	46	47
TIC869	49	50
TIC836	52	53

[0221] 实施例4

[0222] 用于在植物中表达嵌合杀昆虫蛋白质的表达盒

[0223] 本实施例说明包含设计用于在植物中使用的编码嵌合杀昆虫蛋白质的多核苷酸序列的表达盒的构建。

[0224] 用表3中提供的设计用于植物表达的编码嵌合杀昆虫蛋白质的多核苷酸序列构建了多种植物表达盒。此类表达盒可用于在植物原生质体中暂时表达或转化植物细胞。根据蛋白质在细胞内的最终落点来设计典型表达盒。用允许蛋白质被翻译并且保留在细胞溶质中的方式设计一组表达盒。设计具有与毒素蛋白质连续的转运肽以允许靶向细胞的细胞器(诸如叶绿体或质粒)的另一组表达盒。以5'端开始用启动子设计所有表达盒,其可以由可操作地连接以加强转基因的表达的多个启动子元件、增强子元件或本领域技术人员已知的其他表达元件构成。启动子序列通常连续跟随有相对于启动子处于3'的一或多个前导序列。相对于前导序列,内含子序列通常提供在3'以改进转基因的表达。相对于可操作地连接的启动子、前导序列和内含子构形,毒素或转运肽的编码序列和毒素的编码序列通常位于3'。3'UTR序列通常提供在编码序列的3',以促进转录终止并且提供对所得转录产物的聚腺苷酸化非常重要的序列。以上所描述的所有元件都可操作地连接并且依次布置,通常有为了构建表达盒而设的其他序列。

[0225] 实施例5

[0226] 嵌合杀昆虫蛋白质在经稳定转化的玉米中的鳞翅目活性

[0227] 本实施例说明嵌合杀昆虫蛋白质当表达在玉米植物中并且作为食物提供给相应玉米昆虫害虫时对鳞翅目害虫表现出的抑制活性。

[0228] 使用土壤杆菌介导的转化方法,用实施例4中所描述的二元转化载体转化玉米品种LH244。通过本领域中已知的方法诱导经转化的细胞以形成植物。以类似于美国专利No.8,344,207中所描述的那些生物测定的方式,使用植物叶盘进行生物测定。使用未转化的LH244植物来获得用作阴性对照的组织。针对玉米穗虫(CEW,玉米穗夜蛾)、草地粘虫(FAW,草地贪夜蛾)、黑切根虫(BCW,球莴夜蛾)和西南玉米钻心虫(SWCB,西南玉米螟)评价来自每一个二元载体的多个转化事件。

[0229] 对 R_0 和 F_1 代转基因植物进行叶盘生物测定。另外,对受鳞翅目昆虫害虫侵扰的表达某些嵌合杀昆虫蛋白质的完整转基因 F_1 植物,评价叶损伤评级。还评价了表达TIC860和TIC868的 F_1 转基因事件在农田中对FAW、CEW和SWCB的活性。表4中示出了测定结果。‘+’符号表示对特定昆虫害虫观测到的活性。如表4中可见,大部分嵌合杀昆虫蛋白质和许多嵌合杀昆虫蛋白质变体对一种或多种鳞翅目害虫物种显示出活性。

[0230]

表 4. 来自经稳定转化的玉米叶组织的嵌合杀昆虫蛋白质的生物测定活性。

毒素	PRT SEQ ID NO:	昆虫														S B W	EC B
		VB C	SCB	LSCB	CEW SPW CBW	BLAW	TBW	SBL	SAW	FAW	BAW	OBW	OLW	PBW	BCW	SWCB	
TIC1100	4	+	+			+			+		+		+	+			
TIC860	7	+	+	+		+	+	+	+	+	+		+	+		+	+
TIC867	10	+	+			+		+		+	+	+	+			+	
TIC867_2 0	13	NT	NT	NT		NT	NT	NT	NT		NT	NT	NT	NT	NT	NT	NT
TIC867_2 1	16	NT	NT	NT	+	NT	NT	NT	NT		NT	NT	NT	NT	NT	NT	NT
TIC867_2 2	19	NT	NT	NT	+	NT	NT	NT	NT	+	NT	NT	NT	NT	NT	NT	NT
TIC868	28	+	+			+		+	+	+	+		+	+		+	+
TIC868_1 0	33	NT	NT	NT	+	NT	NT	NT	NT	+	NT	NT	NT	NT	NT	NT	NT
TIC868_1 1	36	NT	NT	NT	+	NT	NT	NT	NT	+	NT	NT	NT	NT	NT	NT	NT
TIC868_1 2	39	NT	NT	NT	+	NT	NT	NT	NT	+	NT	NT	NT	NT	NT	NT	NT
TIC869	50	+	+					+	+	+						+	
TIC836	53	+				+		+	+	+							

[0231] 实施例6

[0232] 所述嵌合杀昆虫蛋白质在经稳定转化的大豆中的鳞翅目活性

[0233] 本实施例说明嵌合杀昆虫蛋白质当表达在大豆植物中并且作为食物提供给相应昆虫害虫时对鳞翅目害虫表现出的抑制活性。

[0234] 重新设计所选嵌合杀昆虫蛋白质的编码序列以用于植物表达,克隆至二元植物转化载体中,并且用于转化大豆植物细胞。所述植物转化载体包含用于如实施例4中所描述来

表达嵌合杀昆虫蛋白质的第一转基因盒和用于使用壮观霉素选择来选择经转化的植物细胞的第二转基因盒。在一些情况下,诸如在TIC1100、TIC860和TIC836的情况下,将叶绿体转运肽编码序列可操作地连接至嵌合杀昆虫编码序列。用靶向和非靶向TIC1100、TIC860和TIC836的质粒进行测定。以下表5示出了嵌合杀昆虫蛋白质和TIC867变异嵌合杀昆虫蛋白质以及用于在经稳定转化的大豆中表达的相关编码序列。

[0235] 使用以上所描述的二元转化载体通过土壤杆菌介导的转化来转化大豆植物细胞。诱导所得经转化的植物细胞以形成完整大豆植物。收集叶组织并且用于如实施例5中所描述的生物测定中,或替代地,将冻干组织用于昆虫食物中以用于生物测定。对FAW、南方粘虫(SAW,亚热带粘虫)、大豆尺蠖(SBL,大豆夜蛾)、大豆荚虫(SPW,玉米穗夜蛾)、黎豆毛虫(VBC,黎豆夜蛾)、烟草芽虫(TBW,烟芽夜蛾)、黑粘虫(BLAW,考斯夜蛾)、小玉米茎钻心虫(LSCB,南美玉米苗斑螟)和古棉铃虫(OBW,棉铃实夜蛾)进行生物测定。

[0236] 表5示出了每一种杀昆虫蛋白质在R₀代植物中对所选鳞翅目物种的活性,其中‘+’表示活性。如表5中可见,经稳定转化的大豆中所表达的每一种嵌合杀昆虫蛋白质对多个鳞翅目物种显示出活性。特别值得注意的是,TIC867变体TIC867_23显示了对SPW的活性。

[0237] 表5. 来自经稳定转化的R₀大豆叶组织的嵌合杀昆虫蛋白质的生物测定活性。

杀昆虫 蛋白质	FA W	SA W	SB L	SP W	VB C	TB W	BLA W	LSC B	OB W
TIC1100	+	+	+		+		+	+	+
TIC860	+	+	+		+			+	
TIC867	+	+	+		+	+		+	
TIC867_20		+	+						
TIC867_21		+	+						
[0238] TIC867_22		+	+						
TIC867_23	+	+	+	+					
TIC867_24		+	+						
TIC867_25		+	+						
TIC868	+		+		+		+	+	
TIC869			+		+	+		+	
TIC836	+	+	+		+	+	+		+

[0239] 允许所选转化事件自花授粉,并且种植所得种子。从R₁代植物收获叶组织并且用于饲喂生物测定。测定表达TIC1100、TIC860、TIC867、TIC868、TIC869和TIC836的R₁植物对SAW、SBL、SPW和VBC的活性。表6示出了在这些测试中所观测到的活性。‘+’符号表示对特定昆虫害虫观测到的活性。如表6中所显示,来自R₁代植物的大部分表达的嵌合杀昆虫蛋白质对一种或多种鳞翅目物种显示了活性。

[0240] 表6. 来自经稳定转化的R₁大豆叶组织的嵌合杀昆虫蛋白质的生物测定活性。

[0241] 毒素	SAW	SBL	SPW	VBC
-----------	-----	-----	-----	-----

TIC1100	+	+		+
TIC860	+	+		+
TIC867	+			
TIC868	+	+		+
TIC869	+	+		+
TIC836	+	+		+

[0242] 表7显示在网室中用表达TIC1100、TIC860和TIC836的经稳定转化的R₁代大豆植物进行农田测试的结果。在网室中用于侵扰植物的物种包括SAW、SBL和SPW。抗性定义为小于或等于百分之十五的大豆植物发生脱叶。在这些网笼试验中观测到的抗性与表6中提供的在R₁代大豆叶组织测定中观测到的抗性一致。‘+’符号表示对特定昆虫害虫观测到的活性。

[0243] 表7. 网室农田测试中所测试的R₁代大豆中表达的TIC1100、TIC860和TIC836的活性谱。

毒素	SAW	SBL	SPW
TIC1100	+	+	
TIC860	+	+	
TIC836	+	+	

[0245] 还在阿根廷的两个不同的地方阿塞维多和冯特左拉 (Fontezuela) 在网室中用表达TIC867和TIC869的经稳定转化的R₁代大豆植物进行农田测试。用于在网室中侵扰植物的物种包括南美棉铃虫 (SABW, 南美棉铃虫)、VBC、BLAW和向日葵尺蠖 (SFL, 薄荷灰夜蛾)。抗性定义为小于或等于百分之十五的大豆植物发生脱叶。以下表8示出了观测到的抗性。‘+’符号表示对特定昆虫害虫观测到的活性。如表8中所显示, 表达TIC867的转基因大豆植物对BLAW和VBC显示了抗性。表达TIC869的转基因大豆植物对SABW、SFL、BLAW和VBC显示了抗性。

[0246] 表8. 网室农田测试中所测试的R₁代大豆中表达的TIC867和TIC869的活性谱。

毒素	阿塞维多			冯特左拉		
	SABW	SFL	VBC	SABW	BLAW	VBC
TIC867			+		+	+
TIC869		+	+	+	+	+

[0248] 实施例7

[0249] 嵌合杀昆虫蛋白质在经稳定转化的棉花中的鳞翅目活性

[0250] 本实施例说明嵌合杀昆虫蛋白质当表达在棉花植物中并且作为食物提供给相应昆虫害虫时对鳞翅目害虫表现出的抑制活性。

[0251] 重新设计所选嵌合杀昆虫蛋白质的编码序列以用于植物表达, 克隆至二元植物转化载体中, 并且用于转化棉花植物细胞。所得二元载体类似于实施例4中所描述的那些, 并且被用于表达靶向和非靶向TIC860(编码序列: SEQ ID NO:6; 蛋白质序列: SEQ ID NO:7)、TIC867(编码序列: SEQ ID NO:9; 蛋白质序列: SEQ ID NO:10)、TIC868(编码序列: SEQ ID NO:27; 蛋白质序列: SEQ ID NO:28)和TIC86723(编码序列: SEQ ID NO:20; 蛋白质序列: SEQ ID NO:23)的质粒。

[0252] 通过土壤杆菌介导的转化方法来转化棉花植物细胞。诱导经转化的棉花细胞以形成完整植物。如实施例5中所描述将棉花叶组织用于对棉铃虫 (CBW, 玉米穗夜蛾)、FAW、TBW

和SBL进行生物测定。表9示出了TIC860、TIC867和TIC868在经稳定转化的R₀代棉花中对这些鳞翅目物种的观测活性,其中‘+’表示活性。如表9中可见,TIC860、TIC867和TIC868在经稳定转化的R₀代棉花中对两种或更多种鳞翅目害虫物种显示了活性。

[0253] 表9. 来自经稳定转化的R₀棉花叶组织的TIC860、TIC867和TIC868的生物测定活性。

[0254]

毒素	CBW	FAW	TBW	SBL
TIC860		+		+
TIC867	+	+	+	NT
TIC868		+		+

[0255] 所选转化事件被用于产生R₁种子。测定表达TIC860、TIC867和TIC868的R₁植物对CBW、FAW、TBW和SBL的抗性。将叶、蕾和蒴组织用于测定。表10示出了在这些测试中观测到的活性。‘+’符号表示对特定昆虫害虫观测到的活性。如表10中所显示,TIC860在叶组织中对FAW显示了活性。此外,嵌合杀昆虫蛋白质TIC867在叶、蕾和蒴组织中对CBW和FAW以及在叶中对TBW和SBL显示了活性。嵌合杀昆虫蛋白质TIC868在叶、蕾和蒴组织中对FAW以及在叶中对TBW和SBL显示了活性。

[0256] 表10. 来自经稳定转化的R₁棉花叶组织的嵌合杀昆虫蛋白质的生物测定活性。

[0257]

	CBW			FAW			TBW	SBL
毒素	叶	蕾	蒴	叶	蕾	蒴	叶	叶
TIC860				+				
TIC867	+	+	+	+	+	+	+	+
TIC868				+	+	+	+	+

[0258] 本文中所公开和要求保护的所有组合物都可以在不过度实验的情况下根据本公开内容来制造和执行。尽管已经依据上述说明性实施方案描述了本发明的组合物,但本领域技术人员将显而易见,可以在不背离本发明的真实原理、精神和范围的情况下对本文中所描述的组合物加以变化、改变、修改和变更。更具体来说,将显而易见,在化学上和生理学上相关的某些药剂可以取代本文中所描述的药剂,同时将实现相同或类似的结果。对本领域技术人员显而易见的所有此类取代和修改都被认为在如所附权利要求书所定义的本发明精神、范围和原理内。

[0259] 本说明书中所引用的所有出版物和公开的专利文件都以引用的方式并入本文中,达到与明确地且个别地指示每一个别出版物或专利申请以引用的方式并入相同的程度。

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[1027]	gtgattccgg gtgtcaatgc ggctatttt gaagaattag aagagcgtat ttctactgca	3000
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[1029]	tgctggaacg tgaaagggca tgtagaggta gaagaacaaa acaatcacccg ttcagtcctg	3120
[1030]	gttatcccag aatgggaggc agaagtgtca caagagggtc gtgtctgtcc aggtcgtggc	3180
[1031]	tatatccttc gtgttacagc gtacaaagag ggatatggag aaggttgctg aacgatccat	3240
[1032]	gagatcgaga acaatacaga cgaactgaaa ttcaacaact gtgtagaaga ggaagtatat	3300
[1033]	ccaaacaaca cggtaacgtg tattaattat actgcgactc aagaagaata tgagggtacg	3360
[1034]	tacacttctc gtaatcgagg atatgacgaa gcctatggta ataacccttc cgtaccagct	3420
[1035]	gattatgcgt cagtctatga agaaaaatcg tatacagata gacgaagaga gaatccttgt	3480
[1036]	gaatctaaca gaggatatgg agattacaca ccactaccag ctggttatgt aacaaaggaa	3540
[1037]	ttagagtact tcccagagac cgataaggta tggattgaga ttggagaaac agaaggaaca	3600
[1038]	ttcatcgtgg acagcgtgga attactcctt atggaggaat ag	3642
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[1044]	<223>	设计用于在植物细胞中表达的编码TIC867_20的合成核苷酸序列。
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[1048]	atcgccgagg gcaacaacat cgaccgttc gtgtctgcaa gcacgggtcca gaccggcatc	180
[1049]	aacatcgagg gccgcaccc gggcgtgctc ggcgtgccct tcgcgggtca aatcgccctc	240

