



(12) 发明专利



(10) 授权公告号 CN 112673098 B

(45) 授权公告日 2023. 01. 06

(21) 申请号 201980043453.3

S.佩萨乔维奇 M.何

(22) 申请日 2019.10.31

(74) 专利代理机构 北京市柳沈律师事务所

11105

(65) 同一申请的已公布的文献号

专利代理师 邹宗亮

申请公布号 CN 112673098 A

(43) 申请公布日 2021.04.16

(51) Int.Cl.

(30) 优先权数据

C12N 9/12 (2006.01)

62/753,558 2018.10.31 US

C12Q 1/6844 (2006.01)

(85) PCT国际申请进入国家阶段日

(56) 对比文件

2020.12.25

US 2015024463 A1,2015.01.22

(86) PCT国际申请的申请数据

US 2018119115 A1,2018.05.03

PCT/US2019/059246 2019.10.31

WO 2006037064 A2,2006.04.06

(87) PCT国际申请的公布数据

US 2011301041 A1,2011.12.08

W02020/092830 EN 2020.05.07

HASHIMOTO H 等.Crystal structure of

(73) 专利权人 亿明达股份有限公司

DNA polymerase from hyperthermophilic

地址 美国加利福尼亚州

archaeon pyrococcus kodakaraensis KOD1.

专利权人 亿明达新加坡私人有限公司

《JOURNAL OF MOLECULAR BIOLOGY》.2001,第306

卷(第3期),

(72) 发明人 M.戈莱恩斯基 S.麦克唐纳

审查员 安玉苹

S.尼兰塔 M.凯林格 M.普雷维特

权利要求书1页 说明书26页

序列表92页 附图11页

(54) 发明名称

聚合酶、组合物及使用方法

(57) 摘要

本文提出了用于改善核苷酸和核苷酸类似物的掺入的改变的聚合酶,特别是在减少的掺入时间下保持高保真度的改变的聚合酶,以及使用它们的方法和试剂盒。

1. 重组DNA聚合酶,其选自9°N DNA聚合酶氨基酸序列SEQ ID NO:10-34。
2. 权利要求1的重组DNA聚合酶,其中9°N DNA聚合酶氨基酸序列选自SEQ ID NO:10-11,23-25和27-34。

聚合酶、组合物及使用方法

[0001] 对相关申请的交叉引用

[0002] 本申请要求2018年10月31日提交的美国临时申请系列No.62/753,558的权益,其通过引用完整并入本文。

[0003] 序列表

[0004] 本申请包含通过EFS-Web电子提交给美国专利商标局的序列表,其为ASCII文本文件,名称为“IP-1546-PCT_ST25.txt”,大小为224千字节并且创建于2019年10月31日。序列表中包含的信息通过引用并入本文。

技术领域

[0005] 本公开尤其涉及用于进行核苷酸掺入反应的改变的聚合酶,特别是在核酸合成测序的情况下。

背景技术

[0006] 下一代测序(NGS)技术依赖于DNA聚合酶作为测序过程的关键组成。希望在保持高保真度的同时减少模板测序的时间。减少合成测序(sequencing by synthesis, SBS)过程的每个循环是实现较短测序运行时间的有用步骤。减少循环时间的一种方法是减少掺入步骤的时间。然而,虽然减少掺入时间可以提供整体运行时间的显著改善,但它们通常会以保真度为代价。例如,定相率(phasing rate),预定相率(pre-phasing rate)和/或旁路率(bypass rate)增加,结果是错误率增加。在低错误率下,在测序运行期间,簇中的大多数模板分子都终止于相同的标记核苷酸,并且信号清晰。相反,在降低的保真度下,在测序运行期间,簇中越来越多数目的模板分子终止于不正确的标记核苷酸,并且信号可变得过于嘈杂而无法准确确定掺入了哪个核苷酸。

发明内容

[0007] 本文提供了重组DNA聚合酶。本公开的聚合酶的一个实例包括与9°N DNA聚合酶氨基酸序列SEQ ID NO:1至少80%相同的氨基酸序列。

[0008] 在一个实施方案中,聚合酶还包括在功能上等同于Tyr497的位置处的氨基酸取代突变和在功能上等同于9°N DNA聚合酶氨基酸序列中的Phe152、Val278、Met329、Val471、Thr514、Leu631或Glu734的位置处的至少一个氨基酸取代突变,并且任选地还包括在功能上等同于9°N DNA聚合酶氨基酸序列中的Met129、Asp141、Glu143、Cys223、Leu408、Tyr409、Pro410和Ala485的位置处的氨基酸取代突变。

[0009] 在一个实施方案中,聚合酶还包括在功能上等同于Tyr497的位置处的氨基酸取代突变和在功能上等同于9°N DNA聚合酶氨基酸序列中的Lys476、Lys477、Thr514、Ile521或Thr590的位置处的至少一个氨基酸取代突变,并且任选地还包括在功能上等同于9°N DNA聚合酶氨基酸序列中的Met129、Asp141、Glu143、Cys223、Leu408、Tyr409、Pro410和Ala485的位置处的氨基酸取代突变。

