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(54)发明名称

色氨酸光学探针及其制备方法和应用

(57)摘要

本发明涉及一种色氨酸光学探针及其制备方法和应用。一方面,本发明涉及一种光学探针,包含色氨酸敏感多肽或其功能变体和光学活性多肽或其功能变体,其中光学活性多肽或其功能变体位于色氨酸敏感多肽或其功能变体的序列内。本发明也涉及上述探针的制备方法及其在检测色氨酸中的应用。

1. 一种光学探针, 包含色氨酸敏感多肽和光学活性多肽, 其中光学活性多肽位于色氨酸敏感多肽的序列内。

2. 如权利要求1所述的光学探针, 其中, 色氨酸敏感多肽具有SEQ ID NO:1所示的序列或其功能片段, 或与其有至少35%、至少40%、至少50%、至少60%、至少70%、至少80%、至少85%、至少90%、至少95%、至少99%序列相同性的序列。

3. 如权利要求1或2所述的所述的光学探针, 其中, 光学活性多肽位于色氨酸敏感多肽的选自以下的位置中: 残基114-118、233-235和/或263-268,

优选地, 光学活性多肽位于色氨酸敏感多肽的选自以下的一个或多个位点: 114/115, 114/116, 114/117, 114/118, 115/116, 115/117, 115/118, 116/117, 116/118, 117/118, 233/234, 233/235, 234/235, 263/264, 263/265, 263/266, 263/267, 263/268, 264/265, 264/266, 264/267, 264/268, 265/266, 265/267, 265/268, 266/267, 266/268和/或267/268,

优选地, 所述色氨酸敏感多肽包含选自下述位点处的突变: H13, T71, L84, D93, K105, N117, T130, V170, V192, F202, K214, Y217, K255, T259, I283。

4. 如权利要求3所述的光学探针, 其中, 光学活性多肽位于色氨酸敏感多肽的残基263-268中。

5. 一种核酸序列, 选自

(1) 编码权利要求1-4中任一项所述的光学探针的多核苷酸;

(2) (1) 的片段;

(3) (1) 或 (2) 的互补序列。

6. 一种核酸构建物, 其包含权利要求5所述的核酸序列, 优选地, 所述核酸构建物是表达载体。

7. 一种宿主细胞, 所述宿主细胞

(1) 表达权利要求1-4中任一项所述的光学探针;

(2) 包含权利要求5所述的核酸序列; 或

(3) 包含权利要求6所述的核酸构建物。

8. 一种制备权利要求1-4中任一项所述的光学探针的方法, 包括培养权利要求7所述的宿主细胞, 和由培养物分离所述光学探针。

9. 权利要求1-4中任一项所述的光学探针、权利要求5所述的核酸序列或权利要求6所述的核酸构建物在检测样品中的色氨酸或筛选化合物中的应用, 所述检测包括色氨酸定性、定位或定量检测。

10. 一种检测试剂盒, 其包含

(1) 权利要求1-4中任一项所述的光学探针或权利要求8所述方法制备的光学探针;

(2) 权利要求5所述的核酸序列;

(3) 权利要求6所述的核酸构建物; 或

(4) 权利要求7所述的细胞; 和

任选的利用光学探针检测色氨酸所需的其它试剂。

色氨酸光学探针及其制备方法和应用

技术领域

[0001] 本发明涉及光学探针技术领域,尤其涉及一种色氨酸光学探针及其制备方法和应用。

背景技术

[0002] L-色氨酸是人生长发育不可缺少的必需氨基酸之一。在20种构成蛋白质的氨基酸中,色氨酸在蛋白和胞浆中所占比例最小,除了调节蛋白质的合成外,它和它的代谢物也参与多项生理功能,对身体内许多器官的发育和功能非常重要。

[0003] 不同的组织中色氨酸的代谢与多种生理功能有关。犬尿氨酸途径是人和哺乳动物体内色氨酸代谢的主要途径,大部分发生在肝脏。该途径中,色氨酸在色氨酸2.3-双加氧酶(TDO)(主要位于肝脏)或吲哚胺2.3-双加氧酶(IDO)(广泛存在于多种组织)作用下生成N-甲酰犬尿氨酸和犬尿氨酸,之后再经多级酶促反应生成喹啉酸、烟酸、犬尿喹啉和黄尿酸等活性分子。犬尿氨酸是一种非选择性的N-甲基-D天冬氨酸(NMDA)受体的阻断剂,抑制神经系统的兴奋。喹啉酸与糖原异生作用有关,还能与NMDA受体结合,兴奋神经递质。烟酸是合成辅酶I(nicotinamide adenine dinucleotide,NAD)和辅酶II(nicotinamide adenine dinucleotide phosphate,NADP)的前体,在生物氧化中起递氢体的作用。在肠道和脑中,色氨酸由色氨酸羟化酶(TPH)催化,合成5-HTP(5-羟色胺酸),继而经脱羧酶作用转化为5-HT(5-羟色胺),这是体内色氨酸代谢的另一重要途径。5-羟色胺是一种脑神经递质,血小板凝血因子和遍布全身器官的神经激素,它还会导致血管变窄,大脑中5-羟色胺水平的变化可以改变情绪。在松果体中,5-羟色胺进一步转化为褪黑激素有明显的昼夜节律变化,可调节睡眠和清醒,也参与抗氧化应激和免疫调节。

[0004] 研究发现存在许多以色氨酸缺乏为特征的病症或疾病。例如,果糖吸收不良会导致肠道中色氨酸的吸收不当,这会降低血液中色氨酸的含量并导致抑郁。高玉米或其他色氨酸缺乏的饮食会导致糙皮病,这是一种烟酸-色氨酸缺乏症,症状为皮炎,腹泻和痴呆。Hartnup病是一种无法正确吸收色氨酸和其他氨基酸的疾病,其症状包括皮疹,协调运动困难(小脑性共济失调)和精神症状,如抑郁症或精神病。而在某些情况下,色氨酸也可以是神经毒素和内毒素,在长期高水平下引起不利的健康影响。如在I型戊二酸尿症中发现长期高水平的色氨酸,由于线粒体戊二酰辅酶A脱氢酶(GCDH)的缺乏,身体不能完全分解赖氨酸、羟赖氨酸和色氨酸,过量的中间分解产物累积并对大脑以及其他器官造成损害。高水平的色氨酸也与嗜酸粒细胞增多症-肌痛综合征(EMS)有关,这是一种无法治愈的,有时甚至是致命的流感样神经系统疾病。总而言之,生理条件下的色氨酸浓度或浓度的变化趋势的准确、及时测定,不仅可用来评价营养状态,更对多种疾病的临床诊断及其后的针对性治疗监测具有很重要的价值。

[0005] 目前最常用的检测色氨酸的方法有HPLC法(Sadok,I.等.J Sep Sci 2017,40,(15),3020-3045)、高效毛细管电泳法、质谱法(Chen,T.等.PLoS One 2016,11,(9),e0162192)、层析法、紫外吸收法、可见光光度法(Friedman,M.J Agric Food Chem 2004,

52, (3), 385-406; Wu, Y. 等. Talanta 2018, 176, 604-609)、近红外反射光谱 (Fontaine, J. 等. J Agric Food Chem 2002, 50, (14), 3902-11)、荧光光谱法、流动注射分析法、ELISA等。这些检测分析方法或需要专业分析仪器设备, 或步骤繁琐, 易出现人为误差, 且仅适用于体外检测, 无法实时监控活细胞的色氨酸浓度变化。因此, 亟待开发新的检测方法, 实现在细胞内、外, 简便、快捷、特异性高的实时、定位、定量、高通量检测色氨酸。

发明内容

[0006] 本发明的目的在于提供在细胞内、外实时定位、高通量、定量检测色氨酸的探针和方法。

[0007] 为了实现上述发明目的, 本发明提供以下技术方案:

[0008] 本发明提供一种色氨酸光学探针, 包含色氨酸敏感多肽和光学活性多肽, 其中光学活性多肽位于色氨酸敏感多肽的序列内。色氨酸敏感多肽被光学活性多肽或其功能变体分为第一部分和第二部分。

[0009] 本发明提供了一种色氨酸光学探针, 包括色氨酸敏感多肽B和光学活性多肽A, 其中光学活性多肽A位于色氨酸敏感多肽B的序列内, 将色氨酸敏感多肽B分为第一部分B1和第二部分B2, 形成B1-A-B2式的探针结构。

[0010] 在一个实施方案中, 色氨酸敏感多肽包括色氨酸结合蛋白或其功能片段。在一个实施方案中, 色氨酸敏感多肽源自肺炎链球菌 *Streptococcus pneumoniae*。在一个实施方案中, 色氨酸敏感多肽是色氨酸结合蛋白 3lft。在一个实施方案中, 色氨酸敏感多肽具有 SEQ ID NO:1 所示的序列或其片段, 或与其有至少 35%、至少 40%、至少 50%、至少 60%、至少 70%、至少 80%、至少 85%、至少 90%、至少 95%、至少 99% 序列相同性并保留色氨酸结合功能的序列。

[0011] 在一个实施方案中, 光学活性多肽是荧光蛋白或其功能变体。在一个实施方案中, 荧光蛋白选自黄色荧光蛋白 (如 SEQ ID NO:2 所示的 cpYFP)、橘黄色荧光蛋白、红色荧光蛋白、绿色荧光蛋白 (如 SEQ ID NO:3 所示的 cpGFP)、蓝色荧光蛋白 (如 SEQ ID NO:4 所示的 cpBFP)、苹果红荧光蛋白 (如 SEQ ID NO:5 所示的 cpmApple)。优选地, 光学活性多肽是 cpYFP。在一个实施方案中, 荧光蛋白具有 SEQ ID NO:2-5 中任一所示的序列。

[0012] 在一个实施方案中, 光学探针还包含侧接所述光学活性多肽的一个或多个接头。本发明所述接头可以是任何长度的任何氨基酸序列。在一个实施方案中, 光学活性多肽侧翼包含不超过 5 个氨基酸的接头, 例如 0、1、2、3、4 个氨基酸的接头。在一个实施方案中, 光学活性多肽侧翼的接头包含氨基酸 Y。在一个实施方案中, 接头 Y 位于光学活性多肽的 N 端和/或 C 端。在一个实施方案中, 光学探针如下所示: 色氨酸敏感多肽的第一部分 B1-Y-光学活性多肽 A-色氨酸敏感多肽的第二部分 B2。在一个实施方案中, 本发明光学探针不包含接头。

[0013] 本发明所述光学活性多肽可以位于色氨酸敏感多肽的任何位置。在一个实施方案中, 光学活性多肽位于色氨酸敏感多肽的选自以下的一个或多个位置中: 残基 114-118、233-235 和/或 263-268, 编号对应于色氨酸结合蛋白的全长。在一个实施方案中, 光学活性多肽置换色氨酸敏感多肽的选自以下的一个或多个位置中的一个或多个氨基酸: 残基 114-118、233-235 和/或 263-268, 编号对应于色氨酸结合蛋白的全长。

[0014] 在一个实施方案中, 光学活性多肽位于色氨酸敏感多肽的选自以下的一个或多个

位点:114/115,114/116,114/117,114/118,115/116,115/117,115/118,116/117,116/118,117/118,233/234,233/235,234/235,263/264,263/265,263/266,263/267,263/268,264/265,264/266,264/267,264/268,265/266,265/267,265/268,266/267,266/268和/或267/268。优选地,光学活性多肽位于色氨酸敏感多肽的选自以下的一个或多个位点:263/265,263/266,263/267和263/268。

[0015] 在示例性实施方案中,本发明B1-A-B2式光学探针可为当cpYFP位于色氨酸结合蛋白的选自以下的一个或多个位点的探针:114/115,114/116,114/117,114/118,115/116,115/117,115/118,116/117,116/118,117/118,233/234,233/235,234/235,263/264,263/265,263/266,263/267,263/268,264/265,264/266,264/267,264/268,265/266,265/267,265/268,266/267,266/268或267/268。在示例性实施方案中,本发明B1-A-B2式光学探针可为当cpYFP位于色氨酸结合蛋白的选自以下的一个或多个位点的探针:114/115,114/116,114/118,115/118,116/118,117/118,233/235,263/265,263/266,263/267,263/268,264/265,264/266,264/267,265/266,266/267或266/268。优选地,本发明光学探针可为当cpYFP位于色氨酸结合蛋白的选自以下的一个或多个位点的探针:263/265,263/266,263/267和263/268。

[0016] 在一个或多个实施方案中,本发明光学探针可为当cpGFP位于色氨酸结合蛋白的选自以下的一个或多个位点的探针:114/115,114/116,114/117,114/118,115/116,115/117,115/118,116/117,116/118,117/118,233/234,233/235,234/235,263/264,263/265,263/266,263/267,263/268,264/265,264/266,264/267,264/268,265/266,265/267,265/268,266/267,266/268或267/268。在一个或多个实施方案中,本发明光学探针可为当cpGFP位于色氨酸结合蛋白的选自以下的一个或多个位点的探针:114/117,114/118,115/117,116/117,116/118,117/118,233/234,263/264,263/265,263/266,263/267,263/268,264/265,264/266,264/267,265/267或266/267。在优选的实施方案中,本发明光学探针可为当cpGFP位于色氨酸结合蛋白的选自以下的一个或多个位点的探针:263/265,263/266,263/267和263/268。

[0017] 在一个或多个实施方案中,本发明光学探针可为当cpBFP位于色氨酸结合蛋白的选自以下的一个或多个位点的探针:114/115,114/116,114/117,114/118,115/116,115/117,115/118,116/117,116/118,117/118,233/234,233/235,234/235,263/264,263/265,263/266,263/267,263/268,264/265,264/266,264/267,264/268,265/266,265/267,265/268,266/267,266/268或267/268。在一个或多个实施方案中,本发明光学探针可为当cpBFP位于色氨酸结合蛋白的选自以下的一个或多个位点的探针:114/115,114/117,114/118,115/118,116/118,233/235,263/265,263/266,263/267,263/268,264/265,264/266,265/266,或266/267。在优选的实施方案中,本发明光学探针可为当cpBFP位于色氨酸结合蛋白的选自以下的一个或多个位点的探针:263/265,263/266,263/267和263/268。

[0018] 在一个或多个实施方案中,本发明光学探针可为当cpmApple位于色氨酸结合蛋白的选自以下的一个或多个位点的探针:114/115,114/116,114/117,114/118,115/116,115/117,115/118,116/117,116/118,117/118,233/234,233/235,234/235,263/264,263/265,263/266,263/267,263/268,264/265,264/266,264/267,264/268,265/266,265/267,265/268,266/267,266/268或267/268。在一个或多个实施方案中,本发明光学探针可为当

cpmApple位于色氨酸结合蛋白的选自以下的一个或多个位点的探针:114/115,117/118,263/264,263/265,263/266,263/267,264/268或265/266。在优选的实施方案中,本发明光学探针可为当cpmApple位于色氨酸结合蛋白的选自以下的一个或多个位点的探针:263/264,263/265,263/266。

[0019] 在一个实施方案中,本发明光学探针具有SEQ ID NO:6-9所示序列或由其组成。

[0020] 本发明还提供具有一个或多个突变的色氨酸敏感多肽。所述氨基酸突变包括氨基酸的修饰、取代、缺失或序列的截短。在一个或多个实施方案中,所述突变位于色氨酸结合蛋白的H13,T71,L84,D93,K105,N117,T130,V170,V192,F202,K214,Y217,K255,T259,I283中的1个、2个、3个、4个、5个、6个或多个。示例性地,所述突变选自以下的1个、2个、3个、4个、5个、6个或多个:H13F,H13W,T71F,T71W,L84F,L84W,D93F,D93W,K105F,K105W,N117F,N117W,T130F,T130W,V170F,V170W,V192F,V192W,F202W,K214F,K214W,Y217F,Y217W,K255F,K255W,T259F,T259W,I283F和/或I283W。

[0021] 本发明还提供包含具有一个或多个突变的色氨酸敏感多肽的光学探针。在一个或多个实施方案中,光学探针是如上所述的插入有光学活性多肽的任意光学探针,并且所述光学探针中的色氨酸敏感多肽在选自H13,T71,L84,D93,K105,N117,T130,V170,V192,F202,K214,Y217,K255,T259,I283中的1个、2个、3个、4个、5个、6个或多个位点具有突变。在一个或多个实施方案中,包含突变的色氨酸敏感多肽的光学探针对色氨酸的响应不低于未突变的对应物。优选地,所述突变选自以下的1个、2个、3个、4个、5个、6个或多个:H13F,L84F,L84W,D93F,D93W,K105F,K105W,N117F,N117W,V170F,V170W,V192F,F202W,K214F,K214W,Y217F,Y217W,K255F,K255W,T259F,T259W,I283F和I283W。

[0022] 在示例性实施方案中,本发明光学探针可为色氨酸结合蛋白的选自114/115,114/116,114/117,114/118,115/116,115/117,115/118,116/117,116/118,117/118,233/234,233/235,234/235,263/264,263/265,263/266,263/267,263/268,264/265,264/266,264/267,264/268,265/266,265/267,265/268,266/267,266/268或267/268的一个或多个位点插入有荧光蛋白并且具有选自以下一个或多个突变的融合多肽:H13F,L84F,L84W,D93F,D93W,K105F,K105W,N117F,N117W,V170F,V170W,V192F,F202W,K214F,K214W,Y217F,Y217W,K255F,K255W,T259F,T259W,I283F和I283W。优选地,本发明光学探针是色氨酸结合蛋白的选自263/265,263/266或263/267的一个或多个位点插入有cpYFP并且具有选自以下一个或多个突变的融合多肽:L84F,K105F,K105W,N117F,V170F,F202W,K214F,K214W,Y217F,Y217W,K255F,K255W,T259F,T259W,I283F或I283W。在示例性实施方案中,所述色氨酸结合蛋白是SEQ ID NO:1。优选地,本发明光学探针具有SEQ ID NO:10-39所示序列或由其组成。

[0023] 本发明提供的光学探针包含氨基酸序列SEQ ID NO:6-39中任一或其变体。在一个实施方案中,本发明提供的光学探针包含与氨基酸序列SEQ ID NO:6-39中任一有至少35%、至少40%、至少50%、至少60%、至少70%、至少80%、至少85%、至少90%、至少95%、至少99%序列相同性并保留检测色氨酸功能的序列。在优选实施方案中,本发明提供的光学探针包含与氨基酸序列SEQ ID NO:6-39中任一实质上相似或相同的序列。在更优选的实施方案中,本发明提供的光学探针包含SEQ ID NO:10-39中任一或由其组成。

[0024] 本发明还提供融合多肽,包含本文所述光学探针和其它多肽。在一些实施方案中,其他多肽位于所述光学探针的N端和/或C端。在一些实施方案中,其他多肽包括将光学探针

定位到不同细胞器或亚细胞器的多肽、用于纯化的标签或者用于免疫印迹的标签。

[0025] 本发明还提供编码本文所述多肽、探针或蛋白的核酸序列或其互补序列或片段。在一个实施方案中,本发明的核酸序列选自(1) SEQ ID NO:6-39中任一所示氨基酸序列的编码序列或其互补序列,(2)与(1)具有至少99%、至少95%、至少90%、至少80%、至少70%或至少50%相同性的序列,(3)(1)或(2)的片段。在一个或多个实施方案中,所述片段是引物。在一个实施方案中,本发明核酸序列包含核苷酸序列SEQ ID NO:40或其变体,所述变体编码的氨基酸序列具有检测色氨酸的功能。在优选实施方案中,本发明提供一种核酸序列,包含与核苷酸序列SEQ ID NO:40具有至少99%、至少95%、至少90%、至少80%、至少70%或至少50%相同性的序列。在另一优选实施方案中,本发明提供一种核酸序列,包含与核苷酸序列SEQ ID NO:40实质上相似或相同的核苷酸序列。

[0026] 本发明还涉及上述核酸序列的互补序列或其变体,其可包含编码本发明光学探针或融合多肽的片段、类似物、衍生物、可溶性片段和变体的核酸序列或其互补序列。

[0027] 本发明还提供包含本文所述核酸序列或其互补序列的核酸构建物,该核酸序列编码本发明所述光学探针或融合多肽。在一个或多个实施方案中,所述核酸构建物是克隆载体、表达载体或重组载体。在一个或多个实施方案中,所述核酸序列与表达控制序列操作性连接。在一些实施方案中,表达载体选自原核表达载体、真核表达载体和病毒载体。

[0028] 本发明还提供包含本发明所述核酸序列或核酸构建物的细胞。在一个或多个实施方案中,所述细胞表达本文所述光学探针或融合多肽。

[0029] 本发明还提供包括本文所述色氨酸光学探针或融合多肽或如本文所述方法制备的色氨酸光学探针或融合多肽的检测试剂盒。

[0030] 本发明提供制备本文所述光学探针的方法,包括:提供表达本文所述光学探针或融合多肽的细胞,在所述细胞表达的条件下培养所述细胞,和分离光学探针或融合多肽。在一个实施方案中,制备本文所述色氨酸光学探针或融合多肽的方法包括:1)将编码本文所述色氨酸光学探针的表达载体转移到宿主细胞中;2)在适合所述表达载体表达的条件下培养所述宿主细胞,3)分离色氨酸光学探针。