[1050]	ttctactcat	tcctcgtggg	cgagctgtgg	ccgcgcggac	gtgacccgtg	ggaaatcttc	300
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[1052]	ctggcacggc	tccagggcct	tggcaacagc	ttccgcgcct	accagcagtc	gctggaggac	420
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[1082]	ttcaggggca	tcaacaggca	gcccgaaccg	ggctggcgcg	gctccaccga	catcaccatc	2220
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[1084]	tgttacccca	cctacctcta	ccagaagatc	gacgagtcca	agctgaaggc	ctacaccgcg	2340
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[1087]	gagaaccaga	tcggcccttg	cggcgagcct	aaccgctgcg	cccctcacct	cgagtggaa	2520
[1088]	cctgacctcc	actgctcgtg	cagggacggc	gagaagtgcg	cccacatag	ccaccacttc	2580
[1089]	tctctggaca	tcgacgtggg	ctgcaccgac	ctgaacgagg	acctgggcgt	gtgggttatc	2640
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[1105]	ttggaatact tcccggaaac agataaagtt tggattgaaa tcggagaaac agaaggaaca	3600
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[1112]	<223>	嵌合蛋白质变体TIC867_20的氨基酸序列。
[1113]	<400>	13
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[1116]	Ile Pro Ala Val Ser Asn His Ser Ala Gln Met Asn Leu Ser Thr Asp	
[1117]	20 25 30	
[1118]	Ala Arg Ile Glu Asp Ser Leu Cys Ile Ala Glu Gly Asn Asn Ile Asp	
[1119]	35 40 45	
[1120]	Pro Phe Val Ser Ala Ser Thr Val Gln Thr Gly Ile Asn Ile Ala Gly	
[1121]	50 55 60	
[1122]	Arg Ile Leu Gly Val Leu Gly Val Pro Phe Ala Gly Gln Ile Ala Ser	
[1123]	65 70 75 80	
[1124]	Phe Tyr Ser Phe Leu Val Gly Glu Leu Trp Pro Arg Gly Arg Asp Pro	
[1125]	85 90 95	
[1126]	Trp Glu Ile Phe Leu Glu His Val Glu Gln Leu Ile Arg Gln Gln Val	
[1127]	100 105 110	
[1128]	Thr Glu Asn Thr Arg Asp Thr Ala Leu Ala Arg Leu Gln Gly Leu Gly	
[1129]	115 120 125	
[1130]	Asn Ser Phe Arg Ala Tyr Gln Gln Ser Leu Glu Asp Trp Leu Glu Asn	
[1131]	130 135 140	
[1132]	Arg Asp Asp Ala Arg Thr Arg Ser Val Leu Tyr Thr Gln Tyr Ile Ala	
[1133]	145 150 155 160	

[1134]	Leu Glu Leu Asp Phe Leu Asn Ala Met Pro Leu Phe Ala Ile Arg Asn		
[1135]		165	170
[1136]	Gln Glu Val Pro Leu Leu Met Val Tyr Ala Gln Ala Ala Asn Leu His		175
[1137]		180	185
[1138]	Leu Leu Leu Leu Arg Asp Ala Ser Leu Phe Gly Ser Glu Phe Gly Leu		190
[1139]		195	200
[1140]	Thr Ser Gln Glu Ile Gln Arg Tyr Tyr Glu Arg Gln Val Glu Lys Thr		205
[1141]		210	215
[1142]	Arg Glu Tyr Ser Asp Tyr Cys Ala Arg Trp Tyr Asn Thr Gly Leu Asn		220
[1143]		225	230
[1144]	Asn Leu Arg Gly Thr Asn Ala Glu Ser Trp Leu Arg Tyr Asn Gln Phe		235
[1145]		245	250
[1146]	Arg Arg Asp Leu Thr Leu Gly Val Leu Asp Leu Val Ala Leu Phe Pro		255
[1147]		260	265
[1148]	Ser Tyr Asp Thr Arg Val Tyr Pro Met Asn Thr Ser Ala Gln Leu Thr		270
[1149]		275	280
[1150]	Arg Glu Ile Tyr Thr Asp Pro Ile Gly Arg Thr Asn Ala Pro Ser Gly		285
[1151]		290	295
[1152]	Phe Ala Ser Thr Asn Trp Phe Asn Asn Asn Ala Pro Ser Phe Ser Ala		300
[1153]		305	310
[1154]	Ile Glu Ala Ala Val Ile Arg Pro Pro His Leu Leu Asp Phe Pro Glu		315
[1155]		325	330
[1156]	Gln Leu Thr Ile Phe Ser Val Leu Ser Arg Trp Ser Asn Thr Gln Tyr		335
[1157]		340	345
[1158]	Met Asn Tyr Trp Val Gly His Arg Leu Glu Ser Arg Thr Ile Arg Gly		350
[1159]		355	360
[1160]	Ser Leu Ser Thr Ser Thr His Gly Asn Thr Asn Thr Ser Ile Asn Pro		365
[1161]		370	375
[1162]	Val Thr Leu Gln Phe Thr Ser Arg Asp Val Tyr Arg Thr Glu Ser Phe		380
[1163]		385	390
[1164]	Ala Gly Ile Asn Ile Leu Leu Thr Thr Pro Val Asn Gly Val Pro Trp		395
[1165]		405	410
[1166]	Ala Arg Phe Asn Trp Arg Asn Pro Leu Asn Ser Leu Arg Gly Ser Leu		415
[1167]		420	425
[1168]	Leu Tyr Thr Ile Gly Tyr Thr Gly Val Gly Thr Gln Leu Phe Asp Ser		430
[1169]		435	440
[1170]	Glu Thr Glu Leu Pro Pro Glu Thr Thr Glu Arg Pro Asn Tyr Glu Ser		445
[1171]		450	455
[1172]	Tyr Ser His Arg Leu Ser Asn Ile Arg Leu Ile Ser Gly Asn Thr Leu		460
[1173]		465	470
[1174]	Arg Ala Pro Val Tyr Ser Trp Thr His Arg Ser Ala Asp Arg Thr Asn		475
[1175]		485	490

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[1179]				515					520					525		
[1180]	Gly	Asp	Ile	Leu	Arg	Arg	Thr	Ser	Gly	Gly	Pro	Phe	Ala	Phe	Ser	Asn
[1181]		530					535					540				
[1182]	Val	Asn	Leu	Asp	Phe	Asn	Leu	Ser	Gln	Arg	Tyr	Arg	Ala	Arg	Ile	Arg
[1183]	545					550					555					560
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[1187]				580						585					590	
[1188]	Leu	Thr	Phe	Gln	Ser	Phe	Ser	Tyr	Ala	Thr	Ile	Asn	Thr	Ala	Phe	Thr
[1189]				595						600					605	
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[1191]		610						615					620			
[1192]	Ser	Gly	Asn	Glu	Val	Tyr	Val	Asp	Arg	Phe	Glu	Leu	Ile	Pro	Val	Thr
[1193]	625					630					635					640
[1194]	Ala	Thr	Phe	Glu	Ala	Glu	Tyr	Asp	Leu	Glu	Arg	Ala	Gln	Lys	Val	Val
[1195]				645						650					655	
[1196]	Asn	Ala	Leu	Phe	Thr	Ser	Thr	Asn	Gln	Leu	Gly	Leu	Lys	Thr	Asp	Val
[1197]				660						665					670	
[1198]	Thr	Asp	Tyr	His	Ile	Asp	Gln	Val	Ser	Asn	Leu	Val	Ala	Cys	Leu	Ser
[1199]				675						680					685	
[1200]	Asp	Glu	Phe	Cys	Leu	Asp	Glu	Lys	Arg	Glu	Leu	Ser	Glu	Lys	Val	Lys
[1201]		690						695					700			
[1202]	His	Ala	Lys	Arg	Leu	Ser	Asp	Glu	Arg	Asn	Leu	Leu	Gln	Asp	Pro	Asn
[1203]	705					710						715				720
[1204]	Phe	Arg	Gly	Ile	Asn	Arg	Gln	Pro	Asp	Arg	Gly	Trp	Arg	Gly	Ser	Thr
[1205]				725						730					735	
[1206]	Asp	Ile	Thr	Ile	Gln	Gly	Gly	Asp	Asp	Val	Phe	Lys	Glu	Asn	Tyr	Val
[1207]				740						745					750	
[1208]	Thr	Leu	Pro	Gly	Thr	Phe	Asp	Glu	Cys	Tyr	Pro	Thr	Tyr	Leu	Tyr	Gln
[1209]				755					760					765		
[1210]	Lys	Ile	Asp	Glu	Ser	Lys	Leu	Lys	Ala	Tyr	Thr	Arg	Tyr	Gln	Leu	Arg
[1211]		770						775						780		
[1212]	Gly	Tyr	Ile	Glu	Asp	Ser	Gln	Asp	Leu	Glu	Ile	Tyr	Leu	Ile	Arg	Tyr
[1213]	785					790						795				800
[1214]	Asn	Ala	Lys	His	Glu	Ile	Val	Asn	Val	Pro	Gly	Thr	Gly	Ser	Leu	Trp
[1215]				805						810					815	
[1216]	Pro	Leu	Ser	Val	Glu	Asn	Gln	Ile	Gly	Pro	Cys	Gly	Glu	Pro	Asn	Arg
[1217]				820						825					830	

[1218]	Cys Ala Pro His Leu Glu Trp Asn Pro Asp Leu His Cys Ser Cys Arg		
[1219]	835	840	845
[1220]	Asp Gly Glu Lys Cys Ala His His Ser His His Phe Ser Leu Asp Ile		
[1221]	850	855	860
[1222]	Asp Val Gly Cys Thr Asp Leu Asn Glu Asp Leu Gly Val Trp Val Ile		
[1223]	865	870	875 880
[1224]	Phe Lys Ile Lys Thr Gln Asp Gly His Ala Arg Leu Gly Asn Leu Glu		
[1225]	885	890	895
[1226]	Phe Leu Glu Glu Lys Pro Leu Leu Gly Glu Ala Leu Ala Arg Val Lys		
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[1228]	Arg Ala Glu Lys Lys Trp Arg Asp Lys Arg Glu Thr Leu Gln Leu Glu		
[1229]	915	920	925
[1230]	Thr Thr Ile Val Tyr Lys Glu Ala Lys Glu Ser Val Asp Ala Leu Phe		
[1231]	930	935	940
[1232]	Val Asn Ser Gln Tyr Asp Arg Leu Gln Ala Asp Thr Asn Ile Ala Met		
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[1236]	Pro Glu Leu Ser Val Ile Pro Gly Val Asn Ala Ala Ile Phe Glu Glu		
[1237]	980	985	990
[1238]	Leu Glu Glu Arg Ile Phe Thr Ala Phe Ser Leu Tyr Asp Ala Arg Asn		
[1239]	995	1000	1005
[1240]	Ile Ile Lys Asn Gly Asp Phe Asn Asn Gly Leu Leu Cys Trp Asn		
[1241]	1010	1015	1020
[1242]	Val Lys Gly His Val Glu Val Glu Glu Gln Asn Asn His Arg Ser		
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[1244]	Val Leu Val Ile Pro Glu Trp Glu Ala Glu Val Ser Gln Glu Val		
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[1246]	Arg Val Cys Pro Gly Arg Gly Tyr Ile Leu Arg Val Thr Ala Tyr		
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[1248]	Lys Glu Gly Tyr Gly Glu Gly Cys Val Thr Ile His Glu Ile Glu		
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[1250]	Asn Asn Thr Asp Glu Leu Lys Phe Asn Asn Cys Val Glu Glu Glu		
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[1252]	Val Tyr Pro Asn Asn Thr Val Thr Cys Ile Asn Tyr Thr Ala Thr		
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[1254]	Gln Glu Glu Tyr Glu Gly Thr Tyr Thr Ser Arg Asn Arg Gly Tyr		
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[1256]	Asp Glu Ala Tyr Gly Asn Asn Pro Ser Val Pro Ala Asp Tyr Ala		
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[1258]	Ser Val Tyr Glu Glu Lys Ser Tyr Thr Asp Arg Arg Arg Glu Asn		
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[1260]	Pro Cys Glu Ser Asn Arg Gly Tyr Gly Asp Tyr Thr Pro Leu Pro
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[1262]	Ala Gly Tyr Val Thr Lys Glu Leu Glu Tyr Phe Pro Glu Thr Asp
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[1264]	Lys Val Trp Ile Glu Ile Gly Glu Thr Glu Gly Thr Phe Ile Val
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[1266]	Asp Ser Val Glu Leu Leu Leu Met Glu Glu
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[1270]	<212> DNA
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[1272]	<220>
[1273]	<223> 用于在细菌细胞中表达的编码TIC867_21的重组核苷酸序列。
[1274]	<400> 14
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[1276]	tcgaatcatt ccgcacaaat gaatctatca accgatgctc gtattgagga tagcttgtgt 120
[1277]	atagccgagg ggaacaatat cgatccattt gttagcgcac caacagtcca aacgggtatt 180
[1278]	aacatagctg gtagaatact aggtgtatta ggcgtaccgt ttgctggaca aatagctagt 240
[1279]	ttttatagtt ttcttgttgg tgaattatgg cccgcggca gagatccttg ggaaattttc 300
[1280]	ctagaacatg tcgaacaact tataagacaa caagtaacag aaaatactag ggatacggct 360
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[1417]	Ala Arg Ile Glu Asp Ser Leu Cys Ile Ala Glu Gly Asn Asn Ile Asp	
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[1419]	Pro Phe Val Ser Ala Ser Thr Val Gln Thr Gly Ile Asn Ile Ala Gly	
[1420]	50 55 60	
[1421]	Arg Ile Leu Gly Val Leu Gly Val Pro Phe Ala Gly Gln Ile Ala Ser	
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[1423]	Phe Tyr Ser Phe Leu Val Gly Glu Leu Trp Pro Arg Gly Arg Asp Pro	
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[1425]	Trp Glu Ile Phe Leu Glu His Val Glu Gln Leu Ile Arg Gln Gln Val	
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[1427]	Thr Glu Asn Thr Arg Asp Thr Ala Leu Ala Arg Leu Gln Gly Leu Gly	