[0010] 在一个实施方案中,聚合酶还包含在功能上等同于Tyr497的位置处的氨基酸取代突变和在功能上等同于9°N DNA聚合酶氨基酸序列中的Arg247、Glu599、Lys620、His633或Val661的位置处的至少一个氨基酸取代突变,并且任选地还包括在功能上等同于9°N DNA聚合酶氨基酸序列中的Met129、Asp141、Glu143、Cys223、Leu408、Tyr409、Pro410和Ala485的位置处的氨基酸取代突变。

[0011] 在一个实施方案中,聚合酶还包括(i)在功能上等同于Tyr497的位置处的氨基酸取代突变;(ii)在功能上等同于9°N DNA聚合酶氨基酸序列中的Phe152、Val278、Met329、Val471、Leu631或Glu734的位置处的至少一个氨基酸取代突变,和(iii)在功能上等同于9°N DNA聚合酶氨基酸序列中的Lys476、Lys477、Thr514、Ile521或Thr590的位置处的至少一个氨基酸取代突变,并且任选地还包括在功能上等同于9°N DNA聚合酶氨基酸序列中的Met129、Asp141、Glu143、Cys223、Leu408、Tyr409、Pro410和Ala485的位置处的氨基酸取代突变。

[0012] 在一个实施方案中,聚合酶还包括(i)在功能上等同于Tyr497的位置处的氨基酸取代突变;(ii)在功能上等同于9°N DNA聚合酶氨基酸序列中的Lys476、Lys477、Thr514、Ile521或Thr590的位置处的至少一个氨基酸取代突变,和(iii)在功能上等同于9°N DNA聚合酶氨基酸序列中的Arg247、Glu599、Lys620、His633或Val661的位置处的至少一个氨基酸取代突变,并且任选地还包括在功能上等同于9°N DNA聚合酶氨基酸序列中的Met129、Asp141、Glu143、Cys223、Leu408、Tyr409、Pro410和Ala485的位置处的氨基酸取代突变。

[0013] 在一个实施方案中,聚合酶还包括(i)在功能上等同于Tyr497的位置处的氨基酸取代突变;(ii)在功能上等同于9°N DNA聚合酶氨基酸序列中的Phe152、Val278、Met329、Val471、Leu631或Glu734的位置处的至少一个氨基酸取代突变,(iii)在功能上等同于9°N DNA聚合酶氨基酸序列中的Lys476、Lys477、Thr514、Ile521或Thr590的位置处的至少一个氨基酸取代突变,和(iv)在功能上等同于9°N DNA聚合酶氨基酸序列中的Arg247、Glu599、Lys620、His633或Val661的位置处的至少一个氨基酸取代突变,并且任选地还包括在功能上等同于9°N DNA聚合酶氨基酸序列中的Met129、Asp141、Glu143、Cys223、Leu408、Tyr409、Pro410和Ala485的位置处的氨基酸取代突变。

[0014] 本公开的聚合酶的另一个实例包括与9°N DNA聚合酶氨基酸序列SEQ ID NO:8至少80%相同的氨基酸序列,并且还包括(i)在功能上等同于9°N DNA聚合酶氨基酸序列中的Tyr497、Phe152、Val278、Met329、Val471和Thr514的位置处的氨基酸取代突变;(ii)在功能上等同于9°N DNA聚合酶氨基酸序列中的Tyr497、Met329、Val471和Glu734的位置处的氨基酸取代突变;(iii)在功能上等同于9°N DNA聚合酶氨基酸序列中的Tyr497、Arg247、Glu599和His633的位置处的氨基酸取代突变;(iv)在功能上等同于9°N DNA聚合酶氨基酸序列中的Tyr497、Arg247、Glu599、Lys620和His633的位置处的氨基酸取代突变;(v)在功能上等同于9°N DNA聚合酶氨基酸序列中的Tyr497、Met329、Thr514、Lys620和Val661的位置处的氨基酸取代突变;(vi)在功能上等同于9°N DNA聚合酶氨基酸序列中的Tyr497、Val278、Val471、Arg247、Glu599和His633的位置处的氨基酸取代突变。

[0015] 本文还提供了重组DNA聚合酶,其包含SEQ ID NO:10-34中任一个的氨基酸序列、编码本文所述聚合酶的核酸分子、包括该核酸分子的表达载体、以及包含该载体的宿主细胞。

[0016] 本公开还包括方法。在一个实施方案中,方法用于将修饰的核苷酸掺入正在生长的DNA链中。该方法包括允许以下组分相互作用:(i)本文所述的聚合酶;(ii)DNA模板;和(iii)核苷酸溶液。

