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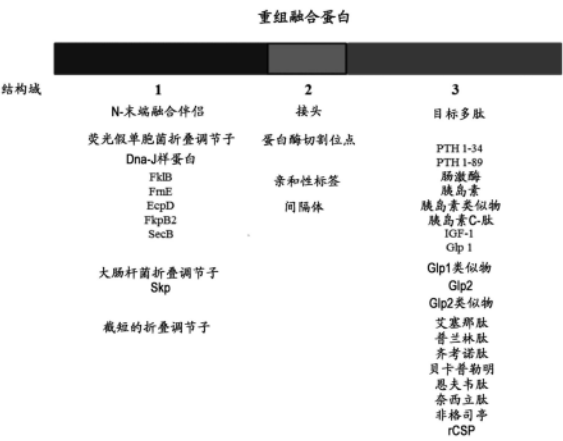
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Kyratsous等.Chaperone-fusion
expression plasmid vectors for improved
solubility of recombinant proteins in
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(54) 发明名称
用于肽生产的融合伴侣

(57) 摘要
本发明涉及医药领域,特别地,涉及作为融合蛋白的部分的大量可溶性重组多肽的生产,所述融合蛋白包含连接于目标多肽的N-末端融合伴侣。



1. 一种重组融合蛋白,其包含:

(i) N-末端融合伴侣,其中所述N-末端融合伴侣是氨基酸序列为SEQ ID NO:2的荧光假单胞菌DnaJ-样蛋白、或氨基酸序列为SEQ ID NO:25的荧光假单胞菌FkbP;

(ii) 选自hPTH1-34、可加工成胰岛素或胰岛素类似物的前胰岛素、Glp1、Glp2、IGF-1、艾塞那肽SEQ ID NO:37、替度鲁肽SEQ ID NO:38、普兰林肽SEQ ID NO:39、齐考诺肽SEQ ID NO:40、贝卡普勒明SEQ ID NO:42、恩夫韦肽SEQ ID NO:43、奈西立肽SEQ ID NO:44、N-met-GCSF、GCSF、恶性疟原虫环孢子蛋白和IFN- β 的目标多肽,其中所述胰岛素类似物是甘精胰岛素、门冬胰岛素、赖脯胰岛素、谷赖胰岛素、地特胰岛素或德谷胰岛素;以及

(iii) 在所述N-末端融合伴侣和所述目标多肽之间的包含蛋白酶切割位点的10至50个氨基酸长的接头。

2. 权利要求1的重组融合蛋白,其中所述蛋白酶切割位点被肠激酶、胰蛋白酶、胰凝乳蛋白酶、因子Xa和弗林蛋白酶组成的组中的裂解酶识别。

3. 权利要求1的重组融合蛋白,其中所述目标多肽是可加工成胰岛素或胰岛素类似物的前胰岛素,并且其中所述重组融合蛋白包含选自SEQ ID NO:122-129的氨基酸序列。

4. 权利要求1的重组融合蛋白,其中所述接头的氨基酸序列选自:

(i) 从N至C端, (G4S)₂间隔体序列SEQ ID NO:59、六组氨酸亲和性标签SEQ ID NO:242、和蛋白酶切割位点DDDDK SEQ ID NO:13;

(ii) 从N至C端, (G4S)₂间隔体序列SEQ ID NO:59、六组氨酸亲和性标签SEQ ID NO:242、和蛋白酶切割位点RKR;

(iii) 从N至C端, (G4S)₂间隔体序列SEQ ID NO:59、六组氨酸亲和性标签SEQ ID NO:242、和蛋白酶切割位点RRR;

(iv) 从N至C端, (G4S)₂间隔体序列SEQ ID NO:59、六组氨酸亲和性标签SEQ ID NO:242、和蛋白酶切割位点LVPR;以及

(v) SEQ ID NO:226。

5. 权利要求1的重组融合蛋白,其中所述目标多肽是可加工成胰岛素或胰岛素类似物的前胰岛素,并且其中所述前胰岛素包含具有选自SEQ ID NO:97、SEQ ID NO:98、SEQ ID NO:99和SEQ ID NO:100的氨基酸序列的C-肽。

6. 权利要求5的重组融合蛋白,其中所述蛋白酶切割位点被肠激酶、胰蛋白酶、胰凝乳蛋白酶、因子Xa和弗林蛋白酶组成的组中的裂解酶识别。

7. 权利要求6的重组融合蛋白,其中所述接头的氨基酸序列选自:

(i) 从N至C端, (G4S)₂间隔体序列SEQ ID NO:59、六组氨酸亲和性标签SEQ ID NO:242、和蛋白酶切割位点DDDDK SEQ ID NO:13;

(ii) 从N至C端, (G4S)₂间隔体序列SEQ ID NO:59、六组氨酸亲和性标签SEQ ID NO:242、和蛋白酶切割位点RKR;

(iii) 从N至C端, (G4S)₂间隔体序列SEQ ID NO:59、六组氨酸亲和性标签SEQ ID NO:242、和蛋白酶切割位点RRR;

(iv) 从N至C端, (G4S)₂间隔体序列SEQ ID NO:59、六组氨酸亲和性标签SEQ ID NO:242、和蛋白酶切割位点LVPR;以及

(v) SEQ ID NO:226。

8. 权利要求1、2、5和6任一项的重组融合蛋白,其中所述接头包含间隔体和亲和性标签。

9. 权利要求8的重组融合蛋白,其中所述接头的氨基酸序列选自:

(i) 从N至C端, (G4S)₂间隔体序列SEQ ID NO:59、六组氨酸亲和性标签SEQ ID NO:242、和蛋白酶切割位点DDDDK SEQ ID NO:13;

(ii) 从N至C端, (G4S)₂间隔体序列SEQ ID NO:59、六组氨酸亲和性标签SEQ ID NO:242、和蛋白酶切割位点RKR;

(iii) 从N至C端, (G4S)₂间隔体序列SEQ ID NO:59、六组氨酸亲和性标签SEQ ID NO:242、和蛋白酶切割位点RRR;

(iv) 从N至C端, (G4S)₂间隔体序列SEQ ID NO:59、六组氨酸亲和性标签SEQ ID NO:242、和蛋白酶切割位点LVPR;以及

(v) SEQ ID NO:226。

10. 权利要求8的重组融合蛋白,其中所述接头包含:

(i) 选自 (G4S)₁、(G4S)₂、(G4S)₃、(G4S)₄和 (G4S)₅的间隔体;和

(ii) 选自麦芽糖结合蛋白标签、聚组氨酸标签、FLAG标签、Myc标签、HA标签和Nus标签的亲和性标签。

11. 权利要求8的重组融合蛋白,其中所述N-末端融合伴侣是氨基酸序列为SEQ ID NO:2的荧光假单胞菌DnaJ-样蛋白,并且其中所述目标多肽是hPTH1-34。

12. 权利要求11的重组融合蛋白,其中所述目标多肽是hPTH1-34,并且其中所述重组融合蛋白的氨基酸序列是SEQ ID NO:45。

13. 一种用于生产目标多肽的方法,包括:

(i) 培养用包含表达构建体的表达载体转化的微生物宿主细胞,其中所述表达构建体包含编码权利要求1至12任一项的重组融合蛋白的核苷酸序列;

(ii) 诱导步骤(i)的宿主细胞来表达所述重组融合蛋白;

(iii) 纯化步骤(ii)诱导的宿主细胞中表达的所述重组融合蛋白;和

(iv) 通过用识别所述接头中的蛋白酶切割位点的裂解酶孵育来切割步骤(iii)纯化的重组融合蛋白,以释放所述目标多肽;由此获得所述目标多肽。

14. 权利要求13的方法,进一步包括测量步骤(ii)中表达的所述重组融合蛋白的表达水平,测量步骤(iii)中纯化的所述重组融合蛋白的量,或测量步骤(iv)中获得的正确释放的所述目标多肽的量,或其组合。

15. 权利要求14的方法,其中步骤(iii)或步骤(iv)中获得的所述目标多肽的量为0.1g/L至25g/L。

16. 权利要求14的方法,其中所获得的正确释放的目标多肽是可溶性的、完整的或两者。

17. 一种用于表达权利要求1至12任一项的重组融合蛋白的表达载体,其中所述表达载体包含编码所述重组融合蛋白的核苷酸序列。

用于肽生产的融合伴侣

[0001] 本申请是申请日为2015年11月30日、申请号为201580064973.4、名称为“用于肽生产的融合伴侣”的专利申请的分案申请。

背景技术

[0002] 由于包括蛋白质水解、低表达水平、不正确的蛋白质折叠(其可能导致差的溶解性)以及宿主细胞的差的分泌的原因,异源重组多肽常常难以在细菌表达系统中高产量地表达。

发明内容

[0003] 本发明提供了包含目标多肽的重组融合蛋白。目标多肽作为所述的重组融合蛋白的部分的表达允许大量地产生高品质多肽。目标多肽包括小的或快速降解的肽,例如,甲状旁腺激素N-末端片段(PTH1-34),具有易于降解的N-末端的蛋白质,例如,GCSF和恶性疟原虫(*P.falciparum*)环孢子蛋白,和通常以不溶形式在微生物表达系统中产生的蛋白质,例如,可以加工成胰岛素或胰岛素类似物的前胰岛素、GCSF或IFN- β 。图1中图示的重组融合蛋白包含N-末端细菌融合伴侣,例如,细菌伴侣蛋白(chaperone)或折叠调节子。目标多肽和N-末端细菌伴侣蛋白或折叠调节子通过含有蛋白酶切割位点的柔性接头序列连接。切割时,目标多肽从N-末端融合伴侣释放。本发明进一步公开了用于表达重组融合蛋白的载体,以及用于在细菌宿主细胞中以高产率生产重组融合蛋白的方法。

[0004] 本发明的重组融合构建体对于生产高产量的重组目标多肽(其由于例如蛋白质水解、低表达水平、差的折叠和/或差的分泌而难以在细菌表达系统中超表达)是有用的。在本发明的实施方案中,本发明的重组融合蛋白以高于0.5g/L的滴定度在细菌宿主细胞中产生。在一些实施方案中,重组目标多肽难以在其中超表达的细菌宿主细胞是大肠杆菌。

[0005] 例如,PTH 1-34蛋白,之前报道为作为包涵体(其需要高浓度的脲(例如,7M)来溶解)中的融合蛋白的部分表达,在本文中描述为作为可溶性PTH 1-34融合蛋白的部分以高滴定度表达(高于0.5g/L)产生。此外,可以在非变性条件下进行纯化,例如,4M或更低浓度的脲,或完全不使用脲。此外,使用本发明的方法,具有容易降解的N-末端的蛋白质,例如,N-met-GCSF或恶性疟原虫环孢子蛋白,可以作为所述的融合蛋白的部分产生并在从融合蛋白制备物中除去宿主细胞蛋白酶后,通过切割与N-末端融合伴侣分离。还如本文中描述的,通常以不溶形式产生的前胰岛素可以在本发明的重组融合蛋白中以可溶形式大量地生产,消除了对重折叠的需求。

[0006] 本发明因此提供了重组融合蛋白,其包含:N-末端融合伴侣,其中N-末端融合伴侣是细菌伴侣蛋白或折叠调节子;目标多肽;以及在N-末端融合伴侣和目标多肽之间的包含切割位点的接头。在一些实施方案中,N-末端融合伴侣选自:DnaJ-样蛋白;Fk1B蛋白或其截短体;EcpD蛋白或其截短体;或Skp蛋白或其截短体。在一些实施方案中,N-末端融合伴侣选自:荧光假单胞菌DnaJ-样蛋白;荧光假单胞菌Fk1B蛋白或其C-末端截短体;荧光假单胞菌FrnE蛋白或其截短体;荧光假单胞菌FkpB2蛋白或其C-末端截短体;或荧光假单胞菌EcpD蛋

白或其C-末端截短体。在某些实施方案中,N-末端融合伴侣是荧光假单胞菌Fk1B蛋白,从C-末端除去1至200个氨基酸的截短体,荧光假单胞菌EcpD蛋白,从C-末端除去1至200个氨基酸的截短体,或荧光假单胞菌FrnE蛋白,从C-末端除去1至180个氨基酸的截短体。在一些实施方案中,目标多肽是选自以下的难表达的蛋白质:小的或快速降解的肽;具有容易降解的N-末端的蛋白质;和通常以不溶形式在细菌表达系统中表达的蛋白质。在一些实施方案中,目标多肽是小的或快速降解的肽,其中目标多肽选自:hPTH1-34、Glp1、Glp2、IGF-1艾塞那肽(Exenatide)(SEQ ID NO:37)、替度鲁肽(Teduglutide)(SEQ ID NO:38)、普兰林肽(Pramlintide)(SEQ ID NO:39)、齐考诺肽(Ziconotide)(SEQ ID NO:40)、贝卡普勒明(Becaplermin)(SEQ ID NO:42)、恩夫韦肽(Enfuvirtide)(SEQ ID NO:43)、奈西立肽(Nesiritide)(SEQ ID NO:44)。在一些实施方案中,目标多肽是具有容易降解的N-末端的蛋白质,其中目标多肽是N-met-GCSF或恶性疟原虫环孢子蛋白。在一些实施方案中,目标多肽是通常作为不溶蛋白在细菌表达系统中表达的蛋白质,其中目标多肽是可加工成胰岛素或胰岛素类似物的前胰岛素、GCSF或IFN- β 。在这些实施方案中的任一个中,前胰岛素C-肽具有选自SEQ ID NO:97;SEQ ID NO:98;SEQ ID NO:99或SEQ ID NO:100的氨基酸序列。在一些实施方案中,胰岛素类似物是甘精胰岛素(insulin glargine)、门冬胰岛素(insulin aspart)、赖脯胰岛素(insulin lispro)、谷赖胰岛素(insulin glulisine)、地特胰岛素(insulin detemir)或德谷胰岛素(insulin degludec)。在某些实施方案中,N-末端融合伴侣是具有SEQ ID NO:2中所示氨基酸序列的荧光假单胞菌DnaJ-样蛋白。在一些实施方案中,N-末端融合伴侣是具有SEQ ID NO:4、SEQ ID NO:28、SEQ ID NO:61或SEQ ID NO:62中所示氨基酸序列的荧光假单胞菌Fk1B蛋白。在一些实施方案中,N-末端融合伴侣是具有SEQ ID NO:3、SEQ ID NO:63或SEQ ID NO:64中所示氨基酸序列的荧光假单胞菌FrnE-样蛋白。在一些实施方案中,N-末端融合伴侣是具有SEQ ID NO:7、SEQ ID NO:65、SEQ ID NO:66或SEQ ID NO:67中所示的氨基酸序列的荧光假单胞菌EcpD蛋白。在一些实施方案中,重组融合蛋白中的切割位点被由以下组成的组中的裂解酶识别:肠激酶、胰蛋白酶、因子Xa和弗林蛋白酶。权利要求1至15任一项的重组融合蛋白,其中接头包含亲和性标签。在某些实施方案中,亲和性标签选自:聚组氨酸;FLAG标签;myc标签;GST标签;NBP标签;钙调蛋白标签;HA标签;E-标签;S-标签;SBP标签;Softtag3;V5标签;和VSV标签。在一些实施方案中,接头具有选自以下的氨基酸序列:SEQ ID NO:9;SEQ ID NO:10;SEQ ID NO:11;SEQ ID NO:12;和SEQ ID NO:226。在一些实施方案中,目标多肽是hPTH1-34,并且重组融合蛋白包含选自以下的氨基酸序列:SEQ ID NO:45;SEQ ID NO:46;和SEQ ID NO:47。在一些实施方案中,目标多肽的等电点比N-末端融合伴侣的等电点高至少约1.5倍。在一些实施方案中,目标多肽的分子量构成重组融合蛋白的分子量的约10%至约50%。

[0007] 本发明还提供了用于重组融合蛋白表达的表达载体。在一些实施方案中,表达载体是用于表达在上述任一个实施方案中的重组融合蛋白。在一些实施方案中,表达载体包含编码以上任一个实施方案的重组融合蛋白的核苷酸序列。

[0008] 本发明进一步提供用于生产目标多肽的方法,包括:

[0009] (i) 培养用包含表达构建体的表达载体转化的微生物宿主细胞,其中该表达构建体包含编码重组融合蛋白的核苷酸序列;(ii) 诱导步骤(i)的宿主细胞以表达重组融合蛋白;(iii) 纯化步骤(ii)的诱导宿主细胞中表达的重组融合蛋白;和(iv) 通过用识别接头中

的切割位点的裂解酶孵育来切割步骤(iii)的纯化的重组融合蛋白,以释放目标多肽;由此获得目标多肽。在一些实施方案中,步骤(i)的重组融合蛋白是上述任一个实施方案中所述的重组融合蛋白。在一些实施方案中,所述方法进一步包括测量步骤(ii)中表达的融合蛋白的表达水平,测量步骤(iii)中纯化的重组融合蛋白的量,或测量步骤(iv)中获得的目标多肽的量,或其组合。在一些实施方案中,步骤(ii)中表达的融合蛋白的表达水平高于0.5g/L。在一些实施方案中,步骤(ii)中表达的融合蛋白的表达水平为约0.5g/L至约25g/L。在一些实施方案中,步骤(ii)中表达的融合蛋白定向到细胞质。在一些实施方案中,步骤(ii)中表达的融合蛋白定向到周质。在一些实施方案中,步骤(iv)的孵育为约一小时至约16小时,并且裂解酶是肠激酶。

[0010] 在一些实施方案中,步骤(iv)的孵育为约一小时至约16小时,裂解酶为肠激酶,且其中步骤(iv)中正确释放的步骤(iii)中所纯化的重组融合蛋白的量为约90%至约100%。在一些实施方案中,步骤(iv)中正确释放的步骤(iii)中所纯化的重组融合蛋白的量为约100%。在一些实施方案中,步骤(iii)或步骤(iv)中获得的目标多肽的量为约0.1g/L至约25g/L。在一些实施方案中,获得的正确释放的目标多肽是可溶的、完整的或两者。在一些实施方案中,步骤(iii)是在非变性条件下进行的。在一些实施方案中,重组融合蛋白在没有使用脲的情况下溶解。在一些实施方案中,非变性条件包括用包含非变性浓度的离液剂 chaotropic agent 的缓冲剂裂解步骤(ii)的经诱导细胞。在一些实施方案中,非变性浓度的离液剂是低于4M的脲。

[0011] 在一些实施方案中,微生物宿主细胞是假单胞菌(Pseudomonad)或大肠杆菌宿主细胞。在一些实施方案中,假单胞菌宿主细胞是假单胞菌属(Pseudomonas)宿主细胞。在一些实施方案中,假单胞菌属宿主细胞是荧光假单胞菌。

[0012] 在特定实施方案中,宿主细胞是至少一种选自以下的蛋白酶缺陷的:Lon(SEQ ID NO:14);Lal(SEQ ID NO:15);AprA(SEQ ID NO:16);HtpX(SEQ ID NO:17);DegP1(SEQ ID NO:18);DegP2(SEQ ID NO:19);Npr(SEQ ID NO:20);Prc1(SEQ ID NO:21);Prc2(SEQ ID NO:22);M50(SEQ ID NO:24);Pr1C(SEQ ID NO:30);Serralysin(RXF04495)(SEQ ID NO:227)和PrtB(SEQ ID NO:23)。在相关实施方案中,宿主细胞是蛋白酶Lon(SEQ ID NO:14)、Lal(SEQ ID NO:15)和AprA(SEQ ID NO:16)缺陷的。在一些实施方案中,宿主细胞是蛋白酶AprA(SEQ ID NO:16)和HtpX(SEQ ID NO:17)缺陷的。在其他实施方案中,宿主细胞是蛋白酶Lon(SEQ ID NO:14)、Lal(SEQ ID NO:15)和DegP2(SEQ ID NO:19)缺陷的。在一些实施方案中,宿主细胞是蛋白酶Npr(SEQ ID NO:20)、DegP1(SEQ ID NO:18)和DegP2(SEQ ID NO:19)缺陷的。在相关实施方案中,宿主细胞是蛋白酶Serralysin(SEQ ID NO:227)和AprA(SEQ ID NO:16)缺陷的。

[0013] 按引用并入

[0014] 本说明书提及的所有出版物、专利和专利申请按引用并入本文中,至如同每篇单独的出版物、专利或专利申请特意和单独表示按引用并入的相同程度。

附图说明

[0015] 列出了本发明的新特征,特别具有所附权利要求中的新特征。通过参考以下给出了说明性实施方案的详细描述和以下附图将获得本发明的特征和优点的更佳理解,在这些

说明性实施方案中利用了本发明的原理:

[0016] 图1.重组融合蛋白的图示。结构域1对应于N-末端融合伴侣,结构域2对应于接头和结构域3对应于目标多肽。N-末端融合伴侣和目标多肽的非限制性实例列于各相应结构域下方。

[0017] 图2A至2C.三个重组融合蛋白氨基酸序列。显示了包含hPTH1-34作为目标多肽的三个重组融合蛋白的氨基酸序列。每个中的hPTH 1-34是斜体的,而N-末端融合伴侣和PTH 1-34之间的接头是加下划线的。2A.包含DnaJ-样蛋白N-末端融合伴侣的重组融合蛋白。(DnaJ-样蛋白,aa 1-77;接头,aa 78-98;hPTH 1-34,aa 99-132) (SEQ ID NO:45)。2B.包含Fk1B N-末端融合伴侣的重组融合蛋白。(Fk1B,aa 1-205;接头,aa 206-226;hPTH 1-34,aa 227-260) (SEQ ID NO:46)。2C.包含FrnE N-末端融合伴侣的重组融合蛋白。(FrnE,aa 1-216;接头,aa 217-237;hPTH 1-34,aa 238-271) (SEQ ID NO:47)。

[0018] 图3.摇瓶表达样品的SDS-CGE分析。样品在三个组中显示:完整细胞培养液(泳道1-6);无细胞培养液(泳道7-12);和可溶性级分(泳道13-18),如图底部所示的。分子量标记显示于图象的每侧(68、48、29、21、16kD,从上往下)。三个组中的各组的泳道从左往右表示:DNAJ-样蛋白-PTH 1-34融合体(STR35970);DNAJ-样蛋白-PTH 1-34融合体(STR35984);Fk1B-PTH 1-34融合体(STR36034);Fk1B-PTH 1-34融合体(STR36085);FrnE-PTH 1-34融合体(STR36150);和FrnE-PTH 1-34融合体(STR36169),如泳道上方所示的。通过实线箭头标记DnaJ-样-PTH融合蛋白条带,通过虚线箭头标记Fk1B-PTH和FrnE-PTH融合蛋白条带。

[0019] 图4.纯化重组融合蛋白的肠激酶切割。样品在三个组中显示:无肠激酶处理(泳道1-6);肠激酶处理40 μ g/ml(泳道7-12);和肠激酶处理10 μ g/ml(泳道13-18)。三个组中各组的泳道从左往右表示:DNAJ-样蛋白-PTH 1-34融合体(STR35970);DNAJ-样蛋白-PTH 1-34融合体(STR35984);Fk1B-PTH 1-34融合体(STR36034);Fk1B-PTH 1-34融合体(STR36085);FrnE-PTH 1-34融合体(STR36150);和FrnE-PTH 1-34融合体(STR36169)。通过下方一对箭头中的实线箭头来表示DnaJ-样融合蛋白的迁移。通过下方一对箭头中的虚线箭头来表示切割的DnaJ-样-蛋白N-末端融合伴侣的迁移。通过上方一对箭头中的实线箭头来表示Fk1B和FrnE融合蛋白的迁移。通过上方一对箭头中的虚线箭头来表示Fk1B和Frn E N-末端融合伴侣的迁移。分子量标记显示于图象的右侧(29、20和16kD,从上到下)。

[0020] 图5.肠激酶切割产物的完整质量分析。显示了用肠激酶消化1小时的从表达菌株STR36970纯化的DnaJ-样蛋白-PTH 1-34融合蛋白的去卷积质谱。通过实线箭头表示对应于PTH 1-34的峰。

[0021] 图6.DnaJ-样蛋白-PTH 1-34融合蛋白纯化级分的肠激酶切割。在常规生物反应器中生长后从表达菌株STR36005纯化DnaJ-样蛋白-PTH融合蛋白。纯化级分用肠激酶孵育1小时(泳道2-4)、16小时(泳道6-8)、不使用肠激酶(对照)1小时(泳道1),或不使用肠激酶(对照)16小时(泳道5)。分析的级分如下:级分1(泳道1、2、5和6);级分2(泳道3和7);和级分3(泳道4和8)。通过黑色实线箭头表示全长DnaJ-样蛋白-PTH 1-34重组融合蛋白条带。通过虚线箭头来表示切割的DnaJ-样蛋白-PTH 1-34融合伴侣条带。分子量标记显示于图象的每侧(49、29、21和16kD,从上到下)。

[0022] 图7A至7C.源自Fk1B-PTH 1-34融合蛋白的PTH 1-34肠激酶切割产物的完整质量分析。这些图显示了针对用肠激酶消化的Fk1B-PTH 1-34融合蛋白纯化级分的去卷积质谱。

通过实线箭头表示对应于PTH 1-34的峰。7A.从STR36034纯化的Fk1B-PTH融合蛋白。7B.从STR36085纯化的Fk1B-PTH融合蛋白。7C.从STR36098纯化的Fk1B-PTH融合蛋白。

[0023] 序列

[0024] 本发明包括核苷酸序列SEQ ID NO:1-237,并且这些核苷酸序列列于权利要求之前的序列表中。

具体实施方式

[0025] 概述

[0026] 本发明涉及用于在细菌表达系统中超表达重组目标多肽的重组融合蛋白、用于表达该重组融合蛋白的构建体和用于高产量地生产可溶形式的重组融合蛋白和重组目标多肽的方法。在一些实施方案中,本发明的方法能够产生在纯化后高于0.5g/L的重组融合蛋白。在一些实施方案中,本发明的方法产生高产量的重组融合蛋白而不需要使用变性浓度的离液剂。在一些实施方案中,本发明的方法产生高产量的重组融合蛋白而不需要使用任何离液剂。

[0027] 如本文中使用的,术语“包含(comprise)”或其变型,如“含有(comprises)”或“包括(comprising)”理解成表示包括任何所述的特征但不排除任何其他特征。因此,如本文中使用的,术语“包含”是包括性的并且不排除其他的未叙述的特征。在本文中提供的任一组合物和方法的实施方案中,“包含”可以用“基本上由……组成”或“由……组成”来替代。短语“基本上由……组成”在本文中用于要求指定的特征以及不实质性地影响所要求的发明的特性或功能的那些特征。如本文中使用的,术语“组成”用于表示仅所叙述的特征(例如,核碱基序列)的存在(使得在由指定核碱基序列组成的反义寡聚物的情况中,排除了其他未叙述的核碱基的存在)。

[0028] 重组融合蛋白

[0029] 本发明的重组融合蛋白包含三个结构域,如图1中一般阐述的。从左起,融合蛋白包含N-末端融合伴侣、接头和目标多肽,其中接头在N-末端融合伴侣之间,而目标多肽在接头的C-末端。在一些实施方案中,接头序列包含蛋白酶切割位点。在一些实施方案中,通过在接头内的蛋白酶切割位点处的切割,目标多肽可以从重组融合蛋白释放出来。

[0030] 在一些实施方案中,重组融合蛋白的分子量为约2kDa至约1000kDa。在一些实施方案中,重组融合蛋白的分子量为约2kDa,约3kDa,约4kDa,约5kDa,约6kDa,约7kDa,约8kDa,约9kDa,约10kDa,约11kDa,约12kDa,约13kDa,约14kDa,约15kDa,约20kDa,约25kDa,约26kDa,约27kDa,约28kDa,约30kDa,约35kDa,约40kDa,约45kDa,约50kDa,约55kDa,约60kDa,约65kDa,约70kDa,约75kDa,约80kDa,约85kDa,约90kDa,约95kDa,约100kDa,约200kDa,约300kDa,约400kDa,约500kDa,约550kDa,约600kDa,约700kDa,约800kDa,约900kDa,约1000kDa或更高。在一些实施方案中,重组融合蛋白的分子量为约2kDa至约1000kDa,约2kDa至约500kDa,约2kDa至约250kDa,约2kDa至约100kDa,约2kDa至约50kDa,约2kDa至约25kDa,约2kDa至约30kDa,约2kDa至约1000kDa,约2kDa至约500kDa,约2kDa至约250kDa,约2kDa至约100kDa,约2kDa至约50kDa,约2kDa至约25kDa,约3kDa至约1000kDa,约3kDa至约500kDa,约3kDa至约250kDa,约3kDa至约100kDa,约3kDa至约50kDa,约3kDa至约25kDa,约3kDa至约30kDa,约4kDa至约1000kDa,约4kDa至约500kDa,约4kDa至约250kDa,约

4kDa至约100kDa,约4kDa至约50kDa,约4kDa至约25kDa,约4kDa至约30kDa,约5kDa至约1000kDa,约5kDa至约500kDa,约5kDa至约250kDa,约5kDa至约100kDa,约5kDa至约50kDa,约5kDa至约25kDa,约5kDa至约30kDa,约10kDa至约1000kDa,约10kDa至约500kDa,约10kDa至约250kDa,约10kDa至约100kDa,约10kDa至约50kDa,约10kDa至约25kDa,约10kDa至约30kDa,约20kDa至约1000kDa,约20kDa至约500kDa,约20kDa至约250kDa,约20kDa至约100kDa,约20kDa至约50kDa,约20kDa至约25kDa,约20kDa至约30kDa,约25kDa至约1000kDa,约25kDa至约500kDa,约25kDa至约250kDa,约25kDa至约100kDa,约25kDa至约50kDa,约25kDa至约25kDa或约25kDa至约30kDa。

[0031] 在一些实施方案中,重组融合蛋白为约50,100,150,200,250,300,350,400,450,470,500,530,560,590,610,640,670,700,750,800,850,900,950,1000,1200,1400,1600,1800,2000,2500或更多个氨基酸长。在一些实施方案中,重组融合蛋白为约50至2500,100至2000,150至1800,200至1600,250至1400,300至1200,350至1000,400至950,450至900,470至850,500至800,530至750,560至700,590至670,或610至640个氨基酸长。

[0032] 在一些实施方案中,重组融合蛋白包含选自以下的N-末端融合伴侣:

[0033] 荧光假单胞菌DnaJ-样蛋白(例如,SEQ ID NO:2)、FrnE(SEQ ID NO:3)、FrnE2(SEQ ID NO:63)、FrnE3(SEQ ID NO:64)、Fk1B(SEQ ID NO:4)、Fk1B3*(SEQ ID NO:28)、Fk1B2(SEQ ID NO:61)、Fk1B3(SEQ ID NO:62)、FkpB2(SEQ ID NO:5)、SecB(SEQ ID NO:6)、SecB的截短体、EcpD(SEQ ID NO:7)、EcpD(SEQ ID NO:65)、EcpD2(SEQ ID NO:66)和EcpD3(SEQ ID NO:67);

[0034] 选自以下的接头:SEQ ID NO:9、10、11、12和226;和

[0035] 选自以下的目标多肽:hPTH 1-34(SEQ ID NO:1)、Met-GCSF(SEQ ID NO:69)、rCSP、前胰岛素(例如,人前胰岛素SEQ ID NO:32、甘精胰岛素、前胰岛素SEQ ID NO:88、89、90或91中的任一个)、赖脯胰岛素SEQ ID NO:33、谷赖胰岛素(SEQ ID NO:34)、胰岛素C-肽(SEQ ID NO:97);美卡舍明(SEQ ID NO:35)、Glp-1(SEQ ID NO:36)、艾塞那肽(SEQ ID NO:37)、替度鲁肽(SEQ ID NO:38)、普兰林肽(SEQ ID NO:39)、齐考诺肽(SEQ ID NO:40)、贝卡普勒明(SEQ ID NO:42)、恩夫韦肽SEQ ID NO:43)、奈西立肽(SEQ ID NO:44)或肠激酶(例如,SEQ ID NO:31)。

[0036] 在一些实施方案中,重组融合蛋白包含荧光假单胞菌DnaJ-样蛋白N-末端融合伴侣和胰蛋白酶切割位点接头,其一起具有SEQ ID NO:101的氨基酸序列。在一些实施方案中,编码SEQ ID NO:101的核苷酸序列是SEQ ID NO:202。

[0037] 在一些实施方案中,重组融合蛋白包含荧光假单胞菌EcpD1蛋白N-末端融合伴侣和胰蛋白酶切割位点接头,其一起具有SEQ ID NO:102或103的氨基酸序列。在一些实施方案中,编码SEQ ID NO:102或103的核苷酸序列分别是SEQ ID NO:202或228。

[0038] 在一些实施方案中,重组融合蛋白包含荧光假单胞菌EcpD2蛋白N-末端融合伴侣和胰蛋白酶切割位点接头,其一起具有SEQ ID NO:104的氨基酸序列。在一些实施方案中,编码SEQ ID NO:104的核苷酸序列是SEQ ID NO:204。

[0039] 在一些实施方案中,重组融合蛋白包含荧光假单胞菌EcpD3蛋白N-末端融合伴侣和胰蛋白酶切割位点接头,其一起具有SEQ ID NO:105的氨基酸序列。在一些实施方案中,编码SEQ ID NO:105的核苷酸序列是SEQ ID NO:205。

[0040] 在一些实施方案中,重组融合蛋白包含荧光假单胞菌Fk1B1蛋白N-末端融合伴侣和胰蛋白酶切割位点接头,其一起具有SEQ ID NO:106的氨基酸序列。在一些实施方案中,编码SEQ ID NO:106的核苷酸序列是SEQ ID NO:206。

[0041] 在一些实施方案中,重组融合蛋白包含荧光假单胞菌Fk1B2蛋白N-末端融合伴侣和胰蛋白酶切割位点接头,其一起具有SEQ ID NO:107的氨基酸序列。在一些实施方案中,编码SEQ ID NO:107的核苷酸序列是SEQ ID NO:207。

[0042] 在一些实施方案中,重组融合蛋白包含荧光假单胞菌Fk1B3蛋白N-末端融合伴侣和胰蛋白酶切割位点接头,其一起具有SEQ ID NO:108的氨基酸序列。在一些实施方案中,编码SEQ ID NO:108的核苷酸序列是SEQ ID NO:208。

[0043] 在一些实施方案中,重组融合蛋白包含荧光假单胞菌FrnE1蛋白N-末端融合伴侣和胰蛋白酶切割位点接头,其一起具有SEQ ID NO:109的氨基酸序列。在一些实施方案中,编码SEQ ID NO:109的核苷酸序列是SEQ ID NO:209。

[0044] 在一些实施方案中,重组融合蛋白包含荧光假单胞菌FrnE2蛋白N-末端融合伴侣和胰蛋白酶切割位点接头,其一起具有SEQ ID NO:110的氨基酸序列。在一些实施方案中,编码SEQ ID NO:110的核苷酸序列是SEQ ID NO:210。

[0045] 在一些实施方案中,重组融合蛋白包含荧光假单胞菌FrnE3蛋白N-末端融合伴侣和胰蛋白酶切割位点接头,其一起具有SEQ ID NO:111的氨基酸序列。在一些实施方案中,编码SEQ ID NO:111的核苷酸序列是SEQ ID NO:211。

[0046] 在一些实施方案中,重组融合蛋白包含荧光假单胞菌DnaJ-样蛋白N-末端融合伴侣和肠激酶切割位点接头,其一起具有SEQ ID NO:112的氨基酸序列。在一些实施方案中,编码SEQ ID NO:112的核苷酸序列是SEQ ID NO:212。

[0047] 在一些实施方案中,重组融合蛋白包含荧光假单胞菌EcpD1蛋白N-末端融合伴侣和肠激酶切割位点接头,其一起具有SEQ ID NO:113的氨基酸序列。在一些实施方案中,编码SEQ ID NO:113的核苷酸序列分别是SEQ ID NO:213。

[0048] 在一些实施方案中,重组融合蛋白包含荧光假单胞菌EcpD2蛋白N-末端融合伴侣和肠激酶切割位点接头,其一起具有SEQ ID NO:114的氨基酸序列。在一些实施方案中,编码SEQ ID NO:114的核苷酸序列是SEQ ID NO:214。

[0049] 在一些实施方案中,重组融合蛋白包含荧光假单胞菌EcpD3蛋白N-末端融合伴侣和肠激酶切割位点接头,其一起具有SEQ ID NO:115的氨基酸序列。在一些实施方案中,编码SEQ ID NO:115的核苷酸序列是SEQ ID NO:215。

[0050] 在一些实施方案中,重组融合蛋白包含荧光假单胞菌Fk1B1蛋白N-末端融合伴侣和肠激酶切割位点接头,其一起具有SEQ ID NO:216的氨基酸序列。在一些实施方案中,编码SEQ ID NO:116的核苷酸序列是SEQ ID NO:216。

[0051] 在一些实施方案中,重组融合蛋白包含荧光假单胞菌Fk1B2蛋白N-末端融合伴侣和肠激酶切割位点接头,其一起具有SEQ ID NO:217的氨基酸序列。在一些实施方案中,编码SEQ ID NO:117的核苷酸序列是SEQ ID NO:217。

[0052] 在一些实施方案中,重组融合蛋白包含荧光假单胞菌Fk1B3蛋白N-末端融合伴侣和肠激酶切割位点接头,其一起具有SEQ ID NO:118的氨基酸序列。在一些实施方案中,编码SEQ ID NO:118的核苷酸序列是SEQ ID NO:218。

[0053] 在一些实施方案中,重组融合蛋白包含荧光假单胞菌FrnE1蛋白N-末端融合伴侣和肠激酶切割位点接头,其一起具有SEQ ID NO:119的氨基酸序列。在一些实施方案中,编码SEQ ID NO:119的核苷酸序列是SEQ ID NO:219。

[0054] 在一些实施方案中,重组融合蛋白包含荧光假单胞菌FrnE2蛋白N-末端融合伴侣和肠激酶切割位点接头,其一起具有SEQ ID NO:120的氨基酸序列。在一些实施方案中,编码SEQ ID NO:120的核苷酸序列是SEQ ID NO:220。

[0055] 在一些实施方案中,重组融合蛋白包含荧光假单胞菌FrnE3蛋白N-末端融合伴侣和肠激酶切割位点接头,其一起具有SEQ ID NO:121的氨基酸序列。在一些实施方案中,编码SEQ ID NO:121的核苷酸序列是SEQ ID NO:221。

[0056] 在一些实施方案中,重组融合蛋白的N-末端融合伴侣、接头和目标多肽分别是:荧光假单胞菌折叠调节子DnaJ-样蛋白(SEQ ID NO:2)、SEQ ID NO:9中所示的接头和人甲状旁腺激素氨基酸1-34(hPTH1-34)(SEQ ID NO:1)。在一些实施方案中,重组融合蛋白的N-末端融合伴侣、接头和目标多肽分别是:荧光假单胞菌折叠调节子FrnE(SEQ ID NO:3)、SEQ ID NO:9中所示的接头和hPTH 1-34(SEQ ID NO:1)。在一些实施方案中,重组融合蛋白的N-末端融合伴侣、接头和目标多肽分别是:荧光假单胞菌折叠调节子Fk1B(SEQ ID NO:4)、SEQ ID NO:9中所示的接头和hPTH 1-34(SEQ ID NO:1)。在一些实施方案中,重组hPTH融合蛋白具有SEQ ID NO:45、46和47中所示的氨基酸序列。

[0057] 在一些实施方案中,重组融合蛋白是具有以下元件的胰岛素融合蛋白:

[0058] 选自荧光假单胞菌的N-末端融合伴侣:DnaJ-样蛋白(SEQ ID NO:2)、FrnE(SEQ ID NO:3)、FrnE2(SEQ ID NO:63)、FrnE3(SEQ ID NO:64)、Fk1B(SEQ ID NO:4)、Fk1B3*(SEQ ID NO:28)、Fk1B2(SEQ ID NO:61)、Fk1B3(SEQ ID NO:62)、FkpB2(SEQ ID NO:5)、EcpD EcpD(SEQ ID NO:65)、EcpD2(SEQ ID NO:66)或EcpD3(SEQ ID NO:67);

[0059] 具有SEQ ID NO:226中所示序列的接头;和

[0060] 选自甘精前胰岛素SEQ ID NO:88、89、90或91的目标多肽。

[0061] 在一些实施方案中,目标多肽是通过SEQ ID NO:80或84所示的核苷酸序列编码的如SEQ ID NO:88所示的甘精前胰岛素。在一些实施方案中,目标多肽是通过SEQ ID NO:81或85所示的核苷酸序列编码的如SEQ ID NO:89所示的甘精前胰岛素。在一些实施方案中,目标多肽是通过SEQ ID NO:82或86所示的核苷酸序列编码的如SEQ ID NO:90所示的甘精前胰岛素。在一些实施方案中,目标多肽是通过SEQ ID NO:83或87所示的核苷酸序列编码的SEQ ID NO:91所示的胰岛素甘精前胰岛素。

[0062] 在一些实施方案中,胰岛素融合蛋白包含荧光假单胞菌DnaJ-样蛋白N-末端融合伴侣和胰蛋白酶切割位点接头,其一起具有SEQ ID NO:101的氨基酸序列。在一些实施方案中,编码SEQ ID NO:101的核苷酸序列是SEQ ID NO:202。

[0063] 在一些实施方案中,胰岛素融合蛋白包含荧光假单胞菌EcpD1蛋白N-末端融合伴侣和胰蛋白酶切割位点接头,其一起具有SEQ ID NO:102或103的氨基酸序列。在一些实施方案中,编码SEQ ID NO:102或103的核苷酸序列分别是SEQ ID NO:202或228。

[0064] 在一些实施方案中,胰岛素融合蛋白包含荧光假单胞菌EcpD2蛋白N-末端融合伴侣和胰蛋白酶切割位点接头,其一起具有SEQ ID NO:104的氨基酸序列。在一些实施方案中,编码SEQ ID NO:104的核苷酸序列是SEQ ID NO:204。

[0065] 在一些实施方案中,胰岛素融合蛋白包含荧光假单胞菌EcpD3蛋白N-末端融合伴侣和胰蛋白酶切割位点接头,其一起具有SEQ ID NO:105的氨基酸序列。在一些实施方案中,编码SEQ ID NO:105的核苷酸序列是SEQ ID NO:205。

[0066] 在一些实施方案中,胰岛素融合蛋白包含荧光假单胞菌Fk1B1蛋白N-末端融合伴侣和胰蛋白酶切割位点接头,其一起具有SEQ ID NO:106的氨基酸序列。在一些实施方案中,编码SEQ ID NO:106的核苷酸序列是SEQ ID NO:206。

[0067] 在一些实施方案中,胰岛素融合蛋白包含荧光假单胞菌Fk1B2蛋白N-末端融合伴侣和胰蛋白酶切割位点接头,其一起具有SEQ ID NO:107的氨基酸序列。在一些实施方案中,编码SEQ ID NO:107的核苷酸序列是SEQ ID NO:207。

[0068] 在一些实施方案中,胰岛素融合蛋白包含荧光假单胞菌Fk1B3蛋白N-末端融合伴侣和胰蛋白酶切割位点接头,其一起具有SEQ ID NO:108的氨基酸序列。在一些实施方案中,编码SEQ ID NO:108的核苷酸序列是SEQ ID NO:208。

[0069] 在一些实施方案中,胰岛素融合蛋白包含荧光假单胞菌FrnE1蛋白N-末端融合伴侣和胰蛋白酶切割位点接头,其一起具有SEQ ID NO:109的氨基酸序列。在一些实施方案中,编码SEQ ID NO:109的核苷酸序列是SEQ ID NO:209。

[0070] 在一些实施方案中,胰岛素融合蛋白包含荧光假单胞菌FrnE2蛋白N-末端融合伴侣和胰蛋白酶切割位点接头,其一起具有SEQ ID NO:110的氨基酸序列。在一些实施方案中,编码SEQ ID NO:110的核苷酸序列是SEQ ID NO:210。

[0071] 在一些实施方案中,胰岛素融合蛋白包含荧光假单胞菌FrnE3蛋白N-末端融合伴侣和胰蛋白酶切割位点接头,其一起具有SEQ ID NO:111的氨基酸序列。在一些实施方案中,编码SEQ ID NO:111的核苷酸序列是SEQ ID NO:211。

[0072] 在一些实施方案中,胰岛素融合蛋白包含荧光假单胞菌DnaJ-样蛋白N-末端融合伴侣和肠激酶切割位点接头,其一起具有SEQ ID NO:112的氨基酸序列。在一些实施方案中,编码SEQ ID NO:112的核苷酸序列是SEQ ID NO:212。

[0073] 在一些实施方案中,胰岛素融合蛋白包含荧光假单胞菌EcpD1蛋白N-末端融合伴侣和肠激酶切割位点接头,其一起具有SEQ ID NO:113的氨基酸序列。在一些实施方案中,编码SEQ ID NO:113的核苷酸序列分别是SEQ ID NO:213。

[0074] 在一些实施方案中,胰岛素融合蛋白包含荧光假单胞菌EcpD2蛋白N-末端融合伴侣和肠激酶切割位点接头,其一起具有SEQ ID NO:114的氨基酸序列。在一些实施方案中,编码SEQ ID NO:114的核苷酸序列是SEQ ID NO:214。

[0075] 在一些实施方案中,胰岛素融合蛋白包含荧光假单胞菌EcpD3蛋白N-末端融合伴侣和肠激酶切割位点接头,其一起具有SEQ ID NO:115的氨基酸序列。在一些实施方案中,编码SEQ ID NO:115的核苷酸序列是SEQ ID NO:215。

[0076] 在一些实施方案中,胰岛素融合蛋白包含荧光假单胞菌Fk1B1蛋白N-末端融合伴侣和肠激酶切割位点接头,其一起具有SEQ ID NO:216的氨基酸序列。在一些实施方案中,编码SEQ ID NO:116的核苷酸序列是SEQ ID NO:216。

[0077] 在一些实施方案中,胰岛素融合蛋白包含荧光假单胞菌Fk1B2蛋白N-末端融合伴侣和肠激酶切割位点接头,其一起具有SEQ ID NO:217的氨基酸序列。在一些实施方案中,编码SEQ ID NO:117的核苷酸序列分别是SEQ ID NO:217。

[0078] 在一些实施方案中,胰岛素融合蛋白包含荧光假单胞菌Fk1B3蛋白N-末端融合伴侣和肠激酶切割位点接头,其一起具有SEQ ID NO:118的氨基酸序列。在一些实施方案中,编码SEQ ID NO:118的核苷酸序列是SEQ ID NO:218。

[0079] 在一些实施方案中,胰岛素融合蛋白包含荧光假单胞菌FrnE1蛋白N-末端融合伴侣和肠激酶切割位点接头,其一起具有SEQ ID NO:119的氨基酸序列。在一些实施方案中,编码SEQ ID NO:119的核苷酸序列是SEQ ID NO:219。

[0080] 在一些实施方案中,胰岛素融合蛋白包含荧光假单胞菌FrnE2蛋白N-末端融合伴侣和肠激酶切割位点接头,其一起具有SEQ ID NO:120的氨基酸序列。在一些实施方案中,编码SEQ ID NO:120的核苷酸序列是SEQ ID NO:220。

[0081] 在一些实施方案中,胰岛素融合蛋白包含荧光假单胞菌FrnE3蛋白N-末端融合伴侣和肠激酶切割位点接头,其一起具有SEQ ID NO:121的氨基酸序列。在一些实施方案中,编码SEQ ID NO:121的核苷酸序列是SEQ ID NO:221。

[0082] 在一些实施方案中,重组胰岛素融合蛋白具有SEQ ID NO:122至201任一个中所示的氨基酸序列。

[0083] 在一些实施方案中,重组融合蛋白是具有以下元件的GCSF融合蛋白:

[0084] 选自荧光假单胞菌的N-末端融合伴侣:DnaJ-样蛋白(SEQ ID NO:2)、FrnE(SEQ ID NO:3)、FrnE2(SEQ ID NO:63)、FrnE3(SEQ ID NO:64)、Fk1B(SEQ ID NO:4)、Fk1B3*(SEQ ID NO:28)、Fk1B2(SEQ ID NO:61)、Fk1B3(SEQ ID NO:62)、FkpB2(SEQ ID NO:5)、EcpD EcpD (SEQ ID NO:65)、EcpD2(SEQ ID NO:66)或EcpD3(SEQ ID NO:67);

[0085] 具有SEQ ID NO:9中所示序列的接头;和

[0086] 具有SEQ ID NO:68中所示序列的目标多肽。

[0087] 目标多肽

[0088] 重组融合蛋白的目标蛋白质或多肽,也称为目标C-末端多肽、重组目标多肽和C-末端融合伴侣,是期望以可溶形式和高产量表达的多肽。在一些实施方案中,目标多肽是已经发现由于例如蛋白质水解、低表达水平、不正确的蛋白质折叠和/或差的宿主细胞分泌而不能在细菌表达系统中以高产量表达的异源多肽。目标多肽包括小的或快速降解的肽、具有易降解的N-末端的蛋白质和通常以不溶形式在微生物或细菌表达系统中产生的蛋白质。在一些实施方案中,将目标多肽的N-末端保护起来免受降解,同时与N-末端融合伴侣融合,形成N-末端完整的蛋白质的更高产量。在一些实施方案中,异源多肽已经被描述为不以高产率和可溶形式在微生物或细菌表达系统中表达。例如,在一些实施方案中,异源多肽已经被描述为不以高产率和可溶形式在大肠杆菌、枯草芽孢杆菌(*B. subtilis*)或植物乳杆菌(*L. plantarum*)、干酪乳杆菌(*L. casei*)、发酵乳杆菌(*L. fermentum*)或谷氨酸棒状杆菌(*corynebacterium glutamicum*)宿主细胞中表达。在一些实施方案中,目标多肽是真核生物多肽或源自真核生物多肽(例如,是其类似物)。在一些实施方案中,目标多肽是哺乳动物多肽或源自哺乳动物多肽。在一些实施方案中,目标多肽是人多肽或源自人多肽。在一些实施方案中,目标多肽是原核生物多肽或源自原核生物多肽。在一些实施方案中,目标多肽是微生物多肽或源自微生物多肽。在一些实施方案中,目标多肽是细菌多肽或源自细菌多肽。“异源”表示目标多肽源自表达宿主细胞以外的生物体。在一些实施方案中,根据本发明的方法,融合蛋白和/或目标多肽以高于另一种微生物表达系统的产率在假单胞菌宿主细胞

(即,假单胞菌目(Pseudomonadales)的宿主细胞)中产生。在一些实施方案中,在基本上相当的条件下,根据本发明的方法,融合蛋白或目标多肽以高于大肠杆菌或其他微生物或细菌表达系统(例如,以上所列那些)中的产率在假单胞菌、假单胞菌属或荧光假单胞菌表达系统中产生,例如,高约1.5倍至约10倍、约1.5倍、约2倍、约2.5倍、约3倍、约5倍或约10倍。在一些实施方案中,融合蛋白或C-末端多肽以低于0.5、低于0.4、低于0.3、低于0.2或低于0.1克/升的产率在大肠杆菌表达系统中产生。

[0089] 在一些实施方案中,目标多肽是小的和/或快速降解的肽。在一些实施方案中,小的和/或快速降解的肽是甲状旁腺激素(PTH)。在一些实施方案中,目标多肽是hPTH 1-34 (SEQ ID NO:1)。PTH是由甲状旁腺分泌的源自115个aa前-原-肽的84个氨基酸(aa)的肽,其用于提高血液中的钙浓度并且已知刺激骨形成。N-末端34个aa的肽批准用于治疗骨质疏松症(**Forteo®**,Eli Lilly and Company;参见包装说明书)。**Forteo®**中的活性成分,PTH 1-34,在大肠杆菌中作为C-末端融合蛋白的部分来产生(对于**Forteo®**,为NDA 21-319;参见,Chemistry Review,Center for Drug Evaluation and Research,2000-2001;还参见Clinical Pharmacology and Biopharmaceutics review,Center for Drug Evaluation and Research,2000-2001)。**Forteo®** (Eli Lilly's LY333334)的纯化描述于例如Jin等("Crystal Structure of Human Parathyroid Hormone 1-34at0.9 Å Resolution" J.Biol.Chem.275(35):27238-44,2000)中,其按引用并入本文中。这篇报道描述了蛋白质作为包涵体的表达,并随后于7M脲中溶解。

[0090] 在一些实施方案中,在细菌表达系统中超表达时,目标多肽通常以不溶形式产生。在一些实施方案中,在细菌表达系统中超表达时通常以不溶形式产生的目标多肽是真核生物多肽或其衍生物或类似物。在一些实施方案中,在细菌表达系统中超表达时通常以不溶形式产生的目标多肽是前胰岛素(胰岛素的前体)。前胰岛素由三个指定的区段组成(从N至C-末端:B-C-A)。通过蛋白酶分裂除去内部C-肽时,前胰岛素加工成胰岛素(或胰岛素类似物,取决于该前胰岛素)。切除C-肽胰岛素后,A和B-肽之间的二硫键合维持其关联。关于此处的胰岛素和胰岛素类似物,"A-肽"和"A-链"可互换使用,以及"B-肽"和"B-链"可互换使用。这些链内的位置通过链以及从链的氨基末端开始的氨基酸编号来提及,例如,"B30"是指B-肽(即,B-链)中的第三十个氨基酸。在一些实施方案中,目标多肽是被加工形成长效胰岛素类似物或速效胰岛素类似物的前胰岛素。

[0091] 在一些实施方案中,目标多肽是被加工形成长效胰岛素类似物的前胰岛素。长效胰岛素类似物包括,例如,甘精胰岛素,43个氨基酸(6050.41Da),作为**Lantus®**销售的长效胰岛素类似物;德谷胰岛素,作为**Tresiba®**销售;以及地特胰岛素,作为**Levemir®**销售。在甘精胰岛素中,N21的天冬酰胺(Asn21)被甘氨酸取代,并且在B-肽的C-末端存在两个精氨酸。在胰岛素中,这两个精氨酸存在于前胰岛素中,但不存在于加工过的成熟分子中。在一些实施方案中,目标多肽被加工成甘精胰岛素,并且目标多肽是SEQ ID NO:88、89、90或91中所示的87个氨基酸的前胰岛素。在非限制性实施方案中,用于SEQ ID NO:88的编码序列是SEQ ID NO:80或84中所示的核苷酸序列。在非限制性实施方案中,用于SEQ ID NO:89的编码序列是SEQ ID NO:81或85中所示的核苷酸序列。在非限制性实施方案中,用于SEQ ID NO:90的编码序列是SEQ ID NO:82或86中所示的核苷酸序列。在非限制性实施方案中,

用于SEQ ID NO:91的编码序列是SEQ ID NO:83或87中所示的核苷酸序列。SEQ ID NO:80-87中的每一个包括5'端的初始15bp克隆位点,因此在这些实施方案中,提及的前胰岛素编码序列是在第一Phe密码子,TTT(在SEQ ID NO:80中)或TTC(在SEQ ID NO:81-87)处开始的序列。德谷胰岛素删除了位置B30处的苏氨酸并且经由 γ -L-谷氨酰间隔体在位置B29的氨基酸赖氨酸处与十六烷二酸偶联。地特胰岛素具有与位置B29的赖氨酸氨基酸结合的脂肪酸(肉豆蔻酸)。

[0092] 在一些实施方案中,目标多肽是被加工以形成速效胰岛素类似物的前胰岛素。速效(或快速起效)胰岛素类似物包括,例如,门冬胰岛素(**NovoLog/NovoRapid®**)(SEQ ID NO:94),其中位置B28的脯氨酸被天冬氨酸替代,以及赖脯胰岛素(**Humalog®**)(赖脯前胰岛素,SEQ ID NO:33),其中出现在B-链的C-末端的端部处的最后赖氨酸和脯氨酸残基反转,和谷赖胰岛素(**Apidra®**)(谷赖前胰岛素,SEQ ID NO:34),其中位置B3的天冬酰胺被赖氨酸替代并且位置B29中的赖氨酸被谷氨酸替代。在所有其他位置,这些分子具有与常规胰岛素(前胰岛素,SEQ ID NO:32;胰岛素A-肽,SEQ ID NO:92;胰岛素B-肽,SEQ ID NO:93)相同的氨基酸序列。

[0093] 在一些实施方案中,在细菌超表达系统中超表达时通常以不溶形式产生的目标多肽是GCSF,例如,Met-GCSF。在一些实施方案中,在细菌超表达系统中超表达时通常以不溶形式产生的目标多肽是IFN- β ,例如,IFN- β -1b。在一些实施方案中,重组目标多肽难以在其中超表达的细菌表达系统是大肠杆菌表达系统。

[0094] 在一些实施方案中,目标多肽是具有易降解N-末端的蛋白质。因为根据本发明方法产生的融合蛋白在切割以释放目标多肽之前与宿主蛋白酶分离,在整个纯化过程中目标多肽的N-末端受到保护。这允许生产高达100%的N-末端完整目标多肽的制备物。

[0095] 在一些实施方案中,具有易降解N-末端的目标多肽是非格司亭,GCSF(粒细胞集落刺激因子,或集落刺激因子3(CSF3))的一种类似物。GCSF是刺激骨髓产生粒细胞和干细胞并将其释放至血流中的174个氨基酸的糖蛋白。非格司亭,其是非糖基化的并且具有N-末端甲硫氨酸,作为**Neupogen®**销售。GCSF(非格司亭)的氨基酸序列示于SEQ ID NO:69中。在一些实施方案中,本发明的方法用于产生具有完整N-末端(包括N-末端甲硫氨酸)的高水平的GCSF(非格司亭)。在蛋白酶缺陷的宿主细胞中生产GCSF描述于美国专利No.8,455,218,“Methods for G-CSF production in a Pseudomonas host cell”中,其全部按引用并入本文中。在本发明的实施方案中,在非蛋白酶缺陷的细菌宿主细胞(例如,假单胞菌属宿主细胞)中,以高水平在融合蛋白内产生包括N-末端甲硫氨酸的完整GCSF。

[0096] 在一些实施方案中,具有易降解N-末端的目标多肽是重组恶性疟原虫环子孢子蛋白(rCSP),描述于例如美国专利No.9,169,304,“Process for Purifying Recombinant Plasmodium Falciparum Circumsporozoite Protein(用于纯化重组恶性疟原虫环子孢子蛋白的方法)”中,全部按引用并入本文中。

[0097] 在一些实施方案中,目标多肽是:试剂蛋白;治疗蛋白;细胞外受体或配体;蛋白酶;激酶;血液蛋白;趋化因子;细胞因子;抗体;基于抗体的药物;抗体片段,例如,单链抗体、抗原结合(ab)片段,例如,F(ab)、F(ab)'、F(ab)'₂、Fv,从IgG或IgM的可变区产生、从抗体的重链恒定区产生的Fc片段、还原的IgG片段(例如,通过还原IgG的铰链区二硫键产生

的)、Fc融合蛋白,例如,包含与目标蛋白或肽融合在一起的IgG的Fc结构域,或本领域中描述的任何其他抗体片段,例如,美国专利No.5,648,237,“Expression of Functional Antibody Fragments”,全部按引用并入本文中;抗凝剂;血液因子;骨形态生产蛋白;工程化蛋白支架;酶;生长因子;干扰素;白细胞介素;血栓溶解剂;或激素。在一些实施方案中,目标多肽选自:人抗血友病因子;人抗血友病因子-血管性血友病因子复合物;重组抗血友病因子(Turoctocog Alfa);Ado-曲妥珠单抗emtansine;阿必鲁肽(Albiglutide);阿葡萄糖苷酶 α ;人 α -1蛋白酶抑制剂;B型肉毒杆菌毒素(Rimabotulinumtoxin B);凝血因子IX Fc融合体;重组凝血因子IX;重组凝血因子VIIa;重组凝血因子XIII A-亚基;人凝血因子VIII-血管性血友病因子复合物;溶组织梭菌胶原酶;人血小板衍生生长因子(Cecaplermin);阿巴西普(Abatacept);阿昔单抗(Abciximab);阿达木单抗(Adalimumab);阿柏西普(Aflibercept); β -半乳糖苷酶;阿地白介素(Aldesleukin);阿法赛特(Alefcept);阿仑单抗(Alemtuzumab);阿葡萄糖苷酶 α ;阿替普酶(Alteplase);阿那白滞素(Anakinra);辛凝血素 α ;重组人抗凝血酶;Azficel-T;巴利昔单抗(Basiliximab);贝拉西普(Belatacept);贝利木单抗(Belimumab);贝伐单抗;A型肉毒杆菌毒素;Brentuximab Vedotin;重组C1酯酶抑制剂;康纳单抗(Canakinumab);赛妥珠单抗(Certolizumab Pegol);西妥昔单抗;诺那凝血素 α ;达克珠单抗(Daclizumab);达贝泊汀(Darbepoetin) α ;狄诺塞麦(Denosumab);地高辛免疫Fab;链道酶(Dornase) α ;艾卡拉肽(Ecallantide);依库丽单抗(Eculizumab);依那西普;纤维蛋白原;非格司亭;加硫酶(Galsulfase);戈利木单抗(Golimumab);替伊莫单抗(Ibritumomab Tiuxetan);艾度硫酸酯酶(Idursulfase);英夫利昔单抗(Infliximab);干扰素 α ;干扰素 α -2b;干扰素Alfacon-1;干扰素 α -2a;干扰素干扰素 α -n3;干扰素 β -1a;干扰素 β -1b;干扰素 γ -1b;戈利木单抗(Ipilimumab);拉罗尼酶(Laronidase);阿法依泊汀;莫罗凝血素 α ;莫罗单抗(Muromonab)-CD3;那他珠单抗;Ocriplasmin;奥法木单抗(Ofatumumab);奥马珠单抗;奥普瑞白介素;帕利夫明(Palifermin);帕利珠单抗;帕尼单抗;PEG化非格司亭;帕妥珠单抗;6;11;16;18-L1型人乳头状瘤病毒(HPV)病毒蛋白病毒样颗粒(VLP);16和18L1型HPV蛋白VLP;兰尼单抗;拉布立酶;瑞西巴库(Raxibacumab);重组因子IX;瑞替普酶;利纳西普(Riloncept);利妥昔单抗;罗米司亭(Romiplostim);沙莫司亭;替奈普酶;托珠单抗;曲妥单抗;优特克单抗(Ustekinumab);阿巴瑞克;西曲瑞克;地西卢定;恩夫韦肽;艾塞那肽;促卵泡素 β ;加尼瑞克;地加瑞克(Degarelix);透明质酸酶;门冬胰岛素;德谷胰岛素;地特胰岛素;甘精胰岛素rDNA注射(长效人胰岛素类似物);重组胰岛素谷赖胰岛素;人胰岛素;赖脯胰岛素(速效胰岛素类似物);重组赖脯胰岛素鱼精蛋白;重组赖脯胰岛素;兰乐肽;利拉鲁肽;Surfaxin(Lucinactant);西那普肽;美卡舍明;胰岛素样生长因子;奈西立肽;普兰林肽;重组替度鲁肽;醋酸替莫瑞林;醋酸齐考诺肽;10.8mg醋酸戈舍瑞林植入;A型肉毒毒素(Abobotulinumtoxin A);半乳糖苷酶 α ;阿利泼金(Alipogene Tiparvovec);安西司亭;阿尼普酶;阿地肝素钠;鸟TB疫苗;巴曲酶;比伐卢定;布舍瑞林(促性腺激素-释放激素激动剂);苹果酸卡博替尼S;卡培立肽;卡妥索单抗;蓝肽(Ceruletide);凝血因子VIII;球虫病疫苗;达特肝素钠;去铁酮;去纤苷;Dibotermine Alfa;Drotrecogin Alfa;依多曲肽;依法利珠单抗;依诺肝素钠;Epoetin Delta;依替巴肽;Eptotermine Alfa;注射用促卵泡素 α ;福米韦生;吉妥单抗奥佐米星;戈那瑞林;重组慢性人戈那瑞林;醋酸组氨瑞林(戈那瑞林释放激素激动剂);HVT IBD疫苗;伊米苷酶;低精蛋

白胰岛素;来格司亭(粒细胞集落刺激因子);来匹卢定(Lepirudin);狗钩端螺旋体疫苗;亮丙瑞林(Leuproprelin);利那洛肽;Lipegfilgrastim;利昔那肽;促黄体素 α (人促黄体生成激素);美泊利单抗;米伐木肽;米泊美生钠;米立司亭(巨噬细胞-集落刺激因子);Mogamulizumab;莫拉司亭(粒细胞巨噬细胞-集落刺激因子);孟替普酶;那屈肝素钙;那法瑞林;奈巴库单抗;奥曲肽;帕米普酶;胰脂肪酶;帕肝素钠;Pasireotide daspartate;Peginesatide acetate;培维索孟;喷曲肽;猪肺磷脂(Poractant alfa);普拉莫瑞林(生长激素释放肽);普罗瑞林;PTH 1-84;rhBMP-2;rhBMP-7;Eptortermin Alfa;罗莫肽;舍莫瑞林;生长激素抑制素;人蛋氨酸生长素(Somatrem);加压素(Vassopressin);去氨加压素;Taliglucerase Alfa;他替瑞林(促甲状腺激素释放激素类似物);他索纳明;他司鲁泰;血栓调节蛋白(Thrombomodulin) Alfa;促甲状腺激素 α ;曲弗明;双羟萘酸曲普瑞林;注射用尿促卵泡素;尿激酶;Velaglucerase Alfa;霍乱毒素B;重组抗血友病因子(Efralotocog Alfa);人 α -1蛋白酶抑制剂;菊欧文氏菌天冬酰胺酶;卡罗单抗;Denileukin Diftitox;羊地高辛免疫Fab;Elosulfase Alfa;促红细胞生成素 α ;因子IX复合物;因子XIII浓缩物;锆(Fanolesomab);血纤维蛋白原;凝血酶;流感血凝集素和神经氨酸苷酶;Glucarpidase;注射用氯高铁血红素(Hemin);乙肝表面抗原;人白蛋白;Incobotulinumtoxin;诺非单抗;阿托珠单抗;L-天冬酰胺酶(来自大肠杆菌;欧文氏菌属;假单胞菌属;等);派姆单抗(Pembrolizumab);蛋白C浓缩物;雷莫芦单抗(Ramucirumab);司妥昔单抗(Siltuximab);Tbo-非格司亭;百日咳毒素亚基A-E;局部牛凝血酶;局部人凝血酶;托西莫单抗;Vedolizumab;Ziv-阿柏西普(Aflibercept);胰高血糖素;促生长激素(Somatropin);恶性疟原虫或间日疟原虫抗原(例如,CSP、Ce1TOS、TRAP、Rh5、AMA-1、LSA-1、LSA-3、Pfs25、MSP-1、MSP-3、STARP、EXP1、pb9、GLURP)。这些多肽的序列,包括变异,是文献中可获得的并且是本领域技术人员已知的。所列的任一个肽的任何已知序列考虑用于本发明的方法中。