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[1431]	Arg Asp Asp Ala Arg Thr Arg Ser Val Leu Tyr Thr Gln Tyr Ile Ala		
[1432]	145	150	155
[1433]	Leu Glu Leu Asp Phe Leu Asn Ala Met Pro Leu Phe Ala Ile Arg Asn		
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[1435]	Gln Glu Val Pro Leu Leu Met Val Tyr Ala Gln Ala Ala Asn Leu His		
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[1437]	Leu Leu Leu Leu Arg Asp Ala Ser Leu Phe Gly Ser Glu Phe Gly Leu		
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[1439]	Thr Ser Gln Glu Ile Gln Arg Tyr Tyr Glu Arg Gln Val Glu Lys Thr		
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[1441]	Arg Glu Tyr Ser Asp Tyr Cys Ala Arg Trp Tyr Asn Thr Gly Leu Asn		
[1442]	225	230	235
[1443]	Asn Leu Arg Gly Thr Asn Ala Glu Ser Trp Leu Arg Tyr Asn Gln Phe		
[1444]	245	250	255
[1445]	Arg Arg Asp Leu Thr Leu Gly Val Leu Asp Leu Val Ala Leu Phe Pro		
[1446]	260	265	270
[1447]	Ser Tyr Asp Thr Arg Val Tyr Pro Met Asn Thr Ser Ala Gln Leu Thr		
[1448]	275	280	285
[1449]	Arg Glu Ile Tyr Thr Asp Pro Ile Gly Arg Thr Asn Ala Pro Ser Gly		
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[1451]	Phe Ala Ser Thr Asn Trp Phe Asn Asn Asn Ala Pro Ser Phe Ser Ala		
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[1455]	Gln Leu Thr Ile Phe Ser Val Leu Ser Arg Trp Ser Asn Thr Gln Tyr		
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[1459]	Ser Leu Ser Thr Ser Thr His Gly Asn Thr Asn Thr Ser Ile Asn Pro		
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[1461]	Val Thr Leu Gln Phe Thr Ser Arg Asp Val Tyr Arg Thr Glu Ser Phe		
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[1463]	Ala Gly Ile Asn Ile Leu Leu Thr Thr Pro Val Asn Gly Val Pro Trp		
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[1465]	Ala Arg Phe Asn Trp Arg Asn Pro Leu Asn Ser Leu Arg Gly Ser Leu		
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[1467]	Leu Tyr Thr Ile Gly Tyr Thr Gly Val Gly Thr Gln Leu Phe Asp Ser		
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[1469]	Glu Thr Glu Leu Pro Pro Glu Thr Thr Glu Arg Pro Asn Tyr Glu Ser		

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[1511]	Lys Pro Tyr Thr Arg Tyr Arg Val Arg Gly Phe Val Gly Ser Ser Lys		

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[1517]	Gly Asp Tyr Arg Cys Glu Ser Ser Ser Gln Phe Leu Val Asn Gln Val			
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[1519]	His Pro Thr Pro Thr Ala Gly Tyr Ala Leu Asp Met Tyr Ala Cys Pro			
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[1521]	Ser Ser Ser Asp Lys Lys His Ile Met Cys His Asp Arg His Pro Phe			
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[1525]	Ile Asp Val Leu Phe Lys Ile Ser Asn Pro Asn Gly Tyr Ala Thr Leu			
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[1527]	Gly Asn Leu Glu Val Ile Glu Glu Gly Pro Leu Thr Asp Glu Ala Leu			
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[1529]	Val His Val Lys Gln Lys Glu Lys Lys Trp Arg Gln His Met Glu Lys			
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[1543]	Gly Trp His Ala Thr Gly Lys Ala Ala Val Gln Gln Ile Asp Gly			
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[1559]	Pro His Thr Gly Asn Met Tyr Glu Gln Ser Tyr Asn Gly Asn Tyr		
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[1561]	Asn Gln Asn Thr Ser Asp Val Tyr His Gln Gly Tyr Ile Asn Asn		
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[1643]	atcgccgagg gcaacaacat cgacccgttc gtgtctgcaa gcacgggtcca gaccggcatc	180
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[1646]	ctggagcacg ttgagcagct catccggcag caagtgaccg agaacaccag ggacaccgca	360
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[1710]	Ala Arg Ile Glu Asp Ser Leu Cys Ile Ala Glu Gly Asn Asn Ile Asp	
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[1716]	Phe Tyr Ser Phe Leu Val Gly Glu Leu Trp Pro Arg Gly Arg Asp Pro	
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[1718]	Trp Glu Ile Phe Leu Glu His Val Glu Gln Leu Ile Arg Gln Gln Val	
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[1721]	115 120 125	

[1722]	Asn Ser Phe Arg Ala Tyr Gln Gln Ser Leu Glu Asp Trp Leu Glu Asn
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[1728]	Gln Glu Val Pro Leu Leu Met Val Tyr Ala Gln Ala Ala Asn Leu His
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[1732]	Thr Ser Gln Glu Ile Gln Arg Tyr Tyr Glu Arg Gln Val Glu Lys Thr
[1733]	210 215 220
[1734]	Arg Glu Tyr Ser Asp Tyr Cys Ala Arg Trp Tyr Asn Thr Gly Leu Asn
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[1736]	Asn Leu Arg Gly Thr Asn Ala Glu Ser Trp Leu Arg Tyr Asn Gln Phe
[1737]	245 250 255
[1738]	Arg Arg Asp Leu Thr Leu Gly Val Leu Asp Leu Val Ala Leu Phe Pro
[1739]	260 265 270
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[1744]	Phe Ala Ser Thr Asn Trp Phe Asn Asn Asn Ala Pro Ser Phe Ser Ala
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[1746]	Ile Glu Ala Ala Val Ile Arg Pro Pro His Leu Leu Asp Phe Pro Glu
[1747]	325 330 335
[1748]	Gln Leu Thr Ile Phe Ser Val Leu Ser Arg Trp Ser Asn Thr Gln Tyr
[1749]	340 345 350
[1750]	Met Asn Tyr Trp Val Gly His Arg Leu Glu Ser Arg Thr Ile Arg Gly
[1751]	355 360 365
[1752]	Ser Leu Ser Thr Ser Thr His Gly Asn Thr Asn Thr Ser Ile Asn Pro
[1753]	370 375 380
[1754]	Val Thr Leu Gln Phe Thr Ser Arg Asp Val Tyr Arg Thr Glu Ser Phe
[1755]	385 390 395 400
[1756]	Ala Gly Ile Asn Ile Leu Leu Thr Thr Pro Val Asn Gly Val Pro Trp
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[1758]	Ala Arg Phe Asn Trp Arg Asn Pro Leu Asn Ser Leu Arg Gly Ser Leu
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[1760]	Leu Tyr Thr Ile Gly Tyr Thr Gly Val Gly Thr Gln Leu Phe Asp Ser
[1761]	435 440 445
[1762]	Glu Thr Glu Leu Pro Pro Glu Thr Thr Glu Arg Pro Asn Tyr Glu Ser
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[1774]	Val Asn Leu Asp Phe Asn Leu Ser Gln Arg Tyr Arg Ala Arg Ile Arg		
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[1776]	Tyr Ala Ser Thr Thr Asn Leu Arg Ile Tyr Val Thr Val Ala Gly Glu		
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[1778]	Arg Ile Phe Ala Gly Gln Phe Asp Lys Thr Met Asp Ala Gly Ala Pro		
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[1808]	Asp Thr Tyr Ser Asp Gly Ser Cys Ser Gly Met Asn Arg Cys Glu Glu
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[1810]	Gln Gln Met Val Asn Ala Gln Leu Glu Thr Glu His His His Pro Met
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[2162]	Ala Arg Ile Glu Asp Ser Leu Cys Ile Ala Glu Gly Asn Asn Ile Asp	
[2163]	35 40 45	
[2164]	Pro Phe Val Ser Ala Ser Thr Val Gln Thr Gly Ile Asn Ile Ala Gly	
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[2166]	Arg Ile Leu Gly Val Leu Gly Val Pro Phe Ala Gly Gln Ile Ala Ser	
[2167]	65 70 75 80	
[2168]	Phe Tyr Ser Phe Leu Val Gly Glu Leu Trp Pro Arg Gly Arg Asp Pro	
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[2170]	Trp Glu Ile Phe Leu Glu His Val Glu Gln Leu Ile Arg Gln Gln Val	
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[2172]	Thr Glu Asn Thr Arg Asp Thr Ala Leu Ala Arg Leu Gln Gly Leu Gly	
[2173]	115 120 125	
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[2176]	Arg Asp Asp Ala Arg Thr Arg Ser Val Leu Tyr Thr Gln Tyr Ile Ala	
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[2180]	Gln Glu Val Pro Leu Leu Met Val Tyr Ala Gln Ala Ala Asn Leu His	
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[2184]	Thr Ser Gln Glu Ile Gln Arg Tyr Tyr Glu Arg Gln Val Glu Lys Thr		
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[2194]	Arg Glu Ile Tyr Thr Asp Pro Ile Gly Arg Thr Asn Ala Pro Ser Gly		
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[2196]	Phe Ala Ser Thr Asn Trp Phe Asn Asn Asn Ala Pro Ser Phe Ser Ala		
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[2204]	Ser Leu Ser Thr Ser Thr His Gly Asn Thr Asn Thr Ser Ile Asn Pro		
[2205]	370	375	380
[2206]	Val Thr Leu Gln Phe Thr Ser Arg Asp Val Tyr Arg Thr Glu Ser Phe		
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[2210]	Ala Arg Phe Asn Trp Arg Asn Pro Leu Asn Ser Leu Arg Gly Ser Leu		
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[2214]	Glu Thr Glu Leu Pro Pro Glu Thr Thr Glu Arg Pro Asn Tyr Glu Ser		
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[2218]	Arg Ala Pro Val Tyr Ser Trp Thr His Arg Ser Ala Asp Arg Thr Asn		
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[2220]	Thr Ile Ser Ser Asp Ser Ile Thr Gln Ile Pro Leu Val Lys Ala His		
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[2224]	Gly Asp Ile Leu Arg Arg Thr Ser Gly Gly Pro Phe Ala Phe Ser Asn		
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[2226]	Val Asn Leu Asp Phe Asn Leu Ser Gln Arg Tyr Arg Ala Arg Ile Arg		
[2227]	545	550	555 560
[2228]	Tyr Ala Ser Thr Thr Asn Leu Arg Ile Tyr Val Thr Val Ala Gly Glu		
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[2230]	Arg Ile Phe Ala Gly Gln Phe Asp Lys Thr Met Asp Ala Gly Ala Pro		
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[2232]	Leu Thr Phe Gln Ser Phe Ser Tyr Ala Thr Ile Asn Thr Ala Phe Thr		
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[2234]	Phe Pro Glu Arg Ser Ser Ser Leu Thr Val Gly Ala Asp Thr Phe Ser		
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[2236]	Ser Gly Asn Glu Val Tyr Val Asp Arg Phe Glu Leu Ile Pro Val Thr		
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[2238]	Ala Thr Thr Ala Thr Phe Glu Ala Glu Ser Asp Leu Glu Arg Ala Arg		
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[2240]	Lys Ala Val Asn Ala Leu Phe Thr Ser Thr Asn Pro Arg Gly Leu Lys		
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[2242]	Thr Asp Val Thr Asp Tyr His Ile Asp Gln Val Ser Asn Leu Val Glu		
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[2246]	Glu Val Lys Tyr Ala Lys Arg Leu Ser Asp Glu Arg Asn Leu Leu Gln		
[2247]	705	710	715 720
[2248]	Asp Pro Thr Phe Thr Ser Ile Ser Gly Gln Thr Asp Arg Gly Trp Ile		
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[2250]	Gly Ser Thr Gly Ile Ser Ile Gln Gly Gly Asp Asp Ile Phe Lys Glu		
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[2252]	Asn Tyr Val Arg Leu Pro Gly Thr Val Asp Glu Cys Tyr Pro Thr Tyr		
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[2254]	Leu Tyr Gln Lys Ile Asp Glu Ser Gln Leu Lys Ser Tyr Thr Arg Tyr		
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[2256]	Gln Leu Arg Gly Tyr Ile Glu Asp Ser Gln Asp Leu Glu Ile Tyr Leu		
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[2258]	Ile Arg Tyr Asn Ala Lys His Glu Thr Leu Ser Val Pro Gly Thr Glu		
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[2260]	Ser Pro Trp Pro Ser Ser Gly Val Tyr Pro Ser Gly Arg Cys Gly Glu		
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[2263]		835	840 845
[2264]	Ser Cys Arg Tyr Gly Glu Lys Cys Val His His Ser His His Phe Ser		
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[2268]	Trp Val Ile Phe Lys Ile Lys Thr Gln Asp Gly His Ala Lys Leu Gly		
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[2413]	Leu Leu Leu Leu Arg Asp Ala Ser Leu Phe Gly Ser Glu Phe Gly Leu		
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[2415]	Thr Ser Gln Glu Ile Gln Arg Tyr Tyr Glu Arg Gln Val Glu Lys Thr		
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[2417]	Arg Glu Tyr Ser Asp Tyr Cys Ala Arg Trp Tyr Asn Thr Gly Leu Asn		
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[2437]	Val Thr Leu Gln Phe Thr Ser Arg Asp Val Tyr Arg Thr Glu Ser Phe		
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[2456]		530	535
[2457]	Val Asn Leu Asp Phe Asn Leu Ser Gln Arg Tyr Arg Ala Arg Ile Arg		540
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[2459]	Tyr Ala Ser Thr Thr Asn Leu Arg Ile Tyr Val Thr Val Ala Gly Glu		560
[2460]		565	570
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[2473]	Thr Asp Val Thr Asp Tyr His Ile Asp Gln Val Ser Asn Leu Val Asp		670
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[2487]	Glu Leu Arg Gly Tyr Ile Glu Asp Ser Gln Asp Leu Glu Ile Tyr Leu			
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[2493]	Pro Asn Arg Cys Ala Pro His Leu Glu Trp Asn Pro Asp Leu Asp Cys			
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[2495]	Ser Cys Arg Asp Gly Glu Lys Cys Ala His His Ser His His Phe Thr			
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[2517]	Cys Trp Asn Val Lys Gly His Val Asp Val Glu Glu Gln Asn Asn			
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[2616]	<400>	27
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[2620]	aacatcgcgg gccgtatcct cggcgtcctc ggtgtcccat tcgccgtca gatcgcgtcc	240
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[2688]	Ala Arg Ile Glu Asp Ser Leu Cys Ile Ala Glu Gly Asn Asn Ile Asp
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[2702]	Arg Asp Asp Ala Arg Thr Arg Ser Val Leu Tyr Thr Gln Tyr Ile Ala
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[2704]	Leu Glu Leu Asp Phe Leu Asn Ala Met Pro Leu Phe Ala Ile Arg Asn
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[2706]	Gln Glu Val Pro Leu Leu Met Val Tyr Ala Gln Ala Ala Asn Leu His
[2707]	180 185 190
[2708]	Leu Leu Leu Leu Arg Asp Ala Ser Leu Phe Gly Ser Glu Phe Gly Leu
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[2710]	Thr Ser Gln Glu Ile Gln Arg Tyr Tyr Glu Arg Gln Val Glu Lys Thr
[2711]	210 215 220
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[2726]	Gln Leu Thr Ile Phe Ser Val Leu Ser Arg Trp Ser Asn Thr Gln Tyr
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[2728]	Met Asn Tyr Trp Val Gly His Arg Leu Glu Ser Arg Thr Ile Arg Gly
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[2730]	Ser Leu Ser Thr Ser Thr His Gly Asn Thr Asn Thr Ser Ile Asn Pro		
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[2732]	Val Thr Leu Gln Phe Thr Ser Arg Asp Val Tyr Arg Thr Glu Ser Phe		
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[2758]	Thr Met Glu Ile Gly Glu Asn Leu Thr Ser Arg Thr Phe Arg Tyr Thr		
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[2760]	Asp Phe Ser Asn Pro Phe Ser Phe Arg Ala Asn Pro Asp Ile Ile Gly		
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[2762]	Ile Ser Glu Gln Pro Leu Phe Gly Ala Gly Ser Ile Ser Ser Gly Glu		
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[2782]	Ser Lys Leu Lys Ala Tyr Thr Arg Tyr Gln Leu Arg Gly Tyr Ile Glu		
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[2798]	Trp Glu Thr Asn Ile Val Tyr Lys Glu Ala Lys Glu Ser Val Asp Ala		
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[2800]	Leu Phe Val Asn Ser Gln Tyr Asp Arg Leu Gln Ala Asp Thr Asn Ile		
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[2804]	Tyr Leu Pro Glu Leu Ser Val Ile Pro Gly Val Asn Ala Ala Ile Phe		
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[2954]	Met Asn Tyr Trp Val Gly His Arg Leu Glu Ser Arg Thr Ile Arg Gly		
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[2956]	Ser Leu Ser Thr Ser Thr His Gly Asn Thr Asn Thr Ser Ile Asn Pro		
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[3004]	Gln Gly Gly Asp Asp Val Phe Lys Glu Asn Tyr Val Thr Leu Leu Gly		
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[3018]	Val Ile Phe Lys Ile Lys Thr Gln Asp Gly His Ala Arg Leu Gly Asn		
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[3026]	Leu Phe Val Asn Ser Gln Tyr Asp Arg Leu Gln Ala Asp Thr Asn Ile		
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[3034]	Arg Asn Val Ile Lys Asn Gly Asp Phe Asn Asn Gly Leu Ser Cys Trp		
[3035]	995	1000	1005
[3036]	Asn Val Lys Gly His Val Asp Val Glu Glu Gln Asn Asn His Arg		
[3037]	1010	1015	1020
[3038]	Ser Val Leu Val Val Pro Glu Trp Glu Ala Glu Val Ser Gln Glu		
[3039]	1025	1030	1035
[3040]	Val Arg Val Cys Pro Gly Arg Gly Tyr Ile Leu Arg Val Thr Ala		
[3041]	1040	1045	1050
[3042]	Tyr Lys Glu Gly Tyr Gly Glu Gly Cys Val Thr Ile His Glu Ile		
[3043]	1055	1060	1065
[3044]	Glu Asn Asn Thr Asp Glu Leu Lys Phe Ser Asn Cys Val Glu Glu		
[3045]	1070	1075	1080
[3046]	Glu Val Tyr Pro Asn Asn Thr Val Thr Cys Asn Asp Tyr Thr Ala		
[3047]	1085	1090	1095
[3048]	Thr Gln Glu Glu Tyr Glu Gly Thr Tyr Thr Ser Arg Asn Arg Gly		
[3049]	1100	1105	1110
[3050]	Tyr Asp Gly Ala Tyr Glu Ser Asn Ser Ser Val Pro Ala Asp Tyr		
[3051]	1115	1120	1125
[3052]	Ala Ser Ala Tyr Glu Glu Lys Ala Tyr Thr Asp Gly Arg Arg Asp		
[3053]	1130	1135	1140
[3054]	Asn Pro Cys Glu Ser Asn Arg Gly Tyr Gly Asp Tyr Thr Pro Leu		
[3055]	1145	1150	1155
[3056]	Pro Ala Gly Tyr Val Thr Lys Glu Leu Glu Tyr Phe Pro Glu Thr		
[3057]	1160	1165	1170
[3058]	Asp Lys Val Trp Ile Glu Ile Gly Glu Thr Glu Gly Thr Phe Ile		
[3059]	1175	1180	1185
[3060]	Val Asp Ser Val Glu Leu Leu Leu Met Glu Glu		
[3061]	1190	1195	
[3062]	<210> 31		
[3063]	<211> 3678		
[3064]	<212> DNA		
[3065]	<213> 人工的		