[0017] 本文还提供了试剂盒。在一个实施方案中,该试剂盒用于进行核苷酸掺入反应。试剂盒可包括例如本文所述的聚合酶和核苷酸溶液。

[0018] 附图简述

[0019] 图1示出了来自热球菌属种(*Thermococcus* sp.) 9°N-7 (9°N, SEQ ID NO:1), 海滨热球菌(*Thermococcus litoralis*) (Vent, SEQ ID NO:2和Deep Vent, SEQ ID NO:3), *Thermococcus waiotapuensis* (Twa, SEQ ID NO:7), 小宝岛热球菌(*Thermococcus kodakaraensis*) (KOD, SEQ ID NO:5), 激烈火球菌(*Pyrococcus furiosus*) (Pfu, SEQ ID NO:4), 深海火球菌(*Pyrococcus abyssi*) (Pab, SEQ ID NO:6)的聚合酶氨基酸序列的比对的示意图。“*” (星号)表示在所有聚合酶之间具有单个完全保守残基的位置。“:” (冒号)表示强相似特性的组之间的保守性,如下所示-大致等于Gonnet PAM 250矩阵中的得分 >0.5 。“.” (句点)表示弱相似特性的组之间的保守性-大致等于Gonnet PAM 250矩阵中的得分 $= < 0.5$ 且 >0 。

[0020] 图2示出了当与Pol 812 (SEQ ID NO:8)对照(左图)进行比较时,由本公开的改变的聚合酶之一Pol 1558 (SEQ ID NO:11)证明的在短掺入时间时减少的定相和累积错误率。两种酶在标准掺入时间时显示出相当的定相和错误率(右图)。

[0021] 图3A示出了当与Pol 812 (SEQ ID NO:8)和Pol 963 (SEQ ID NO:9)对照进行比较时,由本公开的所选的改变的聚合酶,Pol 1558 (SEQ ID NO:11), Pol 1671 (SEQ ID NO:23), Pol 1682 (SEQ ID NO:25)和Pol 1745 (SEQ ID NO:28)证明的在短掺入时间时减少的R1定相和大肠杆菌累积错误率。上部 and 下部图中的虚线表示在标准掺入时间时由Pol 812所证明的累积大肠杆菌错误和R1定相率。图3B参照Pol 812和Pol 963对照比较了相同的改变的聚合酶的定相率和预定相率。

[0022] 图4比较了在长测序读段(2x250个循环)期间在标准和短掺入时间时,Pol 1550 (SEQ ID NO:10)和Pol 1558 (SEQ ID NO:11)与Pol 812 (SEQ ID NO:8)对照的累积PhiX错误率。这两个突变体在配对末端转角(paired-end turn)后均显示出错误率的明显降低。

[0023] 图5A示出了在短掺入时间时证明的本发明的改变的聚合酶之一Pol 1671 (SEQ ID NO:23)的NovaSeq™测序度量和在标准和短的掺入时间时证明的Pol 812 (SEQ ID NO:8)对照的NovaSeq™测序度量之间的比较。上图示出了通过过滤器的簇(“簇PF”)的百分比;下图示出了累积的PhiX错误率。浅色空心圆圈表示在标准掺入时间时的Pol 812度量,而深色空心圆圈表示在短掺入时间时的Pol 812度量。实心圆圈表示的所有Pol 1671度量均处于短掺入时间。图5B总结了在标准和短掺入时间时对于NovaSeq™读段1和2,参照Pol 812对照由Pol 1671所显示的累积PhiX错误率、Q30值和定相率。使用Pol 1671时观察到这两个读段的质量的显著改善。

[0024] 发明详述

[0025] 术语“和/或”意指所列要素中的一个或全部,或所列要素中的任何两个或更多个的组合。

[0026] 词语“优选的”和“优选地”是指在某些情况下可以提供某些益处的本发明的实施

方案。然而,在相同或其他情况下,其他实施方案也可以是优选的。此外,对一个或多个优选实施方案的叙述并不意味着其他实施方案不可用,并且不旨在将其他实施方案排除在本发明的范围之外。

[0027] 术语“包括”及其变型在说明书和权利要求书中出现这些术语时不具有限制的含义。

[0028] 应当理解的是,无论在何处用语言“包括”等描述实施方案,也提供了以“由...组成”和/或“基本上由...组成”的术语描述的其它方面类似的实施方案”。

[0029] 除非另有说明,否则“一个”,“一种”,“该/所述”和“至少一个”可互换使用并且表示一个/种或超过一个/种。

[0030] “适合”于事件发生的条件或“适合”条件是不阻止此类事件发生的条件。因此,这些条件允许、增强、促进和/或有利于该事件。

[0031] 如本文所用,在组合物,制品,核酸或核的情况下“提供”意指制造该组合物,制品,核酸或核,购买该组合物,制品,核酸或核,或以其他方式获得该化合物,组合物,制品或核。