[0098] 在一些实施方案中,目标多肽是肠激酶(例如,SEQ ID NO:31[牛])、胰岛素、前胰岛素(例如,SEQ ID NO:32)、长效胰岛素类似物或经加工以形成长效胰岛素类似物(例如,甘精胰岛素,SEQ ID NO:88,地特胰岛素,或德谷胰岛素)的前胰岛素、速效胰岛素类似物或经加工以形成速效胰岛素类似物(例如,赖脯胰岛素,门冬胰岛素或谷赖胰岛素)的前胰岛素、胰岛素C-肽(例如,SEQ ID NO:97)、IGF-1(例如,美卡舍明,SEQ ID NO:35)、Glp-1(例如,SEQ ID NO:36)、Glp-1类似物(例如,艾塞那肽,SEQ ID NO:37)、Glp-2(例如,SEQ ID NO:38)、Glp-2类似物(例如,替度鲁肽,SEQ ID NO:39)、普兰林肽(例如,SEQ ID NO:40)、齐考诺肽(例如,SEQ ID NO:41)、贝卡普勒明(例如,SEQ ID NO:42)、恩夫韦肽(例如,SEQ ID NO:43)或奈西立肽(例如,SEQ ID NO:44)。

[0099] 在一些实施方案中,目标多肽的分子量为约1kDa,约2kDa,约3kDa,约4kDa,约5kDa,约6kDa,约7kDa,约8kDa,约9kDa,约10kDa,约11kDa,约12kDa,约13kDa,约14kDa,约15kDa,约16kDa,约17kDa,约18kDa,约19kDa,约20kDa,约30kDa,约40kDa,约50kDa,约60kDa,约70kDa,约80kDa,约90kDa,约100kDa,约150kDa,约200kDa,约250kDa,约300kDa,约350kDa,约400kDa,约450kDa,约500kDa或更高。在一些实施方案中,重组多肽的分子量为约1至约10kDa,约1至约20kDa,约1至约30kDa,约1至约40kDa,约1至约50kDa,约1至约60kDa,约1至约70kDa,约1至约80kDa,约1至约90kDa,约1至约100kDa,约1kDa至约200kDa,约1kDa至约300kDa,约1kDa至约400kDa,约1kDa至约500kDa,约2至约10kDa,约2至约20kDa,约2至

约30kDa,约2至约40kDa,约2至约50kDa,约2至约60kDa,约2至约70kDa,约2至约80kDa,约2至约90kDa,约2至约100kDa,约2kDa至约200kDa,约2kDa至约300kDa,约2kDa至约400kDa,约2kDa至约500kDa,约3至约10kDa,约3至约20kDa,约3至约30kDa,约3至约40kDa,约3至约50kDa,约3至约60kDa,约3至约70kDa,约3至约80kDa,约3至约90kDa,约3至约100kDa,约3kDa至约200kDa,约3kDa至约300kDa,约3kDa至约400kDa,或约3kDa至约500kDa。在一些实施方案中,目标多肽的分子量为约4.1kDa。

[0100] 在一些实施方案中,目标多肽是25或更多个氨基酸长。在一些实施方案中,目标多肽是约25或约2000或更多个氨基酸长。在一些实施方案中,目标多肽为约或至少约25、30、35、40、45、50、100、150、200、250、300、350、400、450、475、500、525、550、575、600、625、650、700、750、800、850、900、950、1000、1200、1400、1600、1800或2000个氨基酸长。在一些实施方案中,目标多肽为约:25至约2000,25至约1000,25至约500,25至约250,25至约100,或25至约50个氨基酸长。在一些实施方案中,目标多肽是32,36,39,71,109或110个氨基酸长。在一些实施方案中,目标多肽是34个氨基酸长。

[0101] N-末端融合伴侣

[0102] 重组融合蛋白的N-末端融合伴侣是提高使用细菌表达系统获得的重组融合蛋白的产量的细菌蛋白。在一些实施方案中,N-末端融合伴侣可以在细菌宿主细胞中从重组构建体稳定地超表达。在一些实施方案中,可以通过N-末端融合伴侣的存在提高或改善目标多肽的产量和/或溶解性。在一些实施方案中,N-末端融合伴侣促进重组融合蛋白的正确折叠。在一些实施方案中,N-末端融合伴侣是细菌折叠调节子或伴侣蛋白。

[0103] 在一些实施方案中,N-末端融合伴侣是大尺寸亲和性标签蛋白、折叠调节子、分子伴侣蛋白、核糖体蛋白、翻译相关因子、OB-折叠蛋白(OB-fold protein)(寡肽结合折叠蛋白)或文献中描述的另一种蛋白,例如,Ahn等,2011,“Expression screening of fusion partners from an E.coli genome for soluble expression of recombinant proteins in acell-free protein synthesis system”,PLoS One,6(11):e26875,按引用并入本文中。在一些实施方案中,N-末端融合伴侣是选自MBP、GST、NusA、泛素、IF-2的结构域1和L9的N-末端结构域的大尺寸亲和性标签蛋白。在一些实施方案中,N-末端融合伴侣是来自30S核糖体亚基的核糖体蛋白,或来自50S核糖体亚基的核糖体蛋白。在一些实施方案中,N-末端融合伴侣是大肠杆菌或假单胞菌伴侣蛋白或折叠调节子蛋白。在一些实施方案中,N-末端融合蛋白是荧光假单胞菌伴侣蛋白或折叠调节子蛋白。在一些实施方案中,N-末端融合伴侣是选自表1的伴侣蛋白或折叠调节子蛋白。

[0104] 在一些实施方案中,N-末端融合伴侣是荧光假单胞菌DnaJ-样蛋白(SEQ ID NO:2)、FrnE(SEQ ID NO:3)、FrnE2(SEQ ID NO:63)、FrnE3(SEQ ID NO:64)、Fk1B(SEQ ID NO:4)、Fk1B3*(SEQ ID NO:28)、Fk1B2(SEQ ID NO:61)、Fk1B3(SEQ ID NO:62)、FkpB2(SEQ ID NO:5)、SecB(SEQ ID NO:6)、EcpD(RXF04553.1,SEQ ID NO:7)、EcpD(RXF04296.1,SEQ ID NO:65,在本文中也称为EcpD1)、EcpD2(SEQ ID NO:66)或EcpD3(SEQ ID NO:67)。在一些实施方案中,N-末端融合伴侣是大肠杆菌蛋白Skp(SEQ ID NO:8)。

[0105] 在一些实施方案中,N-末端融合伴侣相对于全长融合伴侣多肽是截短的。在一些实施方案中,N-末端融合伴侣从C-末端截短以除去至少一个C-末端氨基酸。在一些实施方案中,将N-末端融合伴侣截短以从全长多肽的C-末端除去1至300个氨基酸。在一些实施方

案中,将N-末端融合伴侣截短以从多肽的C-末端除去300,290,280,270,260,250,240,230,220,210,200,190,180,170,160,150,140,130,120,110,100,90,80,70,60,50,40,30,20,10,5,1至300,1至295,1至290,1至280,1至270,1至260,1至250,1至240,1至230,1至220,1至210,1至200,1至190,1至180,1至170,1至160,1至150,1至140,1至130,1至120,1至110,1至100,1至90,1至80,1至70,1至60,1至50,1至40,1至30,1至20,1至15,1至10或1至5个氨基酸。在一些实施方案中,N-末端融合伴侣多肽从C-末端截短以保留全长多肽的N-末端前300,290,280,270,260,250,240,230,220,210,200,190,180,170,160,150,140,130,120,110,100,90,80,70,60,50,40,150至40,前150至50,前150至75,前150-100,前100至40,前100至50,前100至75,前75-40,前75-50,前300,前250,前200,前150,前140,前130,前120,前110,前100,前90,前80,前75,前70,前65,前60,前55,前50,或前40个氨基酸。

[0106] 在一些实施方案中,截短的N-末端融合伴侣是Fk1B、FrnE或EcpD1。在一些实施方案中,截短的N-末端融合伴侣是Fk1B,其中Fk1B从C-末端截短以除去148,198,210,200,190,180,170,160,150,140,130,120,110,100,90,80,70,60,50,40,30,20,10,5,1,1至210,1至200,1至190,1至180,1至170,1至160,1至150,1至140,1至130,1至120,1至110,1至100,1至90,1至80,1至70,1至60,1至50,1至40,1至30,1至20,1至15,1至10或1至5个氨基酸。在一些实施方案中,截短的N-末端融合伴侣是EcpD,其中EcpD从C-末端截短以除去148,198,210,200,190,180,170,160,150,140,130,120,110,100,90,80,70,60,50,40,30,20,10,5,1,1至210,1至200,1至190,1至180,1至170,1至160,1至150,1至140,1至130,1至120,1至110,1至100,1至90,1至80,1至70,1至60,1至50,1至40,1至30,1至20,1至15,1至10或1至5个氨基酸。在一些实施方案中,截短的N-末端融合伴侣是FrnE,其中FrnE从C-末端截短以除去118,168,190,180,170,160,150,140,130,120,110,100,90,80,70,60,50,40,30,20,10,5,1,1至190,1至180,1至170,1至160,1至150,1至140,1至130,1至120,1至110,1至100,1至90,1至80,1至70,1至60,1至50,1至40,1至30,1至20,1至15,1至10或1至5个氨基酸。

[0107] 在一些实施方案中,N-末端融合伴侣不是 β -半乳糖苷酶。在一些实施方案中,N-末端融合伴侣不是硫氧还蛋白。在一些实施方案中,N-末端融合伴侣既不是 β -半乳糖苷酶也不是硫氧还蛋白。

[0108] 在一些实施方案中,N-末端融合伴侣的分子量为约1kDa,约2kDa,约3kDa,约4kDa,约5kDa,约6kDa,约7kDa,约8kDa,约9kDa,约10kDa,约11kDa,约12kDa,约13kDa,约14kDa,约15kDa,约16kDa,约17kDa,约18kDa,约19kDa,约20kDa,约30kDa,约40kDa,约50kDa,约60kDa,约70kDa,约80kDa,约90kDa,约100kDa,约150kDa,约200kDa,约250kDa,约300kDa,约350kDa,约400kDa,约450kDa,约500kDa或更高。在一些实施方案中,N-末端融合伴侣的分子量为约1至约10kDa,约1至约20kDa,约1至约30kDa,约1至约40kDa,约1至约50kDa,约1至约60kDa,约1至约70kDa,约1至约80kDa,约1至约90kDa,约1至约100kDa,约1kDa至约200kDa,约1kDa至约300kDa,约1kDa至约400kDa,约1kDa至约500kDa,约2至约10kDa,约2至约20kDa,约2至约30kDa,约2至约40kDa,约2至约50kDa,约2至约60kDa,约2至约70kDa,约2至约80kDa,约2至约90kDa,约2至约100kDa,约2kDa至约200kDa,约2kDa至约300kDa,约2kDa至约400kDa,约2kDa至约500kDa,约3至约10kDa,约3至约20kDa,约3至约30kDa,约3至约40kDa,约3至约50kDa,约3至约60kDa,约3至约70kDa,约3至约80kDa,约3至约90kDa,约3至约

100kDa, 约3kDa至约200kDa, 约3kDa至约300kDa, 约3kDa至约400kDa, 或约3kDa至约500kDa。

[0109] 在一些实施方案中, N-末端融合伴侣或截短的N-末端融合伴侣为25或更多个氨基酸长。在一些实施方案中, N-末端融合伴侣为约25至约2000或更多个氨基酸长。在一些实施方案中, N-末端融合伴侣为约或至少约25, 35, 40, 45, 50, 100, 150, 200, 250, 300, 350, 400, 450, 470, 500, 530, 560, 590, 610, 640, 670, 700, 750, 800, 850, 900, 950, 1000, 1200, 1400, 1600, 1800, 2000个氨基酸长。在一些实施方案中, 目标多肽为约25至约2000, 25至约1000, 25至约500, 25至约250, 25至约100, 或25至约50个氨基酸长。

[0110] 目标多肽和重组融合蛋白的相对尺寸

[0111] 目标多肽的产量与完全重组融合蛋白的产量成比例。该比例取决于目标多肽和重组融合蛋白的相对大小(例如, 分子量和/或以氨基酸计的长度)。例如, 减小融合蛋白中N-末端融合伴侣的尺寸将导致产生的融合蛋白的更高比例是目标多肽。在一些实施方案中, 为了最大化目标多肽的产量, 基于其与目标多肽的相对尺寸来选择N-末端融合伴侣。在一些实施方案中, N-末端融合伴侣选择为相对于目标多肽为特定的最小尺寸(例如, MW或以氨基酸计的长度)。在一些实施方案中, 重组融合蛋白设计为使得目标多肽的分子量构成重组融合蛋白分子量的约10%至约50%。在一些实施方案中, 目标多肽的分子量构成重组融合蛋白分子量的约或至少约: 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 35%, 40%, 45%, 50%。在一些实施方案中, 目标多肽的分子量构成重组融合蛋白分子量的约或至少约: 10%至约50%, 11%至约50%, 12%至约50%, 13%至约50%, 14%至约50%, 15%至约50%, 20%至约50%, 25%至约50%, 30%至约50%, 35%至约50%, 40%至约50%, 13%至约40%, 14%至约40%, 15%至约40%, 20%至约40%, 25%至约40%, 30%至约40%, 35%至约40%, 13%至约30%, 14%至约30%, 15%至约30%, 20%至约30%, 25%至约30%, 13%至约25%, 14%至约25%, 15%至约25%或20%至约25%。在一些实施方案中, 目标多肽是hPTH并且目标多肽的分子量构成重组融合蛋白分子量的约14.6%。在一些实施方案中, 目标多肽是hPTH并且目标多肽的分子量构成重组融合蛋白分子量的约13.6%。在一些实施方案中, 目标多肽是hPTH并且目标多肽的分子量构成重组融合蛋白分子量的约27.3%。在一些实施方案中, 目标多肽是met-GCSF并且目标多肽的分子量构成重组融合蛋白分子量的约39%至约72%。在一些实施方案中, 目标多肽是前胰岛素并且目标多肽的分子量构成重组融合蛋白分子量的约20%至约57%。

[0112] 在一些实施方案中, 目标多肽的长度构成重组融合蛋白总长的约10%至约50%。在一些实施方案中, 目标多肽的长度构成重组融合蛋白总长的约或至少约: 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 35%, 40%, 45%, 50%。在一些实施方案中, 目标多肽的长度构成重组融合蛋白总长的约或至少约: 10%至约50%, 11%至约50%, 12%至约50%, 13%至约50%, 14%至约50%, 15%至约50%, 20%至约50%, 25%至约50%, 30%至约50%, 35%至约50%, 40%至约50%, 13%至约40%, 14%至约40%, 15%至约40%, 20%至约40%, 25%至约40%, 30%至约40%, 35%至约40%, 13%至约30%, 14%至约30%, 15%至约30%, 20%至约30%, 25%至约30%, 13%至约25%, 14%至约25%, 15%至约25%或20%至约25%。在一些实施方案中, 目标多肽是hPTH并且目标多肽的长度构成重组融合蛋白总长的

约13.1%。在一些实施方案中,目标多肽是hPTH并且目标多肽的长度构成重组融合蛋白总长的约12.5%。在一些实施方案中,目标多肽是hPTH并且目标多肽的长度构成重组融合蛋白总长的约25.7%。在一些实施方案中,目标多肽是met-GCSF并且目标多肽的长度构成重组融合蛋白总长的约40%至约72%。在一些实施方案中,目标多肽是前胰岛素并且目标多肽的长度构成重组融合蛋白总长的约19%至约56%。

[0113] 目标多肽和N-末端融合伴侣等电点的差异

[0114] 将蛋白质的等电点(pI)定义为蛋白质没有携带净电荷时的pH。已知pI值影响蛋白质在给定pH下的溶解性。在低于其pI的pH下,蛋白质携带净正电荷,并且在高于其pI的pH下,携带净负电荷。可以根据其等电点(总体电荷)分离蛋白。在一些实施方案中,目标多肽的pI和N-末端融合蛋白的pI实质地不同。这可以促进目标多肽从N-末端融合蛋白的纯化。在一些实施方案中,目标多肽的pI比N-末端融合伴侣的高至少两倍。在一些实施方案中,目标多肽的pI比N-末端融合伴侣的高1.5至3倍。在一些实施方案中,目标多肽的pI比N-末端融合伴侣的高1.5,1.6,1.7,1.8,1.9,2,2.1,2.2,2.3,2.4,2.5,2.6,2.7,2.8,2.9或3倍。在一些实施方案中,N-末端融合伴侣的pI为约4,约4.1,约4.2,约4.3,约4.4,约4.5,约4.6,约4.7,约4.8,约4.9或约5。在一些实施方案中,N-末端融合伴侣的pI为约4至约5,约4.1至约4.9,约4.2至约4.8,约4.3至约4.7,约4.4至约4.6。

[0115] 在一些实施方案中,N-末端融合伴侣是表8或18中所列的一个,具有其中所列的pI。在一些实施方案中,C-末端目标多肽是hPTH1-34,具有8.52的pI和4117.65道尔顿的分子量。在一些实施方案中,C-末端目标多肽是Met-GCSF,具有5.66的pI和18801.9道尔顿的分子量。在一些实施方案中,C-末端目标多肽是SEQ ID NO:88中所示的前胰岛素,具有约5.2的pI和9.34KDa的分子量。在一些实施方案中,C-末端目标多肽是SEQ ID NO:89中所示的前胰岛素,具有约6.07的pI和约8.81KDa的分子量。在一些实施方案中,C-末端目标多肽是SEQ ID NO:90中所示的前胰岛素,具有约5.52的pI和约8.75KDa的分子量。在一些实施方案中,C-末端目标多肽是SEQ ID NO:91中所示的前胰岛素,具有约6.07的pI和约7.3KDa的分子量。可以根据文献中描述的和本领域技术人员已知的任何方法来测定蛋白质的pI。

[0116] 伴侣蛋白和蛋白折叠调节子

[0117] 在非天然宿主细胞(异源蛋白对于其不是天然的细胞)中以高产量生产异源蛋白的障碍是细胞常常未充分地配备来产生可溶的和/或活性形式的异源蛋白。尽管蛋白质的初级结构由其氨基酸序列来限定,但二级结构由 α 螺旋或 β 折叠的存在来限定,而三级结构由蛋白质内(例如,蛋白结构域之间)的氨基酸侧链相互作用来限定。在表达异源蛋白时,特别是在大规模生产中,蛋白质本身的二级和三级结构是非常重要的。蛋白质结构中的任何显著变化可以产生功能上无活性的分子,或具有显著降低的生物活性的蛋白质。在许多情况中,宿主细胞表达适当产生活性异源蛋白需要的伴侣蛋白或蛋白折叠调节子(PFM)。然而,通常在需要产生可用的、经济上令人满意的生物技术产物的高水平表达下,细胞常常不能产生足够的一种或多种天然蛋白折叠调节子来加工异源表达的蛋白。

[0118] 在某些表达系统中,异源蛋白的过度生产可以伴随其错误折叠并隔离成不溶性聚集物。在细菌细胞中,将这些聚集物称为包涵体。在某些情况中,可以通过不溶级分的另外的处理来回收加工成包涵体的蛋白质。包涵体中存在的蛋白质通常必须通过多个步骤来纯化,包括变性和复性。用于包涵体蛋白的典型复性方法包括尝试在浓缩的变性剂中溶解聚

集物,随后通过稀释除去变性剂。在这个阶段常常再次形成聚集物。另外的加工增加了成本,不能保证体外重折叠产生生物活性的产物,并且回收的蛋白质可以包括大量的片段杂质。

[0119] 体内蛋白折叠通过分子伴侣蛋白(其通过与折叠中间体的瞬时相互作用促进其他多肽的正确异构化和细胞靶向)和通过折叠酶(其加速沿着折叠途径的速率限制步骤)来辅助。在某些情况中,已经发现伴侣蛋白的超表达提高了易聚集蛋白的可溶蛋白产量(参见 Baneyx, F., 1999, *Curr. Opin. Biotech.* 10:411-421)。与这些伴侣蛋白的细胞内浓度提高相关的有益效果似乎高度依赖于过度产生的蛋白质的性质,并且可能不是对于所有异源蛋白需要相同蛋白折叠调节子的过度表达。蛋白折叠调节子,包括伴侣蛋白、二硫键异构酶和肽酰-脯氨酰顺-反异构酶(PPI酶)是一类存在于所有细胞中的蛋白质,其帮助新生多肽的折叠、解折叠和降解。

[0120] 伴侣蛋白通过结合新生多肽,使其稳定并且使得它们正确折叠来起作用。蛋白质同时具有疏水性和亲水性残基,前者通常暴露于表面上,而后者包埋在结构内,它们在此与其他亲水性残基相互作用而不是与围绕分子的水相互作用。然而,在折叠多肽链中,亲水性残基常常因为蛋白质以部分折叠或错误折叠的状态存在而暴露一段时间。正是在这个时间段中,形成的多肽可以变成永久错误折叠的或与其他错误折叠的蛋白相互作用并在细胞内形成大的聚集物或包涵体。伴侣蛋白通常通过结合部分折叠链的疏水区并完全防止它们错误折叠或其他蛋白聚集来起作用。伴侣蛋白甚至可以结合包涵体中的蛋白质并使其解聚。折叠调节子的GroES/EL、DnaKJ、Clp、Hsp90和SecB家族全部是具有伴侣蛋白样活性的蛋白质的实例。

[0121] 二硫键异构酶是另一种重要类型的折叠调节子。这些蛋白催化非常特定的一组反应以帮助折叠多肽形成正确的蛋白质内二硫键。具有超过两个半胱氨酸的任何蛋白具有在错误残基之间形成二硫键的风险。二硫键形成家族由Dsb蛋白组成,其在周质的非还原环境中催化二硫键的形成。当周质多肽错误折叠时,二硫键异构酶,DsbC能够重排二硫键并允许蛋白以正确的连接重新形成。

[0122] Fk1B和FrnE蛋白属于折叠调节子的肽酰-脯氨酰顺-反异构酶家族。这是一类催化寡肽中脯氨酸亚氨基酸肽键的顺-反异构的酶。脯氨酸在氨基酸中是独特的,因为紧接其前方的肽键可以采取顺式或反式构象。对于所有其他氨基酸,由于位阻,这是不利的。肽酰-脯氨酰顺-反异构酶(PPI酶)催化这个键从一个形式转变成另一个形式。这种异构化可以加速和/或帮助蛋白质折叠、重折叠、亚基的组装以及在细胞中的运输。

[0123] 除了似乎以非特异性方式与蛋白质相互作用的通用伴侣蛋白外,还存在帮助特定靶标折叠的伴侣蛋白。这些蛋白质特异性伴侣蛋白与其靶标形成复合物,从而防止聚集和降解并留出它们装配成多亚基结构的时间。PapD伴侣蛋白是一个实例(描述于Lombardo等, 1997, *Escherichia coli* PapD中,见Guidebook to Molecular Chaperones and Protein-Folding Catalyst, Gething M-J编辑, Oxford University Press Inc., New York:463-465,按引用并入文本中)。

[0124] 折叠调节子包括,例如,HSP70蛋白、HSP110/SSE蛋白、HSP40 (DnaJ-相关) 蛋白、GRPE-样蛋白、HSP90蛋白、CPN60和CPN10蛋白、细胞溶质伴侣、HSP100蛋白、小HSP、钙联蛋白和钙网蛋白、PDI和硫氧还蛋白相关蛋白、肽酰-脯氨酰异构酶、亲环蛋白PPI酶、FK-506结合

蛋白、细小蛋白 (parvulin) PPI酶、单独伴侣、蛋白特异性伴侣蛋白或分子内伴侣蛋白。折叠调节子一般地描述于“Guidebook to Molecular Chaperones and Protein-Folding Catalysts (分子伴侣蛋白和蛋白折叠催化剂指南”, 1997, 编辑M.Gething, 墨尔本大学, 澳大利亚中, 按引用并入本文中。

[0125] 大肠杆菌细胞质中最佳表征的分子伴侣蛋白是ATP-依赖性DnaK-DnaJ-GrpE和GroEL-GroES系统。在大肠杆菌中, 折叠调节子/伴侣蛋白的网络包括Hsp70家族。主要Hsp70伴侣蛋白, DnaK, 有效防止蛋白质聚集并支持受损蛋白的重折叠。热休克蛋白引入蛋白聚集物中可有助于解聚。基于体外研究和同源性考虑, 已经提出在大肠杆菌中作为分子伴侣蛋白发挥作用的多种另外的胞质蛋白。这些包括C1pB、HtpG和IbpA/B, 其与DnaK-DnaJ-GrpE和GroEL-GroES一样, 是属于应激调节子的热休克蛋白 (Hsp)。

[0126] 荧光假单胞菌DnaJ-样蛋白是属于DnaJ/Hsp40蛋白家族的分子伴侣蛋白, 其特征在于它们高度保守的J-结构域。J-结构域, 其是70个氨基酸的区域, 位于DnaJ蛋白的C末端。N末端具有跨膜 (TM) 结构域, 其促进插入膜中。A-结构域将TM结构域与J-结构域分开。通过与另一种伴侣蛋白DnaK (作为共同伴侣蛋白) 相互作用, DnaJ家族中的蛋白质在蛋白折叠中起着关键作用。高度保守的J-结构域是DnaJ蛋白和DnaK蛋白之间的相互作用位点。据认为I型DnaJ蛋白是真正的DnaJ蛋白, 而II和III型通常称为DnaJ-样蛋白。还已知DnaJ-样蛋白以DnaK依赖性和非DnaK依赖性的两种方式, 通过防止应激变性蛋白的聚集和通过使蛋白解聚积极地参与对高渗和热休克的反应。

[0127] X-Pro键的反式构象在新生蛋白链中是能量有利的; 然而, 发现全部脯氨酰胺键的大约5%在天然蛋白中处于顺式构象中。X-Pro键的顺式-反式异构化在许多肽的折叠中是速率限制的, 并且在体内通过肽酰脯氨酰胺顺/反异构酶 (PPI酶) 来催化。迄今为止, 已经在大肠杆菌中鉴定出三种胞质PPI酶, SlyD、SlpA和引发因子 (TF)。TF, 与50S核糖体亚基相关的48kDa蛋白, 已经推定为与大肠杆菌中的伴侣蛋白合作来确保新合成蛋白质的正确折叠。至少五种蛋白质 (硫氧还蛋白1和2, 以及谷氧还蛋白1、2和3, 分别是trxA、trxC、grxA、grxB和grxC基因的产物) 参与在胞质酶中短暂出现的二硫桥的还原。因此, N-末端融合伴侣可以是允许正确的二硫键形成的二硫键形成蛋白质或伴侣蛋白。

[0128] 本发明的方法中有用的折叠调节子的实例显示于表1中。RXF编号是指开放阅读框。美国专利申请公开号No.200/0269070和2010/0137162 (两者发明名称均为“Method for Rapidly Screening Microbial Hosts to Identify Certain Strains with Improved Yield and/or Quality in the Expression of Heterologous Proteins”, 将其全部按引用并入本文中) 公开了表1中所列蛋白质的开放阅读框序列。蛋白酶和折叠调节子也提供于美国专利No.8,603,824, “Process for improved protein expression by strain engineering”的表A至F中, 将其全部按引用并入本文中。

[0129] 表1. 荧光假单胞菌折叠调节子

[0130]	ORF ID	基因	功能	家族	位置
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[0131]

GroES/EL				
RXF02095.1	groES	伴侣蛋白	Hsp10	细胞质
RXF06767.1:: Rxf02090	groEL	伴侣蛋白	Hsp60	细胞质
RXF01748.1	ibpA	小热休克蛋白 (sHSP) IbpA PA3126 ; 作为用于 GroESL 折叠的支架	Hsp20	细胞质
RXF03385.1	hscB	伴侣蛋白 hscB	Hsp20	细胞质
Hsp70 (DnaK/J)				
RXF05399.1	dnaK	伴侣蛋白	Hsp70	周质
RXF06954.1	dnaK	伴侣蛋白	Hsp70	细胞质
RXF03376.1	hscA	伴侣蛋白	Hsp70	细胞质
RXF03987.2	cbpA	弯曲的 dna-结合蛋白, dnaJ 样活性	Hsp40	细胞质
RXF05406.2	dnaJ	伴侣蛋白 dnaJ	Hsp40	细胞质
RXF03346.2	dnaJ	分子伴侣蛋白 (DnaJ 家 族)	Hsp40	非分泌 性的
RXF05413.1	grpE	热休克蛋白 GrpE PA4762	GrpE	细胞质
Hsp100 (Clp/Hsl)				
RXF04587.1	clpA	atp-依赖性 clp 蛋白酶 atp-结合亚基	Hsp100	细胞质
RXF08347.1	clpB	ClpB 蛋白	Hsp100	细胞质
RXF04654.2	clpX	atp-依赖性 clp 蛋白酶 atp-结合亚基	Hsp100	细胞质
RXF04663.1	clpP	atp-依赖性 Clp 蛋白酶	MEROPS	细胞质

[0132]

		蛋白水解亚基 (ec 3.4.21.92)	肽酶家族 S14	
RXF01957.2	hslU	atp-依赖性 hsl 蛋白酶 atp-结合亚基	Hsp100	细胞质
RXF01961.2	hslV	atp-依赖性 hsl 蛋白酶 蛋 白水解亚基	MEROPS 肽酶 超家族 T1B	细胞质
Hsp33				
RXF04254.2	yrfl	33 kDa 伴侣蛋白(热休克 蛋白 33 同源物 (HSP33)	Hsp33	细胞质
Hsp90				
RXF05455.2	htpG	伴侣蛋白 htpG	Hsp90	细胞质
SecB				
RXF02231.1	secB	分泌特异性伴侣蛋白 SecB	SecB	非分泌 性的
二硫键异构酶				
RXF07017.2	dsbA	二硫化物异构酶	DSBA 氧化-还 原酶	细胞质
RXF08657.2	frnE	二硫化物异构酶	DSBA 氧化-还 原酶	细胞质
RXF01002.1	dsbA 同 源 物	二硫化物异构酶	DSBA 氧化-还 原酶/硫氧还蛋 白	周质
RXF03307.1	dsbC	二硫化物异构酶	谷氧还蛋白/ 硫氧还蛋白	周质
RXF04890.2	dsbG	二硫化物异构酶	谷氧还蛋白/ 硫氧还蛋白	周质
RXF03204.1	dsbB	二硫键形成蛋白 B (二硫 化物氧化还原酶)	DSBA 氧化-还 原酶	周质

[0133]

RXF04886.2	dsbD	硫醇:二硫化物互换蛋白 dsbD	DSBA 氧化-还原酶	周质
肽酰-脯氨酰顺-反异构酶				
RXF03768.1	ppiA	肽酰-脯氨酰顺-反异构酶 A (ec 5.2.1.8)	PPI 酶: 亲环蛋白型	周质
RXF05345.2	ppiB	肽酰-脯氨酰顺-反异构酶 B	PPI 酶: 亲环蛋白型	细胞质
RXF06034.2	fkIB	肽酰-脯氨酰顺-反异构酶 FkIB	PPI 酶: FKBP 型	外膜
RXF06591.1	fkIB/ fkbP	fk506 结合蛋白肽酰-脯氨酰顺-反异构酶 (EC 5.2.1.8)	PPI 酶: FKBP 型	周质
RXF05753.2	fkIB/ fkbP	肽酰-脯氨酰顺-反异构酶(ec 5.2.1.8)	PPI 酶: FKBP 型	外膜
RXF01833.2	slyD	肽酰-脯氨酰顺-反异构酶 SlyD	PPI 酶: FKBP 型	非分泌性的
RXF04655.2	tig	引发因子, ppi 酶 (ec 5.2.1.8)	PPI 酶: FKBP 型	细胞周
RXF05385.1	yaad	可能的 FKBP-型 16 kDa 肽酰-脯氨酰顺-反异构酶(EC 5.2.1.8) (PPi 酶) (旋转异构酶(Rotamase)).	PPI 酶: FKBP 型	非分泌性的
RXF00271.1		肽酰-脯氨酰顺-反异构酶(ec 5.2.1.8)	PPI 酶: FKBP 型	非分泌性的
菌毛(Pili)装配伴侣蛋白(papD-样)				
RXF06068.1	cup	伴侣蛋白 cup	菌毛装配 papD	周质
RXF05719.1	ecpD	伴侣蛋白 ecpD	菌毛装配	信号肽

[0134]

			papD	
RXF05319.1	ecpD	Hnr 蛋白	菌毛装配伴侣蛋白	周质
RXF03406.2	ecpD; csuC	伴侣蛋白 ecpD	菌毛装配 papD	信号肽
RXF04296.1	ecpD; cup	伴侣蛋白 ecpD	菌毛装配 papD	周质
RXF04553.1	ecpD; cup	伴侣蛋白 ecpD	菌毛装配 papD	周质
RXF04554.2	ecpD; cup	伴侣蛋白 ecpD	菌毛装配 papD	周质
RXF05310.2	ecpD; cup	伴侣蛋白 ecpD	菌毛装配 papD	周质
RXF05304.1	ecpD; cup	伴侣蛋白 ecpD	菌毛装配 papD	周质
RXF05073.1	gltF	革兰氏阴性菌毛装配伴侣蛋白 周质功能	菌毛装配 papD	信号肽
II 型分泌复合物				
RXF05445.1	YacJ	组氨醇-磷酸氨基转移酶 (ec2.6.1.9)	II 类吡哆醛-磷酸-依赖性氨基转移酶家族。组氨醇-磷酸氨基-转移酶超家族	膜
RXF05426.1	SecD	蛋白转位酶亚基 secD	II 型分泌复合物	膜
RXF05432.1	SecF	蛋白转位酶亚基 secF	II 型分泌复合物	膜

二硫键还原酶				
[0135]	RXF08122.2	trxC	硫氧还蛋白 2	二硫键还原酶 细胞质
	RXF06751.1	Gor	谷胱甘肽还原酶 (EC 1.8.1.7) (GR) (GRase) PA2025	二硫键还原酶 细胞质
	RXF00922.1	gshA	谷氨酸--半胱氨酸连接酶(ec6.3.2.2) PA5203	二硫键还原酶 细胞质

[0136] 接头

[0137] 本发明的重组融合蛋白在N-末端融合伴侣和C-末端目标多肽之间含有接头。在一些实施方案中,接头包含被裂解酶(即,在内部切割蛋白质的蛋白水解酶)识别的切割位点。在一些实施方案中,接头在切割位点处的切割将目标多肽与N-末端融合伴侣分离。蛋白水解酶可以是本领域已知的或文献中描述的任何蛋白酶,例如,在PCT公开号No.WO 2003/010204,“Process for Preparing Polypeptides of Interest from Fusion Polypeptides”,美国专利No.5,750,374,“Process for Producing Hydrophobic Polypeptides and Proteins,and Fusion Proteins for Use in Producing Same”和美国专利No.5,935,824中所述的,将每篇全部按引用并入本文中。

[0138] 在一些实施方案中,接头包含通过例如丝氨酸蛋白酶、苏氨酸蛋白酶、半胱氨酸蛋白酶、天冬氨酸蛋白酶、谷氨酸蛋白酶、金属蛋白酶、天冬酰胺蛋白酶、混合蛋白酶或未知催化类型的蛋白酶切割的切割位点。在一些实施方案中,丝氨酸蛋白酶例如是胰蛋白酶、胰凝乳蛋白酶、内蛋白酶Arg-C、内蛋白酶Glu-C、内蛋白酶Lys-C、弹性蛋白酶、蛋白酶K、枯草杆菌蛋白酶、羧基肽酶P、羧基肽酶Y、酰基氨基酸释放酶。在一些实施方案中,金属蛋白酶是例如内蛋白酶Asp-N、嗜热菌蛋白酶、羧基肽酶A、羧基肽酶B。在一些实施方案中,半胱氨酸蛋白酶是例如木瓜蛋白酶、梭菌蛋白酶、组织蛋白酶C或焦谷氨酸氨基肽酶。在一些实施方案中,天冬氨酸蛋白酶是例如胃蛋白酶、胃促胰酶、组织蛋白酶D。在一些实施方案中,谷氨酸蛋白酶是例如scytalidoglutamic肽酶。在一些实施方案中,天冬酰胺蛋白酶是例如诺达病毒肽裂解酶、含内含肽叶绿体ATP依赖性肽裂解酶、含内含肽复制DNA螺旋酶前体或1型呼肠孤病毒外壳蛋白。在一些实施方案中,未知催化剂类型的蛋白酶例如是胶原蛋白酶、蛋白P5胞壁质内肽酶、同型多聚肽酶、微菌素-加工肽酶1或Dop异肽酶。

[0139] 在一些实施方案中,接头包含以下酶的切割位点:无色肽酶、氨基肽酶、安克洛酶(Ancrod)、血管紧张素转化酶、菠萝蛋白酶、钙蛋白酶(Calpain)、钙蛋白酶I、钙蛋白酶II、羧基肽酶A、羧基肽酶B、羧基肽酶G、羧基肽酶P、羧基肽酶W、羧基肽酶Y、半胱天冬酶(caspase)(通用)、半胱天冬酶1、半胱天冬酶2、半胱天冬酶3、半胱天冬酶4、半胱天冬酶5、半胱天冬酶6、半胱天冬酶7、半胱天冬酶8、半胱天冬酶9、半胱天冬酶10、半胱天冬酶11、半胱天冬酶12、半胱天冬酶13、组织蛋白酶B、组织蛋白酶C、组织蛋白酶D、组织蛋白酶E、组织蛋白酶G、组织蛋白酶H、组织蛋白酶L、木瓜凝乳蛋白酶、胃促胰酶、胰凝乳蛋白酶、 α -梭菌蛋白酶、胶原蛋白酶、补体C1r、补体C1s、补体因子D、补体因子I、黄瓜素、二肽酰肽酶IV、弹性蛋白酶、白细胞、弹性蛋白酶、内蛋白酶Arg-C、内蛋白酶Asp-N、内蛋白酶Glu-C、内蛋白酶

Lys-C、肠激酶、因子Xa、无花果蛋白酶、弗林蛋白酶、颗粒酶A、颗粒酶B、HIV蛋白酶、IG酶、组织激肽释放酶、亮氨酸氨基肽酶(通用)、亮氨酸氨基肽酶,细胞溶质、亮氨酸氨基肽酶,微粒体、基质金属蛋白酶、甲硫氨酸氨基肽酶、中性蛋白酶、木瓜蛋白酶、胃蛋白酶、血纤维蛋白溶酶、脯氨酰氨基酸二肽酶、链霉蛋白酶E、前列腺特异性抗原、来自灰色链霉菌的嗜碱性蛋白酶、来自曲霉的蛋白酶、来自佐氏曲霉(*Aspergillus saitoi*)的蛋白酶、来自酱油曲霉的蛋白酶、蛋白酶(地衣芽孢杆菌)(碱性)、蛋白酶(地衣芽孢杆菌)(碱性蛋白酶(Alcalase))、来自多粘芽孢杆菌(*Bacillus polymyxa*)的蛋白酶、来自芽孢杆菌的蛋白酶(益瑞蛋白酶(Esperease))、来自根霉的蛋白酶、蛋白酶S、蛋白酶体、来自米曲霉的蛋白酶、蛋白酶3、蛋白酶A、蛋白酶K、蛋白C、焦谷氨酸氨基肽酶、肾素、凝乳酶(rennin)、链激酶、枯草杆菌蛋白酶、嗜热菌蛋白酶、凝血酶、组织血纤维蛋白酶原激活剂、胰蛋白酶、类胰蛋白酶或尿激酶。在一些实施方案中,接头包含肠激酶、因子Xa或弗林蛋白酶识别的切割位点。在一些实施方案中,接头包含肠激酶或胰蛋白酶识别的切割位点。在一些实施方案中,接头包含牛肠激酶识别的切割位点。本发明的方法中有用的这些和其他蛋白酶,及其切割识别位点,是本领域已知的并且描述于文献中,例如,Harlow和Lane,ANTIBODIES:A LABORATORY MANUAL,Cold Spring Harbor Laboratory Pre,Cold Spring Harbor,N.Y. (1988);Walsh,PROTEINS:BIOCHEMISTRY AND BIOTECHNOLOGY,John Wiley&Sons,Ltd.,West Sussex,England (2002),按引用并入本文中。

[0140] 在一些实施方案中,接头包含亲和性标签。亲和性标签是可帮助蛋白质纯化的肽序列。亲和性标签与蛋白质融合以促进蛋白质使用亲和性技术从粗制生物源的纯化。按照需要,可以使用本领域已知的任何合适的亲和性标签。在一些实施方案中,本发明中使用的亲和性标签是例如几丁质结合蛋白、麦芽糖结合蛋白或谷胱甘肽-S-转移酶蛋白、聚组氨酸、FLAG标签(SEQ ID NO:229)、钙调蛋白标签(SEQ ID NO:230)、Myc标签、BP标签、HA-标签(SEQ ID NO:231)、E-标签(SEQ ID NO:232)、S-标签(SEQ ID NO:233)、SBP标签(SEQ ID NO:234)、Softag 1、Softag 3(SEQ ID NO:235)、V5标签(SEQ ID NO:236)、Xpress标签、绿色荧光蛋白、Nus标签、Strep标签、硫氧还蛋白标签、MBP标签、VSV标签(SEQ ID NO:237)或Avi标签。

[0141] 可以通过化学剂或通过酶方式除去亲和性标签,如蛋白质水解。在蛋白质纯化中使用亲和性标签的方法描述于文献中,例如,Lichty等,2005,“Comparison of affinity tags for protein purification”,Protein Expression and Purification 41:98-105。在本发明的接头中有用的其他亲和性标签是本领域已知的并且描述于文献中,例如,以上提及的美国专利No.5,750,374及Terpe K.,2003,“Overview of Tag Protein Fusions: from molecular and biochemical fundamentals to commercial systems”,Applied Microbiology and Biotechnology(60):523-533,两者都全部按引用并入。

[0142] 在一些实施方案中,接头是4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,30,35,40,45,50或更多个氨基酸长。在一些实施方案中,接头是4至50,4至45,4至40,4至35,4至30,4至25,4至20,4至15,4至10,5至50,5至45,5至40,5至35,5至30,5至25,5至20,5至15,5至10,10至50,10至45,10至40,10至35,10至30,10至25,10至20,10至15,15至50,15至45,15至40,15至35,15至30,15至25,15至20,20至50,20至45,20至40,20至35,20至30或20至25个氨基酸长。在一些实施方案中,接头是18个氨基酸长。在一些实施方

案中,接头是19个氨基酸长。

[0143] 在一些实施方案中,接头包括多个甘氨酸残基。在一些实施方案中,接头包括1,2,3,4,5,6,7,8或更多个甘氨酸残基。在一些实施方案中,接头包括1-8,1-7,1-6,1-5或1-4个甘氨酸残基。在一些实施方案中,甘氨酸残基是连续的。在一些实施方案中,接头含有至少一个丝氨酸残基。在一些实施方案中,甘氨酸和/或丝氨酸残基构成间隔体。在一些实施方案中,间隔体是具有10个氨基酸的(G4S)₂间隔体,如SEQ ID NO:59中所示。在一些实施方案中,间隔体是(G4S)₁、(G4S)₂、(G4S)₃、(G4S)₄或(G4S)₅间隔体。在一些实施方案中,接头含有六个组氨酸残基,或His-标签。在一些实施方案中,接头包括肠激酶切割位点,例如,如SEQ ID NO:13(DDDDK)所示的。在一些实施方案中,重组融合蛋白包含表2中所列的如SEQ ID NO:9至12或226任一个所示的接头。SEQ ID NO:9中的肠激酶切割位点是加下划线的。SEQ ID NO:9至12和226每个中斜体的是聚组氨酸亲和性标签。在一些实施方案中,重组融合蛋白包含对应于SEQ ID NO:9的接头。

[0144] 表2:接头序列

SEQ ID NO:	氨基酸序列
9	GGGSGGGGHHHHHHDDDDK
10	GGGSGGGGHHHHHHHRKR
11	GGGSGGGGHHHHHHHRRR
12	GGGSGGGGHHHHHHHLVPR
226	GGGSGGGGSHHHHHHR

[0146] 表达载体

[0147] 在一些实施方案中,将编码重组融合蛋白的基因片段引入合适的表达质粒中来产生用于表达重组融合蛋白的表达载体。表达载体可以例如是质粒。在一些实施方案中,编码重组融合蛋白序列的质粒可以包含选择标记,并且使维持质粒的宿主细胞在选择性条件下生长。在一些实施方案中,质粒不包含选择标记。在一些实施方案中,表达载体可以整合至宿主细胞基因组中。在一些实施方案中,表达载体编码与接头和蛋白融合的hPTH 1-34,所述蛋白可以将表达的融合蛋白指引至细胞质。在一些实施方案中,表达载体编码与接头和蛋白融合的hPTH 1-34,所述蛋白可以将表达的融合蛋白指引至周质。在一些实施方案中,表达载体编码与接头和荧光假单胞菌DnaJ-样蛋白融合的hPTH 1-34。在一些实施方案中,表达载体编码与接头和荧光假单胞菌Fk1B蛋白融合的hPTH 1-34。

[0148] 本文中的序列表中提供了编码PTH 1-34融合蛋白的核苷酸序列的实例。编码包含DnaJ-样蛋白N-末端融合伴侣的融合蛋白的核苷酸序列的实例是指定的基因ID 126203 (SEQ ID NO:52),对应于针对荧光假单胞菌优化的编码序列。指定基因ID 126206的序列 (SEQ ID NO:53)对应于与优化的接头和PTH 1-34编码序列融合的天然荧光假单胞菌DnaJ编码序列。基因序列126203和126206是分别存在于表达质粒p708-001和p708-004中的那些。编码包含Fk1B N-末端融合伴侣的融合蛋白的核苷酸序列的实例是指定的基因ID 126204 (SEQ ID NO:54),对应于针对荧光假单胞菌优化的编码序列。基因ID 126207 (SEQ ID NO:55)对应于与优化的接头和PTH 1-34编码序列融合的天然荧光假单胞菌Fk1B编码序列。基因序列126204和126207分别是表达质粒p708-002和p708-005中存在的那些。编码包含FrnE N-末端融合伴侣的融合蛋白的核苷酸序列的实例是指定的基因ID 126205 (SEQ ID

N0:56), 对应于针对荧光假单胞菌优化的编码序列。指定基因ID 126208的序列 (SEQ ID N0:57) 对应于与优化的接头和PTH 1-34编码序列融合的天然荧光假单胞菌FrnE编码序列。基因序列126205和126208是分别存在于表达质粒p708-003和p708-006中的那些。

[0149] 密码子优化

[0150] 本发明考虑了针对融合蛋白和/或其每个单独的组分, 包括已经针对在所使用的宿主细胞中的表达进行优化的任何序列, 使用任何合适的编码序列。优化密码子以提高在细菌宿主中表达的方法是本领域已知的并且在文献中有描述。例如, 针对在假单胞菌宿主菌株中表达的密码子优化描述于例如美国专利申请公开No. 2007/0292918, “Codon Optimization Method”中, 将其全部按引用并入。针对在大肠杆菌中表达的密码子优化描述于例如Welch等, 2009, PLoS One, “Design Parameters to Control Synthetic Gene Expression in Escherichia coli, 4(9):e7002中, 按引用并入本文中。本文中提供了用于融合蛋白组分的编码序列的非限制性实例, 然而, 应理解可以根据本领域技术人员公知的方法, 按照需要产生任何合适的序列。

[0151] 表达系统

[0152] 基于本文中的教导, 本领域技术人员可以确定对于根据本发明的方法产生目标多肽有用的合适细菌表达系统。在一些实施方案中, 包含编码含目标多肽的重组融合蛋白的核苷酸序列的表达构建体作为诱导型表达载体的部分提供。在一些实施方案中, 培养已经用表达载体转化的宿主细胞, 并且诱导从表达载体表达融合蛋白。表达载体可以例如是质粒。在一些实施方案中, 表达载体是编码进一步包含选择标记的重组融合蛋白编码序列的质粒, 并且将宿主细胞在允许质粒维持的选择性条件下生长。在一些实施方案中, 将表达构建体整合至宿主细胞基因组中。在一些实施方案中, 表达构建体编码与分泌信号融合的重组融合蛋白, 所述分泌信号可以将重组融合蛋白指引至周质中。

[0153] 在本发明的方法中有用的宿主细胞 (包括假单胞菌属宿主细胞) 中表达异源蛋白 (包括有用的调控序列 (例如, 启动子、分泌前导序列和核糖体结合位点)) 的方法描述于例如美国专利申请公开No. 2008/0269070和2010/0137162, 美国专利申请公开No. 2006/0040352, “Expression of Mammalian Proteins in Pseudomonas fluorescens”和美国专利No. 8,603,824中, 其各自全部按引用并入。这些公开还描述了在实施本发明的方法中有用的细菌宿主菌株, 其已经工程化来超表达折叠调节子或其中已经引入蛋白酶突变, 例如, 来消除、灭活或降低蛋白酶的活性, 从而提高异源蛋白表达。前导序列详细描述于美国专利No. 7,618,799, “Bacterial leader sequences for increased expression”和美国专利No. 7,985,564, “Expression systems with Sec-system Secretion”中, 两篇都全部按引用并入本文中, 以及之前提及的美国专利申请公开No. 2010/0137162中。

[0154] 根据本发明使用的启动子可以是组成型启动子或调控型启动子。诱导型启动子的实例包括源自lac启动子家族的那些 (即, lacZ启动子), 例如, 美国专利No. 4,551,433, “Microbial Hybrid Promoters”中描述的tac和trc启动子, 按引用并入, 以及Ptac16、Ptac17、PtacII、PlacUV5和T7lac启动子。在一些实施方案中, 启动子不是源自宿主细胞生物体。在一些实施方案中, 启动子源自大肠杆菌生物体。在一些实施方案中, lac启动子用于调节重组融合蛋白从质粒的表达。在lac启动子衍生物或家族成员的情况中, 例如, tac启动子, 诱导剂是IPTG (异丙基- β -D-1-硫代半乳糖苷, “异丙基硫代半乳糖苷”)。在一些实

施方案中,根据本领域已知的和文献(例如,美国专利公开No.2006/0040352)中所述的方法,将IPTG加入宿主细胞培养物中以诱导重组融合蛋白从假单胞菌属宿主细胞中的lac启动子表达。

[0155] 在根据本发明的表达系统中有用的非-lac启动子的实例包括 P_R (通过高温诱导)、 P_L (通过高温诱导)、 P_m (通过烷基-或卤素-苯甲酸酯诱导)、 P_u (通过烷基-或卤素-甲苯诱导)或 P_{sal} (通过水杨酸酯诱导),例如,描述于J.Sanchez-Romero&V.De Lorenzo(1999)Manual of Industrial Microbiology and Biotechnology (A.Demain&J.Davies编辑) pp.460-74 (ASM Press, Washington, D.C.); H.Schweizer(2001)Current Opinion in Biotechnology, 12:439-445;和R.Slater&R.Williams(2000)Molecular Biology and Biotechnology (J.Walker&R.Rapley编辑) pp.125-54(The Royal Society of Chemistry, Cambridge, UK)中。具有对于选定细菌宿主细胞天然的启动子的核苷酸序列的启动子也可以用于控制编码目标多肽的表达构建体的表达,例如,假单胞菌邻氨基苯甲酸或苯甲酸操纵子启动子(Pant, Pben)。也可以使用串联启动子,其中将超过一个启动子与另一个共价连接,不管序列相同或不同(例如, Pant-Pben串联启动子(启动子间杂合)或Plac-Plac串联启动子),源自相同或不同的生物体。在一些实施方案中,启动子是Pmt1,如例如美国专利No.7,476,532和8,017,355中所述的,两者发明名称均为“Mannitol induced promoter systems in bacterial host cells”,将其全部按引用并入。

[0156] 调控型(诱导型)启动子利用启动子调控蛋白以控制该启动子是其一部分的基因的转录。在本文中使用的调控型启动子的情况中,相应的启动子调控蛋白也将是根据本发明的表达系统的部分。启动子调控蛋白的实例包括:激活剂蛋白,例如,大肠杆菌分解代谢产物激活剂蛋白, MalT蛋白; AraC家族转录激活剂;阻遏蛋白,例如,大肠杆菌Lac蛋白;和双重功能调控蛋白,例如,大肠杆菌NagC蛋白。许多调控型启动子/启动子-调控蛋白对是本领域已知的。

[0157] 启动子调控蛋白与效应化合物相互作用,即,可逆地或不可逆地与调控蛋白结合的化合物,使得该蛋白质能够释放或结合在启动子控制下的基因的至少一个DNA转录调控区,由此允许或阻断转录酶启动基因转录的作用。效应化合物分类为诱导剂或共同阻遏剂,并且这些化合物包括天然效应化合物和安慰诱导剂化合物。许多调控型启动子/启动子-调控蛋白/效应化合物三联组是本领域已知的。尽管效应化合物可以在细胞培养或发酵的整个过程中使用,但在其中使用调控型启动子的优选实施方案中,在所需量或密度的宿主细胞生物量生长后,将合适的效应化合物加入培养物中以直接或间接导致编码目标蛋白质或多肽的所需基因的表达。

[0158] 在其中利用lac家族启动子的实施方案中, lacI基因也可以存在于系统中。lacI基因,其通常是组成型表达的基因,编码Lac阻遏蛋白LacI蛋白,其结合lac家族启动子的lac操作子。因此,在利用lac家族启动子的情况中,也可以包括lac基因并在表达系统中表达。

[0159] 其他调控元件

[0160] 在一些实施方案中,其他调控元件存在于编码重组融合蛋白的表达构建体中。在一些实施方案中,可溶性重组融合蛋白在生产过程中存在于细胞的细胞质或周质中。用于靶向融合蛋白的分泌前导序列描述于本文的别处。在一些实施方案中,本发明的表达构建体编码与分泌前导序列融合的重组融合蛋白,所述分泌前导序列可以将重组融合蛋白转运

至假单胞菌细胞的细胞质。在一些实施方案中,表达构建体编码与分泌前导序列融合的重组融合蛋白,所述分泌前导序列可以将重组融合蛋白转运至假单胞菌细胞的周质。在一些实施方案中,分泌前导序列从重组融合蛋白切除。

[0161] 其他元件包括,但不限于,转录增强子序列、翻译增强子序列、其他启动子、激活子、翻译启示和停止信号、转录终止子、顺反子调控子、多顺反子调控子、标签序列,如核苷酸序列“标签”和“标签”多肽编码序列,其有助于表达多肽的鉴定、分离、纯化和/或分开,如之前所述的。在一些实施方案中,除了蛋白质编码序列外,表达构建体包括与其可操作地连接的以下任一个调控元件:启动子、核糖体结合位点(RBS)、转录终止子以及翻译起始和停止信号。根据之前提及的例如美国专利申请公开No. 2008/0269070和2010/0137162,可以从可在表达系统中用作宿主细胞的任何物种获得有用的RBS。许多特异性的和多种共有RBS是已知的,例如,D.Frishman等, *Gene* 234 (2): 257-65 (1999年7月8日); 和B.E.Suzek等, *Bioinformatics* 17 (12): 1123-30 (2011年12月) 中描述和提及的那些,按引用并入。此外,可以使用天然的或合成的RBS,例如,EP 0207459 (合成的RBS); O. Ikehata等, *Eur. J. Biochem.* 181 (3): 563-70 (1989) 中描述的那些。在一些实施方案中,将“Hi”核糖体结合位点,aggagg, (SEQ ID NO:60) 用于构建体中。核糖体结合位点,包括RBS和翻译起始密码子之间的间隔的优化,描述于文献中,例如,Chen等,1994, “Determination of the optimal aligned spacing between the Shine-Dalgarno sequence and the translation initiation codon of *Escherichia coli* mRNAs”, *Nucleic Acids Research* 22 (23): 4953-4957 和 Ma等,2002, “Correlations between Shine-Dalgarno Sequences and Gene Features Such as Predicted Expression Levels and Operon Structures”, *J. Bact.* 184 (20): 5733-45, 按引用并入。

[0162] 本发明中有用的方法、载体以及翻译和转录元件,和其他元件的其它实例是本领域公知的并且描述于例如:Gilroy等的美国专利No. 5,055,294 和 Gilroy等的美国专利No. 5,128,130; Rammner等的美国专利No. 5,281,532; Barnes等的美国专利No. 4,695,455 和 4,861,595; Gray等的美国专利No. 4,755,465; 和 Wilcox等的美国专利No. 5,169,760 中,全部按引用并入,以及在此按引用并入的其他出版物中。

[0163] 分泌前导序列

[0164] 在一些实施方案中,将分泌信号或前导序列编码序列与编码重组融合蛋白的序列的N-末端融合。分泌信号序列的使用可以提高细菌中重组蛋白的产生。另外,许多类型的蛋白需要使用已知方法不能有效实现的次级修饰。分泌前导序列的使用通过从细胞内环境分泌蛋白质可以提高正确折叠的蛋白质的收获。在革兰氏阴性细菌中,从细胞质分泌的蛋白质可以终止于周质空间中,连接外膜或在胞外培养液中。这些方法也避免了包涵体的形成。蛋白质分泌至周质空间中还具有促进正确二硫键形成的作用 (Bardwell等,1994, *Phosphate Microorg.* 第45章,270-5 和 Manoil,2000, *Methods in Enzymol.* 326:35-47)。重组蛋白分泌的其他益处包括更有效的蛋白质分离、蛋白质的正确折叠和二硫键形成,导致产量提高,所述产量通过例如活性形式的蛋白质的百分比、减少的包涵体形成和降低的对宿主细胞的毒性及增加的可溶性形式重组蛋白质的百分比来表示。用于将目标蛋白质排出至培养基中的潜能还可潜在地促进用于蛋白质生产的连续培养,而不是分批培养。

[0165] 在一些实施方案中,将重组融合蛋白或目标多肽靶向于宿主细胞的周质或靶向到

胞外空间中。在一些实施方案中,表达载体进一步包含编码分泌信号多肽的核苷酸序列,其可操作地连接编码重组融合蛋白或目标多肽的核苷酸序列。

[0166] 因此,在一个实施方案中,重组融合蛋白包含分泌信号、N-末端融合伴侣、接头和目标多肽,其中分泌信号处于融合伴侣的N-末端。在蛋白质靶向于周质时,分泌信号可以从融合重组蛋白切除。在一些实施方案中,分泌信号和蛋白质或多肽之间的连接被修饰以提高分泌信号从融合蛋白的切除。

[0167] 宿主细胞和菌株

[0168] 考虑了将细菌宿主细胞,包括假单胞菌(即,假单胞菌目中的宿主细胞)和密切相关的细菌生物体,用于实施本发明的方法。在某些实施方案中,假单胞菌宿主细胞是荧光假单胞菌。宿主细胞还可以是大肠杆菌。

[0169] 可以使用本领域已知的和文献(例如,美国专利No.8,288,127,“Protein Expression Systems”,按引用全部并入)中描述的试剂和方法来鉴定或制备本发明方法的实施中有用的宿主细胞和构建体。这篇专利描述了通过将核酸构建体引入包含染色体lacI基因插入片段的营养缺陷型荧光假单胞菌宿主细胞中来产生重组多肽。核酸构建体包含可操作地连接能够指引核酸在宿主细胞中表达的启动子的编码重组多肽的核苷酸序列,并且还包含编码营养缺陷选择标记的核苷酸序列。营养缺陷选择标记是将原营养恢复成营养缺陷型宿主细胞的多肽。在一些实施方案中,细胞对于脯氨酸、尿嘧啶或其组合是营养缺陷的。在一些实施方案中,宿主细胞源自MB101(ATCC保藏PTA-7841)。美国专利No.8,288,127,“Protein Expression Systems”和Schneider等,2005,“Auxotrophic markers pyrF and proC can replace antibiotic markers on protein production plasmids in high-cell-density Pseudomonas fluorescens fermentation”,Biotechnol.Progress 21(2): 343-8,将两篇都全部按引用并入,描述了通过删除菌株MB101中的pyrF基因构建的对于尿嘧啶营养缺陷的生产宿主菌株。从菌株MB214(ATCC保藏PTA-7840)克隆pyrF基因来产生可以补偿pyrF删除而恢复原营养的质粒。在特定实施方案中,使用荧光假单胞菌宿主细胞中的双重pyrF-proC双重营养缺陷选择标记系统。鉴于公开的文献,本领域技术人员根据标准重组方法可以产生所述的PyrF生产宿主菌株并用作用于引入其他所需基因组变化的背景,包括本文中所述可用于实施本发明的方法的那些。

[0170] 在一些实施方案中,宿主细胞是假单胞菌目的(在本文中称为“假单胞菌”)。在宿主细胞是假单胞菌目的情况中,其可以是假单胞菌科的成员,包括假单胞菌属。 γ 变形菌(Proteobacteria)宿主包括大肠杆菌物种的成员和荧光假单胞菌物种的成员。其他假单胞菌属生物体也可以是有用的。假单胞菌和密切相关的物种包括革兰氏阴性变形菌亚组1,其包括属于R.E.Buchanan和N.E.Gibbons(编辑),Bergey's Manual of Determinative Bacteriology,pp.217-289(第8版,1974)(The Williams&Wilkins Co.,Baltimore,Md.,USA)(全部按引用并入)中描述为“革兰氏阴性需氧杆菌和球菌”的科和/或属的Proteobacteria组。(即,假单胞菌目的宿主细胞)。表3呈现了这些科和属的生物体。

[0171] 表3:科和属(“革兰氏阴性需氧杆菌和球菌”,“Bergey”,1974)

[0172]

科 I. 假单胞菌科葡萄糖杆菌属 (Pseudomonaceae Gluconobacter)	假单胞菌属 黄单胞菌属 动胶菌属 (Zoogloea)
科 II. 固氮菌科氮单胞菌属 (Azotobacteraceae Azomonas)	固氮菌属 (Azotobacter) 拜叶林克氏菌属 (Beijerinckia) 德克斯氏菌属 (Derxia)
科 III. 根瘤菌科土壤杆菌属 (Rhizobiaceae Agrobacterium)	根瘤菌属 (Rhizobium)
科 IV. 甲基单胞菌科甲基球菌属 (Methylomonadaceae Methylococcus)	甲基单胞菌属 (Methylomonas)
科 V. 盐杆菌科嗜盐杆菌属 (Halobacteriaceae Halobacterium)	盐球菌属 (Halococcus)
其他属醋酸杆菌	产碱杆菌属 (Alcaligenes) 博德特氏菌属 (Bordetella) 布鲁氏菌属 (Brucella) 弗朗西斯氏菌属 (Francisella) 栖热菌属 (Thermus)

[0173] 假单胞菌属和密切相关的细菌通常是定义为“革兰氏(-)变形菌亚组1”或“革兰氏阴性需氧杆菌和球菌”(Buchanan and Gibbons(编辑)(1974)Bergey's Manual of Determinative Bacteriology, pp.217-289)。假单胞菌属宿主菌株描述于文献中,例如,美国专利申请公开No.2006/0040352,全部按引用并入。

[0174] “革兰氏阴性变形菌亚组1”还包括根据分类中使用的标准归入这一标题中的变形菌。该标题还包括之前归入这个部分中但现在不再属于其中的组,如食酸菌属(Acidovorax)、短波单胞菌属(Brevundimonas)、伯克氏菌属(Burkholderia)、氢嗜胞菌属(Hydrogenophaga)、海洋单胞菌属(Oceanimonas)、青枯菌属(Ralstonia)和寡养单胞菌属(Stenotrophomona)、鞘氨醇单胞菌属(Sphingomonas)(和从其衍生的芽单胞菌属(Blastomonas)属),其通过将属于黄单胞菌属(Xanthomonas)(和之前称为黄单胞菌种)的生物体重新分组形成,酸单胞菌属(Acidomona),其通过将属于属醋杆菌属(Acetobacter)的生物体重新分组形成,如Bergey(1974)中定义的。另外,宿主可以包括来自假单胞菌属, Pseudomonas enalia(ATCC 14393)、生黑假单胞菌(Pseudomonas nigrifaciensi)(ATCC19375)和腐败假单胞菌(Pseudomonas putrefaciens)(ATCC 8071)(其分别已经重新归类为河豚毒素交替假单胞菌(Alteromonas haloplanktis)、产黑交替假单胞菌(Alteromonas nigrifaciens)和腐败交替假单胞菌(Alteromonas putrefaciens))的细胞。相似地,例如,食酸假单胞菌(Pseudomonas acidovorans)(ATCC 15668)和睾丸酮假单

胞菌 (*Pseudomonas testosteroni*) (ATCC 11996) 已经分别重新归类为食酸丛毛单胞菌 (*Comamonas acidovorans*) 和睾丸酮丛毛单胞菌 (*Comamonas testosteroni*); 以及生黑假单胞菌 (ATCC 19375) 和杀鱼假单胞菌 (*Pseudomonas piscicida*) (ATCC 15057) 已经分别重新归类为生黑假交替单胞菌 (*Pseudoalteromonas nigrifaciens*) 和杀鱼假交替单胞菌 (*Pseudoalteromonas piscicida*)。“革兰氏阴性变形菌亚组1”还包括归类为属于以下任一个科的变形菌: 假单胞菌科、固氮菌科 (现在常常用同义词假单胞菌科的“固氮菌组”来称呼)、根瘤菌科和甲基单胞菌科 (现在常常用同义词“甲基球菌科”来称呼)。因此, 除了本文中另外描述的那些属, 落入“革兰氏阴性变形菌亚组1”的更多变形菌属包括: 1) 嗜氮根瘤菌属 (*Azorhizophilus*) 的固氮菌组细菌; 2) 纤维弧菌属 (*Cellvibrio*)、寡源杆菌属 (*Oligella*) 和 *Teredinibacter* 的假单胞菌科细菌; 3) 螯合杆菌属 (*Chelatobacter*)、剑菌属 (*Ensifer*)、韧皮杆菌属 (*Liberibacter*) (也称为“*Candidatus Liberibacter*”) 和中华根瘤菌属 (*Sinorhizobium*) 的根瘤菌科细菌; 和 4) 甲基杆菌属 (*Methylobacter*)、甲基暖菌属 (*Methylocaldum*)、甲基微菌属 (*Methylomicrobium*)、甲基八叠球菌属 (*Methylosarcina*) 和甲基球形菌属 (*Methylosphaera*) 的甲基球菌科细菌。