[3066] <220>

[3067] <223> 用于在细菌细胞中表达的编码TIC868_10的重组核苷酸序列。

[3068] <400> 31

[3069] atgacttcaa ataggaaaa tgagaatgaa attataaatg ctttatcgat tccagctgta 60

[3070] tcgaatcatt ccgcacaaat gaatctatca accgatgctc gtattgagga tagcttgtgt 120

[3071] atagccgagg ggaacaatat cgatccattt gttagcgcac caacagtcca aacgggtatt 180

[3072] aacatagctg gtagaatact aggtgtatta ggcgtaccgt ttgctggaca aatagctagt 240

[3073] ttttatagtt ttcttgttgg tgaattatgg ccccgcgga gagatccttg ggaaattttc 300

[3074] ctagaacatg tcgaacaact tataagacaa caagtaacag aaaatactag ggatacggct 360

[3075] cttgctcgat tacaaggttt aggaaattcc ttttaggcct atcaacagtc acttgaagat 420

[3076] tggctagaaa accgtgatga tgcaagaacg agaagtgttc ttataacca atatatagcc 480

[3077] ttagaacttg attttcttaa tgcgatgccg cttttcgcaa ttagaaacca agaagttcca 540

[3078] ttattaatgg tatatgctca agctgcaaat ttacacctat tattattgag agatgcctct 600

[3079] ctttttggtg gtgaatttgg gcttacatcc caagaaattc aacgttatta tgagcgccaa 660

[3080] gtggaaaaaa cgagagaata ttctgattat tgcgcaagat ggtataatac gggtttaaat 720

[3081] aatttgagag ggacaaatgc tgaaagtgtg ttgcgatata atcaattccg tagagactta 780

[3082] acgctaggag tattagatct agtggcacta ttcccaagct atgacacgcg tgtttatcca 840

[3083] atgaatacca gtgctcaatt aacaagagaa atttatacag atccaattgg gagaacaaat 900

[3084] gcaccttcag gatttgcaag tacgaattgg tttaataata atgcaccatc gttttctgcc 960

[3085] atagaggctg ccgttattag gcctccgcac ctacttgatt ttccagaaca gttacaatt 1020

[3086] ttcagcgtat taagtcgatg gagtaatact caatatatga attactgggt gggacataga 1080

[3087] cttgaatcgc gaacaataag ggggtcatta agtacctcga cacacggaaa taccaatact 1140

[3088] tctattaatc ctgtaacatt acagttcaca tctcgagacg tttatagaac agaattcatt 1200

[3089] gcagggataa atatacttct aactactcct gtgaatggag taccttgggc tagatttaat 1260

[3090] tggagaaatc ccctgaattc tcttagaggt agccttctct atactatagg gtatactgga 1320

[3091] gtggggacac aactatttga ttcagaaact gaattaccac cagaaacaac agaacgacca 1380

[3092] aattatgaat cttacagtca tagattatct aatataagac taatatcagg aaacactttg 1440

[3093] agagcaccag tatattcttg gacgcaccgt agtgcagatc gtacaaatac cattagttca 1500

[3094] gatagcatta atcaaatacc tttagtgaag ggatttagag tttggggggg cacctctgtc 1560

[3095] attacaggac caggatttac aggaggggat atccttcgaa gaaatacctt tgggtgatttt 1620

[3096] gtatctctac aagtcaatat taattcacca attacccaaa gataccgttt aagatttcgt 1680

[3097] tacgcttcca gtagggatgc acgagttata gtattaacag gagcggcatc cacaggagt 1740

[3098] ggaggccaag ttagtgtaaa tatgcctctt cagaaaacta tggaatagg ggagaactta 1800

[3099] acatctagaa catttagata taccgatttt agtaatcctt tttcatttag agctaataca 1860

[3100] gatataattg ggataagtga acaacctcta tttggtgcag gttctattag tagcggtgaa 1920

[3101] ctttatatag ataaaattga aattattcta gcagatgcaa catttgaggc agaatatgat 1980

[3102] ttagaaagag cgcaaaagggt ggtgaatgcc ctgtttacgt ctacaaacca actagggtca 2040

[3103] aaaacagatg tgacggatta tcatattgat caggtatcca atctagttgc gtgtttatcg 2100

[3104] gatgaatttt gtctggatga aaagagagaa ttgtccgaga aagttaaaca tgcaaagcga 2160

[3105] ctcagtgatg agcggattt acttcaagat ccaaacttca gagggatcaa taggcaacca 2220

[3106] gaccgtggct ggagaggaag tacggatatt actatccaag gaggagatga cgtattcaaa 2280

[3107] gagaattacg ttacgtacc gggtagcttt gatgagtgtc atccaacgta tttatatcaa 2340

[3108]	aaaatagatg agtcgaaatt aaaagcctat acccgttatc aattaagagg gtatatcgaa	2400
[3109]	gatagtcaag acttagaaat ctatttaatt cgttacaatg caaaacacga aatagtaa	2460
[3110]	gtaccaggta caggaagttt atggcctctt tctgtagaaa atcaaattgg accttgtgga	2520
[3111]	gaaccgaatc gatgcgcgcc acacctgaa tggaatcctg atttacctg ttcctgcaga	2580
[3112]	gacggggaaa aatgtgcaca tcattctcat catttctctt tggacattga tgttggatgt	2640
[3113]	acagacttaa atgaggactt aggtgtatgg gtgatattca agattaagac gcaagatggc	2700
[3114]	cacgcacgac tagggaatct agagtttctc gaagagaaac cattattagg agaagcacta	2760
[3115]	gctcgtgtga aaagagcgga gaaaaaatgg agagacaaac gcgaaacatt acaattggaa	2820
[3116]	acaactatcg ttataaaga ggcaaaagaa tctgtagatg ctttatttgt aaactctcaa	2880
[3117]	tatgatagat tacaagcgga tacgaacatc gcgatgattc atgcggcaga taaacgcgtt	2940
[3118]	catagaattc gagaagcgta tctgccggag ctgtctgtga ttccgggtgt caatgcggct	3000
[3119]	atttttgaag aattagaaga gcgtatttct actgcatttt ccctatatga tgcgagaa	3060
[3120]	attattaaaa atggcgattt caataatggc ttattatgct ggaacgtgaa agggcatgta	3120
[3121]	gaggtagaag aacaaaacaa tcaccgttca gtcctgggta tcccagaatg ggaggcaga	3180
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[3123]	aaagagggat atggagaagg ttgcgtaacg atccatgaga tcgagaacaa tacagacgaa	3300
[3124]	ctgaaattca acaactgtgt agaagaggaa gtatatccaa acaacacggt aacgtgtatt	3360
[3125]	aattatactg cgactcaaga agaataatgag ggtacgtaca cttctcgtaa tcgaggatat	3420
[3126]	gacgaagcct atggtaataa cccttccgta ccagctgatt atgcgtcagt ctatgaagaa	3480
[3127]	aaatcgata cagatagacg aagagagaat cttgtgaat ctaacagagg atatggagat	3540
[3128]	tacacaccac taccagctgg ttatgtaaca aaggaattag agtacttccc agagaccgat	3600
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[3130]	ctccttatgg aggaatag	3678
[3131]	<210>	32
[3132]	<211>	3678
[3133]	<212>	DNA
[3134]	<213>	人工的
[3135]	<220>	
[3136]	<223>	设计用于在植物细胞中表达的编码TIC868_10的合成核苷酸序列。
[3137]	<400>	32
[3138]	atgacgagca accggaagaa cgagaacgag atcatcaacg ccctctcgat ccctgctgtt	60
[3139]	tcaaaccact ccgcgcagat gaacctgtcc accgacgcgc gcatcgagga ctccctctgc	120
[3140]	atagccgagg gcaacaacat cgaccttctc gtgtcggcca gcacggttca gaccggcatc	180
[3141]	aacatcgagg gccgtatcct cggcgctctc ggtgtcccat tcgccgtca gatcgctcc	240
[3142]	ttctactcgt tccttgtggg cgagctgtgg cctcgcggtc gtgaccctg ggagatcttc	300
[3143]	ctggagcatg tggagcagtt gatccggcag caagtcacgg agaacaccg cgatactgct	360
[3144]	ctggccaggc tacagggcct gggaaactcc ttctgggcat accagcagtc actggaggac	420
[3145]	tggttgga acaggatga cgcgcgaaca cgctcggtac tctacacca gtacatcgct	480
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[3147]	ctccttatgg tgtacccca ggccgccaac ttacatctgc tcctgctgcg ggacgccagc	600
[3148]	ctgttcggct ccgagttcgg actcacatct caagaaatcc agcgttacta cgagcgccaa	660
[3149]	gtggagaaga cccgtgagta cagtgaactac tgcgtcgat ggtacaacac agggctcaac	720

[3150]	aacctgcgcg gcaccaacgc tgagtcattg ctccgttaca accagttccg ccgcgacttg	780
[3151]	actttgggtg tcctagacct ggtggcgcta ttcccgtctt acgacacacg ggtgtaccca	840
[3152]	atgaacacta gcgcgcaact cagcgggag atctacacag acccaatcgg ccggacgaac	900
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[3154]	atcgaggccg ccgtcatccg ccctcctcac ctgctcgact ttcccgagca gctcacgac	1020
[3155]	ttctccgtgc tctcacgtg gtccaacaca cagtacatga actactgggt cgggcaccga	1080
[3156]	ttggagagta ggacgatccg tggcagcttg agcaccagta cccacggcaa caccaacacc	1140
[3157]	tccatcaacc cagttacgt acagttcacg agccgcgacg ttaccggac tgagtcgttc	1200
[3158]	gcgggcatta acatccttct gacaacgccc gtcaacggcg tcccgtaggc ccggttcaac	1260
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[3162]	cgtgcgcgg tgtactctg gacacaccg agcgcgacc ggaccaacac gatctcttcc	1500
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[3168]	actagccgaa cttccggta cactgatttc tcgaaccctt tctcattcag agcgaaccct	1860
[3169]	gacatcattg ggatctccga gcaaccgtg ttccgtgtg gctccatcag ctctggcgaa	1920
[3170]	ctgtacatcg acaagattga gatcatctg gcggatgcga cgttcgagc cgagtacgac	1980
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[3190]	gaggtcgagg aacaaaacaa tcatcgtagt gtcctgtca ttcctgagtg ggaggcggag	3180
[3191]	gtctctcaag aggtccgtgt ttgcccggg cgtgggtaca ttcttcgtgt tactgcgtac	3240