[0031] 本发明还提供检测样品中色氨酸的方法,包括:使本文所述光学探针或融合多肽或如本文所述方法制备的光学探针或融合多肽与样品接触,和检测光学活性多肽的变化。所述检测可以在体内、体外、亚细胞或原位进行。所述样品例如血液。

[0032] 本文还提供定量样品中色氨酸的方法,包括:使本文所述光学探针或融合多肽或如本文所述方法制备的光学探针或融合多肽与样品接触,检测光学活性多肽的变化,和根据光学活性多肽的变化定量样品中的色氨酸。

[0033] 本发明还提供筛选化合物(例如药物)的方法,包括:使本文所述光学探针或融合多肽或如本文所述方法制备的光学探针或融合多肽与候选化合物接触,检测光学活性多肽的变化,和根据光学活性多肽的变化筛选化合物。所述方法可以高通量地筛选化合物。

[0034] 本发明还提供本文所述色氨酸光学探针或融合多肽或如本文所述方法制备的色氨酸光学探针或融合多肽在色氨酸细胞内/外定位中的应用。在一个或多个实施方案中,所述定位是实时定位。

[0035] 本发明的有益效果:本发明提供的色氨酸光学探针易于成熟,荧光动态变化大,特异性好,并且能够通过基因操作的方法在细胞中表达,可在细胞内外实时定位、高通量、定

量检测色氨酸,省去了耗时的处理样品步骤。实验效果表明本申请所提供的色氨酸光学探针对色氨酸的最高响应达到对照的9倍以上,并且可以在细胞浆、线粒体、细胞核、内质网、溶酶体和高尔基体等亚细胞结构中对细胞进行定位、定性、定量检测,并且可以进行高通量的化合物筛选以及血液中色氨酸定量检测。

附图说明

[0036] 下面结合附图和实施例对本发明作进一步说明。

[0037] 图1为实施例1所述的示例性色氨酸光学探针的SDS-PAGE图;

[0038] 图2为实施例2所述的示例性的包含cpYFP和色氨酸结合蛋白的色氨酸光学探针对于色氨酸响应变化图;

[0039] 图3为实施例3所述的示例性的包含cpGFP和色氨酸结合蛋白的色氨酸光学探针对于色氨酸响应变化图;

[0040] 图4为实施例4所述的示例性的包含cpBFP和色氨酸结合蛋白的色氨酸光学探针对于色氨酸响应变化图;

[0041] 图5为实施例5所述的示例性的包含cpmApple和色氨酸结合蛋白的色氨酸光学探针对于色氨酸响应变化图;

[0042] 图6为实施例6所述的示例性的色氨酸光学探针对于不同浓度色氨酸的滴定曲线;

[0043] 图7为实施例6所述的示例性色氨酸光学探针对于20种氨基酸的特异性检测的柱状图;

[0044] 图8为实施例7所述的示例性的具有突变的色氨酸光学探针对于色氨酸响应的柱状图;

[0045] 图9A-C为实施例8所述的示例性色氨酸光学探针的荧光光谱性质图;

[0046] 图10为实施例8所述的示例性色氨酸光学探针对于不同浓度色氨酸的滴定曲线;

[0047] 图11为实施例8所述的示例性色氨酸光学探针对于20种氨基酸的特异性检测的柱状图;

[0048] 图12为实施例9所述的示例性色氨酸光学探针在哺乳动物细胞中的亚细胞器定位照片;

[0049] 图13为实施例10所述的对示例性色氨酸光学探针在哺乳动物细胞中对胞浆中的色氨酸浓度进行动态监测的示意图;

[0050] 图14为实施例11所述的示例性色氨酸光学探针在活细胞水平进行高通量化合物筛选的点图;

[0051] 图15为实施例12所述的示例性色氨酸光学探针对于小鼠和人血液中的色氨酸进行定量的柱状图。

具体实施方案

[0052] 在给出数值或范围时,本文所用术语“约”指该数值或范围在给定数值或范围的20%以内、10%以内和5%以内。

[0053] 本文所用术语“色氨酸敏感多肽”或“色氨酸响应多肽”指对色氨酸产生响应的多肽,所述响应包括与敏感多肽的相互作用相关的多肽的化学,生物学,电学或生理学参数的

任何响应。响应包括小的变化,例如,多肽的氨基酸或肽片段的方向的变化以及例如多肽的一级,二级或三级结构的变化,包括例如质子化,电势和/或构象的变化。“构象”是分子中包含侧基的分子的一级,二级和三级结构的三维排列;当分子的三维结构发生变化时,构象发生变化。构象变化的实例包括从 α -螺旋转变为 β -折叠或从 β -折叠转变为 α -螺旋。可以理解的是,只要荧光蛋白部分的荧光被改变,可检测到的改变不需要是构象改变。本文所述色氨酸敏感多肽还可包括其功能变体。色氨酸敏感多肽的功能变体包括但不限于可以与色氨酸相互作用从而发生与亲本色氨酸敏感多肽相同或相似变化的变体。

[0054] 本发明所述色氨酸敏感多肽包括但不限于色氨酸结合蛋白3lft或与其有90%以上同源性的变体。本发明所述示例性色氨酸结合蛋白3lft来源于肺炎链球菌 *Streptococcus pneumoniae*。3lft是ABC转运蛋白家族,由两个结构域组成,两个结构域之间由三个柔性氨基酸肽链进行连接。色氨酸结合蛋白可以感应色氨酸浓度的变化,在色氨酸浓度动态变化的过程中色氨酸结合蛋白的空间构象也会发生改变。示例性3lft蛋白如SEQ ID NO:1所示。在一个或多个实施方案中,色氨酸敏感多肽包含蛋白的色氨酸结合域,而不包括DNA结合域。当描述本发明光学探针或色氨酸结合蛋白时(例如描述插入位点或突变位点时),提及氨基酸残基编号均参考SEQ ID NO:1。

[0055] 本文所用术语“光学探针”是指与光学活性多肽融合的色氨酸敏感多肽。发明人发现,色氨酸敏感多肽例如色氨酸结合蛋白专一性地对生理浓度的色氨酸结合后所产生的构象变化会引起光学活性多肽(例如荧光蛋白)的构象变化,进而导致光学活性多肽的光学性质发生改变。借助不同色氨酸浓度下测定的荧光蛋白的荧光绘制标准曲线,可以检测并分析色氨酸的存在和/或水平。

[0056] 在本发明的光学探针中,光学活性多肽(例如荧光蛋白)可操作地插入色氨酸敏感多肽中。基于蛋白质的“光学活性多肽”是具有发射荧光能力的多肽。荧光是光学活性多肽的一种光学性质,其可用作检测本发明的光学探针的响应性的手段。优选地,选择蛋白质底物以具有在未激活和活化的构象状态下容易区分的荧光特性。本文所述光学活性多肽还可包括其功能变体。光学活性多肽的功能变体包括但不限于可以发生与亲本光学活性多肽相同或相似荧光性质变化的变体。

[0057] 本文所用术语“荧光蛋白”指在激发光照射下发出荧光的蛋白质。荧光蛋白作为生物学领域的基础检测手段,例如生物技术领域常用的绿色荧光蛋白GFP及由该蛋白突变衍生出的环状重排的蓝色荧光蛋白(cpBFP)、环状重排的绿色荧光蛋白(cpGFP)、环状重排的黄色荧光蛋白(cpYFP)等;还有本技术领域常用的红色荧光蛋白RFP,及由该蛋白衍生出来的环状重排的蛋白,如cpmApple, cpmOrange, cpmKate等。本领域知晓可用于本发明的荧光蛋白及其序列。示例性地, cpYFP如SEQ ID NO:2所示; cpGFP如SEQ ID NO:3所示; cpBFP如SEQ ID NO:4所示; cpmApple如SEQ ID NO:5所示。

[0058] “接头”或“连接区”指在本发明多肽、蛋白质或核酸中连接两个部分的氨基酸或核苷酸序列。示例性地,本发明中色氨酸敏感多肽与光学活性多肽的连接区氨基端的氨基酸数目选择的是0-3个,羧基端的氨基酸数目选择的是0-2个;当重组光学探针作为基本单元与功能蛋白连接时,可以融合在重组光学探针的氨基酸或羧基端。接头序列可为一个或多个柔性氨基酸组成的短肽链,如Y。

[0059] 本发明所述的色氨酸光学探针包括色氨酸敏感多肽(B),例如色氨酸结合蛋白或

其变体,和光学活性多肽(A),例如荧光蛋白。光学活性多肽(A)插入到色氨酸敏感多肽(B)中,将B分为B1和B2两个部分,形成B1-A-B2式的探针结构;色氨酸敏感多肽B和色氨酸相互作用导致光学活性多肽(A)的光学信号变强。

[0060] 在本发明的光学探针中,光学活性多肽可以位于色氨酸敏感多肽的任何位置。在一个实施方案中,光学活性多肽以N-C方向位于N-C方向的色氨酸敏感多肽的任何位置。具体地,光学活性多肽位于色氨酸敏感多肽的柔性区域,所述的柔性区域是指蛋白质高级结构中存在的一些特定的如环状结构域等结构,这些结构域相比于蛋白质的其他高级结构具有更高的移动性和柔性,并且该区域可以在该蛋白质和配体结合后,空间结构构象发生动态变化。本发明中所述的柔性区域主要指色氨酸结合蛋白中的插入位点所在区域,如氨基酸残基114-118、233-235和/或263-268区域。示例性地,光学活性多肽位于色氨酸敏感多肽的选自以下的一个或多个位点:114/115,114/116,114/117,114/118,115/116,115/117,115/118,116/117,116/118,117/118,233/234,233/235,234/235,263/264,263/265,263/266,263/267,263/268,264/265,264/266,264/267,264/268,265/266,265/267,265/268,266/267,266/268和/或267/268。优选地,光学活性多肽位于色氨酸敏感多肽的选自以下的一个或多个位点:263/265,263/266,263/267和263/268。本文中,如果以“X/Y”形式表示的位点中的两个数字是连续的整数,则表示光学活性多肽位于该数字所述的氨基酸之间。例如插入位点114/115表示光学活性多肽位于色氨酸敏感多肽的氨基酸114与115之间。如果以“X/Y”形式表示的位点中的两个数字不是连续的整数,则表示光学活性多肽置换该数字所示氨基酸之间的氨基酸。例如插入位点114/116表示光学活性多肽置换色氨酸敏感多肽的氨基酸115。

[0061] 提到某多肽或蛋白时,本发明所用术语“变体”或“突变体”包括具有所述多肽或蛋白相同功能、但序列不同的变体。多肽或蛋白的变体可包括:同源序列、保守性变体、等位变体、天然突变体、诱导突变体。这些变体包括但并不限于:在所述多肽或蛋白的序列中缺失、插入和/或取代一个或多个(通常为1-30个,较佳地1-20个,更佳地1-10个,最佳地1-5个)氨基酸,以及在其羧基末端和/或氨基末端添加一个或数个(通常为20个以内,较佳地为10个以内,更佳地为5个以内)氨基酸获得的序列。这些变体还可包含与所述多肽或蛋白的序列相同性为至少约70%、至少约75%、至少约80%、至少约85%、至少约90%、至少约95%、至少约98%、至少约99%或100%的多肽或蛋白。不希望受理论限制,氨基酸残基发生改变而不改变多肽或蛋白质的总体构型和功能,即功能保守突变。例如,在本领域中,用性能相近或相似的氨基酸进行取代时,通常不会改变多肽或蛋白的功能。在本领域中,性能相似的氨基酸往往指具有相似侧链的氨基酸家族,在本领域已有明确定义。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的氨基酸(例如甘氨酸、天冬酰胺、谷氨酰胺、丝氨酸、苏氨酸、酪氨酸、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、甲硫氨酸、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)和具有芳香侧链的氨基酸(例如酪氨酸、苯丙氨酸、色氨酸、组氨酸)。又比如,在氨基末端和/或羧基末端添加一个或数个氨基酸通常也不会改变多肽或蛋白的功能。对于许多常见已知非遗传性编码氨基酸的保守氨基酸取代本领域已知。其他非编码氨基酸的保守取代可基于其物理性质与遗传上编码的氨基酸的性质的比较来确定。

[0062] 在两种或多种多肽或核酸分子序列中,术语“相同性”或“相同性百分数”指在比较窗口或指定区域上,采用本领域已知方法如序列比较算法,通过手工比对和目测检查来比较和比对最大对应性时,两个或多个序列或子序列相同或其中在指定区域有一定百分数的氨基酸残基或核苷酸相同(例如,至少60%、至少65%、至少70%、至少75%、至少80%、至少85%、至少90%、至少91%、至少92%、至少93%、至少94%、至少95%、至少96%、至少97%、至少98%、至少99%或100%相同)。例如,适合测定序列相同性百分数和序列相似性百分数的优选算法是BLAST和BLAST 2.0算法,分别可参见Altschul等(1977) *Nucleic Acids Res.* 25:3389和Altschul等(1990) *J. Mol. Biol.* 215:403。

[0063] 本领域技术人员公知,在基因克隆操作中,常常需要设计合适的酶切位点,这势必在所表达的多肽或蛋白末端引入了一个或多个不相干的残基,而这并不影响目的多肽或蛋白的活性。又如为了构建融合蛋白、促进重组蛋白的表达、获得自动分泌到宿主细胞外的重组蛋白、或利于重组蛋白的纯化,常常需要将一些氨基酸添加至重组蛋白的N-末端、C-末端或该蛋白内的其它合适区域内,例如,包括但不限于,适合的接头肽、信号肽、前导肽、末端延伸、谷胱甘肽S-转移酶(GST)、麦芽糖E结合蛋白、蛋白A、如6His或Flag的标签,或Xa因子或凝血酶或胰凝乳蛋白酶的蛋白水解酶位点。

[0064] 本发明光学探针可包含具有突变的色氨酸敏感多肽。所述突变例如H13,T71,L84,D93,K105,N117,T130,V170,V192,F202,K214,Y217,K255,T259和/或I283等位点的突变。示例性地,所述突变选自H13F,H13W,T71F,T71W,L84F,L84W,D93F,D93W,K105F,K105W,N117F,N117W,T130F,T130W,V170F,V170W,V192F,V192W,F202W,K214F,K214W,Y217F,Y217W,K255F,K255W,T259F,T259W,I283F和/或I283W中的一个或多个。在一个或多个实施方案中,所述突变选自H13F,L84F,L84W,D93F,D93W,K105F,K105W,N117F,N117W,V170F,V170W,V192F,F202W,K214F,K214W,Y217F,Y217W,K255F,K255W,T259F,T259W,I283F和I283W中的一个或多个。在优选实施方案中,所述突变选自L84F,K105F,K105W,N117F,V170F,F202W,K214F,K214W,Y217F,Y217W,K255F,K255W,T259F,T259W,I283F和/或I283W中的一个或多个。

[0065] 在示例性实施方案中,本发明B1-A-B2式光学探针可为31ft的选自114/115,114/116,114/117,114/118,115/116,115/117,115/118,116/117,116/118,117/118,233/234,233/235,234/235,263/264,263/265,263/266,263/267,263/268,264/265,264/266,264/267,264/268,265/266,265/267,265/268,266/267,266/268或267/268的一个或多个位点插入有cpYFP并且具有选自以下的一个或多个突变的探针:H13F,L84F,L84W,D93F,D93W,K105F,K105W,N117F,N117W,V170F,V170W,V192F,F202W,K214F,K214W,Y217F,Y217W,K255F,K255W,T259F,T259W,I283F和I283W。优选地,光学探针是31ft的选自263/265,263/266或263/267的一个或多个位点插入有cpYFP并且具有选自以下的一个或多个突变的探针:L84F,K105F,K105W,N117F,V170F,F202W,K214F,K214W,Y217F,Y217W,K255F,K255W,T259F,T259W,I283F,I283W,和/或I283W。示例性的序列如SEQ ID NO:10-39所示。

[0066] 本发明提供的光学探针包含氨基酸序列SEQ ID NO:6-39中任一或其变体。在一个实施方案中,本发明提供的光学探针包含与氨基酸序列SEQ ID NO:6-39中任一有至少35%、至少40%、至少50%、至少60%、至少70%、至少80%、至少85%、至少90%、至少95%、至少99%序列相同性的序列。在优选实施方案中,本发明提供的光学探针包含与氨基酸序

列SEQ ID NO:6-39中任一实质上相似或相同的序列。在更优选的实施方案中,本发明提供的光学探针包含SEQ ID NO:10-39中任一或由其组成。

[0067] 本文所用术语“功能片段”、“功能变体”、“衍生物”和“类似物”是指基本上保持与原始多肽或蛋白(例如31ft蛋白或荧光蛋白)相同的生物学功能或活性的蛋白。本发明的多肽或蛋白(例如31ft蛋白或荧光蛋白)的功能变体、衍生物或类似物可以是(i)有一个或多个保守或非保守性氨基酸残基(优选保守性氨基酸残基)被取代的蛋白,而这样的取代的氨基酸残基可以是也可以不是由遗传密码编码的,或(ii)在一个或多个氨基酸残基中具有取代基团的蛋白,或(iii)成熟蛋白与另一个化合物(比如延长蛋白半衰期的化合物,例如聚乙二醇)融合所形成的蛋白,或(iv)附加的氨基酸序列融合到此蛋白序列而形成的蛋白(如分泌序列或用来纯化此蛋白的序列或蛋白原序列,或与抗原IgG片段的形成的融合多肽)。根据本文的教导,这些功能变体、衍生物和类似物属于本领域熟练技术人员公知的范围。

[0068] 所述类似物与原始多肽或蛋白的差别可以是氨基酸序列上的差异,也可以是不影响序列的修饰形式上的差异,或者兼而有之。这些蛋白包括天然或诱导的遗传变体。诱导变体可以通过各种技术得到,如通过辐射或暴露于诱变剂而产生随机诱变,还可通过定点诱变法或其他已知分子生物学的技术得到。

[0069] 本发明融合多肽包含本文所述光学探针和其它多肽。在一些实施方案中,本文所述光学探针还包含与之融合的其它多肽。本文所述其他多肽不影响光学探针的性质。其他多肽可位于所述光学探针的N端和/或C端。在一些实施方案中,其他多肽包括将光学探针定位到不同细胞器或亚细胞器的多肽、用于纯化的标签或者用于免疫印迹的标签。本文所述融合多肽中的光学探针和其它多肽之间可具有接头。

[0070] 本文所述亚细胞器包括细胞浆、线粒体、细胞核、内质网、细胞膜、高尔基体、溶酶体和过氧化物酶体等。在一些实施方案中,用于纯化的标签或者用于免疫印迹的标签包括6组氨酸(6*His)、谷胱甘肽硫转移酶(GST)、Flag。

[0071] 本发明表达载体包含与表达控制序列操作性连接的本发明所述的核酸序列或其互补序列,该核酸序列编码本发明所述光学探针或融合多肽。在一些实施方案中,表达载体选自原核表达载体、真核表达载体和病毒载体。在一些实施方案中,原核表达载体优选由质粒pCDF与本文所述的核酸序列操作性连接得到。在一些实施方案中,表达控制序列包括复制起点、启动子、增强子、操纵子、终止子、核糖体结合位点。

[0072] 本发明还提供了上述色氨酸光学探针的制备方法,包括以下步骤:1)将编码本文所述色氨酸光学探针的核酸序列纳入表达载体;2)将表达载体转移到宿主细胞中;2)在适合所述表达载体表达的条件下培养所述宿主细胞,3)分离色氨酸光学探针。

[0073] 本发明所用术语“核酸”或“核苷酸”或“多核苷酸”或“核酸序列”可以是DNA形式或RNA形式。DNA形式包括cDNA、基因组DNA或人工合成的DNA。DNA可以是单链的或是双链的。DNA可以是编码链或非编码链。提到核酸时,本文所用术语“变体”可以是天然发生的等位变体或非天然发生的变体。这些核苷酸变体包括简并变体、取代变体、缺失变体和插入变体。本发明核酸可包含与所述核酸序列的序列相同性为至少约50%、至少约60%、至少约70%、至少约75%、至少约80%、至少约85%、至少约90%、至少约95%、至少约98%、至少约99%或100%的核苷酸序列。本发明还涉及与上述的序列杂交的核酸片段。在示例性实施方式中,核酸序列如SEQ ID NO:40所示,其表示色氨酸结合蛋白功能片段的263/266位点插入有

cpYFP并具有T259F突变的探针的编码序列。如本文所用,“核酸片段”的长度至少含15个核苷酸,较好是至少30个核苷酸,更好是至少50个核苷酸,最好是至少100个核苷酸以上。核酸片段可用于核酸的扩增技术(如PCR)。

[0074] 本发明光学探针或融合多肽的全长序列或其片段通常可以用PCR扩增法、人工合成法或重组法获得。对于PCR扩增法,可根据本发明所公开的核苷酸序列设计引物,并用市售的cDNA库或按本领域技术人员已知的常规方法所制备的cDNA库作为模板,扩增而得有关序列。当核苷酸序列大于2500bp时,优选的进行2~6次PCR扩增,然后将各次扩增的片段按正确次序拼接在一起。本发明对所述的PCR扩增的程序和体系没有特殊限定,采用本领域常规的PCR扩增程序和体系即可。还可以用重组法来大批量地获得有关序列。这通常是将其克隆入载体,再转入细胞,然后通过常规方法从增殖后的宿主细胞中分离和纯化得到有关多肽或蛋白。此外,还可用人工合成的方法来合成有关序列,尤其是片段长度较短时。在本发明中,在光学探针的核苷酸序列小于2500bp时,可采用人工合成方法来合成。所述人工合成方法为本领域常规的DNA的人工合成方法,无其他特殊要求。通常,通过先合成多个小片段,然后再进行连接可获得序列很长的片段。目前,已经可以完全通过化学合成来得到编码本发明蛋白(或其功能变体、衍生物或类似物)的DNA序列。然后可将该DNA序列引入本领域中已知的各种现有的DNA分子(如载体)和细胞中。可通过突变PCR或化学合成等方法将突变引入本发明蛋白序列中。