[0175] 宿主细胞可以选自“革兰氏阴性变形菌亚组16”。“革兰氏阴性变形菌亚组16”被定义为以下假单胞菌属物种 (具有括号中所示的示例性菌株的 ATCC 或其他保藏号) 的变形菌组: *Pseudomonas abietaniphila* (ATCC 700689); 铜绿假单胞菌 (*Pseudomonas aeruginosa*) (ATCC 10145); 产碱假单胞菌 (*Pseudomonas alcaligenes*) (ATCC 14909); 鳃败血假单胞菌 (*Pseudomonas anguilliseptica*) (ATCC 33660); 香茅醇假单胞菌 (*Pseudomonas citronellolis*) (ATCC 13674); 变黄假单胞菌 (*Pseudomonas flavescens*) (ATCC 51555); 门多萨假单胞菌 (*Pseudomonas mendocina*) (ATCC 25411); 硝基还原假单胞菌 (*Pseudomonas nitroreducens*) (ATCC 33634); 食油假单胞菌 (*Pseudomonas oleovorans*) (ATCC 8062); 类产碱假单胞菌 (*Pseudomonas pseudoalcaligenes*) (ATCC 17440); 食树脂假单胞菌 (*Pseudomonas resinovorans*) (ATCC 14235); 稻草假单胞菌 (*Pseudomonas straminea*) (ATCC 33636); 伞菌假单胞菌 (*Pseudomonas agarici*) (ATCC 25941); 嗜碱性假单胞菌 (*Pseudomonas alcaliphila*); *Pseudomonas alginovora*; *Pseudomonas andersonii*; 铁角蕨假单胞菌 (*Pseudomonas asplenii*) (ATCC 23835); 壬二酸假单胞菌 (*Pseudomonas azelaica*) (ATCC 27162); *Pseudomonas beyerinckii* (ATCC 19372); *Pseudomonas borealis*; 北城假单胞菌 (*Pseudomonas boreopolis*) (ATCC 33662); *Pseudomonas brassicacearum*; 食丁酸假单胞菌 (*Pseudomonas butanovora*) (ATCC 43655); *Pseudomonas cellulosa* (ATCC 55703); 枯黄假单胞菌 (*Pseudomonas aurantiaca*) (ATCC 33663); 绿针假单胞菌 (*Pseudomonas chlororaphis*) (ATCC 9446、ATCC 13985、ATCC 17418、ATCC 17461); 莓实假单胞菌 (*Pseudomonas fragi*) (ATCC 4973); 隆德假单胞菌 (*Pseudomonas lundensis*) (ATCC 49968); 腐臭假单胞菌 (*Pseudomonas taetrolens*) (ATCC 4683); 青紫葛假单胞菌 (*Pseudomonas cissicola*) (ATCC 33616); 晕斑假单胞菌 (*Pseudomonas coronafaciens*); *Pseudomonas diterpeniphila*; 伸长假单胞菌 (*Pseudomonas elongata*) (ATCC 10144); *Pseudomonas flectens* (ATCC 12775); 产氮假单胞菌 (*Pseudomonas azotoformans*); *Pseudomonas brenneri*; *Pseudomonas cedrella*; 皱缩假单胞菌 (*Pseudomonas corrugata*) (ATCC 29736); *Pseudomonas extremorientalis*; 荧光

假单胞菌 (*Pseudomonas fluorescens*) (ATCC35858); *Pseudomonas gessardii*; *Pseudomonas libanensis*; 孟氏假单胞菌 (*Pseudomonas mandelii*) (ATCC 700871); 边缘假单胞菌 (*Pseudomonas marginalis*) (ATCC 10844); 米氏假单胞菌 (*Pseudomonas migulae*); 霉味假单胞菌 (*Pseudomonas mucidolens*) (ATCC 4685); *Pseudomonas orientalis*; *Pseudomonas rhodesiae*; 类黄假单胞菌 (*Pseudomonas synxantha*) (ATCC 9890); 托拉氏假单胞菌 (*Pseudomonas tolaasii*) (ATCC 33618); 维罗纳假单胞菌 (*Pseudomonas veronii*) (ATCC 700474); *Pseudomonas frederiksbergensis*; 弯曲假单胞菌 (*Pseudomonas geniculata*) (ATCC19374); *Pseudomonas gingeri*; *Pseudomonas graminis*; 格氏假单胞菌 (*Pseudomonas grimontii*); *Pseudomonas halodenitrificans*; 嗜盐假单胞菌 (*Pseudomonas halophila*); 栖木槿假单胞菌 (*Pseudomonas hibiscicola*) (ATCC 19867); *Pseudomonas huttiensis* (ATCC 14670); 噬氢假单胞菌 (*Pseudomonas hydrogenovora*); *Pseudomonas jessenii* (ATCC 700870); *Pseudomonas kilonensis*; 柳叶刀形假单胞菌 (*Pseudomonas lanceolata*) (ATCC 14669); *Pseudomonas lini*; *Pseudomonas marginate* (ATCC 25417); *Pseudomonas mephitica* (ATCC33665); 脱氮假单胞菌 (*Pseudomonas denitrificans*) (ATCC 19244); 穿孔素假单胞菌 (*Pseudomonas pertucinogena*) (ATCC 190); *Pseudomonas pictorum* (ATCC 23328); *Pseudomonas psychrophila*; *Pseudomonas filva* (ATCC 31418); 蒙氏假单胞菌 (*Pseudomonas monteilii*) (ATCC 700476); 摩氏假单胞菌 (*Pseudomonas mosselii*); 栖稻假单胞菌 (*Pseudomonas oryzihabitans*) (ATCC 43272); 变形假单胞菌 (*Pseudomonas plecoglossicida*) (ATCC 700383); 恶臭假单胞菌 (*Pseudomonas putida*) (ATCC 12633); *Pseudomonas reactans*; 多刺假单胞菌 (*Pseudomonas spinosa*) (ATCC 14606); 巴利阿里假单胞菌 (*Pseudomonas balearica*); 浅黄假单胞菌 (*Pseudomonas luteola*) (ATCC 43273); 施氏假单胞菌 (*Pseudomonas stutzeri*) (ATCC17588); 扁桃假单胞菌 (*Pseudomonas amygdali*) (ATCC 33614); 榛色假单胞菌 (*Pseudomonas avellanae*) (ATCC 700331); 番木瓜假单胞菌 (*Pseudomonas caricapapayae*) (ATCC 33615); 菊苣假单胞菌 (*Pseudomonas cichorii*) (ATCC 10857); 天仙果假单胞菌 (*Pseudomonas ficuserectae*) (ATCC 35104); 褐鞘假单胞菌 (*Pseudomonas fuscovaginae*); 苦楝假单胞菌 (*Pseudomonas meliae*) (ATCC 33050); 丁香假单胞菌 (*Pseudomonas syringae*) (ATCC19310); 绿黄假单胞菌 (*Pseudomonas viridiflava*) (ATCC 13223); 热食碳酸假单胞菌 (*Pseudomonas thermocarboxydovorans*) (ATCC35961); *Pseudomonas thermotolerans*; *Pseudomonas thivervalensis*; *Pseudomonas vancouverensis* (ATCC 700688); *Pseudomonas wisconsinensis* 和 *Pseudomonas xiamenensis*。在一个实施方案中, 宿主细胞是荧光假单胞菌。

[0176] 宿主细胞也可以选自“革兰氏阴性变形菌亚组17”。“革兰氏阴性变形菌亚组17”定义为本领域称为“荧光假单胞菌”的变形菌组, 包括属于例如以下假单胞菌属的种的那些: 产氮假单胞菌 (*Pseudomonas azotoformans*); *Pseudomonas brenneri*; *Pseudomonas cedrella*; 皱缩假单胞菌 (*Pseudomonas corrugata*); *Pseudomonas extremorientalis*; 荧光假单胞菌; *Pseudomonas gessardii*; *Pseudomonas libanensis*; *Pseudomonas mandelii*; 边缘假单胞菌 (*Pseudomonas marginalis*); *Pseudomonas migulae*; 霉味假单胞菌 (*Pseudomonas mucidolens*); *Pseudomonas orientalis*; *Pseudomonas rhodesiae*; 类黄假

单胞菌 (*Pseudomonas synxantha*); 托拉氏假单胞菌 (*Pseudomonas tolaasii*); 和维罗纳假单胞菌 (*Pseudomonas veronii*)。

[0177] 在一些实施方案中, 本发明的方法中使用的细菌宿主细胞是蛋白酶表达缺陷的。在一些实施方案中, 蛋白酶表达缺陷的细菌宿主细胞是假单胞菌。在一些实施方案中, 蛋白酶表达缺陷的细菌宿主细胞是假单胞菌属。在一些实施方案中, 蛋白酶表达缺陷的细菌宿主细胞是荧光假单胞菌。

[0178] 在一些实施方案中, 本发明的方法中使用的细菌宿主细胞不是蛋白酶表达缺陷的。在一些实施方案中, 不是蛋白酶表达缺陷的细菌宿主细胞是假单胞菌。在一些实施方案中, 不是蛋白酶表达缺陷的细菌宿主细胞是假单胞菌属。在一些实施方案中, 不是蛋白酶表达缺陷的细菌宿主细胞是荧光假单胞菌。

[0179] 在一些实施方案中, 本发明的方法中使用的假单胞菌属宿主细胞是Lon蛋白酶 (例如, SEQ ID NO:14)、Lal蛋白酶 (例如, SEQ ID NO:15)、AprA蛋白酶 (例如, SEQ ID NO:16) 表达缺陷的, 或其组合。在一些实施方案中, 假单胞菌属宿主细胞是AprA (例如, SEQ ID NO:16)、HtpX (例如, SEQ ID NO:17) 表达缺陷的, 或其组合。在一些实施方案中, 假单胞菌属宿主细胞是Lon (例如, SEQ ID NO:14)、Lal (例如, SEQ ID NO:15)、AprA (例如, SEQ ID NO:16)、HtpX (例如, SEQ ID NO:17) 表达缺陷的, 或其组合。在一些实施方案中, 假单胞菌属宿主细胞是Npr (例如, SEQ ID NO:20)、DegP1 (例如, SEQ ID NO:18)、DegP2 (例如, SEQ ID NO:19) 表达缺陷的, 或其组合。在一些实施方案中, 假单胞菌属宿主细胞是Lal (例如, SEQ ID NO:15)、Prc1 (例如, SEQ ID NO:21)、Prc2 (例如, SEQ ID NO:22)、PrtB (例如, SEQ ID NO:23) 表达缺陷的, 或其组合。这些蛋白酶是本领域已知的并且描述于例如美国专利No.8,603,824, “Process for Improved Protein Expression by Strain Engineering”、美国专利申请公开No.2008/0269070和美国专利申请公开No.2010/0137162, 其公开了上述蛋白酶的开放阅读框序列。

[0180] 源自基础菌株MB101 (ATCC保藏PTA-7841) 的荧光假单胞菌宿主菌株的实例在本发明的方法中是有用的。在一些实施方案中, 用于表达hPTH融合蛋白的荧光假单胞菌是例如DC454、DC552、DC572、DC1084、DC1106、DC508、DC992.1、PF1201.9、PF1219.9、PF1326.1、PF1331、PF1345.6或DC1040.1-1。在一些实施方案中, 荧光假单胞菌宿主菌株是PF1326.1。在一些实施方案中, 荧光假单胞菌宿主菌株是PF1345.6。使用本文中提供的信息, 本领域已知的和文献中描述的重组DNA方法以及可利用的材料, 例如, 如所述的在ATCC保藏的荧光假单胞菌菌株MB101, 本领域技术人员可以容易地构建在本发明的方法中有用的这些和其他菌株。

[0181] 表达菌株

[0182] 可以使用本文中和公开文献中所述的方法构建对于实施本发明的方法有用的表达菌株。在一些实施方案中, 本发明方法中有用的表达菌株包含超表达一个或多个荧光假单胞菌伴侣蛋白或折叠调节子蛋白的质粒。例如, DnaJ-样蛋白、FrnE、Fk1B或EcpD可以在表达菌株中超表达。在一些实施方案中, 荧光假单胞菌折叠调节子超表达 (FM0) 质粒编码C1pX、Fk1B3、FrnE、C1pA、Fkbp或ppiA。编码Fkbp的表达质粒的实例是pDOW1384-1。在一些实施方案中, 将不编码折叠调节子的表达质粒引入表达菌株中。在这些实施方案中, 质粒例如是pDOW2247。在一些实施方案中, 用于在本发明的方法中表达hPTH融合蛋白的荧光假单胞

菌表达菌株是STR35970、STR35984、STR36034、STR36085、STR36150、STR36169、STR35949、STR36098或STR35783,如本文中别处所述的。

[0183] 在一些实施方案中,本发明的方法中使用的荧光假单胞菌宿主菌株是DC1106 (mt1DYZ敲除突变体 Δ pyrF Δ proC Δ benAB lsc::lacI^{Q1}),保藏的菌株MB101的衍生物,其中从甘露糖醇 (mt1) 操作子删除基因pyrF、proC、benA、benB和mt1DYZ,并插入大肠杆菌lacI转录阻遏子并与果聚糖蔗糖酶基因 (lsc) 融合。这些基因的序列及其使用方法是本领域已知的并且描述于文献中,例如,美国专利No.8,288,127、8,017,355,“Mannitol induced promoter systems in bacterial host cells”,和7,794,972,“Benzoate-and anthranilate-inducible promoters”,各自按引用并入。

[0184] 可以使用本文中和公开的文献中描述的方法,从MB101构建与DC1106等同的宿主细胞或本文中所述的任一个宿主细胞或表达菌株。在一些实施方案中,使用与DC1106等同的宿主细胞。宿主细胞DC454描述于Schneider等,2005 (在其中称为DC206),和美国专利No.8,569,015,“rPA Optimization”中,全部按引用并入本文中。DC206是与DC454相同的菌株;在无动物培养基中传代三次后,其重命名为DC454。

[0185] 本领域普通技术人员将认识到,在一些实施方案中,可以使用在荧光假单胞菌中不复制的携带待删除基因侧翼的区域的删除质粒,通过例如等位基因交换来进行基因组删除或突变 (例如,灭活或削弱突变)。可以通过PCR扩增待删除基因 (包括待删除基因的上游和下游区域) 来构建删除质粒。可以对通过使用分析引物从基因组DNA扩增的PCR产物测序来验证删除,在通过琼脂糖软凝胶中的电泳分离后进行观察,接着是片段的DNA测序。在一些实施方案中,通过完全删除、部分删除或突变,例如,移码、点突变或插入突变,来使基因灭活。

[0186] 在一些实施方案中,根据本领域已知的方法,用FM0质粒转化使用的菌株,例如,可以用FM0质粒pDOW1384转化DC1106宿主细胞,pDOW1384超表达FkbP (RXF06591.1),这是属于肽酰-脯氨酰顺-反异构酶家族的折叠调节子,以产生表达菌株STR36034。hPTH融合蛋白表达菌株的特定实例的基因型以及用于根据本发明的方法表达hPTH的相应宿主细胞列于表4中。在一些实施方案中,用本文中所述的等同FM0质粒转化与表4中描述的任何宿主细胞等同的宿主细胞,来获得与本文中描述的使用本发明的方法表达hPTH1-34的菌株等同的表达菌株。如所讨论的,根据本文和文献中描述的方法,可以相似地得到合适的表达菌株。

[0187] 表4:用于PTH 1-34融合蛋白生产的荧光假单胞菌宿主细胞和表达菌株

[0188]

宿主菌株	表达菌株	蛋白酶删除	FMO 质粒	融合蛋白
DC508-1	STR35970	M50 S2P 蛋白 酶家族成员金 属蛋白酶	--	DnaJ-样蛋 白-PTH
DC992.1	STR35984	PrlC, AprA	pDOW2247(空 载体; 无折叠调 节子)	DnaJ-样蛋 白-PTH
DC1084-1	STR35949	Lon, Lal, DegP2	pDOW2247	DnaJ-样蛋 白-PTH
PF1201.9	STR35985	AprA, Lon, Lal, DegP1, DegP2, Prc1	pDOW2247	DnaJ-样蛋 白-PTH
PF1326.1	STR36005	HtpX, AprA	pDOW2247	DnaJ-样蛋 白-PTH
DC1106-1	STR36034	AprA, Lon, Lal	pDOW1384-1 FkbP (RXF06591.1)	FklB-PTH
PF1326.1	STR36085	HtpX, AprA	pDOW2247	FklB-PTH
PF1345.6	STR36098	HtpX, AprA, Lon, Lal	pDOW2247	FklB-PTH
DC1040.1-	STR35783	rxsf04495	pDOW2247	FklB-PTH

[0189]

1		(Serralysin) AprA		
PF1219.9	STR36150	Npr, DegP1, DegP2	--	FrnE-PTH
PF1331	STR36169	Lal, Prc1, Prc2, PrtB	--	FrnE-PTH

[0190] 在一些实施方案中,使用本发明的方法,将表4中所列的宿主细胞或菌株,或表4中所述的任何宿主细胞或菌株的等价体,用于表达本文中所述的包含目标多肽的融合蛋白。在一些实施方案中,使用本发明的方法,将表4中所列的宿主细胞或菌株,或表4中所述的任何宿主细胞或菌株的等价体,用于表达如本文中所述的包含hPTH、GCSF或胰岛素多肽(例如,前胰岛素)的融合蛋白。在一些实施方案中,使用本发明的方法,将野生型宿主细胞,例如,DC454或等价体,用于表达本文中所述的包含目标多肽的融合蛋白。

[0191] 用于产生本发明的宿主菌株的这些和其他蛋白酶和折叠调节子的序列是本领域已知的并且公开于文献中,例如,以上所述的美国专利No.8,603,824的表A至F中提供的,并全部按引用并入。例如,M50 S2P蛋白酶家族膜金属蛋白酶开放阅读框序列在其中作为RXF04692来提供。

[0192] 高通量筛选

[0193] 在一些实施方案中,可以进行高通量筛选来确定用于表达可溶性重组融合蛋白的最佳条件。在筛选中可以改变的条件包括,例如,宿主细胞、宿主细胞的遗传背景(例如,不同蛋白酶的删除)、表达构建体中的启动子类型、与编码重组蛋白的序列融合的分泌前导序列的类型、生长温度、使用诱导型启动子时诱导下的OD、使用lacZ启动子时用于诱导的IPTG的浓度、蛋白诱导的持续时间、将诱导剂加入培养物后的生长温度、培养物的搅动速率、用于质粒维持的选择方法、容器中的培养物体积和细胞裂解方法。

[0194] 在一些实施方案中,提供了宿主菌株的文库(或“阵列”),其中文库中的每个菌株(或“宿主细胞群体”)已经被遗传修饰来调节宿主细胞中的一个或多个靶基因的表达。与阵列中表型截然不同的其他宿主细胞群体相比,可以基于表达的重组融合蛋白的数量、质量和/或位置来鉴定或选择“最佳宿主菌株”或“最佳表达系统”。因此,最佳宿主菌株是根据所需规格产生重组融合蛋白的菌株。尽管所需规格将根据待产生的蛋白质而改变,但所述规格包括蛋白质的质量和/或数量,例如,蛋白质是隔离的还是分泌的,并且数量怎么样,蛋白质是否得到正确或所需的加工和/或折叠等。在一些实施方案中,提高的或理想的质量可以是以高滴定度表达和低降解水平产生重组融合蛋白。在一些实施方案中,最佳宿主菌株或最佳表达系统产生特定的绝对水平或相对于指示菌株(即,用于比较的菌株)产生的特定水平的产量,其特征在于可溶性重组融合蛋白的量或质量、可回收的重组融合蛋白的量或质量、正确加工的重组融合蛋白的量或质量、正确折叠的重组融合蛋白的量或质量、活性重组融合蛋白的含量或质量和/或重组融合蛋白的总量或质量。

[0195] 筛选微生物宿主以鉴定在重组融合蛋白的表达中具有改善的产率和/或质量的菌株的方法描述于例如美国专利申请公开No.2008/0269070中。

[0196] 发酵形式

[0197] 本发明的表达菌株可以以任何发酵形式来培养。例如,在本文中可以使用分批、补料-分批、半连续和连续发酵方式。

[0198] 在一些实施方案中,发酵培养基可以选自加富培养基、最低培养基和矿物盐培养基。在其他实施方案中,选择最低培养基或矿物盐培养基。在某些实施方案中,选择矿物盐培养基。

[0199] 矿物盐培养基由矿物盐和碳源组成,所述碳源如例如葡萄糖、蔗糖或甘油。矿物盐培养基的实例包括,例如,M9培养基、假单胞菌培养基(ATCC 179)以及Davis和Mingioli培养基(参见,Davis,B.D.和Mingioli,E.S.,1950,J.Bact.60:17-28)。用于制备矿物盐培养基的矿物盐包括选自例如钾磷酸盐、硫酸铵或氯化铵、硫酸镁或氯化镁和微量矿物质,如氯化钙、硼酸盐以及铁、铜、锰和锌的硫酸盐的那些。通常,矿物盐培养基中不包括有机氮源,如蛋白胨、胰蛋白胨、氨基酸或酵母提取物。替代地,使用无机氮源,并且这可以选自例如铵盐、氨水和气态氨。矿物盐培养基通常含有葡萄糖或甘油作为碳源。与矿物盐培养基相比,最低培养基也可以含有矿物盐和碳源,但可以补充例如低水平的氨基酸、维生素、蛋白胨或

其他成分,尽管这些以非常低的水平的添加。可以使用文献中描述的方法,例如,在以上提及并按引用并入的美国专利申请公开No.2006/0040352中,来制备用于本发明方法中的合适培养基。本发明方法中有用的培养程序和矿物盐培养基的详细内容描述于Riesenberg,D等,1991,“High cell density cultivation of *Escherichia coli* at controlled specific growth rate,”*J.Biotechnol.*20(1):17-27中,按引用并入本文中。

[0200] 在一些实施方案中,可以在生物反应器培养中实现生产。培养物可以生长于例如高达2升的含有矿物盐培养基的生物反应器中,并且通过添加氨维持在32℃和pH 6.5下。通过提高搅拌以及喷入空气和氧气流到发酵器中,可以维持溶解氧过量。可以在整个发酵过程中将甘油递送至培养物以维持过量水平。在一些实施方案中,维持这些条件直至达到用于诱导的目标培养物细胞密度,例如,575nm的光密度(A₅₇₅),并加入IPTG来启动目标蛋白产生。将理解诱导时的细胞密度、IPTG的浓度、pH、温度、CaCl₂浓度、溶解氧流速,各自可以改变来确定用于表达的最佳条件。在一些实施方案中,诱导时的细胞密度可以从A₅₇₅的40改变至200吸光度单位(AU)。IPTG浓度可以在0.02至1.0mM,pH在6至7.5,温度在20至35℃,CaCl₂浓度在0至0.5g/L和溶解氧流速在1LPM(升/分钟)至10LPM的范围中改变。6-48小时后,通过离心收集每个生物反应器的培养物,并将细胞沉淀物在-80℃下冷冻。然后分析样品的产物形成,例如,通过SDS-CGE。

[0201] 可以在任何规模下进行发酵。根据本发明的表达系统对于任何规模下的重组蛋白表达是有用的。因此,例如,可以使用微升规模、毫升规模、厘升规模和分升规模的发酵体积,并且可以使用1升规模和更大的发酵体积。

[0202] 在一些实施方案中,发酵体积为或大于约1升。在一些实施方案中,发酵体积为约1升至约100升。在一些实施方案中,发酵体积为约1升、约2升、约3升、约4升、约5升、约6升、约7升、约8升、约9升或约10升。在一些实施方案中,发酵体积为约1升至约5升、约1升至约10升、约1升至约25升、约1升至约50升、约1升至约75升、约10升至约25升、约25升至约50升或约50升至约100升。在其它实施方案中,发酵体积为或大于5升、10升、15升、20升、25升、50升、75升、100升、200升、250升、300升、500升、1,000升、2,000升、5,000升、10,000升或50,000升。在一些实施方案中,

[0203] 通常,相对于较小培养体积,例如,0.5mL高通量筛选培养,通过较大培养体积,例如,50mL摇瓶培养、1L或更大的培养产生的重组蛋白的量提高。这可能不仅是由于培养物大小的增加,而且可能是例如在大规模发酵中使细胞生长至更高密度的能力(例如,通过培养物吸光度反映的)。例如,来自相同菌株的体积产率从HTP规模到大规模发酵可以提高高达十倍。在一些实施方案中,大规模发酵后,对于相同表达菌株观察到的体积产率比HTP规模生长高2倍至10倍。在一些实施方案中,大规模发酵后针对相同表达菌株观察到的产率量比HTP规模生长高2倍、3倍、4倍、5倍、6倍、7倍、8倍、9倍、2倍至10倍、2倍至9倍、2倍至8倍、2倍至7倍、2倍至6倍、2倍至5倍、2倍至4倍、2倍至3倍、3倍至10倍、3倍至9倍、3倍至8倍、3倍至7倍、3倍至6倍、3倍至5倍、3倍至4倍、4倍至10倍、4倍至9倍、4倍至8倍、4倍至7倍、4倍至6倍、4倍至5倍、5倍至10倍、5倍至9倍、5倍至8倍、5倍至7倍、5倍至6倍、6倍至10倍、6倍至9倍、6倍至8倍、6倍至7倍、7倍至10倍、7倍至9倍、7倍至8倍、8倍至10倍、8倍至9倍、9倍至10倍。参见,例如,Retallack等,2012,“Reliable protein production in a *Pseudomonas fluorescens* expression system,”*Prot.Exp.and Purif.*81:157-165,按引用全部并入本

文中。

[0204] 细菌生长条件

[0205] 本发明提供的方法中有用的生长条件可以包含约4℃至约42℃的温度和约5.7至约8.8的pH。使用具有lacZ启动子的表达构建体时,可以通过以约0.01mM至约1.0mM的终浓度将IPTG添加至培养物中来诱导表达。

[0206] 可以使用本领域技术人员已知的pH缓冲剂和方法来维持培养物的pH。还可以使用氨水来实现培养过程中pH的控制。在一些实施方案中,培养物的pH为约5.7至约8.8。在一些实施方案中,pH为约5.7,5.8,5.9,6.0,6.1,6.2,6.3,6.4,6.5,6.6,6.7,6.8,6.9,7.0,7.1,7.2,7.3,7.4,7.5,7.6,7.7,7.8,7.9,8.0,8.1,8.2,8.3,8.4,8.5,8.6,8.7或8.8。在一些实施方案中,pH为约5.7至约8.8,约5.7至约8.5,约5.7至约8.3,约5.7至约8,约5.7至约7.8,约5.7至约7.6,约5.7至约7.4,约5.7至约7.2,约5.7至约7,约5.7至约6.8,约5.7至约6.6,约5.7至约6.4,约5.7至约6.2,约5.7至约6,约5.9至约8.8,约5.9至约8.5,约5.9至约8.3,约5.9至约8,约5.9至约7.8,约5.9至约7.6,约5.9至约7.4,约5.9至约7.2,约5.9至约7,约5.9至约6.8,约5.9至约6.6,约5.9至约6.4,约5.9至约6.2,约6至约8.8,约6至约8.5,约6至约8.3,约6至约8,约6至约7.8,约6至约7.6,约6至约7.4,约6至约7.2,约6至约7,约6至约6.8,约6至约6.6,约6至约6.4,约6至约6.2,约6.1至约8.8,约6.1至约8.5,约6.1至约8.3,约6.1至约8,约6.1至约7.8,约6.1至约7.6,约6.1至约7.4,约6.1至约7.2,约6.1至约7,约6.1至约6.8,约6.1至约6.6,约6.1至约6.4,约6.2至约8.8,约6.2至约8.5,约6.2至约8.3,约6.2至约8,约6.2至约7.8,约6.2至约7.6,约6.2至约7.4,约6.2至约7.2,约6.2至约7,约6.2至约6.8,约6.2至约6.6,约6.2至约6.4,约6.4至约8.8,约6.4至约8.5,约6.4至约8.3,约6.4至约8,约6.4至约7.8,约6.4至约7.6,约6.4至约7.4,约6.4至约7.2,约6.4至约7,约6.4至约6.8,约6.4至约6.6,约6.6至约8.8,约6.6至约8.5,约6.6至约8.3,约6.6至约8,约6.6至约7.8,约6.6至约7.6,约6.6至约7.4,约6.6至约7.2,约6.6至约7,约6.6至约6.8,约6.8至约8.8,约6.8至约8.5,约6.8至约8.3,约6.8至约8,约6.8至约7.8,约6.8至约7.6,约6.8至约7.4,约6.8至约7.2,约6.8至约7,约7至约8.8,约7至约8.5,约7至约8.3,约7至约8,约7至约7.8,约7至约7.6,约7至约7.4,约7至约7.2,约7.2至约8.8,约7.2至约8.5,约7.2至约8.3,约7.2至约8,约7.2至约7.8,约7.2至约7.6,约7.2至约7.4,约7.4至约8.8,约7.4至约8.5,约7.4至约8.3,约7.4至约8,约7.4至约7.8,约7.4至约7.6,约7.6至约8.8,约7.6至约8.5,约7.6至约8.3,约7.6至约8,约7.6至约7.8,约7.8至约8.8,约7.8至约8.5,约7.8至约8.3,约7.8至约8,约8至约8.8,约8至约8.5,或约8至约8.3。在一些实施方案中,pH为约6.5至约7.2。

[0207] 在一些实施方案中,将生长温度维持在约4℃至约42℃。在一些实施方案中,生长温度为约4℃,约5℃,约6℃,约7℃,约8℃,约9℃,约10℃,约11℃,约12℃,约13℃,约14℃,约15℃,约16℃,约17℃,约18℃,约19℃,约20℃,约21℃,约22℃,约23℃,约24℃,约25℃,约26℃,约27℃,约28℃,约29℃,约30℃,约31℃,约32℃,约33℃,约34℃,约35℃,约36℃,约37℃,约38℃,约39℃,约40℃,约41℃或约42℃。在一些实施方案中,生长温度为约25℃至约32℃。在一些实施方案中,将生长温度维持在约22℃至约27℃,约22℃至约28℃,约22℃至约29℃,约22℃至约30℃,23℃至约27℃,约23℃至约28℃,约23℃至约29℃,约23℃至约30℃,约24℃至约27℃,约24℃至约28℃,约24℃至约29℃,约24℃至约30℃,约25℃至约

27℃,约25℃至约28℃,约25℃至约29℃,约25℃至约30℃,约25℃至约31℃,约25℃至约32℃,约25℃至约33℃,约26℃至约28℃,约26℃至约29℃,约26℃至约30℃,约26℃至约31℃,约26℃至约32℃,约26℃至约33℃,约27℃至约29℃,约27℃至约30℃,约27℃至约31℃,约27℃至约32℃,约27℃至约33℃,约28℃至约30℃,约28℃至约31℃,约28℃至约32℃,约29℃至约31℃,约29℃至约32℃,约29℃至约33℃,约30℃至约32℃,约30℃至约33℃,约31℃至约33℃,约31℃至约32℃,约21℃至约42℃,约22℃至约42℃,约23℃至约42℃,约24℃至约42℃,约25℃至约42℃。在一些实施方案中,生长温度为约25℃至约28.5℃。在一些实施方案中,生长温度高于约20℃,高于约21℃,高于约22℃,高于约23℃,高于约24℃,高于约25℃,高于约26℃,高于约27℃,高于约28℃,高于约29℃或高于约30℃。

[0208] 在一些实施方案中,在培养过程中改变温度。在一些实施方案中,在将试剂(例如,IPTG)加入培养物来诱导从构建体的表达之前,将温度维持在约30℃至约32℃,并且在添加诱导剂后,将温度降至约25℃至约28℃。在一些实施方案中,在将试剂(例如,IPTG)加入培养物来诱导从构建体的表达之前,将温度维持在约30℃,并且在添加诱导剂后,将温度降至约25℃。

[0209] 如本文中别处所述,诱导型启动子可以用于表达构建体中来控制重组融合蛋白的表达,例如,lac启动子。在lac启动子衍生物或家族成员(例如,tac启动子)的情况中,效应化合物是诱导剂,例如,如IPTG的安慰诱导剂。在一些实施方案中,使用lac启动子衍生物,并且在细胞密度达到通过约40至约180的OD₅₇₅确定的水平时,通过添加IPTG至约0.01mM至约1.0mM的终浓度来诱导重组蛋白表达。在一些实施方案中,重组蛋白的培养物诱导时的OD₅₇₅可以为约40,约50,约60,约70,约80,约90,约110,约120,约130,约140,约150,约160,约170,约180。在其它实施方案中,OD₅₇₅为约40至约50,约50至约60,约60至约70,约70至约80,约80至约90,或约90至约100。在其他实施方案中,OD₅₇₅为约40至约100,约100至约120,约120至约130,约130至约140,约140至约150,约150至约160,约160至约170或约170至约180。在其他实施方案中,OD₅₇₅为约40至约140,或约80至约180。可以通过其他方法来测量细胞密度并以其他单位来表示,例如,以细胞/单位体积。例如,荧光假单胞菌培养物的约40至约160的OD₅₇₅等同于大约 4×10^{10} 至约 1.6×10^{10} 集落形成单位/mL或17.5至70g/L干细胞重。在一些实施方案中,培养物诱导时的细胞密度等同于本文中通过OD₅₇₅的吸光度指定的细胞密度,与用于测定细胞密度的方法或测量单位无关。本领域技术人员知道对于任何细胞培养物怎样进行合适的转换。

[0210] 在一些实施方案中,培养物的最终IPTG浓度为约0.01mM,约0.02mM,约0.03mM,约0.04mM,约0.05mM,约0.06mM,约0.07mM,约0.08mM,约0.09mM,约0.1mM,约0.2mM,约0.3mM,约0.4mM,约0.5mM,约0.6mM,约0.7mM,约0.8mM,约0.9mM或约1mM。在一些实施方案中,培养物的最终IPTG浓度为约0.08mM至约0.1mM,约0.1mM至约0.2mM,约0.2mM至约0.3mM,约0.3mM至约0.4mM,约0.2mM至约0.4mM,约0.08至约0.2mM,或约0.1至1mM。

[0211] 如本文中和文献中所述的,在其中使用非lac型启动子的实施方案中,可以使用其他诱导剂或效应物。在一个实施方案中,启动子是组成型启动子。

[0212] 在添加诱导剂后,将培养物生长一段时间,例如,约24小时,在该时间段中,表达重组蛋白。添加诱导剂后,可以将培养物生长约1hr、约2hr,约3hr,约4hr,约5hr,约6hr,约7hr,约8hr,约9hr,约10hr,约11hr,约12hr,约13hr,约14hr,约15hr,约16hr,约17hr,约

18hr,约19hr,约20hr,约21hr,约22hr,约23hr,约24hr,约36hr或约48hr。将诱导剂加入培养物后,可以将培养物生长约1至48hr,约1至24hr,约1至8hr,约10至24hr,约15至24hr或约20至24hr。可以通过离心浓缩细胞培养物,并且将培养物沉淀物重悬浮于适于随后裂解程序的缓冲液或溶液中。

[0213] 在一些实施方案中,使用用于高压机械细胞破坏的设备(其可商业购得,例如, Microfluidics微流化装置、Constant细胞粉碎机、Niro-Soavi均质机或APV-Gaulin均质机)来破坏细胞。例如,可以使用超声处理来破坏表达重组蛋白的细胞。本领域已知的用于裂解细胞的任何合适方法可以用于释放可溶性部分。例如,在一些实施方案中,可以使用化学和/或酶细胞裂解试剂,如细胞壁裂解酶和EDTA。在本发明的方法中,也考虑了使用冷冻或之前储存的培养物。在裂解前,培养物可以OD-标准化。例如,细胞可以标准化至约10、约11、约12、约13、约14、约15、约16、约17、约18、约19或约20的OD₆₀₀。

[0214] 可以使用任何合适的设备和方法来进行离心。用于分离可溶性部分与不溶性部分目的的细胞培养物或裂解物的离心是本领域公知的。例如,裂解的细胞可以在20,800×g下离心20分钟(在4℃下),并且使用人工或自动化液体操作来除去上清液。通过细胞培养物的离心获得的细胞沉淀物,或通过细胞裂解物的离心获得的不溶性部分,可以重悬浮于缓冲溶液中。可以使用,例如,如连接架空混合器、磁搅拌棒、翘板摇床等的叶轮的装置来进行细胞沉淀物或不溶性部分的重悬浮。

[0215] 非变性条件

[0216] 诱导的宿主细胞的裂解在非变性条件下进行。在一些实施方案中,非变性条件包括使用非变性处理缓冲液,例如,以重悬浮细胞沉淀物或细胞糊。在一些实施方案中,非变性处理缓冲液包含磷酸钠或Tris缓冲液、甘油和氯化钠。在其中通过固定化金属亲和色谱(IMAC)进行亲和色谱的实施方案中,非变性处理缓冲液包含咪唑。在一些实施方案中,非变性处理缓冲液包含0至50mM咪唑。在一些实施方案中,非变性处理缓冲液不包含咪唑。在一些实施方案中,非变性处理缓冲液包含25mM咪唑。在一些实施方案中,非变性处理缓冲液包含10-30mM磷酸钠或Tris,pH 7至9。在一些实施方案中,非变性处理缓冲液具有7.3、7.4或7.5的pH。在一些实施方案中,非变性处理缓冲液包含2-10%甘油。在一些实施方案中,非变性处理缓冲液包含50mM至750mM NaCl。在一些实施方案中,将细胞糊重悬浮至10-50%固体。在一些实施方案中,非变性处理缓冲液包含20mM磷酸钠、5%甘油、500mM氯化钠、20mM咪唑,pH 7.4,并且重悬浮至20%固体。在一些实施方案中,非变性处理缓冲液包含20mM Tris、50mM NaCl,pH 7.5,并且重悬浮至20%固体。

[0217] 在一些实施方案中,非变性处理缓冲液不含离液剂。离液剂破坏蛋白或核酸的三维结构,从而引起变性。在一些实施方案中,非变性处理缓冲液包含非变性浓度的离液剂。在一些实施方案中,离液剂例如为脲或盐酸胍。在一些实施方案中,非变性处理缓冲剂包含0至4M脲或盐酸胍。在一些实施方案中,非变性处理缓冲液包含低于4M,低于3.5M,低于3M,低于2.5M,低于2M,低于1.5M,低于1M,低于0.5M,约0.1M,约0.2M,约0.3M,约0.4M,约0.5M,约0.6M,约0.7M,约0.8M,约0.9M,约1.0M,约1.1M,约1.2M,约1.3M,约1.4M,约1.5M,约1.6M,约1.7M,约1.8M,约1.9M,或约2.0M,约2.1M,约2.2M,约2.3M,约2.4M,约2.5M,约2.6M,约2.7M,约2.8M,约2.9M,约3M,约3.1M,约3.2M,约3.3M,约3.4M,约3.5M,约3.6M,约3.7M,约3.8M,约3.9M,约4M,约0.5至约3.5M,约0.5至约3M,约0.5至约2.5M,约0.5至约2M,约0.5至

约1.5M,约0.5至约1M,约1至约4M,约1至约3.5M,约1至约3M,约1至约2.5M,约1至约2M,约1至约1.5M,约1.5至约4M,约1.5至约3.5M,约1.5至约3M,约1.5至约2.5M,约1.5至约2M,约2至约4M,约2至约3.5M,约2至约3M,约2至约2.5M,约2.5至约4M,约2.5至约3.5M,约2.5至约3M,约3至约4M,约3至约3.5M,或约0.5至约1M浓度的脲或盐酸胍。

[0218] 在其中使用非变性处理缓冲剂的实施方案中,将细胞糊在20mM Tris,50mM NaCl,4M脲,pH 7.5中形成20%固体的浆液,在2-8℃下持续1-2.5小时。在一些实施方案中,将细胞糊用Niro均质机进行裂解,例如,在15,000psi下,和在14,000×g下分批离心35分钟或在15,000×g和340mL/min进料下连续离心,用深度过滤器和膜过滤器过滤supe/离心分离液,在重悬浮缓冲液,例如,1XPBS pH 7.4中稀释2X,并且加载于捕获柱上。在一些实施方案中,非变性处理缓冲液包含其他组分,例如,用于IMAC的咪唑,如本文中别处所述的。

[0219] 本领域技术人员将理解离液剂的变性浓度可能受到pH的影响,并且变性水平取决于蛋白质的特征。例如,可以提高pH来引起蛋白质变性,尽管离液剂的浓度较低。

[0220] 产物评价

[0221] 可以通过本领域已知的或文献中描述的任何方法来评价所产生的重组融合蛋白或目标多肽的品质。在一些实施方案中,基于其溶解性或通过缺少或失去生物活性来评价蛋白质的变性。对于许多蛋白质,生物活性测试是商业上可得的。生物活性测试可以包括,例如,抗体结合测试。在一些实施方案中,使用本领域可利用的方法,例如,色谱和分光光度方法,来进行重组融合蛋白或目标多肽的物理表征。目标多肽的评价可以包括确定其已经正确释放(例如,其N-末端是完整的)。

[0222] 可以使用本领域已知的或本文中或文献中描述的任何方法,例如,使用识别蛋白质N-末端的抗体,来评价hPTH(例如,hPTH 1-34或1-84)的活性。所述方法包括,例如,完整质量分析。可以通过,例如,cAMP ELISA、均相时间解析荧光(HTRF)测试(Charles River Laboratories)或按照Nissenson等,1985,“Activation of the Parathyroid Hormone Receptor-Adenylate Cyclase System in Osteosarcoma Cells by a Human Renal Carcinoma Factor,”Cancer Res.45:5358-5363和美国专利No.7,150,974,“Parathyroid Hormone Receptor Binding Method,”中所述的来测量PTH生物活性,将上述每篇文献按引用并入本文中。评价PTH的方法也由Shimizu等,2001,“Parathyroid hormone(1-14) and (1-11)analogs conformationally constrained by α -aminoisobutyric acid mediate full agonist responses via the Juxtamembrane region of the PTH-1receptor,”J.Biol.Chem.276:49003-49012描述,其按引用并入本文中。

[0223] 重组融合蛋白和目标多肽的纯化

[0224] 可以通过本领域技术人员已知的或文献中描述的任何方法,例如,离心方法和/或色谱方法,如大小排阻、阴离子或阳离子交换、疏水性相互作用或亲和性色谱,从其他蛋白质和细胞碎片分离或纯化溶解的重组融合蛋白或目标多肽。在一些实施方案中,使用快速高效液相色谱(FPLC)来纯化溶解的蛋白质。FPLC是用于基于对各种树脂的亲和性分离蛋白质的液相色谱形式。在一些实施方案中,与融合蛋白一起表达的亲和性标签引起溶解于增溶缓冲液中的融合蛋白结合于树脂,同时杂质携带在增溶缓冲液中。随后,使用洗脱缓冲液,以渐递梯度或以逐步方式添加,以从离子交换树脂离解融合蛋白并在洗脱缓冲液中分离纯融合蛋白。

[0225] 在一些实施方案中,完成诱导后,通过离心收集发酵培养液,例如,在 $15,900 \times g$ 下持续60至90分钟。分离细胞糊和上清液,并且将糊状物在 -80°C 下冷冻。按照本文中别处所述的,将冷冻的细胞糊在缓冲液(例如,非变性缓冲液或不含胍的缓冲液)中解冻。在一些实施方案中,将冷冻的细胞糊在20mM磷酸钠,5%甘油,500mM氯化钠,pH 7.4中解冻并重悬浮于其中。在一些实施方案中,缓冲液包含咪唑。在一些实施方案中,将悬浮液的最终体积调节至所需的固体百分比,例如,20%固体。细胞可以通过化学或机械方式裂解,例如,随后可以将材料在15,000psi下通过微流化床来均质化。将裂解物离心,例如,在 $12,000 \times g$ 下持续30分钟,并过滤,例如,通过Sartorius Sartobran 150(0.45/0.2 μm)囊式过滤器。

[0226] 在一些实施方案中,可以将快速蛋白质液相色谱(FPLC)用于纯化,例如,使用配备了Frac-950级分收集器的ÄKTA explorer 100色谱系统(GE Healthcare)。在其中使用His-标签的实施方案中,可以将样品加载于HisTrap FF,10mL柱上(串联连接的两个5mL HisTrap FF筒[GE Healthcare,部件号17-5255-01]),洗涤并洗脱,例如,使用10柱体积线性梯度的洗脱缓冲液(通过将咪唑浓度从0mM改变至200mM),并收集级分。

[0227] 在一些实施方案中,可以如对于目标多肽适宜的进行色谱。例如,可以按照本文中实施例中所所述的,进行固定化金属离子亲和性色谱纯化(例如,使用镍IMAC)。

[0228] 重组融合蛋白的切割

[0229] 在一些实施方案中,纯化的重组融合蛋白级分用裂解酶孵育以将目标多肽与接头和N-末端融合伴侣切割。在一些实施方案中,裂解酶是蛋白酶,例如,丝氨酸蛋白酶,例如,牛肠激酶、猪肠激酶、胰蛋白酶或本文中别处所述的任何其他合适的蛋白酶。可以使用本领域已知的和文献(包括制造商的说明)中描述的任何合适的蛋白酶切割方法。蛋白酶可以商业购得,例如,从Sigma-Aldrich(St.Louis,MO)、ThermoFisher Scientific(Waltham,MA)和Promega(Madison,WI)。例如,在一些实施方案中,可以浓缩牛肠激酶(例如,Novagen目录号#69066-3,批次D00155747)切割融合蛋白纯化级分并重悬浮于含有20mM Tris pH 7.4, 50mM NaCl和2mM CaCl_2 的缓冲液中。将两单位的牛肠激酶加入100 μL 反应中的100 μg 蛋白质中。将融合蛋白纯化级分和肠激酶的混合物孵育合适的时间长度。在一些实施方案中,没有肠激酶的对照反应也进行孵育用于比较。可以通过添加含有盐酸4-苯磺酰氟(AEBSF,Sigma目录号#P8465)的完全蛋白酶抑制剂混合物来停止酶反应。

[0230] 在一些实施方案中,裂解酶孵育进行约1小时至约24小时。在一些实施方案中,孵育进行约1hr,约2hr,约3hr,约4hr,约5hr,约6hr,约7hr,约8hr,约9hr,约10hr,约11hr,约12hr,约13hr,约14hr,约15hr,约16hr,约17hr,约18hr,约19hr,约20hr,约21hr,约22hr,约23hr,约24hr,约1hr至约24hr,约1hr至约23hr,约1hr至约22hr,约1hr至约21hr,约1hr至约20hr,约1hr至约19hr,约1hr至约18hr,约1hr至约17hr,约1hr至约16hr,约1hr至约15hr,约1hr至约14hr,约1hr至约13hr,约1hr至约12hr,约1hr至约11hr,约1hr至约10hr,约1hr至约9hr,约1hr至约8hr,约1hr至约7hr,约1hr至约6hr,约1hr至约5hr,约1hr至约4hr,约1hr至约3hr,约1hr至约2hr,约2hr至约24hr,约2hr至约23hr,约2hr至约22hr,约2hr至约21hr,约2hr至约20hr,约2hr至约19hr,约2hr至约18hr,约2hr至约17hr,约2hr至约16hr,约2hr至约15hr,约2hr至约14hr,约2hr至约13hr,约2hr至约12hr,约2hr至约11hr,约2hr至约10hr,约2hr至约9hr,约2hr至约8hr,约2hr至约7hr,约2hr至约6hr,约2hr至约5hr,约2hr至约4hr,约2hr至约3hr,约3hr至约24hr,约3hr至约23hr,约3hr至约22hr,约3hr至约21hr,约3hr至

约20hr,约3hr至约19hr,约3hr至约18hr,约3hr至约17hr,约3hr至约16hr,约3hr至约15hr,约3hr至约14hr,约3hr至约13hr,约3hr至约12hr,约3hr至约11hr,约3hr至约10hr,约3hr至约9hr,约3hr至约8hr,约3hr至约7hr,约3hr至约6hr,约3hr至约5hr,约3hr至约4hr,约4hr至约24hr,约4hr至约23hr,约4hr至约22hr,约4hr至约21hr,约4hr至约20hr,约4hr至约19hr,约4hr至约18hr,约4hr至约17hr,约4hr至约16hr,约4hr至约15hr,约4hr至约14hr,约4hr至约13hr,约4hr至约12hr,约4hr至约11hr,约4hr至约10hr,约4hr至约9hr,约4hr至约8hr,约4hr至约7hr,约4hr至约6hr,约4hr至约5hr,约5hr至约24hr,约5hr至约23hr,约5hr至约22hr,约5hr至约20hr,约5hr至约21hr,约5hr至约19hr,约5hr至约18hr,约5hr至约17hr,约5hr至约16hr,约5hr至约15hr,约5hr至约14hr,约5hr至约13hr,约5hr至约12hr,约5hr至约11hr,约5hr至约10hr,约5hr至约9hr,约5hr至约8hr,约5hr至约7hr,约5hr至约6hr,约6hr至约24hr,约6hr至约23hr,约6hr至约22hr,约6hr至约21hr,约6hr至约20hr,约6hr至约19hr,约6hr至约18hr,约6hr至约17hr,约6hr至约16hr,约6hr至约15hr,约6hr至约14hr,约6hr至约13hr,约6hr至约12hr,约6hr至约11hr,约6hr至约10hr,约6hr至约9hr,约6hr至约8hr,约6hr至约7hr,约7hr至约24hr,约7hr至约23hr,约7hr至约22hr,约7hr至约21hr,约7hr至约20hr,约7hr至约19hr,约7hr至约18hr,约7hr至约17hr,约7hr至约16hr,约7hr至约15hr,约7hr至约14hr,约7hr至约13hr,约7hr至约12hr,约7hr至约11hr,约7hr至约10hr,约7hr至约9hr,约7hr至约8hr,约8hr至约24hr,约8hr至约23hr,约8hr至约22hr,约8hr至约21hr,约8hr至约20hr,约8hr至约19hr,约8hr至约18hr,约8hr至约17hr,约8hr至约16hr,约8hr至约15hr,约8hr至约14hr,约8hr至约13hr,约8hr至约12hr,约8hr至约11hr,约8hr至约10hr,约8hr至约9hr,约9hr至约24hr,约9hr至约23hr,约9hr至约22hr,约9hr至约21hr,约9hr至约20hr,约9hr至约19hr,约9hr至约18hr,约9hr至约17hr,约9hr至约16hr,约9hr至约15hr,约9hr至约14hr,约9hr至约13hr,约9hr至约12hr,约9hr至约11hr,约9hr至约10hr,约10hr至约24hr,约10hr至约23hr,约10hr至约22hr,约10hr至约21hr,约10hr至约20hr,约10hr至约19hr,约10hr至约18hr,约10hr至约17hr,约10hr至约16hr,约10hr至约15hr,约10hr至约14hr,约10hr至约13hr,约10hr至约12hr,约10hr至约11hr,约11hr至约24hr,约11hr至约23hr,约11hr至约22hr,约11hr至约21hr,约11hr至约20hr,约11hr至约19hr,约11hr至约18hr,约11hr至约17hr,约11hr至约16hr,约11hr至约15hr,约11hr至约14hr,约11hr至约13hr,约11hr至约12hr,约12hr至约24hr,约12hr至约23hr,约12hr至约22hr,约12hr至约21hr,约12hr至约20hr,约12hr至约19hr,约12hr至约18hr,约12hr至约17hr,约12hr至约16hr,约12hr至约15hr,约12hr至约14hr,约12hr至约13hr,约13hr至约24hr,约13hr至约23hr,约13hr至约22hr,约13hr至约21hr,约13hr至约20hr,约13hr至约19hr,约13hr至约18hr,约13hr至约17hr,约13hr至约16hr,约13hr至约15hr,约13hr至约14hr,约14hr至约24hr,约14hr至约23hr,约14hr至约22hr,约14hr至约21hr,约14hr至约20hr,约14hr至约19hr,约14hr至约18hr,约14hr至约17hr,约14hr至约16hr,约14hr至约15hr,约15hr至约24hr,约15hr至约23hr,约15hr至约22hr,约15hr至约21hr,约15hr至约20hr,约15hr至约19hr,约15hr至约18hr,约15hr至约17hr,约16hr至约24hr,约16hr至约23hr,约16hr至约22hr,约16hr至约21hr,约16hr至约20hr,约16hr至约19hr,约16hr至约18hr,或约16hr至约17hr,约17hr至约24hr,约17hr至约23hr,约17hr至约22hr,约17hr至约21hr,约17hr至约20hr,约17hr至约19hr,约17hr至约18hr,约18hr至约24hr,约18hr至约

23hr,约18hr至约22hr,约18hr至约21hr,约18hr至约20hr,约18hr至约19hr,约19hr至约24hr,约19hr至约23hr,约19hr至约22hr,约19hr至约21hr,约19hr至约20hr,约20hr至约24hr,约20hr至约23hr,约20hr至约22hr,约20hr至约21hr,约21hr至约24hr,约21hr至约23hr,约21hr至约22hr,约22hr至约24hr或约22hr至约23hr。

[0231] 在一些实施方案中,用蛋白酶孵育后重组融合蛋白的切割程度为约90%至约100%。在一些实施方案中,用蛋白酶孵育后的切割程度为约90%,约91%,约92%,约93%,约94%,约95%,约96%,约97%,约98%,约99%,约100%,约91%至约100%,约92%至约100%,约93%至约100%,约94%至约100%,约95%至约100%,约96%至约100%,约97%至约100%,约98%至约100%,约99%至约100%,约90%至约99%,约91%至约99%,约92%至约99%,约93%至约99%,约94%至约99%,约95%至约99%,约96%至约99%,约97%至约99%,约98%至约99%,约90%至约98%,约91%至约98%,约92%至约98%,约93%至约98%,约94%至约98%,约95%至约98%,约96%至约98%,约97%至约98%,约90%至约97%,约91%至约97%,约92%至约97%,约93%至约97%,约94%至约97%,约95%至约97%,约96%至约97%,约90%至约96%,约91%至约96%,约92%至约96%,约93%至约96%,约94%至约96%,约95%至约96%,约90%至约95%,约91%至约95%,约92%至约95%,约93%至约95%,约94%至约95%,约90%至约94%,约91%至约94%,约92%至约94%,约93%至约94%,约90%至约93%,约91%至约93%,约92%至约93%,约90%至约92%,约91%至约92%或约90%至约91%。

[0232] 在一些实施方案中,蛋白酶切割导致目标多肽从重组融合蛋白释放。在一些实施方案中,重组融合蛋白被正确切割以正确释放目标多肽。在一些实施方案中,重组融合蛋白的正确切割形成正确释放的具有完整(未降解)N-末端的目标多肽。在一些实施方案中,重组融合蛋白的正确切割形成正确释放的含有第一(N-末端)氨基酸的目标多肽。在一些实施方案中,蛋白酶切割后正确释放的多肽量为约90%至约100%。在一些实施方案中,蛋白酶切割后正确释放的多肽量为约90%,约91%,约92%,约93%,约94%,约95%,约96%,约97%,约98%,约99%,约100%,约91%至约100%,约92%至约100%,约93%至约100%,约94%至约100%,约95%至约100%,约96%至约100%,约97%至约100%,约98%至约100%,约99%至约100%,约90%至约99%,约91%至约99%,约92%至约99%,约93%至约99%,约94%至约99%,约95%至约99%,约96%至约99%,约97%至约99%,约98%至约99%,约90%至约98%,约91%至约98%,约92%至约98%,约93%至约98%,约94%至约98%,约95%至约98%,约96%至约98%,约97%至约98%,约90%至约97%,约91%至约97%,约92%至约97%,约93%至约97%,约94%至约97%,约95%至约97%,约96%至约97%,约90%至约96%,约91%至约96%,约92%至约96%,约93%至约96%,约94%至约96%,约95%至约96%,约90%至约95%,约91%至约95%,约92%至约95%,约93%至约95%,约94%至约95%,约90%至约94%,约91%至约94%,约92%至约94%,约93%至约94%,约90%至约93%,约91%至约93%,约92%至约93%,约90%至约92%,约91%至约92%或约90%至约91%。

[0233] 重组融合蛋白评价和产率

[0234] 使用本领域已知的或文献中描述的用于表征蛋白(例如,用于评价蛋白的产量或质量)的任何合适的测试方法,在任何合适的部分中表征所产生的融合蛋白和/或目标多

肽。

[0235] 在一些实施方案中,将本领域已知的LC-MS或任何其他合适的方法来监测蛋白水解剪切(clipping)、脱酰胺、氧化和断裂,并且用于证实接头切割后目标多肽的N-末端是完整的。可以通过本领域技术人员已知的方法,例如,通过SDS-PAGE、毛细管凝胶电泳(CGE)或Western印迹分析,来测定重组融合蛋白或目标多肽的产率。在一些实施方案中,使用ELISA方法来测量宿主细胞蛋白质。例如,可以根据制造商的实验方案,使用来自Cygnus Technologies, Inc.的“Immunoenzymetric Assay for the Measurement of Pseudomonas fluorescens Host Cell Proteins”试剂盒,目录编号F450,来进行宿主蛋白(HCP)ELISA。平板可以在SPECTRAMax Plus(Molecular Devices)上阅读,使用Softmax Pro v3.1.2软件。

[0236] 使用具有HT Protein Express v2芯片的LabChip GXII仪器(Caliper LifeSciences, Hopkinton, MA)和相应的试剂(部件号分别为760499和760328, Caliper LifeSciences)进行SDS-CGE。可以按照制造商的实验方案(Protein User Guide Document No. 450589, Rev. 3)来准备样品,并在聚丙烯酰胺凝胶上电泳。分离后,可以将凝胶染色、脱色并数字成像。

[0237] 可以通过本领域技术人员已知的和文献中描述的方法,通过吸光度光谱来测定蛋白质的浓度,例如本文中所述的纯化重组融合蛋白或目标多肽。在一些实施方案中,测量(例如,使用Eppendorf BioPhotometer, Eppendorf, 汉堡, 德国)280nm下蛋白质样品的吸光度,并且使用Beer-Lambert定律计算蛋白质浓度。通过已知的方法,例如,Grimsley, G.R.和Pace, C.N., “Spectrophotometric Determination of Protein Concentration”, 见Current Protocols in Protein Science 3.1.1-3.1.9, Copyright © 2003 by John Wiley & Sons, Inc., 按引用并入本文中,来计算蛋白质的准确摩尔吸收系数。

[0238] 表5列出了使用通过Vector NT1, Invitrogen计算的摩尔消光系数测定的在1的 A_{280} 下本文中所述蛋白质的浓度。

[0239] 表5:1的 A_{280} 下的蛋白质浓度

[0240]

氨基酸 SEQ ID NO	蛋白	1的 A ₂₈₀ 下的蛋 白 质 浓 度 (mg/mL)	摩 尔 消 光系数
1	PTH1-34	0.72	
45	DnaJ-样蛋白-PTH 1-34 融合体	0.8	
46	FklB-PTH 1-34 融合体	1.18	
47	FrnE-PTH 1-34 融合体	0.98	
70	DnaJ-样蛋白-EK-GCSF 融合体	1.02	29190
71	EcpD1-EK-GCSF 融合体 (全长 EcpD1)	1.21	39530
72	EcpD2-EK-GCSF 融合体	1.37	23030
73	EcpD3-EK-GCSF 融合体 (50 aa 截短的 EcpD1)	1.51	17430
74	FklB-EK-GCSF 融合体 (全长 FklB)	1.26	33600

[0241]

75	FklB2-EK-GCSF 融合体 (100 aa 截短的 FklB)	1.83	17100
76	FklB3-EK-GCSF 融合体 (500 aa 截短的 FklB)	1.52	17100
77	FrnE-EK-GCSF 融合体 (全长 FrnE)	1.09	40810
78	FrnE2-EK-GCSF 融合体(aa) (100 aa 截短的 FrnE)	1.31	24310
79	FrnE3-EK-GCSF 融合体 (50 aa 截短的 FrnE)	1.21	21750
122	DnaJ-样蛋白 -EK- 前胰岛素 -CP-A	1.06	19210
123	DnaJ-样蛋白 -EK- 前胰岛素 -CP-B	1.04	19210
124	DnaJ-样蛋白 -EK- 前胰岛素 -CP-C	1.03	19210
125	DnaJ-样蛋白-胰蛋白酶-前胰岛素 -CP-A	1.04	19210
126	DnaJ-样蛋白-胰蛋白酶-前胰岛素 -CP-B	1.01	19210
127	DnaJ-样蛋白-胰蛋白酶-前胰岛素 -CP-C	1.01	19210
128	DnaJ-样蛋白 -EK- 前胰岛素 -CP-D	1.05	19210
129	DnaJ-样蛋白-胰蛋白酶-前胰岛素 -CP-D	1.07	19210
130	FklB-EK-前胰岛素-CP-A	1.40	23620
131	FklB-EK-前胰岛素-CP-B	1.38	23620
132	FklB-EK-前胰岛素-CP-C	1.37	23620

[0242]

133	FklB-胰蛋白酶-前胰岛素-CP-A	1.38	23620
134	FklB-胰蛋白酶-前胰岛素-CP-B	1.36	23620
135	FklB-胰蛋白酶-前胰岛素-CP-C	1.35	23620
136	FklB-EK-前胰岛素-CP-D	1.31	23620
137	FklB-胰蛋白酶-前胰岛素-CP-D	1.29	23620
138	FklB2-EK-前胰岛素-CP-A	3.06	7120
139	FklB2-EK-前胰岛素-CP-B	2.99	7120
140	FklB2-EK-前胰岛素-CP-C	2.98	7120
141	FklB2-胰蛋白酶-前胰岛素-CP-A	3.0	7120
142	FklB2-胰蛋白酶-前胰岛素-CP-B	2.93	7120
143	FklB2-胰蛋白酶-前胰岛素-CP-C	2.92	7120
144	FklB2-EK-前胰岛素-CP-D	2.78	7120
145	FklB2-胰蛋白酶-前胰岛素-CP-D	2.72	7120
146	FklB3.1-EK-前胰岛素-CP-A	2.33	7120
147	FklB3-EK-前胰岛素-CP-B	2.26	7120
148	FklB3.1-EK-前胰岛素-CP-C	2.25	7120
149	FklB3-胰蛋白酶-前胰岛素-CP-A	2.27	7120
150	FklB3.1-胰蛋白酶-前胰岛素-CP-B	2.20	7120
151	FklB3.1-胰蛋白酶-前胰岛素-CP-C	2.19	7120
152	FklB-EK-前胰岛素-CP-D	2.04	7120
153	FklB3.1-胰蛋白酶-前胰岛素-CP-D	1.98	7120
154	FrnE-EK-前胰岛素-CP-A	1.14	30830
155	FrnE-EK-前胰岛素-CP-B	1.12	30830
156	FrnE-EK-前胰岛素-CP-C	1.12	30830
157	FrnE-胰蛋白酶-前胰岛素-CP-A	1.13	30830
158	FrnE-胰蛋白酶-前胰岛素-CP-B	1.11	30830

[0243]

159	FrnE-胰蛋白酶-前胰岛素-CP-C	1.11	30830
160	FrnE-EK-前胰岛素-CP-D	1.08	30830
161	FrnE-胰蛋白酶-前胰岛素-CP-D	1.06	30830
162	FrnE2-EK-前胰岛素-CP-A	1.57	14330
163	FrnE2-EK-前胰岛素-CP-B	1.53	14330
164	FrnE2-EK-前胰岛素-CP-C	1.53	14330
165	FrnE2-胰蛋白酶-前胰岛素-CP-A	1.53	14330
166	FrnE2-胰蛋白酶-前胰岛素-CP-B	1.50	14330
167	FrnE2-胰蛋白酶-前胰岛素-CP-C	1.50	14330
168	FrnE2-EK-前胰岛素-CP-D	1.42	14330
169	FrnE2-胰蛋白酶-前胰岛素-CP-D	1.39	14330
170	FrnE3-EK-前胰岛素-CP-A	1.44	11770
171	FrnE3-EK-前胰岛素-CP-B	1.39	11770
172	FrnE3-EK-前胰岛素-CP-C	1.39	11770
173	FrnE3-胰蛋白酶-前胰岛素-CP-A	1.40	11770
174	FrnE3-胰蛋白酶-前胰岛素-CP-B	1.36	11770
175	FrnE3-胰蛋白酶-前胰岛素-CP-C	1.35	11770
176	FrnE3-EK-前胰岛素-CP-D	1.26	11770
177	FrnE3-胰蛋白酶-前胰岛素-CP-D	1.23	11770
178	EcpD1-EK-前胰岛素-CP-A	1.30	29550
179	EcpD1-EK-前胰岛素-CP-B	1.28	29550
180	EcpD1-EK-前胰岛素-CP-C	1.28	29550
181	EcpD1-EK-前胰岛素-CP-D	1.23	29550
182	EcpD1-胰蛋白酶-前胰岛素-CP-A (通过 pFNX4402 编码的 EcpD1-胰蛋白酶不含加下划线的 N 残基)	1.28	29550
183	EcpD1-胰蛋白酶-前胰岛素	1.26	29550

[0244]

	-CP-B (通过 pFNX4402 编码的 EcpD1-胰蛋白酶不含加下划线的 N 残基)		
184	EcpD1-胰蛋白酶-前胰岛素 -CP-C (通过 pFNX4402 编码的 EcpD1-胰蛋白酶不含加下划线的 N 残基)	1.26	29550
185	EcpD1-胰蛋白酶-前胰岛素 -CP-D (通过 pFNX4402 编码的 EcpD1-胰蛋白酶不含加下划线的 N 残基)	1.21	29550
186	EcpD2-EK-前胰岛素-CP-A	1.69	13050
187	EcpD2-EK-前胰岛素-CP-B	1.65	13050
188	EcpD2-EK-前胰岛素-CP-C	1.64	13050
189	EcpD2-胰蛋白酶-前胰岛素 -CP-A	1.65	13050
190	EcpD2-胰蛋白酶-前胰岛素 -CP-B	1.61	13050
191	EcpD2-胰蛋白酶-前胰岛素 -CP-C	1.61	13050
192	EcpD2-EK-前胰岛素-CP-D	1.53	13050
193	EcpD2-胰蛋白酶-前胰岛素 -CP-D	1.50	13050
194	EcpD3-EK-前胰岛素-CP-A	2.28	7360
195	EcpD3-EK-前胰岛素-CP-B	2.21	7360
196	EcpD3-EK-前胰岛素-CP-C	2.20	7360

[0245]	197	EcpD3-胰蛋白酶-前胰岛素-CP-A	2.22	7360
	198	EcpD3-胰蛋白酶-前胰岛素-CP-B	2.15	7360
	199	EcpD3-胰蛋白酶-前胰岛素-CP-C	2.14	7360
	200	EcpD3-EK-前胰岛素-CP-D	2.00	7360
	201	EcpD3-胰蛋白酶-前胰岛素-CP-D	1.95	7360