[3192]	aaggaggggt acggggaggg gtgcgttact attcatgaga ttgagaacaa tactgatgag	3300
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[3194]	aactacacgg caacgcaaga ggaatacgag gggacgtaca cctcgcgtaa tagagggtat	3420
[3195]	gatgaggcgt acggaacaa cccgtcggtt ccagcagatt atgcctcgg tttatgaggag	3480
[3196]	aagtcgtaca cggatagacg acgcgagaat ccatgtgagt caaatcgagg atacggagat	3540
[3197]	tacacaccat taccagcagg atacgttaca aaggagttgg aatacttccc ggaaacagat	3600
[3198]	aaagtttga ttgaaatcg agaaacagaa ggaacattca tcgtcgactc agtagaattg	3660
[3199]	ttgttgatgg aagaatga	3678
[3200]	<210>	33
[3201]	<211>	1225
[3202]	<212>	PRT
[3203]	<213>	人工的
[3204]	<220>	
[3205]	<223>	嵌合蛋白质变体TIC868_10的氨基酸序列。
[3206]	<400>	33
[3207]	Met Thr Ser Asn Arg Lys Asn Glu Asn Glu Ile Ile Asn Ala Leu Ser	
[3208]	1 5 10 15	
[3209]	Ile Pro Ala Val Ser Asn His Ser Ala Gln Met Asn Leu Ser Thr Asp	
[3210]	20 25 30	
[3211]	Ala Arg Ile Glu Asp Ser Leu Cys Ile Ala Glu Gly Asn Asn Ile Asp	
[3212]	35 40 45	
[3213]	Pro Phe Val Ser Ala Ser Thr Val Gln Thr Gly Ile Asn Ile Ala Gly	
[3214]	50 55 60	
[3215]	Arg Ile Leu Gly Val Leu Gly Val Pro Phe Ala Gly Gln Ile Ala Ser	
[3216]	65 70 75 80	
[3217]	Phe Tyr Ser Phe Leu Val Gly Glu Leu Trp Pro Arg Gly Arg Asp Pro	
[3218]	85 90 95	
[3219]	Trp Glu Ile Phe Leu Glu His Val Glu Gln Leu Ile Arg Gln Gln Val	
[3220]	100 105 110	
[3221]	Thr Glu Asn Thr Arg Asp Thr Ala Leu Ala Arg Leu Gln Gly Leu Gly	
[3222]	115 120 125	
[3223]	Asn Ser Phe Arg Ala Tyr Gln Gln Ser Leu Glu Asp Trp Leu Glu Asn	
[3224]	130 135 140	
[3225]	Arg Asp Asp Ala Arg Thr Arg Ser Val Leu Tyr Thr Gln Tyr Ile Ala	
[3226]	145 150 155 160	
[3227]	Leu Glu Leu Asp Phe Leu Asn Ala Met Pro Leu Phe Ala Ile Arg Asn	
[3228]	165 170 175	
[3229]	Gln Glu Val Pro Leu Leu Met Val Tyr Ala Gln Ala Ala Asn Leu His	
[3230]	180 185 190	
[3231]	Leu Leu Leu Leu Arg Asp Ala Ser Leu Phe Gly Ser Glu Phe Gly Leu	
[3232]	195 200 205	
[3233]	Thr Ser Gln Glu Ile Gln Arg Tyr Tyr Glu Arg Gln Val Glu Lys Thr	

[3234]	210	215	220
[3235]	Arg Glu Tyr Ser Asp Tyr Cys Ala Arg Trp Tyr Asn Thr Gly Leu Asn		
[3236]	225	230	235 240
[3237]	Asn Leu Arg Gly Thr Asn Ala Glu Ser Trp Leu Arg Tyr Asn Gln Phe		
[3238]		245	250 255
[3239]	Arg Arg Asp Leu Thr Leu Gly Val Leu Asp Leu Val Ala Leu Phe Pro		
[3240]		260	265 270
[3241]	Ser Tyr Asp Thr Arg Val Tyr Pro Met Asn Thr Ser Ala Gln Leu Thr		
[3242]		275	280 285
[3243]	Arg Glu Ile Tyr Thr Asp Pro Ile Gly Arg Thr Asn Ala Pro Ser Gly		
[3244]	290	295	300
[3245]	Phe Ala Ser Thr Asn Trp Phe Asn Asn Asn Ala Pro Ser Phe Ser Ala		
[3246]	305	310	315 320
[3247]	Ile Glu Ala Ala Val Ile Arg Pro Pro His Leu Leu Asp Phe Pro Glu		
[3248]		325	330 335
[3249]	Gln Leu Thr Ile Phe Ser Val Leu Ser Arg Trp Ser Asn Thr Gln Tyr		
[3250]		340	345 350
[3251]	Met Asn Tyr Trp Val Gly His Arg Leu Glu Ser Arg Thr Ile Arg Gly		
[3252]		355	360 365
[3253]	Ser Leu Ser Thr Ser Thr His Gly Asn Thr Asn Thr Ser Ile Asn Pro		
[3254]		370	375 380
[3255]	Val Thr Leu Gln Phe Thr Ser Arg Asp Val Tyr Arg Thr Glu Ser Phe		
[3256]	385	390	395 400
[3257]	Ala Gly Ile Asn Ile Leu Leu Thr Thr Pro Val Asn Gly Val Pro Trp		
[3258]		405	410 415
[3259]	Ala Arg Phe Asn Trp Arg Asn Pro Leu Asn Ser Leu Arg Gly Ser Leu		
[3260]		420	425 430
[3261]	Leu Tyr Thr Ile Gly Tyr Thr Gly Val Gly Thr Gln Leu Phe Asp Ser		
[3262]		435	440 445
[3263]	Glu Thr Glu Leu Pro Pro Glu Thr Thr Glu Arg Pro Asn Tyr Glu Ser		
[3264]		450	455 460
[3265]	Tyr Ser His Arg Leu Ser Asn Ile Arg Leu Ile Ser Gly Asn Thr Leu		
[3266]	465	470	475 480
[3267]	Arg Ala Pro Val Tyr Ser Trp Thr His Arg Ser Ala Asp Arg Thr Asn		
[3268]		485	490 495
[3269]	Thr Ile Ser Ser Asp Ser Ile Asn Gln Ile Pro Leu Val Lys Gly Phe		
[3270]		500	505 510
[3271]	Arg Val Trp Gly Gly Thr Ser Val Ile Thr Gly Pro Gly Phe Thr Gly		
[3272]		515	520 525
[3273]	Gly Asp Ile Leu Arg Arg Asn Thr Phe Gly Asp Phe Val Ser Leu Gln		
[3274]		530	535 540
[3275]	Val Asn Ile Asn Ser Pro Ile Thr Gln Arg Tyr Arg Leu Arg Phe Arg		

[3276]	545	550	555	560
[3277]	Tyr Ala Ser Ser Arg Asp Ala Arg Val Ile Val Leu Thr Gly Ala Ala			
[3278]		565	570	575
[3279]	Ser Thr Gly Val Gly Gly Gln Val Ser Val Asn Met Pro Leu Gln Lys			
[3280]		580	585	590
[3281]	Thr Met Glu Ile Gly Glu Asn Leu Thr Ser Arg Thr Phe Arg Tyr Thr			
[3282]		595	600	605
[3283]	Asp Phe Ser Asn Pro Phe Ser Phe Arg Ala Asn Pro Asp Ile Ile Gly			
[3284]	610	615	620	
[3285]	Ile Ser Glu Gln Pro Leu Phe Gly Ala Gly Ser Ile Ser Ser Gly Glu			
[3286]	625	630	635	640
[3287]	Leu Tyr Ile Asp Lys Ile Glu Ile Ile Leu Ala Asp Ala Thr Phe Glu			
[3288]		645	650	655
[3289]	Ala Glu Tyr Asp Leu Glu Arg Ala Gln Lys Val Val Asn Ala Leu Phe			
[3290]		660	665	670
[3291]	Thr Ser Thr Asn Gln Leu Gly Leu Lys Thr Asp Val Thr Asp Tyr His			
[3292]		675	680	685
[3293]	Ile Asp Gln Val Ser Asn Leu Val Ala Cys Leu Ser Asp Glu Phe Cys			
[3294]	690	695	700	
[3295]	Leu Asp Glu Lys Arg Glu Leu Ser Glu Lys Val Lys His Ala Lys Arg			
[3296]	705	710	715	720
[3297]	Leu Ser Asp Glu Arg Asn Leu Leu Gln Asp Pro Asn Phe Arg Gly Ile			
[3298]		725	730	735
[3299]	Asn Arg Gln Pro Asp Arg Gly Trp Arg Gly Ser Thr Asp Ile Thr Ile			
[3300]		740	745	750
[3301]	Gln Gly Gly Asp Asp Val Phe Lys Glu Asn Tyr Val Thr Leu Pro Gly			
[3302]		755	760	765
[3303]	Thr Phe Asp Glu Cys Tyr Pro Thr Tyr Leu Tyr Gln Lys Ile Asp Glu			
[3304]	770	775	780	
[3305]	Ser Lys Leu Lys Ala Tyr Thr Arg Tyr Gln Leu Arg Gly Tyr Ile Glu			
[3306]	785	790	795	800
[3307]	Asp Ser Gln Asp Leu Glu Ile Tyr Leu Ile Arg Tyr Asn Ala Lys His			
[3308]		805	810	815
[3309]	Glu Ile Val Asn Val Pro Gly Thr Gly Ser Leu Trp Pro Leu Ser Val			
[3310]		820	825	830
[3311]	Glu Asn Gln Ile Gly Pro Cys Gly Glu Pro Asn Arg Cys Ala Pro His			
[3312]		835	840	845
[3313]	Leu Glu Trp Asn Pro Asp Leu His Cys Ser Cys Arg Asp Gly Glu Lys			
[3314]	850	855	860	
[3315]	Cys Ala His His Ser His His Phe Ser Leu Asp Ile Asp Val Gly Cys			
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[3317]	Thr Asp Leu Asn Glu Asp Leu Gly Val Trp Val Ile Phe Lys Ile Lys			

[3318]	885	890	895
[3319]	Thr Gln Asp Gly His Ala Arg Leu Gly Asn Leu Glu Phe Leu Glu Glu		
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[3325]	Tyr Lys Glu Ala Lys Glu Ser Val Asp Ala Leu Phe Val Asn Ser Gln		
[3326]	945	950	955
[3327]	Tyr Asp Arg Leu Gln Ala Asp Thr Asn Ile Ala Met Ile His Ala Ala		
[3328]	965	970	975
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[3335]	Asn Gly Asp Phe Asn Asn Gly Leu Leu Cys Trp Asn Val Lys Gly		
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[3337]	His Val Glu Val Glu Glu Gln Asn Asn His Arg Ser Val Leu Val		
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[3339]	Ile Pro Glu Trp Glu Ala Glu Val Ser Gln Glu Val Arg Val Cys		
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[3341]	Pro Gly Arg Gly Tyr Ile Leu Arg Val Thr Ala Tyr Lys Glu Gly		
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[3343]	Tyr Gly Glu Gly Cys Val Thr Ile His Glu Ile Glu Asn Asn Thr		
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[3345]	Asp Glu Leu Lys Phe Asn Asn Cys Val Glu Glu Glu Val Tyr Pro		
[3346]	1100	1105	1110
[3347]	Asn Asn Thr Val Thr Cys Ile Asn Tyr Thr Ala Thr Gln Glu Glu		
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[3349]	Tyr Glu Gly Thr Tyr Thr Ser Arg Asn Arg Gly Tyr Asp Glu Ala		
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[3351]	Tyr Gly Asn Asn Pro Ser Val Pro Ala Asp Tyr Ala Ser Val Tyr		
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[3353]	Glu Glu Lys Ser Tyr Thr Asp Arg Arg Arg Glu Asn Pro Cys Glu		
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[3355]	Ser Asn Arg Gly Tyr Gly Asp Tyr Thr Pro Leu Pro Ala Gly Tyr		
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[3357]	Val Thr Lys Glu Leu Glu Tyr Phe Pro Glu Thr Asp Lys Val Trp		
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[3366]	<213>	人工的	
[3367]	<220>		
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[3373]	aacatagctg gtagaatact aggtgtatta ggcgtaccgt ttgctggaca aatagctagt 240		
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[3407]	ttttcagatg gatttgagga aaacggatgg acgacaagtg ataatatctt aatccaggcg	2280
[3408]	gataatcccc tttttaaggg gaattattta aaaatgtttg gggcaagaga tattgatgga	2340
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[3442]	atagccgagg gcaacaacat cgaccttctc gtgtcggcca gcacggttca gaccggcatc	180
[3443]	aacatcgagg gccgtatcct cggcgtcttc ggtgtcccat tcgccgtca gatcgcttcc	240

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[3509]	<400>	36
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[3512]	Ile Pro Ala Val Ser Asn His Ser Ala Gln Met Asn Leu Ser Thr Asp	
[3513]	20 25 30	
[3514]	Ala Arg Ile Glu Asp Ser Leu Cys Ile Ala Glu Gly Asn Asn Ile Asp	
[3515]	35 40 45	
[3516]	Pro Phe Val Ser Ala Ser Thr Val Gln Thr Gly Ile Asn Ile Ala Gly	
[3517]	50 55 60	
[3518]	Arg Ile Leu Gly Val Leu Gly Val Pro Phe Ala Gly Gln Ile Ala Ser	
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[3521]	85 90 95	
[3522]	Trp Glu Ile Phe Leu Glu His Val Glu Gln Leu Ile Arg Gln Gln Val	
[3523]	100 105 110	
[3524]	Thr Glu Asn Thr Arg Asp Thr Ala Leu Ala Arg Leu Gln Gly Leu Gly	
[3525]	115 120 125	
[3526]	Asn Ser Phe Arg Ala Tyr Gln Gln Ser Leu Glu Asp Trp Leu Glu Asn	
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[3528]	Arg Asp Asp Ala Arg Thr Arg Ser Val Leu Tyr Thr Gln Tyr Ile Ala		
[3529]	145	150	155 160
[3530]	Leu Glu Leu Asp Phe Leu Asn Ala Met Pro Leu Phe Ala Ile Arg Asn		
[3531]		165 170	175
[3532]	Gln Glu Val Pro Leu Leu Met Val Tyr Ala Gln Ala Ala Asn Leu His		
[3533]		180 185	190
[3534]	Leu Leu Leu Leu Arg Asp Ala Ser Leu Phe Gly Ser Glu Phe Gly Leu		
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[3536]	Thr Ser Gln Glu Ile Gln Arg Tyr Tyr Glu Arg Gln Val Glu Lys Thr		
[3537]		210 215	220
[3538]	Arg Glu Tyr Ser Asp Tyr Cys Ala Arg Trp Tyr Asn Thr Gly Leu Asn		
[3539]		225 230	235 240
[3540]	Asn Leu Arg Gly Thr Asn Ala Glu Ser Trp Leu Arg Tyr Asn Gln Phe		
[3541]		245 250	255
[3542]	Arg Arg Asp Leu Thr Leu Gly Val Leu Asp Leu Val Ala Leu Phe Pro		
[3543]		260 265	270
[3544]	Ser Tyr Asp Thr Arg Val Tyr Pro Met Asn Thr Ser Ala Gln Leu Thr		
[3545]		275 280	285
[3546]	Arg Glu Ile Tyr Thr Asp Pro Ile Gly Arg Thr Asn Ala Pro Ser Gly		
[3547]		290 295	300
[3548]	Phe Ala Ser Thr Asn Trp Phe Asn Asn Asn Ala Pro Ser Phe Ser Ala		
[3549]		305 310	315 320
[3550]	Ile Glu Ala Ala Val Ile Arg Pro Pro His Leu Leu Asp Phe Pro Glu		
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[3552]	Gln Leu Thr Ile Phe Ser Val Leu Ser Arg Trp Ser Asn Thr Gln Tyr		
[3553]		340 345	350
[3554]	Met Asn Tyr Trp Val Gly His Arg Leu Glu Ser Arg Thr Ile Arg Gly		
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[3556]	Ser Leu Ser Thr Ser Thr His Gly Asn Thr Asn Thr Ser Ile Asn Pro		
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[3558]	Val Thr Leu Gln Phe Thr Ser Arg Asp Val Tyr Arg Thr Glu Ser Phe		
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[3564]	Leu Tyr Thr Ile Gly Tyr Thr Gly Val Gly Thr Gln Leu Phe Asp Ser		
[3565]		435 440	445
[3566]	Glu Thr Glu Leu Pro Pro Glu Thr Thr Glu Arg Pro Asn Tyr Glu Ser		
[3567]		450 455	460
[3568]	Tyr Ser His Arg Leu Ser Asn Ile Arg Leu Ile Ser Gly Asn Thr Leu		
[3569]		465 470	475 480