[0075] 本发明还提供包括本文所述光学探针或融合多肽或多核苷酸或如本文所述方法制备的光学探针或融合多肽的检测试剂盒。该试剂盒还任选包含利用光学探针检测色氨酸所需的其它试剂。本领域知晓常规的所述其它试剂。

[0076] 本发明也涉及核酸构建物,该核酸构建物含有本文所述的多核苷酸,以及与这些序列操作性连接的一个或多个调控序列。本发明所述的多核苷酸可以多种方式被操作以保证所述多肽或蛋白的表达。在将核酸构建物插入载体之前可根据表达载体的不同或要求而对核酸构建物进行操作。利用重组DNA方法来改变多核苷酸序列的技术是本领域已知的。

[0077] 在某些实施方案中,所述核酸构建物是载体。载体可以是克隆载体,也可以是表达载体,或者是基因敲入载体,例如同源重组载体。本发明的多核苷酸可被克隆入许多类型的载体,例如,质粒、噬菌粒、噬菌体衍生物、动物病毒和粘粒。克隆载体可用于提供本发明蛋白或多肽的编码序列。表达载体可以以细菌载体或病毒载体形式提供给细胞。通常通过可操作地连接本发明的多核苷酸至启动子,并将构建体并入表达载体,实现本发明多核苷酸的表达。该载体对于复制和整合真核细胞可为合适的。典型的表达载体包含可用于调节期望核酸序列表达的表达控制序列。基因敲入载体用于将本文所述的表达框敲入或整合到宿主基因组中。

[0078] 本文所用术语“表达控制序列”指调控目的基因的转录、翻译和表达的可以与目的基因操作性连接的元件,可以是复制起点、启动子、标记基因或翻译控制元件,包括增强子、操纵子、终止子、核糖体结合位点等,表达控制序列的选择取决于所用的宿主细胞。在重组表达载体中,“操作性连接”是指目的的核苷酸序列与调节序列以允许核苷酸序列表达的方式连接。本领域的技术人员熟知能用于构建含本发明融合多肽的编码序列和合适的转录/翻译控制信号的表达载体的方法。这些方法包括体外重组DNA技术、DNA合成技术、体内重组技术等。所述的DNA序列可有效连接到表达载体中的适当启动子上,以指导mRNA合成。这些

启动子的代表性例子有：大肠杆菌的lac或trp启动子；λ噬菌体PL启动子；真核启动子包括CMV立即早期启动子、HSV胸苷激酶启动子、早期和晚期SV40启动子、反转录病毒的LTR和其他一些已知的可控制基因在原核或真核细胞或其病毒中表达的启动子。表达载体还包括翻译起始用的核糖体结合位点和转录终止子。在一个实施方案中，表达载体可采用市售的pCDF载体，无其他特殊要求。示例性地，采用HindIII和XhoI分别对编码所述光学探针的核苷酸序列和表达载体进行双酶切，然后将二者的酶切产物连接得到重组表达载体。本发明对酶切和连接的具体步骤和参数没有特殊限定，采用本领域常规的步骤和参数即可。

[0079] 在获得重组表达载体后，将该载体转化到宿主细胞中，以产生包括融合蛋白的蛋白或肽。此种转移过程可用转化或转染等本领域技术人员熟知的常规技术进行。本发明所述的宿主细胞是指能够接收和容纳重组DNA分子的细胞，是重组基因扩增的场所，理想的受体细胞应该满足易于获取和增殖两个条件。本发明的“宿主细胞”可包括原核细胞和真核细胞，具体包括细菌细胞、酵母细胞、昆虫细胞和哺乳动物细胞。所述宿主细胞优选各种利于基因产物表达或发酵生产的细胞，此类细胞已为本领域熟知并常用。本领域一般技术人员都清楚如何选择适当的载体、启动子、增强子和宿主细胞。

[0080] 本发明所述的转移到宿主细胞的方法为本领域常规的方法，包括磷酸钙或氯化钙共沉淀、DEAE-甘露聚糖-介导的转染、脂转染、天然感受态、化学介导的转移或电穿孔。当宿主为原核生物如大肠杆菌时，所述方法优选的为CaCl₂法或MgCl₂法处理，所用的步骤为本领域公知。当宿主细胞是真核细胞时，可选用如下的DNA转染方法：磷酸钙共沉淀法，常规机械方法如显微注射、电穿孔、脂质体包装等。

[0081] 本发明在将表达载体转入宿主细胞后，对转入表达载体的宿主细胞进行扩增表达培养，分离得到色氨酸光学探针。所述宿主细胞扩增表达培养采用常规的方法即可。根据所用的宿主细胞种类，培养中所用的培养基可以是各种常规培养基。在适于宿主细胞生长的条件下进行培养。

[0082] 在本发明中，光学探针在细胞内、细胞膜上表达、或分泌到细胞外。如果需要，可利用其物理的、化学的和其它特性通过各种分离方法分离或纯化重组的蛋白。本发明对分离所述色氨酸荧光蛋白的方法没有特殊限定，采用本领域常规的融合蛋白的分离方法即可。这些方法是本领域技术人员所熟知的，包括但并不限于：常规的复性处理、盐析方法、离心、渗透破菌、超声处理、超离心、分子筛层析、吸附层析、离子交换层析、高效液相层析(HPLC)和其它各种液相层析技术及这些方法的结合。在一个实施方案中，利用His标签的亲亲和层析法进行光学探针的分离。

[0083] 本发明还提供了所述色氨酸光学探针在色氨酸实时定位、定量检测以及高通量化合物筛选中的应用。在一个方面，所述的色氨酸光学探针优选与细胞不同部位的信号肽连接，转入到细胞中，通过检测细胞中荧光信号的强弱，进行色氨酸的实时定位；通过色氨酸标准滴加曲线进行相应色氨酸的定量检测。本发明所述的色氨酸标准滴加曲线是根据色氨酸光学探针在不同浓度色氨酸的情况下的荧光信号绘制而成。本发明所述色氨酸光学探针直接转入细胞中，在色氨酸实时定位和定量检测过程中，不需要耗时的样品处理过程，更加准确。本发明色氨酸光学探针在进行高通量化合物筛选时，将不同的化合物添加到细胞培养液中，测定色氨酸含量的变化，从而筛选出对色氨酸含量变化有影响的化合物。在本发明中所述的色氨酸光学探针在色氨酸实时定位、定量检测以及高通量化合物筛选中的应用，

均是非诊断和治疗目的,不涉及疾病的诊断和治疗。

[0084] 在本文中,浓度、含量、百分数和其它数值均可用范围的形式表示。也应理解,使用这种范围形式只是为了方便和简洁,应该被弹性地解读为包括范围上下限所明确提及的数值,还应包括该范围内包括的所有单个数值或子范围。

[0085] 实施例

[0086] 下面结合实施例对本发明提供的色氨酸光学探针进行详细的说明,但是不能把它们理解为对本发明保护范围的限定。

[0087] I. 实验材料和试剂

[0088] 实施例中主要采用常规的基因工程分子生物学克隆方法和细胞培养以及成像方法等,这些方法是本领域普通技术人员所熟知的,例如:简·罗斯凯姆斯等的《分子生物学实验参考手册》,J. 萨姆布鲁克,D.W. 拉塞尔著,黄培堂等译:《分子克隆实验指南》(第三版,2002年8月,科学出版社出版,北京);费雷谢尼等的《动物细胞培养:基本技术指南》(第五版),章静波,徐存拴等译;J.S. 博尼费斯农,M. 达索等的《精编细胞生物学实验指南》,章静波等译。

[0089] 实施例中所用的基于pCDF-cpYFP,pCDF-色氨酸结合蛋白质粒由华东理工大学蛋白质实验室构建,pCDF质粒载体购自Invitrogen公司。所有用于PCR的引物均由上海捷瑞生物工程有限公司合成、纯化和经质谱法鉴定正确。实施例中构建的表达质粒都经过序列测定,序列测定由华大基因公司和杰李测序公司完成。各实施例所用的Taq DNA聚合酶购自东盛生物,pfu DNA聚合酶购自天根生化科技(北京)有限公司,primeSTAR DNA聚合酶购自TaKaRa公司,三种聚合酶购买时都附带赠送对应聚合酶缓冲液和dNTP。BamHI、BglII、HindIII、NdeI、XhoI、EcoRI、SpeI等限制性内切酶、T4连接酶、T4磷酸化酶(T4 PNK)购自Fermentas公司,购买时附带有相对应的缓冲液等。转染试剂Lip2000 Kit购于Invitrogen公司。色氨酸等氨基酸均购自Sigma公司。除非特别声明,无机盐类等化学试剂均购自Sigma-Aldrich公司。HEPES盐,氨苄青霉素(Amp)和嘌呤霉素购自Ameresco公司。96孔检测黑板、384孔荧光检测黑板购自Grenier公司。

[0090] 实施例中所用的DNA纯化试剂盒购自BBI公司(加拿大),普通质粒小抽试剂盒购自天根生化科技(北京)有限公司。克隆菌株Mach1购自Invitrogen公司。镍柱亲和层析柱和脱盐柱填料均来自GE healthcare公司。

[0091] 实施例中用到的主要仪器包括:Biotek Synergy 2多功能酶标仪(美国Bio-Tek公司),X-15R高速冷冻离心机(美国Beckman公司),Microfuge22R台式高速冷冻离心机(美国Beckman公司),PCR扩增仪(德国Biometra公司),超声破碎仪(宁波新芝公司),核酸电泳仪(申能博彩公司),荧光分光光度计(美国Varian公司),CO₂恒温细胞培养箱(SANYO),倒置荧光显微镜(日本尼康公司)。

[0092] II. 分子生物学方法和细胞实验方法

[0093] II.1 聚合酶链式反应(PCR):

[0094] 1. 目的片段扩增PCR:

[0095] 该方法主要用于基因片段扩增和菌落PCR鉴定阳性克隆。所述PCR扩增的反应体系如下:模板序列0.5-1μL,正向引物(25μM)0.5μL,反向引物(25μM)0.5μL,10×pfu缓冲液5μL,pfu DNA聚合酶0.5μL,dNTP(10mM)1μL,灭菌超纯水(ddH₂O)41.5-42μL,总体积50μL。PCR

扩增程序如下:95℃变性2-10分钟,30轮循环(94-96℃持续30-45秒,50-65℃持续30-45秒,72℃持续一定时间(600bp/min)),72℃延伸10分钟。

[0096] 2.长片段(>2500bp)扩增PCR:

[0097] 本发明中使用的长片段扩增,主要是反向PCR扩增载体,在下述实施例中用于获得定点突变的一种技术。在变异部位设计反向PCR引物,其中一条引物的5'端包含变异的核苷酸序列。扩增后的产物就含有相应的突变位点。长片段扩增PCR反应体系如下:模板序列(10pg-1ng) 1μL,正向引物(25μM) 0.5μL,反向引物(25μM) 0.5μL,5×PrimerSTAR缓冲液10μL,PrimerSTAR DNA聚合酶0.5μL,dNTP(2.5mM) 4μL,灭菌超纯水(ddH₂O) 33.5μL,总体积50μL。PCR扩增程序如下:95℃变性5分钟,30轮循环(98℃持续10秒,50-68℃持续5-15秒,72℃持续一定时间(1000bp/min)),72℃延伸10分钟;或者95℃变性5分钟,30轮循环(98℃持续10秒,68℃持续一定时间(1000bp/min)),72℃延伸10分钟。

[0098] II.2核酸内切酶酶切反应:

[0099] 对质粒载体进行双酶切的体系如下:质粒载体20μL(约1.5μg),10×缓冲液5μL,限制性内切酶11-2μL,限制性内切酶21-2μL,用灭菌超纯水补至总体积50μL。反应条件37℃,1-7小时。

[0100] II.3DNA片段5'端磷酸化反应

[0101] 从微生物中抽提出的质粒或者基因组末端都含有磷酸基团,而PCR产物没有,故需对PCR产物的5'端碱基进行磷酸基团加成反应,只有末端含有磷酸基团DNA分子才能发生连接反应。磷酸化反应体系如下:PCR产物片段DNA序列5-8μL,10×T4连接酶缓冲液1μL,T4多聚核苷酸激酶(T4 PNK) 1μL,灭菌超纯水0-3μL,总体积10μL。反应条件37℃,30分钟-2小时后72℃灭活20分钟。

[0102] II.4目的片段和载体的连接反应

[0103] 不同的片段和载体之间的连接方法有所差异,本发明中使用了三种连接方法

[0104] 1.平末端短片段和线性化载体的平末端连接

[0105] 该方法的原理是PCR获得的平末端产物在T4 PNK作用下对DNA片段的5'末端进行磷酸化反应后,与线性化的载体在PEG4000和T4 DNA连接酶的作用下连接获得重组质粒。同源重组连接体系如下:T4 PNK处理的DNA片段4μL,线性化载体片段4μL,PEG4000 1μL,10×T4连接酶缓冲液1μL,T4 DNA连接酶1μL,总计10μL。反应条件22℃,30分钟。

[0106] 2.含有粘性末端的DNA片段和含有粘性末端载体片段的连接

[0107] 通过限制性内切酶切割的DNA片段通常会产生突出的粘性末端,因此可以和含有序列互补的粘性末端载体片段连接,形成重组质粒。连接反应体系如下:酶切后的PCR产物片段DNA 1-7μL,酶切后的质粒0.5-7μL,10×T4连接酶缓冲液1μL,T4 DNA连接酶1μL,灭菌超纯水补至总体积10μL。反应条件16℃,4-8小时。

[0108] 3.反向PCR引入定点突变后5'端磷酸化的DNA片段产物自身环化的连接反应

[0109] 将5'端磷酸化的DNA片段通过自身环化连接反应将线性化载体的3'端和5'端连接反应得到重组质粒。自身环化连接反应体系如下:磷酸化反应体系10μL,T4连接酶(5U/μL) 0.5μL,总体积10.5μL。反应条件16℃,4-16小时。

[0110] II.5感受态细胞的制备与转化

[0111] 感受态细胞的制备:

- [0112] 1.挑取单菌落(如Mach1)接种于5mL LB培养基中,37℃摇床过夜。
- [0113] 2.取0.5-1mL过夜培养的菌液转种到50mL LB培养基中,37℃,220rpm培养3至5小时,直到OD600达到0.5。
- [0114] 3.冰浴预冷细胞2小时。
- [0115] 4.4℃,4000rpm离心10分钟。
- [0116] 5.弃上清,用5mL预冷的缓冲液重悬细胞,待均匀后再加入重悬缓冲液至终体积为50mL。
- [0117] 6.冰浴45分钟。
- [0118] 7.4℃ 4000rpm离心10分钟,用5mL冰预冷的储存缓冲液重悬细菌。
- [0119] 8.每个EP管中放100μL菌液,-80℃或液氮冻存。
- [0120] 重悬缓冲液:CaCl₂ (100mM)、MgCl₂ (70mM)、NaAc (40mM)
- [0121] 储存缓冲液:0.5mL DMSO、1.9mL 80%甘油、1mL 10×CaCl₂ (1M)、1mL 10×MgCl₂ (700mM)、1mL 10×NaAc (400mM)、4.6mL ddH₂O
- [0122] 感受态细胞的转化:
- [0123] 1.取100μL感受态细胞于冰浴上融化。
- [0124] 2.加入适当体积的连接产物,轻轻吹打混匀,冰浴30分钟。通常加入的连接产物的体积少于感受态细胞体积的1/10。
- [0125] 3.将菌液放入42℃水浴中热激90秒,迅速转移至冰浴中放置5分钟。
- [0126] 4.加入500μL LB,于37℃恒温摇床上200rpm培养1小时。
- [0127] 5.将菌液4000rpm离心3分钟,留200μL上清将菌体吹匀,均匀涂布于含适当抗生素的琼脂平板表面,平板于37℃恒温培养箱内倒置过夜。
- [0128] II.6蛋白质的表达,纯化和荧光检测
- [0129] 1.将表达载体(例如以pCDF为基础的色氨酸光学探针表达载体)转化到BL21 (DE3)细胞中,倒置培养过夜,从平板上挑取克隆到250mL锥形瓶中,置于37℃摇床,220rpm培养至OD=0.4-0.8,加入1/1000 (v/v)的IPTG (1M),18℃诱导表达24-36小时。
- [0130] 2.诱导表达完成后,4000rpm,30分钟离心收菌,加入50mM的磷酸盐缓冲液重悬菌体沉淀,超声破碎至菌体澄清。9600rpm,4℃离心20分钟。
- [0131] 3.离心上清通过自装的镍柱亲和层析柱纯化获得蛋白,镍柱亲和层析后的蛋白再通过自装的脱盐柱获得溶解在100mM HEPES缓冲液(pH 7.4)中的蛋白。
- [0132] 4.纯化的蛋白经过SDS-PAGE鉴定后,使用测定缓冲液(100mM HEPES,100mM NaCl,pH 7.4)稀释探针成终浓度为0.2-5μM的蛋白溶液。用测定缓冲液(100mM HEPES,100mM NaCl,pH 7.4)将色氨酸配制成终浓度为50mM的储液。
- [0133] 5.取100μL 1μM的蛋白溶液,37℃温育10分钟,加入色氨酸滴定,测定蛋白的420nm光激发后528nm发射和485nm光激发后528nm发射的荧光强度。对样品的荧光激发、发射测定利用多功能荧光酶标仪完成。
- [0134] 6.取100μL 1μM的蛋白溶液,37℃温育10分钟,加入色氨酸,测定蛋白的吸收光谱和荧光光谱。对样品的吸收光谱和荧光光谱的测定通过分光光度计和荧光分光光度计完成。
- [0135] II.7哺乳动物细胞的转染和荧光检测

[0136] 1.将pCDNA3.1+为基础的色氨酸光学探针质粒通过转染试剂Lipofectamine2000 (Invitrogen) 转染到HeLa中,置于37℃,5%CO₂的细胞培养箱中培养。待外源基因充分表达24~36h后进行荧光检测。

[0137] 2.诱导表达完成后,将贴壁的HeLa细胞,用PBS冲洗三次,置于HBSS溶液中分别进行荧光显微镜和酶标仪检测。

[0138] 实施例1:色氨酸结合蛋白质粒

[0139] 通过PCR扩增肺炎链球菌基因中的3lft基因,PCR产物凝胶电泳后回收后用HindIII和XhoI酶切,同时对pCDF载体进行相应的双酶切。用T4 DNA连接酶连接后,用产物转化DH5α,转化的DH5α涂布于LB平板(链霉素100ug/mL),置于37℃培养过夜。将生长DH5α转化子进行质粒抽提后,进行PCR鉴定。阳性质粒经过测序正确后进行后续的质粒构建。

[0140] 实施例2:不同插入位点的cpYFP光学探针的表达和检测

[0141] 本实施例中,以pCDF-3lft为基础根据色氨酸结合蛋白晶体结构选择了下述位点插入cpYFP,得到相应pCDF-3lft-cpYFP质粒:114/115,114/116,114/117,114/118,115/116,115/117,115/118,116/117,116/118,117/118,233/234,233/235,234/235,263/264,263/265,263/266,263/267,263/268,264/265,264/266,264/267,264/268,265/266,265/267,265/268,266/267,266/268或267/268。示例性的序列如表1所示。

[0142] 表1,光学探针的序列

[0143]

序列	插入位点
SEQ ID NO:6	263/265
SEQ ID NO:7	263/266
SEQ ID NO:8	263/267
SEQ ID NO:9	263/268

[0144] 利用PCR产生cpYFP的DNA片段,同时通过引物5'端引入cpYFP末端同源序列,PCR扩增产生pCDF-色氨酸结合蛋白线性化载体,其5'和3'最末端分别带有和cpYFP两末端对应的完全一致的序列(15bp~20bp)。将线性化的pCDF-3lft和cpYFP片段在Hieff Clone Enzyme的作用下发生同源重组。产物转化DH5α,转化的DH5α涂布于LB平板(链霉素100ug/mL),置于37℃培养过夜。PCR鉴定的阳性克隆抽质粒后测序。由杰李测序公司完成测序。

[0145] 经过测序正确后,将重组质粒转化到BL21(DE3)中诱导表达,并纯化蛋白质,通过SDS-PAGE电泳大小在63Kda附近。该大小符合pCDF-3lft-cpYFP表达出的含His-tag纯化标签的3lft-cpYFP融合蛋白质的大小。结果如图1所示。

[0146] 用表达3lft-cpYFP融合蛋白质的大肠杆菌的破碎上清进行色氨酸响应筛选,将含有1mM色氨酸的融合荧光蛋白质的检测信号除以无色氨酸的融合荧光蛋白质的检测信号。结果如图2所示,检测结果显示对色氨酸响应超过1.5倍的光学探针有在263/265,263/266,263/267和263/268位点或者其家族蛋白的对应氨基酸位点实施插入的光学探针。