[0246] 通过将SDS-PAGE凝胶上分离的蛋白质转移至硝基纤维素膜上并用目标多肽特异性的单克隆抗体孵育膜,可以根据本领域已知的任何合适的方法来进行测定目标多肽的产率或纯度的Western印迹分析。可以通过本领域技术人员已知的合适程序来产生对于本文中所述的任何分析方法有用的抗体。

[0247] 如本文中所述的和已知的活性测试也可以提供关于蛋白质产率的信息。在一些实施方案中,本领域已知的这些或任何其他方法用于评价蛋白质的正确加工,例如,正确的分泌前导序列切割。

[0248] 重组融合蛋白产率的有用量度包括,例如,每培养体积的可溶性重组融合蛋白的量(例如,克或毫克蛋白/升培养物)、获得的可溶性重组融合蛋白的百分比或分数(例如,可溶性重组融合蛋白的量/总重组融合蛋白的量)、总细胞蛋白(tcp)的百分比或分数和干生物质的百分比或比例。在一些实施方案中,按照本文中所述的重组融合蛋白产率的测量是基于获得的可溶性重组融合蛋白的量。在一些实施方案中,可溶性重组融合蛋白的测量是在细胞裂解后获得的可溶性部分中进行的,例如,在一个或多个离心步骤后或重组融合蛋白的纯化后获得的可溶性部分。

[0249] 目标多肽产率的有用测量包括,例如,每培养物体积获得的可溶性目标多肽的量(例如,克或毫克蛋白/升培养物)、获得的可溶性目标多肽的百分比或分数(例如,可溶性目标多肽的量/总目标多肽的量)、获得的活性目标多肽的百分比或分数(例如,活性测试中活性目标多肽的量/目标多肽总量)、总细胞蛋白(tcp)的百分比或分数和干生物质的百分比或比例。

[0250] 在其中产率根据培养物体积来表示的实施方案中,可以考虑培养细胞密度,特别是在比较不同培养物之间的产率时。在一些实施方案中,本发明的方法可以用于获得约0.5克/升至约25克/升的可溶性和/或活性和/或正确加工(例如,具有正确切割的分泌前导序列)的重组融合蛋白产率。在一些实施方案中,重组融合蛋白包含N-末端融合伴侣,其是来自热休克蛋白家族的细胞质伴侣蛋白或折叠调节子并且在表达后将融合蛋白指引至细胞质。在一些实施方案中,重组融合蛋白包含N-末端融合伴侣,其是来自周质肽酰脯氨酰异构酶家族的周质伴侣蛋白或折叠调节子,并且在表达后将融合蛋白指引至周质。在一些实施方案中,融合蛋白(细胞质表达的融合蛋白或周质表达的融合蛋白)的产率为约0.5g/L,约1g/L,约1.5g/L,约2g/L,约2.5g/L,约3g/L,约3.5g/L,约4g/L,约4.5g/L,约5g/L,约6g/L,

约7g/L,约8g/L,约9g/L,约10g/L,约11g/L,约12g/L,约13g/L,约14g/L,约15g/L,约16g/L,约17g/L,约18g/L,约19g/L,约20g/L,约21g/L,约22g/L,约23g/L,约24g/L,约25g/L,约0.5g/L至约25g/L,约0.5g/L至约23g/L,约1g/L至约23g/L,约1.5g/L至约23g/L,约2g/L至约23g/L,约2.5g/L至约23g/L,约3g/L至约23g/L,约3.5g/L至约23g/L,约4g/L至约23g/L,约4.5g/L至约23g/L,约5g/L至约23g/L,约6g/L至约23g/L,约7g/L至约23g/L,约8g/L至约23g/L,约9g/L至约23g/L,约10g/L至约23g/L,约15g/L至约23g/L,约20g/L至约23g/L,约0.5g/L至约20g/L,约1g/L至约20g/L,约1.5g/L至约20g/L,约2g/L至约20g/L,约2.5g/L至约20g/L,约3g/L至约20g/L,约3.5g/L至约20g/L,约4g/L至约20g/L,约4.5g/L至约20g/L,约5g/L至约20g/L,约6g/L至约20g/L,约7g/L至约20g/L,约8g/L至约20g/L,约9g/L至约20g/L,约10g/L至约20g/L,约15g/L至约20g/L,约0.5g/L至约15g/L,约1g/L至约15g/L,约1.5g/L至约15g/L,约2g/L至约15g/L,约2.5g/L至约15g/L,约3g/L至约15g/L,约3.5g/L至约15g/L,约4g/L至约15g/L,约4.5g/L至约15g/L,约5g/L至约15g/L,约6g/L至约15g/L,约7g/L至约15g/L,约8g/L至约15g/L,约9g/L至约15g/L,约10g/L至约15g/L,约0.5g/L至约12g/L,约1g/L至约12g/L,约1.5g/L至约12g/L,约2g/L至约12g/L,约2.5g/L至约12g/L,约3g/L至约12g/L,约3.5g/L至约12g/L,约4g/L至约12g/L,约4.5g/L至约12g/L,约5g/L至约12g/L,约6g/L至约12g/L,约7g/L至约12g/L,约8g/L至约12g/L,约9g/L至约12g/L,约10g/L至约12g/L,约0.5g/L至约10g/L,约1g/L至约10g/L,约1.5g/L至约10g/L,约2g/L至约10g/L,约2.5g/L至约10g/L,约3g/L至约10g/L,约3.5g/L至约10g/L,约4g/L至约10g/L,约4.5g/L至约10g/L,约5g/L至约10g/L,约6g/L至约10g/L,约7g/L至约10g/L,约8g/L至约10g/L,约9g/L至约10g/L,约0.5g/L至约9g/L,约1g/L至约9g/L,约1.5g/L至约9g/L,约2g/L至约9g/L,约2.5g/L至约9g/L,约3g/L至约9g/L,约3.5g/L至约9g/L,约4g/L至约9g/L,约4.5g/L至约9g/L,约5g/L至约9g/L,约6g/L至约9g/L,约7g/L至约9g/L,约8g/L至约9g/L,约0.5g/L至约8g/L,约1g/L至约8g/L,约1.5g/L至约8g/L,约2g/L至约8g/L,约2.5g/L至约8g/L,约3g/L至约8g/L,约3.5g/L至约8g/L,约4g/L至约8g/L,约4.5g/L至约8g/L,约5g/L至约8g/L,约6g/L至约8g/L,约7g/L至约8g/L,约0.5g/L至约7g/L,约1g/L至约7g/L,约1.5g/L至约7g/L,约2g/L至约7g/L,约2.5g/L至约7g/L,约3g/L至约7g/L,约3.5g/L至约7g/L,约4g/L至约7g/L,约4.5g/L至约7g/L,约5g/L至约7g/L,约6g/L至约7g/L,约0.5g/L至约6g/L,约1g/L至约6g/L,约1.5g/L至约6g/L,约2g/L至约6g/L,约2.5g/L至约6g/L,约3g/L至约6g/L,约3.5g/L至约6g/L,约4g/L至约6g/L,约4.5g/L至约6g/L,约5g/L至约6g/L,约0.5g/L至约5g/L,约1g/L至约5g/L,约1.5g/L至约5g/L,约2g/L至约5g/L,约2.5g/L至约5g/L,约3g/L至约5g/L,约3.5g/L至约5g/L,约4g/L至约5g/L,约4.5g/L至约5g/L,约0.5g/L至约4g/L,约1g/L至约4g/L,约1.5g/L至约4g/L,约2g/L至约4g/L,约2.5g/L至约4g/L,约3g/L至约4g/L,约0.5g/L至约3g/L,约1g/L至约3g/L,约1.5g/L至约3g/L,约2g/L至约3g/L,约0.5g/L至约2g/L,约1g/L至约2g/L或约0.5g/L至约1g/L。

[0251] 在一些实施方案中,目标多肽是hPTH并且指引至细胞质的重组融合蛋白的产率为约0.5g/L至约2.4g/升。

[0252] 在一些实施方案中,目标多肽是hPTH并且指引至周质的重组融合蛋白的产量为约0.5g/L至约6.7g/升。

[0253] 目标多肽的产率

[0254] 在一些实施方案中,目标多肽通过接头内的蛋白酶切割从完全重组融合蛋白释放。在一些实施方案中,用蛋白酶切割后获得的目标多肽是正确释放的目标多肽。在一些实施方案中,目标多肽的产率-基于正确释放的蛋白的测量或基于已知目标多肽与总融合蛋白的比例计算的-为约0.7克/升至约25.0克/升。在一些实施方案中,在0.5mL至100L, 0.5mL, 50mL, 100mL, 1L, 2L或更大规模下,目标多肽的产率为约0.5g/L (500mg/L), 约1g/L, 约1.5g/L, 约2g/L, 约2.5g/L, 约3g/L, 约3.5g/L, 约4g/L, 约4.5g/L, 约5g/L, 约6g/L, 约7g/L, 约8g/L, 约9g/L, 约10g/L, 约11g/L, 约12g/L, 约13g/L, 约14g/L, 约15g/L, 约16g/L, 约17g/L, 约18g/L, 约19g/L, 约20g/L, 约21g/L, 约22g/L, 约23g/L, 约24g/L, 约25g/L, 约0.5g/L至约23g/L, 约1g/L至约23g/L, 约1.5g/L至约23g/L, 约2g/L至约23g/L, 约2.5g/L至约23g/L, 约3g/L至约23g/L, 约3.5g/L至约23g/L, 约4g/L至约23g/L, 约4.5g/L至约23g/L, 约5g/L至约23g/L, 约6g/L至约23g/L, 约7g/L至约23g/L, 约8g/L至约23g/L, 约9g/L至约23g/L, 约10g/L至约23g/L, 约15g/L至约23g/L, 约20g/L至约23g/L, 约0.5g/L至约20g/L, 约1g/L至约20g/L, 约1.5g/L至约20g/L, 约2g/L至约20g/L, 约2.5g/L至约20g/L, 约3g/L至约20g/L, 约3.5g/L至约20g/L, 约4g/L至约20g/L, 约4.5g/L至约20g/L, 约5g/L至约20g/L, 约6g/L至约20g/L, 约7g/L至约20g/L, 约8g/L至约20g/L, 约9g/L至约20g/L, 约10g/L至约20g/L, 约15g/L至约20g/L, 约0.5g/L至约15g/L, 约1g/L至约15g/L, 约1.5g/L至约15g/L, 约2g/L至约15g/L, 约2.5g/L至约15g/L, 约3g/L至约15g/L, 约3.5g/L至约15g/L, 约4g/L至约15g/L, 约4.5g/L至约15g/L, 约5g/L至约15g/L, 约6g/L至约15g/L, 约7g/L至约15g/L, 约8g/L至约15g/L, 约9g/L至约15g/L, 约10g/L至约15g/L, 约0.5g/L至约12g/L, 约1g/L至约12g/L, 约1.5g/L至约12g/L, 约2g/L至约12g/L, 约2.5g/L至约12g/L, 约3g/L至约12g/L, 约3.5g/L至约12g/L, 约4g/L至约12g/L, 约4.5g/L至约12g/L, 约5g/L至约12g/L, 约6g/L至约12g/L, 约7g/L至约12g/L, 约8g/L至约12g/L, 约9g/L至约12g/L, 约10g/L至约12g/L, 约0.5g/L至约10g/L, 约1g/L至约10g/L, 约1.5g/L至约10g/L, 约2g/L至约10g/L, 约2.5g/L至约10g/L, 约3g/L至约10g/L, 约3.5g/L至约10g/L, 约4g/L至约10g/L, 约4.5g/L至约10g/L, 约5g/L至约10g/L, 约6g/L至约10g/L, 约7g/L至约10g/L, 约8g/L至约10g/L, 约9g/L至约10g/L, 约0.5g/L至约9g/L, 约1g/L至约9g/L, 约1.5g/L至约9g/L, 约2g/L至约9g/L, 约2.5g/L至约9g/L, 约3g/L至约9g/L, 约3.5g/L至约9g/L, 约4g/L至约9g/L, 约4.5g/L至约9g/L, 约5g/L至约9g/L, 约6g/L至约9g/L, 约7g/L至约9g/L, 约8g/L至约9g/L, 约0.5g/L至约8g/L, 约1g/L至约8g/L, 约1.5g/L至约8g/L, 约2g/L至约8g/L, 约2.5g/L至约8g/L, 约3g/L至约8g/L, 约3.5g/L至约8g/L, 约4g/L至约8g/L, 约4.5g/L至约8g/L, 约5g/L至约8g/L, 约6g/L至约8g/L, 约7g/L至约8g/L, 约0.5g/L至约7g/L, 约1g/L至约7g/L, 约1.5g/L至约7g/L, 约2g/L至约7g/L, 约2.5g/L至约7g/L, 约3g/L至约7g/L, 约3.5g/L至约7g/L, 约4g/L至约7g/L, 约4.5g/L至约7g/L, 约5g/L至约7g/L, 约6g/L至约7g/L, 约0.5g/L至约6g/L, 约1g/L至约6g/L, 约1.5g/L至约6g/L, 约2g/L至约6g/L, 约2.5g/L至约6g/L, 约3g/L至约6g/L, 约3.5g/L至约6g/L, 约4g/L至约6g/L, 约4.5g/L至约6g/L, 约5g/L至约6g/L, 约0.5g/L至约5g/L, 约1g/L至约5g/L, 约1.5g/L至约5g/L, 约2g/L至约5g/L, 约2.5g/L至约5g/L, 约3g/L至约5g/L, 约3.5g/L至约5g/L, 约4g/L至约5g/L, 约4.5g/L至约5g/L, 约0.5g/L至约4g/L, 约1g/L至约4g/L, 约1.5g/L至约4g/L, 约2g/L至约4g/L, 约2.5g/L至约4g/L, 约3g/L至约4g/L, 约0.5g/L至约3g/L, 约1g/L至约3g/L, 约1.5g/L至约3g/L, 约2g/L至约3g/L, 约0.5g/L至约2g/L, 约1g/L至约2g/L或约0.5g/L至约

1g/L。

[0255] 在一些实施方案中,作为如表8中所述的具有N-末端融合伴侣的融合蛋白和hPTH构建体来生产hPTH。在一些实施方案中,在0.5mL至100L,0.5mL,50mL,100mL,1L,2L或更大规模下,hPTH融合蛋白的表达产生至少100,至少125,至少150,至少175,至少200,至少250,至少300,至少350,至少400,至少450,至少500,至少550,至少600,至少650或至少1000mg/L的总hPTH融合蛋白。

[0256] 在一些实施方案中,作为如表19中所述的具有N-末端融合伴侣的前胰岛素融合蛋白和包含C-肽序列的前胰岛素构建体来产生前胰岛素,例如,用于胰岛素类似物(例如,甘精胰岛素)的前胰岛素。在一些实施方案中,在0.5mL至100L,50mL,100mL,1L,2L或更大规模下,根据本发明方法的前胰岛素融合蛋白的表达产生至少约10,至少约20,至少约30,至少约40,至少约50,至少约60,至少约70,至少约80,至少约90,至少约100,至少约110,至少约120,至少约130,至少约140,至少约150,至少约200或至少约250mg/L的可溶性前胰岛素,如正确释放时测量的或基于其已知的融合蛋白的比例计算的。

[0257] 在一些实施方案中,在0.5mL至100L,0.5mL,50mL,100mL,1L,2L或更大规模下,根据本发明方法的前胰岛素融合蛋白的表达产生约10至约500,约15至约500,约20至约500,约30至约500,约40至约500,约50至约500,约60至约500,约70至约500,约80至约500,约90至约500,约100至约500,约200至约500,约10至约400,约15至约400,约20至约400,约30至约400,约40至约400,约50至约400,约60至约400,约70至约400,约80至约400,约90至约400,约100至约400,约200至约400,约10至约300,约15至约300,约20至约300,约30至约300,约40至约300,约50至约300,约60至约300,约70至约300,约80至约300,约90至约300,约100至约300,约200至约300,约10至约250,约15至约250,约20至约250,约30至约250,约40至约250,约50至约250,约60至约250,约70至约250,约80至约250,约90至约250,约100至约250,约10至约200,约15至约200,约20至约200,约30至约200,约40至约200,约50至约200,约60至约200,约70至约200,约80至约200,约90至约200或约100至约200mg/L可溶性前胰岛素,如正确释放时测量的或基于其已知的融合蛋白比例计算的。

[0258] 在一些实施方案中,前胰岛素融合蛋白的表达产生至少约100,至少约125,至少约150,至少约175,至少约200,至少约250,至少约300,至少约350,至少约400,至少约450,至少约500,至少约550,至少约600,至少约650或至少约1000mg/L的总可溶性和不溶性前胰岛素。在一些实施方案中,在0.5mL至100L,0.5mL,50mL,100mL,1L,2L或更大规模下,前胰岛素融合蛋白的表达产生约100至约2000mg/L,约100至约1500mg/L,约100至约1000mg/L,约100至约900mg/L,约100至约800mg/L,约100至约700mg/L,约100至约600mg/L,约100至约500mg/L,约100至约400mg/L,约200至约2000mg/L,约200至约1500mg/L,约200至约1000mg/L,约200至约900mg/L,约200至约800mg/L,约200至约700mg/L,约200至约600mg/L,约200至约500mg/L,约300至约2000mg/L,约300至约1500mg/L,约300至约1000mg/L,约300至约900mg/L,约300至约800mg/L,约300至约700mg/L或约300至约600mg/L的总可溶性和不溶性前胰岛素。在一些实施方案中,前胰岛素被切割以释放C-肽并产生成熟胰岛素。在一些实施方案中,在0.5mL至100L,0.5mL,50mL,100mL,1L,2L或更大规模下,前胰岛素融合蛋白的表达产生至少约100,至少约200,至少约250,至少约300,至少约400,至少约500,约100至约2000mg/L,约200至约2000mg/L,约300至约2000mg/L,约400至约2000mg/L,约500至约

2000mg/L, 约100至约1000mg/L, 约200至约1000mg/L, 约300至约1000mg/L, 约400至约1000mg/L, 约500至约1000mg/L的成熟胰岛素, 如正确释放时测量的或基于其已知的融合蛋白比例计算的。

[0259] 在一些实施方案中, 作为如表21中所述的具有N-末端融合伴侣的GCSF融合蛋白来产生GCSF。在一些实施方案中, 在0.5mL至100L, 0.5mL, 50mL, 100mL, 1L, 2L或更大规模下, 根据本发明方法的GCSF融合体的表达产生可溶性融合蛋白, 其包含至少100, 至少200, 至少250, 至少300, 至少400, 至少500, 或至少1000, 约100至约1000, 约200至约1000, 约300至约1000, 约400至约1000或约500至约1000mg/L的可溶性GCSF, 如正确释放时测量的或基于其已知的融合蛋白比例计算的。在一些实施方案中, 根据本发明方法的GCSF融合体的表达产生至少100, 至少200, 至少250, 至少300, 至少400, 至少500或至少1000mg/L的可溶性GCSF。在一些实施方案中, 在0.5mL至100L, 0.5mL, 50mL, 100mL, 1L, 2L或更大规模下, GCSF融合体的表达产生至少300, 至少350, 至少400, 至少450, 至少500, 至少550, 至少600, 至少650, 至少700, 至少850, 至少, 至少550, 至少600, 至少650, 约100至约1000, 约200至约1000, 约300至约1000, 约400至约1000或约500至约1000mg/L的总可溶性和不溶性GCSF。

[0260] 在一些实施方案中, 产生的重组融合蛋白的量为总细胞蛋白质的约1%至约75%。在特定实施方案中, 产生的重组融合蛋白的量为总细胞蛋白质的约1%, 约2%, 约3%, 约4%, 约5%, 约10%, 约15%, 约20%, 约25%, 约30%, 约35%, 约40%, 约45%, 约50%, 约55%, 约60%, 约65%, 约70%, 约75%, 约1%至约5%, 约1%至约10%, 约1%至约20%, 约1%至约30%, 约1%至约40%, 约1%至约50%, 约1%至约60%, 约1%至约75%, 约2%至约5%, 约2%至约10%, 约2%至约20%, 约2%至约30%, 约2%至约40%, 约2%至约50%, 约2%至约60%, 约2%至约75%, 约3%至约5%, 约3%至约10%, 约3%至约20%, 约3%至约30%, 约3%至约40%, 约3%至约50%, 约3%至约60%, 约3%至约75%, 约4%至约10%, 约4%至约20%, 约4%至约30%, 约4%至约40%, 约4%至约50%, 约4%至约60%, 约4%至约75%, 约5%至约10%, 约5%至约20%, 约5%至约30%, 约5%至约40%, 约5%至约50%, 约5%至约60%, 约5%至约75%, 约10%至约20%, 约10%至约30%, 约10%至约40%, 约10%至约50%, 约10%至约60%, 约10%至约75%, 约20%至约30%, 约20%至约40%, 约20%至约50%, 约20%至约60%, 约20%至约75%, 约30%至约40%, 约30%至约50%, 约30%至约60%, 约30%至约75%, 约40%至约50%, 约40%至约60%, 约40%至约75%, 约50%至约60%, 约50%至约75%, 约60%至约75%或约70%至约75%。

[0261] 溶解性和活性

[0262] 通常通过不同方式来测定蛋白质的“溶解性”和“活性”, 尽管还存在相关品质。蛋白质的溶解性, 特别是疏水性蛋白质, 指示疏水性氨基酸残基没有正确地位于折叠蛋白质的外部。蛋白质活性(其可以由本领域技术人员使用确定为适用于目标多肽的方法来评价)是正确的蛋白质构象的另一个指示。如本文中使用的“可溶的、活性的或两者”是指通过本领域技术人员已知的方法确定是可溶性的、活性的或同时可溶和活性的蛋白质。

[0263] 通常, 关于氨基酸序列, 术语“修饰”包括单独或组合的置换、插入、延长、删除和衍生。在一些实施方案中, 重组融合蛋白可以包括“非必需”氨基酸残基的一个或多个修饰。在这种情况下, “非必需”氨基酸残基是在新氨基酸序列中可以改变(例如, 删除或置换)而不消除或实质性地降低重组融合蛋白的活性(例如, 激动剂活性)的残基。例如, 重组融合蛋白

可以包括1,2,3,4,5,6,7,8,9,10或更多个置换,以连续方式或在整个重组融合蛋白分子中间隔的。单独地或与置换结合,重组融合蛋白可以包括1,2,3,4,5,6,7,8,9,10或更多个插入,同样以连续方式或在整个重组融合蛋白分子中间隔的。单独地或与置换和/或插入结合,重组融合蛋白还可以包括1,2,3,4,5,6,7,8,9,10或更多个删除,同样以连续方式或在整个重组融合蛋白分子中间隔的。单独地或与置换、插入和/或删除结合,重组融合蛋白还可以包括1,2,3,4,5,6,7,8,9,10或更多个氨基酸添加。

[0264] 置换包括保守性氨基酸置换。“保守性氨基酸置换”是其中氨基酸残基被具有相似侧链或物理化学特征(例如,静电、氢键结合、电子等排性、疏水性特征)的氨基酸替代的置换。氨基酸可以是天然存在的或非成熟的(natural)(非天然的)。具有相似侧链的氨基酸残基的家族是本领域已知的。这些家族包括具有碱性侧链的氨基酸(例如,赖氨酸、精氨酸、组氨酸)、具有酸性侧链的氨基酸(例如,天冬氨酸、谷氨酸)、具有不带电荷的极性侧链(例如,甘氨酸、天冬酰胺、谷氨酰胺、丝氨酸、苏氨酸、酪氨酸、甲硫氨酸、半胱氨酸)、具有非极性侧链的氨基酸(例如,丙氨酸、缬氨酸、亮氨酸、异亮氨酸、脯氨酸、苯丙氨酸、色氨酸)、具有 β -分支侧链的氨基酸(例如,苏氨酸、缬氨酸、异亮氨酸)和具有芳香族侧链的氨基酸(例如,酪氨酸、苯丙氨酸、色氨酸、组氨酸)。置换还可以包括非保守性变化。

[0265] 尽管本文中已经显示和描述了本发明的优选实施方案,但本领域技术人员将清楚这些实施方案只是通过举例的方式提供。本领域技术人员现在可想到各种改变、变化和置换而不脱离本发明。应当理解本文中所述的本发明实施方案的各种替代方式可以用于实施本发明。旨在以下的权利要求限定了本发明的范围并且由此涵盖这些权利要求及其等价体范围内的方法和结构。

[0266] 实施例

[0267] 实施例I:表达hPTH 1-34融合体的菌株的高通量筛选

[0268] 进行这个研究来测试由表达hPTH 1-34融合蛋白的荧光假单胞菌菌株产生的重组蛋白水平,所述融合蛋白包含DNAJ-样蛋白、Fk1B或FrnE作为N-末端融合伴侣。

[0269] 材料和方法

[0270] PTH 1-34融合蛋白表达质粒的构建:使用DNA2.0(一种基因设计和合成服务(Menlo Park,CA))合成了编码PTH 1-34融合蛋白的基因片段。每个基因片段包括荧光假单胞菌折叠调节子(DnaJ-样、Fk1B或FrnE)的编码序列,其与用于PTH 1-34和接头的编码序列融合。每个基因片段还包括用于限制酶SpeI和XhoI的识别序列、“Hi”核糖体结合位点和18碱基对的间隔体,其包括在编码序列上游添加的核糖体结合位点和限制位点(SEQ ID NO:58)以及三个终止密码子。作为SEQ ID NO:52-57提供了编码这些PTH 1-34融合蛋白的核苷酸序列。

[0271] 为了产生表达质粒p708-004、-005和-006(列于表6中),使用SpeI和XhoI限制酶消化PTH 1-34融合蛋白基因片段,并亚克隆至表达载体pDOW1169中,其含有pTac启动子和rrnT1T2转录终止子。pDOW1169在文献中有描述,例如,美国专利申请公开No.7,833,752,“Bacterial Leader Sequences for Increased Expression”和Schneider等,2005,“Auxotrophic markers pyrF and proC can replace antibiotic markers on protein production plasmids in high-cell-density Pseudomonas fluorescens fermentation”,Biotechnol.Progress 21(2):343-8,将两篇文献都按引用并入本文中。将

质粒电穿孔至感受态荧光假单胞菌DC454宿主细胞(pyrF⁺ lsc::lacI^{Q1})中。

[0272] 表6: PTH 1-34融合蛋白质粒

[0273]	质粒编号	N-末端融合伴侣	融合蛋白
	p708-004	DnaJ-样蛋白	DnaJ-样蛋白-PTH
	p708-005	FklB	FklB-PTH
	p708-006	FrnE	FrnE-PTH

[0274] DNA测序:使用BigDye® Terminator v3.1循环测序试剂盒(Applied Biosystems, 4337455),通过DNA测序证实了融合蛋白表达质粒中克隆片段的存在。通过混合1μL测序预混物、0.5μL的100μM引物原液、3.5μL测序缓冲液和添加至20μL终体积的水来制备含有待分析的50fmol质粒DNA的DNA测序反应物。使用Sequencher™软件(Gene Codes)来组合并分析结果。

[0275] 96-孔形式的生长和表达(HTP):将融合蛋白表达质粒转化至阵列形式的荧光假单胞菌宿主菌株中。通过混合35μL荧光假单胞菌感受态细胞和10μL体积的质粒DNA(2.5ng)来启动转化反应。将25μL等份的混合物转移至96孔多孔Nucleovette®平板(Lonza)中。使用Nucleofector™96-孔Shuttle™系统(Lonza AG)进行电穿孔,并随后将电穿孔的细胞转移至新鲜的96-孔深孔平板中,其含有500μL补充了1%葡萄糖培养基和微量元素的M9盐。将平板在30℃下振荡孵育48小时以产生种子培养物。

[0276] 将10μL等份的种子培养物一式两份地转移至96-孔深孔平板中。每个孔含有500μL HTP-YE培养基(Teknova),补充了微量元素和5%甘油。将放置在补充甘油的HTP培养基中的种子培养物在摇床中在30℃下孵育24小时。将0.3mM终浓度的异丙基-β-D-1-硫代半乳糖苷(IPTG)加入每个孔中以诱导PTH 1-34融合蛋白的表达。对于含有折叠调节子超表达质粒的菌株(参见表4),IPTG补充有1%终浓度的甘露糖醇(Sigma,M1902)以诱导折叠调节子的表达。此外,在诱导时,每孔添加0.01μL的250单位/μL储备Benzonase(Novagen,70746-3)以降低培养物粘度的可能。24小时诱导后,通过测量600nm下的光密度(OD₆₀₀)来计算细胞密度。随后收集细胞,并用1×磷酸盐缓冲盐水(PBS)1:3稀释至400μL的终体积,并冷冻用于之后的处理。

[0277] 用于分析表征的可溶性裂解物样品制备:将收集的细胞样品稀释并使用24探头尖角,通过使用细胞裂解自动化超声系统(CLASS,Scinomix)的超声处理来裂解。将裂解物在8℃下在5,500×g下离心15分钟。收集上清液,并标记为可溶性部分。收集沉淀物,通过另一轮超声处理重悬浮于400μL 1×PBS pH 7.4中,并且标记为不溶性部分。

[0278] SDS-CGE分析:使用具有HT Protein Express v2芯片的LabChip GXII仪器(Caliper LifeSciences)和相应的试剂(部件号分别为760499和760328,Caliper LifeSciences),通过HTP微芯片SDS毛细管凝胶电泳分析了可溶性和不溶性部分。按照制造商的实验方案(Protein User Guide Document No.450589,Rev.3)制备了样品。简而言之,将4μL等份的可溶性或不溶性部分样品与14μL缓冲液在95℃下加热的96-孔聚丙烯锥形孔PCR平板中混合5分钟(使用或不用二硫苏糖醇(DTT)还原剂),并用70μL去离子水稀释。将来自没有用融合蛋白表达质粒转化的空白宿主菌株的裂解物作为对照与测试样品平行运行,并且使用系统内部标准来定量。

[0279] 摇瓶表达:将用于待评价的每个融合蛋白表达菌株的种子培养物生长于M9 Glucose (Teknova) 中以产生中间培养物,并且将5mL体积的每个中间培养物用于接种含有250mL HTP培养基 (Teknova3H1129) 的四个1升带挡板底烧瓶中的各一个。在30℃下生长24小时后,用0.3mM IPTG和1%甘露糖醇诱导培养物,并在30℃下再孵育24小时。然后将烧瓶培养液离心以收集细胞,并将收集的细胞糊冷冻用于将来的使用。

[0280] 机械释放和纯化:将5克或10克量的冷冻细胞糊解冻并重悬浮于3×PBS,5%甘油,50mM咪唑pH7.4中以分别制备50mL或100mL的终体积。随后将悬浮液以15,000psi两次通过微流化床 (Microfluidics, Inc., 型号M 110Y) 均质化。将裂解物在12,000×g下离心30分钟并通过Sartorius Sartobran 150 (0.45/0.2μm) 囊式过滤器过滤。

[0281] 色谱:使用配备了Frac-950级分收集器的ÄKTA explorer 100色谱系统 (GE Healthcare) 进行快速蛋白质液相色谱 (FPLC) 操作。将从HTP表达培养液制备的可溶性部分样品加载于预先用3×PBS,5%甘油,50mM咪唑pH 7.4平衡的5mL HisTrap FF柱 (GE Healthcare, 部件号17-5248-02) 上。用4柱体积的平衡缓冲液洗涤柱子,并使用10柱体积的洗脱缓冲液,应用从50mM至200mM的咪唑线性梯度,从HisTrap柱洗脱融合蛋白。整个过程以100cm/h运行,其等于1.5分钟停留时间。使用上述的SDS-CGE分析方法,通过SDS-CGE分析了纯化级分。

[0282] 肠激酶切割:使用7000分子量截止 (MWC0) Slide-A-Lyzer盒 (Pierce),通过将含有融合蛋白的纯化级分相对补充了2mM CaCl₂的1×PBS pH 7.4在4℃下渗析过夜来制备第一组样品。将渗析的样品保持在约1mg/mL的浓度。用水通过含有融合蛋白的纯化级分的2×稀释来制备第二组样品,并以0.5mg/mL浓度储存于包含1.5×PBS、2.5%甘油和~30-70mM咪唑的缓冲液中。将猪肠激酶 (Sigma E0632-1.5KU) 的原液以5×或20×稀释 (分别对应于40μg/mL和10μg/mL的肠激酶浓度) 加入样品中。还添加CaCl₂至2mM终浓度,并且将反应混合物在室温下孵育过夜。

[0283] 液相色谱-质谱:将与配备了自动进样器、柱加热器和UV检测器的Agilent 1100HPLC偶联的具有电喷雾接口 (ESI) 的Q-ToF_{micro}质谱仪 (Waters) 用于液相色谱-质谱 (LC-MS) 分析。与保护柱 (Agilent, 目录号820950-923) 一起使用CN-反向柱,其具有2.1mm ID的内径、150mm的长度、5μm的粒径和300Å的孔径 (Agilent, 目录号883750-905)。在50℃的温度下进行了HPLC运行,并且在2℃下维持流速。HPLC缓冲液为0.1%甲酸 (流动相A) 和含有0.1%甲酸的90%乙腈 (流动相B)。将大约4μg融合蛋白样品加载至HPLC柱上。HPLC运行条件设定为加载样品时为95%流动相A。使用表7中示例的反相梯度来洗脱融合蛋白。

[0284] 表7:用于纯化的蛋白样品的质谱分析的反相梯度

[0285]

时间	%流动相A	%流动相B	流速 (ml/min)	曲线
0.0	95.0	5	0.2	--
10.0	90	10	0.2	线性
50.0	35	65	0.2	线性
52.0	0	100.0	0.2	线性
57.0	0	100.0	0.2	保持
57.1	95.0	5.0	0.2	阶梯

65.0	95.0	5.0	0.2	保持
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[0286] 在MS之前,从180nm至500nm收集UV吸收谱。ESI-MS源在2.5kV下以正离子模式使用。以每秒2次扫描,使用600-2600m/z范围进行MS扫描。使用MassLynx软件(Waters)分析了MS和UV数据。产生了UV色谱图和MS总离子电流(TIC)色谱图。总结了目标峰的MS谱。使用MaxEnt1(Waters)扫描针对2,800-6,000分子量范围(对于PTH 1-34,其具有4118kDa的理论分子量,以及对于融合蛋白或N-末端融合蛋白具有较高的窗口),1Da/通道的分辨率和0.25Da的高斯宽度,对这些谱去卷积。

[0287] 结果

[0288] PTH 1-34基因融合片段的设计:为了促进PTH 1-34融合蛋白的高水平表达,基于高可溶性表达、低于25kDa的分子量和显著不同于PTH 1-34(其具有8.52的pI)的等电点(pI),选择了来自荧光假单胞菌的三个折叠调节子,DnaJ-样蛋白(SEQ ID NO:2,细胞质伴侣蛋白)、FrnE(SEQ ID NO:3,细胞质PPI酶)和Fk1B(SEQ ID NO:4,周质PPI酶)。折叠调节子的特征显示于表8中。如表8中所示,DnaJ-样蛋白、Fk1B和FrnE的pI在4.6至4.8之间,与PTH 1-34的pI充分分开。这使得可用于通过离子交换分离。为了进一步帮助融合蛋白的纯化,在接头中包括六-组氨酸标签。接头还含有肠激酶切割位点(DDDDK)以促进N-末端融合伴侣与所需的PTH 1-34目标多肽的分离。PTH 1-34融合蛋白的氨基酸序列显示于图2A(DnaJ-样蛋白,SEQ ID NO:45)、2B(Fk1B-PTH,SEQ ID NO:46)和2C(FrnE-PTH,SEQ ID NO:47)中。在图2A、B和C中,对应于接头的氨基酸是加下划线的,以及对应于PTH 1-34的那些氨基酸是斜体的。

[0289] 表8:选定的N-末端融合伴侣的物理化学特性

融合伴侣	分子量 (Da)	1 μ g 的摩尔当量 (pM)	摩尔消光系数	1mg/mL 的 A[280nm] (AU-吸光度单位)	等电点 (pI)	pH 7 下的电荷
[0290] DnaJ-样蛋白(79 aa)	9176.27 Da	108.977	13370	1.46	4.83	-5.04
Fk1B(206 aa)	21770.89	45.933	17780	1.22	4.71	-9.94
[0291] FrnE(218 aa)	23945	41.762	24990	1.04	4.62	-14.77

[0292] PTH融合表达载体的构建和HTP表达:通过DNA 2.0合成编码表6中所列的三个PTH融合蛋白中每一个的合成基因片段。用SpeI和XhoI消化合成的基因片段并连接到pDOW1169(用相同的酶消化的),产生表达质粒p708-004、p708-005和p708-006。证实插入片段后,将质粒用于电穿孔一系列荧光假单胞菌宿主菌株并产生表4中所列的表达菌株。按照材料和方法中所述的程序,所得到的转化菌株生长并用IPTG和甘露糖醇诱导。诱导后,收集细胞,超声处理,并离心以分离可溶性和不溶性部分。收集可溶性和不溶性部分。使用还原的SDS-CGE来分析可溶性和不溶性部分以测量PTH 1-34融合蛋白表达水平。选择总共六个菌株,包括对于三种PTH 1-34融合蛋白中每一种的两个高HTP表达菌株,用于摇瓶表达。使用摇瓶表

达方法筛选的菌株列于表9中。

[0293] 摇瓶表达:按照材料和方法(摇瓶表达)部分中所述的,将六个菌株中的每一个以250mL培养规模(每个4×250mL培养物)生长并诱导。诱导后,保留来自每个培养样品(全细胞培养液,WCB);将样品亚组用PBS稀释3×,超声处理并离心以产生可溶性和不溶性部分。将每个培养的其余部分离心以产生细胞糊和无细胞培养液(CFB)上清液。保留细胞糊用于纯化。通过还原的SDS-CGE评价WCB、CFB和可溶性部分(图3)。

[0294] 在WCB和可溶性部分中观察到了融合蛋白(对于DnaJ-样蛋白-PTH融合体,对应于约14kDa分子量的条带;以及对于FrnE-PTH和Fk1B-PTH融合体,约26kDa分子量的条带);在CFB中没有观察到融合蛋白。STR35984、STR36085和STR36169的摇瓶表达滴度为HTP表达滴度的50%,而菌株STR35970、STR36034和STR36150的摇瓶表达滴度为HTP规模下观察到的滴度的70-100%。HTP和摇瓶表达滴度列于表9中。

[0295] 表9:选定的PTH 1-34融合蛋白表达菌株的HTP和摇瓶表达滴度

菌株条形码	质粒	宿主细胞	融合伴侣	大 小	HTP 表达滴度 (g/L)	摇瓶表达滴度 (g/L)
				(kDa)		
STR35970	p708-004	DC508-1	DnaJ-样蛋白	14	0.552	0.382
STR35984	p708-004	DC992.1-1	DnaJ-样蛋白	14	0.490	0.266
STR36034	p708-005	DC1106-1	Fk1B	26	0.672	0.573
STR36085	p708-005	PF1326.1-1	Fk1B	26	0.670	0.233
STR36150	p708-006	PF1219.9-1	FrnE	26	0.577	0.651
STR36169	p708-006	PF1331-1	FrnE	26	0.551	0.284

[0297] HTP和摇瓶规模下生长的PTH融合蛋白表达菌株的IMAC纯化以分离PTH融合蛋白:将六个菌株的细胞糊接受机械裂解和IMAC纯化。每个纯化运行获得了高度富集的级分。源自DnaJ-样蛋白-PTH表达菌株STR35970的峰级分为60-80%纯的,来自Fk1B-PTH表达菌株STR36034的那些峰级分为60-90%纯的,以及来自FrnE-PTH表达菌株STR36150的那些峰级分为90-95%纯的。

[0298] PTH融合蛋白的肠激酶切割:选择来自IMAC纯化运行的含有融合蛋白的高纯度、浓缩的级分用于肠激酶切割反应,以证实N-末端融合伴侣可以从PTH 1-34切割。将猪来源的肠激酶用于研究。由于4kDa PTH 1-34目标多肽不容易通过SDS-PAGE来检测,因此对于DnaJ-样蛋白-PTH融合蛋白14kDa至10kDa及对于Fk1B-PTH和FrnE-PTH融合蛋白26kDa至22kDa的总融合蛋白的分子量偏移接受为肠激酶切割的证据。用40μg/mL或10μg/mL肠激酶处理样品过夜。肠激酶处理后,通过SDS-CGE分析样品。如图4中与未切割样品(泳道1-6)相比的MW偏移所示的,在使用40μg/mL肠激酶时,观察到融合伴侣与PTH 1-34完全切割(泳道7-12),和在使用10μg/mL肠激酶时,观察到了部分切割(泳道13-18)。

[0299] 肠激酶切割后PTH融合蛋白的完整质量分析:将从菌株STR35970纯化的DnaJ-样蛋白-PTH融合蛋白用于另外的肠激酶切割实验和完整质量分析。用猪肠激酶将源自STR35970的含有DnaJ-样蛋白-PTH融合蛋白的纯化级分在室温下孵育1至3小时,接着立即进行完整质量分析。如图5中所示,检测到了C-末端PTH 1-34多肽。完整质量分析的详细内容概括于

表10中。除了全长PTH 1-34外,还检测到了对应于5或8个氨基酸的N-末端删除的片段。所观察到的蛋白水解很可能是由于宿主细胞蛋白污染物或猪肠激酶制备物中的污染物。通过相似的步骤,重组肠激酶也可以用于评价切割。对于通过完整质量分析检测到的主要物质,观察到的和理论的分子量(MW)显示于表10中。与接受肠激酶切割1至3小时的融合蛋白的27分钟的平均停留时间相比,未切割的融合蛋白的停留时间为约33分钟。

[0300] 表10:完整质量结果

			理论 MW:	DnaJ-样蛋白 -PTH	PTH 1-34
				15207.95	4117.8
样品名	观察的主要物质的 MW			观察 - 理论 MW	观察-理论 MW
[0301] DnaJ-样蛋白-PTH 级分(约 3 hrs 切割反应)		4118			0.2
		4118			0.2
		4119			1.2
	DnaJ-样蛋白-PTH 级分(无切割 反应)	15207		-1.0	
[0302]	140116 PTH (试剂蛋白 Cat # RAB-391)	4117			-0.8

[0303] 实施例II.大规模发酵和PTH 1-34融合蛋白的表达

[0304] 还针对荧光假单胞菌中的大规模表达评价了实施例1中所述的PTH 1-34融合蛋白,以鉴定用于大规模PTH 1-34生产的高生产力表达菌株。这个研究中筛选的荧光假单胞菌菌株是DnaJ-样蛋白-PTH融合表达菌株STR35970、STR35984、STR35949、STR36005、STR35985、FklB-PTH融合蛋白表达菌株STR36034、STR36085、STR36098以及FrnE-PTH融合蛋白表达菌株STR36150、STR36169,列于表11和12中。

[0305] 表11:用于大规模发酵的DnaJ-样蛋白-PTH融合表达菌株

菌株	质粒	宿主
STR35949	p708-004	DC1084
STR35970	p708-004	DC508
STR35984	p708-004	DC992.1
STR35985	p708-004	PF1201.9
STR36005	p708-004	PF1326.1

[0307] 表12:用于大规模发酵的FrnE-PTH和FklB-PTH融合表达菌株

[0308]	菌株	质粒	宿主
	STR36034	p708-005	DC1106
	STR36085	p708-005	PF1326.1
	STR36098	p708-005	PF1345.6
	STR36150	p708-006	PF1219.9
	STR36169	p708-006	PF1331

[0309] 材料和方法

[0310] MBR发酵:含有补充了酵母提取物的培养基的摇瓶接种选定菌株的冷冻的培养物储备物。对于微型生物反应器 (MBR), 使用含有50mL补充了酵母提取物的化学限定培养基的250mL摇瓶。将摇瓶培养物在30℃下振荡孵育16至24小时。将来自摇瓶培养物的等份试样用于接种MBR (Pall Micro-24)。将MBR培养物在pH、温度和溶解氧受控的条件下在一次性微型生物反应器盒的每个10mL孔中以4mL体积操作。当培养基中含有的初始含量的甘油耗尽时, 用IPTG诱导培养物。发酵持续16小时, 并且收集样品和冷冻用于分析。

[0311] CBR发酵:通过用选定菌株的冷冻培养物储备物接种含有600mL补充了酵母提取物和甘油的化学限定培养基的摇瓶来产生用于1升CBR (常规生物反应器) 发酵罐培养的接种物。在32℃下16至24小时振荡孵育后, 然后将来自每个摇瓶培养物的等份的等份试样无菌地转移至包含2升生物反应器 (1升工作体积) 的8单位多重发酵系统的每个中。补料-分批高细胞密度发酵过程由生长期接着诱导期组成, 一旦培养物达到目标光密度, 则诱导期通过添加IPTG来启动。

[0312] 允许发酵的诱导期进行8小时, 并且从发酵罐取出分析样品以测定575nm下的细胞密度 (OD_{575})。将分析样品冷冻用于随后的分析来测定融合蛋白表达水平。完成8小时诱导后, 通过在15,900×g离心60至90分钟来收集每个容器的整个发酵培养液 (每个2L生物反应器大约0.8L培养液)。分离细胞糊和上清液, 并且将细胞糊在-80℃下冷冻。

[0313] 机械均质化和纯化:将如上所述从CBR发酵过程获得的冷冻细胞糊 (20g) 解冻并重悬浮于20mM磷酸钠、5%甘油、500mM氯化钠、20mM咪唑pH 7.4中。调节悬浮液的终体积以确保固体物质的浓度为20%。然后将材料在15,000psi下两次通过微流化床 (Microfluidics, Inc., 型号M110Y) 来均质化。将裂解物在12,000×g下离心30分钟, 并通过Sartorius Sartobran 150 (0.45/0.2μm) 囊式过滤器过滤。

[0314] 色谱:使用配备了Frac-950级分收集器的ÄKTA explorer 100色谱系统 (GE Healthcare) 进行快速蛋白质液相色谱 (FPLC) 操作。将样品加载于HisTrap FF, 10mL柱上 (串联连接的两个5mL HisTrap FF筒 [GE Healthcare, 部件号17-5255-01]), 洗涤并通过将咪唑浓度从0mM改变至200mM, 使用10柱体积线性梯度的洗脱缓冲液洗脱。收集了2毫升体积的级分。

[0315] 使用镍IMAC (GE Healthcare, 部件号17-5318-01) 进行固定化金属离子亲和色谱 (IMAC) 纯化。将CBR发酵后收集的分析样品分离成可溶性和不溶性部分。用100μL IMAC树脂在摇床上在室温下孵育600μL等份可溶性部分一小时, 并在12,000×g下离心一分钟来沉淀树脂。取出上清液, 并标记为流过物。然后用1mL含有20mM磷酸钠pH 7.3, 500mM NaCl, 5%甘油和20mM咪唑的洗涤缓冲液将树脂洗涤三次。第三次洗涤后, 将树脂重悬浮于200μL含有

400mM咪唑的洗涤缓冲液中并离心。收集上清液并标记为洗脱液。

[0316] 肠激酶切割:将PTH 1-34融合蛋白纯化级分浓缩并重悬浮于含有20mM Tris pH 7.4、50mM NaCl和2mM CaCl₂的缓冲液中。将两个单位的肠激酶(Novagen cat#69066-3,批次D00155747)加入100μL反应的100μg蛋白中。将融合蛋白纯化级分和肠激酶的混合物在室温下孵育一小时或过夜。还将不含肠激酶的对照反应在室温下孵育一小时或过夜。通过添加含有4-盐酸苯磺酰氯(AEBSF, Sigma cat#P8465)的完全蛋白酶抑制剂混合物来停止酶反应。

[0317] 结果

[0318] DnaJ-样蛋白-PTH、Fk1B-PTH和FrnE-PTH融合表达菌株的发酵评价:针对发酵,首先在微型生物反应器(MBR)中,并且随后在常规生物反应器(CBR)中,各自评价了表9和10中所列的前五个表达DnaJ-样蛋白-PTH融合菌株、三个Fk1B-PTH表达菌株和两个FrnE-PTH表达菌株。

[0319] 按照实施例1的材料和方法部分中描述的实验方案,通过SDS-CGE分析了来自DnaJ-样蛋白-PTH融合表达菌株的每个MBR发酵的可溶性部分。DnaJ-样蛋白-PTH融合表达菌株的MBR发酵产率列于表13中。总体地,具有可溶性融合蛋白的最高MBR表达水平的菌株为STR35949,为2.1g/L。

[0320] 表13:MBR发酵罐中测试的DnaJ-样蛋白-hPTH融合菌株的可溶性融合蛋白产率

菌株	可溶性融合蛋白产率
STR35949	0.6-2.1g/L
STR36005	1.5g/L
STR35970	1.1g/L
STR35985	0.9g/L

[0322] 在常规生物反应器(CBR)中,评价1L规模的发酵的DnaJ-样蛋白PTH融合菌株。DnaJ-样蛋白-PTH融合蛋白菌株的CBR表达水平与MBR水平相当,如表14中所示。在诱导后8小时的时间点,表达水平高于诱导后24小时的时间点。

[0323] 表14:在诱导后8(I8)和24(I24)小时,在CBR发酵罐中评价的DnaJ-样-hPTH 1-34融合菌株的可溶性融合蛋白产率

菌株	可溶性融合蛋白产率-(I8)	可溶性融合蛋白产率-(I24)
STR35949	1.5-2.4g/L	1.1-1.9g/L
STR35970	2.0g/L	0.9g/L
STR35985	1.7-2.4g/L	0.3-0.6g/L
STR36005	2.1g/L	1.4g/L

[0325] 在还原条件下,通过SDS-CGE分析了来自Fk1B-PTH和FrnE-PTH融合表达菌株的MBR发酵的可溶性部分(结果显示于表15中)。

[0326] 表15:在MBR发酵罐中评价的Fk1B-hPTH 1-34和FrnE-hPTH1-34融合菌株的可溶性融合蛋白产率

菌株	可溶性融合蛋白产率
STR36085	6.4g/L

STR36034	3.4-5.8g/L
STR36098	3.4-4.7g/L
STR36150	0.8-2.2g/L

[0328] 总体地,具有可溶性融合蛋白的最高表达水平的菌株是STR36034,为6.4g/L。相同的菌株还针对常规生物反应器(CBR)中的大规模发酵进行评价(结果显示于表16中)。在CBR发酵中,具有最大产率的菌株为STR36034,其在24小时的诱导时间段后,以6.7g/L表达Fk1B-PTH融合蛋白。

[0329] 表16:在诱导后24小时(I24),在CBR发酵罐中评价的Fk1B-hPTH 1-34和FrnE-hPTH 1-34融合菌株的可溶性融合蛋白产率

[0330]	菌株	可溶性融合蛋白产率(I24)
	STR36034	4.9-6.7g/L
	STR36085	4.6-4.9g/L
	STR36098	2.9-5.2g/L
	STR36150	2.6-3.8g/L

[0331] DnaJ-样蛋白-PTH和Fk1B-PTH融合蛋白的纯化和肠激酶切割的评价:如材料和方法中所述的,将DnaJ-样蛋白-PTH融合表达菌株STR36005诱导表达和生长后获得的细胞糊接受机械裂解和IMAC纯化。每个纯化运行获得高度富集的级分。峰级分的纯度为90%或更高。

[0332] 将从菌株36005纯化的DnaJ-样蛋白-PTH融合蛋白的高纯度浓缩级分用于肠激酶切割测试,以证实N-末端融合伴侣可以与PTH 1-34目标多肽切割。将重组牛肠激酶用于切割反应。使用IMAC树脂将来自分析规模样品的可溶性部分用于融合蛋白的小规模批次富集(图6)。用肠激酶孵育一小时后,观察到了DnaJ-样蛋白融合伴侣的部分切割。过夜孵育后,切割完成(泳道6-8)。

[0333] Fk1B-PTH融合菌株显示出在1升规模下是强健的。进一步分析纯化样品来证实融合蛋白可以富集和用肠激酶切割。使用IMAC树脂,将来自分析规模样品的可溶性部分用于融合蛋白的小规模批次富集。用肠激酶处理三个表达菌株STR36034、STR36085和STR36098每一个的一个富集样品,并使用实施例1中所述的方法进行完整质量分析。对于每个样品,确认了PTH 1-34目标多肽并观察为正确的质量,~4118Da,如图7中所示的。

[0334] 实施例III.肠激酶融合体的构建

[0335] 设计了DnaJ-样蛋白、Fk1B和FrnE N-末端融合伴侣-肠激酶融合蛋白并产生了表达构建体,其用于表达重组肠激酶(SEQ ID NO:31)。

[0336] 肠激酶融合表达质粒的构建:评价的肠激酶(EK)融合编码区列于表17中。通过DNA 2.0合成了编码融合蛋白的基因片段。该片段包括SpeI和XhoI限制酶位点、“Hi”核糖体结合位点、编码序列上游添加的18碱基对间隔体(5'-actagtaggaggtctaga-3')和三个终止密码子。

[0337] 使用标准克隆方法来构建表达质粒。使用SpeI和XhoI限制酶消化了含有各个肠激酶融合编码序列的质粒DNA,然后亚克隆至SpeI-XhoI消化的含有pTac启动子和IrrnT1T2转录终止子的pDOW1169表达载体中。用T4 DNA连接酶(Fermentas EL0011)连接插入片段和载体过夜,形成肠激酶融合蛋白表达质粒。将质粒电穿孔至感受态荧光假单胞菌DC454宿主细

胞中。通过使用Ptac和Term序列引物(来自Quanta的AccuStart II,PCR SuperMix,95137-500)的PCR,针对肠激酶融合蛋白序列插入片段的存在筛选阳性克隆。

[0338] 表17:肠激酶融合蛋白

[0339]	基因 ID	融合伴侣	融合蛋白
	<u>EK1</u>	DnaJ-样蛋白(SEQ ID NO: 2)	DnaJ-样蛋白肠激酶(SEQ ID NO: 48)
	<u>EK2</u>	Fk1B(SEQ ID NO: 4)	Fk1B-肠激酶(SEQ ID NO: 49)
	<u>EK4</u>	EcpD(SEQ ID NO: 65)	EcpD-肠激酶(SEQ ID NO: 50)
	<u>EK5</u>	无	肠激酶 SEQ ID NO: 51

[0340] 实施例IV.肠激酶融合蛋白(DNAJ-样、Fk1B、FrnE N-末端伴侣)的大规模发酵

[0341] 按照与实施例I中所述相似的那些方法,通过HTP分析,针对重组蛋白的表达测试了实施例III中所述的表达菌株。

[0342] 基于可溶性融合蛋白表达水平,选择表达菌株用于发酵研究。如以上针对PTH 1-34融合蛋白所描述的,将选定的菌株生长并诱导,并将诱导的细胞离心、裂解和再次离心。使用上述提取条件提取所得到的不溶性部分和可溶性部分,并使用SDS-CGE定量EK融合蛋白提取物上清液。

[0343] 实施例V.表达胰岛素融合蛋白的菌株的高通量筛选

[0344] 进行这一研究来测试通过表达前胰岛素融合蛋白的荧光假单胞菌菌株产生的重组蛋白水平,所述融合蛋白包含DNAJ-样蛋白、EcpD、Fk1B、FrnE或者EcpD、Fk1B、FrnE的截短体作为N-末端融合伴侣。

[0345] 材料和方法

[0346] 前胰岛素表达载体的构建:通过DNA 2.0(Menlo Park,CA)合成了编码前胰岛素(甘精胰岛素)的优化基因片段。基因片段和通过基因片段内所含的前胰岛素编码序列编码的前胰岛素氨基酸序列列于表18中。每个基因片段含有肽A和B编码序列,以及四种不同甘精C肽序列中的一种:CP-A(MW=9336.94Da;pI=5.2;65%的A+B甘精)、CP-B(MW=8806.42Da;69%的A+B甘精)、CP-C(MW=8749.32Da;69%的A+B甘精)和CP-D(MW=7292.67Da;83%的A+B甘精)。该基因片段设计在前胰岛素编码序列上游和下游添加SapI限制酶位点,以使得能够将基因片段快速克隆至各种表达载体中。基因片段还在5'侧翼区内包括赖氨酸氨基酸密码子(AAG)或精氨酸氨基酸密码子(CGA),以促进连接分别含有肠激酶切割位点或胰蛋白酶切割位点的表达载体中。此外,在所有基因片段的3'侧翼区内包括三个终止密码子(TGA、TAA、TAG)。

[0347] 表18:前胰岛素基因片段和C-肽氨基酸序列

[0348]

基因片段	核苷酸序列	甘精 B-肽	甘精 C-肽	甘精 A-肽	前胰岛素氨基酸序列	pI	MW KDa
G737-001	SEQ ID NO: 80	SEQ ID NO: 93	CP-A SEQ ID NO: 97	SEQ ID NO: 92	SEQ ID NO: 88	5.2	9.34
G737-002	SEQ ID NO: 81	SEQ ID NO: 93	CP-B SEQ ID NO: 98	SEQ ID NO: 92	SEQ ID NO: 89	6.07	8.81
G737-003	SEQ ID NO: 82	SEQ ID NO: 93	CP-C SEQ ID NO: 99	SEQ ID NO: 92	SEQ ID NO: 90	5.52	8.75
G737-007	SEQ ID NO: 83	SEQ ID NO: 93	CP-D SEQ ID NO: 100	SEQ ID NO: 92	SEQ ID NO: 91	6.07	7.29
G737-009	SEQ ID NO: 84	SEQ ID NO: 93	CP-A SEQ ID NO: 97	SEQ ID NO: 92	SEQ ID NO: 88	5.2	9.34
G737-017	SEQ ID NO: 85	SEQ ID NO: 93	CP-B SEQ ID NO: 98	SEQ ID NO: 92	SEQ ID NO: 89	6.07	8.81
G737-018	SEQ ID NO: 86	SEQ ID NO: 93	CP-C SEQ ID NO: 99	SEQ ID NO: 92	SEQ ID NO: 90	5.52	8.75
G737-031	SEQ ID NO: 87	SEQ ID NO: 93	CP-D SEQ ID NO: 100	SEQ ID NO: 92	SEQ ID NO: 91	6.07	7.29

[0349] 然后通过使用T4 DNA连接酶(New England Biolabs,M0202S),将编码序列连接至表达载体中,将前胰岛素克隆序列亚克隆至含有不同融合伴侣的表达载体中(表19)。将连接的载体以96-孔形式电穿孔至感受态DC454荧光假单胞菌细胞中。

[0350] 表19:用于甘精前胰岛素融合蛋白表达的载体

[0351]

表达载体	N-末端融合伴侣-切割位点	氨基酸序列 (SEQ ID NO)	核酸序列 (SEQ ID NO)	蛋白质大小 (KDa)	pI
pFNX4401	DnaJ-样蛋白-胰蛋白酶	SEQ ID NO: 101	SEQ ID NO: 202	10.67	6.03
pFNX4402	EcpD1-胰蛋白酶	SEQ ID NO: 102	SEQ ID NO: 203	28.52	9.15
pFNX4403	EcpD2-胰蛋白酶	SEQ ID NO: 104	SEQ ID NO: 204	12.25	9.78
pFNX4404	EcpD3-胰蛋白酶	SEQ ID NO: 105	SEQ ID NO: 205	7.04	9.70
pFNX4405	FklB-胰蛋白酶	SEQ ID NO: 106	SEQ ID NO: 206	23.27	5.41
pFNX4406	FklB2-胰蛋白酶	SEQ ID NO: 107	SEQ ID NO: 207	12.07	6.04
pFNX4407	FklB3-胰蛋白酶	SEQ ID NO: 108	SEQ ID NO: 208	6.85	6.28
pFNX4408	FrnE-胰蛋白酶	SEQ ID NO: 109	SEQ ID NO: 209	25.44	5.12
pFNX4409	FrnE2-胰蛋白酶	SEQ ID NO: 110	SEQ ID NO: 210	12.7	5.85
pFNX4410	FrnE3-胰蛋白酶	SEQ ID NO: 111	SEQ ID NO: 211	7.17	5.90
pFNX4411	DnaJ-样蛋白-EK	SEQ ID NO: 112	SEQ ID NO: 212	11.11	5.32

[0352]

pFNX4412	EcpD1-EK	SEQ ID NO: 113	SEQ ID NO: 213	28.95	7.26
pFNX4413	EcpD2-EK	SEQ ID NO: 114	SEQ ID NO: 214	12.68	8.05
pFNX4414	EcpD3-EK	SEQ ID NO: 115	SEQ ID NO: 215	7.48	7.22
pFNX4415	FklB-EK	SEQ ID NO: 116	SEQ ID NO: 216	23.70	4.99
pFNX4416	FklB2-EK	SEQ ID NO: 117	SEQ ID NO: 217	12.49	5.19
pFNX4417	FklB3-EK	SEQ ID NO: 118	SEQ ID NO: 218	7.28	5.22
pFNX4418	FrnE-EK	SEQ ID NO: 119	SEQ ID NO: 219	25.88	4.84
pFNX4419	FrnE2-EK	SEQ ID NO: 120	SEQ ID NO: 220	13.13	5.17
pFNX4420	FrnE3-EK	SEQ ID NO: 121	SEQ ID NO: 221	7.60	4.99

[0353] 以96孔形式生长和表达 (HTP): 将含有前胰岛素编码序列和融合伴侣的质粒转化至荧

光假单胞菌DC454宿主菌株中。将25微升感受态细胞解冻,转移至96孔多孔Nucleovette®平板(Lonza VHNP-1001)中并与之前的步骤中制备的连接混合物混合。使用Nucleovette™96-孔Shuttle™系统(Lonza AG)进行了电穿孔,并且随后将转化的细胞转移至含有400μL的M9盐1%葡萄糖培养基和微量元素的96-孔深孔平板(种子平板)中。将种子平板在30℃下振荡孵育48小时以产生种子培养物。

[0354] 将10微升种子培养物一式两份转移至新鲜的96-孔深孔平板中,每孔含有500μL HTP培养基(Teknova 3H1129)(补充了微量元素和5%甘油),并在30℃下振荡孵育24小时。将异丙基-β-D-1-硫代半乳糖苷(IPTG)以0.3mM的终浓度加入每个孔中以诱导前胰岛素融合蛋白的表达。此外,在诱导时,每孔添加0.01μL的250单位/μl储备Benzonase(Novagen,70746-3)以降低培养物粘度的潜能。诱导后24小时,通过测量600nm下的光密度(OD_{600})来定量细胞密度。诱导后24小时,收集细胞,并用1×PBS 1:3稀释至400μL终体积,并随后冷冻用于之后的处理。

[0355] 用于分析表征的可溶性裂解物样品制备:将按照以上所述制备并冷冻储存的培养液样品解冻、稀释和超声处理。将通过超声处理获得的裂解物在8℃的温度下在5,500×g下离心15分钟以分离可溶性(上清液)和不溶性(沉淀)部分。使用超声处理将不溶性部分重悬浮于PBS中。

[0356] SDS-CGE分析:使用具有HT Protein Express v2芯片的LabChip GXII仪器(PerkinElmer)和相应的试剂(部件号分别为760499和760328,PerkinElmer),通过HTP微芯片SDS毛细管凝胶电泳分析了按照以上的讨论制备的测试蛋白质样品。按照制造商的实验方案(Protein User Guide Document No.450589,Rev.3)制备了样品。在96-孔锥形孔PCR平板中,将4μL样品与14μL缓冲液混合,具有或不具有二硫苏糖醇(DTT)还原剂。将混合物在95℃下加热5分钟,并通过添加70μL去离子水来稀释。

[0357] 基于融合蛋白滴度乘以由前胰岛素组成的融合蛋白的百分比测定96-孔规模下的前胰岛素滴度。总滴度表示可溶性和不溶性靶表达的总和(mg/L)。

[0358] 结果

[0359] 如表20中所示,具有DnaJ-样蛋白作为N-末端融合伴侣的甘精前胰岛素融合蛋白显示出前胰岛素表达的最高水平。令人惊讶地,含有最小形式的EcpD融合伴侣(50个氨基酸的融合伴侣EcpD3)的前胰岛素融合蛋白与全长融合伴侣EcpD1和100个氨基酸的截短形式EcpD2相比,显示出较高的表达水平。对于含有Fk1B或FrnE末端融合伴侣的前胰岛素融合蛋白,分别与最小融合伴侣片段Fk1B3和FrnE3融合的前胰岛素的表达等于或略低于具有较长N-末端融合伴侣的构建体的表达。表20概括了在高通量表达研究过程中观察到的前胰岛素蛋白滴度,可溶性的和总的。

[0360] 因此,成熟甘精胰岛素确定为在胰蛋白酶切割后成功地从纯化的融合蛋白(和C-肽)释放出来。对选定的融合蛋白(DnaJ构建体G737-031和Fk1B构建体G737-009,在非变性浓度的脲存在下纯化的,和FrnE1构建体G737-018,在没有脲的情况下纯化的)进行的IMAC富集接着胰蛋白酶切割证明了融合蛋白切割以,产生成熟胰岛素,如通过SDS-PAGE或SDS-CGE评价的,与甘精胰岛素标准品相比较。受体结合测试进一步表明了活性。

[0361] 表20:示例性前胰岛素融合蛋白的HTP表达滴度

[0362]

前胰岛素基 因片段(表 18 中的 SEQ ID NO)	N-末端融合伴侣-切 割位点(表 19 中的 SEQ ID NO)	C-肽序列 (表 18 中 的 SEQ ID NO)	可溶性前 胰岛素滴 度(mg/L)	总前胰岛素滴 度(mg/L) (可 溶性+不溶性 部分)
G737-001	DnaJ-样蛋白-EK	CP-A	66	235
G737-002	DnaJ-样蛋白-EK	CP-B	81	241
G737-003	DnaJ-样蛋白-EK	CP-C	88	267
G737-007	DnaJ-样蛋白-EK	CP-D	50	499
G737-009	DnaJ-样蛋白-胰蛋 白酶	CP-A	9	136
G737-017	DnaJ-样蛋白-胰蛋 白酶	CP-B	7	81
G737-018	DnaJ-样蛋白-胰蛋 白酶	CP-C	21	331
G737-031	DnaJ-样蛋白-胰蛋 白酶	CP-D	10	487
G737-001	FklB-EK	CP-A	50	445
G737-002	FklB-EK	CP-B	38	321
G737-003	FklB-EK	CP-C	33	210
G737-007	FklB-EK	CP-D	10	343
G773-009	FklB-胰蛋白酶	CP-A	8	578
G737-017	FklB-胰蛋白酶	CP-B	23	375

[0363]