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[3575]																
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[3579]																
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[3581]																
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[3587]																
[3588]	Ile	Ser	Glu	Gln	Pro	Leu	Phe	Gly	Ala	Gly	Ser	Ile	Ser	Ser	Gly	Glu
[3589]																
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[3591]																
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[3593]																
[3594]	Ala	Leu	Asn	Ala	Leu	Phe	Thr	Asp	Gly	Thr	Asn	Gly	Tyr	Leu	Gln	Met
[3595]																
[3596]	Asp	Ala	Thr	Asp	Tyr	Asp	Ile	Asn	Gln	Thr	Ala	Asn	Leu	Ile	Glu	Cys
[3597]																
[3598]	Val	Ser	Asp	Glu	Leu	Tyr	Ala	Lys	Glu	Lys	Ile	Val	Leu	Leu	Asp	Glu
[3599]																
[3600]	Val	Lys	Tyr	Ala	Lys	Arg	Leu	Ser	Ile	Ser	Arg	Asn	Leu	Leu	Leu	Asn
[3601]																
[3602]	Asp	Asp	Leu	Glu	Phe	Ser	Asp	Gly	Phe	Gly	Glu	Asn	Gly	Trp	Thr	Thr
[3603]																
[3604]	Ser	Asp	Asn	Ile	Ser	Ile	Gln	Ala	Asp	Asn	Pro	Leu	Phe	Lys	Gly	Asn
[3605]																
[3606]	Tyr	Leu	Lys	Met	Phe	Gly	Ala	Arg	Asp	Ile	Asp	Gly	Thr	Leu	Phe	Pro
[3607]																
[3608]	Thr	Tyr	Leu	Tyr	Gln	Lys	Ile	Asp	Glu	Ser	Arg	Leu	Lys	Pro	Tyr	Thr
[3609]																
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[3611]																

[3612]	Val Val Thr Arg Tyr Glu Lys Glu Ile Asp Ala Ile Met Asn Val Pro		
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[3616]	Cys Glu Ser Ser Ser Gln Phe Leu Val Asn Gln Val His Pro Thr Pro		
[3617]		850	855 860
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[3619]		865	870 875 880
[3620]	Lys Lys His Ile Met Cys His Asp Arg His Pro Phe Asp Phe His Ile		
[3621]		885	890 895
[3622]	Asp Thr Gly Glu Leu Asn Pro Asn Thr Asn Leu Gly Ile Asp Val Leu		
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[3624]	Phe Lys Ile Ser Asn Pro Asn Gly Tyr Ala Thr Leu Gly Asn Leu Glu		
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[3626]	Val Ile Glu Glu Gly Pro Leu Thr Asp Glu Ala Leu Val His Val Lys		
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[3628]	Gln Lys Glu Lys Lys Trp Arg Gln His Met Glu Lys Lys Arg Met Glu		
[3629]		945	950 955 960
[3630]	Thr Gln Gln Ala Tyr Asp Pro Ala Lys Gln Ala Val Asp Ala Leu Phe		
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[3632]	Thr Asn Glu Gln Glu Leu Asp Tyr His Thr Thr Leu Asp His Ile Gln		
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[3634]	Asn Ala Asp Gln Leu Val Gln Ala Ile Pro Tyr Val His His Ala Trp		
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[3636]	Leu Pro Asp Ala Pro Gly Met Asn Tyr Asp Val Tyr Gln Gly Leu		
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[3638]	Asn Ala Arg Ile Met Gln Ala Tyr Asn Leu Tyr Asp Ala Arg Asn		
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[3640]	Val Ile Ile Asn Gly Asp Phe Thr Gln Gly Leu Gln Gly Trp His		
[3641]		1040	1045 1050
[3642]	Ala Thr Gly Lys Ala Ala Val Gln Gln Ile Asp Gly Ala Ser Val		
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[3644]	Leu Val Leu Ser Asn Trp Ser Ala Glu Val Ser Gln Asn Leu His		
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[3646]	Ala Gln Asp His His Gly Tyr Met Leu Arg Val Ile Ala Lys Lys		
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[3648]	Glu Gly Pro Gly Lys Gly Tyr Val Met Met Met Asp Phe Asn Gly		
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[3650]	Lys Gln Glu Thr Leu Thr Phe Thr Ser Cys Glu Glu Gly Tyr Ile		
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[3652]	Thr Lys Thr Ile Glu Val Phe Pro Glu Ser Asp Arg Ile Arg Ile		
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[3654]	Glu Met Gly Glu Thr Glu Gly Thr Phe Tyr Val Asp Ser Ile Glu
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[3656]	Leu Leu Cys Met Gln Gly Tyr Ala Ser Asp Asn Asn Pro His Thr
[3657]	1160 1165 1170
[3658]	Gly Asn Met Tyr Glu Gln Ser Tyr Asn Gly Asn Tyr Asn Gln Asn
[3659]	1175 1180 1185
[3660]	Thr Ser Asp Val Tyr His Gln Gly Tyr Ile Asn Asn Tyr Asn Gln
[3661]	1190 1195 1200
[3662]	Asn Ser Ser Ser Met Tyr Asn Gln Asn Tyr Ile Asn Asn Asp Asp
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[3664]	Leu His Ser Gly Cys Thr Cys Asn Gln Gly His Asn Ser Gly Cys
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[3666]	Thr Cys Asn Gln Gly Tyr Asn Arg
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[3669]	<211> 3468
[3670]	<212> DNA
[3671]	<213> 人工的
[3672]	<220>
[3673]	<223> 用于在细菌细胞中表达的编码TIC868_12的重组核苷酸序列。
[3674]	<400> 37
[3675]	atgacttcaa ataggaaaa tgagaatgaa attataaatg ctttatcgat tccagctgta 60
[3676]	tcgaatcatt ccgcacaaat gaatctatca accgatgctc gtattgagga tagcttgtgt 120
[3677]	atagccgagg ggaacaatat cgatccattht gttagcgcac caacagtcca aacgggtatt 180
[3678]	aacatagctg gtagaatact aggtgtatta ggcgtaccgt ttgctggaca aatagctagt 240
[3679]	ttttatagtt ttcttgttgg tgaattatgg ccccgcgga gagatccttg ggaaattttc 300
[3680]	ctagaacatg tcgaacaact tataagacaa caagtaacag aaaataactag ggatacggct 360
[3681]	cttgctcgat tacaaggttt aggaaattcc tttagagcct atcaacagtc acttgaagat 420
[3682]	tggtagaaaa accgtgatga tgcaagaacg agaagtgttc ttataacca atatatagcc 480
[3683]	ttagaacttg attttcttaa tgcgatgccg cttttcgcaa ttagaaacca agaagttcca 540
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[3685]	ctttttggta gtgaatttgg gcttacatcc caagaaattc aacgttatta tgagcgccaa 660
[3686]	gtggaaaaaa cgagagaata ttctgattat tgcgcaagat ggtataatac gggtttaaat 720
[3687]	aatttgagag ggacaaatgc tgaaagtgg ttgcgatata atcaattccg tagagactta 780
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[3689]	atgaatacca gtgctcaatt aacaagagaa atttatacag atccaattgg gagaacaaat 900
[3690]	gcaccttcag gatttgcaag tacgaattgg tttaataata atgcaccatc gttttctgcc 960
[3691]	atagaggetg ccgttattag gcctccgcac ctacttgatt ttccagaaca gcttacaatt 1020
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[3693]	cttgaatcgc gaacaataag ggggtcatta agtacctcga cacacggaaa taccaatact 1140
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[3700]	gatagcatta atcaaatacc tttagtgaag ggatttagag tttggggggg cacctctgtc	1560
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[3710]	ttatcagatg aacaatatgg gcatgacaaa aagatgttat tggaagcggg aagagcggca	2160
[3711]	aaacgcctca gccgagaacg caacttactt caggatccag attttaatac aatcaatagt	2220
[3712]	acagaagaaa atggatggaa agcaagtaac ggcgttacta ttagcgaggg cgggtccattc	2280
[3713]	tataaaggcc gtgcgcttca gctagcaagc gcaagagaaa attacccaac atacatttat	2340
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[3718]	gattgctgtg aagcggctca aacacatgag ttttcttcct atattaatac aggcgatcta	2640
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[3723]	caacaattaa atccagaaat agggatggca gatattattg acgctcaaaa tcttgtcgca	2940
[3724]	tcaatttcag atgtgtatag cgatgcagta ctgcaaatcc ctggaattaa ctatgagatt	3000
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[3727]	gtacaacagg atggcaatac gcatcttcta gttctttctc attgggatgc acaagtttct	3180
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[3730]	tttaatgcat gtgattatga tataaatggc acgtacgtga ctgataatac gtatctaaca	3360
[3731]	aaagaagtgg tattctattc acatacagaa cacatgtggg tagaggtaag tgaaacagaa	3420
[3732]	ggtgcatttc atatagatag tattgaattc gttgaaacag aaaagtag	3468
[3733]	<210>	38
[3734]	<211>	3468
[3735]	<212>	DNA
[3736]	<213>	人工的
[3737]	<220>	

[3738] <223> 设计用于在植物细胞中表达的编码TIC868_12的合成核苷酸序列。

[3739] <400> 38

[3740] atgacgagca accggaagaa cgagaacgag atcatcaacg cctctctgat ccttgcgtgtt 60

[3741] tcaaaccact ccgcgcagat gaacctgtcc accgacgcgc gcatcgagga ctccctctgc 120

[3742] atagccgagg gcaacaacat cgacccattc gtgtcggcca gcacggttca gaccggcatc 180

[3743] aacatcgagg gccgtatcct cggcgtcctc ggtgtcccat tcgccgttca gatcgcgctc 240

[3744] ttctactcgt tccttgtggg cgagctgtgg cctcgcggtc gtgacccgtg ggagatcttc 300

[3745] ctggagcatg tggagcagtt gatccggcag caagtcacgg agaacaccg cgatactgct 360

[3746] ctggccaggc tacaggcct gggaaactcc ttctgggcat accagcagtc actggaggac 420

[3747] tggttggaga acagggatga cgcgcaaca cgctcggtag tctacacca gtacatcgct 480

[3748] ctcgaaactc acttctgaa cgctatgccg ctgttcgcca tcaggaacca ggaagtcca 540

[3749] ctccctatgg tgtacgcca gcccgccaac ttacatctgc tctgtctgcg ggacgccagc 600

[3750] ctgttcggct ccgagttcgg actcacatct caagaaatcc agcgttacta cgagcgccaa 660

[3751] gtggagaaga cccgtgagta cagtactac tgcgctgat ggtacaacac agggctcaac 720

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[3753] actttgggtg tcttagacct ggtggcgcta ttccgtctt acgacacacg ggtgtacca 840

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[3759] tccatcaacc cagttacgt acagttcacg agccgcgacg tttaccggac tgagtcgttc 1200

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[3761] tggcgtaacc cgttgaaact cctgcgcggg tcattgtctt acaccatcgg gtacacgggc 1320

[3762] gtccgcaccc agctcttga cagtgaact gagctgccg ccgagaccac ggaacgccc 1380

[3763] aactacgagt cctacagcca ccgcctgtcc aacatccggc tcattctctg caacacgtg 1440

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[3765] gactccatta accagatccc gtcctgaag ggcttccgtg tgtggggtg cagcagcgtc 1560

[3766] atcaccggtc cgggttcac cgggtggagac atactgcggc gcaacacttt cggcgacttc 1620

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[3775] ctgagcgacg agcagtacgg tcacgacaag aaaatgctg tggaggccgt ccgggccgcc 2160

[3776] aagcggtgt ccaggagcg taacctgta caagatccc actttaacac gatcaacagc 2220

[3777] acagaggaga atggctggaa ggccagcaac ggagttacga taagcgagg cggtccgttc 2280

[3778] tacaagggtc gtgccctcca gctcgctct gcaagggaga actatccaac ctacatctat 2340

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[3796]	aaggaggtgg tggtctattc tcacaccgag cacatgtggg ttgaggtcag cgagaccgag	3420
[3797]	ggagccttcc acattgacag catcgagttc gtggagactg agaagtga	3468
[3798]	<210>	39
[3799]	<211>	1155
[3800]	<212>	PRT
[3801]	<213>	人工的
[3802]	<220>	
[3803]	<223>	嵌合蛋白质变体TIC868_12的氨基酸序列。
[3804]	<400>	39
[3805]	Met Thr Ser Asn Arg Lys Asn Glu Asn Glu Ile Ile Asn Ala Leu Ser	
[3806]	1 5 10 15	
[3807]	Ile Pro Ala Val Ser Asn His Ser Ala Gln Met Asn Leu Ser Thr Asp	
[3808]	20 25 30	
[3809]	Ala Arg Ile Glu Asp Ser Leu Cys Ile Ala Glu Gly Asn Asn Ile Asp	
[3810]	35 40 45	
[3811]	Pro Phe Val Ser Ala Ser Thr Val Gln Thr Gly Ile Asn Ile Ala Gly	
[3812]	50 55 60	
[3813]	Arg Ile Leu Gly Val Leu Gly Val Pro Phe Ala Gly Gln Ile Ala Ser	
[3814]	65 70 75 80	
[3815]	Phe Tyr Ser Phe Leu Val Gly Glu Leu Trp Pro Arg Gly Arg Asp Pro	
[3816]	85 90 95	
[3817]	Trp Glu Ile Phe Leu Glu His Val Glu Gln Leu Ile Arg Gln Gln Val	
[3818]	100 105 110	
[3819]	Thr Glu Asn Thr Arg Asp Thr Ala Leu Ala Arg Leu Gln Gly Leu Gly	
[3820]	115 120 125	
[3821]	Asn Ser Phe Arg Ala Tyr Gln Gln Ser Leu Glu Asp Trp Leu Glu Asn	