[0032] 还在本文,通过端点叙述数值范围包括该范围内包含的所有数字(例如1至5包括1、1.5、2、2.75、3、3.80、4、5等)。

[0033] 在整个说明书中,对“一个实施方案”,“实施方案”,“某些实施方案”或“一些实施方案”等的引用意指结合该实施方案描述的特定特征、构造、组成或特性包括在本公开的至少一个实施方案中。因此,在整个说明书中这些短语在各个地方的出现不一定是指本公开的相同实施方案。此外,在一个或多个实施方案中,可以以任何合适的方式组合特定的特征,构造,组成或特性。

[0034] 新一代的聚合酶可以帮助以更快的掺入时间维持或超过当前的性能水平。本文提出的是在合成测序(SBS)的快速循环时间条件下具有显著改善的性能的聚合酶。令人惊讶地,发明人鉴定了某些改变的聚合酶,其在短的掺入时间期间表现出改善的特性,包括提高的准确性。提高的准确性包括降低错误率和降低定相。改变的聚合酶具有许多其他相关的优点,包括在SBS反应中降低的预定相,降低的旁路率和提高的质量指标。即使以较低的浓度使用聚合酶时,也可以保持此种改善。因此,在一个实施方案中,SBS反应中DNA聚合酶的浓度可以从120ng/ μ l至80ng/ μ l。在一个实施方案中,SBS反应中DNA聚合酶的浓度可以不大于120ng/ μ l,不大于110ng/ μ l,不大于100ng/ μ l,或不大于90ng/ μ l。在一个实施方案中,SBS反应中DNA聚合酶的浓度可以为至少80ng/ μ l,至少90ng/ μ l,至少100ng/ μ l或至少110ng/ μ l。

[0035] 错误率是指在测序反应期间正确碱基,即特定位置处的模板序列的互补物的鉴定中错误频率的测量。测序文库与原始基因组序列匹配的保真度可以随从核酸提取到其在测序平台上测序的任何阶段发生的碱基突变的频率而变化。该频率对测序的碱基正确的可能性设置了上限。在一些实施方案中,质量得分表示为数值。例如,质量得分可以用QXX提及,其中XX是得分并且其意指该特定调用(call)的错误概率为 $10^{-xx/10}$ 。因此,作为例子,Q30等于1/1000的错误率,或0.1%,并且Q40等于1/10,000的错误率,即0.01%。

[0036] 定相和预定相是本领域技术人员已知的术语,并且用于描述簇的序列拷贝的读出(readout)中的同步性损失。定相和预定相导致特定循环的提取的强度包括当前循环的信号以及前面和后面循环的噪声。因此,如本文所用,术语“定相”是指在SBS中的现象,该现象

是由在给定的测序循环时在簇内DNA链的某些部分中聚合酶不完全掺入核苷酸引起的,因此是簇内的单一分子彼此失去同步的比率的度量。可以在每个循环检测簇信号期间测量定相,并且可以将其报告为与簇中的信号不同步的来自簇的可检测信号的百分比。作为例子,在循环N期间通过“绿色”荧光团信号检测簇。在随后的循环(循环N+1)中,在“红色”通道中检测到99.9%的簇信号,并且0.1%的信号从先前的循环保留并在“绿色”通道中检测到。该结果将表明正在发生定相,并且可以将其报告为数值,例如定相值为0.1,这表明簇中的分子的0.1%在每个循环中都落下。

[0037] 如本文所用,术语“预定相”是指SBS中的现象,其由在没有有效的3'终止剂的情况下的核苷酸掺入引起,造成掺入事件提前一个循环进行。随着循环数的增加,受定相影响的每个簇的序列分数增加,从而妨碍了对正确碱基的鉴定。可以通过测序仪器检测到预定相,并以数值报告,例如预定相值为0.1,这表明簇中的分子中的0.1%在每个循环时提前运行。

[0038] 可以根据本领域中已知的任何合适的方法来进行定相和预定相的检测并报告,例如,如美国专利号8,965,076中所述。例如,如下面的实施例所述,在SBS测序运行期间,例如在来自Illumina公司(San Diego,CA)的HiSeqTM,Genome AnalyzerTM,NextSeqTM,NovaSeqTM,iSeqTM,MiniSeqTM或MiSeqTM测序平台等测序仪器或本领域已知的任何其他合适的仪器上,定期检测并定期报告。

[0039] 减少的循环时间可以增加定相、预定相和/或旁路率的发生,每种情况都导致错误率。即便在快速循环时间条件下使用时也降低定相,预定相和/或旁路率发生的改变的聚合酶的发现是令人惊讶的,并且在SBS应用中提供了很大的优势。例如,改变的聚合酶可以提供更快的SBS循环时间,更低的定相和预定相值和/或更长的测序读段长度。如本文所提供的改变的聚合酶的错误率和定相的表征在下面的实施例部分中阐述。