G737-018	FklB-胰蛋白酶	CP-C	18	59
G737-031	FklB-胰蛋白酶	CP-D	10	321
G737-001	FklB2-EK	CP-A	7	528
G737-002	FklB2-EK	CP-B	46	60
G737-003	FklB2-EK	CP-C	36	69
G737-007	FklB2-EK	CP-D	22	339
G773-009	FklB2-胰蛋白酶	CP-A	10	658
G737-017	FklB2-胰蛋白酶	CP-B	6	92
G737-018	FklB2-胰蛋白酶	CP-C	16	20
G737-031	FklB2-胰蛋白酶	CP-D	11	193
G737-001	FklB3-EK	CP-A	13	565
G737-002	FklB3-EK	CP-B	10	109
G737-003	FklB3-EK	CP-C	11	26
G737-007	FklB3-EK	CP-D	10	12
G737-009	FklB3-胰蛋白酶	CP-A	12	222
G737-017	FklB3-胰蛋白酶	CP-B	9	108
G737-018	FklB3-胰蛋白酶	CP-C	17	70
G737-031	FklB3-胰蛋白酶	CP-D	15	457
G737-001	FrnE-EK	CP-A	132	258
G737-007	FrnE-EK	CP-D	16	52
G737-009	FrnE-胰蛋白酶	CP-A	30	65
G737-017	FrnE-胰蛋白酶	CP-B	41	63
G737-018	FrnE-胰蛋白酶	CP-C	43	56
G737-031	FrnE-胰蛋白酶	CP-D	13	218
G737-009	FrnE2-胰蛋白酶	CP-A	20	96
G737-017	FrnE2-胰蛋白酶	CP-B	6	39
G737-018	FrnE2-胰蛋白酶	CP-C	13	53
G737-007	FrnE2-EK	CP-D	10	219
G737-031	FrnE2-胰蛋白酶	CP-D	5	201

[0364]

G737-001	FrnE3-EK	CP-A	18	266
G737-002	FrnE3-EK	CP-B	10	248
G737-003	FrnE3-EK	CP-C	9	171
G737-007	FrnE3-EK	CP-D	13	161
G773-009	FrnE3-胰蛋白酶	CP-A	8	144
G737-017	FrnE3-胰蛋白酶	CP-B	8	49
G737-018	FrnE3-胰蛋白酶	CP-C	17	22
G737-031	FrnE3-胰蛋白酶	CP-D	7	307
G737-001	EcpD1-EK	CP-A	9	194
G737-002	EcpD1-EK	CP-B	5	131
G737-003	EcpD1-EK	CP-B	5	132
G737-007	EcpD1-EK	CP-D	5	22
G773-009	EcpD1-胰蛋白酶	CP-A	21	86
G737-017	EcpD1-胰蛋白酶	CP-B	16	39
G737-018	EcpD1-胰蛋白酶	CP-C	27	74
G737-031	EcpD1-胰蛋白酶	CP-D	4	206
G737-001	EcpD2-EK	CP-A	16	21
G737-002	EcpD2-EK	CP-B	9	24
G737-003	EcpD2-EK	CP-C	9	29
G737-007	EcpD2-EK	CP-D	9	60
G773-009	EcpD2-胰蛋白酶	CP-A	18	125
G737-017	EcpD2-胰蛋白酶	CP-B	6	9
G737-018	EcpD2-胰蛋白酶	CP-C	7	34
G737-031	EcpD2-胰蛋白酶	CP-D	5	33
G737-001	EcpD3-EK	CP-A	8	81
G737-002	EcpD3-EK	CP-B	15	18
G737-003	EcpD3-EK	CP-C	17	64
G737-007	EcpD3-EK	CP-D	10	169
G773-009	EcpD3-胰蛋白酶	CP-A	8	40

[0365]	G737-017	EcpD3-胰蛋白酶	CP-B	9	9
	G737-018	EcpD3-胰蛋白酶	CP-C	10	12
	G737-031	EcpD3-胰蛋白酶	CP-D	7	57

[0366] 实施例VI.GCSF融合蛋白的高通量筛选

[0367] 进行这个研究来测试由表达GCSF融合蛋白的荧光假单胞菌菌株产生的重组GCSF蛋白的水平,所述融合蛋白含有DnaJ-样蛋白、不同长度的FklB(FklB、FklB2或FklB3)、FrnE(FrnE、FrnE2或FrnE3)或EcpD(EcpD1、EcpD3或EcpD3)作为N-末端融合伴侣。

[0368] 材料和方法

[0369] GCSF表达载体的构建:通过DNA2.0(Menlo Park,CA)合成了AGCSF基因片段(SEQ ID NO.68),其含有优化的gcsf编码序列、编码序列下游和上游的用于限制酶SapI的识别序列和编码序列下游的三个终止子。用限制酶SapI消化质粒pJ201:207232的GCSF基因片段以产生含有优化的gcsf编码序列的片段。然后通过使用T4 DNA连接酶(Fermentas EL0011)连接GCSF基因片段和表达载体将gcsf编码序列亚克隆至含有不同融合伴侣的表达载体中,并以96-孔形式电穿孔至感受态荧光假单胞菌DC454宿主细胞中。在GCSF和各N-末端融合伴侣之间的接头中包括六组氨酸标签,连同肠激酶切割位点(DDDK),用于从GCSF释放N-末端融合伴侣。所得到的含有融合蛋白构建体的质粒列于表21的第三栏中。

[0370] 表21:用于GCSF融合蛋白表达的质粒

[0371]	表达载体	融合伴侣-切割位点	GCSF 表达质粒	大 小 (kDa)	GCSF 大小	融合体的 %GCSF	融合蛋白大小
	pFNX4411	DnaJ-样蛋白-EK	p529-301	11	19	0.63	30
	pFNX4412	EcpD1-EK	p529-302	29	19	0.40	48

[0372]	表达载体	融合伴侣-切割位点	GCSF 表达质粒	大 小 (kDa)	GCSF 大小	融合体的 %GCSF	融合蛋白大小
	pFNX4413	EcpD2-EK	p529-303	13	19	0.60	32
	pFNX4414	EcpD3-EK	p529-304	7	19	0.72	27
	pFNX4415	FklB-EK	p529-305	24	19	0.45	43
	pFNX4416	FklB2-EK	p529-306	12	19	0.61	32
	pFNX4417	FklB3-EK	p529-307	7	19	0.73	27
	pFNX4418	FrnE-EK	p529-308	26	19	0.43	45
	pFNX4419	FrnE2-EK	p529-309	13	19	0.59	32
	pFNX4420	FrnE3-EK	p529-310	8	19	0.72	27

[0373] 96孔形式的生长和表达(HTP):将含有用于gcsf基因和N-末端融合伴侣的编码序列的质粒转化至一系列荧光假单胞菌宿主菌株中。将35微升荧光假单胞菌感受态细胞解冻并与10 μ L的10 $\times$ 稀释的质粒DNA(2.5ng)混合。使用NucleofectorTM96-孔ShuttleTM系统

(Lonza AG) 将25微升混合物转移至96孔多孔 **Nucleovette®** 平板 (Lonza VHNP-1001) 中用于通过电穿孔转化, 并随后将转化的细胞转移至96-孔深孔平板 (种子平板) 中, 其含有500 μ L的M9盐1%葡萄糖培养基和微量元素。将种子平板在30℃下振荡孵育48小时以产生种子培养物。

[0374] 将10微升种子培养物一式两份地转移至新鲜的96-孔深孔平板中, 每个孔含有500 μ L HTP培养基 (Teknova 3H1129) (补充了微量元素和5%甘油), 并在30℃下振荡孵育24小时。将异丙基- β -D-1-硫代半乳糖苷 (IPTG) 以0.3mM终浓度加入每个孔中以诱导GCSF融合蛋白的表达。在超表达折叠调节子的假单胞菌菌株 (FM0菌株) 中, 连同IPTG一起加入1%终浓度的甘露糖醇 (Sigma, M1902) 以诱导折叠调节子的表达。此外, 在诱导时, 每孔添加0.01 μ L 250单位/ μ L储备Benzonase (Novagen, 70746-3) 以降低培养物粘度的可能。诱导后24小时, 通过测量600nm下的光密度 (OD_{600}) 来定量细胞密度。诱导后24小时, 收集细胞, 并用1 $\times$ PBS 1:3稀释至400 μ L的终体积, 随后冷冻用于之后的处理。

[0375] 用于分析表征的可溶性裂解物样品制备: 将按照以上所述制备并冷冻的培养液样品解冻、稀释并使用具有24探头尖角的细胞裂解自动化超声系统 (CLASS, Scinomix) 超声处理。将通过超声处理获得的裂解物在8℃的温度下, 在5,500 $\times$ g下离心15分钟以分离可溶性 (上清液) 和不溶性 (沉淀) 部分。还是通过超声处理, 将不溶性部分重悬浮于400 μ L的PBS, pH 7.4中。

[0376] SDS-CGE分析: 使用具有HT Protein Express v2芯片的LabChip GXII仪器 (Caliper LifeSciences) 和相应的试剂 (部件号分别为760499和760328, Caliper LifeSciences), 通过HTP微芯片SDS毛细管凝胶电泳分析了按照以上的讨论制备的测试蛋白质样品。按照制造商的实验方案 (Protein User Guide Document No.450589, Rev.3) 制备了样品。在96-孔锥形孔PCR平板中, 将4 μ L样品与14 μ L缓冲液混合, 具有或不具有二硫苏糖醇 (DTT) 还原剂。将混合物在95℃下加热5分钟, 并通过添加70 μ L去离子水来稀释。与测试蛋白质样品平行, 还分析了来自不含融合蛋白的菌株 (空白菌株) 的裂解物。使用系统内部标准定量空白菌株裂解物而没有背景减除。在HTP筛选过程中, 定量了每个菌株的一个样品; 通常, SDS-CGE方法的标准偏差为 $\sim 10\%$ 。

[0377] 结果

[0378] 使用融合伴侣方法, 在96-孔规模下获得了GCSF的高水平表达, 所述方法呈现了筛选蛋白酶缺陷宿主以鉴定能够高水平表达N-末端Met-GCSF的菌株的可替代方案。融合蛋白和GCSF滴度 (基于总融合蛋白的GCSF百分比计算的, 按MW计) 显示于表22中。野生型菌株DC454产生了484mg/L融合蛋白, 和305mg/L具有dnaJ融合伴侣的GCSF。所有融合伴侣构建体产生了超过100mg/L的融合蛋白滴度, 如表22中所示的。在HTP规模下观察到的这些高水平显示出在摇瓶或发酵规模下表达的巨大前景。此外, 普遍观察到在HTP和较大规模培养之间体积滴度的显著提高。在之前的研究中, prtB蛋白酶缺陷菌株显示出在0.5mL规模下能够表达 ~ 247 mg/L Met-GCSF (H.Jin等, 2011, Protein Expression and Purification 78:69-77和美国专利No.8,455,218)。在本研究中, 如所述的, 即使在不具有蛋白酶缺陷的宿主细胞中, 也观察到了作为融合蛋白的部分的高水平Met-GCSF的表达。注意到通过作为任一个所述融合蛋白的部分表达并通过蛋白酶切割释放而获得的Met-GCSF制备物实际上含有100%Met-GCSF (并且没有des-Met-GCSF), 因为切割是在除去任何蛋白酶后进行的。

[0379] 表22:GCSF融合蛋白的HTP表达滴度

[0380]	融合伴侣-切割位点	融合体滴度 (mg/L)	融合体中的靶标%	GCSF 滴度 (mg/L)
	DnaJ-样蛋白 EK	155-758	63	98-478
	EcpD1-EK (FL EcpD)	247-542	40	96-211
	EcpD2-EK	101-112	60	61-67
	EcpD3-EK	137-249	72	99-179
	FklB1-EK(FL FklB)	226-565	44	99-249
	FklB2-EK	171-362	60	103-217
	FklB3-EK	79-145	72	57-104
	FrnE1-EK (FL FrnE)	241-763	42	101-320
	FrnE2-EK	---	59	---
	FrnE3-EK	141-260	71	100-185

[0381] 尽管本文中已经显示和描述了本发明的优选实施方案,但本领域技术人员清楚只是通过举例来提供这样的实施方案。本领域技术人员现在可想到各种改变、变化和置换而不脱离本发明。应当理解本文中所述的本发明的实施方案的各种替换方案可以用于实施本发明。确定以下的权利要求限定了本发明的范围并且由此涵盖这些权利要求及其等价体的范围内的方法和结构。

[0382] 序列表

[0383]

SEQ ID NO.	蛋白/基因名称	序列
1	PTH 1-34	SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNF
2	DnaJ-样蛋白 (荧光假单胞菌)	MKVEFGLYQHYKGPQYRVFSVARHSETEEEVVFYQALYGEYGF WVRPLSMFLETVEVDGEQVPRFALVTAEPSLFTGQ
3	FrnE (荧光假单胞菌)	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAESQANRARI RDMGAA LGFAFRTDGQSRIYNTFDAHRLHWHAGLEGLQYNLKEALFKAY FSDGQDPSDHATLAI IAESVGLDLARAAEILASDEYAAEVREQ EQLWVSRGVSSVPTIVFNDQYAVSGGQPAEAFVGAI RQIINES KS
4	FklB (荧光假单胞菌) RXF6034.1 (全长)	MSEVNLSTDETRVSYGIGRQLGDQLRDNPFGVSLDAILAGLT DAFAGKPSRVDQE QMAASFKVIREIMQAEAAKAEAAAGAGLA FLAENAKRDGITT LASGLQFEVLTAGTGAKPTREDQVRTHYHG TLIDGTVFDSSYERGQPAEFVGGVIAGWTEALQLMNAGSKWR VYVPSELAYGAQGVGSI PPHSVLVFDVELLDVL
5	FklB/FkbP RXF05753.2	MSRYLFLVFLAICVADASEQPSSNITDATPHDLAYSLGASLG ERLRQEV PDLQIQALLDGLKQAYQGKPLALDKARIEQILSQHE AQNTADAQLPQSEKALAAEQQFLTREKAAAGVRQLADGILLTE LAPGTGNKPLASDEVQVKYVGRLPDGT VFDKSTQPQWFRVNSV ISGWSSALQQMPVGAKWRLVIPSAQAYGADGAGELIPPYTPLV FEIELLGTRH
6	SecB RXF02231.1 (荧光假单胞菌)	MTDQQNTEAAQDQGPFSLQRIYVRDLSFEAPKSPAIFRQEW PSVALDLNTRQKSLEGDFHEVVLTLSVTVKNGEEVAFIAEVQQ AGIFLIQGLDEASMSHTLGAFCPNILFPYARETLD SLVTRGSF PALMLAPVNF DALYAQELQRMQQEGAPT VQ
7	EcpD RXF04553.1	MGCVPLPDHGITVFMFLRMVLLACGLLV LAPPPADAALKIEG TRLIYFGQDKAAGISVVNQASREV VVQTWITGEDESADRTVPF

[0384]

	(荧光假单胞菌)	AATEPLVQLGAGEHHKLRILYAGEGLPSDRESLFWLNIMEIPL KPEDPNSVQFAIRQLKLFYRPPALQGGSAEAVQQLVWSSDGR TVTVNNPSAFHLSLVNLRIDSQTLSDYLLLKPKHERKTLTALDA VPKGATLHFTEITDIGLQARHSTALN
8	Skp (大肠杆菌, 成熟形式)	ADKIAIVNMGSLFQQVAQKTGVSNTLENEFKGRASELQRMETD LQAKMKKLQSMKAGSDRTKLEKDVMAQRQTFAQKAQAFEQDRA RRSNEERGKLVTRIQTAVKSVANSQDIDLVDANAVAYNSSDV KIDITADV LKQVK
9	接头	GGGSGGGGHHHHHDDDDK
10	接头	GGGSGGGGHHHHHHRKR
11	接头	GGGSGGGGHHHHHRRR
12	接头	GGGSGGGGHHHHHHLVPR
13	肠激酶切割序列	DDDDK
14	Lon 蛋白酶 (荧光假单胞菌) RXF 04653.2	MKTTIELPLPLRDVVVPHMVIPLFVGREKSIEALEAAMTGD KQILLLAQKNPADDPPGEDALYRVGTIATVLQLLKLDPDGTVKV LVEGEQRGAVFERFMEVDGHLRAEVALIEEVEAPERERESEVFVRS LLSQFEQYVQLGKKVPAEVLSSLNSIDEPSRLVDTMAAHMALK IEQKQDILEIIDLSARVEHVLAMLDGEIDLLQVEKRIRGRVKK QMERSQREYYLNEQMKAIQKELGDGEEGHNEIEELKKRIDAAG LPKDALTKATAELNKLKQMSPMSEATVVRSYIDWLQVVPWKA QTKVRLDLARAEIILDADHYGLEEVKERILEYLAVQKRVKKIR GPVLCVLGPPGVGKTS LAESI ASATNRKFVRMALGGVRDEAEI RGHRRTYIGSMPGR LIQKMTKVGVRNPLFLLEIDKMGSDMRG DPASALLEVLDPEQNHNFNDHYLEVDYDLS DVMFLCTS NSMNI PPALLDRMEVIRLPGYTEDEKINI AVKYLAPKQI SANG LKKGE IEFEVEAIRDIVRYYTREAGVRGLERQIAKICRKAVKEHALEK RFSVKVVADSLEHFLGVKKFRYGLAEQQDQVGQVTGLAWTQVG GELLTIEAAVIPGKGQLIKTGS LGDVMVESITAAQTVVRSRAR SLGIPLD FHEKHDTHIHMPEGATPKDGPSAGVGMCTALVSALT GIPVRADVAMTGEITLRGQVLAIGGLKEKLLAAHRGGIKTVII PEENV RDLKEIPDNIKQDLQIKPVKWIDEVLQIALQYAPEPLP DVAPEIVAKDEKRESDSKERISTH
15	La1 RXF08653.1 ATP-依赖性蛋白	MSDQQEFPDYDLNDYADPENAEAPSSNTGLALPGQNLPDKVYI IPIHNRPFPPAQVLPVIVNEEPWAETLELVSKSDHHSALFFM DTPPDDPRHFDTSALPLYGTLVKVHHASRENGKLQFVAQGLTR

[0385]

	酶	VRIKTWLKHHRPPYLVEVEYPHQPSEPTDEVKAYGMALINAIK ELLPLNPLYSEELKNYLNRFSPNDPSPLTDFAAALTSATGNEL QEVLDCVPMLKRMEKVLPLMRKEVEVARLQKELSAEVNRKIGE HQREFFLKEQLKVIQQELGLTKDDRSADVEQFEQRLQGKVLPA QAQKRIDEELNKLSEILETGSPYAVTRNYLDWATSVPGVYGA DKLDLKHARKVLDKHHAGLDDIKSRILEFLAVGAYKGEVAGSI VLLVGPPPGVGKTSVGKSLAESLGRPFYRFSVGGMRDEAEIKGH RRTYIGALPGKLVQALKDVEVMNPVIMLDEIDKMGQSFQGDPA SALLETLDPEQNVEFLDHYLDLRLDLSKVLVCTANTLDSIPG PLLDRMEVIRLSGYITEEKVAIAKRHLWPKQLEKAGVAKNSLT ISDGALRALIDGYAREAGVRQLEKQLGKLVRKAVVKLLDEPDS VIKIGNKDLESSLGMPVFRNEQVLSGTGVITGLAWTSMGGATL PIEATRIHTLNRGFKLTGQLGEVMKESAEIAYSYSISNLKSFG GDAKFFDEAFVHLHVPEGATPKDGPSAGVTMASALLSLARNQP PPKGVA MTGELTLTGHVLP IGGVREKVIAARRQKIHELILPEP NRGSFEELPDYLKEGMTVHFAKRFADVAKVLF
16	AprA RXF04304.1	MSKVKDKAIVSAAQASTAYSQIDSFHLYDRGGNLTVNGKPSY TVDQAATQLLRDGAAYRDFDNGKIDLTYYTFLTSATQSTMNKH GISGFSQFNTQQKAQAALAMQSWADVANTFTTEKASGGDGHMT FGNYSQGQDGAFAFAYLPGTGAGYDGTSWYLTNNSYTPNKTPD LNNYGRQTLTHEIGHTLGLAHPGDYNAGNGNPTYNDATYGQDT RGYSLMSYWSESNTNQNFSGGVEAYASGPLIDDI AAIQKLYG ANLSTRATDTTYGFNSNTGRDFLSATSNADKLVSVDGGGND TLDFSGFTQNQKINLTATSFSVDVGGVGNVSIAGVVTIENAFG GAGNDLIIGNQVANTIKGGAGNDLIYGGGGADQLWGAGSDTF VYGASSDSKPGAADKIFDFTSGSDKIDLSGITKGAGVTFVNAF TGHAGDAVLSYASGTNLGTLAVDFSGHGVADFLVTTVGQAAAS DIVA
17	HtpX RXF05137.1	MMRILLFLATNLAVVLIASVTLSLFGFNGFMAANGVDLNLNQL LIFCAVFGFAGSLFSLFISKWMAKMSTSTQIITQPRTRHEQWL MQTVEQLSQEAGIKMPEVGIFPAYEANAFATGWNKN DALVAVS QGLLERFSPDEVKAVLAHEIGHVANGDMVTALVQGVVNTFVM FFARIIGNFVDKVIKNEEGRGIAYFVATIFAELVLGFLASAI VMWFSRKREFRADEAGARLAGTSAMIGALQRLRSEQGLPVHMP DSLTAFGINGGIKQGLARLFMSHPPLEERIDALRRRG

[0386]

18	DegP1 RXF01250.2	MLKALRFFGWPLLAGVLIAMLI IQRYPQWVGLPTLDVNLQQAP QTNTVVQGPVTYADAVVIAAPAVVNLYTTKVINKPAHPLFEDP QFRRYFGDNGPKQRRMESSLGSGVIMSPEGYILTNNHVTTGAD QIVVALRDGRETARVVGSDPETDLAVLKIDLKKNLPAITLGRS DGLRVGDVALAIGNPFGVGQTVTMGII SATGRNQGLNSYEDF IQTDAAINPGNSGGALVDANGNLTGINTAIFSKSGGSQGIGFA IPVKLAMEVMKSI IEHGQVIRGWLGIEVQPLTKELAESFGLTG RPGIVVAGIFRDGPAQKAGLQLGDVILSIDGAPAGDGRKSMNQ VARIKPTDKVAILVMRNGKEIKLSAEIGLRPPPATAPVKEEQ
19	DegP2 RXF07210.1	MSIPRLKSYLSIVATVVLVGLQALPAQAVELPDFTQLVEQASPA VVNISTTQKLPDRKVSNOQMPDLEGLPMLREFFERGMPPQRS PRGGGGQREAAQSLGSGFII SPDGYILTNNHVIADADEILVRLA DRSELKAKLIGTDPRSDVALLKIEGKDLVVLKLGKSQDLKAGQ WVVAIGSPFGFDHTVTQGIVSAIGRSLPNENYVPFIQTDVPIN PGNSGGPLFNLAGEVVGINSQIYTRSGGFMGVSFAPIDVAMD VSNQLKSGGKVSRLGVLGVVIEVNKDLAESFGLDKPAGALVAQ IQDNGPAAKGGLKVGDVILSMNGQPI IMSADLPHLVGALKAGG KAKLEVIRDGKRQNVELTVGAI PEEGATLDALGNAKPGAERSS NRLGIAVVELTAEQKKTFDLQSGVVIKEVQDGPAAALIGLQPGD VITHLNNQAIDTTKEFADIAKALPKNRSVSMRVLROGRASFIT FKLAE
20	Npr RXF05113.2	MCVRQPRNP I FCLIPPYMLDQIARHGDKAQREVALRTRAKDST FRSLRMVAVPAKGPARMALAVGAEKQRSIYSAENTDSLPGKLI RGEGQPASGDAAVDEAYDGLGATFDFFDQVFDNRNSIDDAGMAL DATVHFGQDYNNAFWNSTQMVF GDGDQQLFNRFTVALDVIGHE LAHGVTEDEAKLMYFNQSGALNESLSDVFGSLIKQYALKQTAE DADWLIGKGLFTKKIKGTALRSMKAPGTAFDDKLLGKDPQPGH MDDFVQTYEDNGGVHINS GIPNHAFYQVAINIGGFAWERAGRI WYDALRDSRLRPNSGFLRFARITHDIAGQLYGVNKA EQKAVKE GWKAVGINV
21	Prc1 RXF06586.1	MRYQLPPRRISMKHLFPSTALAFFIGLGFASMSTNTFAANSWD NLQPDRDEVIASLNVVELLKRHHYSKPPLDDARSVIIYDSYLK LLDPSRSYFLASDIAEFDKWKTQFDDFLKSGDLQPGFTIYKRY LDRVKARLDFALGELNKGVDKLDFTQKETLLVDRKDAPWLST AALDDLWRKRVKDEVLR LK IAGKEPKAIQELLTKRYKNQLARL DQTRAEDIFQAYINTFAMSYPHTNYLSPDNAENFDINMSLSL

[0387]

		EGIGAVLQSDNDQVKIVRLVPAGPADKTKQVAPADKIIIGVAQA DKEMVDVVGWRLDEVVKLIRGPKGSVVRLEVI PHTNAPNDQTS KIVSITREAVKLEDQAVQKKVLNLKQDGKDYKLGVIEIPAFYL DFKAFRAGDPDYKSTTRDVKKILTELQKEKVDGVVIDLRNNGG GSLQEATELTSLFIDKGPTVLVRNADGRVDVLEDENPGAFYKG PMALLVNRLSASASEIFAGAMQDYHRALIIGGQTFGKGTVQTI QPLNHGELKLTAKFYRVSGQSTQHOGVLPDIDFPSIIDTKEI GESALPEAMPWDTIRPAIKPASDPFKPFLAQLKADHDTRSAKD AEFVFIRDKLALAKKLMEEKTVSLNEADRRQAQHSSENQQLVL ENTRRKAKGEDPLKELKKEDEDALPTEADKTKPEDDAYLAETG RILLDYLKITKQVAKQ
22	Prc2 RXF01037.1	MLHLSRLTSLALTIALVIGAPLAFADQAAPAAPATAATTKAPL PLDELRTFAEVMMDRIKAAYVEPVDDKALLENAIKGMLSNLDPH SAYLGPEDFAELQESTSGEFGGLGIEVGSEDGQIKVVSPIDDT PASKAGIQAGDLIVKINGQPTRGQTMTEAVDKMRGKLGQKITL TLVRDGGNPFVDVTLARATITVKS VKSQLLES GYGYIRITQFQV KTGDEVAKALAKLRKDNGKKLNGIVLDRNNPGGVLSAVEVV DHFVTKGLIVYTKGRIANSELRF SATGN DLSENVPLAVLINGG SASASEIVAGALQDLKRGVLMGTTSFGKGSVQTVLPLNNERAL KIT TALLYTPNGRSIQAGGIVPDIEVRRAKITNEIDGEYYKEA DLQGH LGNGNGGADQPTGSRKAKPMPQDDDYQLAQALSLLKG LSITRSR
23	PrtB RXF08627.2	MDVAGNGFTVSQRNRTPRFKTTPLTPIALGLALWLGHGSVARA DDNPYTPQVLES AFRTAVASFGPETAVYKNLRFAYADIVDLAA KDFAAQSGKFDSALKQNYELQPENLTIGAMLGDRRPLDYASR LDYYRSRLFSNSGRYTTNILD FSKAI IANLPAAKPYTYVEPGV SSNLNGQLNAGQSWAGATRDWSANAQTWKTPEAQVNSGLDRTN AYYAYALGITGKGVNVGVLD SGIFTEHSEFQGKNAQGQDRVQA VTSTGEYYATHPRYRLEVPSGEFKQGEHFSIPGEYDPAFNDGH GTEMSGVLAANRNGTGMHGIAFDANL FVANTGGSDNDRYQGSN DL DYN AFMASYNALAAKNVAIVNQSWGQSSRD DENHFGNVGD SAAQNLRDMTAA YRPFWDKAHAGHKTWMDAMADAARQNTFIQI ISAGNDSHG ANPD TNSNL PFFKPDIEAKFLSITGYDETS AQVY NRCGTSKWVCVMGISGIP SAGPEGEIIPNANGTSAAAPSVSGA LALVMQRFPYMTASQARDVLLTSSLQAPDGPDPVGTLTGGR TYDNLQPVHDAAPGLPQVPGVVSGWGLPNLQKAMQGPGQFLGA

[0388]

		VAVALPSGTRDIWANPISDEAIRARRVEDAAEQATWAATKQQK GWLSGLPANASADDQFEYDIGHAREQATLTRGQDVLGTSTYVG SLVKSGDGELVLEGQNTYSGSTWVRGGKLSVDGALTSVTVDS SAVGTRNADNGVMTTLGGTLAGNGTVGALTVNNGGRVAPGHSI GTLRTGDVTFNPGSVYAVEVGADGRSDQLQSSGVATLNGGVVS VSLNSPNLLTATEARSLLGQQFNILSASQGIQGQFAAFAPNY LFIGTALNYQPNQLTLAIARNQTTFASVAQTRNERSVATVAET LGAGSPVYESLLASDSAAQAREGFKQLSGQLHSDVAAAQMADS RYLREAVNARLQQAQALDSSAQIDSRDNGGWVQLLGGRRNVSG DNNASGYSSSTSGVLLGLDTEVNDGWRVGAATGYTQSHLNGQS ASADSDNYHLSVYGGKRFEAIALRLGGASTWHRLDTSRRVAYA NQSDHAKADYNARTDQVFAEIGYTQWTVFEPFANLTYLNYQSD SFKEKGGAAALHASQQSQDATLSTLGVRGHTQLPLTSTSAVTL RGELGWEHQFGDTEASLKFAGSDTAFVNSVPVARDGAVIK ASAEMALTKDTLVSLNYSGLLSNRGNNGINAGFTFLF
24	M50 (S2P 蛋白酶 家族)RXF04692.1	MSALYMIVGTLVALGVLVTFHEFGHFWARRCGVKVLRFSVGF GMPLLRWHDRRGTEFVIAAIPGGYVKMLDEREAGEVPADQLDQ SFNRKTVRQRIAIVAAGPIANFLLAMVFFWVLAMLGSQQVRPV IGAVEADSIAAKAGLTAGQEIVSIDGEPTTGWGAVNLQLVRRL GESGTVNVVVRDQDSSAETPRALALDHWLKGADPEPDIKSLGI RPWRPALPPVLAELDPKGPAQAAGLKTGDRLLALDGQALGDWQ QVVDLVRVRPDTKIVLKVEREGAQIDVPVTLVSRGEAKAAGGY LGAGVKGVVEWPPSMVREVSYGPLAAIGEGAKRTWTMSVLTLES LKKMLFGELSVKNLSGPITIAKVAGASAQSGVADFLNFLAYLS ISLGVLNLLPIPVLDGGHLLFYLVVEWVRGRPLSDRVQGWGIQI GISLVVGVMLLALVNDLGRL
25	FkbP RXF06591.1	MKQHRLAAVALVSLVLAGCDSQTSVELKTPAQKASYGIGLNM GKSLAQEGMDDLDSKAVAQGIEDAVGKKEQKLKDELVEAFAA LQKRAEERMTKMSEESAAAGKKFLEDNAKKDGVVTTASGLQYK IVKKADGAQPKPTDVVTVHYTGKLTNGTTFDSSVDRGSPIDL VSGVIPGWVEGLQLMHVGEKVELYIPSDLAYGAQSPSPAIPAN SVLVFDLELLGIKDKPAKAEAADAPAAPAAKK
26	ClpX RXF04654.2	MTDTRNGEDNGKLLYCSFCGKSQHEVRKLIAGPSVFICDECVD LCNDIIREEVQEAQAESSAHKLPSPEISGILDQYVIGQERAK KVLAVAVYNHYKRLNQDKKGDEVELGKSNILLIGPTGSGKTL

[0389]

		LAETLARLLNVPFTIADATTLTEAGYVGEDVENIIQKLLQKCD YDVEKAQMGIVYIDEIDKISRKSDNPSITRDVSGEGVQQALLK LIEGTVASVPPQGGKHPQQEFLQVDTRNLFICGGAFFSGLEK VIQQRSTRGGIGFSAEVRSKKEGKKVGESLREVEPDDLVKFGL IPEFVGRPLVLATLDELDEAALIQILTEPKNALTKQYGKLFEM EGVDLEFRTDALKSVAKRALERKTGARGRLRSILEGVLLDTMYE IPSQSEVSKVVIDESVIEGKSKPLYIYENSEPAAKAAPDA
27	ClpA RXF04587.1	MLNRELEVTLNLAFKEARSKRHEFMTVEHLLLALLDNEAAATV LRACGANLDKLDLQEFIDSTTPLIPVHDEDRETQPTLGFQR VLQRAVFHVQSSGKREVTGANVLVAIFSEQESQAVFLLKQQSV ARIDVVNYIAHGISKVPGHGDHSEGEQDMQDEEGGESSSSSNP LDAYASNLNEMARQGRIDPLVGREHEVERVAQILARRRKNPL LVGEAGVGKTAIAEGLAKRIVDNQVPDLLASSVVYSLDLGALL AGTKYRGDFEKRFKALLGELKKRPQAILFIDEIHTIIGAGAAS GGVMDASNLLKPLLSSGDIRCIGSTTFQEFRGIFEKDRALARR FQKVDVSEPSVEDTIGILRGLKGRFEAHHGIEYTDEALRAAAE LASRYINDRHMPDKAIDVIDEAGAYQRLQPVKEKRVKRIDVPQV EDIVAKIARIIPKHVTSSDKELLRNLERDLKLTVFGQDAAIDS LSTAIKLSRAGLKSPDKPVGSFLFAGPTGVGKTEAARQLAKAM GIELVRFDMSYMERHTVSRIGAPPGYVGFDQGGLLTEAITK QPHCVLLLDEIEKAHPEVFNLLLQVMDHGTLTDNNGRKADFRN VIVIMTTNAGAETAARASIGFTHQDHSSDAMEVIKKSFTPEFR NRLDTIIQFGRLSHEVIKSVVDKFLTELQAQLEDKRVQLDVTE AARSWIAEGGYDAAMGARPMARLIQDKIKRPLAEIILFGELSD HGGVVHIDLKDGELTFEFETTAEMA
28	FkIB3* RXF06034.2 (全长)	MSEVNLSTDETRVSYGIGRQLGDQLRDNPFGVSLDAILAGLT DAFAGKPSRVDQEQMAASFVKVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITTSLASGLQFEVLTAGTGAKPTREDQVRTHYHG TLIDGTVFDSSYERGQPAEFFVGGVIAGWTEALQLMNAGSKWR VYVPSELAYGAQGVGSIPPHSVLYSTSSCSTFCKTCWLPVGTN AFAPTGVCQFLHGWNLPLSSVAGRNARA
29	PpiA RXF03768.1	MLKKIALFAGSALFAANLMAAEPKAPHVLLDTTNGQIEIELD PVKAPISTKNFLEYVDSGFYTNTIFHRVIPGFMVQGGGFTQQM QQKDTKAPIKNEASNLHNVRGTLSMARTSNPNSATSQFFINV ADNAFLDPGRDAGYAVFAKVVKGMDVVDIIVNSQTTTKQGMQN VPIDPVLIKSARID

[0390]

30	PrlC RXF04631.2	MPESNPLLLPYDLPPFSAIRAEHLVPAIEQIITESRNTTATII ASQTPFPPTWDDLQAVEALEARLDGVLKIIELLDSPQGPWT LASHRSYELAMQYRVELAGNNDLYQLHRQLADSPIATLFNEQR HSALRKILRKYHLAGLDLSPEKQRRKALNLQIDFSHEFLRR VSDSSDAWRKHIQDKALLSGLPDAALARLEFAARDAGLGGWLL TLSKQSFQEVMSYADHRALRQEMMLAYYSRAVGTGPDATDN EAVLTVLLDSRHQKAQLLGYNFAELALVEQMAETTDEVTACV HQQIDQARTTFAHDAQQLQRYAAQRGVDALEPWDYDFFAEKIR QDVAGVSQDAVRLYFPLETVLQRLCTFTQTFLGVELIEQATVD TWHPDVRVFELREYAQPIGHLFIDPYRRVAGGEIGAAMGLRNH RMTAEGRPQRPIAVLRSQLPRTAAQPCLLDHLQLRVLLHEFG HCLQHLLSAAPYRAISGMGQLSHDTTEFFGLVLEQFCLTPSFL IYLSGHVQTGDPLPDKMATQMSRFAHTQTSQETASILLTGLVD FELHRTYGDGRTPHEVFTDANVEVGHLQWPDGARPINSFEQPM GSYGAKLYSYTWSGVLARQAFAFERFERDGLFNPQTGKAFRDAFI TEGDTGTLLSALALFRGDGAGCVGHSTGV
31	肠激酶 (aa, 牛) GenBank AY682203.1	IVGGSDSREGAWPVVVALYFDDQQVCGASLVSRDWLVSAAHCV YGRNMEPSKWKAVLGLHMASNLTSPIETRLIDQIVINRHYNK RRKNNDIAMMHLEMKVNYTDYIQPICLPEENQVFPPGRICSIA GWGALIYQGSTADVLQEADVPLLSNEKCQQQMPEYNITENMVC AGYDAGGVDSQCQDGGPLMCQENNRWLLAGVTSFGYQCALPN RPGVIARVPRFTEWIIQSFLH
32	人前胰岛素 (下划线: B-肽) (粗体: C-肽) (普通: A-肽)	FVNQHLCGSHLVEALYLVCGERGFFYTPKTRR EAEDLQVGQVE LGGPGAGSLQPLALEGSLQKR GIVEQCCTSICSLYQLENYCN
33	赖脯胰岛素前胰 岛素 (下划线: B-肽) (粗体: C-肽) (普通: A-肽)	FVNQHLCGSHLVEALYLVCGERGFFYTPKPT EAEDLQVGQVELG GPGAGSLQPLALEGSLQKR GIVEQCCTSICSLYQLENYCN
34	谷赖胰岛素前胰 岛素 (下划线: B-肽)	FVKQHLCGSHLVEALYLVCGERGFFYTPETRR EAEDLQVGQVE LGGPGAGSLQPLALEGSLQKR GIVEQCCTSICSLYQLENYCN

[0391]

	(粗体: C-肽) (普通: A-肽) (高亮: 相对于胰 岛素的改变)	
35	IGF-1(美卡舍明)	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGI VDECCFRSCDLRRLEMYCAPLKPAKSA
36	Glp-1	HAEGTFTSDVSSYLEGQAAKEFIAWLVKGRG
37	Glp-1 类似物(艾 塞那肽)	HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS
38	Glp-2	HADGSFSDEMNTILDNLAARDFINWLIQTKITD
39	Glp-2 类似物(替 度鲁肽)	HGDGSFSDEMNTILDNLAARDFINWLIQTKITD
40	普兰林肽	KCNTATCATQRLANFLVHSSNNFGPILPPTNVGSNTY
41	齐考诺肽	MKLTCVVIVAVLLLTACQLITADDSRGTQKHRALRSTTKLSTS TRCKGKGAKCSRLMYDCCTGSCRSKGCG
42	贝卡普勒明	SLGSLTIAEPAMIAECKTRTEVF EISRRLIDRTNANFLVWPPC VEVQRCSGCCNNRNVQCRPTQVQLRPVQVRKIEIVRKKPIFKK ATVTLEDHLACKCETVAAARPVT
43	恩夫韦肽	YTSLIHSLIEESQNQOEKNEQEELLELDKWASLWNWF
44	奈西立肽	SPKMVQSGSGCFGRKMDRISSSSGLGCKVLRH
45	DnaJ-样蛋白-PTH 1-34 融合体(aa)	MKVEPGLYQHYKGPQYRVFSVARHSETEEEVVIFYQALYGEYGF WVRPLSMFLETVEVDGEQVPRFALVTAEPSLFTGQGGGGSGGG GSHHHHHHDDDDKSVSEIQLMHNLGKHLNSMERVEWLRKKLQD VHNF
46	FkIB-PTH 1-34 融合体(aa)	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPSRVDQE QMAASFKVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITT LASGLQFEVLTAGTGAKPTREDQVRTHYHG TLIDGTVFDSSYERGQPAEFFVGGVIAGWTEALQLMNAGSKWR VYVPSELAYGAQGVGSI PPHSVLVFDVELLDVLGGGGSGGGGS HHHHHHHDDDDKSVSEIQLMHNLGKHLNSMERVEWLRKKLQDVH NF
47	FrnE-PTH 1-34 融 合体(aa)	MSTPLKIDFVSDVSCPWCII GLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAEESQANRARIRDMGAA

[0392]

		LGFAFRTDGQSRIYNTFDAHRLHWHAGLEGLQYNLKEALFKAY FSDGQDPSDHATLAI IAESVGLDLARAAEILASDEYAAEVREQ EQLWVSRGVSSVPTIVFNDQYAVSGGQPAEAFVGAIRQI INES KSGGGGSGGGGSHHHHHDDDDKSVSEIQLMHNLGKHLNSMER VEWLRKKLQDVHNF
48	EK1: DnaJ- 样蛋白-肠激酶融合体(aa)	MKVEPGLYQHYKGPQYRVFSVARHSETEEEVVIFYQALYGEYGF WVRPLSMFLETVEVDGEQVPRFALVTAEPSLFTGQGGGSGGG GSDDDDKIVGGSDSREGAWPWVVALYFDDQQVCGASLVS RDWL VSAAHCVYGRNMEPSKWKAVLGLHMASNLTS PQIETRLIDQIV INRHYNKRRKNNDIAMMHLEMKNYTDYIQPICLPEENQVFPP GRICSIAGWGALIYQGSTADVLQEADVPLLSNEKCQQQMPEYN ITENMVCAGYDAGGVDSCQGD SGGPLMCQENNRWLLAGVTSFG YQCALPNRPGVYARVPRFTEW IQSFLHhhhhhh
49	EK2: FkIB- 肠激酶融合蛋白(aa)	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPSRVDQE QMAASFKVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITTLASGLQFEVLTAGTGAKPTREDQVRTHYHG TLIDGTVFDSSYERGQPAEFFVGGVIAGWTEALQLMNAGSKWR VYVPSELAYGAQGVGSI PPHSVLVFDVELLDVLGGGGSGGGGS DDDDKIVGGSDSREGAWPWVVALYFDDQQVCGASLVS RDWLVS AAHCVYGRNMEPSKWKAVLGLHMASNLTS PQIETRLIDQIVIN RHYNKRRKNNDIAMMHLEMKNYTDYIQPICLPEENQVFPPGR ICSIAGWGALIYQGSTADVLQEADVPLLSNEKCQQQMPEYNIT ENMVCAGYDAGGVDSCQGD SGGPLMCQENNRWLLAGVTSFGYQ CALPNRPGVYARVPRFTEW IQSFLHhhhhhh
50	EK4: EcpD- 肠激酶融合蛋白(aa)	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGAPLAQDKESVFWLNVLEIPPKPEAGADLN TLQMAFRSRIKLFYRPVGLPGNPNEAVEQVQWQLVTARDGQGL ALKAYNPFAFHVSLIELDLVAGNQRYRSEDGMVGPGETRQFAL PTLKARPSSQAQVEFSAINDYGALVPTRNTLQPGGGSDDDDK IVGGSDSREGAWPWVVALYFDDQQVCGASLVS RDWLVSAAHCV YGRNMEPSKWKAVLGLHMASNLTS PQIETRLIDQIVINRHYNK RRKNNDIAMMHLEMKNYTDYIQPICLPEENQVFPPGRICSI AGWGALIYQGSTADVLQEADVPLLSNEKCQQQMPEYNITENMVC AGYDAGGVDSCQGD SGGPLMCQENNRWLLAGVTSFGYQCALPN RPGVYARVPRFTEW IQSFLHhhhhhh

[0393]

51	EK5: 肠激酶 (aa, 没有 N-末端融合伴侣)(带有 His-标签)	IVGGSDSREGAWPWVVALYFDDQQVCGASLVSRDWLVSAAHCV YGRNMEPSKWKAVLGLHMASNLTSQPQIETRLIDQIVINRHYNK RRKNNDIAMMHLEMKVNYTDYIQPICLPEENQVFPPGRICSLA GWGALIIYQGSTADVLQEADVPLLSNEKCQQQMPEYNITENMVC AGYDAGGVDSQCQDGGGFLMCQENNRWLLAGVTSFGYQCALPN RPGVIARVPRFTEWISFLHhhhhhh
52	DnaJ-样蛋白-PTH 1-34 融合基因片段 126203(优化的 荧光假单胞菌 nt 序列, 具有克隆位 点) (下划线: 起始密 码子)	ACTAGTAGGAGGTCTAGAAATGAAAGTCGAACCGGGTCTGTACC AGCATTACAAGGGTCCCCAATATCGCGTGTTTTTCGGTAGCGCG GCACAGCGAAACCGAAGAAGAAGTGGTGTCTACCAAGCGCTC TACGGCGAGTACGGCTTCTGGGTGCGTCCGCTGTGATGTTCC TGGAGACTGTGAGGTAGACGGTGAGCAAGTCCCAGCGCTTCGC CCTGGTGACGGCCGAGCCCAGCCTGTTACCGGCCAGGGCGGG GGCGGCAGCGGCGTGGGGGCTCGCATCACCACCACCATCACGA CGACGACGATAAGAGCGTGTCGAGATCCAGCTCATGCATAAT CTGGGCAAGCACTTGAACAGCATGGAGCGCGTGGAGTGGCTCC GGAAGAACTGCAAGATGTCCACAACCTTTTAATGATAGCTCGA G
53	DnaJ-样蛋白-PTH 1-34 融合基因片段 126206(优化的 荧光假单胞菌 nt 序列, 具有克隆位 点) (下划线: 起始密 码子)	ACTAGTAGGAGGTCTAGAAATGAAAGTCGAACCGGGTCTTACC AGCATTACAAGGGGCCGAGTACCGTGTTTTTCAGCGTGGCGCG CCACTCTGAAACCGAAGAAGAAGTGGTGTTTTACCAAGCGCTG TATGGCGAATACGGCTTTTGGGTGCGCCCTTTGAGCATGTTCC TGGAGACCGTCGAAGTTGACGGCGAGCAGGTCCCAGCGCTTTGC TTTGGTCACGGCCGAACCCAGTCTTTTTACAGGGCAAGGTGGC GGTGGTTCGGGCGTGGCGGCAGCCATCATCACCACCACCACGA CGACGATGATAAGAGCGTGTCGAGATCCAAGTATGCATAAT CTGGGCAAGCACCTGAACTCGATGGAGCGGGTAGAGTGGCTCC GGAAAAAGCTCCAAGACGTGCACAACCTTCTAATGATAGCTCGA G
54	FkIB-PTH 1-34 融合基因片段 126204(优化的荧光假单胞菌 nt 序列, 具有克隆位 点) (下划线: 起始密 码子)	ACTAGTAGGAGGTCTAGAAATGAGCGAAGTCAACTTGAGCACTG ATGAAACCCGGGTAAGCTATGGTATTGGGCGGCAGCTGGGGGA CCAAGTGCGGGACAACCCGCTCCCGGCGTGAGCCTCGACGCG ATCCTCGCGGGTCTGACCGACGCCCTTCGCCGGCAAGCCGAGCC GCGTGGACCAAGAACAGATGGCCGCCTCGTTCAAGGTCATCCG CGAAATCATGCAGGCCGAAGCGGCAGCGAAGGCCGAGGCCGA GCGGGTGCCGGCCTGGCGTTCCTGGCCGAGAACGCCAAGCGTG ACGGCATCACGACCCTGGCGTCGGGCCTCCAATTCGAAGTCCT

[0394]

	码子)	GACGGCCGGTACTGGCGCGAAGCCCACTCGCGAGGATCAGGTG CGCACCCACCTACCATGGCACGCTGATCGATGGCACCGTATTC GACAGCAGCTACGAGCGTGGCCAACCGGCGGAGTTTCCGGTGG GCGGTGTGATCGCCGGCTGGACCGAGGCCCTGCAACTCATGAA CGCGGGGCTCGAAGTGGCGCGTGTACGTCCCCAGCGAGCTGGC GTACGGTGC GCAAGGCGTGGGCTCGATTCCGCCCCACAGCGTA CTCGTCTTTGACGTGGAAGTGTGCTGGGCGGTGGCG GGAGTGGGGGTGGCGGCTCCCACCACCATCACCACCATGATGA CGATGACAAGTCCGTGTCGGAGATCCAGCTGATGCATAATCTC GGCAAGCACCTGAACTCGATGGAGCGCGTCGAGTGGCTCCGCA AAAAGCTCCAAGACGTGCACAACCTTCTAATGATAGCTCGAG
55	FklB-PTH 1-34 融合基因片段 126207(天然荧光假单胞菌 nt 序列, 具有克隆位点) (下划线: 起始密码子)	ACTAGTAGGAGGTCTAGAA <u>TG</u> TCCGAAGTTAATCTGTCCACCG ACGAAACCCGCGTCAGCTACGGTATCGGCCGTGAGTTGGGCGA CCAAGTGCCTGACAACCCGCCACCGGGCGTCAGCCTGGACGCG ATCCTGGCCGGCCTGACCGACGCGTTCGAGGCAAGCCAAGCC GTGTTGACCAAGAGCAAATGGCGGCCAGCTTCAAAGTGATCCG CGAAATCATGCAAGCCGAAGCCGCTGCCAAGGCTGAAGCTGCA GCAGGCGCTGGCCTGGCTTTCTGGCGGAAAACGCCAAGCGTG ATGGCATCACCAACCTGGCTTCCGGCCTGCAATTTGAAGTGCT GACGGCTGGTACCGGCGCCAAGCCGACCGTGAAGACCAAGTG CGTACTCACCTACCACGGCACCCCTGATCGACGGCACTGTGTTT GACAGCTCCTACGAGCGCGGCCAGCCTGCAGAATTCCCGGTTG GCGGCGTGATCGCCGGCTGGACCGAAGCCCTGCAACTGATGAA TGCCGGGCGAGCAAATGGCGCGTGTACGTGCCGAGCGAACTGGC TTACGGCGCTCAAGGCGTTGGCAGCATCCCGCCGACAGCGTT CTGGTATTCGACGTCGAGCTGCTCGACGTTCTGGGTGGGGGTG GGTCGGGTGGTGGTGGGTCGCATCATCATCACCACCACGATGA TGATGATAAGAGTGTCTCGGAGATTCAGCTCATGCACAACCTC GGTAAGCATCTCAACTCGATGGAGCGGGTAGAGTGGCTCCGGA AGAAACTCCAAGATGTGCACAACCTTTAATGATAGCTCGAG
56	FrnE-PTH 1-34 融合基因片段 126205(优化的荧光假单胞菌 nt 序列)	ACTAGTAGGAGGTCTAGAA <u>TG</u> TCCACCCCCCTGAAGATTGATT TTGTCTCCGACGTATCGTGCCGTGGTGTATCATCGGCCTGCG TGGCCTGACTGAAGCCCTCGACCAACTGGGCAGCGAAGTCCAG GCCGAGATCCACTTCCAACCGTTTGAGCTGAACCCCAACATGC CTGCCGAGGGCCAAAACATCGTGGAGCATATCACGGAGAAGTA CGGCAGCACCGCCGAGGAATCGCAGGCGAACCGTGCGCGGATC

[0395]

	(下划线: 起始密码子)	CGGGATATGGGTCCGCACTCGGGTTCGCGTTCCGCACGGACGG CCAGTCGCGCATCTACAATACTTTCGATGCCCACCGGCTCCTG CATTGGGCCGGTCTGGAAGGCCTGCAATACAACCTGAAAGAAG CGCTGTTCAAGGCCTACTTCTCGGACGGCCAAGACCCGTCGGA CCACGCGACCCTCGCGATCATCGCCGAGAGTGTAGGGCTGGAC TTGGCCCGCGCGGCCGAAATTCTCGCGAGCGACGAGTATGCCG CGGAAGTCCGGGAGCAAGAGCAGCTCTGGGTGAGCCGCGGTGT GAGCAGCGTCCCCACCATCGTGTTCAACGATCAGTACGCCGTG AGCGGTGGCCAACCCGCGGAAGCCTTCGTGGGCGCGATCCGCC AGACATCAACGAGTCAAAGTCGGGCGGTGGCGGCAGCGGCGGT GGTGGCAGCCATCACCATCATCACCACGACGACGATGATAAGT CCGTGTCGGAGATCCAACCTGATGCACAATCTCGGGAAGCACCT GAACAGCATGGAGCGCGTGAATGGCTGCGCAAGAACTGCAA GACGTGCACAACCTTTTAATGATAGCTCGAG
57	FrnE-PTH 1-34 融合基因片段 126208(天然荧光假单胞菌 nt 序列) (下划线: 起始密码子)	ACTAGTAGGAGGTCTAGAA <u>T</u> GAGTACTCCCCTGAAAATCGATT TCGTCAGCGACGTATCCTGCCCCCTGGTGCATCATCGGCCTGCG CGGCTTGACCGAAGCCCTCGACCAGCTCGGCAGCGAGGTGCAG GCCGAGATTCATTTTCAACCGTTCGAACTGAACCCGAACATGC CCGCCGAAGGTCAGAACATCGTCGAGCACATTACCGAAAAGTA CGGCTCCACGGCTGAAGAGTCCCAGGCTAATCGTGCGCGTATC CGTGACATGGGCCC GCGTTGGGCTTTGCTTTTCGCACCGATGG CCAGAGCCGTATCTACAACACCTTCGACGCGCACCGTCTGTTG CACTGGGCCGGGTTGGAAGGCTTGCAGTACAACCTCAAGGAAG CGCTGTTCAAGGCGTACTTCAGCGATGGCCAGGACCCCTCCGA CCACGCGACCTTGCGATCATCGCCGAAAGCGTCGGGCTGGAC CTTGCGCGCGCCGCGAGATTCTTGCCAGCGATGAATACGCCG CCGAGGTCCGCGAGCAGGAGCAGCTGTGGGTTTCCCGTGGGGT GAGTTCGGTGCCGACCATTGTCTTCAATGACCAATATGCGGTG AGCGGTGGGCAACCGGCTGAAGCCTTCGTGGGTGCGATTGCGC AGATCATCAACGAATCCAAATCCGGTGGTGGCGGCTCGGGCGG TGGTGGCTCGCATCATCATCACCACCAGATGACGATGACAAG AGCGTATCGGAGATCCAACCTATGCACAACCTGGGCAAGCACCC TCAACTCGATGGAGCGGGTGGAGTGGCTGCGGAAGAACTGCA AGACGTGCATAACTTCTAATGATAGCTCGAG
58	18 碱基对间隔体	5' ACTAGTAGGAGGTCTAGA3'
59	(G4S) ₂ 间隔体序	GGGSGGGGS

[0396]

	列	
60	“Hi”核糖体结合位点	AGGAGG
61	FklB2 (荧光假单胞菌)	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPSRVDQEQMAASFVKVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITTL
62	FklB3 (荧光假单胞菌)	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKP
63	FrnE2 (荧光假单胞菌)	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAEESQANRARIRDMGAA LGFAFRTDGQSRIY
64	FrnE3 (荧光假单胞菌)	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPN
65	EcpD RXF04296.1 (荧光假单胞菌)	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGAPLAQDKESVFWLNVLEIPPKPEAGADLN TLQMAFRSRIKLFYRPVGLPGNPNEAVEQVQWQLVTARDGQGL ALKAYNPFAFHVSLIELDLVAGNQRYRSEDGMVGPGETRQFAL PTLKARPSSQAQVEFSAINDYGALVPTRNTLQP
66	EcpD2 (荧光假单胞菌)	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFT
67	EcpD3 (荧光假单胞菌)	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNN
68	gcsf 基因片段的 核苷酸序列(包括 克隆位点; met 起 始密码子加下划 线)	ATATGCTCTTCAAAGATGACTCCTCTGGGTCCTGCAAGTAGTC TGCCGCAAAGTTTTCTCCTGAAGTGCCTGGAACAGGTGCGCAA AATTCAGGGCGACGGCGCAGCACTGCAGGAAAACTGTGCGCG ACCTATAAGTTGTGCCACCCCGAAGAACTGGTGTCTGCTGGGCC ATAGCCTGGGGATTCCATGGGCGCCGCTGTCGTCCTGTCTTAG TCAAGCCTTGCAATTGGCCGGTGCCTCTCGCAACTGCATAGC GGCCTGTTCTGTACCAAGGCCTGCTGCAGGCCTTGGAAGGCA TCTCCCCGGAAGTGGGCCCCGACGCTGGATACCCTGCAACTGGA CGTAGCAGATTTGCCACGACCATCTGGCAGCAGATGGAAGAA CTGGGCATGGCCCCGGCCCTCCAGCCCACGCAAGGCGCGATGC

[0397]

		CTGCATTTCGCTCGGCGTTTCAACGCCGTGCGGGTGGCGTGCT GGTAGCCAGCCATTTGCAGAGCTTTCTGGAGGTGAGCTATCGC GTCCTCCGTCTCTCGCCCAACCGTGATAATAGTTCAGAAGAG CATAT
69	通过 <i>gcsf</i> 基因片段 (SEQ ID NO: 68) 编码的 GCSF 的氨基酸序列	MTPLGPASSLPQSFLKCLEQVRKIQGDGAALQEKLCAATYKLC HPEELVLLGHSLGIPWAPLSSCPSQALQLAGCLSGLHSGFLY QGLLQALEGISPELGPTLDTLQLDVADFATTIWQQMEELGMAP ALQPTQGAMPAFASAFQRRAGGVLVASHLQSFLEVSYRVLRLH AQP
70	DnaJ- 样蛋白 -EK-GCSF 融合体	MKVEPGLYQHYKGPQYRVFSVARHSETEEVVFYQALYGEYGF WVRPLSMFLETVEVDGEQVPRFALVTAEPSLFTGQGGGSGGG GSHHHHHHDDDDKMTPLGPASSLPQSFLKCLEQVRKIQGDGA ALQEKLCAATYKLCHEPELVLLGHSLGIPWAPLSSCPSQALQLA GCLSGLHSGFLYQGLLQALEGISPELGPTLDTLQLDVADFAT TIWQQMEELGMAPALQPTQGAMPAFASAFQRRAGGVLVASHLQ SFLEVSYRVLRLHLAQP
71	EcpD1-EK-GCSF 融合体 (全长 EcpD1)	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGAPLAQDKESVFWLNLEIPPKPEAGADLN TLQMAFRSRIKLFYRPVGLPGNPNEAVEQVQWQLVTARDGQGL ALKAYNPFAFHVSLIELDLVAGNQRYRSEDGMVGPGETRQFAL PTLKARPSSQAQVEFSAINDYGALVPTRNTLQPGGGSGGGGS HHHHHHHDDDDKMTPLGPASSLPQSFLKCLEQVRKIQGDGAAL QEKLCAATYKLCHEPELVLLGHSLGIPWAPLSSCPSQALQLAGC LSGLHSGFLYQGLLQALEGISPELGPTLDTLQLDVADFATTI WQQMEELGMAPALQPTQGAMPAFASAFQRRAGGVLVASHLQSF LEVSYRVLRLHLAQP
72	EcpD2-EK-GCSF 融合体	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGGGGSGGGGSHHHHHHDDDDKMTPLGPAS SLPQSFLKCLEQVRKIQGDGAALQEKLCAATYKLCHEPELVLL GHSLGIPWAPLSSCPSQALQLAGCLSGLHSGFLYQGLLQALE GISPELGPTLDTLQLDVADFATTIWQQMEELGMAPALQPTQGA MPAFASAFQRRAGGVLVASHLQSFLEVSYRVLRLHLAQP
73	EcpD3-EK-GCSF 融合体 (50 个 aa 截	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGGGSGGGGSHHHHHHDDDDKMTPLGPASSLPQSFL

[0398]

	短的 EcpD1)	LKCLEQVRKIQGDGAALQEKLCATYKLCHPEELVLLGHSLGIP WAPLSSCPSQALQLAGCLS QLHSGFLYQGLLQALEGISPELG PTLDTLQLDVADFATTIWQQMEELGMAPALQPTQGAMPAFASA FQRRAGGVLVASHLQSFLEVSYRVLRLHLAQP
74	FkIB-EK-GCSF 融 合体(全长 FkIB)	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPSRVDQEQMAASFVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITTLLASGLQFEVLTAGTGAKPTREDQVRTHYHG TLIDGTVFDSSYERGQPAEFFVGGVIAGWTEALQLMNAGSKWR VYVPSELAYGAQGVGSIPPHSVLVFDVELLDVLGGGGSGGGGS HHHHHHDDDDKMTPLGPASSLPQSFLKCLEQVRKIQGDGAAL QEKLCATYKLCHPEELVLLGHSLGIPWAPLSSCPSQALQLAGC LSQLHSGFLYQGLLQALEGISPELGPTLDTLQLDVADFATTI WQQMEELGMAPALQPTQGAMPAFASAFQRRAGGVLVASHLQSF LEVSYRVLRLHLAQP
75	FkIB2-EK-GCSF 融合体(100 个 aa 截短的 FkIB)	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPSRVDQEQMAASFVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITTLLGGGGSGGGGSHHHHHHDDDDKMTPLGPAS SLPQSFLKCLEQVRKIQGDGAALQEKLCATYKLCHPEELVLL GHSLGIPWAPLSSCPSQALQLAGCLS QLHSGFLYQGLLQALE GISPELGPTLDTLQLDVADFATTIWQQMEELGMAPALQPTQGA MPAFASAFQRRAGGVLVASHLQSFLEVSYRVLRLHLAQP
76	FkIB3-EK-GCSF 融合体 (500 个 aa 截短的 FkIB)	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPGGGSGGGGSHHHHHHDDDDKMTPLGPASSLPQSFL LKCLEQVRKIQGDGAALQEKLCATYKLCHPEELVLLGHSLGIP WAPLSSCPSQALQLAGCLS QLHSGFLYQGLLQALEGISPELG PTLDTLQLDVADFATTIWQQMEELGMAPALQPTQGAMPAFASA FQRRAGGVLVASHLQSFLEVSYRVLRLHLAQP
77	FrnE-EK-GCSF 融 合体(全长 FrnE)	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHQ PFELNPNMPAEGQNIVEHITEKYGSTAESQANRARIRDMGAA LGFAFRTDGQSRIYNTFDAHRLHGWAGLEGLQYNLKEALFKAY FSDGQDPSDHATLAI IAESVGLDLARAAEILASDEYAAEVREQ EQLWVSRGVSSVPTIVFNDQYAVSGGQPAEAFVGAIQIINES KSGGGSGGGGSHHHHHHDDDDKMTPLGPASSLPQSFLKCLE QVRKIQGDGAALQEKLCATYKLCHPEELVLLGHSLGIPWAPLS SCPSQALQLAGCLS QLHSGFLYQGLLQALEGISPELGPTLDT

[0399]

		LQLDVADFATTIWQQMEELGMAPALQPTQGAMPAFASAFQRRAGGVLVASHLQSFLEVSYRVLRLHLAQP
78	FrnE2-EK-GCSF 融合体(100 个 aa 截短的 FrnE)	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAESQANRARIRDMGAA LGFAFRTDGQSRIYGGGSGGGGSHHHHHHDDDDKMTPLGPAS SLPQSFLLKCLEQVRKIQGDGAALQEKLCAATYKLCHEPEELVLL GHSLGIPWAPLSSCPSQALQLAGCLSQLHSGFLFYQGLLQALE GISPELGPTLDTLQLDVADFATTIWQQMEELGMAPALQPTQGA MPAFASAFQRRAGGVLVASHLQSFLEVSYRVLRLHLAQP
79	FrnE3-EK-GCSF 融合体(50 个 aa 截 短的 FrnE)	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNNGGGSGGGGSHHHHHHDDDDKMTPLGPASSLPQSFL LKCLEQVRKIQGDGAALQEKLCAATYKLCHEPEELVLLGHSLGIP WAPLSSCPSQALQLAGCLSQLHSGFLFYQGLLQALEGISPELG PTLDTLQLDVADFATTIWQQMEELGMAPALQPTQGAMPAFASA FQRRAGGVLVASHLQSFLEVSYRVLRLHLAQP
80	前 胰 岛 素 G737-001 基因片 段(CP-A)的核苷 酸序列(包括克隆 位点;下划线是起 始 Phe 密码子)	ATATGCTCTTCAAAGTTTGTAAACCAACACCTGTGTGGCTCCC ATCTCGTCGAAGCCCTGTACCTCGTCTGCGGTGAGCGCGGCTT CTTCTACACTCCCAAGACCCGGCGTGAAGCCGAGGACTTGCAA GTGGGCCAAGTGGAGCTCGGCGGTGGTCCCGGTGCGGGCAGCC TGCAACCGCTCGCGCTGGAAGGGTCGCTGCAGAAGCGCGGCAT CGTGGAGCAGTGTGTCACGAGCATCTGCTCGCTGTACCAGCTG GAGAACTACTGCGGCTGATAATAGTTCAGAAGAGCATAT
81	前 胰 岛 素 G737-002 基因片 段(CP-B)的核苷 酸序列(包括克隆 位点;下划线是起 始 Phe 密码子)	ATATGCTCTTCAAAGTTTCGTAAACCAACATCTGTGTGGCTCCC ACCTCGTCGAAGCCCTGTACCTCGTCTGCGGTGAGCGCGGCTT CTTTTACACGCCCAAGACCCGGCGTGACGTGCCGCAAGTGGAG CTGGGGGGTGGCCCCGGCGCGGGTAGCCTGCAGCCGCTGGCCC TGGAAGGCTCGCTCCAAAAGCGCGGCATCGTGGAGCAGTGTG CACTAGCATCTGCTCGCTGTACCAGTTGGAGAACTACTGCGGC TGATAATAGTTCAGAAGAGCATAT
82	前 胰 岛 素 G737-003 基因片 段(CP-C)的核苷 酸序列(包括克隆 位点;下划线是起 始 Phe 密码子)	ATATGCTCTTCAAAGTTTCGTCAACCAACACCTGTGCGGCTCCC ATCTCGTCGAAGCCCTGTACCTCGTATGCGGTGAGCGCGGGTT TTTCTACACGCCCAAGACTCGCCGGGACGTGCCGCAAGTGGAG CTGGGCGGTGGCCCCGGCGCGGGCTCGCTGCAGCCCCCTGGCGC TGGAAGGCAGCTTGCAAGCCCGTGGCATCGTGGAGCAGTGTG TACCTCGATCTGCAGCCTCTACCAGCTGGAGAACTACTGCGGT TGATAATAGTTCAGAAGAGCATAT

[0400]