[3822]	130	135	140
[3823]	Arg Asp Asp Ala Arg Thr Arg Ser Val Leu Tyr Thr Gln Tyr Ile Ala		
[3824]	145	150	155
[3825]	Leu Glu Leu Asp Phe Leu Asn Ala Met Pro Leu Phe Ala Ile Arg Asn		
[3826]	165	170	175
[3827]	Gln Glu Val Pro Leu Leu Met Val Tyr Ala Gln Ala Ala Asn Leu His		
[3828]	180	185	190
[3829]	Leu Leu Leu Leu Arg Asp Ala Ser Leu Phe Gly Ser Glu Phe Gly Leu		
[3830]	195	200	205
[3831]	Thr Ser Gln Glu Ile Gln Arg Tyr Tyr Glu Arg Gln Val Glu Lys Thr		
[3832]	210	215	220
[3833]	Arg Glu Tyr Ser Asp Tyr Cys Ala Arg Trp Tyr Asn Thr Gly Leu Asn		
[3834]	225	230	235
[3835]	Asn Leu Arg Gly Thr Asn Ala Glu Ser Trp Leu Arg Tyr Asn Gln Phe		
[3836]	245	250	255
[3837]	Arg Arg Asp Leu Thr Leu Gly Val Leu Asp Leu Val Ala Leu Phe Pro		
[3838]	260	265	270
[3839]	Ser Tyr Asp Thr Arg Val Tyr Pro Met Asn Thr Ser Ala Gln Leu Thr		
[3840]	275	280	285
[3841]	Arg Glu Ile Tyr Thr Asp Pro Ile Gly Arg Thr Asn Ala Pro Ser Gly		
[3842]	290	295	300
[3843]	Phe Ala Ser Thr Asn Trp Phe Asn Asn Asn Ala Pro Ser Phe Ser Ala		
[3844]	305	310	315
[3845]	Ile Glu Ala Ala Val Ile Arg Pro Pro His Leu Leu Asp Phe Pro Glu		
[3846]	325	330	335
[3847]	Gln Leu Thr Ile Phe Ser Val Leu Ser Arg Trp Ser Asn Thr Gln Tyr		
[3848]	340	345	350
[3849]	Met Asn Tyr Trp Val Gly His Arg Leu Glu Ser Arg Thr Ile Arg Gly		
[3850]	355	360	365
[3851]	Ser Leu Ser Thr Ser Thr His Gly Asn Thr Asn Thr Ser Ile Asn Pro		
[3852]	370	375	380
[3853]	Val Thr Leu Gln Phe Thr Ser Arg Asp Val Tyr Arg Thr Glu Ser Phe		
[3854]	385	390	395
[3855]	Ala Gly Ile Asn Ile Leu Leu Thr Thr Pro Val Asn Gly Val Pro Trp		
[3856]	405	410	415
[3857]	Ala Arg Phe Asn Trp Arg Asn Pro Leu Asn Ser Leu Arg Gly Ser Leu		
[3858]	420	425	430
[3859]	Leu Tyr Thr Ile Gly Tyr Thr Gly Val Gly Thr Gln Leu Phe Asp Ser		
[3860]	435	440	445
[3861]	Glu Thr Glu Leu Pro Pro Glu Thr Thr Glu Arg Pro Asn Tyr Glu Ser		
[3862]	450	455	460
[3863]	Tyr Ser His Arg Leu Ser Asn Ile Arg Leu Ile Ser Gly Asn Thr Leu		

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[3865]	Arg Ala Pro Val Tyr Ser Trp Thr His Arg Ser Ala Asp Arg Thr Asn			
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[3867]	Thr Ile Ser Ser Asp Ser Ile Asn Gln Ile Pro Leu Val Lys Gly Phe			
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[3869]	Arg Val Trp Gly Gly Thr Ser Val Ile Thr Gly Pro Gly Phe Thr Gly			
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[3871]	Gly Asp Ile Leu Arg Arg Asn Thr Phe Gly Asp Phe Val Ser Leu Gln			
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[3873]	Val Asn Ile Asn Ser Pro Ile Thr Gln Arg Tyr Arg Leu Arg Phe Arg			
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[3875]	Tyr Ala Ser Ser Arg Asp Ala Arg Val Ile Val Leu Thr Gly Ala Ala			
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[3877]	Ser Thr Gly Val Gly Gly Gln Val Ser Val Asn Met Pro Leu Gln Lys			
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[3881]	Asp Phe Ser Asn Pro Phe Ser Phe Arg Ala Asn Pro Asp Ile Ile Gly			
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[3903]	Ala Ser Glu Leu Lys Pro Tyr Thr Arg Tyr Arg Leu Asp Gly Phe Val			
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[3905]	Lys Ser Ser Gln Asp Leu Glu Ile Asp Leu Ile His His His Lys Val			

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[3915]	Asn Ser Ser Val Asp Gln Gly Ile Trp Val Val Leu Lys Val Arg Thr		
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[3917]	Thr Asp Gly Tyr Ala Thr Leu Gly Asn Leu Glu Leu Val Glu Val Gly		
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[3919]	Pro Leu Ser Gly Glu Ser Leu Glu Arg Glu Gln Arg Asp Asn Ala Lys		
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[3921]	Trp Ser Ala Glu Leu Gly Arg Lys Arg Ala Glu Thr Asp Arg Val Tyr		
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[3943]	Leu Thr Phe Asn Ala Cys Asp Tyr Asp Ile Asn Gly Thr Tyr Val		
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[4038]	Phe Tyr Ser Phe Leu Val Gly Glu Leu Trp Pro Arg Gly Arg Asp Pro		
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[4042]	Thr Glu Asn Thr Arg Asp Thr Ala Leu Ala Arg Leu Gln Gly Leu Gly		
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[4044]	Asn Ser Phe Arg Ala Tyr Gln Gln Ser Leu Glu Asp Trp Leu Glu Asn		
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[4046]	Arg Asp Asp Ala Arg Thr Arg Ser Val Leu Tyr Thr Gln Tyr Ile Ala		
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[4048]	Leu Glu Leu Asp Phe Leu Asn Ala Met Pro Leu Phe Ala Ile Arg Asn		
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[4062]	Ser Tyr Asp Thr Arg Val Tyr Pro Met Asn Thr Ser Ala Gln Leu Thr		
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[4080]	Ala Arg Phe Asn Trp Arg Asn Pro Leu Asn Ser Leu Arg Gly Ser Leu		
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[4082]	Leu Tyr Thr Ile Gly Tyr Thr Gly Val Gly Thr Gln Leu Phe Asp Ser		
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[4086]	Tyr Ser His Arg Leu Ser Asn Ile Arg Leu Ile Ser Gly Asn Thr Leu		
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[4191]	<223> 设计用于在植物细胞中表达的编码TIC868_14的合成核苷酸序列。	
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[4196]	aacatcgcg gccgtatcct cggcgctctc ggtgtcccat tcgccgtca gatcgctcc	240
[4197]	ttctactcgt tccttgtggg cgagctgtgg cctcgcggtc gtgaccgtg ggagatcttc	300
[4198]	ctggagcatg tggagcagtt gatccggcag caagtcacgg agaacaccg cgatactgct	360
[4199]	ctggccaggc tacagggcct gggaaactcc tttcgggcat accagcagtc actggaggac	420

[4200]	tggttgaga acagggatga cgcggaaca cgctcggtac tctacacca gtacatcgct	480
[4201]	ctcgaactcg acttcctgaa cgctatgccg ctgttcgcca tcaggaaacca ggaagttcca	540
[4202]	ctccttatgg tgtacgcca gcccgccaac ttacatctgc tctgtctgcg ggacgccagc	600
[4203]	ctgttcggct ccgagttcgg actcacatct caagaaatcc agcgttacta cgagcgccaa	660
[4204]	gtggagaaga cccgtgagta cagtgaactac tgcgctcgat ggtacaacac agggctcaac	720
[4205]	aacctgcgcg gcaccaacgc tgagtcattg ctccgttaca accagttccg ccgcgacttg	780
[4206]	actttgggtg tcttagacct ggtggcgcta ttcccgcttt acgacacacg ggtgtacca	840
[4207]	atgaacacta gcgcgcaact cagcgggag atctacacag acccaatcgg ccggacgaac	900
[4208]	gcaccctccg gtttcgcatc caggaattgg ttcaacaaca acgcaccctc cttctcggca	960
[4209]	atcgaggccg ccgtcatccg ccctctctac ctgctcgact ttcccgagca gctcacgac	1020
[4210]	ttctccgtgc tctcacgtg gtccaacaca cagtacatga actactgggt cgggcaccga	1080
[4211]	ttggagagta ggacgatccg tggcagcttg agcaccagta cccacggcaa caccaacacc	1140
[4212]	tccatcaacc cagttacgct acagttcacg agccgcgacg ttaccggac tgagtcgttc	1200
[4213]	gcgggcatta acatccttct gacaacgccc gtcaacggcg tcccgtaggc ccggttcaac	1260
[4214]	tggcgtaacc cgttgaactc cctgcgcggg tcattgctct acaccatcgg gtacacgggc	1320
[4215]	gtcggcacc acgtcttga cagtgaact gagctgccg ccgagaccac ggaacgccc	1380
[4216]	aactacgagt cctacagcca ccgcctgtcc aacatccggc tcattctctg caacacgtg	1440
[4217]	cgtgcgccg tgtactcctg gacacaccgc agcgcgacc ggaccaacac gatctcttc	1500
[4218]	gactccatta accagatccc gctcgtgaag ggcttccgtg tgtggggtgg cagcagcgtc	1560
[4219]	atcacccggtc cgggcttcac cgggtggagac atactgcggc gcaacacttt cggcgacttc	1620
[4220]	gtttcggtgc aagtgaacat caactcgccg atcaccagc gttaccgtct gaggttccgc	1680
[4221]	tacgttcaa gccgcgacgc gaggtcatt gtctgaccg gagccgcgtc cacaggcgtg	1740
[4222]	ggaggccaag tctcagtaa catgcctctc cagaagacga tggagatagg cgagaacttg	1800
[4223]	actagccgaa cttccggtg cactgatttc tcgaaccctt tctcattcag agcgaacct	1860
[4224]	gacatcattg ggatctccga gcaaccgtg ttccgtgctg gctccatcag ctctggcgaa	1920
[4225]	ctgtacatcg acaagattga gatcatctg gcggatgcga cgaccgcgac gtttgaagct	1980
[4226]	gaatccgacc tcgagcgtg gcgcaaggcg gtgaacgctc tgttcacgag caccaacct	2040
[4227]	cgtggcttga agacggatgt gacggactac cacatcgacc aagtctcga cctcgtggag	2100
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[4229]	gccaaagccc tctccgatga gcgcaacctg ctccaagatc ctaccttcac gtcgatttc	2220
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[4231]	atcttcaagg agaactatgt tcggctgccg ggcacgggtg acgagtgtta cccgacgtac	2340
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[4239]	aaggcacttt ccagggtcaa gcgggccgag aagaaatgga gggacaagta cgagaaactc	2820
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[4241]	gactctcagt acgacaagct ccaggcgaac acaaacattg gcatcatcca cgggtcggac	2940

[4242]	aagcaagtgc acaggatacg ggagccttac ctctcggagc tgccggtgat tccctcgatc	3000
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[4253]	ctggaatact ttcccagac agaacaagtg tggatagaga tcggcgagac cgagggcaca	3660
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[4258]	<213>	人工的
[4259]	<220>	
[4260]	<223>	嵌合蛋白质变体TIC868_14的氨基酸序列。
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[4264]	Ile Pro Ala Val Ser Asn His Ser Ala Gln Met Asn Leu Ser Thr Asp	
[4265]	20 25 30	
[4266]	Ala Arg Ile Glu Asp Ser Leu Cys Ile Ala Glu Gly Asn Asn Ile Asp	
[4267]	35 40 45	
[4268]	Pro Phe Val Ser Ala Ser Thr Val Gln Thr Gly Ile Asn Ile Ala Gly	
[4269]	50 55 60	
[4270]	Arg Ile Leu Gly Val Leu Gly Val Pro Phe Ala Gly Gln Ile Ala Ser	
[4271]	65 70 75 80	
[4272]	Phe Tyr Ser Phe Leu Val Gly Glu Leu Trp Pro Arg Gly Arg Asp Pro	
[4273]	85 90 95	
[4274]	Trp Glu Ile Phe Leu Glu His Val Glu Gln Leu Ile Arg Gln Gln Val	
[4275]	100 105 110	
[4276]	Thr Glu Asn Thr Arg Asp Thr Ala Leu Ala Arg Leu Gln Gly Leu Gly	
[4277]	115 120 125	
[4278]	Asn Ser Phe Arg Ala Tyr Gln Gln Ser Leu Glu Asp Trp Leu Glu Asn	
[4279]	130 135 140	
[4280]	Arg Asp Asp Ala Arg Thr Arg Ser Val Leu Tyr Thr Gln Tyr Ile Ala	
[4281]	145 150 155 160	
[4282]	Leu Glu Leu Asp Phe Leu Asn Ala Met Pro Leu Phe Ala Ile Arg Asn	
[4283]	165 170 175	