[0040] 聚合酶

[0041] 本文提供了聚合酶,包括聚合酶的组合物以及使用聚合酶的方法。本文所述的聚合酶是DNA聚合酶。在一个实施方案中,本公开的聚合酶,在本文中也称为“改变的聚合酶”,是基于参照聚合酶的氨基酸序列。与参照聚合酶相比时,改变的聚合酶在一个或多个残基处包括取代突变。与参照聚合酶相比,取代突变可以在相同位置或功能上等同的位置处。参照聚合酶和功能上等同的位置在本文中详细描述。技术人员将容易理解,本文所述的改变的聚合酶不是天然存在的。

[0042] 本文所述的参照聚合酶具有对SBS反应有用的错误率;然而,以缩短的掺入时间在SBS反应中使用参照聚合酶增加错误率。本文所述的改变的聚合酶即使在较短的掺入时间用于SBS反应中时也保持用参照聚合酶观察到的优异的错误率。在一个实施方案中,当使用快速掺入时间测试改变的聚合酶时,发生降低的错误率。掺入是指DNA聚合酶与模板接触的时间量。如本文所用,缓慢的掺入时间是使用MiniSeqTM台式测序系统在标准循环下使用的掺入时间。缓慢的掺入时间包括40秒至50秒。如本文所用,快速循环时间是指从10秒到40秒的掺入步骤。在一个实施方案中,快速循环时间是不大于40秒,不大于30秒,不大于20秒,不大于18秒,不大于16秒,不大于14秒或不大于12秒的掺入时间。在一个实施方案中,快速循环时间是至少10秒,至少12秒,至少14秒,至少16秒,至少18秒,至少20秒或至少30秒的掺入时间。在一个实施方案中,快速循环时间是少于40秒,少于30秒,少于20秒,少于18秒,少于16秒,少于14秒,少于12秒或少于10秒的掺入时间。

[0043] 本文所述的改变的聚合酶可以用于SBS反应中达不同长度的运行。“运行”是指在模板上鉴定的核苷酸的数目。运行通常包括读取模板的一条链的基于第一引物(例如,读段1引物)的运行和读取模板的互补链的基于第二引物(例如,读段2引物)的运行。在一个实施方案中,使用第一引物或第二引物鉴定的核苷酸的数目可以是10至150个核苷酸。在一个实施方案中,使用第一引物或第二引物鉴定的核苷酸数目可以不大于150个核苷酸,不大于130个核苷酸,不大于110个核苷酸,不大于90个核苷酸,不大于70个核苷酸,大于50个核苷酸,不大于30个核苷酸或不大于20个核苷酸。在一个实施方案中,使用第一引物或第二引物鉴定的核苷酸的数目可以是至少10,至少20,至少30,至少50,至少70,至少90,至少110或至少130个核苷酸。

[0044] 在某些实施方案中,改变的聚合酶基于B族型DNA聚合酶。改变的聚合酶可以基于例如B族古细菌 (archaeal) DNA聚合酶,人DNA聚合酶- α 或噬菌体聚合酶。

[0045] B族古细菌DNA聚合酶在本领域中是公知的,以美国专利号8,283,149的公开为例。在某些实施方案中,古细菌DNA聚合酶来自嗜嗜热古菌 (archaeon),并且是热稳定的。

[0046] 在某些实施方案中,B族古细菌DNA聚合酶来自例如,诸如热球菌属 (Thermococcus),火球菌属 (Pyrococcus) 或甲烷球菌属 (Methanococcus) 的属。热球菌属的成员在本领域中是公知的,包括但不限于T.4557,嗜压热球菌 (T.barophilus),耐 γ 射线热球菌 (T.gammatolerans), T.onnurineus,西伯利亚热球菌 (T.sibiricus),小宝岛热球菌 (T.kodakarensis), T.gorgonarius和T.waiotapuensis。嗜热球菌属的成员在本领域中是公知的,包括但不限于P.NA2,深海火球菌,激烈火球菌,堀越火球菌 (P.horikoshii), P.yayanosii, P.endeavori, P.glycervorans和P.woesei。甲烷球菌属的成员在本领域中是公知的,包括但不限于:M.aeolicus, M.maripaludis, M.vannielii, M.voltae, M.thermolithotrophicus和M.jannaschii。

[0047] 在一个实施方案中,改变的聚合酶基于**Vent®**,**DeepVent®**,9°N,Pfu,KOD或Pab聚合酶。**Vent®**和**DeepVent®**是用于从嗜热古菌Thercococcus litoralis中分离的B族DNA聚合酶的商品名。9°N聚合酶是从热球菌属种分离的B族聚合酶。Pfu聚合酶是从激烈火球菌分离的B族聚合酶。KOD聚合酶是从小宝岛热球菌分离的B族聚合酶。Pab聚合酶是从深海火球菌分离的B族聚合酶。Twa是从T.waiotapuensis分离的B族聚合酶。图1中公开了**Vent®**, **DeepVent®**,9°N,Pfu,KOD,Pab和Twa聚合酶的实例。