[0147] 实施例3:不同插入位点的cpGFP光学探针的表达和检测

[0148] 按照实施例2中的方法将cpYFP替换为cpGFP,构建色氨酸绿色荧光蛋白荧光探针。如图3所示,破碎上清检测结果显示对色氨酸响应超过1.5倍的光学探针有在263/265,263/266,263/267和263/268位点或者其家族蛋白的对应氨基酸位点实施插入的光学探针。

[0149] 实施例4:不同插入位点的cpBFP光学探针的表达和检测

[0150] 按照实施例2中的方法将cpYFP替换为cpBFP,构建色氨酸蓝色荧光蛋白荧光探针。如图4所示,破碎上清检测结果显示对色氨酸响应超过1.5倍的光学探针有在263/266,263/267和263/268位点或者其家族蛋白的对应氨基酸位点实施插入的光学探针。

[0151] 实施例5:不同插入位点的cpmApple光学探针的表达和检测

[0152] 按照实施例2中的方法将cpYFP替换为cpmApple,构建色氨酸红色荧光蛋白荧光探针。如图5所示,破碎上清检测结果显示对色氨酸响应超过1.5倍的光学探针有在263/264,263/265和263/266位点或者其家族蛋白的对应氨基酸位点实施插入的光学探针。

[0153] 实施例6:光学探针的性能

[0154] 对于实施例2中所得的对色氨酸响应超过1.5倍的光学探针,即在263/265,263/266,263/267和263/268位点实施插入的4种光学探针,进行蛋白纯化和浓度梯度的色氨酸检测,检测420nm激发528nm发射处荧光强度和485nm激发528nm发射处荧光强度比值的变化。插入位点为263/265,263/266,263/267和263/268的4种色氨酸光学探针的 K_d (结合常数)分别为1.6 μ M、7.2 μ M、48 μ M和152 μ M,变化幅度分别为4.2倍、5.3倍、3.3和3.0倍,结果如图6所示。

[0155] 对于插入位点为263/265、263/266、263/267和263/268的探针(即3lft-263/265-cpYFP、3lft-263/266-cpYFP、3lft-263/267-cpYFP和3lft-263/268-cpYFP),进行其对各氨基酸的特异性检测。

[0156] 结果表明插入位点为263/265、263/266的探针对苯丙氨酸和酪氨酸有相似程度的响应,263/265分别为1.37倍、1.25倍,263/266分别为1.34倍、1.26倍,对色氨酸、苯丙氨酸和酪氨酸之外的其他氨基酸均无响应,263/267和263/268对色氨酸之外的其他氨基酸均无响应,如图7所示。

[0157] 实施例7:突变的cpYFP光学探针的表达和检测

[0158] 在3lft-263/265-cpYFP、3lft-263/266-cpYFP、3lft-263/267-cpYFP的基础上构建光学探针突变体。通过PCR线性化质粒pCDF-3lft-263/265-cpYFP、pCDF-3lft-263/266-cpYFP、pCDF-3lft-263/267-cpYFP,引物中含有所要突变位点的碱基序列,对得到的PCR产物在PNK、T4 DNA连接酶和PEG4000的作用下加磷连接,得到H13,T71,L84,D93,K105,N117,T130,V170,V192,F202,K214,Y217,K255,T259,I283这15个位点的突变质粒,并由杰李测序公司完成测序。部分突变的光学探针的序列如表2所示。示例性的核酸序列如SEQ ID NO:40(263/266-T259F)所示。

[0159] 表2,突变的光学探针的序列

	序列	插入位点	突变位点
[0160]	SEQ ID NO:10	263/265	L84F
	SEQ ID NO:11	263/265	K105F
	SEQ ID NO:12	263/265	F202W
	SEQ ID NO:13	263/265	K214F
	SEQ ID NO:14	263/265	K214W
	SEQ ID NO:15	263/265	Y217F
	SEQ ID NO:16	263/265	Y217W
	SEQ ID NO:17	263/265	K255F
	SEQ ID NO:18	263/265	K255W
	SEQ ID NO:19	263/265	T259F
	SEQ ID NO:20	263/265	T259W
	SEQ ID NO:21	263/265	I283F
	SEQ ID NO:22	263/265	I283W
	SEQ ID NO:23	263/266	L84F
	SEQ ID NO:24	263/266	K105F
	SEQ ID NO:25	263/266	K105W
	SEQ ID NO:26	263/266	F202W
	SEQ ID NO:27	263/266	K214F
	SEQ ID NO:28	263/266	K214W
	SEQ ID NO:29	263/266	Y217F
	SEQ ID NO:30	263/266	K255W
	SEQ ID NO:31	263/266	T259F
	SEQ ID NO:32	263/266	T259W
	SEQ ID NO:33	263/266	I283F
[0161]	SEQ ID NO:34	263/267	L84F
	SEQ ID NO:35	263/267	K105F
	SEQ ID NO:36	263/267	K105W
	SEQ ID NO:37	263/267	F202W
	SEQ ID NO:38	263/267	Y217F
	SEQ ID NO:39	263/267	T259F

[0162] 结果如图8所示。纯化蛋白的荧光检测结果显示对色氨酸响应超过2.5倍的有L84F, F202W, K214F, K214W, Y217F, Y217W, K255F, K255W, T259F, T259W, I283F和I283W突变体。

[0163] 实施例8. 光学探针突变体的光谱性能、滴定曲线和特异性

[0164] 将纯化的色氨酸光学探针31ft-263/266-cpYFP-T259F分别进行0mM和1mM色氨酸处理10分钟后, 使用荧光分光光度计进行荧光谱的检测。

[0165] 对激发光谱的测定: 以370nm至510nm的激发范围和530nm的发射波长记录激发光谱, 每5nm读取一次。结果显示, 探针在约410和490nm处有两个激发峰, 如图9A所示。

[0166] 对发射光谱的测定: 固定激发波长分别为420nm和460nm, 记录470-600nm和490-

600nm的发射光谱,每5nm读取一次。结果显示,探针在添加1mM色氨酸后,在420nm激发下荧光强度略增为添加0mM色氨酸的1.1倍;在460nm激发下荧光强度降低为添加0mM色氨酸的0.26倍。如图9B和9C所示。

[0167] 对纯化的3lft-263/266-cpYFP-T259F等30个对色氨酸响应超过2.5倍的蛋白进行浓度梯度(0~5mM)的色氨酸检测。对纯化的探针处理10分钟后,检测420nm激发528nm发射处荧光强度和485nm激发528nm发射处荧光强度比值的变化。结果如图10所示,30个色氨酸光学探针的 K_d (结合常数)从1.1 μ M至133 μ M不等。

[0168] 将纯化的3lft-263/266-cpYFP-T259F等30个蛋白与20种氨基酸进行反应性检测,结果表明其具有很好的特异性,如图11所示。

[0169] 实施例9:光学探针的亚细胞器定位和在亚细胞器内的性能

[0170] 本实施例中,使用不同的定位信号肽与光学探针3lft-263/266-cpYFP-T259F融合,将光学探针定位到不同的细胞器中。

[0171] 用融合不同定位信号肽的光学探针质粒转染HeLa细胞36小时后,使用PBS冲洗,置于HBSS溶液中使用倒置荧光显微镜进行FITC通道下进行荧光检测。结果如图12所示。色氨酸光学探针通过与不同的特异定位信号肽融合能够定位到包括细胞浆、细胞外膜、细胞核、内质网、线粒体和核排阻等亚细胞器中。不同的亚细胞结构中都显示有荧光,并且荧光的分布和强度各不相同。

[0172] 实施例10:色氨酸跨膜运输的动态监测

[0173] 用胞浆表达的光学探针质粒转染HeLa细胞36小时后,使用PBS冲洗,置于HBSS溶液中,检测30min时间段内420nm激发528nm发射处荧光强度和485nm激发528nm发射处荧光强度比值的变化。结果如图13所示。添加1mM色氨酸,继续检测30分钟。添加色氨酸的样品的485/420逐渐增加,最高可以达到初始值的1.3倍,而不添加色氨酸的对照组的485/420为0.38保持不变。

[0174] 实施例11:在活细胞中基于光学探针进行高通量化合物筛选

[0175] 本实施例中,我们使用胞浆表达3lft-263/266-cpYFP-T259F的HeLa细胞进行高通量化合物筛选。

[0176] 经转染的HeLa细胞使用PBS冲洗,置于HBSS溶液中(无色氨酸)处理1小时,然后使用10 μ M的化合物处理1小时。各样品中分别滴加色氨酸。使用酶标仪记录420nm激发528nm发射处荧光强度和485nm激发528nm发射处荧光强度比值变化。以未用任何化合物处理的样品作为对照进行标准化。结果如图14所示。在使用的2000种化合物中,绝大部分的化合物对色氨酸进入细胞影响极小。有12种化合物能够提高细胞对色氨酸的摄取能力,另外有9种化合物能够明显降低细胞对色氨酸的摄取。

[0177] 实施例12:光学探针定量检测血液中的色氨酸

[0178] 在本实施中,使用纯化的3lft-263/265-cpYFP-F202W对小鼠和人的血液上清中的色氨酸进行分析。

[0179] 将3lft-263/265-cpYFP-F202W与稀释的血液上清混合处理10分钟后,使用酶标仪检测420nm激发528nm发射处荧光强度和485nm激发528nm发射处荧光强度比值。结果如图15所示,小鼠血液中的色氨酸含量在100 μ M左右,人血液中的色氨酸含量在48 μ M左右。

[0180] 由以上实施例可知,本发明提供的色氨酸光学探针,蛋白分子量相对较小且易于

成熟, 荧光动态变化大, 特异性好, 并且能够通过基因操作的方法在细胞中表达, 可在细胞内外实时定位、定量检测色氨酸; 并且能够进行高通量的化合物筛选。

[0181] 其它实施方案

[0182] 本说明书描述了许多实施方案。然而应理解, 本领域技术人员通过阅读本说明书获知的不背离本发明的构思和范围的各种改进, 也应包括在所附权利要求书的范围内。

序列表

<110> 华东理工大学

<120> 色氨酸光学探针及其制备方法和应用

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 Gln Phe Met Tyr Gly Ser Lys Ala Tyr Ile Lys His Pro Ala Asp Ile
 165 170 175
 Pro Asp Tyr Phe Lys Leu Ser Phe Pro Glu Gly Phe Arg Trp Glu Arg
 180 185 190
 Val Met Asn Phe Glu Asp Gly Gly Ile Ile His Val Asn Gln Asp Ser
 195 200 205
 Ser Leu Gln Asp Gly Val Phe Ile Tyr Lys Val Lys Leu Arg Gly Thr
 210 215 220
 Asn Phe Pro Pro Asp Gly Pro Val Met Gln Lys Lys Thr Met Gly Trp
 225 230 235 240
 Glu Ala

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

Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His		
275	280	285
Asn Val Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn		
290	295	300
Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu		
305	310	315
Ser Phe Gln Ser Val Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His		
	325	330
Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met		
	340	345
Asp Glu Leu Tyr Asn Val Asp Gly Gly Ser Gly Gly Thr Gly Ser Lys		
	355	360
Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp		
	370	375
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly		
385	390	395
Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys Thr Thr Gly		
	405	410
Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Gly Tyr Gly		
	420	425
Leu Lys Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe		
	435	440
Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe		
	450	455
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu		
465	470	475
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys		
	485	490
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Ser Thr Gly		
	500	505
Lys Ser Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile		
	515	520
Pro Glu Ser Val Leu Lys Glu Ala Gly Gln Val Ile		
	530	535
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<211> 539		
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<213> Artificial Sequence		
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Ser	Asn	Ala	Lys	Ile	Gly	Val	Leu	Gln	Phe	Val	Ser	His	Pro	Ser	Leu
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Asp	Leu	Ile	Tyr	Lys	Gly	Ile	Gln	Asp	Gly	Leu	Ala	Glu	Glu	Gly	Tyr
			20					25						30	
Lys	Asp	Asp	Gln	Val	Lys	Ile	Asp	Phe	Met	Asn	Ser	Glu	Gly	Asp	Gln
			35					40						45	
Ser	Lys	Val	Ala	Thr	Met	Ser	Lys	Gln	Leu	Val	Ala	Asn	Gly	Asn	Asp
			50					55						60	
Leu	Val	Val	Gly	Ile	Ala	Thr	Pro	Ala	Ala	Gln	Gly	Leu	Ala	Ser	Ala
65					70					75					80
Thr	Lys	Asp	Leu	Pro	Val	Ile	Met	Ala	Ala	Ile	Thr	Asp	Pro	Ile	Gly
					85					90					95
Ala	Asn	Leu	Val	Lys	Asp	Leu	Lys	Lys	Pro	Gly	Gly	Asn	Val	Thr	Gly
			100						105					110	
Val	Ser	Asp	His	Asn	Pro	Ala	Gln	Gln	Gln	Val	Glu	Leu	Ile	Lys	Ala
			115						120					125	
Leu	Thr	Pro	Asn	Val	Lys	Thr	Ile	Gly	Ala	Leu	Tyr	Ser	Ser	Ser	Glu
			130						135					140	
Asp	Asn	Ser	Lys	Thr	Gln	Val	Glu	Glu	Phe	Lys	Ala	Tyr	Ala	Glu	Lys
145					150					155					160
Ala	Gly	Leu	Thr	Val	Glu	Thr	Phe	Ala	Val	Pro	Ser	Thr	Asn	Glu	Ile
					165					170				175	
Ala	Ser	Thr	Val	Thr	Val	Met	Thr	Ser	Lys	Val	Asp	Ala	Ile	Trp	Val
			180						185					190	
Pro	Ile	Asp	Asn	Thr	Ile	Ala	Ser	Gly	Phe	Pro	Thr	Val	Val	Ser	Ser
			195						200					205	
Asn	Gln	Ser	Ser	Lys	Lys	Pro	Ile	Tyr	Pro	Ser	Ala	Thr	Ala	Met	Val
			210						215					220	
Glu	Val	Gly	Gly	Leu	Ala	Ser	Val	Val	Ile	Asp	Gln	His	Asp	Leu	Gly
225					230					235					240
Val	Ala	Thr	Gly	Lys	Met	Ile	Val	Gln	Val	Leu	Lys	Gly	Ala	Lys	Pro
					245					250				255	
Ala	Asp	Thr	Pro	Val	Asn	Val	Tyr	Asn	Ser	Asp	Asn	Val	Tyr	Ile	Met
			260						265					270	
Ala	Asp	Lys	Gln	Lys	Asn	Gly	Ile	Lys	Ala	Asn	Phe	Lys	Ile	Arg	His
			275						280					285	
Asn	Val	Glu	Asp	Gly	Ser	Val	Gln	Leu	Ala	Asp	His	Tyr	Gln	Gln	Asn
			290						295					300	
Thr	Pro	Ile	Gly	Asp	Gly	Pro	Val	Leu	Leu	Pro	Asp	Asn	His	Tyr	Leu

305	310	315	320
Ser Phe Gln Ser Val Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His			
	325	330	335
Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met			
	340	345	350
Asp Glu Leu Tyr Asn Val Asp Gly Gly Ser Gly Gly Thr Gly Ser Lys			
	355	360	365
Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp			
	370	375	380
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly			
385	390	395	400
Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys Thr Thr Gly			
	405	410	415
Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Gly Tyr Gly			
	420	425	430
Leu Lys Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe			
	435	440	445
Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe			
	450	455	460
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu			
465	470	475	480
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys			
	485	490	495
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Thr Gly Lys			
	500	505	510
Ser Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile Pro			
	515	520	525
Glu Ser Val Leu Lys Glu Ala Gly Gln Val Ile			
	530	535	
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<212> PRT			
<213> Artificial Sequence			
<400> 8			
Ser Asn Ala Lys Ile Gly Val Leu Gln Phe Val Ser His Pro Ser Leu			
1	5	10	15
Asp Leu Ile Tyr Lys Gly Ile Gln Asp Gly Leu Ala Glu Glu Gly Tyr			
	20	25	30
Lys Asp Asp Gln Val Lys Ile Asp Phe Met Asn Ser Glu Gly Asp Gln			

35	40	45
Ser Lys Val Ala Thr Met	Ser Lys Gln Leu Val	Ala Asn Gly Asn Asp
50	55	60
Leu Val Val Gly Ile Ala Thr	Pro Ala Ala Gln Gly	Leu Ala Ser Ala
65	70	75
Thr Lys Asp Leu Pro Val	Ile Met Ala Ala Ile	Thr Asp Pro Ile Gly
85	90	95
Ala Asn Leu Val Lys Asp	Leu Lys Lys Pro Gly	Gly Asn Val Thr Gly
100	105	110
Val Ser Asp His Asn Pro	Ala Gln Gln Gln Val	Glu Leu Ile Lys Ala
115	120	125
Leu Thr Pro Asn Val Lys	Thr Ile Gly Ala Leu	Tyr Ser Ser Ser Glu
130	135	140
Asp Asn Ser Lys Thr Gln	Val Glu Glu Phe Lys	Ala Tyr Ala Glu Lys
145	150	155
Ala Gly Leu Thr Val Glu	Thr Phe Ala Val Pro	Ser Thr Asn Glu Ile
165	170	175
Ala Ser Thr Val Thr Val	Met Thr Ser Lys Val	Asp Ala Ile Trp Val
180	185	190
Pro Ile Asp Asn Thr Ile	Ala Ser Gly Phe Pro	Thr Val Val Ser Ser
195	200	205
Asn Gln Ser Ser Lys Lys	Pro Ile Tyr Pro Ser	Ala Thr Ala Met Val
210	215	220
Glu Val Gly Gly Leu Ala	Ser Val Val Ile Asp	Gln His Asp Leu Gly
225	230	235
Val Ala Thr Gly Lys Met	Ile Val Gln Val Leu	Lys Gly Ala Lys Pro
245	250	255
Ala Asp Thr Pro Val Asn	Val Tyr Asn Ser Asp	Asn Val Tyr Ile Met
260	265	270
Ala Asp Lys Gln Lys Asn	Gly Ile Lys Ala Asn	Phe Lys Ile Arg His
275	280	285
Asn Val Glu Asp Gly Ser	Val Gln Leu Ala Asp	His Tyr Gln Gln Asn
290	295	300
Thr Pro Ile Gly Asp Gly	Pro Val Leu Leu Pro	Asp Asn His Tyr Leu
305	310	315
Ser Phe Gln Ser Val Leu	Ser Lys Asp Pro Asn	Glu Lys Arg Asp His
325	330	335
Met Val Leu Leu Glu Phe	Val Thr Ala Ala Gly	Ile Thr Leu Gly Met
340	345	350

Asp	Glu	Leu	Tyr	Asn	Val	Asp	Gly	Gly	Ser	Gly	Gly	Thr	Gly	Ser	Lys		
355						360						365					
Gly	Glu	Glu	Leu	Phe	Thr	Gly	Val	Val	Pro	Ile	Leu	Val	Glu	Leu	Asp		
370						375						380					
Gly	Asp	Val	Asn	Gly	His	Lys	Phe	Ser	Val	Ser	Gly	Glu	Gly	Glu	Gly		
385						390						395				400	
Asp	Ala	Thr	Tyr	Gly	Lys	Leu	Thr	Leu	Lys	Leu	Ile	Cys	Thr	Thr	Gly		
405						410						415					
Lys	Leu	Pro	Val	Pro	Trp	Pro	Thr	Leu	Val	Thr	Thr	Leu	Gly	Tyr	Gly		
420						425						430					
Leu	Lys	Cys	Phe	Ala	Arg	Tyr	Pro	Asp	His	Met	Lys	Gln	His	Asp	Phe		
435						440						445					
Phe	Lys	Ser	Ala	Met	Pro	Glu	Gly	Tyr	Val	Gln	Glu	Arg	Thr	Ile	Phe		
450						455						460					
Phe	Lys	Asp	Asp	Gly	Asn	Tyr	Lys	Thr	Arg	Ala	Glu	Val	Lys	Phe	Glu		
465						470						475				480	
Gly	Asp	Thr	Leu	Val	Asn	Arg	Ile	Glu	Leu	Lys	Gly	Ile	Gly	Phe	Lys		
485						490						495					
Glu	Asp	Gly	Asn	Ile	Leu	Gly	His	Lys	Leu	Glu	Tyr	Asn	Gly	Lys	Ser		
500						505						510					
Val	Ile	Asn	Lys	Lys	Ile	Ala	Gln	Glu	Leu	Gly	Ile	Thr	Ile	Pro	Glu		
515						520						525					
Ser	Val	Leu	Lys	Glu	Ala	Gly	Gln	Val	Ile								
530						535											
<210> 9																	
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<212> PRT																	
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<400> 9																	
Ser	Asn	Ala	Lys	Ile	Gly	Val	Leu	Gln	Phe	Val	Ser	His	Pro	Ser	Leu		
1				5				10				15					
Asp	Leu	Ile	Tyr	Lys	Gly	Ile	Gln	Asp	Gly	Leu	Ala	Glu	Glu	Gly	Tyr		
20						25						30					
Lys	Asp	Asp	Gln	Val	Lys	Ile	Asp	Phe	Met	Asn	Ser	Glu	Gly	Asp	Gln		
35						40						45					
Ser	Lys	Val	Ala	Thr	Met	Ser	Lys	Gln	Leu	Val	Ala	Asn	Gly	Asn	Asp		
50						55						60					
Leu	Val	Val	Gly	Ile	Ala	Thr	Pro	Ala	Ala	Gln	Gly	Leu	Ala	Ser	Ala		
65						70						75				80	