[4284]	Gln Glu Val Pro Leu Leu Met Val Tyr Ala Gln Ala Ala Asn Leu His		
[4285]	180	185	190
[4286]	Leu Leu Leu Leu Arg Asp Ala Ser Leu Phe Gly Ser Glu Phe Gly Leu		
[4287]	195	200	205
[4288]	Thr Ser Gln Glu Ile Gln Arg Tyr Tyr Glu Arg Gln Val Glu Lys Thr		
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[4290]	Arg Glu Tyr Ser Asp Tyr Cys Ala Arg Trp Tyr Asn Thr Gly Leu Asn		
[4291]	225	230	235
[4292]	Asn Leu Arg Gly Thr Asn Ala Glu Ser Trp Leu Arg Tyr Asn Gln Phe		
[4293]	245	250	255
[4294]	Arg Arg Asp Leu Thr Leu Gly Val Leu Asp Leu Val Ala Leu Phe Pro		
[4295]	260	265	270
[4296]	Ser Tyr Asp Thr Arg Val Tyr Pro Met Asn Thr Ser Ala Gln Leu Thr		
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[4298]	Arg Glu Ile Tyr Thr Asp Pro Ile Gly Arg Thr Asn Ala Pro Ser Gly		
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[4300]	Phe Ala Ser Thr Asn Trp Phe Asn Asn Asn Ala Pro Ser Phe Ser Ala		
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[4302]	Ile Glu Ala Ala Val Ile Arg Pro Pro His Leu Leu Asp Phe Pro Glu		
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[4304]	Gln Leu Thr Ile Phe Ser Val Leu Ser Arg Trp Ser Asn Thr Gln Tyr		
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[4306]	Met Asn Tyr Trp Val Gly His Arg Leu Glu Ser Arg Thr Ile Arg Gly		
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[4308]	Ser Leu Ser Thr Ser Thr His Gly Asn Thr Asn Thr Ser Ile Asn Pro		
[4309]	370	375	380
[4310]	Val Thr Leu Gln Phe Thr Ser Arg Asp Val Tyr Arg Thr Glu Ser Phe		
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[4312]	Ala Gly Ile Asn Ile Leu Leu Thr Thr Pro Val Asn Gly Val Pro Trp		
[4313]	405	410	415
[4314]	Ala Arg Phe Asn Trp Arg Asn Pro Leu Asn Ser Leu Arg Gly Ser Leu		
[4315]	420	425	430
[4316]	Leu Tyr Thr Ile Gly Tyr Thr Gly Val Gly Thr Gln Leu Phe Asp Ser		
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[4318]	Glu Thr Glu Leu Pro Pro Glu Thr Thr Glu Arg Pro Asn Tyr Glu Ser		
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[4320]	Tyr Ser His Arg Leu Ser Asn Ile Arg Leu Ile Ser Gly Asn Thr Leu		
[4321]	465	470	475
[4322]	Arg Ala Pro Val Tyr Ser Trp Thr His Arg Ser Ala Asp Arg Thr Asn		
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[4324]	Thr Ile Ser Ser Asp Ser Ile Asn Gln Ile Pro Leu Val Lys Gly Phe		
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[4329]			530				535					540				
[4330]	Val	Asn	Ile	Asn	Ser	Pro	Ile	Thr	Gln	Arg	Tyr	Arg	Leu	Arg	Phe	Arg
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[4339]	610					615					620					
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[4341]	625					630				635						640
[4342]	Leu	Tyr	Ile	Asp	Lys	Ile	Glu	Ile	Ile	Leu	Ala	Asp	Ala	Thr	Thr	Ala
[4343]				645				650					655			
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[4345]			660					665					670			
[4346]	Ala	Leu	Phe	Thr	Ser	Thr	Asn	Pro	Arg	Gly	Leu	Lys	Thr	Asp	Val	Thr
[4347]			675					680					685			
[4348]	Asp	Tyr	His	Ile	Asp	Gln	Val	Ser	Asn	Leu	Val	Glu	Cys	Leu	Ser	Asp
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[4351]	705					710					715					720
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[4353]				725						730					735	
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[4355]			740					745					750			
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[4357]			755					760					765			
[4358]	Leu	Pro	Gly	Thr	Val	Asp	Glu	Cys	Tyr	Pro	Thr	Tyr	Leu	Tyr	Gln	Lys
[4359]			770					775					780			
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[4361]	785					790					795					800
[4362]	Tyr	Ile	Glu	Asp	Ser	Gln	Asp	Leu	Glu	Ile	Tyr	Leu	Ile	Arg	Tyr	Asn
[4363]				805						810					815	
[4364]	Ala	Lys	His	Glu	Thr	Leu	Ser	Val	Pro	Gly	Thr	Glu	Ser	Pro	Trp	Pro
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[4368]	Ala Pro Arg Ile Glu Trp Asn Pro Asp Leu Asp Cys Ser Cys Arg Tyr
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[4370]	Gly Glu Lys Cys Val His His Ser His His Phe Ser Leu Asp Ile Asp
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[4372]	Val Gly Cys Thr Asp Leu Asn Glu Asp Leu Gly Val Trp Val Ile Phe
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[4375]	900 905 910
[4376]	Ile Glu Glu Lys Pro Leu Leu Gly Lys Ala Leu Ser Arg Val Lys Arg
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[4419]	<223> 设计用于在植物细胞中表达的编码TIC868_15的合成核苷酸序列。
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[4792]	Ser Thr Gly Val Gly Gly Gln Val Ser Val Asn Met Pro Leu Gln Lys		
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[4794]	Thr Met Glu Ile Gly Glu Asn Leu Thr Ser Arg Thr Phe Arg Tyr Thr		
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[4796]	Asp Phe Ser Asn Pro Phe Ser Phe Arg Ala Asn Pro Asp Ile Ile Gly		
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[5067]	Val Lys Ala Phe Asn Leu His Ser Gly Ala Thr Val Val Arg Gly Pro					
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[5071]	Ala Asp Ile Arg Val Asn Val Pro Ser Ser Leu Phe Ser Gln Arg Tyr					
[5072]		500		505		510
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[5082]	580	585	590
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[5089]	Glu Cys Leu Ser Asp Glu Phe Cys Leu Asp Glu Lys Lys Glu Leu Ser		
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[5091]	Glu Lys Val Lys His Ala Lys Arg Leu Ser Asp Glu Arg Asn Leu Leu		
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[5095]	Arg Gly Ser Thr Asp Ile Thr Ile Gln Gly Gly Asp Asp Val Phe Lys		
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[5107]	His His Ser His His Phe Ser Leu Asp Ile Asp Val Gly Cys Thr Asp		
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[5109]	Leu Asn Glu Asp Leu Gly Val Trp Val Ile Phe Lys Ile Lys Thr Gln		
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[5127]	Phe Asn Asn Gly Leu Ser Cys Trp Asn Val Lys Gly His Val Asp Val		
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[5133]	Leu Arg Val Thr Ala Tyr Lys Glu Gly Tyr Gly Glu Gly Cys Val Thr		
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[5157]	<220>		
[5158]	<223> 用于在细菌细胞中表达的编码TIC836的重组核苷酸序列。		
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[5164]	ttgattgagc aaagaataga aacattggaa aggaaccggg caattactac attacgaggg 300		
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[5222]	<213>	人工的
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[5291]	<400>	53

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[5316]	Arg	Leu	Ile	Asn	Leu	Ile	His	Arg	Tyr	Thr	Lys	His	Cys	Leu	Asp	Thr
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[5325]				260						265					270	
[5326]	Asp	Ser	Pro	Val	Ser	Ala	Asn	Ile	Pro	Asn	Gly	Phe	Asn	Arg	Ala	Glu
[5327]			275					280					285			
[5328]	Phe	Gly	Val	Arg	Pro	Pro	His	Leu	Met	Asp	Phe	Met	Asn	Ser	Leu	Phe
[5329]		290					295					300				
[5330]	Val	Thr	Ala	Glu	Thr	Val	Arg	Ser	Gln	Thr	Val	Trp	Gly	Gly	His	Leu
[5331]	305					310					315				320	
[5332]	Val	Ser	Ser	Arg	Asn	Thr	Ala	Gly	Asn	Arg	Ile	Asn	Phe	Pro	Ser	Tyr
[5333]				325						330					335	

[5334]	Gly Val Phe Asn Pro Gly Gly Ala Ile Trp Ile Ala Asp Glu Asp Pro		
[5335]		340	345 350
[5336]	Arg Pro Phe Tyr Arg Thr Leu Ser Asp Pro Val Phe Val Arg Gly Gly		
[5337]		355	360 365
[5338]	Phe Gly Asn Pro His Tyr Val Leu Gly Leu Arg Gly Val Ala Phe Gln		
[5339]		370	375 380
[5340]	Gln Thr Gly Thr Asn His Thr Arg Thr Phe Arg Asn Ser Gly Thr Ile		
[5341]		385	390 395 400
[5342]	Asp Ser Leu Asp Glu Ile Pro Pro Gln Asp Asn Ser Gly Ala Pro Trp		
[5343]		405	410 415
[5344]	Asn Asp Tyr Ser His Val Leu Asn His Val Thr Phe Val Arg Trp Pro		
[5345]		420	425 430
[5346]	Gly Glu Ile Ser Gly Ser Asp Ser Trp Arg Ala Pro Met Phe Ser Trp		
[5347]		435	440 445
[5348]	Thr His Arg Ser Ala Thr Pro Thr Asn Thr Ile Asp Pro Glu Arg Ile		
[5349]		450	455 460
[5350]	Thr Gln Ile Pro Leu Thr Lys Ser Thr Asn Leu Gly Ser Gly Thr Ser		
[5351]		465	470 475 480
[5352]	Val Val Lys Gly Pro Gly Phe Thr Gly Gly Asp Ile Leu Arg Arg Thr		
[5353]		485	490 495
[5354]	Ser Pro Gly Gln Ile Ser Thr Leu Arg Val Asn Ile Thr Ala Pro Leu		
[5355]		500	505 510
[5356]	Ser Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr Asn Leu		
[5357]		515	520 525
[5358]	Gln Phe His Thr Ser Ile Asp Gly Arg Pro Ile Asn Gln Gly Asn Phe		
[5359]		530	535 540
[5360]	Ser Ala Thr Met Ser Ser Gly Ser Asn Leu Gln Ser Gly Ser Phe Arg		
[5361]		545	550 555 560
[5362]	Thr Val Gly Phe Thr Thr Pro Phe Asn Phe Ser Asn Gly Ser Ser Val		
[5363]		565	570 575
[5364]	Phe Thr Leu Ser Ala His Val Phe Asn Ser Gly Asn Glu Val Tyr Ile		
[5365]		580	585 590
[5366]	Asp Arg Ile Glu Phe Val Pro Ala Glu Val Thr Phe Glu Ala Glu Tyr		
[5367]		595	600 605
[5368]	Asp Leu Glu Arg Ala Gln Lys Ala Val Asn Ala Leu Phe Thr Ser Thr		
[5369]		610	615 620
[5370]	Asn Gln Leu Gly Leu Lys Thr Asn Val Thr Asp Tyr His Ile Asp Gln		
[5371]		625	630 635 640
[5372]	Val Ser Asn Leu Val Thr Tyr Leu Ser Asp Glu Phe Cys Leu Asp Glu		
[5373]		645	650 655
[5374]	Lys Arg Glu Leu Ser Glu Lys Val Lys His Ala Lys Arg Leu Ser Asp		
[5375]		660	665 670

[5376]	Glu Arg Asn Leu Leu Gln Asp Ser Asn Phe Lys Asp Ile Asn Arg Gln		
[5377]	675	680	685
[5378]	Pro Glu Arg Gly Trp Gly Gly Ser Thr Gly Ile Thr Ile Gln Gly Gly		
[5379]	690	695	700
[5380]	Asp Asp Val Phe Lys Glu Asn Tyr Val Thr Leu Ser Gly Thr Phe Asp		
[5381]	705	710	715
[5382]	Glu Cys Tyr Pro Thr Tyr Leu Tyr Gln Lys Ile Asp Glu Ser Lys Leu		
[5383]	725	730	735
[5384]	Lys Ala Phe Thr Arg Tyr Gln Leu Arg Gly Tyr Ile Glu Asp Ser Gln		
[5385]	740	745	750
[5386]	Asp Leu Glu Ile Tyr Leu Ile Arg Tyr Asn Ala Lys His Glu Thr Val		
[5387]	755	760	765
[5388]	Asn Val Pro Gly Thr Gly Ser Leu Trp Pro Leu Ser Ala Gln Ser Pro		
[5389]	770	775	780
[5390]	Ile Gly Lys Cys Gly Glu Pro Asn Arg Cys Ala Pro His Leu Glu Trp		
[5391]	785	790	795
[5392]	Asn Pro Asp Leu Asp Cys Ser Cys Arg Asp Gly Glu Lys Cys Ala His		
[5393]	805	810	815
[5394]	His Ser His His Phe Ser Leu Asp Ile Asp Val Gly Cys Thr Asp Leu		
[5395]	820	825	830
[5396]	Asn Glu Asp Leu Gly Val Trp Val Ile Phe Lys Ile Lys Thr Gln Asp		
[5397]	835	840	845
[5398]	Gly His Ala Arg Leu Gly Asn Leu Glu Phe Leu Glu Glu Lys Pro Leu		
[5399]	850	855	860
[5400]	Val Gly Glu Ala Leu Ala Arg Val Lys Arg Ala Glu Lys Lys Trp Arg		
[5401]	865	870	875
[5402]	Asp Lys Arg Glu Lys Leu Glu Trp Glu Thr Asn Ile Val Tyr Lys Glu		
[5403]	885	890	895
[5404]	Ala Lys Glu Ser Val Asp Ala Leu Phe Val Asn Ser Gln Tyr Asp Gln		
[5405]	900	905	910
[5406]	Leu Gln Ala Asp Thr Asn Ile Ala Met Ile His Ala Ala Asp Lys Arg		
[5407]	915	920	925
[5408]	Val His Ser Ile Arg Glu Ala Tyr Leu Pro Glu Leu Ser Val Ile Pro		
[5409]	930	935	940
[5410]	Gly Val Asn Ala Ala Ile Phe Glu Glu Leu Glu Gly Arg Ile Phe Thr		
[5411]	945	950	955
[5412]	Ala Phe Ser Leu Tyr Asp Ala Arg Asn Val Ile Lys Asn Gly Asp Phe		
[5413]	965	970	975
[5414]	Asn Asn Gly Leu Ser Cys Trp Asn Val Lys Gly His Val Asp Val Glu		
[5415]	980	985	990
[5416]	Glu Gln Asn Asn Gln Arg Ser Val Leu Val Val Pro Glu Trp Glu Ala		
[5417]	995	1000	1005

[5418]	Glu Val Ser Gln Glu Val Arg Val Cys Pro Gly Arg Gly Tyr Ile
[5419]	1010 1015 1020
[5420]	Leu Arg Val Thr Ala Tyr Lys Glu Gly Tyr Gly Glu Gly Cys Val
[5421]	1025 1030 1035
[5422]	Thr Ile His Glu Ile Glu Asn Asn Thr Asp Glu Leu Lys Phe Ser
[5423]	1040 1045 1050
[5424]	Asn Cys Val Glu Glu Glu Ile Tyr Pro Asn Asn Thr Val Thr Cys
[5425]	1055 1060 1065
[5426]	Asn Asp Tyr Thr Val Asn Gln Glu Glu Tyr Gly Gly Ala Tyr Thr
[5427]	1070 1075 1080
[5428]	Ser Arg Asn Arg Gly Tyr Asn Glu Ala Pro Ser Val Pro Ala Asp
[5429]	1085 1090 1095
[5430]	Tyr Ala Ser Val Tyr Glu Glu Lys Ser Tyr Thr Asp Gly Arg Arg
[5431]	1100 1105 1110
[5432]	Glu Asn Pro Cys Glu Phe Asn Arg Gly Tyr Arg Asp Tyr Thr Pro
[5433]	1115 1120 1125
[5434]	Leu Pro Val Gly Tyr Val Thr Lys Glu Leu Glu Tyr Phe Pro Glu
[5435]	1130 1135 1140
[5436]	Thr Asp Lys Val Trp Ile Glu Ile Gly Glu Thr Glu Gly Thr Phe
[5437]	1145 1150 1155
[5438]	Ile Val Asp Ser Val Glu Leu Leu Leu Met Glu Glu
[5439]	1160 1165 1170