[0048] 在某些实施方案中,B族古细菌DNA聚合酶来自噬菌体,例如T4,RB69或phi29噬菌体。

[0049] 图1示出了具有SEQ ID NO:1-7所示的氨基酸序列的蛋白质的序列比对。比对指示在不同的B族聚合酶中保守的氨基酸。技术人员将理解,保守氨基酸和保守区最可能是保守的,因为它们对于聚合酶的功能是重要的,因此显示出聚合酶的结构和功能之间的相关性。比对还显示了跨不同B族聚合酶的可变性区域。本领域普通技术人员可以从聚合酶的此类数据推断出可以允许取代,特别是保守取代,而不过度影响改变的聚合酶的生物学活性的聚合酶区域。

[0050] 本文所述的改变的聚合酶是基于已知聚合酶(本文也称为参照聚合酶)的氨基酸序列,并且还包括一个或多个残基处的取代突变。在一个实施方案中,取代突变在功能上等同于参照聚合酶的氨基酸的位置。“功能上等同的”是意指改变的聚合酶在参照聚合酶的氨

基酸位置处具有在参照聚合酶和改变的聚合酶中均具有相同的功能性作用的氨基酸取代。

[0051] 通常,两种或更多种不同聚合酶中功能上等同的取代突变发生在聚合酶氨基酸序列中的同源氨基酸位置处。因此,本文中使用的术语“功能上等同的”也包括与给定突变“位置上等同”或“同源”的突变,而不管突变氨基酸的具体功能是否已知。基于序列比对和/或分子建模,可以鉴定两种或更多种不同聚合酶的氨基酸序列中功能上等同和位置上等同的氨基酸残基的位置。图1中列出了鉴定位置上等同和/或功能上等同的残基的序列比对的例子。例如,垂直比对的图1的Twa,KOD,Pab,Pfu,Deep Vent和Vent聚合酶中的残基被认为与9°N聚合酶氨基酸序列中的相应残基在位置上等同以及在功能上等同。因此,例如9°N,Twa,KOD,Pfu,Deep Vent和Pab聚合酶的残基349和Vent聚合酶的残基351是功能上等同的且位置上等同的。同样,例如9°N,Twa,KOD和Pab聚合酶的残基633,Pfu和Deep Vent聚合酶的残基634,以及Vent聚合酶的残基636是功能上等同的且位置上等同的。技术人员可以容易地鉴定DNA聚合酶中功能上等同的残基。

[0052] 在某些实施方案中,取代突变包括突变为具有非极性侧链的残基的突变。具有非极性侧链的氨基酸是本领域公知的,并且包括例如:丙氨酸,甘氨酸,异亮氨酸,亮氨酸,甲硫氨酸,苯丙氨酸,脯氨酸,色氨酸和缬氨酸。

[0053] 在某些实施方案中,取代突变包括突变为具有极性侧链的残基的突变。具有极性侧链的氨基酸在本领域中是公知的,包括例如:精氨酸,天冬酰胺,天冬氨酸,谷氨酰胺,谷氨酸,组氨酸,赖氨酸,丝氨酸,半胱氨酸,酪氨酸和苏氨酸。

[0054] 在某些实施方案中,取代突变包括突变为具有疏水性侧链的残基的突变。具有疏水性侧链的氨基酸在本领域中是公知的,包括例如:甘氨酸,丙氨酸,缬氨酸,亮氨酸,异亮氨酸,脯氨酸,苯丙氨酸,甲硫氨酸和色氨酸。

[0055] 在某些实施方案中,取代突变包括突变为具有不带电荷的侧链的残基的突变。具有不带电荷的侧链的氨基酸是本领域公知的,并且包括例如:甘氨酸,丝氨酸,半胱氨酸,天冬酰胺,谷氨酰胺,酪氨酸和苏氨酸等。

[0056] 在一个实施方案中,改变的聚合酶具有与本文公开的参照聚合酶在结构上相似的氨基酸序列。在一个实施方案中,参照聚合酶是包括9°N的氨基酸序列(SEQ ID NO:1)的参照聚合酶。任选地,参照聚合酶是SEQ ID NO:1,具有以下取代突变:Met129Ala,Asp141Ala,Glu143Ala,Cys223Ser,Leu408Ala,Tyr409Ala,Pro410Ile和Ala485Val。具有9°N氨基酸序列(SEQ ID NO:1)且具有取代突变Met129Ala,Asp141Ala,Glu143Ala,Cys223Ser,Leu408Ala,Tyr409Ala,Pro410Ile和Ala485Val的聚合酶公开于SEQ ID NO:8,并且在本文中也称为Pol812聚合酶。其他参照序列包括SEQ ID NO:2、3、4、5、6或7。任选地,参照聚合酶是SEQ ID NO:2、3、4、5、6或7,其取代突变在功能上和位置上等同于SEQ ID NO:1中的以下取代突变:Met129Ala,Asp141Ala,Glu143Ala,Cys223Ser,Leu408Ala,Tyr409Ala,Pro410Ile和Ala485Val。