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

385	390	395	400
Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys Thr Thr Gly			
	405	410	415
Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Gly Tyr Gly			
	420	425	430
Leu Lys Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe			
	435	440	445
Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe			
	450	455	460
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu			
465	470	475	480
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys			
	485	490	495
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Lys Ser Val			
	500	505	510
Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile Pro Glu Ser			
	515	520	525
Val Leu Lys Glu Ala Gly Gln Val Ile			
	530	535	
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<213> Artificial Sequence			
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Ser Asn Ala Lys Ile Gly Val Leu Gln Phe Val Ser His Pro Ser Leu			
1	5	10	15
Asp Leu Ile Tyr Lys Gly Ile Gln Asp Gly Leu Ala Glu Glu Gly Tyr			
	20	25	30
Lys Asp Asp Gln Val Lys Ile Asp Phe Met Asn Ser Glu Gly Asp Gln			
	35	40	45
Ser Lys Val Ala Thr Met Ser Lys Gln Leu Val Ala Asn Gly Asn Asp			
	50	55	60
Leu Val Val Gly Ile Ala Thr Pro Ala Ala Gln Gly Leu Ala Ser Ala			
65	70	75	80
Thr Lys Asp Phe Pro Val Ile Met Ala Ala Ile Thr Asp Pro Ile Gly			
	85	90	95
Ala Asn Leu Val Lys Asp Leu Lys Lys Pro Gly Gly Asn Val Thr Gly			
	100	105	110
Val Ser Asp His Asn Pro Ala Gln Gln Gln Val Glu Leu Ile Lys Ala			

115	120	125
Leu Thr Pro Asn Val Lys Thr	Ile Gly Ala Leu Tyr Ser Ser Ser Glu	
130	135	140
Asp Asn Ser Lys Thr Gln Val Glu Glu Phe Lys Ala Tyr Ala Glu Lys		
145	150	155
Ala Gly Leu Thr Val Glu Thr Phe Ala Val Pro Ser Thr Asn Glu Ile		
165	170	175
Ala Ser Thr Val Thr Val Met Thr Ser Lys Val Asp Ala Ile Trp Val		
180	185	190
Pro Ile Asp Asn Thr Ile Ala Ser Gly Phe Pro Thr Val Val Ser Ser		
195	200	205
Asn Gln Ser Ser Lys Lys Pro Ile Tyr Pro Ser Ala Thr Ala Met Val		
210	215	220
Glu Val Gly Gly Leu Ala Ser Val Val Ile Asp Gln His Asp Leu Gly		
225	230	235
Val Ala Thr Gly Lys Met Ile Val Gln Val Leu Lys Gly Ala Lys Pro		
245	250	255
Ala Asp Thr Pro Val Asn Val Tyr Asn Ser Asp Asn Val Tyr Ile Met		
260	265	270
Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His		
275	280	285
Asn Val Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn		
290	295	300
Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu		
305	310	315
Ser Phe Gln Ser Val Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His		
325	330	335
Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met		
340	345	350
Asp Glu Leu Tyr Asn Val Asp Gly Gly Ser Gly Gly Thr Gly Ser Lys		
355	360	365
Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp		
370	375	380
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly		
385	390	395
Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys Thr Thr Gly		
405	410	415
Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Gly Tyr Gly		
420	425	430

Leu Lys Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe
 435 440 445
 Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe
 450 455 460
 Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu
 465 470 475 480
 Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys
 485 490 495
 Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Ser Thr Gly
 500 505 510
 Lys Ser Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile
 515 520 525
 Pro Glu Ser Val Leu Lys Glu Ala Gly Gln Val Ile
 530 535 540
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 Ser Asn Ala Lys Ile Gly Val Leu Gln Phe Val Ser His Pro Ser Leu
 1 5 10 15
 Asp Leu Ile Tyr Lys Gly Ile Gln Asp Gly Leu Ala Glu Glu Gly Tyr
 20 25 30
 Lys Asp Asp Gln Val Lys Ile Asp Phe Met Asn Ser Glu Gly Asp Gln
 35 40 45
 Ser Lys Val Ala Thr Met Ser Lys Gln Leu Val Ala Asn Gly Asn Asp
 50 55 60
 Leu Val Val Gly Ile Ala Thr Pro Ala Ala Gln Gly Leu Ala Ser Ala
 65 70 75 80
 Thr Lys Asp Leu Pro Val Ile Met Ala Ala Ile Thr Asp Pro Ile Gly
 85 90 95
 Ala Asn Leu Val Lys Asp Leu Lys Phe Pro Gly Gly Asn Val Thr Gly
 100 105 110
 Val Ser Asp His Asn Pro Ala Gln Gln Gln Val Glu Leu Ile Lys Ala
 115 120 125
 Leu Thr Pro Asn Val Lys Thr Ile Gly Ala Leu Tyr Ser Ser Ser Glu
 130 135 140
 Asp Asn Ser Lys Thr Gln Val Glu Glu Phe Lys Ala Tyr Ala Glu Lys
 145 150 155 160

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

465	470	475	480
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys			
	485	490	495
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Ser Thr Gly			
	500	505	510
Lys Ser Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile			
	515	520	525
Pro Glu Ser Val Leu Lys Glu Ala Gly Gln Val Ile			
	530	535	540
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<211> 540			
<212> PRT			
<213> Artificial Sequence			
<400> 12			
Ser Asn Ala Lys Ile Gly Val Leu Gln Phe Val Ser His Pro Ser Leu			
1	5	10	15
Asp Leu Ile Tyr Lys Gly Ile Gln Asp Gly Leu Ala Glu Glu Gly Tyr			
	20	25	30
Lys Asp Asp Gln Val Lys Ile Asp Phe Met Asn Ser Glu Gly Asp Gln			
	35	40	45
Ser Lys Val Ala Thr Met Ser Lys Gln Leu Val Ala Asn Gly Asn Asp			
	50	55	60
Leu Val Val Gly Ile Ala Thr Pro Ala Ala Gln Gly Leu Ala Ser Ala			
65	70	75	80
Thr Lys Asp Leu Pro Val Ile Met Ala Ala Ile Thr Asp Pro Ile Gly			
	85	90	95
Ala Asn Leu Val Lys Asp Leu Lys Lys Pro Gly Gly Asn Val Thr Gly			
	100	105	110
Val Ser Asp His Asn Pro Ala Gln Gln Gln Val Glu Leu Ile Lys Ala			
	115	120	125
Leu Thr Pro Asn Val Lys Thr Ile Gly Ala Leu Tyr Ser Ser Ser Glu			
	130	135	140
Asp Asn Ser Lys Thr Gln Val Glu Glu Phe Lys Ala Tyr Ala Glu Lys			
145	150	155	160
Ala Gly Leu Thr Val Glu Thr Phe Ala Val Pro Ser Thr Asn Glu Ile			
	165	170	175
Ala Ser Thr Val Thr Val Met Thr Ser Lys Val Asp Ala Ile Trp Val			
	180	185	190
Pro Ile Asp Asn Thr Ile Ala Ser Gly Trp Pro Thr Val Val Ser Ser			

195	200	205
Asn Gln Ser Ser Lys Lys Pro Ile Tyr Pro Ser Ala Thr Ala Met Val		
210	215	220
Glu Val Gly Gly Leu Ala Ser Val Val Ile Asp Gln His Asp Leu Gly		
225	230	235
Val Ala Thr Gly Lys Met Ile Val Gln Val Leu Lys Gly Ala Lys Pro		
245	250	255
Ala Asp Thr Pro Val Asn Val Tyr Asn Ser Asp Asn Val Tyr Ile Met		
260	265	270
Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His		
275	280	285
Asn Val Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn		
290	295	300
Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu		
305	310	315
Ser Phe Gln Ser Val Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His		
325	330	335
Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met		
340	345	350
Asp Glu Leu Tyr Asn Val Asp Gly Gly Ser Gly Gly Thr Gly Ser Lys		
355	360	365
Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp		
370	375	380
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly		
385	390	395
Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys Thr Thr Gly		
405	410	415
Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Gly Tyr Gly		
420	425	430
Leu Lys Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe		
435	440	445
Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe		
450	455	460
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu		
465	470	475
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys		
485	490	495
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Ser Thr Gly		
500	505	510

Lys Ser Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile
 515 520 525
 Pro Glu Ser Val Leu Lys Glu Ala Gly Gln Val Ile
 530 535 540
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 Asp Leu Ile Tyr Lys Gly Ile Gln Asp Gly Leu Ala Glu Glu Gly Tyr
 20 25 30
 Lys Asp Asp Gln Val Lys Ile Asp Phe Met Asn Ser Glu Gly Asp Gln
 35 40 45
 Ser Lys Val Ala Thr Met Ser Lys Gln Leu Val Ala Asn Gly Asn Asp
 50 55 60
 Leu Val Val Gly Ile Ala Thr Pro Ala Ala Gln Gly Leu Ala Ser Ala
 65 70 75 80
 Thr Lys Asp Leu Pro Val Ile Met Ala Ala Ile Thr Asp Pro Ile Gly
 85 90 95
 Ala Asn Leu Val Lys Asp Leu Lys Lys Pro Gly Gly Asn Val Thr Gly
 100 105 110
 Val Ser Asp His Asn Pro Ala Gln Gln Gln Val Glu Leu Ile Lys Ala
 115 120 125
 Leu Thr Pro Asn Val Lys Thr Ile Gly Ala Leu Tyr Ser Ser Ser Glu
 130 135 140
 Asp Asn Ser Lys Thr Gln Val Glu Glu Phe Lys Ala Tyr Ala Glu Lys
 145 150 155 160
 Ala Gly Leu Thr Val Glu Thr Phe Ala Val Pro Ser Thr Asn Glu Ile
 165 170 175
 Ala Ser Thr Val Thr Val Met Thr Ser Lys Val Asp Ala Ile Trp Val
 180 185 190
 Pro Ile Asp Asn Thr Ile Ala Ser Gly Phe Pro Thr Val Val Ser Ser
 195 200 205
 Asn Gln Ser Ser Lys Phe Pro Ile Tyr Pro Ser Ala Thr Ala Met Val
 210 215 220
 Glu Val Gly Gly Leu Ala Ser Val Val Ile Asp Gln His Asp Leu Gly
 225 230 235 240

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

<210> 14

<211> 540

<212> PRT

<213> Artificial Sequence

<400> 14

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Ser Asn Ala Lys Ile Gly Val Leu Gln Phe Val Ser His Pro Ser Leu
1           5           10           15
Asp Leu Ile Tyr Lys Gly Ile Gln Asp Gly Leu Ala Glu Glu Gly Tyr
          20           25           30
Lys Asp Asp Gln Val Lys Ile Asp Phe Met Asn Ser Glu Gly Asp Gln
          35           40           45
Ser Lys Val Ala Thr Met Ser Lys Gln Leu Val Ala Asn Gly Asn Asp
          50           55           60
Leu Val Val Gly Ile Ala Thr Pro Ala Ala Gln Gly Leu Ala Ser Ala
65           70           75           80
Thr Lys Asp Leu Pro Val Ile Met Ala Ala Ile Thr Asp Pro Ile Gly
          85           90           95
Ala Asn Leu Val Lys Asp Leu Lys Lys Pro Gly Gly Asn Val Thr Gly
          100          105          110
Val Ser Asp His Asn Pro Ala Gln Gln Gln Val Glu Leu Ile Lys Ala
          115          120          125
Leu Thr Pro Asn Val Lys Thr Ile Gly Ala Leu Tyr Ser Ser Ser Glu
          130          135          140
Asp Asn Ser Lys Thr Gln Val Glu Glu Phe Lys Ala Tyr Ala Glu Lys
145          150          155          160
Ala Gly Leu Thr Val Glu Thr Phe Ala Val Pro Ser Thr Asn Glu Ile
          165          170          175
Ala Ser Thr Val Thr Val Met Thr Ser Lys Val Asp Ala Ile Trp Val
          180          185          190
Pro Ile Asp Asn Thr Ile Ala Ser Gly Phe Pro Thr Val Val Ser Ser
          195          200          205
Asn Gln Ser Ser Lys Trp Pro Ile Tyr Pro Ser Ala Thr Ala Met Val
          210          215          220
Glu Val Gly Gly Leu Ala Ser Val Val Ile Asp Gln His Asp Leu Gly
225          230          235          240
Val Ala Thr Gly Lys Met Ile Val Gln Val Leu Lys Gly Ala Lys Pro
          245          250          255
Ala Asp Thr Pro Val Asn Val Tyr Asn Ser Asp Asn Val Tyr Ile Met
          260          265          270
Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His

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275	280	285
Asn Val Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn		
290	295	300
Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu		
305	310	315
Ser Phe Gln Ser Val Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His		
325	330	335
Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met		
340	345	350
Asp Glu Leu Tyr Asn Val Asp Gly Gly Ser Gly Gly Thr Gly Ser Lys		
355	360	365
Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp		
370	375	380
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly		
385	390	395
Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys Thr Thr Gly		
405	410	415
Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Gly Tyr Gly		
420	425	430
Leu Lys Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe		
435	440	445
Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe		
450	455	460
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu		
465	470	475
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys		
485	490	495
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Ser Thr Gly		
500	505	510
Lys Ser Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile		
515	520	525
Pro Glu Ser Val Leu Lys Glu Ala Gly Gln Val Ile		
530	535	540
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<213> Artificial Sequence		
<400> 15		
Ser Asn Ala Lys Ile Gly Val Leu Gln Phe Val Ser His Pro Ser Leu		

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

<210> 16
<211> 540
<212> PRT
<213> Artificial Sequence
<400> 16
Ser Asn Ala Lys Ile Gly Val Leu Gln Phe Val Ser His Pro Ser Leu
1 5 10 15
Asp Leu Ile Tyr Lys Gly Ile Gln Asp Gly Leu Ala Glu Glu Gly Tyr
 20 25 30
Lys Asp Asp Gln Val Lys Ile Asp Phe Met Asn Ser Glu Gly Asp Gln
 35 40 45

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

355	360	365
Gly Glu Glu Leu Phe Thr	Gly Val Val Pro Ile	Leu Val Glu Leu Asp
370	375	380
Gly Asp Val Asn Gly His	Lys Phe Ser Val Ser	Gly Glu Gly Glu Gly
385	390	395
Asp Ala Thr Tyr Gly Lys	Leu Thr Leu Lys Leu	Ile Cys Thr Thr Gly
405	410	415
Lys Leu Pro Val Pro Trp	Pro Thr Leu Val Thr	Thr Leu Gly Tyr Gly
420	425	430
Leu Lys Cys Phe Ala Arg	Tyr Pro Asp His Met	Lys Gln His Asp Phe
435	440	445
Phe Lys Ser Ala Met Pro	Glu Gly Tyr Val Gln	Glu Arg Thr Ile Phe
450	455	460
Phe Lys Asp Asp Gly Asn	Tyr Lys Thr Arg Ala	Glu Val Lys Phe Glu
465	470	475
Gly Asp Thr Leu Val Asn	Arg Ile Glu Leu Lys	Gly Ile Gly Phe Lys
485	490	495
Glu Asp Gly Asn Ile Leu	Gly His Lys Leu Glu	Tyr Asn Ser Thr Gly
500	505	510
Lys Ser Val Ile Asn Lys	Lys Ile Ala Gln Glu	Leu Gly Ile Thr Ile
515	520	525
Pro Glu Ser Val Leu Lys	Glu Ala Gly Gln Val	Ile
530	535	540
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<211> 540		
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<213> Artificial Sequence		
<400> 17		
Ser Asn Ala Lys Ile Gly	Val Leu Gln Phe Val	Ser His Pro Ser Leu
1	5	10
Asp Leu Ile Tyr Lys Gly	Ile Gln Asp Gly Leu	Ala Glu Glu Gly Tyr
20	25	30
Lys Asp Asp Gln Val Lys	Ile Asp Phe Met Asn	Ser Glu Gly Asp Gln
35	40	45
Ser Lys Val Ala Thr Met	Ser Lys Gln Leu Val	Ala Asn Gly Asn Asp
50	55	60
Leu Val Val Gly Ile Ala	Thr Pro Ala Ala Gln	Gly Leu Ala Ser Ala
65	70	75
Thr Lys Asp Leu Pro Val	Ile Met Ala Ala Ile	Thr Asp Pro Ile Gly

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

Asp	Ala	Thr	Tyr	Gly	Lys	Leu	Thr	Leu	Lys	Leu	Ile	Cys	Thr	Thr	Gly
				405					410						415
Lys	Leu	Pro	Val	Pro	Trp	Pro	Thr	Leu	Val	Thr	Thr	Leu	Gly	Tyr	Gly
			420					425					430		
Leu	Lys	Cys	Phe	Ala	Arg	Tyr	Pro	Asp	His	Met	Lys	Gln	His	Asp	Phe
		435					440					445			
Phe	Lys	Ser	Ala	Met	Pro	Glu	Gly	Tyr	Val	Gln	Glu	Arg	Thr	Ile	Phe
	450					455					460				
Phe	Lys	Asp	Asp	Gly	Asn	Tyr	Lys	Thr	Arg	Ala	Glu	Val	Lys	Phe	Glu
465				470					475						480
Gly	Asp	Thr	Leu	Val	Asn	Arg	Ile	Glu	Leu	Lys	Gly	Ile	Gly	Phe	Lys
			485					490					495		
Glu	Asp	Gly	Asn	Ile	Leu	Gly	His	Lys	Leu	Glu	Tyr	Asn	Ser	Thr	Gly
		500						505					510		
Lys	Ser	Val	Ile	Asn	Lys	Lys	Ile	Ala	Gln	Glu	Leu	Gly	Ile	Thr	Ile
	515						520					525			
Pro	Glu	Ser	Val	Leu	Lys	Glu	Ala	Gly	Gln	Val	Ile				
	530					535					540				
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Ser	Asn	Ala	Lys	Ile	Gly	Val	Leu	Gln	Phe	Val	Ser	His	Pro	Ser	Leu
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Asp	Leu	Ile	Tyr	Lys	Gly	Ile	Gln	Asp	Gly	Leu	Ala	Glu	Glu	Gly	Tyr
		20					25					30			
Lys	Asp	Asp	Gln	Val	Lys	Ile	Asp	Phe	Met	Asn	Ser	Glu	Gly	Asp	Gln
	35					40					45				
Ser	Lys	Val	Ala	Thr	Met	Ser	Lys	Gln	Leu	Val	Ala	Asn	Gly	Asn	Asp
	50				55					60					
Leu	Val	Val	Gly	Ile	Ala	Thr	Pro	Ala	Ala	Gln	Gly	Leu	Ala	Ser	Ala
65				70					75					80	
Thr	Lys	Asp	Leu	Pro	Val	Ile	Met	Ala	Ala	Ile	Thr	Asp	Pro	Ile	Gly
			85					90				95			
Ala	Asn	Leu	Val	Lys	Asp	Leu	Lys	Lys	Pro	Gly	Gly	Asn	Val	Thr	Gly
	100						105					110			
Val	Ser	Asp	His	Asn	Pro	Ala	Gln	Gln	Gln	Val	Glu	Leu	Ile	Lys	Ala
	115					120					125				

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

435	440	445
Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe		
450	455	460
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu		
465	470	475
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys		480
485	490	495
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Ser Thr Gly		
500	505	510
Lys Ser Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile		
515	520	525
Pro Glu Ser Val Leu Lys Glu Ala Gly Gln Val Ile		
530	535	540
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Asp Leu Ile Tyr Lys Gly Ile Gln Asp Gly Leu Ala Glu Glu Gly Tyr		15
20	25	30
Lys Asp Asp Gln Val Lys Ile Asp Phe Met Asn Ser Glu Gly Asp Gln		
35	40	45
Ser Lys Val Ala Thr Met Ser Lys Gln Leu Val Ala Asn Gly Asn Asp		
50	55	60
Leu Val Val Gly Ile Ala Thr Pro Ala Ala Gln Gly Leu Ala Ser Ala		
65	70	75
Thr Lys Asp Leu Pro Val Ile Met Ala Ala Ile Thr Asp Pro Ile Gly		80
85	90	95
Ala Asn Leu Val Lys Asp Leu Lys Lys Pro Gly Gly Asn Val Thr Gly		
100	105	110
Val Ser Asp His Asn Pro Ala Gln Gln Gln Val Glu Leu Ile Lys Ala		
115	120	125
Leu Thr Pro Asn Val Lys Thr Ile Gly Ala Leu Tyr Ser Ser Ser Glu		
130	135	140
Asp Asn Ser Lys Thr Gln Val Glu Glu Phe Lys Ala Tyr Ala Glu Lys		
145	150	155
Ala Gly Leu Thr Val Glu Thr Phe Ala Val Pro Ser Thr Asn Glu Ile		160