	始 Phe 密码子)	
83	前 胰 岛 素 G737-007 基因片 段 (CP-D) 的 核 苷 酸 序 列 (包 括 克 隆 位 点; 下 划 线 是 起 始 Phe 密码子)	ATATGCTCTTCAAAGTTCGTCAACCAACACCTGTGTGGCTCCC ATCTCGTCGAAGCGCTGTACCTCGTATGCGGTGAGCGGGGTTT CTTTTACACGCCCCAAGACCCGTCGCGAGGCCGAGGACCAGGGC TCGCTGCAGAAGCGCGGGATCGTGGAACAATGCTGCACTAGCA TCTGCAGCCTGTACCAACTGGAGAATACTGCGGCTGATAATA GTTTCAGAAGAGCATAT
84	前 胰 岛 素 G737-009 基因片 段 (CP-A) 的 核 苷 酸 序 列 (包 括 克 隆 位 点; 下 划 线 是 起 始 Phe 密码子)	ATATGCTCTTACGATTCGTCAACCAACACCTCTGCGGCAGCC ATCTCGTCGAAGCCCTCTACCTCGTATGTGGCGAACGGGGCTT CTTTTACACCCCCAAGACGCGCCGTGAGGCCGAGGACTTGCAA GTGGGCCAAGTGGAGCTGGGCGGTGGTCCCGGTGCGGGCTCGC TGCAACCGCTGGCGCTGGAAGGGTCGCTGCAGAAGCGCGGCAT CGTGGAGCAGTGTGCACTAGCATCTGCTCCCTGTACCAGCTG GAGAACTACTGCGGCTGATAATAGTTTCAGAAGAGCATAT
85	前 胰 岛 素 G737-017 基因片 段 (CP-B) 的 核 苷 酸 序 列 (包 括 克 隆 位 点; 下 划 线 是 起 始 Phe 密码子)	ATATGCTCTTACGATTCGTAAACCAACACCTCTGCGGCTCCC ATTTGGTCGAAGCCCTCTACCTCGTCTGCGGTGAGCGGGGGTT TTTCTACACTCCCAAGACCCGTCGCGACGTGCCGCAAGTGGAG CTGGGCGGTGGCCCCGGCGCCGGCTCGCTGCAACCGCTGGCGC TGGAAGGTTTCGCTGCAGAAGCGCGGCATCGTGGAGCAGTGCTG CACGAGCATCTGCAGCCTGTACCAGCTGGAGAACTACTGTGGC TGATAATAGTTTCAGAAGAGCATAT
86	前 胰 岛 素 G737-018 基因片 段 (CP-C) 的 核 苷 酸 序 列 (包 括 克 隆 位 点; 下 划 线 是 起 始 Phe 密码子)	ATATGCTCTTACGATTCGTCAACCAACATCTCTGCGGCTCCC ACCTGGTCGAAGCCCTCTACCTCGTATGCGGCGAACGCGGCTT TTTCTACACCCCCAAGACTCGGCGCGACGTGCCGCAAGTGGAG CTGGGCGGTGGTCCCGGTGCGGGCTCGCTGCAGCCGTTGGCCC TGGAAGGGAGCCTGCAGGCGCGTGGCATCGTGGAGCAATGCTG CACGTCGATCTGTAGCCTGTACCAGCTGGAGAACTACTGCGGC TGATAATAGTTTCAGAAGAGCATAT
87	前 胰 岛 素 G737-031 基因片 段 (CP-D) 的 核 苷 酸 序 列 (包 括 克 隆 位 点; 下 划 线 是 起	ATATGCTCTTACGATTCGTCAACCAACACCTGTGCGGCTCCC ATCTGGTCGAAGCCCTCTACCTCGTATGCGGCGAGCGCGGCTT CTTTTACACCCCCAAGACGCGTCGGGAAGCGGAAGATCAGGGT AGCCTGCAAAAGCGCGGTATCGTGGAGCAGTGCTGCACTTCGA TCTGTAGCCTGTACCAACTGGAGAATACTGCGGGTGATAATA GTTTCAGAAGAGCATAT

[0401]

	始 Phe 密码子)	
88	甘精前胰岛素(由 G737-001 和 G737-009 基因片段编码)(B-肽下划线)(C-肽粗体)(A-肽普通)(高亮: 相对于胰岛素的改变)	FVNQHLCGSHLVEALYLVCGERGFFYTPKTRR EAEDLQVGQVE LGGPGAGSLQPLALEGSLQKR GIVEQCCTSICSLYQLENYCG
89	由 G737-002 和 G737-017 基因片段编码的甘精前胰岛素(C-肽粗体)	FVNQHLCGSHLVEALYLVCGERGFFYTPKTRR DVPQVELGGGP GAGSLQPLALEGSLQKR GIVEQCCTSICSLYQLENYCG
90	由 G737-003 和 G737-018 基因片段编码的甘精前胰岛素(C-肽粗体)	FVNQHLCGSHLVEALYLVCGERGFFYTPKTRR DVPQVELGGGP GAGSLQPLALEGSLQAR GIVEQCCTSICSLYQLENYCG
91	由 G737-007 和 G737-031 基因片段编码的甘精前胰岛素(C-肽粗体)	FVNQHLCGSHLVEALYLVCGERGFFYTPKTRR EAEDQGS LQKR GIVEQCCTSICSLYQLENYCG
92	甘精胰岛素 A-肽	GIVEQCCTSICSLYQLENYCG
93	甘精胰岛素 B-肽	FVNQHLCGSHLVEALYLVCGERGFFYTPKTRR
94	门冬胰岛素(B-肽下划线)(C-肽粗体)(A-肽普通)(高亮: 相对于胰岛素的改变)	FVNQHLCGSHLVEALYLVCGERGFFYTDKTE EAEDLQVGQVELG GGPGAGSLQPLALEGSLQKR GIVEQCCTSICSLYQLENYCN
95	人胰岛素 A-肽	GIVEQCCTSICSLYQLENYCN
96	人胰岛素 B-肽	FVNQHLCGSHLVEALYLVCGERGFFYTPKT

[0402]

97	前胰岛素 (CP-A) 的 C-肽	EAEDLQVGQVELGGGPGAGSLQPLALEGSLQKR
98	前胰岛素 (CP-B) 的 C-肽	DVPQVELGGGPGAGSLQPLALEGSLQKR
99	前胰岛素 (CP-C) 的 C-肽	DVPQVELGGGPGAGSLQPLALEGSLQAR
100	前胰岛素 (CP-D) 的 C-肽	EAEDQGSLSQKR
101	DnaJ-样蛋白-胰 蛋白酶 (aa)(由 pFNX4401 编码)	MKVEPGLYQHYKGPQYRVFSVARHSETEEVVVFYQALYGEYGF WVRPLSMFLETVEVDGEQVPRFALVTAEPSLFTGQGGGSGGG GSHHHHHHR
102	EcpD1-胰蛋白酶 (aa)(由 pFNX4402 编码, 其缺乏 EcpD1 RXF04296.1 中下 划线的 Y 和 P 之 间出现的 N 残基)	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGAPLAQDKESVFWLNVLEIPPKPEAGADLN TLQMAFRSRIKLFYRPVGLPGNPNEAVEQVQWQLVTARDGQGL ALKAYPSAFHVSLIELDLVAGNQRYRSEDGMVGPGETRQFALP TLKARPSSQAQVEFSAINDYGALVPTRNTLQPGGGSGGGGSH HHHHHR
103	EcpD1-胰蛋白酶 (aa)	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGAPLAQDKESVFWLNVLEIPPKPEAGADLN TLQMAFRSRIKLFYRPVGLPGNPNEAVEQVQWQLVTARDGQGL ALKAYNPSAFHVSLIELDLVAGNQRYRSEDGMVGPGETRQFAL PTLKARPSSQAQVEFSAINDYGALVPTRNTLQPGGGSGGGGS HHHHHR
104	EcpD2-胰蛋白酶 (aa)(由 pFNX4403 编码)	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGGGGSGGGGSHHHHHHR
105	EcpD3-胰蛋白酶 (aa)(由 pFNX4404 编码)	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGGGSGGGGSHHHHHHR

[0403]

106	FkIB- 胰蛋白酶 (aa)(由 pFNX4405 编码)	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPSRVDQEQMAASFVKVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITTTLASGLQFEVLTAGTGAKPTREDQVRTHYHG TLIDGTVFDSSYERGQPAEFVGGVIAGWTEALQLMNAGSKWR VYVPSELAYGAQGVGSIIPHSVLVFDVELLDVLGGGGSGGGGS HHHHHHR
107	FkIB2- 胰蛋白酶 (aa)(由 pFNX4406 编码)	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPSRVDQEQMAASFVKVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITTTLGGGGSGGGGSHHHHHR
108	FkIB3- 胰蛋白酶 (aa)(由 pFNX4407 编码)	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPGGGSGGGGSHHHHHR
109	FrnE- 胰蛋白酶 (aa)(由 pFNX4408 编码)	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAEESQANRARIRDMGAA LGFAFRTDGQSRIYNTFDAHRLHWHAGLEGLQYNLKEALFKAY FSDGQDPSDHATLAI IAE SVGLDLARAAEILASDEYAAEVREQ EQLWVSRGVSSVPTIVFNDQYAVSGGQPAEAFVGAIRQIINES KSGGGSGGGGSHHHHHR
110	FrnE2- 胰蛋白酶 (aa)(由 pFNX4409 编码)	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAEESQANRARIRDMGAA LGFAFRTDGQSRIYGGGGSGGGGSHHHHHR
111	FrnE3- 胰蛋白酶 (aa)(由 pFNX4410 编码)	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNGGGSGGGGSHHHHHR
112	DnaJ- 样蛋白-EK (aa)(由 pFNX4411 编码)	MKVEPGLYQHYKGPQYRVFSVARHSETEEEVVIFYQALYGEYGF WVRPLSMFLETVEVDGEQVPRFALVTAEPSLFTGQGGGGSGGG GSHHHHHHDDDDK
113	EcpD1-EK(aa)(由 pFNX4412 编码)	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGAPLAQDKESVFWLNVLEIPPKPEAGADLN TLQMAFRSRIKLFYRPVGLPGNPNEAVEQVQWQLVTARDGQGL ALKAYNPFAFHVSLIELDLVAGNQRYRSEDGMVGPGETRQFAL PTLKARPSSQAQVEFSAINDYGALVPTRNTLQPGGGSGGGGS

[0404]

		HHHHHHDDDDK
114	EcpD2-EK(aa)(由 pFNX4413 编码)	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGGGGSGGGGSHHHHHHDDDDK
115	EcpD3-EK(aa)(由 pFNX4414 编码)	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGGGGSGGGGSHHHHHHDDDDK
116	FklB-EK(aa)(由 pFNX4415 编码)	MSEVNLSTDETRVSYGIGRQLGDQLRDNPFGVSLDAILAGLT DAFAGKPSRVDQEQAASFKVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITT LASGLQFEVLTAGTGAKPTREDQVRTHYHG TLIDGTVFDSSYERGQPAEFFVGGVIAGWTEALQLMNAGSKWR VYVPSELAYGAQGVGSI PPHSVLVFDVELLDVLGGGGSGGGGS HHHHHHDDDDK
117	FklB2-EK(aa)(由 pFNX4416 编码)	MSEVNLSTDETRVSYGIGRQLGDQLRDNPFGVSLDAILAGLT DAFAGKPSRVDQEQAASFKVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITT LGGGGSGGGGSHHHHHHDDDDK
118	FklB3-EK(aa)(由 pFNX4417 编码)	MSEVNLSTDETRVSYGIGRQLGDQLRDNPFGVSLDAILAGLT DAFAGKPGGGSGGGGSHHHHHHDDDDK
119	FrnE-EK(aa)(由 pFNX4418 编码)	MSTPLKIDFVSDVSCPWCII GLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAESQANRARIRDMGAA LGFAFRTDGQSRIYNTFDAHRLHWHAGLEGLQYNLKEALFKAY FSDGQDPSDHATLAI I AESVGLDLARAAEILASDEYAAEVREQ EQLWVSRGVSSVPTIVFNDQYAVSGGQPAEAFVGAIRQI INES KSGGGSGGGGSHHHHHHDDDDK
120	FrnE2-EK(aa)(由 pFNX4419 编码)	MSTPLKIDFVSDVSCPWCII GLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAESQANRARIRDMGAA LGFAFRTDGQSRIYGGGGSGGGGSHHHHHHDDDDK
121	FrnE3-EK(aa)(由 pFNX4420 编码)	MSTPLKIDFVSDVSCPWCII GLRGLTEALDQLGSEVQAEIHFQ PFELNPNGGGGSGGGGSHHHHHHDDDDK
122	DnaJ-样蛋白-EK- 前胰岛素-CP-A	MKVEPGLYQHYKGPQYRVFSVARHSETEEEVVIFYQALYGEYGF WVRPLSMFLETVEVDGEQVPRFALVTAEPSLFTGQGGGGSGGG GSHHHHHHDDDDKFVNQHLGSHLVEALYLVCGERGFFYTPKT RREAEDLQVGQVELGGGPGAGSLQPLALEGSLQKRGIVEQCCT SICSLYQLENYCG
123	DnaJ-样蛋白-EK-	MKVEPGLYQHYKGPQYRVFSVARHSETEEEVVIFYQALYGEYGF WVRPLSMFLETVEVDGEQVPRFALVTAEPSLFTGQGGGGSGGG

[0405]

	前胰岛素-CP-B	GSHHHHHHDDDDKFVNQHLCGSHLVEALYLVCGERGFFYTPKT RRDVPQVELGGGPGAGSLQPLALEGSLQKRGIVEQCCTSI CSL YQLENYCG
124	DnaJ-样蛋白-EK- 前胰岛素-CP-C	MKVEPGLYQHYKGPQYRVFSVARHSETEEVVVFYQALYGEYGF WVRPLSMFLETVEVDGEQVPRFALVTAEPSLFTGQGGGSGGG GSHHHHHHDDDDKFVNQHLCGSHLVEALYLVCGERGFFYTPKT RRDVPQVELGGGPGAGSLQPLALEGSLQARGIVEQCCTSI CSL YQLENYCG
125	DnaJ-样蛋白-胰 蛋白酶-前胰岛素 -CP-A	MKVEPGLYQHYKGPQYRVFSVARHSETEEVVVFYQALYGEYGF WVRPLSMFLETVEVDGEQVPRFALVTAEPSLFTGQGGGSGGG GSHHHHHHR FVNQHLCGSHLVEALYLVCGERGFFYTPKTRREAEDLQVGQVE LGGGPGAGSLQPLALEGSLQKRGIVEQCCTSI CSLYQLENYCG
126	DnaJ-样蛋白-胰 蛋白酶-前胰岛素 -CP-B	MKVEPGLYQHYKGPQYRVFSVARHSETEEVVVFYQALYGEYGF WVRPLSMFLETVEVDGEQVPRFALVTAEPSLFTGQGGGSGGG GSHHHHHHR FVNQHLCGSHLVEALYLVCGERGFFYTPKTRRDVPQVELGGGP GAGSLQPLALEGSLQKRGIVEQCCTSI CSLYQLENYCG
127	DnaJ-样蛋白-胰 蛋白酶-前胰岛素 -CP-C	MKVEPGLYQHYKGPQYRVFSVARHSETEEVVVFYQALYGEYGF WVRPLSMFLETVEVDGEQVPRFALVTAEPSLFTGQGGGSGGG GSHHHHHHR FVNQHLCGSHLVEALYLVCGERGFFYTPKTRRDVPQVELGGGP GAGSLQPLALEGSLQARGIVEQCCTSI CSLYQLENYCG
128	DnaJ-样蛋白-EK- 前胰岛素-CP-D	MKVEPGLYQHYKGPQYRVFSVARHSETEEVVVFYQALYGEYGF WVRPLSMFLETVEVDGEQVPRFALVTAEPSLFTGQGGGSGGG GSHHHHHHDDDDKFVNQHLCGSHLVEALYLVCGERGFFYTPKT RREAEDQGSLQKRGIVEQCCTSI CSLYQLENYCG
129	DnaJ-样蛋白-胰 蛋白酶-前胰岛素 -CP-D	MKVEPGLYQHYKGPQYRVFSVARHSETEEVVVFYQALYGEYGF WVRPLSMFLETVEVDGEQVPRFALVTAEPSLFTGQGGGSGGG GSHHHHHHR FVNQHLCGSHLVEALYLVCGERGFFYTPKTRREAEDQGSLQKR GIVEQCCTSI CSLYQLENYCG
130	FklB-EK- 前胰岛 素-CP-A	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPSRVDQE QMAASFVKVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITTLASGLQFEVLTAGTGAKPTREDQVRTHYHG TLIDGTVFDSSYERGQPAEFFVGGVIAGWTEALQLMNAGSKWR

[0406]

		VYVPSELAYGAQGVGSIPPHSVLVFDVELLDVLGGGSGGGGS HHHHHHDDDDKFVNQHLCGSHLVEALYLVCGERGFFYTPKTRR EAEDLQVGQVELGGGPGAGSLQPLALEGSLQKRGIVEQCCTSI CSLYQLENYCG
131	FkIB-EK- 前胰 素-CP-B	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPSRVDQEOMAASFVKVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITTTLASGLQFEVLTAGTGAKPTREDQVRTHYHG TLIDGTVFDSSYERGQPAEFFVGGVIAGWTEALQLMNAGSKWR VYVPSELAYGAQGVGSIPPHSVLVFDVELLDVLGGGSGGGGS HHHHHHDDDDKFVNQHLCGSHLVEALYLVCGERGFFYTPKTRR DVPQVELGGGPGAGSLQPLALEGSLQKRGIVEQCCTSICSLYQ LENYCG
132	FkIB-EK- 前胰 素-CP-C	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPSRVDQEOMAASFVKVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITTTLASGLQFEVLTAGTGAKPTREDQVRTHYHG TLIDGTVFDSSYERGQPAEFFVGGVIAGWTEALQLMNAGSKWR VYVPSELAYGAQGVGSIPPHSVLVFDVELLDVLGGGSGGGGS HHHHHHDDDDKFVNQHLCGSHLVEALYLVCGERGFFYTPKTRR DVPQVELGGGPGAGSLQPLALEGSLQARGIVEQCCTSICSLYQ LENYCG
133	FkIB-胰蛋白酶-前 胰岛素-CP-A	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPSRVDQEOMAASFVKVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITTTLASGLQFEVLTAGTGAKPTREDQVRTHYHG TLIDGTVFDSSYERGQPAEFFVGGVIAGWTEALQLMNAGSKWR VYVPSELAYGAQGVGSIPPHSVLVFDVELLDVLGGGSGGGGS HHHHHHRFVNQHLCGSHLVEALYLVCGERGFFYTPKTRREAED LQVGQVELGGGPGAGSLQPLALEGSLQKRGIVEQCCTSICSLY QLENYCG
134	FkIB-胰蛋白酶-前 胰岛素-CP-B	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPSRVDQEOMAASFVKVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITTTLASGLQFEVLTAGTGAKPTREDQVRTHYHG TLIDGTVFDSSYERGQPAEFFVGGVIAGWTEALQLMNAGSKWR VYVPSELAYGAQGVGSIPPHSVLVFDVELLDVLGGGSGGGGS HHHHHHRFVNQHLCGSHLVEALYLVCGERGFFYTPKTRRDVPQ VELGGGPGAGSLQPLALEGSLQKRGIVEQCCTSICSLYQLENY CG

[0407]

135	FlkB-胰蛋白酶-前胰岛素-CP-C	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPSRVDQEQMAASFVKVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITTLLASGLQFEVLTAGTGAKPTREDQVRTHYHG TLIDGTVFDSSYERGQPAEFPVGGVIAGWTEALQLMNAGSKWR VYVPSELAYGAQGVGSIPPHSVLVFDVELLDVLGGGGSGGGGS HHHHHHRFVNQHLCGSHLVEALYLVCGERGFFYTPKTRRDVPQ VELGGGPGAGSLQPLALEGSLQARGIVEQCCTSICSLYQLENY CG
136	FlkB-EK-前胰岛素-CP-D	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPSRVDQEQMAASFVKVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITTLLASGLQFEVLTAGTGAKPTREDQVRTHYHG TLIDGTVFDSSYERGQPAEFPVGGVIAGWTEALQLMNAGSKWR VYVPSELAYGAQGVGSIPPHSVLVFDVELLDVLGGGGSGGGGS HHHHHHDDDDKFVNQHLCGSHLVEALYLVCGERGFFYTPKTRR EAEDQGSLLQKRGIVEQCCTSICSLYQLENYCG
137	FlkB-胰蛋白酶-前胰岛素-CP-D	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPSRVDQEQMAASFVKVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITTLLASGLQFEVLTAGTGAKPTREDQVRTHYHG TLIDGTVFDSSYERGQPAEFPVGGVIAGWTEALQLMNAGSKWR VYVPSELAYGAQGVGSIPPHSVLVFDVELLDVLGGGGSGGGGS HHHHHHRFVNQHLCGSHLVEALYLVCGERGFFYTPKTRREAED QGSLLQKRGIVEQCCTSICSLYQLENYCG
138	FlkB2-EK-前胰岛素-CP-A	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPSRVDQEQMAASFVKVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITTLLGGGGSGGGGSHHHHHHDDDDKFVNQHLCG SHLVEALYLVCGERGFFYTPKTRREAEDLQVGQVELGGGPGAG SLQPLALEGSLQKRGIVEQCCTSICSLYQLENYCG
139	FlkB2-EK-前胰岛素-CP-B	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPSRVDQEQMAASFVKVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITTLLGGGGSGGGGSHHHHHHDDDDKFVNQHLCG SHLVEALYLVCGERGFFYTPKTRRDVPQVELGGGPGAGSLQPL ALEGSLQKRGIVEQCCTSICSLYQLENYCG
140	FlkB2-EK-前胰岛素-CP-C	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPSRVDQEQMAASFVKVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITTLLGGGGSGGGGSHHHHHHDDDDKFVNQHLCG SHLVEALYLVCGERGFFYTPKTRRDVPQVELGGGPGAGSLQPL

[0408]

		ALEGLSLQARGIVEQCCTSI CSLYQLENYCG
141	FkIB2-胰蛋白酶- 前胰岛素-CP-A	MSEVNLSTDETRVSYGIGRQLGDQLRDNP PPGVSLDAILAGLT DAFAGKPSRVDQEQMAASF KVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITT LGGGGSGGGGSHHHHHHRFVNQHLCGSHLV EALYLVCGERGFFYTPKTRREAEDLQVGQVELGGGPGAGSLQP LALEGLSLQKRGIVEQCCTSI CSLYQLENYCG
142	FkIB2-胰蛋白酶- 前胰岛素-CP-B	MSEVNLSTDETRVSYGIGRQLGDQLRDNP PPGVSLDAILAGLT DAFAGKPSRVDQEQMAASF KVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITT LGGGGSGGGGSHHHHHHRFVNQHLCGSHLV EALYLVCGERGFFYTPKTRRDVPQVELGGGPGAGSLQPLALEG SLQKRGIVEQCCTSI CSLYQLENYCG
143	FkIB2-胰蛋白酶- 前胰岛素-CP-C	MSEVNLSTDETRVSYGIGRQLGDQLRDNP PPGVSLDAILAGLT DAFAGKPSRVDQEQMAASF KVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITT LGGGGSGGGGSHHHHHHRFVNQHLCGSHLV EALYLVCGERGFFYTPKTRRDVPQVELGGGPGAGSLQPLALEG SLQARGIVEQCCTSI CSLYQLENYCG
144	FkIB2-EK-前胰 素-CP-D	MSEVNLSTDETRVSYGIGRQLGDQLRDNP PPGVSLDAILAGLT DAFAGKPSRVDQEQMAASF KVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITT LGGGGSGGGGSHHHHHHDDDDKFVNQHLCG SHLVEALYLVCGERGFFYTPKTRREAEDQGS LQKRGIVEQCCT SI CSLYQLENYCG
145	FkIB2-胰蛋白酶- 前胰岛素-CP-D	MSEVNLSTDETRVSYGIGRQLGDQLRDNP PPGVSLDAILAGLT DAFAGKPSRVDQEQMAASF KVIREIMQAEAAAKAEAAAGAGLA FLAENAKRDGITT LGGGGSGGGGSHHHHHHRFVNQHLCGSHLV EALYLVCGERGFFYTPKTRREAEDQGS LQKRGIVEQCCTSI CS LYQLENYCG
146	FkIB3.1-EK-前胰 岛素-CP-A	MSEVNLSTDETRVSYGIGRQLGDQLRDNP PPGVSLDAILAGLT DAFAGKPGGGGSGGGGSHHHHHHDDDDKFVNQHLCGSHLVEAL YLVCGERGFFYTPKTRREAEDLQVGQVELGGGPGAGSLQPLAL EGSLQKRGIVEQCCTSI CSLYQLENYCG
147	FkIB3-EK-前胰 素-CP-B	MSEVNLSTDETRVSYGIGRQLGDQLRDNP PPGVSLDAILAGLT DAFAGKPGGGGSGGGGSHHHHHHDDDDKFVNQHLCGSHLVEAL YLVCGERGFFYTPKTRRDVPQVELGGGPGAGSLQPLALEGSLQ KRGIVEQCCTSI CSLYQLENYCG
148	FkIB3.1-EK-前胰 岛素-CP-C	MSEVNLSTDETRVSYGIGRQLGDQLRDNP PPGVSLDAILAGLT DAFAGKPGGGGSGGGGSHHHHHHDDDDKFVNQHLCGSHLVEAL

[0409]

		YLVCGERGGFFYTPKTRRDVPQVELGGGPGAGSLQPLALEGSLQ ARGIVEQCCTSICSLYQLENYCG
149	FklB3-胰蛋白酶- 前胰岛素-CP-A	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPGGGGSGGGGSHHHHHHRFVNQHLCGSHLVEALYLVC GERGGFFYTPKTRREAEDLQVGQVELGGGPGAGSLQPLALEGSL QKRGIVEQCCTSICSLYQLENYCG
150	FklB3.1-胰蛋白酶 -前胰岛素-CP-B	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPGGGGSGGGGSHHHHHHRFVNQHLCGSHLVEALYLVC GERGGFFYTPKTRRDVPQVELGGGPGAGSLQPLALEGSLQKRG I VEQCCTSICSLYQLENYCG
151	FklB3.1-胰蛋白酶 -前胰岛素-CP-C	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPGGGGSGGGGSHHHHHHRFVNQHLCGSHLVEALYLVC GERGGFFYTPKTRRDVPQVELGGGPGAGSLQPLALEGSLQARG I VEQCCTSICSLYQLENYCG
152	FklB-EK- 前胰岛 素-CP-D	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPGGGGSGGGGSHHHHHHDDDDKFVNQHLCGSHLVEAL YLVCGERGGFFYTPKTRREAEDQGS LQKRGIVEQCCTSICSLYQ LENYCG
153	FklB3.1-胰蛋白酶 -前胰岛素-CP-D	MSEVNLSTDETRVSYGIGRQLGDQLRDNPPPGVSLDAILAGLT DAFAGKPGGGGSGGGGSHHHHHHRFVNQHLCGSHLVEALYLVC GERGGFFYTPKTRREAEDQGS LQKRGIVEQCCTSICSLYQLENY CG
154	FrnE-EK- 前胰岛 素-CP-A	MSTPLKIDFVSDVSCPWCII GLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAESQANRARIRDMGAA LGFAFRTDGQSRIYNTFDAHRLH WAGLEGLQYNLKEALFKAY FSDGQDPSDHATLAI I AESVGLDLARAAEILASDEYAAEVREQ EQLWVSRGVSSVPTIVFNDQYAVSGGQPAEAFVGAI RQI INES KSGGGGSGGGGSHHHHHHDDDDKFVNQHLCGSHLVEALYLVC GERGGFFYTPKTRREAEDLQVGQVELGGGPGAGSLQPLALEGSLQ KRGIVEQCCTSICSLYQLENYCG
155	FrnE-EK- 前胰岛 素-CP-B	MSTPLKIDFVSDVSCPWCII GLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAESQANRARIRDMGAA LGFAFRTDGQSRIYNTFDAHRLH WAGLEGLQYNLKEALFKAY FSDGQDPSDHATLAI I AESVGLDLARAAEILASDEYAAEVREQ EQLWVSRGVSSVPTIVFNDQYAVSGGQPAEAFVGAI RQI INES KSGGGGSGGGGSHHHHHHDDDDKFVNQHLCGSHLVEALYLVC

[0410]

		ERGFFYTPKTRRDVPQVELGGGPGAGSLQPLALEGSLQKRGIV EQCCTSICSLYQLENYCG
156	FrnE-EK- 前胰 素-CP-C	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAEESQANRARIRDMGAA LGFAFRTDGQSRIYNTFDAHRLHWHAGLEGLQYNLKEALFKAY FSDGQDPSDHATLAI IAESVGLDLARAAEILASDEYAAEVREQ EQLWVSRGVSSVPTIVFNDQYAVSGGQPAEAFVGAI RQI INES KSGGGGSGGGGSHHHHHHDDDDKFVNQHL CGSHLVEALYLVCG ERGFFYTPKTRRDVPQVELGGGPGAGSLQPLALEGSLQARGIV EQCCTSICSLYQLENYCG
157	FrnE-胰蛋白酶-前 胰岛素-CP-A	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAEESQANRARIRDMGAA LGFAFRTDGQSRIYNTFDAHRLHWHAGLEGLQYNLKEALFKAY FSDGQDPSDHATLAI IAESVGLDLARAAEILASDEYAAEVREQ EQLWVSRGVSSVPTIVFNDQYAVSGGQPAEAFVGAI RQI INES KSGGGGSGGGGSHHHHHHRFVNQHL CGSHLVEALYLVCGGERGF FYTPKTRREAEDLQVGQVELGGGPGAGSLQPLALEGSLQKRG I VEQCCTSICSLYQLENYCG
158	FrnE-胰蛋白酶-前 胰岛素-CP-B	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAEESQANRARIRDMGAA LGFAFRTDGQSRIYNTFDAHRLHWHAGLEGLQYNLKEALFKAY FSDGQDPSDHATLAI IAESVGLDLARAAEILASDEYAAEVREQ EQLWVSRGVSSVPTIVFNDQYAVSGGQPAEAFVGAI RQI INES KSGGGGSGGGGSHHHHHHRFVNQHL CGSHLVEALYLVCGGERGF FYTPKTRRDVPQVELGGGPGAGSLQPLALEGSLQKRGIVEQCC TSICSLYQLENYCG
159	FrnE-胰蛋白酶-前 胰岛素-CP-C	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAEESQANRARIRDMGAA LGFAFRTDGQSRIYNTFDAHRLHWHAGLEGLQYNLKEALFKAY FSDGQDPSDHATLAI IAESVGLDLARAAEILASDEYAAEVREQ EQLWVSRGVSSVPTIVFNDQYAVSGGQPAEAFVGAI RQI INES KSGGGGSGGGGSHHHHHHRFVNQHL CGSHLVEALYLVCGGERGF FYTPKTRRDVPQVELGGGPGAGSLQPLALEGSLQARGIVEQCC TSICSLYQLENYCG
160	FrnE-EK- 前胰 素-CP-D	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAEESQANRARIRDMGAA

[0411]

		LGFAFRTDGQSRIYNTFDAHRLHWHAGLEGLQYNLKEALFKAY FSDGQDPSDHATLAI IAESVGLDLARAAEILASDEYAAEVREQ EQLWVSRGVSSVPTIVFNDQYAVSGGQPAAEFVGAIRQI INES KSGGGGSGGGGSHHHHHHDDDDKFVNQHLCGSHLVEALYLVCG ERGFFYTPKTRREAEDQGS LQKRGIVEQCCTSI CSLYQLENYC G
161	FrnE-胰蛋白酶-前 胰岛素-CP-D	MSTPLKIDFVSDVSCPWCII GLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAEESQANRARIRDMGAA LGFAFRTDGQSRIYNTFDAHRLHWHAGLEGLQYNLKEALFKAY FSDGQDPSDHATLAI IAESVGLDLARAAEILASDEYAAEVREQ EQLWVSRGVSSVPTIVFNDQYAVSGGQPAAEFVGAIRQI INES KSGGGGSGGGGSHHHHHHRFVNQHLCGSHLVEALYLVCGERGF FYTPKTRREAEDQGS LQKRGIVEQCCTSI CSLYQLENYCG
162	FrnE2-EK-前胰岛 素-CP-A	MSTPLKIDFVSDVSCPWCII GLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAEESQANRARIRDMGAA LGFAFRTDGQSRIYGGGGSGGGGSHHHHHHDDDDKFVNQHLCG SHLVEALYLVCGERGFFYTPKTRREAEDLQVGQVELGGGPGAG SLQPLALEGSLQKRGIVEQCCTSI CSLYQLENYCG
163	FrnE2-EK-前胰岛 素-CP-B	MSTPLKIDFVSDVSCPWCII GLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAEESQANRARIRDMGAA LGFAFRTDGQSRIYGGGGSGGGGSHHHHHHDDDDKFVNQHLCG SHLVEALYLVCGERGFFYTPKTRRDVPQVELGGGPGAGSLQPL ALEGSLQKRGIVEQCCTSI CSLYQLENYCG
164	FrnE2-EK-前胰岛 素-CP-C	MSTPLKIDFVSDVSCPWCII GLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAEESQANRARIRDMGAA LGFAFRTDGQSRIYGGGGSGGGGSHHHHHHDDDDKFVNQHLCG SHLVEALYLVCGERGFFYTPKTRRDVPQVELGGGPGAGSLQPL ALEGSLQARGIVEQCCTSI CSLYQLENYCG
165	FrnE2-胰蛋白酶- 前胰岛素-CP-A	MSTPLKIDFVSDVSCPWCII GLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAEESQANRARIRDMGAA LGFAFRTDGQSIYGGGGSGGGGSHHHHHHRFVNQHLCGSHLVE ALYLVCGERGFFYTPKTRREAEDLQVGQVELGGGPGAGSLQPL ALEGSLQKRGIVEQCCTSI CSLYQLENYCG
166	FrnE2-胰蛋白酶- 前胰岛素-CP-B	MSTPLKIDFVSDVSCPWCII GLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAEESQANRARIRDMGAA LGFAFRTDGQSRIYGGGGSGGGGSHHHHHHRFVNQHLCGSHLV

[0412]

		EALYLVCGERGFFYTPKTRRDVPQVELGGGPGAGSLQPLALEG SLQKRGIVEQCCTSICSLYQLENYCG
167	FrnE2-胰蛋白酶- 前胰岛素-CP-C	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAEESQANRARIRDMGAA LGFAFRTDGQSRIYGGGGSGGGGSHHHHHHRFVNQHLGSHLV EALYLVCGERGFFYTPKTRRDVPQVELGGGPGAGSLQPLALEG SLQARGIVEQCCTSICSLYQLENYCG
168	FrnE2-EK-前胰岛 素-CP-D	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAEESQANRARIRDMGAA LGFAFRTDGQSRIYGGGGSGGGGSHHHHHHDDDDKFVNQHLG SHLVEALYLVCGERGFFYTPKTRREAEDQGSQKRGIVEQCCT SICSLYQLENYCG
169	FrnE2-胰蛋白酶- 前胰岛素-CP-D	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNMPAEGQNIVEHITEKYGSTAEESQANRARIRDMGAA LGFAFRTDGQSRIYGGGGSGGGGSHHHHHHRFVNQHLGSHLV EALYLVCGERGFFYTPKTRREAEDQGSQKRGIVEQCCTSICS LYQLENYCG
170	FrnE3-EK-前胰岛 素-CP-A	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNGGGGSGGGGSHHHHHHDDDDKFVNQHLGSHLVEAL YLVCGERGFFYTPKTRREAEDLQVGQVELGGGPGAGSLQPLAL EGSLQKRGIVEQCCTSICSLYQLENYCG
171	FrnE3-EK-前胰岛 素-CP-B	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNGGGGSGGGGSHHHHHHDDDDKFVNQHLGSHLVEAL YLVCGERGFFYTPKTRRDVPQVELGGGPGAGSLQPLALEGSLQ KRGIVEQCCTSICSLYQLENYCG
172	FrnE3-EK-前胰岛 素-CP-C	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNGGGGSGGGGSHHHHHHDDDDKFVNQHLGSHLVEAL YLVCGERGFFYTPKTRRDVPQVELGGGPGAGSLQPLALEGSLQ ARGIVEQCCTSICSLYQLENYCG
173	FrnE3-胰蛋白酶- 前胰岛素-CP-A	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNGGGGSGGGGSHHHHHHRFVNQHLGSHLVEALYLVC GERGFFYTPKTRREAEDLQVGQVELGGGPGAGSLQPLALEGSL QKRGIVEQCCTSICSLYQLENYCG
174	FrnE3-胰蛋白酶- 前胰岛素-CP-B	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNGGGGSGGGGSHHHHHHRFVNQHLGSHLVEALYLVC GERGFFYTPKTRRDVPQVELGGGPGAGSLQPLALEGSLQKRG

[0413]

		VEQCCTSICSLYQLENYCG
175	FrnE3-胰蛋白酶- 前胰岛素-CP-C	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNGGGGSGGGGSHHHHHHRFVNQHLCGSHLVEALYLVC GERGFFYTPKTRRDVPQVELGGGPGAGSLQPLALEGSLQARGI VEQCCTSICSLYQLENYCG
176	FrnE3-EK-前胰岛 素-CP-D	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNGGGGSGGGGSHHHHHHDDDDKFVNQHLCGSHLVEAL YLVCGERGFFYTPKTRREAEDQGSQKRGIVEQCCTSICSLYQ LENYCG
177	FrnE3-胰蛋白酶- 前胰岛素-CP-D	MSTPLKIDFVSDVSCPWCIIIGLRGLTEALDQLGSEVQAEIHFQ PFELNPNGGGGSGGGGSHHHHHHRFVNQHLCGSHLVEALYLVC GERGFFYTPKTRREAEDQGSQKRGIVEQCCTSICSLYQLENY CG
178	EcpD1-EK- 前胰 岛素-CP-A	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGAPLAQDKESVFWLNVLEIPPKPEAGADLN TLQMAFRSRIKLFYRPVGLPGNPNEAVEQVQWQLVTARDGQGL ALKAYNPFAFHVSLIELDLVAGNQRYRSEDGMVGPGETRQFAL PTLKARPSSQAQVEFSAINDYGALVPTRNTLQPGGGGSGGGGS HHHHHHDDDDKFVNQHLCGSHLVEALYLVCGERGFFYTPKTRR EAEDLQVGQVELGGGPGAGSLQPLALEGSLQKRGIVEQCCTSI CSLYQLENYCG
179	EcpD1-EK- 前胰 岛素-CP-B	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGAPLAQDKESVFWLNVLEIPPKPEAGADLN TLQMAFRSRIKLFYRPVGLPGNPNEAVEQVQWQLVTARDGQGL ALKAYNPFAFHVSLIELDLVAGNQRYRSEDGMVGPGETRQFAL PTLKARPSSQAQVEFSAINDYGALVPTRNTLQPGGGGSGGGGS HHHHHHDDDDKFVNQHLCGSHLVEALYLVCGERGFFYTPKTRR DVPQVELGGGPGAGSLQPLALEGSLQKRGIVEQCCTSICSLYQ LENYCG
180	EcpD1-EK- 前胰 岛素-CP-C	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGAPLAQDKESVFWLNVLEIPPKPEAGADLN TLQMAFRSRIKLFYRPVGLPGNPNEAVEQVQWQLVTARDGQGL ALKAYNPFAFHVSLIELDLVAGNQRYRSEDGMVGPGETRQFAL

[0414]

		PTLKARPSSQAQVEFSAINDYGALVPTRNTLQPGGGSGGGGS HHHHHHDDDDKFVNQHLCGSHLVEALYLVCGERGFFYTPKTRR DVPQVELGGGPGAGSLQPLALEGSLQARGIVEQCCTSICSLYQ LENYCG
181	EcpD1-EK- 前胰 岛素-CP-D	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGAPLAQDKESVFWLNVL EIPPKPEAGADLN TLQMAFRSRIKLFYRPVGLPGNPNEAVEQVQWQLVTARDGQGL ALKAYNP ^u SAFHVSLIELDLVAGNQRYS ^u EDGMVGPGETRQFAL PTLKARPSSQAQVEFSAINDYGALVPTRNTLQPGGGSGGGGS HHHHHHDDDDKFVNQHLCGSHLVEALYLVCGERGFFYTPKTRR EAEDQGS ^u LQKRGIVEQCCTSICSLYQLENYCG
182	EcpD1-胰蛋白酶- 前胰岛素-CP-A (如 通 过 pFNX4402 编码的 不含下划线 N 残 基的 EcpD1-胰蛋 白酶)	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGAPLAQDKESVFWLNVL EIPPKPEAGADLN TLQMAFRSRIKLFYRPVGLPGNPNEAVEQVQWQLVTARDGQGL ALKAYNP ^u SAFHVSLIELDLVAGNQRYS ^u EDGMVGPGETRQFAL PTLKARPSSQAQVEFSAINDYGALVPTRNTLQPGGGSGGGGS HHHHHHRFVNQHLCGSHLVEALYLVCGERGFFYTPKTRREAED LQVGQVELGGGPGAGSLQPLALEGSLQKRGIVEQCCTSICSLY QLENYCG
183	EcpD1-胰蛋白酶- 前胰岛素-CP-B (如 通 过 pFNX4402 编码的 不含下划线 N 残 基的 EcpD1-胰蛋 白酶)	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGAPLAQDKESVFWLNVL EIPPKPEAGADLN TLQMAFRSRIKLFYRPVGLPGNPNEAVEQVQWQLVTARDGQGL ALKAYNP ^u SAFHVSLIELDLVAGNQRYS ^u EDGMVGPGETRQFAL PTLKARPSSQAQVEFSAINDYGALVPTRNTLQPGGGSGGGGS HHHHHHRFVNQHLCGSHLVEALYLVCGERGFFYTPKTRRDVPQ VELGGGPGAGSLQPLALEGSLQKRGIVEQCCTSICSLYQLENY CG
184	EcpD1-胰蛋白酶- 前胰岛素-CP-C (如 通 过 pFNX4402 编码的 不含下划线 N 残	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGAPLAQDKESVFWLNVL EIPPKPEAGADLN TLQMAFRSRIKLFYRPVGLPGNPNEAVEQVQWQLVTARDGQGL ALKAYNP ^u SAFHVSLIELDLVAGNQRYS ^u EDGMVGPGETRQFAL PTLKARPSSQAQVEFSAINDYGALVPTRNTLQPGGGSGGGGS

[0415]

	基的 EcpD1-胰蛋白酶)	HHHHHHRFVNQHLCGSHLVEALYLVCGERGFFYTPKTRRDVPQ VELGGGPGAGSLQPLALEGSLQARGIVEQCCTSICSLYQLENY CG
185	EcpD1-胰蛋白酶- 前胰岛素-CP-D (如 通 过 pFNX4402 编码的 不含下划线 N 残 基的 EcpD1-胰蛋 白酶)	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGAPLAQDKESVFWLNVEIIPKPEAGADLN TLQMAFRSRIKLFYRPVGLPGNPNEAVEQVQWQLVTARDGQGL ALKAYNP ^u SAFHVSLIELDLVAGNQRYRSEDGMVGPGETRQFAL PTLKARPSSQAQVEFSAINDYGALVPTRNTLQPGGGSGGGGS HHHHHHRFVNQHLCGSHLVEALYLVCGERGFFYTPKTRREAED QGSLQKRGIVEQCCTSICSLYQLENYCG
186	EcpD2-EK- 前胰 岛素-CP-A	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGGGGSGGGGSHHHHHHDDDDKFVNQHLCG SHLVEALYLVCGERGFFYTPKTRREAEDLQVGQVELGGGPGAG SLQPLALEGSLQKRGIVEQCCTSICSLYQLENYCG
187	EcpD2-EK- 前胰 岛素-CP-B	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGGGGSGGGGSHHHHHHDDDDKFVNQHLCG SHLVEALYLVCGERGFFYTPKTRRDVPQVELGGGPGAGSLQPL ALEGSLQKRGIVEQCCTSICSLYQLENYCG
188	EcpD2-EK- 前胰 岛素-CP-C	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGGGGSGGGGSHHHHHHDDDDKFVNQHLCG SHLVEALYLVCGERGFFYTPKTRRDVPQVELGGGPGAGSLQPL ALEGSLQARGIVEQCCTSICSLYQLENYCG
189	EcpD2-胰蛋白酶- 前胰岛素-CP-A	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGGGGSGGGGSHHHHHHRFVNQHLCGSHLV EALYLVCGERGFFYTPKTRREAEDLQVGQVELGGGPGAGSLQP LALEGSLQKRGIVEQCCTSICSLYQLENYCG
190	EcpD2-胰蛋白酶- 前胰岛素-CP-B	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGGGGSGGGGSHHHHHHRFVNQHLCGSHLV EALYLVCGERGFFYTPKTRRDVPQVELGGGPGAGSLQPLALEG SLQKRGIVEQCCTSICSLYQLENYCG

[0416]

191	EcpD2-胰蛋白酶- 前胰岛素-CP-C	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGGGGSGGGGSHHHHHHRFVNQHLCGSHLV EALYLVCGERGFFYTPKTRRDVPQVELGGGPGAGSLQPLALEG SLQARGIVEQCCTSICSLYQLENYCG
192	EcpD2-EK- 前胰 岛素-CP-D	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGGGGSGGGGSHHHHHHDDDDKFVNQHLCG SHLVEALYLVCGERGFFYTPKTRREAEDQGS LQKRGIVEQCCT SICSLYQLENYCG
193	EcpD2-胰蛋白酶- 前胰岛素-CP-D	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGTLPALVQSWIDTGSVESTPTSSKAPFLLSPPVARI DPTKGQSLRVLFTGGGGSGGGGSHHHHHHRFVNQHLCGSHLV EALYLVCGERGFFYTPKTRREAEDQGS LQKRGIVEQCCTSICS LYQLENYCG
194	EcpD3-EK- 前胰 岛素-CP-A	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGGGGSGGGGSHHHHHHDDDDKFVNQHLCGSHLVEAL YLVCGERGFFYTPKTRREAEDLQVGQVELGGGPGAGSLQPLAL EGSLQKRGIVEQCCTSICSLYQLENYCG
195	EcpD3-EK- 前胰 岛素-CP-B	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGGGGSGGGGSHHHHHHDDDDKFVNQHLCGSHLVEAL YLVCGERGFFYTPKTRRDVPQVELGGGPGAGSLQPLALEGSLQ KRGIVEQCCTSICSLYQLENYCG
196	EcpD3-EK- 前胰 岛素-CP-C	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGGGGSGGGGSHHHHHHDDDDKFVNQHLCGSHLVEAL YLVCGERGFFYTPKTRRDVPQVELGGGPGAGSLQPLALEGSLQ ARGIVEQCCTSICSLYQLENYCG
197	EcpD3-胰蛋白酶- 前胰岛素-CP-A	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGGGGSGGGGSHHHHHHRFVNQHLCGSHLVEALYLVC GERGFFYTPKTRREAEDLQVGQVELGGGPGAGSLQPLALEGSL QKRGIVEQCCTSICSLYQLENYCG
198	EcpD3-胰蛋白酶- 前胰岛素-CP-B	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGGGGSGGGGSHHHHHHRFVNQHLCGSHLVEALYLVC GERGFFYTPKTRRDVPQVELGGGPGAGSLQPLALEGSLQKRG I VEQCCTSICSLYQLENYCG
199	EcpD3-胰蛋白酶-	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI

[0417]

	前胰岛素-CP-C	TVKLNNNGGGSGGGSHHHHHHRFVNQHLCGSHLVEALYLVC GERGFFYTPKTRRDVPQVELGGGPGAGSLQPLALEGSLQARGI VEQCCTSICSLYQLENYCG
200	EcpD3-EK- 前胰 岛素-CP-D	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGGGSGGGSHHHHHHDDDDKFVNQHLCGSHLVEAL YLVCGERGFFYTPKTRREAEDQGS LQKRGIVEQCCTSICSLYQ LENYCG
201	EcpD3-胰蛋白酶- 前胰岛素-CP-D	MSCTRAFKPLLLIGLATLMCSHAFAAVVITGTRLVYPADQKEI TVKLNNNGGGSGGGSHHHHHHRFVNQHLCGSHLVEALYLVC GERGFFYTPKTRREAEDQGS LQKRGIVEQCCTSICSLYQLENY CG
202	pFNX4401 DnaJ- 样蛋白-胰蛋白酶 编码序列	ATGAAAGTCGAACCAGGGCTCTACCAGCATTACAAGGGGCCGC AGTACCGTGTTTTTCAGCGTGGCGGCCACTCTGAAACCGAAGA AGAAGTGGTGTTTTACCAAGCGCTGTATGGCGAATACGGCTTT TGGGTGCGCCCTTTGAGCATGTTCTGGAGACCGTCGAAGTTG ACGGCGAGCAGGTCCCGCGCTTTGCTTTGGTCACGGCCGAACC CAGTCTTTTTACAGGGCAAGGTGGGGGTGGGTGCGGTGGTGGT GGGTGCGATCATCATCACCACCACCGA
203	pFNX4402 EcpD1-胰蛋白酶 编码序列 (pFNX4402 不含 下划线的 C 残基 之间出现的 N 密 码子 CAA)	ATGTCGTGCACACGTGCATTCAAACCACTGCTGCTGATCGGCC TGGCCACACTGATGTGTTCCCATGCATTCGCTGCAGTGGTGAT TACCGGTACGCGCCTGGTCTATCCGGCGGACCAGAAAGAAATC ACCGTAAACTGAACAATAACGGCACGTTGCCCGCACTGGTCC AATCATGGATCGACACCGGCAGCGTCGAATCGACACCCACCAG CTCCAAGGCGCCGTTCTATTGTGCGCCCCCGGTGGCGCGCAT GACCCGACCAAGGGCCAAAGCTTGCGAGTGCTCTTTACCGGCG CGCCTTTGGCGCAGGACAAAGAGTCGGTGTTCTGGCTCAACGT TCTCGAAATCCCGCCCAAACCGAGGCGGGTGCAGACCTCAAC ACGCTGCAAATGGCTTTCCGTTTCGCGCATCAAGCTGTTCTATC GCCCCGTGCGCTTGCTGGAAATCCCAATGAGGCGGTTGAGCA GGTGCAGTGGCAATTGGTTACGGCACGCGATGGCCAAGGCCTG GCGCTGAAGGCGT <u>ACCC</u> GTGCGCGTTCCACGTCTCGCTGATCG AGTTGGACCTGGTGGCGGGTAACCAACGCTATCGCAGTGAGGA CGGCATGGTCGGCCCTGGGGAAACCGGCAGTTTCGCGCTGCCC ACGCTCAAGGCCAGGCCGTCGAGCCAGGCACAAGTGAGATTCA GCGCCATCAACGATTACGGCGCGTTGGTCCCGACCCGCAACAC GCTGCAGCCCGGTGGGGGTGGGTGCGGTGGTGGTGGGTGCGCAT

		CATCATCACCACCACCGA
204	pFNX4403 EcpD2-胰蛋白酶 编码序列	ATGTCGTGCACACGTGCATTCAAACCACTGCTGCTGATCGGCC TGGCCACACTGATGTGTTCCCATGCATTTCGCTGCAGTGGTGAT TACCGGTACGCGCCTGGTCTATCCGGCGGACCAGAAAGAAATC ACCGTAAAACTGAACAATAACGGCACGTTGCCCGCACTGGTCC AATCATGGATCGACACCGGCAGCGTCGAATCGACACCCACCAG CTCCAAGGCGCCGTTCTTATTGTGCCCCCGGTGGCGCGCATT GACCCGACCAAGGGCCAAAGCTTGCGAGTGCTCTTTACCGGCG GTGGGGGTGGGTCTGGGTGGTGGTGGGTGCGATCATCATCACC CCACCGA
205	pFNX4404 EcpD3-胰蛋白酶 编码序列	ATGTCGTGCACACGTGCATTCAAACCACTGCTGCTGATCGGCC TGGCCACACTGATGTGTTCCCATGCATTTCGCTGCAGTGGTGAT TACCGGTACGCGCCTGGTCTATCCGGCGGACCAGAAAGAAATC ACCGTAAAACTGAACAATAACGGTGGGGGTGGGTCTGGGTGGTG GTGGGTGCGATCATCATCACCACCACCGA
206	pFNX4405 FkIB1- 胰蛋白酶编码序 列	ATGTCCGAAGTTAATCTGTCCACCGACGAAACCCGCGTCAGCT ACGGTATCGGCCGTCAGTTGGGCGACCAACTGCGTGACAACCC GCCACCGGGCGTCAGCCTGGACGCGATCCTGGCCGGCCTGACC GACGCGTTTCGCAGGCAAGCCAAGCCGTGTTGACCAAGAGCAAA TGGCGGCCAGCTTCAAAGTGATCCGCGAAATCATGCAAGCCGA AGCCGCTGCCAAGGCTGAAGCTGCAGCAGGCGCTGGCCTGGCT TTCCTGGCGGAAAACGCCAAGCGTGATGGCATCACCACCCTGG CTTCCGGCCTGCAATTTGAAGTGCTGACGGCTGGTACCGGCGC CAAGCCGACCCGTGAAGACCAAGTGCGTACTACTACCACGGC ACCCTGATCGACGGCACTGTGTTTCGACAGCTCCTACGAGCGCG GCCAGCCTGCAGAAATCCCGGTTGGCGGCGTGATCGCCGGCTG GACCGAAGCCCTGCAACTGATGAATGCCGGCAGCAAATGGCGC GTGTACGTGCCGAGCGAACTGGCTTACGGCGCTCAAGGCGTTG GCAGCATCCCGCCGCACAGCGTTCTGGTATTTCGACGTCGAGCT GCTCGACGTTCTGGGTGGGGGTGGGTCTGGGTGGTGGGTGCG CATCATCATCACCACCACCGA
207	pFNX4406 FkIB2- 胰蛋白酶编码序 列	ATGTCCGAAGTTAATCTGTCCACCGACGAAACCCGCGTCAGCT ACGGTATCGGCCGTCAGTTGGGCGACCAACTGCGTGACAACCC GCCACCGGGCGTCAGCCTGGACGCGATCCTGGCCGGCCTGACC GACGCGTTTCGCAGGCAAGCCAAGCCGTGTTGACCAAGAGCAAA TGGCGGCCAGCTTCAAAGTGATCCGCGAAATCATGCAAGCCGA

[0419]

		AGCCGCTGCCAAGGCTGAAGCTGCAGCAGGCGCTGGCCTGGCT TTCTTGGCGGAAAACGCCAAGCGTGATGGCATCACCAACCTGG GTGGGGGTGGGTTCGGGTGGTGGTGGGTTCGCATCATCATCACCA CCACCGA
208	pFNX4407 FklB3- 胰蛋白酶编码序 列	ATGTCCGAAGTTAATCTGTCCACCGACGAAACCCGCGTCAGCT ACGGTATCGGCCGTCAGTTGGGCGACCAACTGCGTGACAACCC GCCACCGGGCGTCAGCCTGGACGCGATCCTGGCCGGCCTGACC GACGCGTTTCGACAGCAAGCCAGGTGGGGGTGGGTTCGGGTGGTG GTGGGTTCGCATCATCATCACCAACCGA
209	pFNX4408 FrnE1- 胰蛋白酶编码序 列	ATGAGTACTCCCCTGAAAAATCGATTTTCGTCAGCGACGTATCCT GCCCCCTGGTGCATCATCGGCCTGCGCGGCTTGACCGAAGCCCT CGACCAGCTCGGCAGCGAGGTGCAGGCCGAGATTCAATTTTCAA CCGTTCGAACTGAACCCGAACATGCCCGCCGAAGGTCAGAACA TCGTCGAGCACATTACCGAAAAAGTACGGCTCCACGGCTGAAGA GTCCCAGGCTAATCGTGCGCGTATCCGTGACATGGGCGCCGCG TTGGGCTTTGCTTTTCGCACCGATGGCCAGAGCCGTATCTACA ACACCTTCGACGCGCACCGTCTGTTGCACTGGGCGGGGTGGA AGGCTTGCAGTACAACCTCAAGGAAGCGCTGTTCAAGGCGTAC TTCAGCGATGGCCAGGACCCCTCCGACCACGCGACCTTGCGCA TCATCGCCGAAAGCGTCGGGCTGGACCTGCGCGCGCCGCCGA GATTCTTGCCAGCGATGAATACGCCGCCGAGGTCCGCGAGCAG GAGCAGCTGTGGGTTTCCCGTGGGGTGAGTTCCGTGCCGACCA TTGTCTTCAATGACCAATATGCGGTGAGCGGTGGGCAACCGGC TGAAGCCTTCGTGGGTGCGATTTCGCCAGATCATCAACGAATCC AAATCCGGTGGGGGTGGGTTCGGGTGGTGGTGGGTTCGCATCATC ATCACCAACCGA
210	pFNX4409 FrnE2- 胰蛋白酶编码序 列	ATGAGTACTCCCCTGAAAAATCGATTTTCGTCAGCGACGTATCCT GCCCCCTGGTGCATCATCGGCCTGCGCGGCTTGACCGAAGCCCT CGACCAGCTCGGCAGCGAGGTGCAGGCCGAGATTCAATTTTCAA CCGTTCGAACTGAACCCGAACATGCCCGCCGAAGGTCAGAACA TCGTCGAGCACATTACCGAAAAAGTACGGCTCCACGGCTGAAGA GTCCCAGGCTAATCGTGCGCGTATCCGTGACATGGGCGCCGCG TTGGGCTTTGCTTTTCGCACCGATGGCCAGAGCCGTATCTACG GTGGGGGTGGGTTCGGGTGGTGGTGGGTTCGCATCATCATCACCA CCACCGA
211	pFNX4410 FrnE3-	ATGAGTACTCCCCTGAAAAATCGATTTTCGTCAGCGACGTATCCT

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	胰蛋白酶编码序列	GCCCCCTGGTGCATCATCGGCCCTGCGCGGCTTGACCGAAGCCCTCGACCAGCTCGGCAGCGAGGTGCAGGCCGAGATTCATTTTCAA CCGTTCGAACTGAACCCGAACGGTGGGGGTGGGTGCGGTGGTG GTGGGTGCGATCATCATCACCACCACCGA
212	pFNX4411 DnaJ-EK 编码序列	ATGAAAGTGAACAGGGCTCTACCAGCATTACAAGGGCCGC AGTACCGTGTTTTCAGCGTGGCGGCCACTCTGAAACCGAAGA AGAAGTGGTGTTTTACCAAGCGCTGTATGGCGAATACGGCTTT TGGGTGCGCCCTTTGAGCATGTTCTGGAGACCGTCGAAGTTG ACGGCGAGCAGGTCCCGCGCTTTGCTTTGGTCACGGCCGAACC CAGTCTTTTACAGGGCAAGGTGGGGGTGGGTGCGGTGGTGGT GGGTGCGATCATCATCACCACCACGATGATGATGATAAG
213	pFNX4412 EcpD1-EK- 接头 编码序列	ATGTCGTGCACACGTGCATTCAAACCACTGCTGCTGATCGGCC TGGCCACACTGATGTGTTCCCATGCATTCGCTGCAGTGGTGAT TACCGGTACGCGCCTGGTCTATCCGGCGGACCAGAAAGAAATC ACCGTAAACTGAACAATAACGGCACGTTGCCCGCACTGGTCC AATCATGGATCGACACCGGCAGCGTCGAATCGACACCCACCAG CTCCAAGGCGCCGTTCTATTGTCGCCCCCGGTGGCGCGCATT GACCCGACCAAGGGCCAAAGCTTGCGAGTGCTCTTTACCGGCG CGCCTTTGGCGCAGGACAAAGAGTCGGTGTTCTGGCTCAACGT TCTCGAAATCCCGCCCAAACCCGAGGCGGGTGCAGACCTCAAC ACGCTGCAAATGGCTTTCCGTTTCGCGCATCAAGCTGTTCTATC GCCCCGTTCGGCTTGCTGGAATCCCAATGAGGCGGTTGAGCA GGTGCAGTGGCAATTGGTTACGGCACGCGATGGCCAAGGCCTG GCGCTGAAGGCGTACAACCCGTCGCGGTTCCACGTCTCGCTGA TCGAGTTGGACCTGGTGGCGGGTAACCAACGCTATCGCAGTGA GGACGGCATGGTCGGCCCTGGGGAAACCCGGCAGTTCGCGCTG CCCACGCTCAAGGCCAGGCCGTCGAGCCAGGCACAAGTGAGGT TCAGCGCCATCAACGATTACGGCGCGTTGGTCCCGACCCGCAA CACGCTGCAGCCCGGTGGGGGTGGGTGCGGTGGTGGTGGGTGCG CATCATCATCACCACCACGATGATGATGATAAG
214	pFNX4413 EcpD2-EK 编码序列	ATGTCGTGCACACGTGCATTCAAACCACTGCTGCTGATCGGCC TGGCCACACTGATGTGTTCCCATGCATTCGCTGCAGTGGTGAT TACCGGTACGCGCCTGGTCTATCCGGCGGACCAGAAAGAAATC ACCGTAAACTGAACAATAACGGCACGTTGCCCGCACTGGTCC AATCATGGATCGACACCGGCAGCGTCGAATCGACACCCACCAG CTCCAAGGCGCCGTTCTATTGTCGCCCCCGGTGGCGCGCATT

[0421]

		GACCCGACCAAGGGCCAAAGCTTGCGAGTGCTCTTTACCGGCG GTGGGGGTGGGTTCGGGTGGTGGTGGGTTCGCATCATCATCACCA CCACGATGATGATGATAAG
215	pFNX4414 EcpD3-EK 编码序 列	ATGTCGTGCACACGTGCATTCAAACCACTGCTGCTGATCGGCC TGGCCACACTGATGTGTTCCCATGCATTCGCTGCAGTGGTGAT TACCGGTACGCGCCTGGTCTATCCGGCGGACCAGAAAGAAATC ACCGTAAAACTGAACAATAACGGTGGGGGTGGGTTCGGGTGGTG GTGGGTTCGCATCATCATCACCACCACGATGATGATGATAAG
216	pFNX4415 FklB1-EK 编码序 列	ATGTCCGAAGTTAATCTGTCCACCGACGAAACCCGCGTCAGCT ACGGTATCGGCCGTGAGTTGGGCGACCAACTGCGTGACAACCC GCCACCGGGCGTCAGCCTGGACGCGATCCTGGCCGGCCTGACC GACGCGTTCGCAGGCAAGCCAAGCCGTGTTGACCAAGAGCAAA TGGCGGCCAGCTTCAAAGTGATCCGCGAAATCATGCAAGCCGA AGCCGCTGCCAAGGCTGAAGCTGCAGCAGGCGCTGGCCTGGCT TTCTTGGCGGAAAACGCCAAGCGTGATGGCATCACCACCTGG CTTCCGGCCTGCAATTTGAAGTGCTGACGGCTGGTACCGGCGC CAAGCCGACCCGTGAAGACCAAGTGCGTACTACTACCACGGC ACCCTGATCGACGGCACTGTGTTGACAGCTCCTACGAGCGCG GCCAGCCTGCAGAATTCCCGGTTGGCGGCGTGATCGCCGGCTG GACCGAAGCCCTGCAACTGATGAATGCCGCGAGCAAATGGCGC GTGTACGTGCCGAGCGAACTGGCTTACGGCGCTCAAGGCGTTG GCAGCATCCCGCCGCACAGCGTTCTGGTATTGACGTCGAGCT GCTCGACGTTCTGGGTGGGGGTGGGTTCGGGTGGTGGTGGTTCG CATCATCATCACCACCACGATGATGATGATAAG
217	pFNX4416 FklB2-EK 编码序 列	ATGTCCGAAGTTAATCTGTCCACCGACGAAACCCGCGTCAGCT ACGGTATCGGCCGTGAGTTGGGCGACCAACTGCGTGACAACCC GCCACCGGGCGTCAGCCTGGACGCGATCCTGGCCGGCCTGACC GACGCGTTCGCAGGCAAGCCAAGCCGTGTTGACCAAGAGCAAA TGGCGGCCAGCTTCAAAGTGATCCGCGAAATCATGCAAGCCGA AGCCGCTGCCAAGGCTGAAGCTGCAGCAGGCGCTGGCCTGGCT TTCTTGGCGGAAAACGCCAAGCGTGATGGCATCACCACCTGG GTGGGGGTGGGTTCGGGTGGTGGTGGGTTCGCATCATCATCACA CCACGATGATGATGATAAG
218	pFNX4417 FklB3-EK 编码序 列	ATGTCCGAAGTTAATCTGTCCACCGACGAAACCCGCGTCAGCT ACGGTATCGGCCGTGAGTTGGGCGACCAACTGCGTGACAACCC GCCACCGGGCGTCAGCCTGGACGCGATCCTGGCCGGCCTGACC

[0422]

		GACGCGTTCGCAGGCAAGCCAGGTGGGGGTGGGTCGGGTGGTG GTGGGTCGCATCATCATCACCACCACGATGATGATGATAAG
219	pFNX4418 FrnE1-EK 编码序列	ATGAGTACTCCCCTGAAAATCGATTTCGTCAGCGACGTATCCT GCCCCCTGGTGCATCATCGGCCTGCGCGGCTTGACCGAAGCCCT CGACCAGCTCGGCAGCGAGGTGCAGGCCGAGATTTCATTTTCAA CCGTTCGAACTGAACCCGAACATGCCCGCCGAAGGTCAGAACA TCGTCGAGCACATTACCGAAAAGTACGGCTCCACGGCTGAAGA GTCCCAGGCTAATCGTGCGCGTATCCGTGACATGGGCGCCGCG TTGGGCTTTGCTTTTCGCACCGATGGCCAGAGCCGTATCTACA ACACCTTCGACGCGCACCGTCTGTTGCACTGGGCCGGGTGGA AGGCTTGCAGTACAACCTCAAGGAAGCGCTGTTCAAGGCGTAC TTCAGCGATGGCCAGGACCCCTCCGACCACGCGACCTTGCGCA TCATCGCCGAAAGCGTCGGGCTGGACCTTGCGCGCGCCGCCGA GATTCTTGCCAGCGATGAATACGCCGCCGAGGTCCGCGAGCAG GAGCAGCTGTGGGTTTCCCGTGGGGTGAGTTTCGGTGCCGACCA TTGTCTTCAATGACCAATATGCGGTGAGCGGTGGGCAACCGGC TGAAGCCTTCGTGGGTGCGATTGCGCAGATCATCAACGAATCC AAATCCGGTGGGGGTGGGTGGGTGGGTGGGTGGGTGCGATCATC ATCACCACCACGATGATGATGATAAG
220	pFNX4419 FrnE2-EK 编码序列	ATGAGTACTCCCCTGAAAATCGATTTCGTCAGCGACGTATCCT GCCCCCTGGTGCATCATCGGCCTGCGCGGCTTGACCGAAGCCCT CGACCAGCTCGGCAGCGAGGTGCAGGCCGAGATTTCATTTTCAA CCGTTCGAACTGAACCCGAACATGCCCGCCGAAGGTCAGAACA TCGTCGAGCACATTACCGAAAAGTACGGCTCCACGGCTGAAGA GTCCCAGGCTAATCGTGCGCGTATCCGTGACATGGGCGCCGCG TTGGGCTTTGCTTTTCGCACCGATGGCCAGAGCCGTATCTACG GTGGGGGTGGGTGGGTGGGTGGGTGGGTGCGATCATCATCACCA CCACGATGATGATGATAAG
221	pFNX4420 FrnE3-EK 编码序列	ATGAGTACTCCCCTGAAAATCGATTTCGTCAGCGACGTATCCT GCCCCCTGGTGCATCATCGGCCTGCGCGGCTTGACCGAAGCCCT CGACCAGCTCGGCAGCGAGGTGCAGGCCGAGATTTCATTTTCAA CCGTTCGAACTGAACCCGAACGGTGGGGGTGGGTGGGTGGTG GTGGGTGCGATCATCATCACCACCACGATGATGATGATAAG
222	人胰岛素 B-肽	FVNQHLCGSHLVEALYLVCGERGFFYTPKT
223	人胰岛素 A-肽	GIVEQCCTSICSLYQLENYCN