[0057] 如本文所用,如果改变的聚合酶的氨基酸序列与参照聚合酶相比具有指定量的序列相似性和/或序列同一性,则改变的聚合酶可以与参照聚合酶“在结构上相似”。

[0058] 两个氨基酸序列的结构相似性可通过比对两个序列的残基(例如,本文所述的候选聚合酶和参照聚合酶)以沿着其序列长度优化相同氨基酸的数目来确定;为了使相同氨基酸的数目优化,在进行比对中允许一个或两个序列中的缺口,尽管每个序列中的氨基酸

仍然必须保持其适当顺序。候选聚合酶是与参照聚合酶比较的聚合酶。具有与参照聚合酶的结构相似性和聚合酶活性的候选聚合酶是改变的聚合酶。

[0059] 除非本文另外进行修饰,否则氨基酸序列或核苷酸序列的成对比较分析可以例如通过Smith&Waterman, *Adv. Appl. Math.* 2:482 (1981) 的局部同源性算法,通过Needleman&Wunsch, *J. Mol. Biol.* 48:443 (1970) 的同源性比对算法,通过Pearson&Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988) 的寻找相似性方法,通过这些算法的计算机实施 (GAP, BESTFIT, FASTA和TFASTA, Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.) 或通过目视检查 (一般参见 *Current Protocols in Molecular Biology*, Ausubel 等人编辑, *Current Protocols*, Greensub Publishing Associates, Inc. 和 John Wiley&Sons, Inc. 合资, 到2004年的补充) 来进行。

[0060] 适用于确定结构相似性的算法的一个示例是BLAST算法,该算法描述于Altschul et al., *J. Mol. Biol.* 215:403-410 (1990)。可通过国家生物技术信息中心公开获得进行BLAST分析的软件。该算法涉及首先通过鉴定查询序列中长度W的短单词来鉴定高分序列对 (HSP), 这些短单词在与数据库序列中相同长度的单词比对时匹配或满足一些正值阈值得分T。T被称为邻近单词得分阈值 (Altschul et al., *J. Mol. Biol.* 215:403-410 (1990))。这些最初的邻近单词命中充当启动搜索以查找包含它们的较长HSP的种子。然后将单词命中沿着每个序列在两个方向上延伸,直到可以增加累积比对得分。对于核苷酸序列,使用参数M (一对匹配残基的奖励得分;始终>0) 和N (错配残基的罚分;始终<0) 来计算累积得分。对于氨基酸序列,使用得分矩阵来计算累积得分。在以下情况下,将停止单词命中在每个方向上的延伸:累积比对得分从其最大实现值降低数量X;由于一个或多个负得分残基比对的累积,累积得分变为零或之下;或到达任一序列的末尾。BLAST算法参数W, T和X确定比对的灵敏度和速度。BLASTN程序 (用于核苷酸序列) 使用字长 (W) 为11,期望 (E) 为10,截留为100, M=5, N=-4以及两条链的比较作为默认值。对于氨基酸序列,BLASTP程序使用字长 (W) 为3,期望 (E) 为10和BLOSUM62评分矩阵作为默认值 (参见Henikoff&Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915)。

[0061] 除了计算百分比序列同一性之外,BLAST算法还对两个序列之间的相似性进行统计分析 (参见,例如, Karlin&Altschul, *Proc. Nat'l. Acad. Sci. USA* 90:5873-5877 (1993))。BLAST算法提供的一种相似性度量是最小总和概率 (P (N)), 它表示偶然发生两个核苷酸或氨基酸序列之间匹配的概率。例如,如果在测试核酸与参照核酸的比较中最小总和概率小于约0.1,更优选小于约0.01,最优选小于约0.001,则认为核酸与参照序列相似。

[0062] 在两个氨基酸序列的比较中,结构相似性可以用“同一性”百分比来提及,或者可以用“相似性”百分比来提及。“同一性”是指存在相同氨基酸。“相似性”是指不仅存在相同的氨基酸,而且还存在保守的取代。蛋白质中氨基酸的保守取代可以选自该氨基酸所属类别的其他成员。例如,在蛋白质生物化学领域中公知,属于具有特定大小或特征 (例如电荷,疏水性或亲水性) 的氨基酸分组的氨基酸可以在不改变蛋白质活性,特别是在与生物活性不直接相关的蛋白质区域的情况下替代另一种氨基酸。例如,非极性氨基酸包括丙氨酸,甘氨酸,异亮氨酸,亮氨酸,甲硫氨酸,苯丙氨酸,脯氨酸,色氨酸和缬氨酸。疏水氨基酸包括甘氨酸,丙氨酸,缬氨酸,亮氨酸,异亮氨酸,脯氨酸,苯丙氨酸,甲硫氨酸和色氨酸。极性氨基酸包括精氨酸,天冬酰胺,天冬氨酸,谷氨酰胺,谷氨酸,组氨酸,赖氨酸,丝氨酸,半胱氨酸,