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

Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys
 485 490 495
 Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Ser Thr Gly
 500 505 510
 Lys Ser Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile
 515 520 525
 Pro Glu Ser Val Leu Lys Glu Ala Gly Gln Val Ile
 530 535 540
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 Asp Leu Ile Tyr Lys Gly Ile Gln Asp Gly Leu Ala Glu Glu Gly Tyr
 20 25 30
 Lys Asp Asp Gln Val Lys Ile Asp Phe Met Asn Ser Glu Gly Asp Gln
 35 40 45
 Ser Lys Val Ala Thr Met Ser Lys Gln Leu Val Ala Asn Gly Asn Asp
 50 55 60
 Leu Val Val Gly Ile Ala Thr Pro Ala Ala Gln Gly Leu Ala Ser Ala
 65 70 75 80
 Thr Lys Asp Leu Pro Val Ile Met Ala Ala Ile Thr Asp Pro Ile Gly
 85 90 95
 Ala Asn Leu Val Lys Asp Leu Lys Lys Pro Gly Gly Asn Val Thr Gly
 100 105 110
 Val Ser Asp His Asn Pro Ala Gln Gln Gln Val Glu Leu Ile Lys Ala
 115 120 125
 Leu Thr Pro Asn Val Lys Thr Ile Gly Ala Leu Tyr Ser Ser Ser Glu
 130 135 140
 Asp Asn Ser Lys Thr Gln Val Glu Glu Phe Lys Ala Tyr Ala Glu Lys
 145 150 155 160
 Ala Gly Leu Thr Val Glu Thr Phe Ala Val Pro Ser Thr Asn Glu Ile
 165 170 175
 Ala Ser Thr Val Thr Val Met Thr Ser Lys Val Asp Ala Ile Trp Val
 180 185 190
 Pro Ile Asp Asn Thr Ile Ala Ser Gly Phe Pro Thr Val Val Ser Ser
 195 200 205

Asn	Gln	Ser	Ser	Lys	Lys	Pro	Ile	Tyr	Pro	Ser	Ala	Thr	Ala	Met	Val
210						215					220				
Glu	Val	Gly	Gly	Leu	Ala	Ser	Val	Val	Ile	Asp	Gln	His	Asp	Leu	Gly
225					230					235					240
Val	Ala	Thr	Gly	Lys	Met	Ile	Val	Gln	Val	Leu	Lys	Gly	Ala	Lys	Pro
				245					250					255	
Ala	Asp	Trp	Pro	Val	Asn	Val	Tyr	Asn	Ser	Asp	Asn	Val	Tyr	Ile	Met
			260					265					270		
Ala	Asp	Lys	Gln	Lys	Asn	Gly	Ile	Lys	Ala	Asn	Phe	Lys	Ile	Arg	His
		275					280					285			
Asn	Val	Glu	Asp	Gly	Ser	Val	Gln	Leu	Ala	Asp	His	Tyr	Gln	Gln	Asn
290						295					300				
Thr	Pro	Ile	Gly	Asp	Gly	Pro	Val	Leu	Leu	Pro	Asp	Asn	His	Tyr	Leu
305					310					315					320
Ser	Phe	Gln	Ser	Val	Leu	Ser	Lys	Asp	Pro	Asn	Glu	Lys	Arg	Asp	His
				325					330					335	
Met	Val	Leu	Leu	Glu	Phe	Val	Thr	Ala	Ala	Gly	Ile	Thr	Leu	Gly	Met
				340				345					350		
Asp	Glu	Leu	Tyr	Asn	Val	Asp	Gly	Gly	Ser	Gly	Gly	Thr	Gly	Ser	Lys
		355					360					365			
Gly	Glu	Glu	Leu	Phe	Thr	Gly	Val	Val	Pro	Ile	Leu	Val	Glu	Leu	Asp
370						375					380				
Gly	Asp	Val	Asn	Gly	His	Lys	Phe	Ser	Val	Ser	Gly	Glu	Gly	Glu	Gly
385					390					395					400
Asp	Ala	Thr	Tyr	Gly	Lys	Leu	Thr	Leu	Lys	Leu	Ile	Cys	Thr	Thr	Gly
				405					410					415	
Lys	Leu	Pro	Val	Pro	Trp	Pro	Thr	Leu	Val	Thr	Thr	Leu	Gly	Tyr	Gly
			420					425					430		
Leu	Lys	Cys	Phe	Ala	Arg	Tyr	Pro	Asp	His	Met	Lys	Gln	His	Asp	Phe
		435					440					445			
Phe	Lys	Ser	Ala	Met	Pro	Glu	Gly	Tyr	Val	Gln	Glu	Arg	Thr	Ile	Phe
450						455					460				
Phe	Lys	Asp	Asp	Gly	Asn	Tyr	Lys	Thr	Arg	Ala	Glu	Val	Lys	Phe	Glu
465					470					475					480
Gly	Asp	Thr	Leu	Val	Asn	Arg	Ile	Glu	Leu	Lys	Gly	Ile	Gly	Phe	Lys
				485					490				495		
Glu	Asp	Gly	Asn	Ile	Leu	Gly	His	Lys	Leu	Glu	Tyr	Asn	Ser	Thr	Gly
			500					505					510		
Lys	Ser	Val	Ile	Asn	Lys	Lys	Ile	Ala	Gln	Glu	Leu	Gly	Ile	Thr	Ile

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

	245		250		255
Ala Asp Thr Pro Val Asn Val Tyr Asn Ser Asp Asn Val Tyr Ile Met					
	260		265		270
Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His					
	275		280		285
Asn Val Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn					
	290		295		300
Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu					
305		310		315	320
Ser Phe Gln Ser Val Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His					
	325		330		335
Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met					
	340		345		350
Asp Glu Leu Tyr Asn Val Asp Gly Gly Ser Gly Gly Thr Gly Ser Lys					
	355		360		365
Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp					
	370		375		380
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly					
385		390		395	400
Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys Thr Thr Gly					
	405		410		415
Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Gly Tyr Gly					
	420		425		430
Leu Lys Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe					
	435		440		445
Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe					
	450		455		460
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu					
465		470		475	480
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys					
	485		490		495
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Ser Thr Gly					
	500		505		510
Lys Ser Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Phe					
	515		520		525
Pro Glu Ser Val Leu Lys Glu Ala Gly Gln Val Ile					
	530		535		540
<210> 22					
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<212> PRT

<213> Artificial Sequence

<400> 22

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

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Asn Val Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn			
290	295	300	
Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu			
305	310	315	320
Ser Phe Gln Ser Val Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His			
	325	330	335
Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met			
	340	345	350
Asp Glu Leu Tyr Asn Val Asp Gly Gly Ser Gly Gly Thr Gly Ser Lys			
	355	360	365
Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp			
	370	375	380
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly			
385	390	395	400
Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys Thr Thr Gly			
	405	410	415
Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Gly Tyr Gly			
	420	425	430
Leu Lys Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe			
	435	440	445
Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe			
	450	455	460
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu			
465	470	475	480
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys			
	485	490	495
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Ser Thr Gly			
	500	505	510
Lys Ser Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Trp			
	515	520	525
Pro Glu Ser Val Leu Lys Glu Ala Gly Gln Val Ile			
	530	535	540
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Ser Asn Ala Lys Ile Gly Val Leu Gln Phe Val Ser His Pro Ser Leu			
1	5	10	15

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

	325		330		335
Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met					
	340		345		350
Asp Glu Leu Tyr Asn Val Asp Gly Gly Ser Gly Gly Thr Gly Ser Lys					
	355		360		365
Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp					
	370		375		380
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly					
385		390		395	400
Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys Thr Thr Gly					
	405		410		415
Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Gly Tyr Gly					
	420		425		430
Leu Lys Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe					
	435		440		445
Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe					
	450		455		460
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu					
465		470		475	480
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys					
	485		490		495
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Thr Gly Lys					
	500		505		510
Ser Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile Pro					
	515		520		525
Glu Ser Val Leu Lys Glu Ala Gly Gln Val Ile					
	530		535		
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Ser Asn Ala Lys Ile Gly Val Leu Gln Phe Val Ser His Pro Ser Leu					
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Asp Leu Ile Tyr Lys Gly Ile Gln Asp Gly Leu Ala Glu Glu Gly Tyr					
	20		25		30
Lys Asp Asp Gln Val Lys Ile Asp Phe Met Asn Ser Glu Gly Asp Gln					
	35		40		45
Ser Lys Val Ala Thr Met Ser Lys Gln Leu Val Ala Asn Gly Asn Asp					

50	55	60
Leu Val Val Gly Ile Ala Thr Pro Ala Ala Gln Gly Leu Ala Ser Ala		
65	70	75
Thr Lys Asp Leu Pro Val Ile Met Ala Ala Ile Thr Asp Pro Ile Gly		
85	90	95
Ala Asn Leu Val Lys Asp Leu Lys Phe Pro Gly Gly Asn Val Thr Gly		
100	105	110
Val Ser Asp His Asn Pro Ala Gln Gln Gln Val Glu Leu Ile Lys Ala		
115	120	125
Leu Thr Pro Asn Val Lys Thr Ile Gly Ala Leu Tyr Ser Ser Ser Glu		
130	135	140
Asp Asn Ser Lys Thr Gln Val Glu Glu Phe Lys Ala Tyr Ala Glu Lys		
145	150	155
Ala Gly Leu Thr Val Glu Thr Phe Ala Val Pro Ser Thr Asn Glu Ile		
165	170	175
Ala Ser Thr Val Thr Val Met Thr Ser Lys Val Asp Ala Ile Trp Val		
180	185	190
Pro Ile Asp Asn Thr Ile Ala Ser Gly Phe Pro Thr Val Val Ser Ser		
195	200	205
Asn Gln Ser Ser Lys Lys Pro Ile Tyr Pro Ser Ala Thr Ala Met Val		
210	215	220
Glu Val Gly Gly Leu Ala Ser Val Val Ile Asp Gln His Asp Leu Gly		
225	230	235
Val Ala Thr Gly Lys Met Ile Val Gln Val Leu Lys Gly Ala Lys Pro		
245	250	255
Ala Asp Thr Pro Val Asn Val Tyr Asn Ser Asp Asn Val Tyr Ile Met		
260	265	270
Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His		
275	280	285
Asn Val Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn		
290	295	300
Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu		
305	310	315
Ser Phe Gln Ser Val Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His		
325	330	335
Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met		
340	345	350
Asp Glu Leu Tyr Asn Val Asp Gly Gly Ser Gly Gly Thr Gly Ser Lys		
355	360	365

Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp
 370 375 380
 Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly
 385 390 395 400
 Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys Thr Thr Gly
 405 410 415
 Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Gly Tyr Gly
 420 425 430
 Leu Lys Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe
 435 440 445
 Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe
 450 455 460
 Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu
 465 470 475 480
 Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys
 485 490 495
 Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Thr Gly Lys
 500 505 510
 Ser Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile Pro
 515 520 525
 Glu Ser Val Leu Lys Glu Ala Gly Gln Val Ile
 530 535
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 20 25 30
 Lys Asp Asp Gln Val Lys Ile Asp Phe Met Asn Ser Glu Gly Asp Gln
 35 40 45
 Ser Lys Val Ala Thr Met Ser Lys Gln Leu Val Ala Asn Gly Asn Asp
 50 55 60
 Leu Val Val Gly Ile Ala Thr Pro Ala Ala Gln Gly Leu Ala Ser Ala
 65 70 75 80
 Thr Lys Asp Leu Pro Val Ile Met Ala Ala Ile Thr Asp Pro Ile Gly
 85 90 95

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

				405				410				415				
Lys	Leu	Pro	Val	Pro	Trp	Pro	Thr	Leu	Val	Thr	Thr	Leu	Gly	Tyr	Gly	
				420				425				430				
Leu	Lys	Cys	Phe	Ala	Arg	Tyr	Pro	Asp	His	Met	Lys	Gln	His	Asp	Phe	
				435				440				445				
Phe	Lys	Ser	Ala	Met	Pro	Glu	Gly	Tyr	Val	Gln	Glu	Arg	Thr	Ile	Phe	
				450				455				460				
Phe	Lys	Asp	Asp	Gly	Asn	Tyr	Lys	Thr	Arg	Ala	Glu	Val	Lys	Phe	Glu	
465				470				475				480				
Gly	Asp	Thr	Leu	Val	Asn	Arg	Ile	Glu	Leu	Lys	Gly	Ile	Gly	Phe	Lys	
				485				490				495				
Glu	Asp	Gly	Asn	Ile	Leu	Gly	His	Lys	Leu	Glu	Tyr	Asn	Thr	Gly	Lys	
				500				505				510				
Ser	Val	Ile	Asn	Lys	Lys	Ile	Ala	Gln	Glu	Leu	Gly	Ile	Thr	Ile	Pro	
				515				520				525				
Glu	Ser	Val	Leu	Lys	Glu	Ala	Gly	Gln	Val	Ile						
530				535												
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<213> Artificial Sequence																
<400> 26																
Ser	Asn	Ala	Lys	Ile	Gly	Val	Leu	Gln	Phe	Val	Ser	His	Pro	Ser	Leu	
1				5				10				15				
Asp	Leu	Ile	Tyr	Lys	Gly	Ile	Gln	Asp	Gly	Leu	Ala	Glu	Glu	Gly	Tyr	
				20				25				30				
Lys	Asp	Asp	Gln	Val	Lys	Ile	Asp	Phe	Met	Asn	Ser	Glu	Gly	Asp	Gln	
				35				40				45				
Ser	Lys	Val	Ala	Thr	Met	Ser	Lys	Gln	Leu	Val	Ala	Asn	Gly	Asn	Asp	
50				55				60								
Leu	Val	Val	Gly	Ile	Ala	Thr	Pro	Ala	Ala	Gln	Gly	Leu	Ala	Ser	Ala	
65				70				75				80				
Thr	Lys	Asp	Leu	Pro	Val	Ile	Met	Ala	Ala	Ile	Thr	Asp	Pro	Ile	Gly	
				85				90				95				
Ala	Asn	Leu	Val	Lys	Asp	Leu	Lys	Lys	Pro	Gly	Gly	Asn	Val	Thr	Gly	
				100				105				110				
Val	Ser	Asp	His	Asn	Pro	Ala	Gln	Gln	Gln	Val	Glu	Leu	Ile	Lys	Ala	
				115				120				125				
Leu	Thr	Pro	Asn	Val	Lys	Thr	Ile	Gly	Ala	Leu	Tyr	Ser	Ser	Ser	Glu	

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

Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe
 450 455 460
 Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu
 465 470 475 480
 Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys
 485 490 495
 Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Thr Gly Lys
 500 505 510
 Ser Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile Pro
 515 520 525
 Glu Ser Val Leu Lys Glu Ala Gly Gln Val Ile
 530 535
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 Ser Asn Ala Lys Ile Gly Val Leu Gln Phe Val Ser His Pro Ser Leu
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 Asp Leu Ile Tyr Lys Gly Ile Gln Asp Gly Leu Ala Glu Glu Gly Tyr
 20 25 30
 Lys Asp Asp Gln Val Lys Ile Asp Phe Met Asn Ser Glu Gly Asp Gln
 35 40 45
 Ser Lys Val Ala Thr Met Ser Lys Gln Leu Val Ala Asn Gly Asn Asp
 50 55 60
 Leu Val Val Gly Ile Ala Thr Pro Ala Ala Gln Gly Leu Ala Ser Ala
 65 70 75 80
 Thr Lys Asp Leu Pro Val Ile Met Ala Ala Ile Thr Asp Pro Ile Gly
 85 90 95
 Ala Asn Leu Val Lys Asp Leu Lys Lys Pro Gly Gly Asn Val Thr Gly
 100 105 110
 Val Ser Asp His Asn Pro Ala Gln Gln Gln Val Glu Leu Ile Lys Ala
 115 120 125
 Leu Thr Pro Asn Val Lys Thr Ile Gly Ala Leu Tyr Ser Ser Ser Glu
 130 135 140
 Asp Asn Ser Lys Thr Gln Val Glu Glu Phe Lys Ala Tyr Ala Glu Lys
 145 150 155 160
 Ala Gly Leu Thr Val Glu Thr Phe Ala Val Pro Ser Thr Asn Glu Ile
 165 170 175

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

	485		490		495
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Thr Gly Lys					
	500		505		510
Ser Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile Pro					
	515		520		525
Glu Ser Val Leu Lys Glu Ala Gly Gln Val Ile					
	530		535		
<210> 28					
<211> 539					
<212> PRT					
<213> Artificial Sequence					
<400> 28					
Ser Asn Ala Lys Ile Gly Val Leu Gln Phe Val Ser His Pro Ser Leu					
1	5		10		15
Asp Leu Ile Tyr Lys Gly Ile Gln Asp Gly Leu Ala Glu Glu Gly Tyr					
	20		25		30
Lys Asp Asp Gln Val Lys Ile Asp Phe Met Asn Ser Glu Gly Asp Gln					
	35		40		45
Ser Lys Val Ala Thr Met Ser Lys Gln Leu Val Ala Asn Gly Asn Asp					
	50		55		60
Leu Val Val Gly Ile Ala Thr Pro Ala Ala Gln Gly Leu Ala Ser Ala					
65	70		75		80
Thr Lys Asp Leu Pro Val Ile Met Ala Ala Ile Thr Asp Pro Ile Gly					
	85		90		95
Ala Asn Leu Val Lys Asp Leu Lys Lys Pro Gly Gly Asn Val Thr Gly					
	100		105		110
Val Ser Asp His Asn Pro Ala Gln Gln Gln Val Glu Leu Ile Lys Ala					
	115		120		125
Leu Thr Pro Asn Val Lys Thr Ile Gly Ala Leu Tyr Ser Ser Ser Glu					
	130		135		140
Asp Asn Ser Lys Thr Gln Val Glu Glu Phe Lys Ala Tyr Ala Glu Lys					
145	150		155		160
Ala Gly Leu Thr Val Glu Thr Phe Ala Val Pro Ser Thr Asn Glu Ile					
	165		170		175
Ala Ser Thr Val Thr Val Met Thr Ser Lys Val Asp Ala Ile Trp Val					
	180		185		190
Pro Ile Asp Asn Thr Ile Ala Ser Gly Phe Pro Thr Val Val Ser Ser					
	195		200		205
Asn Gln Ser Ser Lys Trp Pro Ile Tyr Pro Ser Ala Thr Ala Met Val					

210	215	220
Glu Val Gly Gly Leu Ala Ser Val Val Ile Asp Gln His Asp Leu Gly		
225	230	235
Val Ala Thr Gly Lys Met Ile Val Gln Val Leu Lys Gly Ala Lys Pro		240
245	250	255
Ala Asp Thr Pro Val Asn Val Tyr Asn Ser Asp Asn Val Tyr Ile Met		
260	265	270
Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His		
275	280	285
Asn Val Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn		
290	295	300
Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu		
305	310	315
Ser Phe Gln Ser Val Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His		320
325	330	335
Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met		
340	345	350
Asp Glu Leu Tyr Asn Val Asp Gly Gly Ser Gly Gly Thr Gly Ser Lys		
355	360	365
Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp		
370	375	380
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly		
385	390	395
Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys Thr Thr Gly		400
405	410	415
Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Gly Tyr Gly		
420	425	430
Leu Lys Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe		
435	440	445
Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe		
450	455	460
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu		
465	470	475
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys		480
485	490	495
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Thr Gly Lys		
500	505	510
Ser Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile Pro		
515	520	525

Glu Ser Val Leu Lys Glu Ala Gly Gln Val Ile
 530 535
 <210> 29
 <211> 539
 <212> PRT
 <213> Artificial Sequence
 <400> 29
 Ser Asn Ala Lys Ile Gly Val Leu Gln Phe Val Ser His Pro Ser Leu
 1 5 10 15
 Asp Leu Ile Tyr Lys Gly Ile Gln Asp Gly Leu Ala Glu Glu Gly Tyr
 20 25 30
 Lys Asp Asp Gln Val Lys Ile Asp Phe Met Asn Ser Glu Gly Asp Gln
 35 40 45
 Ser Lys Val Ala Thr Met Ser Lys Gln Leu Val Ala Asn Gly Asn Asp
 50 55 60
 Leu Val Val Gly Ile Ala Thr Pro Ala Ala Gln Gly Leu Ala Ser Ala
 65 70 75 80
 Thr Lys Asp Leu Pro Val Ile Met Ala Ala Ile Thr Asp Pro Ile Gly
 85 90 95
 Ala Asn Leu Val Lys Asp Leu Lys Lys Pro Gly Gly Asn Val Thr Gly
 100 105 110
 Val Ser Asp His Asn Pro Ala Gln Gln Gln Val Glu Leu Ile Lys Ala
 115 120 125
 Leu Thr Pro Asn Val Lys Thr Ile Gly Ala Leu Tyr Ser Ser Ser Glu
 130 135 140
 Asp Asn Ser Lys Thr Gln Val Glu Glu Phe Lys Ala Tyr Ala Glu Lys
 145 150 155 160
 Ala Gly Leu Thr Val Glu Thr Phe Ala Val Pro Ser Thr Asn Glu Ile
 165 170 175
 Ala Ser Thr Val Thr Val Met Thr Ser Lys Val Asp Ala Ile Trp Val
 180 185 190
 Pro Ile Asp Asn Thr Ile Ala Ser Gly Phe Pro Thr Val Val Ser Ser
 195 200 205
 Asn Gln Ser Ser Lys Lys Pro Ile Phe Pro Ser Ala Thr Ala Met Val
 210 215 220
 Glu Val Gly Gly Leu Ala Ser Val Val Ile Asp Gln His Asp Leu Gly
 225 230 235 240
 Val Ala Thr Gly Lys Met Ile Val Gln Val Leu Lys Gly Ala Lys Pro
 245 250 255