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224	甘精胰岛素 B-肽 (高亮: 相对于胰 岛素的改变)	FVNQHLCGSHLVEALYLVCGERGFFYTPKTRR
225	甘精胰岛素 A-肽 (高亮: 相对于胰 岛素的改变)	GIVEQCCTSICSLYQLENYCG
226	具有胰蛋白酶切 割位点的接头	GGGSGGGGSHHHHHR
227	Serralysin (RXF04495)	MTVVKVFMSWELRADNGAVGIGNSHIWTVNFPLFRVSKHMH PVRQSSYSRPSDKLQPDLSPEHQVVLWANNKKSFTTDQAAKH ITRGGFKFHDRNNDGKIVVGYNFAGGFNAAQKERARQALQYWA DVANIEFVENGPNTDGTISIKGVPGSAGVAGLPNKYNSNVQAN IGTQGGQNPAMGSHFLGLLIHELGH TLGLSHPGKYDQG GFNYD RAAEYAQDTKARSVMSYWTETHQPGHNFAGRSPGAPMMDDIAA AQRLYGANTKTRNTDTTYGFNSNSGREAYSLKQGS DKPIFTVW DGGGNDTLDFSGFTQNQTINLKAESFSDVGGLRGNVSIAGVS VENAIGGTGNDTLTGNEGNRLTGKGADKLHGGAGADTFVYR RASDSTPQAPDIIQDFQSGSDKIDLTGVVQEAGLKSLSFVEKF SGKAGEAVLGQDAKTGRFTLAVDTTGNGTADLLVASQSQIKQA DVIWNGQAPTPTPTPEPTVVPVSDPVPTPTSEPTPEPTPEPA PLPVPTPRPGGGFIGKIFSSFKGFIKKVWSIFR
228	EcpD1-胰蛋白酶 编码序列(包括用 于 EcpD1 中存在的 N 残基的下划线 CAA 密码子)	ATGTCGTGCACACGTGCATTCAAACCACTGCTGCTGATCGGCC TGGCCACACTGATGTGTTCCCATGCATTCGCTGCAGTGGTGAT TACCGGTACGCGCCTGGTCTATCCGGCGGACCAGAAAGAAATC ACCGTAAACTGAACAATAACGGCACGTTGCCCCGCACTGGTCC AATCATGGATCGACACCGGCAGCGTCGAATCGACACCCACCAG CTCCAAGGCGCCGTTCTATTGTCGCCCCCGGTGGCGCGCATT GACCCGACCAAGGGCCAAAGCTTGCGAGTGCTCTTTACCGGCG CGCCTTTGGCGCAGGACAAAGAGTCGGTGTTCTGGCTCAACGT TCTCGAAATCCCGCCCAAACCCGAGGCGGTGCAGACCTCAAC ACGCTGCAAATGGCTTTCCGTTTCGCGCATCAAGCTGTTCTATC GCCCCGTTCGGCTTGCTTGAAATCCCAATGAGGCGGTTGAGCA GGTGCACTGGCAATTGGTTACGGCACGCGATGGCCAAGGCCTG GCGCTGAAGGCGTACA <u>ACCCGTCGGCGTTCCACGTCTCGCTGA</u>

[0424]

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229	FLAG 标签	DYKDDDDK
230	钙调蛋白标签	KRRWKKNFIAVSAANRFKKISSSGAL
231	HA 标签	YPYDVPDYA
232	E-标签	GAPVPYPDPLEPR
233	S-标签	KETAAAKFERQHMS
234	SBP 标签	MDEKTTGWRGGHVVEGLAGELEQLRARLEHHPQGQREP
235	Softag 3	TQDPSRVG
236	V5 标签	GKPIPNPLLGLDST
237	VSV 标签	YTDIEMNRLGK

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127

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[0384]	Arg Pro Phe Phe Pro Ala Gln Val Leu Pro Val Ile Val Asn Glu Glu
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[0444]	Gly Tyr Ile Thr Glu Glu Lys Val Ala Ile Ala Lys Arg His Leu Trp					
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[0446]	Pro Lys Gln Leu Glu Lys Ala Gly Val Ala Lys Asn Ser Leu Thr Ile					
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[0454]	Lys Asp Leu Glu Ser Ser Leu Gly Met Pro Val Phe Arg Asn Glu Gln					
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[0456]	Val Leu Ser Gly Thr Gly Val Ile Thr Gly Leu Ala Trp Thr Ser Met					
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[0458]	Gly Gly Ala Thr Leu Pro Ile Glu Ala Thr Arg Ile His Thr Leu Asn					
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[0460]	Arg Gly Phe Lys Leu Thr Gly Gln Leu Gly Glu Val Met Lys Glu Ser					
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[0462]	Ala Glu Ile Ala Tyr Ser Tyr Ile Ser Ser Asn Leu Lys Ser Phe Gly					
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[0464]	Gly Asp Ala Lys Phe Phe Asp Glu Ala Phe Val His Leu His Val Pro					
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[0466]	Glu Gly Ala Thr Pro Lys Asp Gly Pro Ser Ala Gly Val Thr Met Ala					
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[0471]						740				745					750	
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[0474]	Leu	Ile	Leu	Pro	Glu	Pro	Asn	Arg	Gly	Ser	Phe	Glu	Glu	Leu	Pro	Asp
[0475]						770				775					780	
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[0510]		195	200
[0511]	Ala Thr Tyr Gly Gln Asp Thr Arg Gly Tyr Ser Leu Met Ser Tyr Trp		205
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[0520]		275	280
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[0525]	Phe Ser Asp Val Gly Gly Leu Val Gly Asn Val Ser Ile Ala Lys Gly		315
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[0535]	Asp Lys Ile Phe Asp Phe Thr Ser Gly Ser Asp Lys Ile Asp Leu Ser		395
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[0558]	Met	Ala	Lys	Met	Ser	Thr	Ser	Thr	Gln	Ile	Ile	Thr	Gln	Pro	Arg	Thr
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[0627]	Glu	His	Gly	Gln	Val	Ile	Arg	Gly	Trp	Leu	Gly	Ile	Glu	Val	Gln	Pro
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[0660]	Gln	Ser	Leu	Gly	Ser	Gly	Phe	Ile	Ile	Ser	Pro	Asp	Gly	Tyr	Ile	Leu
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[0668]	Lys Leu Gly Lys Ser Gln Asp	Leu Lys Ala Gly Gln Trp Val Val Ala	
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[0670]	Ile Gly Ser Pro Phe Gly Phe	Asp His Thr Val Thr Gln Gly Ile Val	
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[0674]	Gln Thr Asp Val Pro Ile Asn	Pro Gly Asn Ser Gly Gly Pro Leu Phe	
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[0678]	Ser Gly Gly Phe Met Gly Val	Ser Phe Ala Ile Pro Ile Asp Val Ala	
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[0690]	Val Gly Ala Leu Lys Ala Gly	Gly Lys Ala Lys Leu Glu Val Ile Arg	
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[0694]	Glu Gly Ala Thr Leu Asp Ala	Leu Gly Asn Ala Lys Pro Gly Ala Glu	
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[0696]	Arg Ser Ser Asn Arg Leu Gly	Ile Ala Val Val Glu Leu Thr Ala Glu	
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[0698]	Gln Lys Lys Thr Phe Asp Leu	Gln Ser Gly Val Val Ile Lys Glu Val	
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[0735]	Val Thr Glu Asp Glu Ala Lys Leu Met Tyr Phe Asn Gln Ser Gly Ala
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[0753]	Ala	Arg	Ile	Thr	His	Asp	Ile	Ala	Gly	Gln	Leu	Tyr	Gly	Val	Asn	Lys
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[0775]	Ala	Ser	Asp	Ile	Ala	Glu	Phe	Asp	Lys	Trp	Lys	Thr	Gln	Phe	Asp	Asp
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[0778]			115					120					125			
[0779]	Tyr	Leu	Asp	Arg	Val	Lys	Ala	Arg	Leu	Asp	Phe	Ala	Leu	Gly	Glu	Leu

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[0782]	145	150	155
[0783]	Val Asp Arg Lys Asp Ala Pro Trp Leu Thr Ser Thr Ala Ala Leu Asp		
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[0785]	Asp Leu Trp Arg Lys Arg Val Lys Asp Glu Val Leu Arg Leu Lys Ile		
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[0787]	Ala Gly Lys Glu Pro Lys Ala Ile Gln Glu Leu Leu Thr Lys Arg Tyr		
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[0789]	Lys Asn Gln Leu Ala Arg Leu Asp Gln Thr Arg Ala Glu Asp Ile Phe		
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[0793]	Tyr Leu Ser Pro Asp Asn Ala Glu Asn Phe Asp Ile Asn Met Ser Leu		
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[0795]	Ser Leu Glu Gly Ile Gly Ala Val Leu Gln Ser Asp Asn Asp Gln Val		
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[0803]	Arg Gly Pro Lys Gly Ser Val Val Arg Leu Glu Val Ile Pro His Thr		
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[0805]	Asn Ala Pro Asn Asp Gln Thr Ser Lys Ile Val Ser Ile Thr Arg Glu		
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[0813]	Ser Thr Thr Arg Asp Val Lys Lys Ile Leu Thr Glu Leu Gln Lys Glu		
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[0823]	Arg Leu Ser Ala Ser Ala Ser Glu Ile Phe Ala Gly Ala Met Gln Asp
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[0827]	Val Gln Thr Ile Gln Pro Leu Asn His Gly Glu Leu Lys Leu Thr Leu
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[0833]	Glu Ser Ala Leu Pro Glu Ala Met Pro Trp Asp Thr Ile Arg Pro Ala
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[0837]	Ala Asp His Asp Thr Arg Ser Ala Lys Asp Ala Glu Phe Val Phe Ile
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[0839]	Arg Asp Lys Leu Ala Leu Ala Lys Lys Leu Met Glu Glu Lys Thr Val
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[0843]	Gln Gln Leu Val Leu Glu Asn Thr Arg Arg Lys Ala Lys Gly Glu Asp
[0844]	645 650 655
[0845]	Pro Leu Lys Glu Leu Lys Lys Glu Asp Glu Asp Ala Leu Pro Thr Glu
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[0847]	Ala Asp Lys Thr Lys Pro Glu Asp Asp Ala Tyr Leu Ala Glu Thr Gly
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[0849]	Arg Ile Leu Leu Asp Tyr Leu Lys Ile Thr Lys Gln Val Ala Lys Gln
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[0862]	Arg	Thr	Phe	Ala	Glu	Val	Met	Asp	Arg	Ile	Lys	Ala	Ala	Tyr	Val	Glu
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[0864]	Pro	Val	Asp	Asp	Lys	Ala	Leu	Leu	Glu	Asn	Ala	Ile	Lys	Gly	Met	Leu
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[0880]	Val	Lys	Ser	Val	Lys	Ser	Gln	Leu	Leu	Glu	Ser	Gly	Tyr	Gly	Tyr	Ile
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[0935]	Thr	Thr	Asn	Ile	Leu	Asp	Phe	Ser	Lys	Ala	Ile	Ile	Ala	Asn	Leu	Pro	

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[0937]	Ala Ala Lys Pro Tyr Thr Tyr Val Glu Pro Gly Val Ser Ser Asn Leu			
[0938]		165	170	175
[0939]	Asn Gly Gln Leu Asn Ala Gly Gln Ser Trp Ala Gly Ala Thr Arg Asp			
[0940]		180	185	190
[0941]	Trp Ser Ala Asn Ala Gln Thr Trp Lys Thr Pro Glu Ala Gln Val Asn			
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[0943]	Ser Gly Leu Asp Arg Thr Asn Ala Tyr Tyr Ala Tyr Ala Leu Gly Ile			
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[0945]	Thr Gly Lys Gly Val Asn Val Gly Val Leu Asp Ser Gly Ile Phe Thr			
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[0947]	Glu His Ser Glu Phe Gln Gly Lys Asn Ala Gln Gly Gln Asp Arg Val			
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[0949]	Gln Ala Val Thr Ser Thr Gly Glu Tyr Tyr Ala Thr His Pro Arg Tyr			
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[0951]	Arg Leu Glu Val Pro Ser Gly Glu Phe Lys Gln Gly Glu His Phe Ser			
[0952]		275	280	285
[0953]	Ile Pro Gly Glu Tyr Asp Pro Ala Phe Asn Asp Gly His Gly Thr Glu			
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[0955]	Met Ser Gly Val Leu Ala Ala Asn Arg Asn Gly Thr Gly Met His Gly			
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[0963]	Ser Trp Gly Gln Ser Ser Arg Asp Asp Val Glu Asn His Phe Gly Asn			
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[0965]	Val Gly Asp Ser Ala Ala Gln Asn Leu Arg Asp Met Thr Ala Ala Tyr			
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[0967]	Arg Pro Phe Trp Asp Lys Ala His Ala Gly His Lys Thr Trp Met Asp			
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[0969]	Ala Met Ala Asp Ala Ala Arg Gln Asn Thr Phe Ile Gln Ile Ile Ser			
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[0971]	Ala Gly Asn Asp Ser His Gly Ala Asn Pro Asp Thr Asn Ser Asn Leu			
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[0973]	Pro Phe Phe Lys Pro Asp Ile Glu Ala Lys Phe Leu Ser Ile Thr Gly			
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[0979]	Gly	Glu	Ile	Ile	Pro	Asn	Ala	Asn	Gly	Thr	Ser	Ala	Ala	Ala	Pro	Ser
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[0983]	Ala	Ser	Gln	Ala	Arg	Asp	Val	Leu	Leu	Thr	Thr	Ser	Ser	Leu	Gln	Ala
[0984]		530					535					540				
[0985]	Pro	Asp	Gly	Pro	Asp	Thr	Pro	Val	Gly	Thr	Leu	Thr	Gly	Gly	Arg	Thr
[0986]	545					550					555					560
[0987]	Tyr	Asp	Asn	Leu	Gln	Pro	Val	His	Asp	Ala	Ala	Pro	Gly	Leu	Pro	Gln
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[0996]	625					630					635					640
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[1025]	Thr Ala Leu Asn Tyr Gln Pro Asn Gln Leu Thr Leu Ala Ile Ala Arg		860
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[1037]	Gln Ala Leu Asp Ser Ser Ala Gln Ile Asp Ser Arg Asp Asn Gly Gly		960
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[1039]	Trp Val Gln Leu Leu Gly Gly Arg Asn Asn Val Ser Gly Asp Asn Asn		975
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[1051]	Ser Arg Arg Val Ala Tyr Ala Asn Gln Ser Asp His Ala Lys Ala		1065
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			1080

[1053]	Asp Tyr Asn Ala Arg Thr Asp Gln Val Phe Ala Glu Ile Gly Tyr
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[1055]	Thr Gln Trp Thr Val Phe Glu Pro Phe Ala Asn Leu Thr Tyr Leu
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[1057]	Asn Tyr Gln Ser Asp Ser Phe Lys Glu Lys Gly Gly Ala Ala Ala
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[1061]	Gly Val Arg Gly His Thr Gln Leu Pro Leu Thr Ser Thr Ser Ala
[1062]	1145 1150 1155
[1063]	Val Thr Leu Arg Gly Glu Leu Gly Trp Glu His Gln Phe Gly Asp
[1064]	1160 1165 1170
[1065]	Thr Asp Arg Glu Ala Ser Leu Lys Phe Ala Gly Ser Asp Thr Ala
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[1071]	Leu Asn Tyr Ser Gly Leu Leu Ser Asn Arg Gly Asn Asn Asn Gly
[1072]	1220 1225 1230
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[1074]	1235 1240
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[1078]	<213> ö ¢ (Pseudomonas fluorescens)
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[1084]	Gly Val Lys Val Leu Arg Phe Ser Val Gly Phe Gly Met Pro Leu Leu
[1085]	35 40 45
[1086]	Arg Trp His Asp Arg Arg Gly Thr Glu Phe Val Ile Ala Ala Ile Pro
[1087]	50 55 60
[1088]	Leu Gly Gly Tyr Val Lys Met Leu Asp Glu Arg Glu Gly Glu Val Pro
[1089]	65 70 75 80
[1090]	Ala Asp Gln Leu Asp Gln Ser Phe Asn Arg Lys Thr Val Arg Gln Arg
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[1108]	Val	Leu	Ala	Glu	Leu	Asp	Pro	Lys	Gly	Pro	Ala	Gln	Ala	Ala	Gly	Leu
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[1184]	Pro Ser Val Phe Ile Cys Asp Glu Cys Val Asp Leu Cys Asn Asp Ile		
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[1188]	Leu Pro Ser Pro Lys Glu Ile Ser Gly Ile Leu Asp Gln Tyr Val Ile		
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[1194]	Gly Lys Ser Asn Ile Leu Leu Ile Gly Pro Thr Gly Ser Gly Lys Thr		
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[1202]	Lys Ala Gln Met Gly Ile Val Tyr Ile Asp Glu Ile Asp Lys Ile Ser		
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[1204]	Arg Lys Ser Asp Asn Pro Ser Ile Thr Arg Asp Val Ser Gly Glu Gly		
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[1206]	Val Gln Gln Ala Leu Leu Lys Leu Ile Glu Gly Thr Val Ala Ser Val		
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[1208]	Pro Pro Gln Gly Gly Arg Lys His Pro Gln Gln Glu Phe Leu Gln Val		

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[1216]	Arg Glu Val Glu Pro Asp Asp Leu Val Lys Phe Gly Leu Ile Pro Glu			
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[1218]	Phe Val Gly Arg Leu Pro Val Leu Ala Thr Leu Asp Glu Leu Asp Glu			
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[1220]	Ala Ala Leu Ile Gln Ile Leu Thr Glu Pro Lys Asn Ala Leu Thr Lys			
[1221]		325	330	335
[1222]	Gln Tyr Gly Lys Leu Phe Glu Met Glu Gly Val Asp Leu Glu Phe Arg			
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[1224]	Thr Asp Ala Leu Lys Ser Val Ala Lys Arg Ala Leu Glu Arg Lys Thr			
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[1226]	Gly Ala Arg Gly Leu Arg Ser Ile Leu Glu Gly Val Leu Leu Asp Thr			
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[1228]	Met Tyr Glu Ile Pro Ser Gln Ser Glu Val Ser Lys Val Val Ile Asp			
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[1230]	Glu Ser Val Ile Glu Gly Lys Ser Lys Pro Leu Tyr Ile Tyr Glu Asn			
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[1237]	<213> ö ¢ (Pseudomonas fluorescens)			
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[1241]	Ala Arg Ser Lys Arg His Glu Phe Met Thr Val Glu His Leu Leu Leu			
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[1243]	Ala Leu Leu Asp Asn Glu Ala Ala Ala Thr Val Leu Arg Ala Cys Gly			
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[1245]	Ala Asn Leu Asp Lys Leu Lys His Asp Leu Gln Glu Phe Ile Asp Ser			
[1246]		50	55	60
[1247]	Thr Thr Pro Leu Ile Pro Val His Asp Glu Asp Arg Glu Thr Gln Pro			

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[1249]	Thr Leu Gly Phe Gln Arg Val Leu Gln Arg Ala Val Phe His Val Gln			
[1250]		85	90	95
[1251]	Ser Ser Gly Lys Arg Glu Val Thr Gly Ala Asn Val Leu Val Ala Ile			
[1252]		100	105	110
[1253]	Phe Ser Glu Gln Glu Ser Gln Ala Val Phe Leu Leu Lys Gln Gln Ser			
[1254]		115	120	125
[1255]	Val Ala Arg Ile Asp Val Val Asn Tyr Ile Ala His Gly Ile Ser Lys			
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[1257]	Val Pro Gly His Gly Asp His Ser Glu Gly Glu Gln Asp Met Gln Asp			
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[1259]	Glu Glu Gly Gly Glu Ser Ser Ser Ser Ser Asn Pro Leu Asp Ala Tyr			
[1260]		165	170	175
[1261]	Ala Ser Asn Leu Asn Glu Met Ala Arg Gln Gly Arg Ile Asp Pro Leu			
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[1263]	Val Gly Arg Glu His Glu Val Glu Arg Val Ala Gln Ile Leu Ala Arg			
[1264]		195	200	205
[1265]	Arg Arg Lys Asn Asn Pro Leu Leu Val Gly Glu Ala Gly Val Gly Lys			
[1266]		210	215	220
[1267]	Thr Ala Ile Ala Glu Gly Leu Ala Lys Arg Ile Val Asp Asn Gln Val			
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[1269]	Pro Asp Leu Leu Ala Ser Ser Val Val Tyr Ser Leu Asp Leu Gly Ala			
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[1271]	Leu Leu Ala Gly Thr Lys Tyr Arg Gly Asp Phe Glu Lys Arg Phe Lys			
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[1273]	Ala Leu Leu Gly Glu Leu Lys Lys Arg Pro Gln Ala Ile Leu Phe Ile			
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[1275]	Asp Glu Ile His Thr Ile Ile Gly Ala Gly Ala Ala Ser Gly Gly Val			
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[1277]	Met Asp Ala Ser Asn Leu Leu Lys Pro Leu Leu Ser Ser Gly Asp Ile			
[1278]	305	310	315	320
[1279]	Arg Cys Ile Gly Ser Thr Thr Phe Gln Glu Phe Arg Gly Ile Phe Glu			
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[1281]	Lys Asp Arg Ala Leu Ala Arg Arg Phe Gln Lys Val Asp Val Ser Glu			
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[1283]	Pro Ser Val Glu Asp Thr Ile Gly Ile Leu Arg Gly Leu Lys Gly Arg			
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[1285]	Phe Glu Ala His His Gly Ile Glu Tyr Thr Asp Glu Ala Leu Arg Ala			
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[1287]	Ala Ala Glu Leu Ala Ser Arg Tyr Ile Asn Asp Arg His Met Pro Asp
[1288]	385 390 395 400
[1289]	Lys Ala Ile Asp Val Ile Asp Glu Ala Gly Ala Tyr Gln Arg Leu Gln
[1290]	405 410 415
[1291]	Pro Val Glu Lys Arg Val Lys Arg Ile Asp Val Pro Gln Val Glu Asp
[1292]	420 425 430
[1293]	Ile Val Ala Lys Ile Ala Arg Ile Pro Pro Lys His Val Thr Ser Ser
[1294]	435 440 445
[1295]	Asp Lys Glu Leu Leu Arg Asn Leu Glu Arg Asp Leu Lys Leu Thr Val
[1296]	450 455 460
[1297]	Phe Gly Gln Asp Ala Ala Ile Asp Ser Leu Ser Thr Ala Ile Lys Leu
[1298]	465 470 475 480
[1299]	Ser Arg Ala Gly Leu Lys Ser Pro Asp Lys Pro Val Gly Ser Phe Leu
[1300]	485 490 495
[1301]	Phe Ala Gly Pro Thr Gly Val Gly Lys Thr Glu Ala Ala Arg Gln Leu
[1302]	500 505 510
[1303]	Ala Lys Ala Met Gly Ile Glu Leu Val Arg Phe Asp Met Ser Glu Tyr
[1304]	515 520 525
[1305]	Met Glu Arg His Thr Val Ser Arg Leu Ile Gly Ala Pro Pro Gly Tyr
[1306]	530 535 540
[1307]	Val Gly Phe Asp Gln Gly Gly Leu Leu Thr Glu Ala Ile Thr Lys Gln
[1308]	545 550 555 560
[1309]	Pro His Cys Val Leu Leu Leu Asp Glu Ile Glu Lys Ala His Pro Glu
[1310]	565 570 575
[1311]	Val Phe Asn Leu Leu Leu Gln Val Met Asp His Gly Thr Leu Thr Asp
[1312]	580 585 590
[1313]	Asn Asn Gly Arg Lys Ala Asp Phe Arg Asn Val Ile Val Ile Met Thr
[1314]	595 600 605
[1315]	Thr Asn Ala Gly Ala Glu Thr Ala Ala Arg Ala Ser Ile Gly Phe Thr
[1316]	610 615 620
[1317]	His Gln Asp His Ser Ser Asp Ala Met Glu Val Ile Lys Lys Ser Phe
[1318]	625 630 635 640
[1319]	Thr Pro Glu Phe Arg Asn Arg Leu Asp Thr Ile Ile Gln Phe Gly Arg
[1320]	645 650 655
[1321]	Leu Ser His Glu Val Ile Lys Ser Val Val Asp Lys Phe Leu Thr Glu
[1322]	660 665 670
[1323]	Leu Gln Ala Gln Leu Glu Asp Lys Arg Val Gln Leu Asp Val Thr Glu
[1324]	675 680 685
[1325]	Ala Ala Arg Ser Trp Ile Ala Glu Gly Gly Tyr Asp Ala Ala Met Gly

[1326]	690	695	700
[1327]	Ala Arg Pro Met Ala Arg Leu Ile Gln Asp Lys Ile Lys Arg Pro Leu		
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[1329]	Ala Glu Glu Ile Leu Phe Gly Glu Leu Ser Asp His Gly Gly Val Val		
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[1331]	His Ile Asp Leu Lys Asp Gly Glu Leu Thr Phe Glu Phe Glu Thr Thr		
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[1333]	Ala Glu Met Ala		
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[1342]	Ile Gly Arg Gln Leu Gly Asp Gln Leu Arg Asp Asn Pro Pro Pro Gly		
[1343]	20	25	30
[1344]	Val Ser Leu Asp Ala Ile Leu Ala Gly Leu Thr Asp Ala Phe Ala Gly		
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[1346]	Lys Pro Ser Arg Val Asp Gln Glu Gln Met Ala Ala Ser Phe Lys Val		
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[1348]	Ile Arg Glu Ile Met Gln Ala Glu Ala Ala Ala Lys Ala Glu Ala Ala		
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[1350]	Ala Gly Ala Gly Leu Ala Phe Leu Ala Glu Asn Ala Lys Arg Asp Gly		
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[1352]	Ile Thr Thr Leu Ala Ser Gly Leu Gln Phe Glu Val Leu Thr Ala Gly		
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[1354]	Thr Gly Ala Lys Pro Thr Arg Glu Asp Gln Val Arg Thr His Tyr His		
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[1356]	Gly Thr Leu Ile Asp Gly Thr Val Phe Asp Ser Ser Tyr Glu Arg Gly		
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[1358]	Gln Pro Ala Glu Phe Pro Val Gly Gly Val Ile Ala Gly Trp Thr Glu		
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[1360]	Ala Leu Gln Leu Met Asn Ala Gly Ser Lys Trp Arg Val Tyr Val Pro		
[1361]	165	170	175
[1362]	Ser Glu Leu Ala Tyr Gly Ala Gln Gly Val Gly Ser Ile Pro Pro His		
[1363]	180	185	190
[1364]	Ser Val Leu Tyr Ser Thr Ser Ser Cys Ser Thr Phe Cys Lys Thr Cys		

[1365]	195	200	205
[1366]	Trp Leu Pro Val Gly Thr Asn Ala Phe Ala Pro Thr Gly Val Cys Gln		
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[1368]	Phe Leu His Gly Trp Asn Leu Pro Leu Ser Ser Val Ala Gly Arg Asn		
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[1370]	Ala Arg Ala		
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[1374]	<213> ö ¢ (Pseudomonas fluorescens)		
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[1378]	Asn Leu Met Ala Ala Glu Pro Ala Lys Ala Pro His Val Leu Leu Asp		
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[1380]	Thr Thr Asn Gly Gln Ile Glu Ile Glu Leu Asp Pro Val Lys Ala Pro		
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[1382]	Ile Ser Thr Lys Asn Phe Leu Glu Tyr Val Asp Ser Gly Phe Tyr Thr		
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[1384]	Asn Thr Ile Phe His Arg Val Ile Pro Gly Phe Met Val Gln Gly Gly		
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[1386]	Gly Phe Thr Gln Gln Met Gln Gln Lys Asp Thr Lys Ala Pro Ile Lys		
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[1388]	Asn Glu Ala Ser Asn Gly Leu His Asn Val Arg Gly Thr Leu Ser Met		
[1389]	100	105	110
[1390]	Ala Arg Thr Ser Asn Pro Asn Ser Ala Thr Ser Gln Phe Phe Ile Asn		
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[1392]	Val Ala Asp Asn Ala Phe Leu Asp Pro Gly Arg Asp Ala Gly Tyr Ala		
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[1394]	Val Phe Ala Lys Val Val Lys Gly Met Asp Val Val Asp Ile Ile Val		
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[1396]	Asn Ser Gln Thr Thr Thr Lys Gln Gly Met Gln Asn Val Pro Ile Asp		
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[1398]	Pro Val Leu Ile Lys Ser Ala Lys Arg Ile Asp		
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[1415]	Gln	Gly	Pro	Ala	Trp	Thr	Leu	Ala	Ser	His	Arg	Ser	Tyr	Glu	Leu	Ala
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[1417]	Met	Gln	Tyr	Arg	Val	Glu	Leu	Ala	Gly	Asn	Asn	Asp	Leu	Tyr	Gln	Leu
[1418]				100					105				110			
[1419]	His	Arg	Gln	Leu	Ala	Asp	Ser	Pro	Ile	Ala	Thr	Leu	Phe	Asn	Glu	Gln
[1420]			115					120				125				
[1421]	Arg	His	Ser	Ala	Leu	Arg	Lys	Ile	Leu	Arg	Lys	Tyr	His	Leu	Ala	Gly
[1422]		130					135					140				
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[1424]	145					150				155						160
[1425]	Gln	Ile	Asp	Glu	Phe	Ser	His	Glu	Phe	Leu	Arg	Arg	Val	Ser	Asp	Ser
[1426]					165					170					175	
[1427]	Ser	Asp	Ala	Trp	Arg	Lys	His	Ile	Gln	Asp	Lys	Ala	Leu	Leu	Ser	Gly
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[1429]	Leu	Pro	Asp	Ala	Ala	Leu	Ala	Arg	Leu	Glu	Phe	Ala	Ala	Arg	Asp	Ala
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[1431]	Gly	Leu	Gly	Gly	Trp	Leu	Leu	Thr	Leu	Ser	Lys	Gln	Ser	Phe	Gln	Glu
[1432]		210					215					220				
[1433]	Val	Met	Ser	Tyr	Ala	Asp	His	Arg	Ala	Leu	Arg	Gln	Glu	Met	Met	Leu
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[1435]	Ala	Tyr	Tyr	Ser	Arg	Ala	Val	Gly	Thr	Gly	Pro	Asp	Ala	Ile	Ala	Thr
[1436]					245					250					255	
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[1443]	Ile Asp Gln Ala Arg Thr Thr Phe Ala His Asp Ala Gln Gln Leu Gln
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[1447]	Asp Phe Phe Ala Glu Lys Ile Arg Gln Asp Val Ala Gly Val Ser Gln
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[1449]	Asp Ala Val Arg Leu Tyr Phe Pro Leu Glu Thr Val Leu Gln Arg Leu
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[1451]	Cys Thr Phe Thr Gln Thr Leu Phe Gly Val Glu Leu Ile Glu Gln Ala
[1452]	370 375 380
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[1455]	Tyr Ala Gln Pro Ile Gly His Leu Phe Ile Asp Pro Tyr Arg Arg Val
[1456]	405 410 415
[1457]	Ala Gly Gly Glu Ile Gly Ala Ala Met Gly Leu Arg Asn His Arg Met
[1458]	420 425 430
[1459]	Thr Ala Glu Gly Arg Pro Gln Arg Pro Ile Ala Val Leu Arg Ser Gln
[1460]	435 440 445
[1461]	Leu Pro Arg Pro Thr Ala Ala Gln Pro Cys Leu Leu Asp His Leu Gln
[1462]	450 455 460
[1463]	Leu Arg Val Leu Leu His Glu Phe Gly His Cys Leu Gln His Leu Leu
[1464]	465 470 475 480
[1465]	Ser Ala Ala Pro Tyr Arg Ala Ile Ser Gly Met Gly Gln Leu Ser His
[1466]	485 490 495
[1467]	Asp Thr Thr Glu Phe Phe Gly Leu Val Leu Glu Gln Phe Cys Leu Thr
[1468]	500 505 510
[1469]	Pro Ser Phe Leu Ile Tyr Leu Ser Gly His Val Gln Thr Gly Asp Pro
[1470]	515 520 525
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[1474]	545 550 555 560
[1475]	Glu Leu His Arg Thr Tyr Gly Asp Gly Arg Thr Pro His Glu Val Phe
[1476]	565 570 575
[1477]	Thr Asp Ala Asn Val Glu Val Gly His Leu Gln Trp Pro Asp Gly Ala
[1478]	580 585 590
[1479]	Arg Pro Ile Asn Ser Phe Glu Gln Pro Met Gly Ser Tyr Gly Ala Lys
[1480]	595 600 605
[1481]	Leu Tyr Ser Tyr Thr Trp Ser Gly Val Leu Ala Arg Gln Ala Phe Glu

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[1483]	Arg Phe Glu Arg Asp Gly Leu Phe Asn Pro Gln Thr Gly Lys Ala Phe		
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[1485]	Arg Asp Ala Phe Ile Thr Glu Gly Asp Thr Gly Thr Leu Leu Ser Ala		
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[1487]	Leu Ala Leu Phe Arg Gly Asp Gly Ala Gly Cys Val Gly His Ser Thr		
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[1489]	Gly Val		
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[1494]	<400> 31		
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[1497]	Ala Leu Tyr Phe Asp Asp Gln Gln Val Cys Gly Ala Ser Leu Val Ser		
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[1499]	Arg Asp Trp Leu Val Ser Ala Ala His Cys Val Tyr Gly Arg Asn Met		
[1500]	35	40	45
[1501]	Glu Pro Ser Lys Trp Lys Ala Val Leu Gly Leu His Met Ala Ser Asn		
[1502]	50	55	60
[1503]	Leu Thr Ser Pro Gln Ile Glu Thr Arg Leu Ile Asp Gln Ile Val Ile		
[1504]	65	70	75
[1505]	Asn Arg His Tyr Asn Lys Arg Arg Lys Asn Asn Asp Ile Ala Met Met		
[1506]	85	90	95
[1507]	His Leu Glu Met Lys Val Asn Tyr Thr Asp Tyr Ile Gln Pro Ile Cys		
[1508]	100	105	110
[1509]	Leu Pro Glu Glu Asn Gln Val Phe Pro Pro Gly Arg Ile Cys Ser Ile		
[1510]	115	120	125
[1511]	Ala Gly Trp Gly Ala Leu Ile Tyr Gln Gly Ser Thr Ala Asp Val Leu		
[1512]	130	135	140
[1513]	Gln Glu Ala Asp Val Pro Leu Leu Ser Asn Glu Lys Cys Gln Gln Gln		
[1514]	145	150	155
[1515]	Met Pro Glu Tyr Asn Ile Thr Glu Asn Met Val Cys Ala Gly Tyr Asp		
[1516]	165	170	175
[1517]	Ala Gly Gly Val Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Met		
[1518]	180	185	190
[1519]	Cys Gln Glu Asn Asn Arg Trp Leu Leu Ala Gly Val Thr Ser Phe Gly		
[1520]	195	200	205

[1521]	Tyr	Gln	Cys	Ala	Leu	Pro	Asn	Arg	Pro	Gly	Val	Tyr	Ala	Arg	Val	Pro
[1522]	210						215					220				
[1523]	Arg	Phe	Thr	Glu	Trp	Ile	Gln	Ser	Phe	Leu	His					
[1524]	225						230					235				
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[1526]	<211>	86														
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[1531]	1				5					10					15	
[1532]	Leu	Val	Cys	Gly	Glu	Arg	Gly	Phe	Phe	Tyr	Thr	Pro	Lys	Thr	Arg	Arg
[1533]				20					25					30		
[1534]	Glu	Ala	Glu	Asp	Leu	Gln	Val	Gly	Gln	Val	Glu	Leu	Gly	Gly	Gly	Pro
[1535]			35					40					45			
[1536]	Gly	Ala	Gly	Ser	Leu	Gln	Pro	Leu	Ala	Leu	Glu	Gly	Ser	Leu	Gln	Lys
[1537]		50					55					60				
[1538]	Arg	Gly	Ile	Val	Glu	Gln	Cys	Cys	Thr	Ser	Ile	Cys	Ser	Leu	Tyr	Gln
[1539]	65					70					75				80	
[1540]	Leu	Glu	Asn	Tyr	Cys	Asn										
[1541]					85											
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[1543]	<211>	84														
[1544]	<212>	PRT														
[1545]	<213>	(Artificial Sequence)														
[1546]	<220>															
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[1549]	Phe	Val	Asn	Gln	His	Leu	Cys	Gly	Ser	His	Leu	Val	Glu	Ala	Leu	Tyr
[1550]	1				5					10					15	
[1551]	Leu	Val	Cys	Gly	Glu	Arg	Gly	Phe	Phe	Tyr	Thr	Lys	Pro	Thr	Glu	Ala
[1552]				20					25					30		
[1553]	Glu	Asp	Leu	Gln	Val	Gly	Gln	Val	Glu	Leu	Gly	Gly	Gly	Pro	Gly	Ala
[1554]			35					40					45			
[1555]	Gly	Ser	Leu	Gln	Pro	Leu	Ala	Leu	Glu	Gly	Ser	Leu	Gln	Lys	Arg	Gly
[1556]		50					55					60				
[1557]	Ile	Val	Glu	Gln	Cys	Cys	Thr	Ser	Ile	Cys	Ser	Leu	Tyr	Gln	Leu	Glu
[1558]	65					70					75				80	
[1559]	Asn	Tyr	Cys	Asn												

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[1565]	<223>	e j æ
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[1567]	Phe Val Lys Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr	
[1568]	1 5 10 15	
[1569]	Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Glu Thr Arg Arg	
[1570]	20 25 30	
[1571]	Glu Ala Glu Asp Leu Gln Val Gly Gln Val Glu Leu Gly Gly Gly Pro	
[1572]	35 40 45	
[1573]	Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln Lys	
[1574]	50 55 60	
[1575]	Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln	
[1576]	65 70 75 80	
[1577]	Leu Glu Asn Tyr Cys Asn	
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[1580]	<211>	70
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[1584]	<223>	e j æ
[1585]	<400>	35
[1586]	Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe	
[1587]	1 5 10 15	
[1588]	Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly	
[1589]	20 25 30	
[1590]	Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys	
[1591]	35 40 45	
[1592]	Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu	
[1593]	50 55 60	
[1594]	Lys Pro Ala Lys Ser Ala	
[1595]	65 70	
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[1597]	<211>	31
[1598]	<212>	PRT

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[1602]	<400>	36	
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[1604]	1	5	10 15
[1605]	Gln	Ala	Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly
[1606]		20	25 30
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[1614]	His	Gly	Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
[1615]	1	5	10 15
[1616]	Glu	Ala	Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
[1617]		20	25 30
[1618]	Ser	Gly	Ala Pro Pro Pro Ser
[1619]		35	
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[1623]	<213>	δ _v	
[1624]	<220>		
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[1626]	<400>	38	
[1627]	His	Ala	Asp Gly Ser Phe Ser Asp Glu Met Asn Thr Ile Leu Asp Asn
[1628]	1	5	10 15
[1629]	Leu	Ala	Ala Arg Asp Phe Ile Asn Trp Leu Ile Gln Thr Lys Ile Thr
[1630]		20	25 30
[1631]	Asp		
[1632]	<210>	39	
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[1635]	<213>	⋅	(Artificial Sequence)
[1636]	<220>		
[1637]	<223>	⋅ e	j æ

[1638]	<400> 39
[1639]	His Gly Asp Gly Ser Phe Ser Asp Glu Met Asn Thr Ile Leu Asp Asn
[1640]	1 5 10 15
[1641]	Leu Ala Ala Arg Asp Phe Ile Asn Trp Leu Ile Gln Thr Lys Ile Thr
[1642]	20 25 30
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[1644]	<210> 40
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[1651]	Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
[1652]	1 5 10 15
[1653]	Val His Ser Ser Asn Asn Phe Gly Pro Ile Leu Pro Pro Thr Asn Val
[1654]	20 25 30
[1655]	Gly Ser Asn Thr Tyr
[1656]	35
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[1662]	<223> e j æ
[1663]	<400> 41
[1664]	Met Lys Leu Thr Cys Val Val Ile Val Ala Val Leu Leu Leu Thr Ala
[1665]	1 5 10 15
[1666]	Cys Gln Leu Ile Thr Ala Asp Asp Ser Arg Gly Thr Gln Lys His Arg
[1667]	20 25 30
[1668]	Ala Leu Arg Ser Thr Thr Lys Leu Ser Thr Ser Thr Arg Cys Lys Gly
[1669]	35 40 45
[1670]	Lys Gly Ala Lys Cys Ser Arg Leu Met Tyr Asp Cys Cys Thr Gly Ser
[1671]	50 55 60
[1672]	Cys Arg Ser Gly Lys Cys Gly
[1673]	65 70
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 [1680] <400> 42
 [1681] Ser Leu Gly Ser Leu Thr Ile Ala Glu Pro Ala Met Ile Ala Glu Cys
 [1682] 1 5 10 15
 [1683] Lys Thr Arg Thr Glu Val Phe Glu Ile Ser Arg Arg Leu Ile Asp Arg
 [1684] 20 25 30
 [1685] Thr Asn Ala Asn Phe Leu Val Trp Pro Pro Cys Val Glu Val Gln Arg
 [1686] 35 40 45
 [1687] Cys Ser Gly Cys Cys Asn Asn Arg Asn Val Gln Cys Arg Pro Thr Gln
 [1688] 50 55 60
 [1689] Val Gln Leu Arg Pro Val Gln Val Arg Lys Ile Glu Ile Val Arg Lys
 [1690] 65 70 75 80
 [1691] Lys Pro Ile Phe Lys Lys Ala Thr Val Thr Leu Glu Asp His Leu Ala
 [1692] 85 90 95
 [1693] Cys Lys Cys Glu Thr Val Ala Ala Ala Arg Pro Val Thr
 [1694] 100 105
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 [1703] 1 5 10 15
 [1704] Glu Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu
 [1705] 20 25 30
 [1706] Trp Asn Trp Phe
 [1707] 35
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 [1712] <220>
 [1713] <223> ' e j æ
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 [1715] Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Lys Met Asp

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[1727]	1	5	10	15
[1728]	Arg Val Phe Ser Val Ala Arg His Ser Glu Thr Glu Glu Glu Val Val			
[1729]	20	25	30	
[1730]	Phe Tyr Gln Ala Leu Tyr Gly Glu Tyr Gly Phe Trp Val Arg Pro Leu			
[1731]	35	40	45	
[1732]	Ser Met Phe Leu Glu Thr Val Glu Val Asp Gly Glu Gln Val Pro Arg			
[1733]	50	55	60	
[1734]	Phe Ala Leu Val Thr Ala Glu Pro Ser Leu Phe Thr Gly Gln Gly Gly			
[1735]	65	70	75	80
[1736]	Gly Gly Ser Gly Gly Gly Gly Ser His His His His His His Asp Asp			
[1737]	85	90	95	
[1738]	Asp Asp Lys Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys			
[1739]	100	105	110	
[1740]	His Leu Asn Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln			
[1741]	115	120	125	
[1742]	Asp Val His Asn Phe			
[1743]	130			
[1744]	<210> 46			
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[1749]	<223> ' e j æ			
[1750]	<400> 46			
[1751]	Met Ser Glu Val Asn Leu Ser Thr Asp Glu Thr Arg Val Ser Tyr Gly			
[1752]	1	5	10	15
[1753]	Ile Gly Arg Gln Leu Gly Asp Gln Leu Arg Asp Asn Pro Pro Pro Gly			
[1754]	20	25	30	

[1755]	Val Ser Leu Asp Ala Ile Leu Ala Gly Leu Thr Asp Ala Phe Ala Gly
[1756]	35 40 45
[1757]	Lys Pro Ser Arg Val Asp Gln Glu Gln Met Ala Ala Ser Phe Lys Val
[1758]	50 55 60
[1759]	Ile Arg Glu Ile Met Gln Ala Glu Ala Ala Ala Lys Ala Glu Ala Ala
[1760]	65 70 75 80
[1761]	Ala Gly Ala Gly Leu Ala Phe Leu Ala Glu Asn Ala Lys Arg Asp Gly
[1762]	85 90 95
[1763]	Ile Thr Thr Leu Ala Ser Gly Leu Gln Phe Glu Val Leu Thr Ala Gly
[1764]	100 105 110
[1765]	Thr Gly Ala Lys Pro Thr Arg Glu Asp Gln Val Arg Thr His Tyr His
[1766]	115 120 125
[1767]	Gly Thr Leu Ile Asp Gly Thr Val Phe Asp Ser Ser Tyr Glu Arg Gly
[1768]	130 135 140
[1769]	Gln Pro Ala Glu Phe Pro Val Gly Gly Val Ile Ala Gly Trp Thr Glu
[1770]	145 150 155 160
[1771]	Ala Leu Gln Leu Met Asn Ala Gly Ser Lys Trp Arg Val Tyr Val Pro
[1772]	165 170 175
[1773]	Ser Glu Leu Ala Tyr Gly Ala Gln Gly Val Gly Ser Ile Pro Pro His
[1774]	180 185 190
[1775]	Ser Val Leu Val Phe Asp Val Glu Leu Leu Asp Val Leu Gly Gly Gly
[1776]	195 200 205
[1777]	Gly Ser Gly Gly Gly Gly Ser His His His His His His Asp Asp Asp
[1778]	210 215 220
[1779]	Asp Lys Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His
[1780]	225 230 235 240
[1781]	Leu Asn Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp
[1782]	245 250 255
[1783]	Val His Asn Phe
[1784]	260
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[1790]	<223> ' e j æ
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[1792]	Met Ser Thr Pro Leu Lys Ile Asp Phe Val Ser Asp Val Ser Cys Pro
[1793]	1 5 10 15

[1794]	Trp Cys Ile Ile Gly Leu Arg Gly Leu Thr Glu Ala Leu Asp Gln Leu
[1795]	20 25 30
[1796]	Gly Ser Glu Val Gln Ala Glu Ile His Phe Gln Pro Phe Glu Leu Asn
[1797]	35 40 45
[1798]	Pro Asn Met Pro Ala Glu Gly Gln Asn Ile Val Glu His Ile Thr Glu
[1799]	50 55 60
[1800]	Lys Tyr Gly Ser Thr Ala Glu Glu Ser Gln Ala Asn Arg Ala Arg Ile
[1801]	65 70 75 80
[1802]	Arg Asp Met Gly Ala Ala Leu Gly Phe Ala Phe Arg Thr Asp Gly Gln
[1803]	85 90 95
[1804]	Ser Arg Ile Tyr Asn Thr Phe Asp Ala His Arg Leu Leu His Trp Ala
[1805]	100 105 110
[1806]	Gly Leu Glu Gly Leu Gln Tyr Asn Leu Lys Glu Ala Leu Phe Lys Ala
[1807]	115 120 125
[1808]	Tyr Phe Ser Asp Gly Gln Asp Pro Ser Asp His Ala Thr Leu Ala Ile
[1809]	130 135 140
[1810]	Ile Ala Glu Ser Val Gly Leu Asp Leu Ala Arg Ala Ala Glu Ile Leu
[1811]	145 150 155 160
[1812]	Ala Ser Asp Glu Tyr Ala Ala Glu Val Arg Glu Gln Glu Gln Leu Trp
[1813]	165 170 175
[1814]	Val Ser Arg Gly Val Ser Ser Val Pro Thr Ile Val Phe Asn Asp Gln
[1815]	180 185 190
[1816]	Tyr Ala Val Ser Gly Gly Gln Pro Ala Glu Ala Phe Val Gly Ala Ile
[1817]	195 200 205
[1818]	Arg Gln Ile Ile Asn Glu Ser Lys Ser Gly Gly Gly Gly Ser Gly Gly
[1819]	210 215 220
[1820]	Gly Gly Ser His His His His His Asp Asp Asp Asp Lys Ser Val
[1821]	225 230 235 240
[1822]	Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn Ser Met
[1823]	245 250 255
[1824]	Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe
[1825]	260 265 270
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[1835]	Arg	Val	Phe	Ser	Val	Ala	Arg	His	Ser	Glu	Thr	Glu	Glu	Glu	Val	Val
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[1837]	Phe	Tyr	Gln	Ala	Leu	Tyr	Gly	Glu	Tyr	Gly	Phe	Trp	Val	Arg	Pro	Leu
[1838]			35					40					45			
[1839]	Ser	Met	Phe	Leu	Glu	Thr	Val	Glu	Val	Asp	Gly	Glu	Gln	Val	Pro	Arg
[1840]		50						55				60				
[1841]	Phe	Ala	Leu	Val	Thr	Ala	Glu	Pro	Ser	Leu	Phe	Thr	Gly	Gln	Gly	Gly
[1842]	65					70					75				80	
[1843]	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Asp	Asp	Asp	Asp	Lys	Ile	Val	Gly
[1844]				85					90						95	
[1845]	Gly	Ser	Asp	Ser	Arg	Glu	Gly	Ala	Trp	Pro	Trp	Val	Val	Ala	Leu	Tyr
[1846]				100					105						110	
[1847]	Phe	Asp	Asp	Gln	Gln	Val	Cys	Gly	Ala	Ser	Leu	Val	Ser	Arg	Asp	Trp
[1848]			115						120					125		
[1849]	Leu	Val	Ser	Ala	Ala	His	Cys	Val	Tyr	Gly	Arg	Asn	Met	Glu	Pro	Ser
[1850]		130						135					140			
[1851]	Lys	Trp	Lys	Ala	Val	Leu	Gly	Leu	His	Met	Ala	Ser	Asn	Leu	Thr	Ser
[1852]	145					150					155				160	
[1853]	Pro	Gln	Ile	Glu	Thr	Arg	Leu	Ile	Asp	Gln	Ile	Val	Ile	Asn	Arg	His
[1854]				165						170					175	
[1855]	Tyr	Asn	Lys	Arg	Arg	Lys	Asn	Asn	Asp	Ile	Ala	Met	Met	His	Leu	Glu
[1856]			180						185					190		
[1857]	Met	Lys	Val	Asn	Tyr	Thr	Asp	Tyr	Ile	Gln	Pro	Ile	Cys	Leu	Pro	Glu
[1858]			195						200				205			
[1859]	Glu	Asn	Gln	Val	Phe	Pro	Pro	Gly	Arg	Ile	Cys	Ser	Ile	Ala	Gly	Trp
[1860]		210						215				220				
[1861]	Gly	Ala	Leu	Ile	Tyr	Gln	Gly	Ser	Thr	Ala	Asp	Val	Leu	Gln	Glu	Ala
[1862]	225					230					235				240	
[1863]	Asp	Val	Pro	Leu	Leu	Ser	Asn	Glu	Lys	Cys	Gln	Gln	Gln	Met	Pro	Glu
[1864]				245						250					255	
[1865]	Tyr	Asn	Ile	Thr	Glu	Asn	Met	Val	Cys	Ala	Gly	Tyr	Asp	Ala	Gly	Gly
[1866]			260						265					270		
[1867]	Val	Asp	Ser	Cys	Gln	Gly	Asp	Ser	Gly	Gly	Pro	Leu	Met	Cys	Gln	Glu
[1868]			275						280					285		
[1869]	Asn	Asn	Arg	Trp	Leu	Leu	Ala	Gly	Val	Thr	Ser	Phe	Gly	Tyr	Gln	Cys
[1870]		290						295				300				
[1871]	Ala	Leu	Pro	Asn	Arg	Pro	Gly	Val	Tyr	Ala	Arg	Val	Pro	Arg	Phe	Thr

[1872]	305	310	315	320
[1873]	Glu Trp Ile Gln Ser Phe Leu His His His His His His His			
[1874]	325	330		
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[1879]	<220>			
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[1881]	<400> 49			
[1882]	Met Ser Glu Val Asn Leu Ser Thr Asp Glu Thr Arg Val Ser Tyr Gly			
[1883]	1 5 10 15			
[1884]	Ile Gly Arg Gln Leu Gly Asp Gln Leu Arg Asp Asn Pro Pro Pro Gly			
[1885]	20 25 30			
[1886]	Val Ser Leu Asp Ala Ile Leu Ala Gly Leu Thr Asp Ala Phe Ala Gly			
[1887]	35 40 45			
[1888]	Lys Pro Ser Arg Val Asp Gln Glu Gln Met Ala Ala Ser Phe Lys Val			
[1889]	50 55 60			
[1890]	Ile Arg Glu Ile Met Gln Ala Glu Ala Ala Lys Ala Glu Ala Ala			
[1891]	65 70 75 80			
[1892]	Ala Gly Ala Gly Leu Ala Phe Leu Ala Glu Asn Ala Lys Arg Asp Gly			
[1893]	85 90 95			
[1894]	Ile Thr Thr Leu Ala Ser Gly Leu Gln Phe Glu Val Leu Thr Ala Gly			
[1895]	100 105 110			
[1896]	Thr Gly Ala Lys Pro Thr Arg Glu Asp Gln Val Arg Thr His Tyr His			
[1897]	115 120 125			
[1898]	Gly Thr Leu Ile Asp Gly Thr Val Phe Asp Ser Ser Tyr Glu Arg Gly			
[1899]	130 135 140			
[1900]	Gln Pro Ala Glu Phe Pro Val Gly Gly Val Ile Ala Gly Trp Thr Glu			
[1901]	145 150 155 160			
[1902]	Ala Leu Gln Leu Met Asn Ala Gly Ser Lys Trp Arg Val Tyr Val Pro			
[1903]	165 170 175			
[1904]	Ser Glu Leu Ala Tyr Gly Ala Gln Gly Val Gly Ser Ile Pro Pro His			
[1905]	180 185 190			
[1906]	Ser Val Leu Val Phe Asp Val Glu Leu Leu Asp Val Leu Gly Gly Gly			
[1907]	195 200 205			
[1908]	Gly Ser Gly Gly Gly Gly Ser Asp Asp Asp Asp Lys Ile Val Gly Gly			
[1909]	210 215 220			
[1910]	Ser Asp Ser Arg Glu Gly Ala Trp Pro Trp Val Val Ala Leu Tyr Phe			

[1911]	225	230	235	240
[1912]	Asp Asp Gln Gln Val Cys Gly Ala Ser Leu Val Ser Arg Asp Trp Leu			
[1913]		245	250	255
[1914]	Val Ser Ala Ala His Cys Val Tyr Gly Arg Asn Met Glu Pro Ser Lys			
[1915]		260	265	270
[1916]	Trp Lys Ala Val Leu Gly Leu His Met Ala Ser Asn Leu Thr Ser Pro			
[1917]		275	280	285
[1918]	Gln Ile Glu Thr Arg Leu Ile Asp Gln Ile Val Ile Asn Arg His Tyr			
[1919]		290	295	300
[1920]	Asn Lys Arg Arg Lys Asn Asn Asp Ile Ala Met Met His Leu Glu Met			
[1921]	305	310	315	320
[1922]	Lys Val Asn Tyr Thr Asp Tyr Ile Gln Pro Ile Cys Leu Pro Glu Glu			
[1923]		325	330	335
[1924]	Asn Gln Val Phe Pro Pro Gly Arg Ile Cys Ser Ile Ala Gly Trp Gly			
[1925]		340	345	350
[1926]	Ala Leu Ile Tyr Gln Gly Ser Thr Ala Asp Val Leu Gln Glu Ala Asp			
[1927]		355	360	365
[1928]	Val Pro Leu Leu Ser Asn Glu Lys Cys Gln Gln Gln Met Pro Glu Tyr			
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[1930]	Asn Ile Thr Glu Asn Met Val Cys Ala Gly Tyr Asp Ala Gly Gly Val			
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[1936]	Leu Pro Asn Arg Pro Gly Val Tyr Ala Arg Val Pro Arg Phe Thr Glu			
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[1969]	Tyr Asn Pro Ser Ala Phe His Val Ser Leu Ile Glu Leu Asp Leu Val		
[1970]	180	185	190
[1971]	Ala Gly Asn Gln Arg Tyr Arg Ser Glu Asp Gly Met Val Gly Pro Gly		
[1972]	195	200	205
[1973]	Glu Thr Arg Gln Phe Ala Leu Pro Thr Leu Lys Ala Arg Pro Ser Ser		
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[1975]	Gln Ala Gln Val Glu Phe Ser Ala Ile Asn Asp Tyr Gly Ala Leu Val		
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[1977]	Pro Thr Arg Asn Thr Leu Gln Pro Gly Gly Gly Gly Ser Asp Asp Asp		
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[1979]	Asp Lys Ile Val Gly Gly Ser Asp Ser Arg Glu Gly Ala Trp Pro Trp		
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[1981]	Val Val Ala Leu Tyr Phe Asp Asp Gln Gln Val Cys Gly Ala Ser Leu		
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[1983]	Val Ser Arg Asp Trp Leu Val Ser Ala Ala His Cys Val Tyr Gly Arg		
[1984]	290	295	300
[1985]	Asn Met Glu Pro Ser Lys Trp Lys Ala Val Leu Gly Leu His Met Ala		
[1986]	305	310	315
[1987]	Ser Asn Leu Thr Ser Pro Gln Ile Glu Thr Arg Leu Ile Asp Gln Ile		
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[2029]	His Leu Glu Met Lys Val Asn Tyr Thr Asp Tyr Ile Gln Pro Ile Cys		
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[2031]	Leu Pro Glu Glu Asn Gln Val Phe Pro Pro Gly Arg Ile Cys Ser Ile		
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[2033]	Ala Gly Trp Gly Ala Leu Ile Tyr Gln Gly Ser Thr Ala Asp Val Leu		
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[2035]	Gln Glu Ala Asp Val Pro Leu Leu Ser Asn Glu Lys Cys Gln Gln Gln		
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[2037]	Met Pro Glu Tyr Asn Ile Thr Glu Asn Met Val Cys Ala Gly Tyr Asp		
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[2039]	Ala Gly Gly Val Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Met		
[2040]	180	185	190
[2041]	Cys Gln Glu Asn Asn Arg Trp Leu Leu Ala Gly Val Thr Ser Phe Gly		
[2042]	195	200	205
[2043]	Tyr Gln Cys Ala Leu Pro Asn Arg Pro Gly Val Tyr Ala Arg Val Pro		
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[2045]	Arg Phe Thr Glu Trp Ile Gln Ser Phe Leu His His His His His His		
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[2196]	Ile Gly Arg Gln Leu Gly Asp Gln Leu Arg Asp Asn Pro Pro Pro Gly			
[2197]		20	25	30
[2198]	Val Ser Leu Asp Ala Ile Leu Ala Gly Leu Thr Asp Ala Phe Ala Gly			
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[2200]	Lys Pro Ser Arg Val Asp Gln Glu Gln Met Ala Ala Ser Phe Lys Val			
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[2202]	Ile Arg Glu Ile Met Gln Ala Glu Ala Ala Ala Lys Ala Glu Ala Ala			
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[2204]	Ala Gly Ala Gly Leu Ala Phe Leu Ala Glu Asn Ala Lys Arg Asp Gly			
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[2230]	Gly Ser Glu Val Gln Ala Glu Ile His Phe Gln Pro Phe Glu Leu Asn	
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[2436]	Trp	Gln	Leu	Val	Thr	Ala	Arg	Asp	Gly	Gln	Gly	Leu	Ala	Leu	Lys	Ala
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[2643]	Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro Ser Gln Ala Leu Gln
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[2645]	Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly Leu Phe Leu Tyr Gln
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[3070]	Glu Ala Glu Asp Leu Gln Val Gly Gln Val Glu Leu Gly Gly Gly Pro
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[3072]	Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln Lys
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[3081]	<400> 98
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[3083]	1 5 10 15
[3084]	Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln Lys Arg
[3085]	20 25
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[3115]	Arg Val Phe Ser Val Ala Arg His Ser Glu Thr Glu Glu Glu Val Val
[3116]	20 25 30
[3117]	Phe Tyr Gln Ala Leu Tyr Gly Glu Tyr Gly Phe Trp Val Arg Pro Leu
[3118]	35 40 45
[3119]	Ser Met Phe Leu Glu Thr Val Glu Val Asp Gly Glu Gln Val Pro Arg

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[3139]	50	55	60
[3140]	Ser Val Glu Ser Thr Pro Thr Ser Ser Lys Ala Pro Phe Leu Leu Ser		
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[3142]	Pro Pro Val Ala Arg Ile Asp Pro Thr Lys Gly Gln Ser Leu Arg Val		80
[3143]	85	90	95
[3144]	Leu Phe Thr Gly Ala Pro Leu Ala Gln Asp Lys Glu Ser Val Phe Trp		
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[3146]	Leu Asn Val Leu Glu Ile Pro Pro Lys Pro Glu Ala Gly Ala Asp Leu		
[3147]	115	120	125
[3148]	Asn Thr Leu Gln Met Ala Phe Arg Ser Arg Ile Lys Leu Phe Tyr Arg		
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[3150]	Pro Val Gly Leu Pro Gly Asn Pro Asn Glu Ala Val Glu Gln Val Gln		
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[3152]	Trp Gln Leu Val Thr Ala Arg Asp Gly Gln Gly Leu Ala Leu Lys Ala		160
[3153]	165	170	175
[3154]	Tyr Pro Ser Ala Phe His Val Ser Leu Ile Glu Leu Asp Leu Val Ala		
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[3156]	Gly Asn Gln Arg Tyr Arg Ser Glu Asp Gly Met Val Gly Pro Gly Glu		
[3157]	195	200	205
[3158]	Thr Arg Gln Phe Ala Leu Pro Thr Leu Lys Ala Arg Pro Ser Ser Gln		

[3159]	210	215	220
[3160]	Ala Gln Val Glu Phe Ser Ala Ile Asn Asp Tyr Gly Ala Leu Val Pro		
[3161]	225	230	235
[3162]	Thr Arg Asn Thr Leu Gln Pro Gly Gly Gly Gly Ser Gly Gly Gly Gly		
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[3164]	Ser His His His His His His Arg		
[3165]	260		
[3166]	<210> 103		
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[3173]	Met Ser Cys Thr Arg Ala Phe Lys Pro Leu Leu Leu Ile Gly Leu Ala		
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[3175]	Thr Leu Met Cys Ser His Ala Phe Ala Ala Val Val Ile Thr Gly Thr		
[3176]	20 25 30		
[3177]	Arg Leu Val Tyr Pro Ala Asp Gln Lys Glu Ile Thr Val Lys Leu Asn		
[3178]	35 40 45		
[3179]	Asn Asn Gly Thr Leu Pro Ala Leu Val Gln Ser Trp Ile Asp Thr Gly		
[3180]	50 55 60		
[3181]	Ser Val Glu Ser Thr Pro Thr Ser Ser Lys Ala Pro Phe Leu Leu Ser		
[3182]	65 70 75 80		
[3183]	Pro Pro Val Ala Arg Ile Asp Pro Thr Lys Gly Gln Ser Leu Arg Val		
[3184]	85 90 95		
[3185]	Leu Phe Thr Gly Ala Pro Leu Ala Gln Asp Lys Glu Ser Val Phe Trp		
[3186]	100 105 110		
[3187]	Leu Asn Val Leu Glu Ile Pro Pro Lys Pro Glu Ala Gly Ala Asp Leu		
[3188]	115 120 125		
[3189]	Asn Thr Leu Gln Met Ala Phe Arg Ser Arg Ile Lys Leu Phe Tyr Arg		
[3190]	130 135 140		
[3191]	Pro Val Gly Leu Pro Gly Asn Pro Asn Glu Ala Val Glu Gln Val Gln		
[3192]	145 150 155 160		
[3193]	Trp Gln Leu Val Thr Ala Arg Asp Gly Gln Gly Leu Ala Leu Lys Ala		
[3194]	165 170 175		
[3195]	Tyr Asn Pro Ser Ala Phe His Val Ser Leu Ile Glu Leu Asp Leu Val		
[3196]	180 185 190		
[3197]	Ala Gly Asn Gln Arg Tyr Arg Ser Glu Asp Gly Met Val Gly Pro Gly		