Ala Asp Thr Pro Val Asn Val Tyr Asn Ser Asp Asn Val Tyr Ile Met		
260	265	270
Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His		
275	280	285
Asn Val Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn		
290	295	300
Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu		
305	310	315
Ser Phe Gln Ser Val Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His		
325	330	335
Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met		
340	345	350
Asp Glu Leu Tyr Asn Val Asp Gly Gly Ser Gly Gly Thr Gly Ser Lys		
355	360	365
Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp		
370	375	380
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly		
385	390	395
Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys Thr Thr Gly		
405	410	415
Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Gly Tyr Gly		
420	425	430
Leu Lys Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe		
435	440	445
Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe		
450	455	460
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu		
465	470	475
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys		
485	490	495
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Thr Gly Lys		
500	505	510
Ser Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile Pro		
515	520	525
Glu Ser Val Leu Lys Glu Ala Gly Gln Val Ile		
530	535	
<210> 30		
<211> 539		
<212> PRT		

<213> Artificial Sequence

<400> 30

Ser	Asn	Ala	Lys	Ile	Gly	Val	Leu	Gln	Phe	Val	Ser	His	Pro	Ser	Leu
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Asp	Leu	Ile	Tyr	Lys	Gly	Ile	Gln	Asp	Gly	Leu	Ala	Glu	Glu	Gly	Tyr
				20				25						30	
Lys	Asp	Asp	Gln	Val	Lys	Ile	Asp	Phe	Met	Asn	Ser	Glu	Gly	Asp	Gln
				35				40						45	
Ser	Lys	Val	Ala	Thr	Met	Ser	Lys	Gln	Leu	Val	Ala	Asn	Gly	Asn	Asp
								55						60	
Leu	Val	Val	Gly	Ile	Ala	Thr	Pro	Ala	Ala	Gln	Gly	Leu	Ala	Ser	Ala
65					70					75					80
Thr	Lys	Asp	Leu	Pro	Val	Ile	Met	Ala	Ala	Ile	Thr	Asp	Pro	Ile	Gly
					85					90					95
Ala	Asn	Leu	Val	Lys	Asp	Leu	Lys	Lys	Pro	Gly	Gly	Asn	Val	Thr	Gly
Val	Ser	Asp	His	Asn	Pro	Ala	Gln	Gln	Gln	Val	Glu	Leu	Ile	Lys	Ala
Leu	Thr	Pro	Asn	Val	Lys	Thr	Ile	Gly	Ala	Leu	Tyr	Ser	Ser	Ser	Glu
Asp	Asn	Ser	Lys	Thr	Gln	Val	Glu	Glu	Phe	Lys	Ala	Tyr	Ala	Glu	Lys
145					150					155					160
Ala	Gly	Leu	Thr	Val	Glu	Thr	Phe	Ala	Val	Pro	Ser	Thr	Asn	Glu	Ile
					165					170					175
Ala	Ser	Thr	Val	Thr	Val	Met	Thr	Ser	Lys	Val	Asp	Ala	Ile	Trp	Val
					180					185					190
Pro	Ile	Asp	Asn	Thr	Ile	Ala	Ser	Gly	Phe	Pro	Thr	Val	Val	Ser	Ser
Asn	Gln	Ser	Ser	Lys	Lys	Pro	Ile	Tyr	Pro	Ser	Ala	Thr	Ala	Met	Val
Glu	Val	Gly	Gly	Leu	Ala	Ser	Val	Val	Ile	Asp	Gln	His	Asp	Leu	Gly
225					230					235					240
Val	Ala	Thr	Gly	Lys	Met	Ile	Val	Gln	Val	Leu	Lys	Gly	Ala	Trp	Pro
					245					250					255
Ala	Asp	Thr	Pro	Val	Asn	Val	Tyr	Asn	Ser	Asp	Asn	Val	Tyr	Ile	Met
					260					265					270
Ala	Asp	Lys	Gln	Lys	Asn	Gly	Ile	Lys	Ala	Asn	Phe	Lys	Ile	Arg	His
					275					280					285
Asn	Val	Glu	Asp	Gly	Ser	Val	Gln	Leu	Ala	Asp	His	Tyr	Gln	Gln	Asn

290	295	300
Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu		
305	310	315
Ser Phe Gln Ser Val Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His		320
	325	330
Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met		335
	340	345
Asp Glu Leu Tyr Asn Val Asp Gly Gly Ser Gly Gly Thr Gly Ser Lys		350
	355	360
Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp		365
	370	375
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly		380
385	390	395
Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys Thr Thr Gly		400
	405	410
Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Gly Tyr Gly		415
	420	425
Leu Lys Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe		430
	435	440
Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe		445
	450	455
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu		460
465	470	475
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys		480
	485	490
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Thr Gly Lys		495
	500	505
Ser Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile Pro		510
	515	520
Glu Ser Val Leu Lys Glu Ala Gly Gln Val Ile		525
	530	535
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<212> PRT		
<213> Artificial Sequence		
<400> 31		
Ser Asn Ala Lys Ile Gly Val Leu Gln Phe Val Ser His Pro Ser Leu		
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Asp Leu Ile Tyr Lys Gly Ile Gln Asp Gly Leu Ala Glu Glu Gly Tyr		15

20	25	30
Lys Asp Asp Gln Val Lys Ile Asp Phe Met Asn Ser Glu Gly Asp Gln		
35	40	45
Ser Lys Val Ala Thr Met Ser Lys Gln Leu Val Ala Asn Gly Asn Asp		
50	55	60
Leu Val Val Gly Ile Ala Thr Pro Ala Ala Gln Gly Leu Ala Ser Ala		
65	70	75
Thr Lys Asp Leu Pro Val Ile Met Ala Ala Ile Thr Asp Pro Ile Gly		
85	90	95
Ala Asn Leu Val Lys Asp Leu Lys Lys Pro Gly Gly Asn Val Thr Gly		
100	105	110
Val Ser Asp His Asn Pro Ala Gln Gln Gln Val Glu Leu Ile Lys Ala		
115	120	125
Leu Thr Pro Asn Val Lys Thr Ile Gly Ala Leu Tyr Ser Ser Ser Glu		
130	135	140
Asp Asn Ser Lys Thr Gln Val Glu Glu Phe Lys Ala Tyr Ala Glu Lys		
145	150	155
Ala Gly Leu Thr Val Glu Thr Phe Ala Val Pro Ser Thr Asn Glu Ile		
165	170	175
Ala Ser Thr Val Thr Val Met Thr Ser Lys Val Asp Ala Ile Trp Val		
180	185	190
Pro Ile Asp Asn Thr Ile Ala Ser Gly Phe Pro Thr Val Val Ser Ser		
195	200	205
Asn Gln Ser Ser Lys Lys Pro Ile Tyr Pro Ser Ala Thr Ala Met Val		
210	215	220
Glu Val Gly Gly Leu Ala Ser Val Val Ile Asp Gln His Asp Leu Gly		
225	230	235
Val Ala Thr Gly Lys Met Ile Val Gln Val Leu Lys Gly Ala Lys Pro		
245	250	255
Ala Asp Phe Pro Val Asn Val Tyr Asn Ser Asp Asn Val Tyr Ile Met		
260	265	270
Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His		
275	280	285
Asn Val Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn		
290	295	300
Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu		
305	310	315
Ser Phe Gln Ser Val Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His		
325	330	335

Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met		
340	345	350
Asp Glu Leu Tyr Asn Val Asp Gly Gly Ser Gly Gly Thr Gly Ser Lys		
355	360	365
Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp		
370	375	380
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly		
385	390	395
Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys Thr Thr Gly		
405	410	415
Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Gly Tyr Gly		
420	425	430
Leu Lys Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe		
435	440	445
Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe		
450	455	460
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu		
465	470	475
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys		
485	490	495
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Thr Gly Lys		
500	505	510
Ser Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile Pro		
515	520	525
Glu Ser Val Leu Lys Glu Ala Gly Gln Val Ile		
530	535	
<210> 32		
<211> 539		
<212> PRT		
<213> Artificial Sequence		
<400> 32		
Ser Asn Ala Lys Ile Gly Val Leu Gln Phe Val Ser His Pro Ser Leu		
1	5	10
Asp Leu Ile Tyr Lys Gly Ile Gln Asp Gly Leu Ala Glu Glu Gly Tyr		
20	25	30
Lys Asp Asp Gln Val Lys Ile Asp Phe Met Asn Ser Glu Gly Asp Gln		
35	40	45
Ser Lys Val Ala Thr Met Ser Lys Gln Leu Val Ala Asn Gly Asn Asp		
50	55	60

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

370	375	380
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly		
385	390	395
Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys Thr Thr Gly		400
405	410	415
Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Gly Tyr Gly		
420	425	430
Leu Lys Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe		
435	440	445
Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe		
450	455	460
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu		
465	470	475
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys		
485	490	495
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Thr Gly Lys		
500	505	510
Ser Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile Pro		
515	520	525
Glu Ser Val Leu Lys Glu Ala Gly Gln Val Ile		
530	535	
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Ser Asn Ala Lys Ile Gly Val Leu Gln Phe Val Ser His Pro Ser Leu		
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Asp Leu Ile Tyr Lys Gly Ile Gln Asp Gly Leu Ala Glu Glu Gly Tyr		
20	25	30
Lys Asp Asp Gln Val Lys Ile Asp Phe Met Asn Ser Glu Gly Asp Gln		
35	40	45
Ser Lys Val Ala Thr Met Ser Lys Gln Leu Val Ala Asn Gly Asn Asp		
50	55	60
Leu Val Val Gly Ile Ala Thr Pro Ala Ala Gln Gly Leu Ala Ser Ala		
65	70	75
Thr Lys Asp Leu Pro Val Ile Met Ala Ala Ile Thr Asp Pro Ile Gly		
85	90	95
Ala Asn Leu Val Lys Asp Leu Lys Lys Pro Gly Gly Asn Val Thr Gly		

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

Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Gly Tyr Gly
 420 425 430
 Leu Lys Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe
 435 440 445
 Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe
 450 455 460
 Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu
 465 470 475 480
 Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys
 485 490 495
 Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Thr Gly Lys
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 Ser Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Phe Pro
 515 520 525
 Glu Ser Val Leu Lys Glu Ala Gly Gln Val Ile
 530 535
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 <211> 538
 <212> PRT
 <213> Artificial Sequence
 <400> 34
 Ser Asn Ala Lys Ile Gly Val Leu Gln Phe Val Ser His Pro Ser Leu
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 Asp Leu Ile Tyr Lys Gly Ile Gln Asp Gly Leu Ala Glu Glu Gly Tyr
 20 25 30
 Lys Asp Asp Gln Val Lys Ile Asp Phe Met Asn Ser Glu Gly Asp Gln
 35 40 45
 Ser Lys Val Ala Thr Met Ser Lys Gln Leu Val Ala Asn Gly Asn Asp
 50 55 60
 Leu Val Val Gly Ile Ala Thr Pro Ala Ala Gln Gly Leu Ala Ser Ala
 65 70 75 80
 Thr Lys Asp Phe Pro Val Ile Met Ala Ala Ile Thr Asp Pro Ile Gly
 85 90 95
 Ala Asn Leu Val Lys Asp Leu Lys Lys Pro Gly Gly Asn Val Thr Gly
 100 105 110
 Val Ser Asp His Asn Pro Ala Gln Gln Gln Val Glu Leu Ile Lys Ala
 115 120 125
 Leu Thr Pro Asn Val Lys Thr Ile Gly Ala Leu Tyr Ser Ser Ser Glu
 130 135 140

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

450	455	460
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu		
465	470	475
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys		
485	490	495
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Gly Lys Ser		
500	505	510
Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile Pro Glu		
515	520	525
Ser Val Leu Lys Glu Ala Gly Gln Val Ile		
530	535	
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<213> Artificial Sequence		
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Asp Leu Ile Tyr Lys Gly Ile Gln Asp Gly Leu Ala Glu Glu Gly Tyr		
20	25	30
Lys Asp Asp Gln Val Lys Ile Asp Phe Met Asn Ser Glu Gly Asp Gln		
35	40	45
Ser Lys Val Ala Thr Met Ser Lys Gln Leu Val Ala Asn Gly Asn Asp		
50	55	60
Leu Val Val Gly Ile Ala Thr Pro Ala Ala Gln Gly Leu Ala Ser Ala		
65	70	75
Thr Lys Asp Leu Pro Val Ile Met Ala Ala Ile Thr Asp Pro Ile Gly		
85	90	95
Ala Asn Leu Val Lys Asp Leu Lys Phe Pro Gly Gly Asn Val Thr Gly		
100	105	110
Val Ser Asp His Asn Pro Ala Gln Gln Gln Val Glu Leu Ile Lys Ala		
115	120	125
Leu Thr Pro Asn Val Lys Thr Ile Gly Ala Leu Tyr Ser Ser Ser Glu		
130	135	140
Asp Asn Ser Lys Thr Gln Val Glu Glu Phe Lys Ala Tyr Ala Glu Lys		
145	150	155
Ala Gly Leu Thr Val Glu Thr Phe Ala Val Pro Ser Thr Asn Glu Ile		
165	170	175
Ala Ser Thr Val Thr Val Met Thr Ser Lys Val Asp Ala Ile Trp Val		

180	185	190
Pro Ile Asp Asn Thr Ile Ala Ser Gly Phe Pro Thr Val Val Ser Ser		
195	200	205
Asn Gln Ser Ser Lys Lys Pro Ile Tyr Pro Ser Ala Thr Ala Met Val		
210	215	220
Glu Val Gly Gly Leu Ala Ser Val Val Ile Asp Gln His Asp Leu Gly		
225	230	235
Val Ala Thr Gly Lys Met Ile Val Gln Val Leu Lys Gly Ala Lys Pro		
245	250	255
Ala Asp Thr Pro Val Asn Val Tyr Asn Ser Asp Asn Val Tyr Ile Met		
260	265	270
Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His		
275	280	285
Asn Val Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn		
290	295	300
Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu		
305	310	315
Ser Phe Gln Ser Val Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His		
325	330	335
Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met		
340	345	350
Asp Glu Leu Tyr Asn Val Asp Gly Gly Ser Gly Gly Thr Gly Ser Lys		
355	360	365
Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp		
370	375	380
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly		
385	390	395
Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys Thr Thr Gly		
405	410	415
Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Gly Tyr Gly		
420	425	430
Leu Lys Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe		
435	440	445
Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe		
450	455	460
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu		
465	470	475
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys		
485	490	495

Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Gly Lys Ser
 500 505 510
 Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile Pro Glu
 515 520 525
 Ser Val Leu Lys Glu Ala Gly Gln Val Ile
 530 535
 <210> 36
 <211> 538
 <212> PRT
 <213> Artificial Sequence
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 Asp Leu Ile Tyr Lys Gly Ile Gln Asp Gly Leu Ala Glu Glu Gly Tyr
 20 25 30
 Lys Asp Asp Gln Val Lys Ile Asp Phe Met Asn Ser Glu Gly Asp Gln
 35 40 45
 Ser Lys Val Ala Thr Met Ser Lys Gln Leu Val Ala Asn Gly Asn Asp
 50 55 60
 Leu Val Val Gly Ile Ala Thr Pro Ala Ala Gln Gly Leu Ala Ser Ala
 65 70 75 80
 Thr Lys Asp Leu Pro Val Ile Met Ala Ala Ile Thr Asp Pro Ile Gly
 85 90 95
 Ala Asn Leu Val Lys Asp Leu Lys Trp Pro Gly Gly Asn Val Thr Gly
 100 105 110
 Val Ser Asp His Asn Pro Ala Gln Gln Gln Val Glu Leu Ile Lys Ala
 115 120 125
 Leu Thr Pro Asn Val Lys Thr Ile Gly Ala Leu Tyr Ser Ser Ser Glu
 130 135 140
 Asp Asn Ser Lys Thr Gln Val Glu Glu Phe Lys Ala Tyr Ala Glu Lys
 145 150 155 160
 Ala Gly Leu Thr Val Glu Thr Phe Ala Val Pro Ser Thr Asn Glu Ile
 165 170 175
 Ala Ser Thr Val Thr Val Met Thr Ser Lys Val Asp Ala Ile Trp Val
 180 185 190
 Pro Ile Asp Asn Thr Ile Ala Ser Gly Phe Pro Thr Val Val Ser Ser
 195 200 205
 Asn Gln Ser Ser Lys Lys Pro Ile Tyr Pro Ser Ala Thr Ala Met Val
 210 215 220

Glu Val Gly Gly Leu Ala Ser Val Val Ile Asp Gln His Asp Leu Gly			
225	230	235	240
Val Ala Thr Gly Lys Met Ile Val Gln Val Leu Lys Gly Ala Lys Pro			
	245	250	255
Ala Asp Thr Pro Val Asn Val Tyr Asn Ser Asp Asn Val Tyr Ile Met			
	260	265	270
Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His			
	275	280	285
Asn Val Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn			
	290	295	300
Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu			
305	310	315	320
Ser Phe Gln Ser Val Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His			
	325	330	335
Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met			
	340	345	350
Asp Glu Leu Tyr Asn Val Asp Gly Gly Ser Gly Gly Thr Gly Ser Lys			
	355	360	365
Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp			
	370	375	380
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly			
385	390	395	400
Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys Thr Thr Gly			
	405	410	415
Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Gly Tyr Gly			
	420	425	430
Leu Lys Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe			
	435	440	445
Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe			
	450	455	460
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu			
465	470	475	480
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys			
	485	490	495
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Gly Lys Ser			
	500	505	510
Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile Pro Glu			
	515	520	525
Ser Val Leu Lys Glu Ala Gly Gln Val Ile			

530 535
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 <211> 538
 <212> PRT
 <213> Artificial Sequence
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 Ser Asn Ala Lys Ile Gly Val Leu Gln Phe Val Ser His Pro Ser Leu
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 Asp Leu Ile Tyr Lys Gly Ile Gln Asp Gly Leu Ala Glu Glu Gly Tyr
 20 25 30
 Lys Asp Asp Gln Val Lys Ile Asp Phe Met Asn Ser Glu Gly Asp Gln
 35 40 45
 Ser Lys Val Ala Thr Met Ser Lys Gln Leu Val Ala Asn Gly Asn Asp
 50 55 60
 Leu Val Val Gly Ile Ala Thr Pro Ala Ala Gln Gly Leu Ala Ser Ala
 65 70 75 80
 Thr Lys Asp Leu Pro Val Ile Met Ala Ala Ile Thr Asp Pro Ile Gly
 85 90 95
 Ala Asn Leu Val Lys Asp Leu Lys Lys Pro Gly Gly Asn Val Thr Gly
 100 105 110
 Val Ser Asp His Asn Pro Ala Gln Gln Gln Val Glu Leu Ile Lys Ala
 115 120 125
 Leu Thr Pro Asn Val Lys Thr Ile Gly Ala Leu Tyr Ser Ser Ser Glu
 130 135 140
 Asp Asn Ser Lys Thr Gln Val Glu Glu Phe Lys Ala Tyr Ala Glu Lys
 145 150 155 160
 Ala Gly Leu Thr Val Glu Thr Phe Ala Val Pro Ser Thr Asn Glu Ile
 165 170 175
 Ala Ser Thr Val Thr Val Met Thr Ser Lys Val Asp Ala Ile Trp Val
 180 185 190
 Pro Ile Asp Asn Thr Ile Ala Ser Gly Trp Pro Thr Val Val Ser Ser
 195 200 205
 Asn Gln Ser Ser Lys Lys Pro Ile Tyr Pro Ser Ala Thr Ala Met Val
 210 215 220
 Glu Val Gly Gly Leu Ala Ser Val Val Ile Asp Gln His Asp Leu Gly
 225 230 235 240
 Val Ala Thr Gly Lys Met Ile Val Gln Val Leu Lys Gly Ala Lys Pro
 245 250 255
 Ala Asp Thr Pro Val Asn Val Tyr Asn Ser Asp Asn Val Tyr Ile Met

260	265	270
Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His		
275	280	285
Asn Val Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn		
290	295	300
Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu		
305	310	315
Ser Phe Gln Ser Val Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His		
325	330	335
Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met		
340	345	350
Asp Glu Leu Tyr Asn Val Asp Gly Gly Ser Gly Gly Thr Gly Ser Lys		
355	360	365
Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp		
370	375	380
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly		
385	390	395
Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys Thr Thr Gly		
405	410	415
Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Gly Tyr Gly		
420	425	430
Leu Lys Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe		
435	440	445
Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe		
450	455	460
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu		
465	470	475
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys		
485	490	495
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Gly Lys Ser		
500	505	510
Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile Pro Glu		
515	520	525
Ser Val Leu Lys Glu Ala Gly Gln Val Ile		
530	535	
<210> 38		
<211> 538		
<212> PRT		
<213> Artificial Sequence		

<400> 38

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

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Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu			
305	310	315	320
Ser Phe Gln Ser Val Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His			
	325	330	335
Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met			
	340	345	350
Asp Glu Leu Tyr Asn Val Asp Gly Gly Ser Gly Gly Thr Gly Ser Lys			
	355	360	365
Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp			
	370	375	380
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly			
385	390	395	400
Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys Thr Thr Gly			
	405	410	415
Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Gly Tyr Gly			
	420	425	430
Leu Lys Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe			
	435	440	445
Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe			
	450	455	460
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu			
465	470	475	480
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Gly Phe Lys			
	485	490	495
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Gly Lys Ser			
	500	505	510
Val Ile Asn Lys Lys Ile Ala Gln Glu Leu Gly Ile Thr Ile Pro Glu			
	515	520	525
Ser Val Leu Lys Glu Ala Gly Gln Val Ile			
	530	535	
<210> 39			
<211> 538			
<212> PRT			
<213> Artificial Sequence			
<400> 39			
Ser Asn Ala Lys Ile Gly Val Leu Gln Phe Val Ser His Pro Ser Leu			
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Asp Leu Ile Tyr Lys Gly Ile Gln Asp Gly Leu Ala Glu Glu Gly Tyr			
	20	25	30

Lys	Asp	Asp	Gln	Val	Lys	Ile	Asp	Phe	Met	Asn	Ser	Glu	Gly	Asp	Gln				
35					40					45									
Ser	Lys	Val	Ala	Thr	Met	Ser	Lys	Gln	Leu	Val	Ala	Asn	Gly	Asn	Asp				
50					55					60									
Leu	Val	Val	Gly	Ile	Ala	Thr	Pro	Ala	Ala	Gln	Gly	Leu	Ala	Ser	Ala				
65					70					75					80				
Thr	Lys	Asp	Leu	Pro	Val	Ile	Met	Ala	Ala	Ile	Thr	Asp	Pro	Ile	Gly				
85					90					95									
Ala	Asn	Leu	Val	Lys	Asp	Leu	Lys	Lys	Pro	Gly	Gly	Asn	Val	Thr	Gly				
100					105					110									
Val	Ser	Asp	His	Asn	Pro	Ala	Gln	Gln	Gln	Val	Glu	Leu	Ile	Lys	Ala				
115					120					125									
Leu	Thr	Pro	Asn	Val	Lys	Thr	Ile	Gly	Ala	Leu	Tyr	Ser	Ser	Ser	Glu				
130					135					140									
Asp	Asn	Ser	Lys	Thr	Gln	Val	Glu	Glu	Phe	Lys	Ala	Tyr	Ala	Glu	Lys				
145					150					155					160				
Ala	Gly	Leu	Thr	Val	Glu	Thr	Phe	Ala	Val	Pro	Ser	Thr	Asn	Glu	Ile				
165					170					175									
Ala	Ser	Thr	Val	Thr	Val	Met	Thr	Ser	Lys	Val	Asp	Ala	Ile	Trp	Val				
180					185					190									
Pro	Ile	Asp	Asn	Thr	Ile	Ala	Ser	Gly	Phe	Pro	Thr	Val	Val	Ser	Ser				
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325					330					335									
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370	375	380
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385	390	395
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Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu		
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485	490	495
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Gly Lys Ser		
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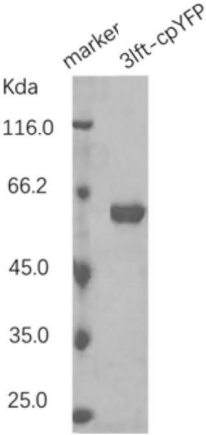


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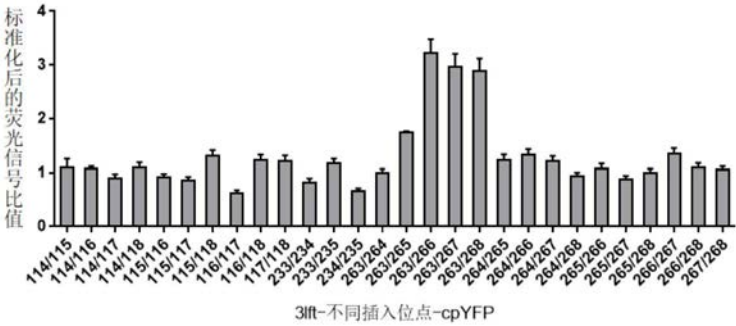


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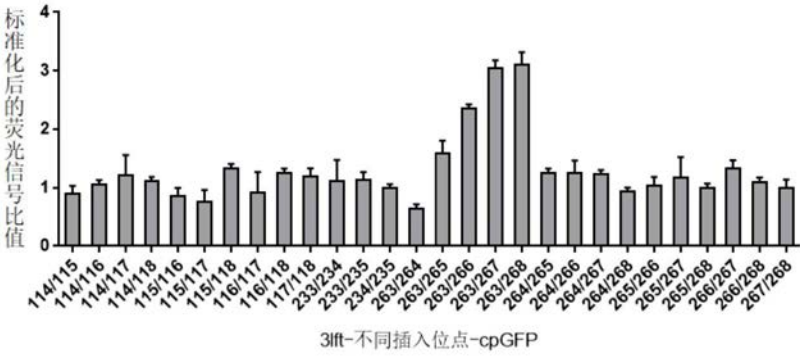


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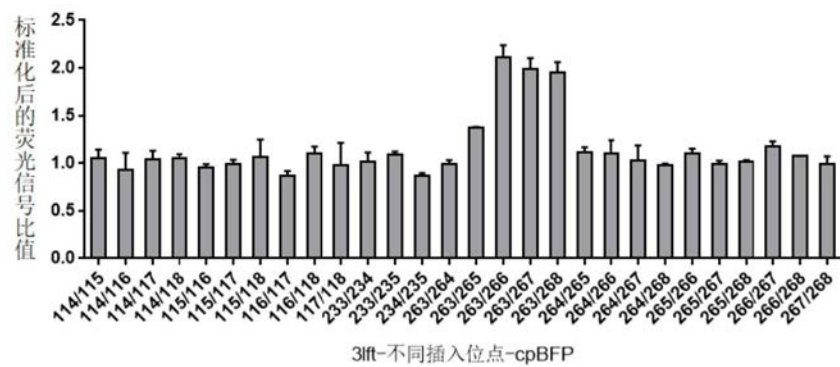


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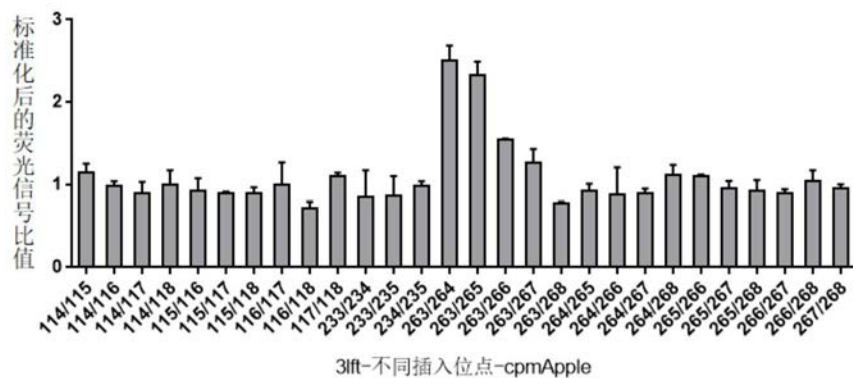


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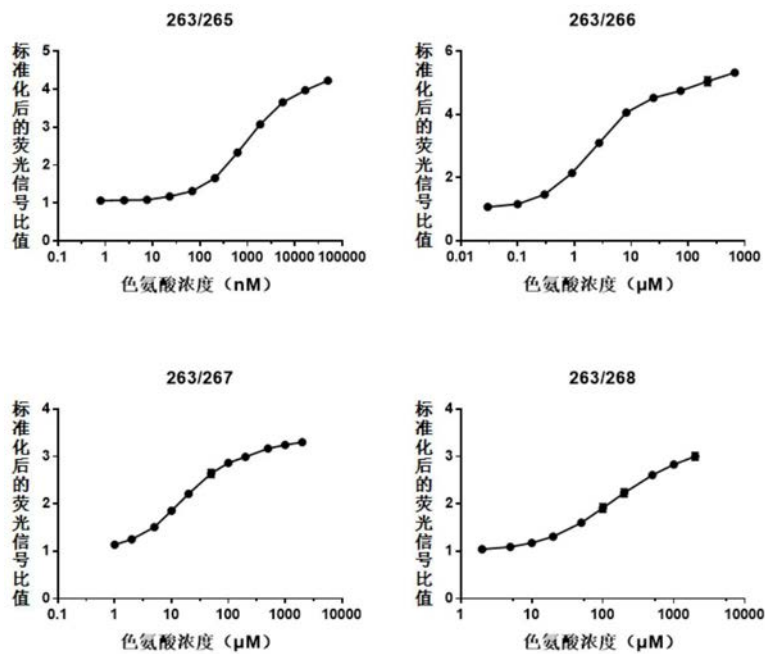


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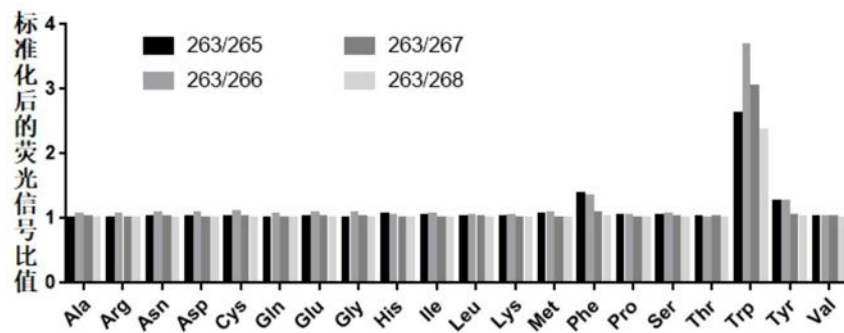


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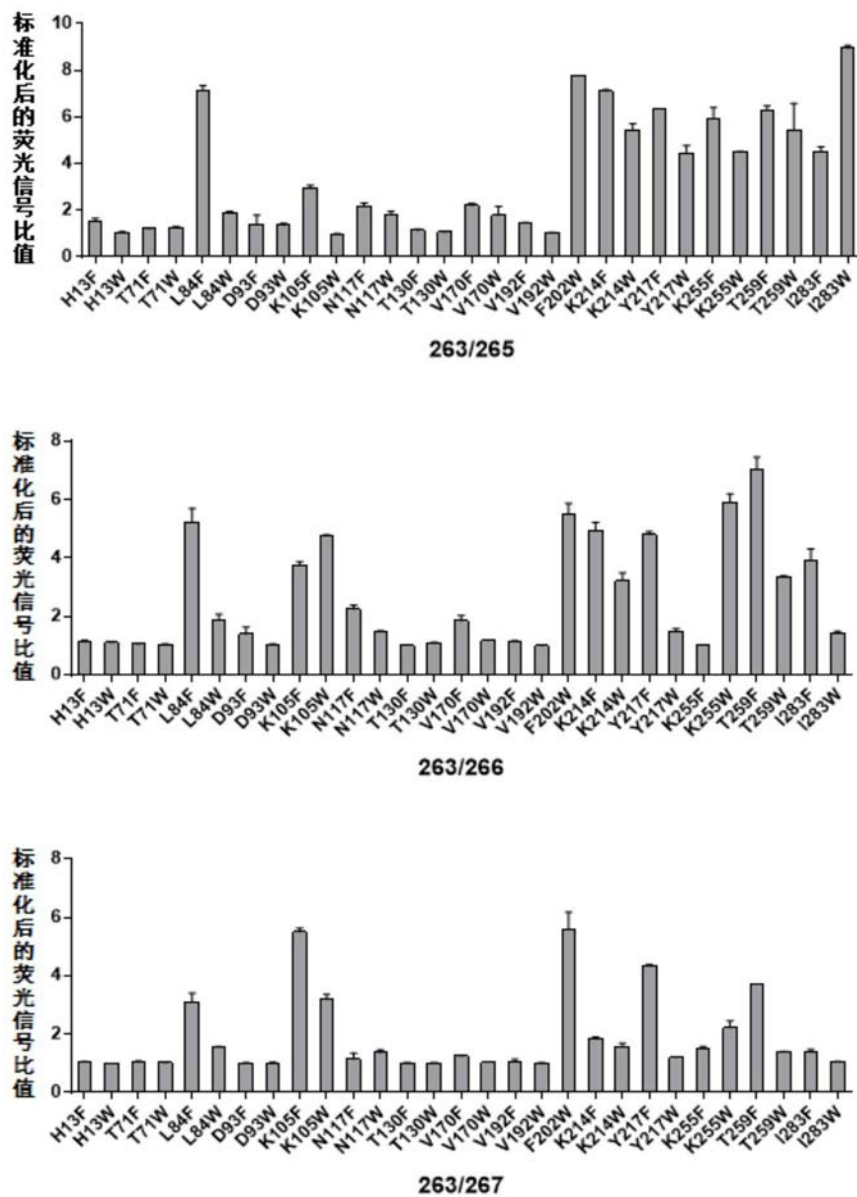


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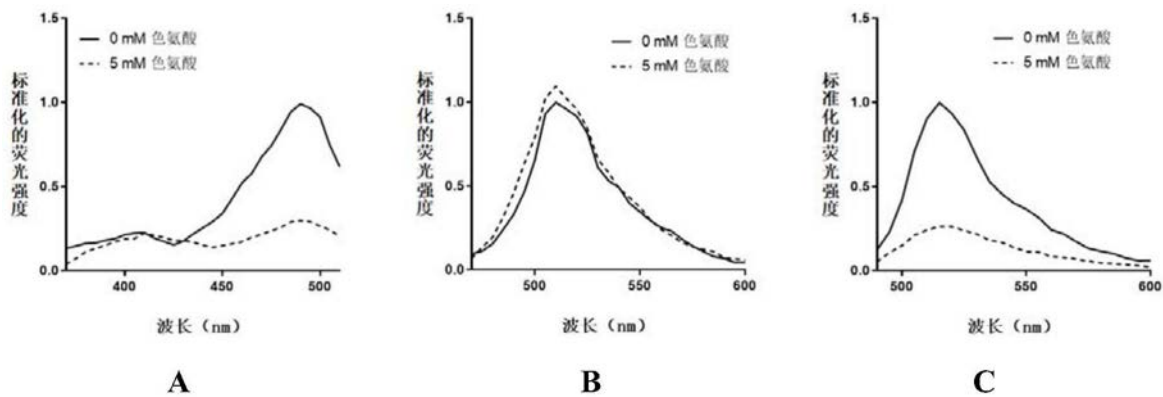


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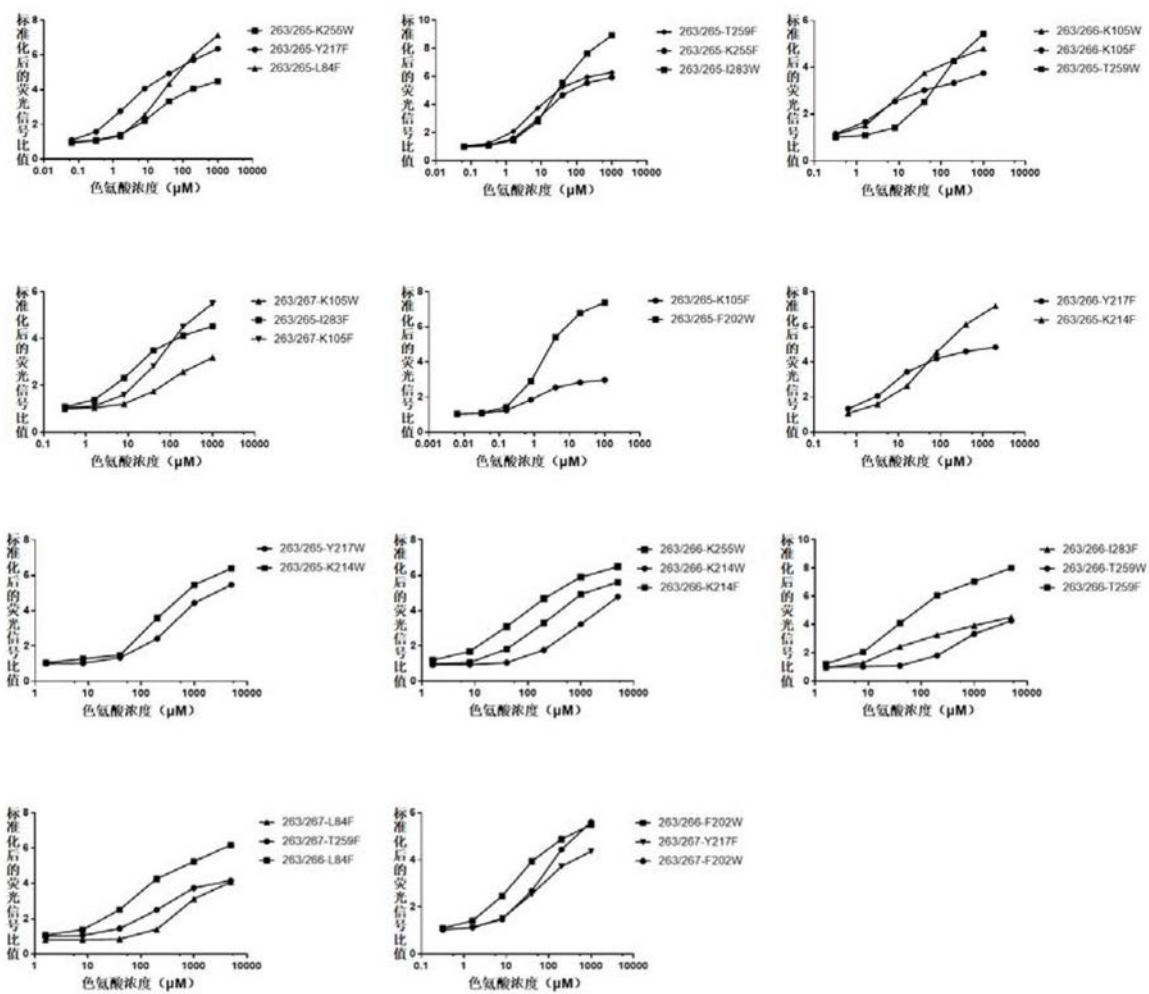


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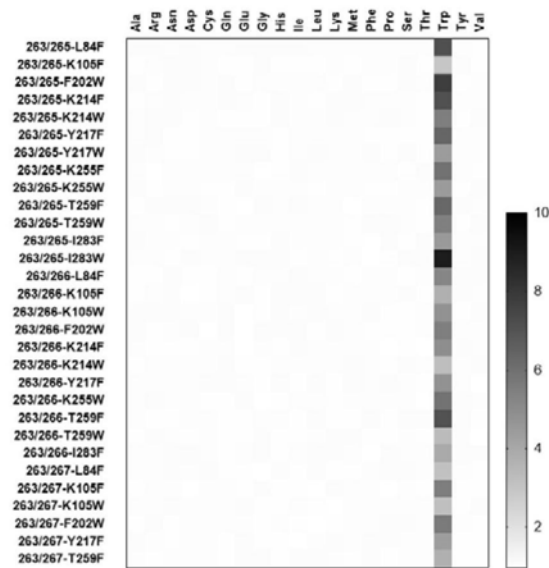


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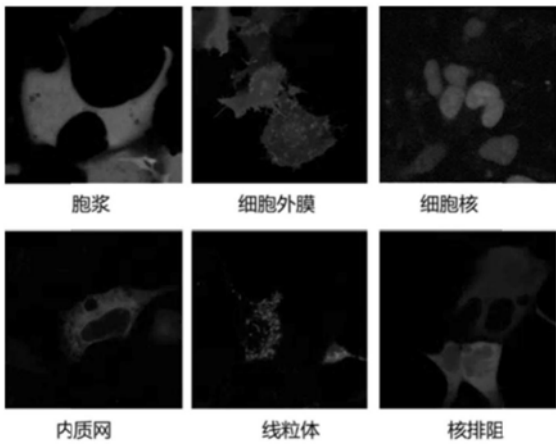


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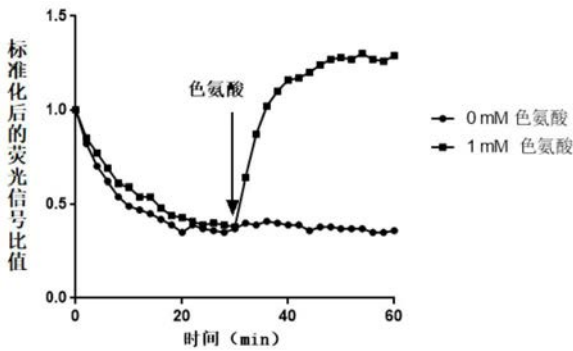


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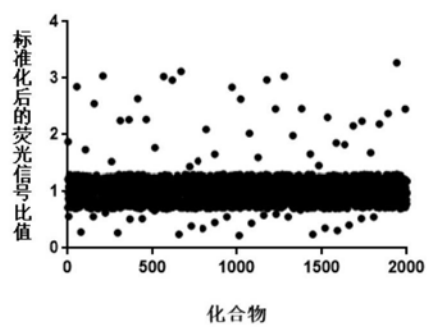


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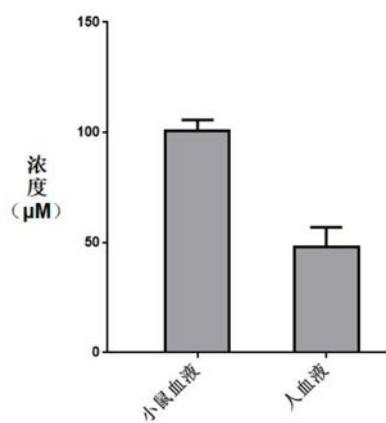


图15