[3198]	195	200	205
[3199]	Glu Thr Arg Gln Phe Ala Leu Pro Thr Leu Lys Ala Arg Pro Ser Ser		
[3200]	210	215	220
[3201]	Gln Ala Gln Val Glu Phe Ser Ala Ile Asn Asp Tyr Gly Ala Leu Val		
[3202]	225	230	235
[3203]	Pro Thr Arg Asn Thr Leu Gln Pro Gly Gly Gly Gly Ser Gly Gly Gly		
[3204]	245	250	255
[3205]	Gly Ser His His His His His His Arg		
[3206]	260	265	
[3207]	<210> 104		
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[3211]	<220>		
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[3214]	Met Ser Cys Thr Arg Ala Phe Lys Pro Leu Leu Leu Ile Gly Leu Ala		
[3215]	1 5 10 15		
[3216]	Thr Leu Met Cys Ser His Ala Phe Ala Ala Val Val Ile Thr Gly Thr		
[3217]	20 25 30		
[3218]	Arg Leu Val Tyr Pro Ala Asp Gln Lys Glu Ile Thr Val Lys Leu Asn		
[3219]	35 40 45		
[3220]	Asn Asn Gly Thr Leu Pro Ala Leu Val Gln Ser Trp Ile Asp Thr Gly		
[3221]	50 55 60		
[3222]	Ser Val Glu Ser Thr Pro Thr Ser Ser Lys Ala Pro Phe Leu Leu Ser		
[3223]	65 70 75 80		
[3224]	Pro Pro Val Ala Arg Ile Asp Pro Thr Lys Gly Gln Ser Leu Arg Val		
[3225]	85 90 95		
[3226]	Leu Phe Thr Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His		
[3227]	100 105 110		
[3228]	His His His His Arg		
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[3237]	Met Ser Cys Thr Arg Ala Phe Lys Pro Leu Leu Leu Ile Gly Leu Ala
[3238]	1 5 10 15
[3239]	Thr Leu Met Cys Ser His Ala Phe Ala Ala Val Val Ile Thr Gly Thr
[3240]	20 25 30
[3241]	Arg Leu Val Tyr Pro Ala Asp Gln Lys Glu Ile Thr Val Lys Leu Asn
[3242]	35 40 45
[3243]	Asn Asn Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His His His
[3244]	50 55 60
[3245]	His His Arg
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[3251]	<220>
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[3254]	Met Ser Glu Val Asn Leu Ser Thr Asp Glu Thr Arg Val Ser Tyr Gly
[3255]	1 5 10 15
[3256]	Ile Gly Arg Gln Leu Gly Asp Gln Leu Arg Asp Asn Pro Pro Pro Gly
[3257]	20 25 30
[3258]	Val Ser Leu Asp Ala Ile Leu Ala Gly Leu Thr Asp Ala Phe Ala Gly
[3259]	35 40 45
[3260]	Lys Pro Ser Arg Val Asp Gln Glu Gln Met Ala Ala Ser Phe Lys Val
[3261]	50 55 60
[3262]	Ile Arg Glu Ile Met Gln Ala Glu Ala Ala Lys Ala Glu Ala Ala
[3263]	65 70 75 80
[3264]	Ala Gly Ala Gly Leu Ala Phe Leu Ala Glu Asn Ala Lys Arg Asp Gly
[3265]	85 90 95
[3266]	Ile Thr Thr Leu Ala Ser Gly Leu Gln Phe Glu Val Leu Thr Ala Gly
[3267]	100 105 110
[3268]	Thr Gly Ala Lys Pro Thr Arg Glu Asp Gln Val Arg Thr His Tyr His
[3269]	115 120 125
[3270]	Gly Thr Leu Ile Asp Gly Thr Val Phe Asp Ser Ser Tyr Glu Arg Gly
[3271]	130 135 140
[3272]	Gln Pro Ala Glu Phe Pro Val Gly Gly Val Ile Ala Gly Trp Thr Glu
[3273]	145 150 155 160
[3274]	Ala Leu Gln Leu Met Asn Ala Gly Ser Lys Trp Arg Val Tyr Val Pro
[3275]	165 170 175

[3276]	Ser Glu Leu Ala Tyr Gly Ala Gln Gly Val Gly Ser Ile Pro Pro His
[3277]	180 185 190
[3278]	Ser Val Leu Val Phe Asp Val Glu Leu Leu Asp Val Leu Gly Gly Gly
[3279]	195 200 205
[3280]	Gly Ser Gly Gly Gly Gly Ser His His His His His His Arg
[3281]	210 215 220
[3282]	<210> 107
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[3284]	<212> PRT
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[3286]	<220>
[3287]	<223> e j æ
[3288]	<400> 107
[3289]	Met Ser Glu Val Asn Leu Ser Thr Asp Glu Thr Arg Val Ser Tyr Gly
[3290]	1 5 10 15
[3291]	Ile Gly Arg Gln Leu Gly Asp Gln Leu Arg Asp Asn Pro Pro Pro Gly
[3292]	20 25 30
[3293]	Val Ser Leu Asp Ala Ile Leu Ala Gly Leu Thr Asp Ala Phe Ala Gly
[3294]	35 40 45
[3295]	Lys Pro Ser Arg Val Asp Gln Glu Gln Met Ala Ala Ser Phe Lys Val
[3296]	50 55 60
[3297]	Ile Arg Glu Ile Met Gln Ala Glu Ala Ala Lys Ala Glu Ala Ala
[3298]	65 70 75 80
[3299]	Ala Gly Ala Gly Leu Ala Phe Leu Ala Glu Asn Ala Lys Arg Asp Gly
[3300]	85 90 95
[3301]	Ile Thr Thr Leu Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His
[3302]	100 105 110
[3303]	His His His His Arg
[3304]	115
[3305]	<210> 108
[3306]	<211> 67
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[3309]	<220>
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[3312]	Met Ser Glu Val Asn Leu Ser Thr Asp Glu Thr Arg Val Ser Tyr Gly
[3313]	1 5 10 15
[3314]	Ile Gly Arg Gln Leu Gly Asp Gln Leu Arg Asp Asn Pro Pro Pro Gly

[3315]	20	25	30
[3316]	Val Ser Leu Asp Ala Ile Leu Ala Gly Leu Thr Asp Ala Phe Ala Gly		
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[3318]	Lys Pro Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His His His		
[3319]	50	55	60
[3320]	His His Arg		
[3321]	65		
[3322]	<210> 109		
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[3327]	<223> e j æ		
[3328]	<400> 109		
[3329]	Met Ser Thr Pro Leu Lys Ile Asp Phe Val Ser Asp Val Ser Cys Pro		
[3330]	1 5 10 15		
[3331]	Trp Cys Ile Ile Gly Leu Arg Gly Leu Thr Glu Ala Leu Asp Gln Leu		
[3332]	20 25 30		
[3333]	Gly Ser Glu Val Gln Ala Glu Ile His Phe Gln Pro Phe Glu Leu Asn		
[3334]	35 40 45		
[3335]	Pro Asn Met Pro Ala Glu Gly Gln Asn Ile Val Glu His Ile Thr Glu		
[3336]	50 55 60		
[3337]	Lys Tyr Gly Ser Thr Ala Glu Glu Ser Gln Ala Asn Arg Ala Arg Ile		
[3338]	65 70 75 80		
[3339]	Arg Asp Met Gly Ala Ala Leu Gly Phe Ala Phe Arg Thr Asp Gly Gln		
[3340]	85 90 95		
[3341]	Ser Arg Ile Tyr Asn Thr Phe Asp Ala His Arg Leu Leu His Trp Ala		
[3342]	100 105 110		
[3343]	Gly Leu Glu Gly Leu Gln Tyr Asn Leu Lys Glu Ala Leu Phe Lys Ala		
[3344]	115 120 125		
[3345]	Tyr Phe Ser Asp Gly Gln Asp Pro Ser Asp His Ala Thr Leu Ala Ile		
[3346]	130 135 140		
[3347]	Ile Ala Glu Ser Val Gly Leu Asp Leu Ala Arg Ala Ala Glu Ile Leu		
[3348]	145 150 155 160		
[3349]	Ala Ser Asp Glu Tyr Ala Ala Glu Val Arg Glu Gln Glu Gln Leu Trp		
[3350]	165 170 175		
[3351]	Val Ser Arg Gly Val Ser Ser Val Pro Thr Ile Val Phe Asn Asp Gln		
[3352]	180 185 190		
[3353]	Tyr Ala Val Ser Gly Gly Gln Pro Ala Glu Ala Phe Val Gly Ala Ile		

[3354]	195	200	205
[3355]	Arg Gln Ile Ile Asn Glu Ser Lys Ser Gly Gly Gly Gly Ser Gly Gly		
[3356]	210	215	220
[3357]	Gly Gly Ser His His His His His His Arg		
[3358]	225	230	
[3359]	<210> 110		
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[3363]	<220>		
[3364]	<223> ' e j æ		
[3365]	<400> 110		
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[3367]	1 5 10 15		
[3368]	Trp Cys Ile Ile Gly Leu Arg Gly Leu Thr Glu Ala Leu Asp Gln Leu		
[3369]	20 25 30		
[3370]	Gly Ser Glu Val Gln Ala Glu Ile His Phe Gln Pro Phe Glu Leu Asn		
[3371]	35 40 45		
[3372]	Pro Asn Met Pro Ala Glu Gly Gln Asn Ile Val Glu His Ile Thr Glu		
[3373]	50 55 60		
[3374]	Lys Tyr Gly Ser Thr Ala Glu Glu Ser Gln Ala Asn Arg Ala Arg Ile		
[3375]	65 70 75 80		
[3376]	Arg Asp Met Gly Ala Ala Leu Gly Phe Ala Phe Arg Thr Asp Gly Gln		
[3377]	85 90 95		
[3378]	Ser Arg Ile Tyr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His		
[3379]	100 105 110		
[3380]	His His His His Arg		
[3381]	115		
[3382]	<210> 111		
[3383]	<211> 67		
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[3387]	<223> ' e j æ		
[3388]	<400> 111		
[3389]	Met Ser Thr Pro Leu Lys Ile Asp Phe Val Ser Asp Val Ser Cys Pro		
[3390]	1 5 10 15		
[3391]	Trp Cys Ile Ile Gly Leu Arg Gly Leu Thr Glu Ala Leu Asp Gln Leu		
[3392]	20 25 30		

[3393]	Gly Ser Glu Val Gln Ala Glu Ile His Phe Gln Pro Phe Glu Leu Asn
[3394]	35 40 45
[3395]	Pro Asn Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His His His
[3396]	50 55 60
[3397]	His His Arg
[3398]	65
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[3404]	<223> e j æ
[3405]	<400> 112
[3406]	Met Lys Val Glu Pro Gly Leu Tyr Gln His Tyr Lys Gly Pro Gln Tyr
[3407]	1 5 10 15
[3408]	Arg Val Phe Ser Val Ala Arg His Ser Glu Thr Glu Glu Glu Val Val
[3409]	20 25 30
[3410]	Phe Tyr Gln Ala Leu Tyr Gly Glu Tyr Gly Phe Trp Val Arg Pro Leu
[3411]	35 40 45
[3412]	Ser Met Phe Leu Glu Thr Val Glu Val Asp Gly Glu Gln Val Pro Arg
[3413]	50 55 60
[3414]	Phe Ala Leu Val Thr Ala Glu Pro Ser Leu Phe Thr Gly Gln Gly Gly
[3415]	65 70 75 80
[3416]	Gly Gly Ser Gly Gly Gly Gly Ser His His His His His His Asp Asp
[3417]	85 90 95
[3418]	Asp Asp Lys
[3419]	<210> 113
[3420]	<211> 269
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[3423]	<220>
[3424]	<223> e j æ
[3425]	<400> 113
[3426]	Met Ser Cys Thr Arg Ala Phe Lys Pro Leu Leu Leu Ile Gly Leu Ala
[3427]	1 5 10 15
[3428]	Thr Leu Met Cys Ser His Ala Phe Ala Ala Val Val Ile Thr Gly Thr
[3429]	20 25 30
[3430]	Arg Leu Val Tyr Pro Ala Asp Gln Lys Glu Ile Thr Val Lys Leu Asn
[3431]	35 40 45

[3432]	Asn Asn Gly Thr Leu Pro Ala Leu Val Gln Ser Trp Ile Asp Thr Gly
[3433]	50 55 60
[3434]	Ser Val Glu Ser Thr Pro Thr Ser Ser Lys Ala Pro Phe Leu Leu Ser
[3435]	65 70 75 80
[3436]	Pro Pro Val Ala Arg Ile Asp Pro Thr Lys Gly Gln Ser Leu Arg Val
[3437]	85 90 95
[3438]	Leu Phe Thr Gly Ala Pro Leu Ala Gln Asp Lys Glu Ser Val Phe Trp
[3439]	100 105 110
[3440]	Leu Asn Val Leu Glu Ile Pro Pro Lys Pro Glu Ala Gly Ala Asp Leu
[3441]	115 120 125
[3442]	Asn Thr Leu Gln Met Ala Phe Arg Ser Arg Ile Lys Leu Phe Tyr Arg
[3443]	130 135 140
[3444]	Pro Val Gly Leu Pro Gly Asn Pro Asn Glu Ala Val Glu Gln Val Gln
[3445]	145 150 155 160
[3446]	Trp Gln Leu Val Thr Ala Arg Asp Gly Gln Gly Leu Ala Leu Lys Ala
[3447]	165 170 175
[3448]	Tyr Asn Pro Ser Ala Phe His Val Ser Leu Ile Glu Leu Asp Leu Val
[3449]	180 185 190
[3450]	Ala Gly Asn Gln Arg Tyr Arg Ser Glu Asp Gly Met Val Gly Pro Gly
[3451]	195 200 205
[3452]	Glu Thr Arg Gln Phe Ala Leu Pro Thr Leu Lys Ala Arg Pro Ser Ser
[3453]	210 215 220
[3454]	Gln Ala Gln Val Glu Phe Ser Ala Ile Asn Asp Tyr Gly Ala Leu Val
[3455]	225 230 235 240
[3456]	Pro Thr Arg Asn Thr Leu Gln Pro Gly Gly Gly Gly Ser Gly Gly Gly
[3457]	245 250 255
[3458]	Gly Ser His His His His His His Asp Asp Asp Asp Lys
[3459]	260 265
[3460]	<210> 114
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[3464]	<220>
[3465]	<223> ' e j æ
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[3467]	Met Ser Cys Thr Arg Ala Phe Lys Pro Leu Leu Leu Ile Gly Leu Ala
[3468]	1 5 10 15
[3469]	Thr Leu Met Cys Ser His Ala Phe Ala Ala Val Val Ile Thr Gly Thr
[3470]	20 25 30

[3471]	Arg	Leu	Val	Tyr	Pro	Ala	Asp	Gln	Lys	Glu	Ile	Thr	Val	Lys	Leu	Asn
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[3473]	Asn	Asn	Gly	Thr	Leu	Pro	Ala	Leu	Val	Gln	Ser	Trp	Ile	Asp	Thr	Gly
[3474]			50					55					60			
[3475]	Ser	Val	Glu	Ser	Thr	Pro	Thr	Ser	Ser	Lys	Ala	Pro	Phe	Leu	Leu	Ser
[3476]	65					70				75						80
[3477]	Pro	Pro	Val	Ala	Arg	Ile	Asp	Pro	Thr	Lys	Gly	Gln	Ser	Leu	Arg	Val
[3478]					85					90					95	
[3479]	Leu	Phe	Thr	Gly	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	His	His
[3480]				100					105					110		
[3481]	His	His	His	His	Asp	Asp	Asp	Asp	Lys							
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[3492]	Thr	Leu	Met	Cys	Ser	His	Ala	Phe	Ala	Ala	Val	Val	Ile	Thr	Gly	Thr
[3493]				20					25					30		
[3494]	Arg	Leu	Val	Tyr	Pro	Ala	Asp	Gln	Lys	Glu	Ile	Thr	Val	Lys	Leu	Asn
[3495]				35					40					45		
[3496]	Asn	Asn	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	His	His	His	His
[3497]			50						55					60		
[3498]	His	His	Asp	Asp	Asp	Asp	Lys									
[3499]	65					70										
[3500]	<210>	116														
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[3508]	1				5				10					15		
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[3510]	20	25	30
[3511]	Val Ser Leu Asp Ala Ile Leu Ala Gly Leu Thr Asp Ala Phe Ala Gly		
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[3513]	Lys Pro Ser Arg Val Asp Gln Glu Gln Met Ala Ala Ser Phe Lys Val		
[3514]	50	55	60
[3515]	Ile Arg Glu Ile Met Gln Ala Glu Ala Ala Ala Lys Ala Glu Ala Ala		
[3516]	65	70	75
[3517]	Ala Gly Ala Gly Leu Ala Phe Leu Ala Glu Asn Ala Lys Arg Asp Gly		
[3518]	85	90	95
[3519]	Ile Thr Thr Leu Ala Ser Gly Leu Gln Phe Glu Val Leu Thr Ala Gly		
[3520]	100	105	110
[3521]	Thr Gly Ala Lys Pro Thr Arg Glu Asp Gln Val Arg Thr His Tyr His		
[3522]	115	120	125
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[3527]	Ala Leu Gln Leu Met Asn Ala Gly Ser Lys Trp Arg Val Tyr Val Pro		
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[3531]	Ser Val Leu Val Phe Asp Val Glu Leu Leu Asp Val Leu Gly Gly Gly		
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[3533]	Gly Ser Gly Gly Gly Gly Ser His His His His His His Asp Asp Asp		
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[3554]	Ala Gly Ala Gly Leu Ala Phe Leu Ala Glu Asn Ala Lys Arg Asp Gly		
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[3556]	Ile Thr Thr Leu Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His		
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[3573]	Lys Pro Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His His His		
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[3590]	Pro Asn Met Pro Ala Glu Gly Gln Asn Ile Val Glu His Ile Thr Glu
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[3596]	Ser Arg Ile Tyr Asn Thr Phe Asp Ala His Arg Leu Leu His Trp Ala
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[3600]	Tyr Phe Ser Asp Gly Gln Asp Pro Ser Asp His Ala Thr Leu Ala Ile
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[3602]	Ile Ala Glu Ser Val Gly Leu Asp Leu Ala Arg Ala Ala Glu Ile Leu
[3603]	145 150 155 160
[3604]	Ala Ser Asp Glu Tyr Ala Ala Glu Val Arg Glu Gln Glu Gln Leu Trp
[3605]	165 170 175
[3606]	Val Ser Arg Gly Val Ser Ser Val Pro Thr Ile Val Phe Asn Asp Gln
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[3608]	Tyr Ala Val Ser Gly Gly Gln Pro Ala Glu Ala Phe Val Gly Ala Ile
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[3610]	Arg Gln Ile Ile Asn Glu Ser Lys Ser Gly Gly Gly Gly Ser Gly Gly
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[3612]	Gly Gly Ser His His His His His His Asp Asp Asp Asp Lys
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[3627]	Pro Asn Met Pro Ala Glu Gly Gln Asn Ile Val Glu His Ile Thr Glu
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[3631]	Arg Asp Met Gly Ala Ala Leu Gly Phe Ala Phe Arg Thr Asp Gly Gln
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[3633]	Ser Arg Ile Tyr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His
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[3648]	Gly Ser Glu Val Gln Ala Glu Ile His Phe Gln Pro Phe Glu Leu Asn
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[3650]	Pro Asn Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His His His
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[3652]	His His Asp Asp Asp Asp Lys
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[3669]	Phe Ala Leu Val Thr Ala Glu Pro Ser Leu Phe Thr Gly Gln Gly Gly		
[3670]	65	70	75
[3671]	Gly Gly Ser Gly Gly Gly Gly Ser His His His His His His Asp Asp		
[3672]	85	90	95
[3673]	Asp Asp Lys Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu		
[3674]	100	105	110
[3675]	Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys		
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[3677]	Thr Arg Arg Glu Ala Glu Asp Leu Gln Val Gly Gln Val Glu Leu Gly		
[3678]	130	135	140
[3679]	Gly Gly Pro Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser		
[3680]	145	150	155
[3681]	Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser		
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[3683]	Leu Tyr Gln Leu Glu Asn Tyr Cys Gly		
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[3703]	85	90	95
[3704]	Asp Asp Lys Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu		

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[3708]	Thr Arg Arg Asp Val Pro Gln Val Glu Leu Gly Gly Gly Pro Gly Ala		
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[3737]	Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys		
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[3739]	Thr Arg Arg Asp Val Pro Gln Val Glu Leu Gly Gly Gly Pro Gly Ala		
[3740]	130 135 140		
[3741]	Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln Ala Arg Gly		
[3742]	145 150 155 160		
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[3881]	Gly Gly Ser Gly Gly Gly Gly Ser His His His His His His Arg Phe
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[3883]	Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu
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[3886]	115 120 125
[3887]	Ala Glu Asp Gln Gly Ser Leu Gln Lys Arg Gly Ile Val Glu Gln Cys
[3888]	130 135 140
[3889]	Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Gly
[3890]	145 150 155
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[3910]	Ile Thr Thr Leu Ala Ser Gly Leu Gln Phe Glu Val Leu Thr Ala Gly		95
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[3914]	Gly Thr Leu Ile Asp Gly Thr Val Phe Asp Ser Ser Tyr Glu Arg Gly		125
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[3918]	Ala Leu Gln Leu Met Asn Ala Gly Ser Lys Trp Arg Val Tyr Val Pro		160
[3919]		165	170
[3920]	Ser Glu Leu Ala Tyr Gly Ala Gln Gly Val Gly Ser Ile Pro Pro His		175
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[3922]	Ser Val Leu Val Phe Asp Val Glu Leu Leu Asp Val Leu Gly Gly Gly		190
[3923]		195	200
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[3934]	Gln Lys Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu		285
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[4004]	Ile Thr Thr Leu Ala Ser Gly Leu Gln Phe Glu Val Leu Thr Ala Gly		
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[4006]	Thr Gly Ala Lys Pro Thr Arg Glu Asp Gln Val Arg Thr His Tyr His		
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[4008]	Gly Thr Leu Ile Asp Gly Thr Val Phe Asp Ser Ser Tyr Glu Arg Gly		
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[4010]	Gln Pro Ala Glu Phe Pro Val Gly Gly Val Ile Ala Gly Trp Thr Glu		
[4011]	145 150 155 160		
[4012]	Ala Leu Gln Leu Met Asn Ala Gly Ser Lys Trp Arg Val Tyr Val Pro		
[4013]	165 170 175		
[4014]	Ser Glu Leu Ala Tyr Gly Ala Gln Gly Val Gly Ser Ile Pro Pro His		
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[4016]	Ser Val Leu Val Phe Asp Val Glu Leu Leu Asp Val Leu Gly Gly Gly		

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[4018]	Gly Ser Gly Gly Gly Gly Ser His His His His His His Asp Asp Asp		
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[4022]	Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr		
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[4024]	Arg Arg Asp Val Pro Gln Val Glu Leu Gly Gly Gly Pro Gly Ala Gly		
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[4028]	Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn		
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[4043]	Val Ser Leu Asp Ala Ile Leu Ala Gly Leu Thr Asp Ala Phe Ala Gly		
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[4049]	Ala Gly Ala Gly Leu Ala Phe Leu Ala Glu Asn Ala Lys Arg Asp Gly		
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[4051]	Ile Thr Thr Leu Ala Ser Gly Leu Gln Phe Glu Val Leu Thr Ala Gly		
[4052]	100 105 110		
[4053]	Thr Gly Ala Lys Pro Thr Arg Glu Asp Gln Val Arg Thr His Tyr His		
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[4055]	Gly Thr Leu Ile Asp Gly Thr Val Phe Asp Ser Ser Tyr Glu Arg Gly		

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[4057]	Gln Pro Ala Glu Phe Pro Val Gly Gly Val Ile Ala Gly Trp Thr Glu		
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[4059]	Ala Leu Gln Leu Met Asn Ala Gly Ser Lys Trp Arg Val Tyr Val Pro		160
[4060]		165	170
[4061]	Ser Glu Leu Ala Tyr Gly Ala Gln Gly Val Gly Ser Ile Pro Pro His		175
[4062]		180	185
[4063]	Ser Val Leu Val Phe Asp Val Glu Leu Leu Asp Val Leu Gly Gly Gly		190
[4064]		195	200
[4065]	Gly Ser Gly Gly Gly Gly Ser His His His His His His Arg Phe Val		205
[4066]		210	215
[4067]	Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val		220
[4068]	225	230	235
[4069]	Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr Arg Arg Glu Ala		240
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[4071]	Glu Asp Leu Gln Val Gly Gln Val Glu Leu Gly Gly Gly Pro Gly Ala		255
[4072]		260	265
[4073]	Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln Lys Arg Gly		270
[4074]		275	280
[4075]	Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu		285
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[4088]	Ile Gly Arg Gln Leu Gly Asp Gln Leu Arg Asp Asn Pro Pro Pro Gly		15
[4089]		20	25
[4090]	Val Ser Leu Asp Ala Ile Leu Ala Gly Leu Thr Asp Ala Phe Ala Gly		30
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[4096]	Ala Gly Ala Gly Leu Ala Phe Leu Ala Glu Asn Ala Lys Arg Asp Gly			
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[4102]	Gly Thr Leu Ile Asp Gly Thr Val Phe Asp Ser Ser Tyr Glu Arg Gly			
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[4104]	Gln Pro Ala Glu Phe Pro Val Gly Gly Val Ile Ala Gly Trp Thr Glu			
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[4106]	Ala Leu Gln Leu Met Asn Ala Gly Ser Lys Trp Arg Val Tyr Val Pro			
[4107]		165	170	175
[4108]	Ser Glu Leu Ala Tyr Gly Ala Gln Gly Val Gly Ser Ile Pro Pro His			
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[4110]	Ser Val Leu Val Phe Asp Val Glu Leu Leu Asp Val Leu Gly Gly Gly			
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[4112]	Gly Ser Gly Gly Gly Gly Ser His His His His His His Arg Phe Val			
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[4114]	Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val			
[4115]	225	230	235	240
[4116]	Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr Arg Arg Asp Val			
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[4118]	Pro Gln Val Glu Leu Gly Gly Gly Pro Gly Ala Gly Ser Leu Gln Pro			
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[4122]	Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Gly			
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[4137]	Lys Pro Ser Arg Val Asp Gln Glu Gln Met Ala Ala Ser Phe Lys Val		
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[4139]	Ile Arg Glu Ile Met Gln Ala Glu Ala Ala Ala Lys Ala Glu Ala Ala		
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[4141]	Ala Gly Ala Gly Leu Ala Phe Leu Ala Glu Asn Ala Lys Arg Asp Gly		
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[4143]	Ile Thr Thr Leu Ala Ser Gly Leu Gln Phe Glu Val Leu Thr Ala Gly		
[4144]	100	105	110
[4145]	Thr Gly Ala Lys Pro Thr Arg Glu Asp Gln Val Arg Thr His Tyr His		
[4146]	115	120	125
[4147]	Gly Thr Leu Ile Asp Gly Thr Val Phe Asp Ser Ser Tyr Glu Arg Gly		
[4148]	130	135	140
[4149]	Gln Pro Ala Glu Phe Pro Val Gly Gly Val Ile Ala Gly Trp Thr Glu		
[4150]	145	150	155
[4151]	Ala Leu Gln Leu Met Asn Ala Gly Ser Lys Trp Arg Val Tyr Val Pro		
[4152]	165	170	175
[4153]	Ser Glu Leu Ala Tyr Gly Ala Gln Gly Val Gly Ser Ile Pro Pro His		
[4154]	180	185	190
[4155]	Ser Val Leu Val Phe Asp Val Glu Leu Leu Asp Val Leu Gly Gly Gly		
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[4158]	210	215	220
[4159]	Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val		
[4160]	225	230	235
[4161]	Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr Arg Arg Asp Val		
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[4163]	Pro Gln Val Glu Leu Gly Gly Gly Pro Gly Ala Gly Ser Leu Gln Pro		
[4164]	260	265	270
[4165]	Leu Ala Leu Glu Gly Ser Leu Gln Ala Arg Gly Ile Val Glu Gln Cys		
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[4167]	Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Gly		
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[4182]	Lys	Pro	Ser	Arg	Val	Asp	Gln	Glu	Gln	Met	Ala	Ala	Ser	Phe	Lys	Val	
[4183]		50					55					60					
[4184]	Ile	Arg	Glu	Ile	Met	Gln	Ala	Glu	Ala	Ala	Ala	Lys	Ala	Glu	Ala	Ala	
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[4186]	Ala	Gly	Ala	Gly	Leu	Ala	Phe	Leu	Ala	Glu	Asn	Ala	Lys	Arg	Asp	Gly	
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[4188]	Ile	Thr	Thr	Leu	Ala	Ser	Gly	Leu	Gln	Phe	Glu	Val	Leu	Thr	Ala	Gly	
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[4190]	Thr	Gly	Ala	Lys	Pro	Thr	Arg	Glu	Asp	Gln	Val	Arg	Thr	His	Tyr	His	
[4191]			115					120					125				
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[4198]	Ser	Glu	Leu	Ala	Tyr	Gly	Ala	Gln	Gly	Val	Gly	Ser	Ile	Pro	Pro	His	
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[4227]	Lys Pro Ser Arg Val Asp Gln Glu Gln Met Ala Ala Ser Phe Lys Val
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[4229]	Ile Arg Glu Ile Met Gln Ala Glu Ala Ala Lys Ala Glu Ala Ala
[4230]	65 70 75 80
[4231]	Ala Gly Ala Gly Leu Ala Phe Leu Ala Glu Asn Ala Lys Arg Asp Gly
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[4233]	Ile Thr Thr Leu Ala Ser Gly Leu Gln Phe Glu Val Leu Thr Ala Gly
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[4235]	Thr Gly Ala Lys Pro Thr Arg Glu Asp Gln Val Arg Thr His Tyr His
[4236]	115 120 125
[4237]	Gly Thr Leu Ile Asp Gly Thr Val Phe Asp Ser Ser Tyr Glu Arg Gly
[4238]	130 135 140
[4239]	Gln Pro Ala Glu Phe Pro Val Gly Gly Val Ile Ala Gly Trp Thr Glu
[4240]	145 150 155 160
[4241]	Ala Leu Gln Leu Met Asn Ala Gly Ser Lys Trp Arg Val Tyr Val Pro
[4242]	165 170 175
[4243]	Ser Glu Leu Ala Tyr Gly Ala Gln Gly Val Gly Ser Ile Pro Pro His
[4244]	180 185 190
[4245]	Ser Val Leu Val Phe Asp Val Glu Leu Leu Asp Val Leu Gly Gly Gly
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[4247]	Gly Ser Gly Gly Gly Gly Ser His His His His His His Arg Phe Val
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[4249]	Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val
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[4251]	Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr Arg Arg Glu Ala
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[4253]	Glu Asp Gln Gly Ser Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys
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[4269]	35 40 45
[4270]	Lys Pro Ser Arg Val Asp Gln Glu Gln Met Ala Ala Ser Phe Lys Val
[4271]	50 55 60
[4272]	Ile Arg Glu Ile Met Gln Ala Glu Ala Ala Lys Ala Glu Ala Ala
[4273]	65 70 75 80
[4274]	Ala Gly Ala Gly Leu Ala Phe Leu Ala Glu Asn Ala Lys Arg Asp Gly
[4275]	85 90 95
[4276]	Ile Thr Thr Leu Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His
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[4282]	Phe Phe Tyr Thr Pro Lys Thr Arg Arg Glu Ala Glu Asp Leu Gln Val
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[4284]	Gly Gln Val Glu Leu Gly Gly Gly Pro Gly Ala Gly Ser Leu Gln Pro
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[4286]	Leu Ala Leu Glu Gly Ser Leu Gln Lys Arg Gly Ile Val Glu Gln Cys
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[4303]	Lys Pro Ser Arg Val Asp Gln Glu Gln Met Ala Ala Ser Phe Lys Val	
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[4309]	Ile Thr Thr Leu Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His	
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[4313]	Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly	
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[4315]	Phe Phe Tyr Thr Pro Lys Thr Arg Arg Asp Val Pro Gln Val Glu Leu	
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[4333]	20 25 30	
[4334]	Val Ser Leu Asp Ala Ile Leu Ala Gly Leu Thr Asp Ala Phe Ala Gly	
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[4336]	Lys Pro Ser Arg Val Asp Gln Glu Gln Met Ala Ala Ser Phe Lys Val	
[4337]	50 55 60	
[4338]	Ile Arg Glu Ile Met Gln Ala Glu Ala Ala Ala Lys Ala Glu Ala Ala	
[4339]	65 70 75 80	
[4340]	Ala Gly Ala Gly Leu Ala Phe Leu Ala Glu Asn Ala Lys Arg Asp Gly	
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[4342]	Ile Thr Thr Leu Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His	
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[4377]	His His His His Arg Phe Val Asn Gln His Leu Cys Gly Ser His Leu		
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[4381]	Pro Lys Thr Arg Arg Glu Ala Glu Asp Leu Gln Val Gly Gln Val Glu		
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[4385]	Gly Ser Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile		
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[4451]	Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln		
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[4507]	His His His His Arg Phe Val Asn Gln His Leu Cys Gly Ser His Leu			
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[4526]	Ile Gly Arg Gln Leu Gly Asp Gln Leu Arg Asp Asn Pro Pro Pro Gly
[4527]	20 25 30
[4528]	Val Ser Leu Asp Ala Ile Leu Ala Gly Leu Thr Asp Ala Phe Ala Gly
[4529]	35 40 45
[4530]	Lys Pro Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His His His
[4531]	50 55 60
[4532]	His His Asp Asp Asp Asp Lys Phe Val Asn Gln His Leu Cys Gly Ser
[4533]	65 70 75 80
[4534]	His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe
[4535]	85 90 95
[4536]	Tyr Thr Pro Lys Thr Arg Arg Glu Ala Glu Asp Leu Gln Val Gly Gln
[4537]	100 105 110
[4538]	Val Glu Leu Gly Gly Gly Pro Gly Ala Gly Ser Leu Gln Pro Leu Ala
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[4540]	Leu Glu Gly Ser Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys Thr
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[4554]	20 25 30
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[4556]	35 40 45
[4557]	Lys Pro Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His His His
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[4559]	His His Asp Asp Asp Asp Lys Phe Val Asn Gln His Leu Cys Gly Ser
[4560]	65 70 75 80
[4561]	His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe
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[4563]	Tyr Thr Pro Lys Thr Arg Arg Asp Val Pro Gln Val Glu Leu Gly Gly
[4564]	100 105 110
[4565]	Gly Pro Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu
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[4567]	Gln Lys Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu
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[4583]	35 40 45
[4584]	Lys Pro Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His His His
[4585]	50 55 60
[4586]	His His Asp Asp Asp Asp Lys Phe Val Asn Gln His Leu Cys Gly Ser
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[4588]	His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe
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[4590]	Tyr Thr Pro Lys Thr Arg Arg Asp Val Pro Gln Val Glu Leu Gly Gly
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[4592]	Gly Pro Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu
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[4594]	Gln Ala Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu
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[4610]	35 40 45
[4611]	Lys Pro Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His His His
[4612]	50 55 60
[4613]	His His Arg Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu
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[4615]	Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys
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[4617]	Thr Arg Arg Glu Ala Glu Asp Leu Gln Val Gly Gln Val Glu Leu Gly
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[4619]	Gly Gly Pro Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser
[4620]	115 120 125
[4621]	Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser
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[4623]	Leu Tyr Gln Leu Glu Asn Tyr Cys Gly
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[4634]	Ile Gly Arg Gln Leu Gly Asp Gln Leu Arg Asp Asn Pro Pro Pro Gly
[4635]	20 25 30
[4636]	Val Ser Leu Asp Ala Ile Leu Ala Gly Leu Thr Asp Ala Phe Ala Gly
[4637]	35 40 45
[4638]	Lys Pro Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His His His
[4639]	50 55 60
[4640]	His His Arg Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu

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[4646]	Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln Lys Arg Gly			
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[4667]	His His Arg Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu			
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[4671]	Thr Arg Arg Asp Val Pro Gln Val Glu Leu Gly Gly Gly Pro Gly Ala			
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[4691]	35 40 45	
[4692]	Lys Pro Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His His His	
[4693]	50 55 60	
[4694]	His His Asp Asp Asp Asp Lys Phe Val Asn Gln His Leu Cys Gly Ser	
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[4696]	His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe	
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[4698]	Tyr Thr Pro Lys Thr Arg Arg Glu Ala Glu Asp Gln Gly Ser Leu Gln	
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[4715]	Val Ser Leu Asp Ala Ile Leu Ala Gly Leu Thr Asp Ala Phe Ala Gly	
[4716]	35 40 45	
[4717]	Lys Pro Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His His His	
[4718]	50 55 60	

[4719]	His His Arg Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu
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[4721]	Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys
[4722]	85 90 95
[4723]	Thr Arg Arg Glu Ala Glu Asp Gln Gly Ser Leu Gln Lys Arg Gly Ile
[4724]	100 105 110
[4725]	Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn
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[4747]	85 90 95
[4748]	Ser Arg Ile Tyr Asn Thr Phe Asp Ala His Arg Leu Leu His Trp Ala
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[4754]	Ile Ala Glu Ser Val Gly Leu Asp Leu Ala Arg Ala Ala Glu Ile Leu
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[4756]	Ala Ser Asp Glu Tyr Ala Ala Glu Val Arg Glu Gln Glu Gln Leu Trp
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[4758]	Val Ser Arg Gly Val Ser Ser Val Pro Thr Ile Val Phe Asn Asp Gln
[4759]	180 185 190
[4760]	Tyr Ala Val Ser Gly Gly Gln Pro Ala Glu Ala Phe Val Gly Ala Ile
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[4762]	Arg Gln Ile Ile Asn Glu Ser Lys Ser Gly Gly Gly Gly Ser Gly Gly
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[4766]	Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val
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[4768]	Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr Arg Arg Glu Ala
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[4774]	Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu
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[4785]	1 5 10 15
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[4790]	Pro Asn Met Pro Ala Glu Gly Gln Asn Ile Val Glu His Ile Thr Glu
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[4792]	Lys Tyr Gly Ser Thr Ala Glu Glu Ser Gln Ala Asn Arg Ala Arg Ile
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[4794]	Arg Asp Met Gly Ala Ala Leu Gly Phe Ala Phe Arg Thr Asp Gly Gln
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[4796]	Ser Arg Ile Tyr Asn Thr Phe Asp Ala His Arg Leu Leu His Trp Ala

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[4806]	Val Ser Arg Gly Val Ser Ser Val Pro Thr Ile Val Phe Asn Asp Gln		
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[4808]	Tyr Ala Val Ser Gly Gly Gln Pro Ala Glu Ala Phe Val Gly Ala Ile		
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[4810]	Arg Gln Ile Ile Asn Glu Ser Lys Ser Gly Gly Gly Gly Ser Gly Gly		
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[4818]	Pro Gln Val Glu Leu Gly Gly Gly Pro Gly Ala Gly Ser Leu Gln Pro		
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[4853]	Val Ser Arg Gly Val Ser Ser Val Pro Thr Ile Val Phe Asn Asp Gln		
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[4855]	Tyr Ala Val Ser Gly Gly Gln Pro Ala Glu Ala Phe Val Gly Ala Ile		
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[4865]	Pro Gln Val Glu Leu Gly Gly Gly Pro Gly Ala Gly Ser Leu Gln Pro		
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[4977]	35 40 45
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[4982]	Arg Asp Met Gly Ala Ala Leu Gly Phe Ala Phe Arg Thr Asp Gly Gln
[4983]	85 90 95
[4984]	Ser Arg Ile Tyr Asn Thr Phe Asp Ala His Arg Leu Leu His Trp Ala
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[4986]	Gly Leu Glu Gly Leu Gln Tyr Asn Leu Lys Glu Ala Leu Phe Lys Ala
[4987]	115 120 125
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[5091]	210 215 220
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[5121]	Ser	Arg	Ile	Tyr	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	His	His
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[5218]	Arg Asp Met Gly Ala Ala Leu Gly Phe Ala Phe Arg Thr Asp Gly Gln		
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[5244]	20 25 30
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[5246]	35 40 45
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[5256]	115 120 125
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[5285]	85 90 95
[5286]	Ser Arg Ile Tyr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His
[5287]	100 105 110
[5288]	His His His His Arg Phe Val Asn Gln His Leu Cys Gly Ser His Leu
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[5290]	Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr
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[5292]	Pro Lys Thr Arg Arg Asp Val Pro Gln Val Glu Leu Gly Gly Gly Pro
[5293]	145 150 155 160
[5294]	Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln Ala
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[5296]	Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln
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[5314]	50 55 60	
[5315]	Lys Tyr Gly Ser Thr Ala Glu Glu Ser Gln Ala Asn Arg Ala Arg Ile	
[5316]	65 70 75 80	
[5317]	Arg Asp Met Gly Ala Ala Leu Gly Phe Ala Phe Arg Thr Asp Gly Gln	
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[5319]	Ser Arg Ile Tyr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His	
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[5325]	Phe Phe Tyr Thr Pro Lys Thr Arg Arg Glu Ala Glu Asp Gln Gly Ser	
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[5350]	Ser Arg Ile Tyr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His		
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[5352]	His His His His Arg Phe Val Asn Gln His Leu Cys Gly Ser His Leu		
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[5357]	145	150	155
[5358]	Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu		
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[5375]	Pro Asn Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His His His		
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[5377]	His His Asp Asp Asp Asp Lys Phe Val Asn Gln His Leu Cys Gly Ser		
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[5379]	His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe		
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[5401]	35 40 45		
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[5412]	Gln Lys Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu		
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[5414]	Tyr Gln Leu Glu Asn Tyr Cys Gly		
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[5465]	115 120 125
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[5467]	130 135 140
[5468]	Leu Tyr Gln Leu Glu Asn Tyr Cys Gly
[5469]	145 150
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[5482]	35 40 45
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[5484]	50 55 60
[5485]	His His Arg Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu
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[5507]	20 25 30	
[5508]	Gly Ser Glu Val Gln Ala Glu Ile His Phe Gln Pro Phe Glu Leu Asn	
[5509]	35 40 45	
[5510]	Pro Asn Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His His His His	
[5511]	50 55 60	
[5512]	His His Arg Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu	
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[5514]	Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys	
[5515]	85 90 95	
[5516]	Thr Arg Arg Asp Val Pro Gln Val Glu Leu Gly Gly Gly Pro Gly Ala	
[5517]	100 105 110	
[5518]	Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln Ala Arg Gly	
[5519]	115 120 125	
[5520]	Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu	
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[5534]	20 25 30	
[5535]	Gly Ser Glu Val Gln Ala Glu Ile His Phe Gln Pro Phe Glu Leu Asn	
[5536]	35 40 45	
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[5542]		85	90
[5543]	Tyr Thr Pro Lys Thr Arg Arg Glu Ala Glu Asp Gln Gly Ser Leu Gln		95
[5544]		100	105
[5545]	Lys Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr		110
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[5547]	Gln Leu Glu Asn Tyr Cys Gly		125
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[5560]	Gly Ser Glu Val Gln Ala Glu Ile His Phe Gln Pro Phe Glu Leu Asn		30
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[5567]		85	90
[5568]	Thr Arg Arg Glu Ala Glu Asp Gln Gly Ser Leu Gln Lys Arg Gly Ile		95
[5569]		100	105
[5570]	Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn		110
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[5593]	Leu Phe Thr Gly Ala Pro Leu Ala Gln Asp Lys Glu Ser Val Phe Trp		
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[5601]	Trp Gln Leu Val Thr Ala Arg Asp Gly Gln Gly Leu Ala Leu Lys Ala		
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[5615]	Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys		

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[5638]	Arg Leu Val Tyr Pro Ala Asp Gln Lys Glu Ile Thr Val Lys Leu Asn		
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[5644]	Pro Pro Val Ala Arg Ile Asp Pro Thr Lys Gly Gln Ser Leu Arg Val		
[5645]	85 90 95		
[5646]	Leu Phe Thr Gly Ala Pro Leu Ala Gln Asp Lys Glu Ser Val Phe Trp		
[5647]	100 105 110		
[5648]	Leu Asn Val Leu Glu Ile Pro Pro Lys Pro Glu Ala Gly Ala Asp Leu		
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[5652]	Pro Val Gly Leu Pro Gly Asn Pro Asn Glu Ala Val Glu Gln Val Gln		
[5653]	145 150 155 160		
[5654]	Trp Gln Leu Val Thr Ala Arg Asp Gly Gln Gly Leu Ala Leu Lys Ala		

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[5748]	Leu	Phe	Thr	Gly	Ala	Pro	Leu	Ala	Gln	Asp	Lys	Glu	Ser	Val	Phe	Trp
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[5843]	50 55 60
[5844]	Ser Val Glu Ser Thr Pro Thr Ser Ser Lys Ala Pro Phe Leu Leu Ser
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[5899]	Leu	Phe	Thr	Gly	Ala	Pro	Leu	Ala	Gln	Asp	Lys	Glu	Ser	Val	Phe	Trp
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[5971]	260	265	270	
[5972]	Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly			
[5973]	275	280	285	
[5974]	Phe Phe Tyr Thr Pro Lys Thr Arg Arg Glu Ala Glu Asp Gln Gly Ser			
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[5976]	Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser			
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[6063]	Pro Pro Val Ala Arg Ile Asp Pro Thr Lys Gly Gln Ser Leu Arg Val	
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[6106]	Leu	Gly	Gly	Gly	Pro	Gly	Ala	Gly	Ser	Leu	Gln	Pro	Leu	Ala	Leu	Glu
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[6108]	Gly	Ser	Leu	Gln	Lys	Arg	Gly	Ile	Val	Glu	Gln	Cys	Cys	Thr	Ser	Ile
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[6223]	50 55 60
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[6226]	Pro Pro Val Ala Arg Ile Asp Pro Thr Lys Gly Gln Ser Leu Arg Val
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[6263]	Leu Glu Gly Ser Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys Thr	
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[6726]	cacattaccg aaaagtacgg ctccacggct gaagagtcgc aggctaatacg tgcgcgtatc	240
[6727]	cgtgacatgg ggcgcgcgtt gggctttgct tttcgcaccg atggccagag ccgtatctac	300
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[6803]		50						55				60					
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[6817]					165					170					175		
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[6823]		210						215				220					
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[6830]	Asp Thr Thr Tyr Gly Phe Asn Ser Asn Ser Gly Arg Glu Ala Tyr Ser			
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[6836]	Asn Leu Lys Ala Glu Ser Phe Ser Asp Val Gly Gly Leu Arg Gly Asn			
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[6838]	Val Ser Ile Ala Lys Gly Val Ser Val Glu Asn Ala Ile Gly Gly Thr			
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[6842]	Gly Lys Gly Ala Asp Lys Leu His Gly Gly Ala Gly Ala Asp Thr Phe			
[6843]		370	375	380
[6844]	Val Tyr Arg Arg Ala Ser Asp Ser Thr Pro Gln Ala Pro Asp Ile Ile			
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[6846]	Gln Asp Phe Gln Ser Gly Ser Asp Lys Ile Asp Leu Thr Gly Val Val			
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[6848]	Gln Glu Ala Gly Leu Lys Ser Leu Ser Phe Val Glu Lys Phe Ser Gly			
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[6850]	Lys Ala Gly Glu Ala Val Leu Gly Gln Asp Ala Lys Thr Gly Arg Phe			
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[6860]	Pro Glu Pro Ala Pro Leu Pro Val Pro Thr Pro Arg Pro Gly Gly Gly			
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[6862]	Phe Ile Gly Lys Ile Phe Ser Ser Phe Lys Gly Phe Ile Lys Lys Val			
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[6875]	aaagaaatca ccgtaaaact gaacaataac ggcacgttgc ccgcactggt ccaatcatgg	180
[6876]	atcgacaccg gcagcgtcga atcgacaccc accagctcca aggcgccgtt cctattgtcg	240
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[6878]	gcgccttttg cgcaggacaa agagtcggtg ttctggctca acgttctcga aatcccggcc	360
[6879]	aaacccgagg cgggtgcaga cctcaacacg ctgcaaattg ctttccgttc gcgcatcaag	420
[6880]	ctgtttctatc gcccggtcgg cttgcctgga aatcccaatg aggcggttga gcaggtgcag	480
[6881]	tggcaattgg ttacggcacg cgatggccaa ggcttggcgc tgaaggcgta caacccgtcg	540
[6882]	gcgttccacg tctcgtgat cgagttggac ctggtggcgg gtaaccaacg ctatcgcagt	600
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[6884]	aggccgtcga gccaggcaca agtggagttc agcgccatca acgattacgg cgcgttggtc	720
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[6905]	Phe Lys Lys Ile Ser Ser Ser Gly Ala Leu
[6906]	20 25
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[6974]	<210>	238		
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[7010]	Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
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[7013]	20 25
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[7020]	<400>	242
[7021]	His His His His His His	
[7022]	1	5



图1

Dna-J样蛋白-PTH 1-34融合体 (SEQ ID NO: 45)

mkvepglyqhykgpqyrvfsvarhseteeevfyqalygeygfwvrplsmfletvevdgeqvprfalvtae
pslftgqggggsggggshhhhhdddksvseiqlmhnlgkhlnsmervewlrkklqdvhnf

图2A

FkIB-PTH 1-34融合体 (SEQ ID NO: 46)

msevnlstetrsvsygigrqlgdqlrdnpppgvslldailagltdefagkpsrvdqeemaasfkvireimqae
aaakaeaaagaglaflaenakrdgittlasglqfevltagtgakptredqvrthyhgtlidgtvfdssyergqpa
efpvggviagwtealqlmnagskwrvypselaygaqgvgsipphsvlvfdvllvgggsggggshh
hhhhdddksvseiqlmhnlgkhlnsmervewlrkklqdvhnf

图2B

FrnE-PTH 1-34融合体 (SEQ ID NO: 47)

mstplkidfvsvscpwciiglrgrltealdqlgsevqaeihfqpfeInpnmpaegqnivehitekygstaees
qanrarirdmgaalgfaftrtdgqsriyntfdahrllhwagleglqynlkealfkayfsdgqdpdhatlaiiaes
vgldlaraaeilasdeyaaevreqeqlwvsrgvssvptivfndqyavsggqpaeafvgairqiinesksggg
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图2C

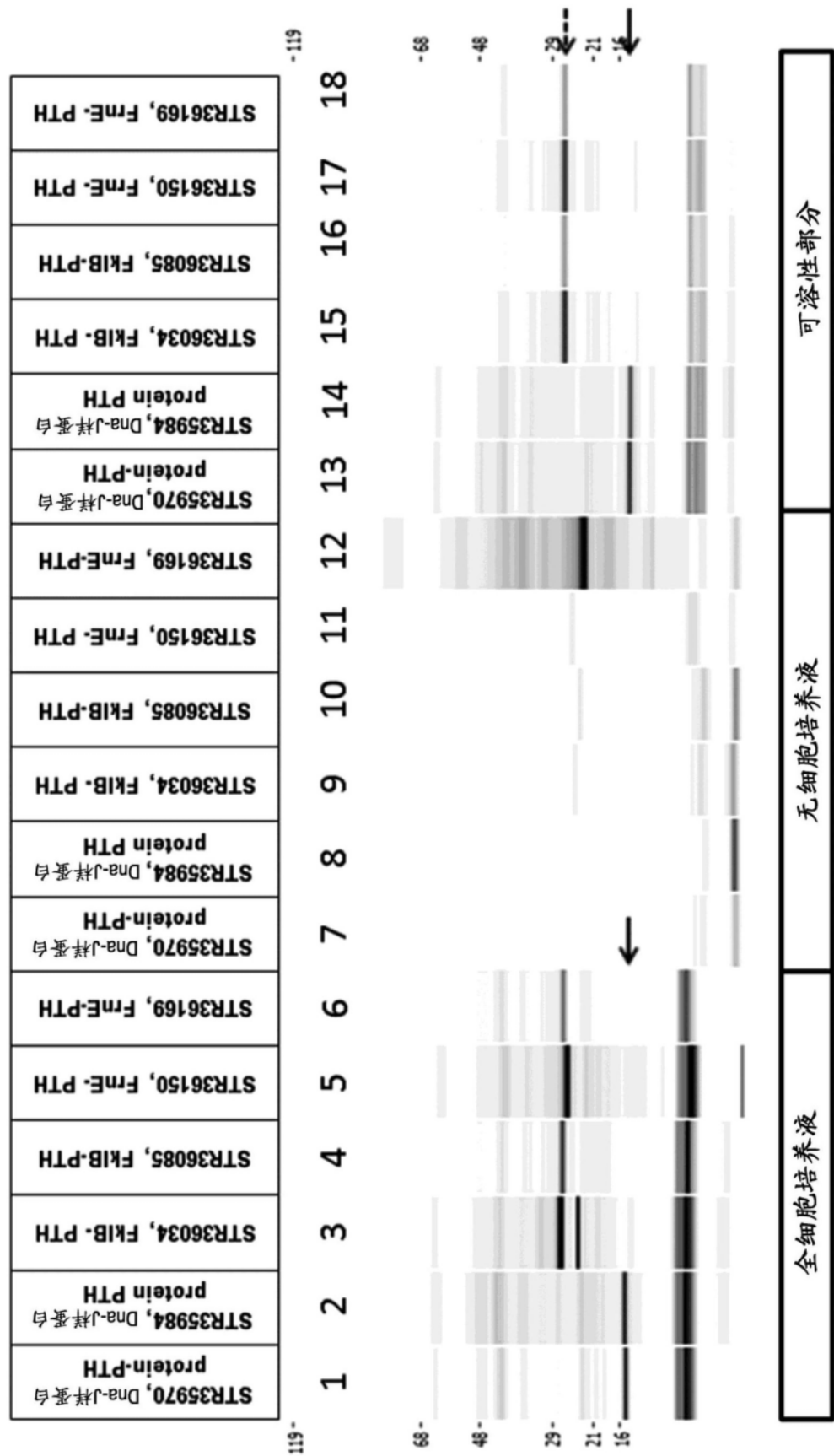


图3

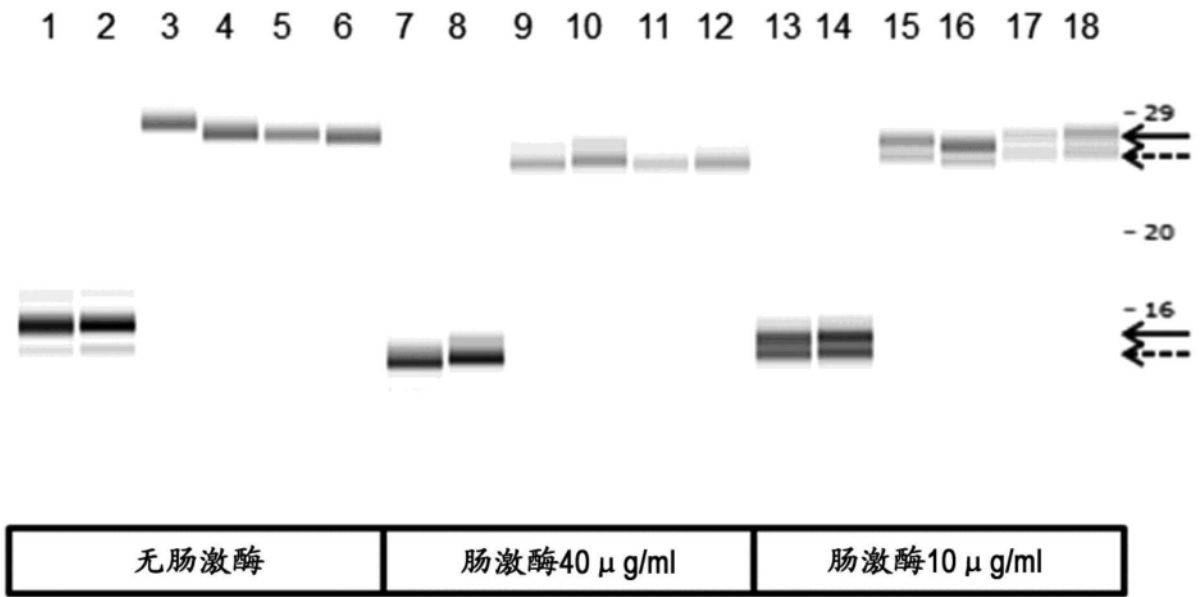


图4

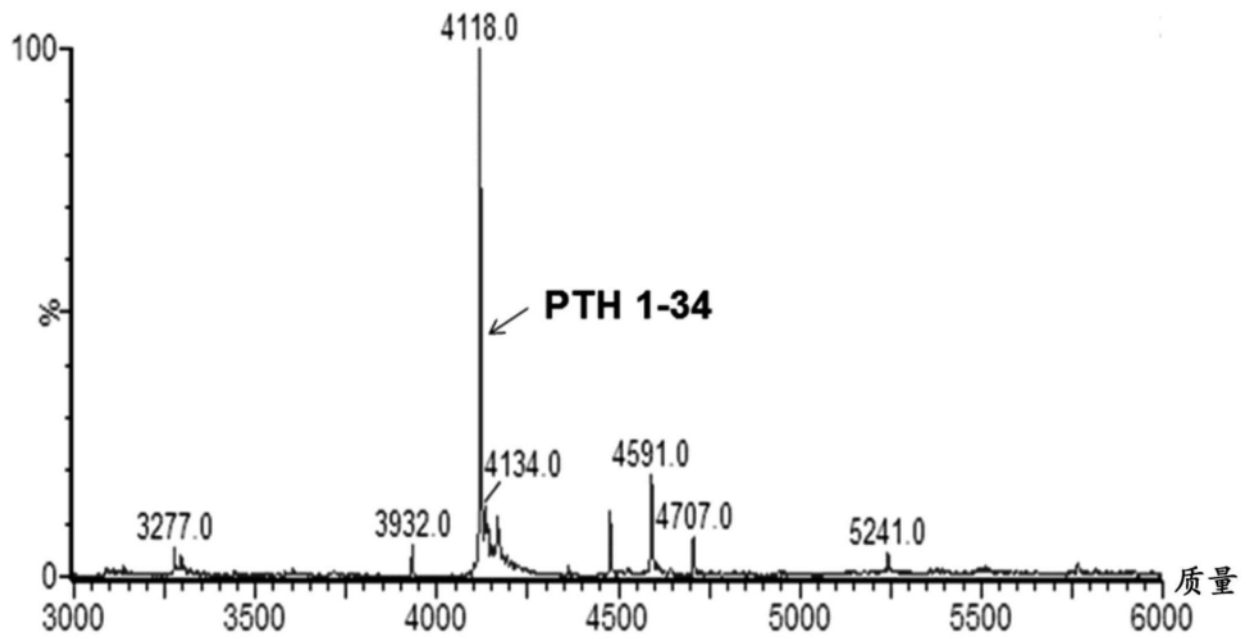


图5

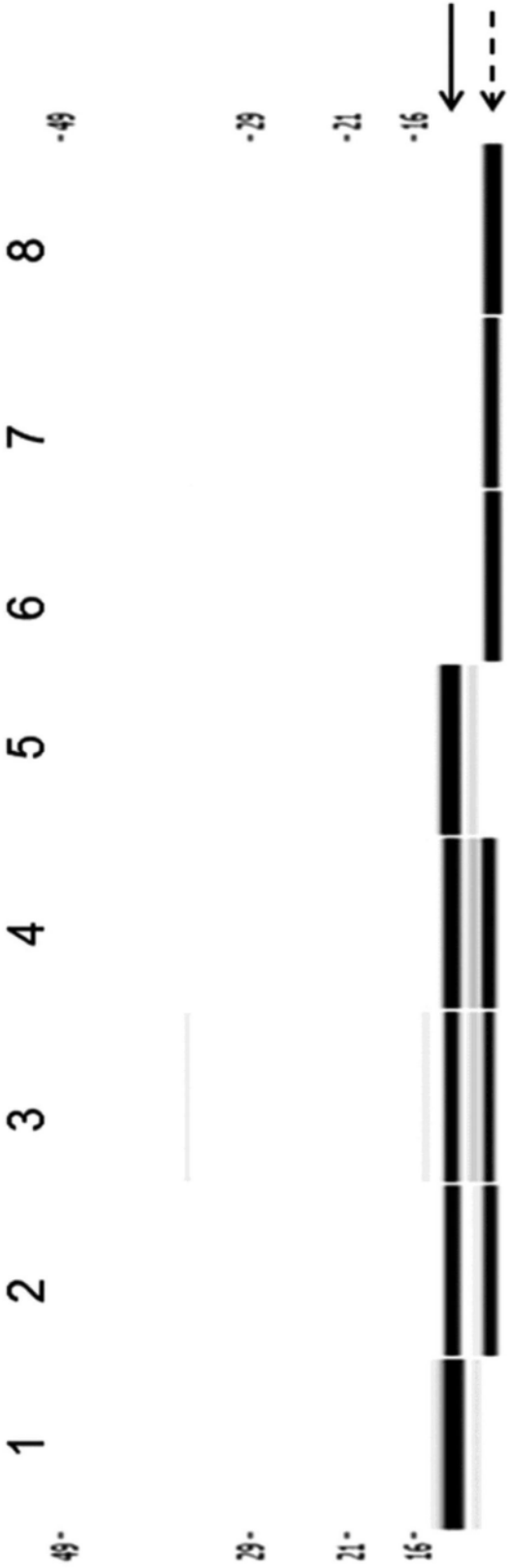


图6

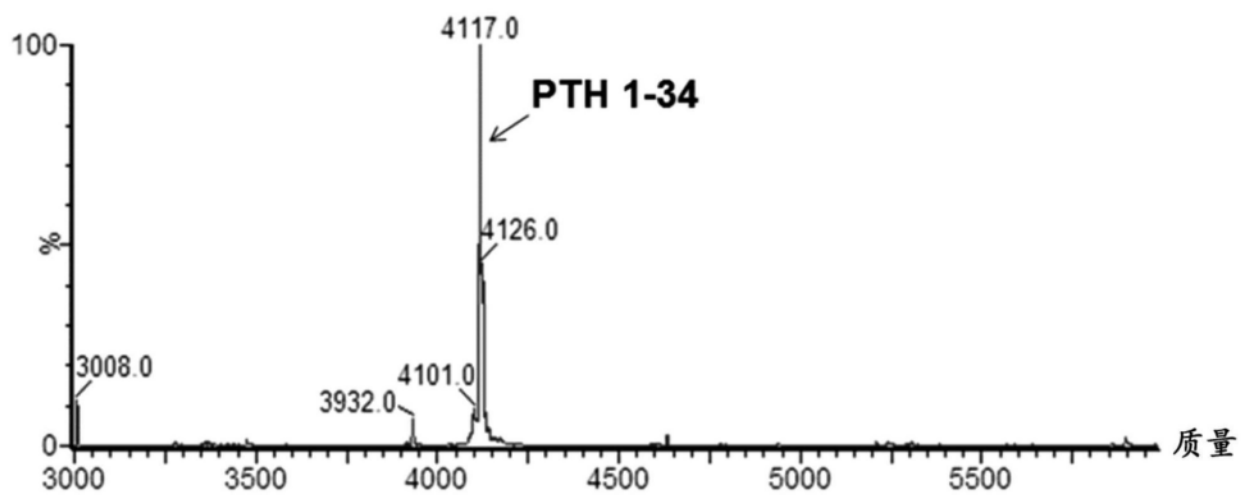


图7A

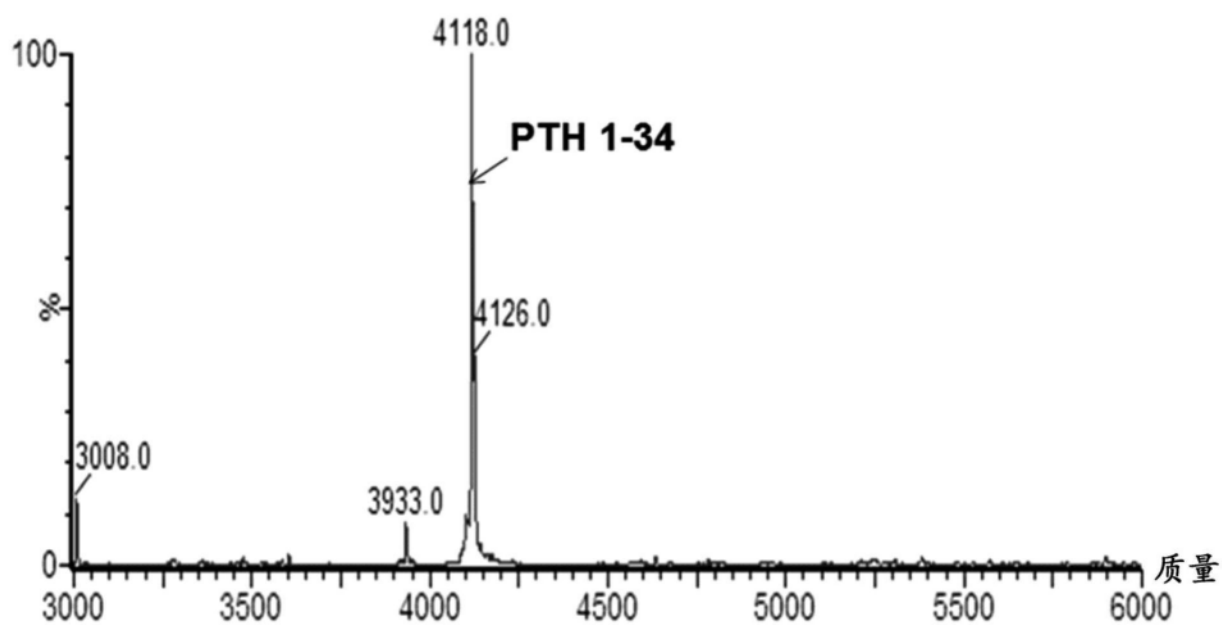


图7B

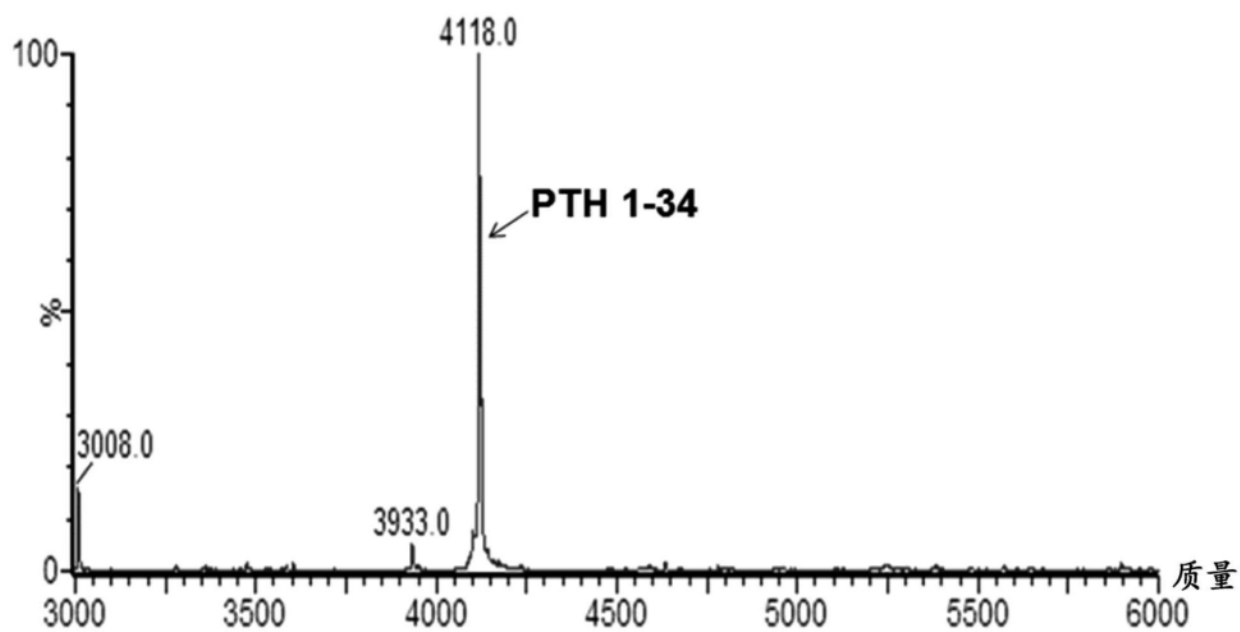


图7C