酪氨酸和苏氨酸。不带电荷的氨基酸包括甘氨酸,丝氨酸,半胱氨酸,天冬酰胺,谷氨酰胺,酪氨酸和苏氨酸等。

[0063] 因此,如本文所用,提及如本文所述的聚合酶,例如提及本文所述的一种或多种 SEQ ID NO的氨基酸序列,可以包括与参照聚合酶具有至少80%,至少85%,至少86%,至少87%,至少88%,至少89%,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%或至少99%的氨基酸序列相似性的蛋白质。

[0064] 或者,如本文所用,提及如本文所述的聚合酶,例如提及本文所述的一种或多种 SEQ ID NO的氨基酸序列,可以包括与参照聚合酶具有至少80%,至少85%,至少86%,至少87%,至少88%,至少89%,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%或至少99%的氨基酸序列同一性的蛋白质。

[0065] 本公开描述了导致具有一种或多种本文所述活性的聚合酶的突变的集合。本文所述的聚合酶可以包括相比于参照聚合酶,例如SEQ ID NO:1或SEQ ID NO:8的任何数量的突变,例如至少1,至少2,至少3,至少4,至少5,至少6,至少7,至少8,至少9,至少10,至少11,至少12,至少13,至少14,至少15,至少16,至少17或至少18个突变。同样地,本文所述的聚合酶可以包括任何组合的突变。例如,表1列出了包括本文所述突变的不同组合的特定的改变的聚合酶的实例。选取标记(√)表示存在列出的突变。列出的突变,例如Y497G,F152G,V278L等,是在SEQ ID NO:1上的位置处的突变。

[0066]

表1：改变的聚合酶的实例

突变																		
Pol (SEQ ID NO:)	Y497 G	F152 G	V278 L	M329 H	V471 S	T514 A	L631 M	E734 R	K476 W	K477 M	T514 S	I521 L	T590 I	R247 Y	E599 D	K620 R	H633 G	V661 D
812 (8)																		
963 (9)										✓	✓	✓						
1550 (10)	✓	✓	✓	✓	✓	✓												
1558 (11)	✓			✓	✓	✓												
1563 (12)	✓			✓	✓	✓	✓											
1565 (13)	✓		✓	✓	✓			✓										
1630 (14)	✓		✓	✓	✓													
1634 (15)	✓				✓	✓												
1641 (16)	✓	✓	✓		✓													
1573 (17)	✓					✓			✓									
1576 (18)	✓										✓	✓	✓					
1584 (19)	✓								✓	✓	✓	✓						

[0067]

				✓			✓							
			✓	✓	✓	✓		✓	✓		✓		✓	✓
					✓		✓		✓	✓		✓		✓
			✓		✓			✓	✓			✓		✓
			✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓
✓	✓	✓												
	✓	✓												
		✓												
							✓			✓	✓	✓		
								✓	✓	✓	✓	✓	✓	✓
	✓						✓				✓		✓	✓
								✓	✓				✓	✓
									✓					
✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
1586 (20)	1601 (21)	1611 (22)	1671 (23)	1677 (24)	1682 (25)	1700 (26)	1680 (27)	1745 (28)	1758 (29)	1761 (30)	1762 (31)	1765 (32)	1769 (33)	1770 (34)

[0068] 本公开的改变的聚合酶包括在功能上等同于9°N聚合酶(SEQ ID NO:1)中的Tyr497的位置处的取代突变。在一个实施方案中,在功能上等同于Tyr497的位置处的取代突变是突变为非极性,疏水或不带电荷的氨基酸,例如Gly的突变。

[0069] 在一个实施方案中,改变的聚合酶包括在功能上等同于9°N聚合酶(SEQ ID NO:1)中的Phe152的位置处的取代突变。在一个实施方案中,在功能上等同于Phe152的位置处的取代突变是突变为非极性,疏水或不带电荷的氨基酸,例如Gly的突变。

[0070] 在一个实施方案中,改变的聚合酶包括在功能上等同于9°N聚合酶(SEQ ID NO:1)中的Val278的位置处的取代突变。在一个实施方案中,在功能上等同于Val278的位置处的取代突变是突变为非极性或疏水的氨基酸,例如Leu的突变。

[0071] 在一个实施方案中,改变的聚合酶包括在功能上等同于9°N聚合酶(SEQ ID NO:1)中的Met329的位置处的取代突变。在一个实施方案中,在功能上等同于Met329的位置处的取代突变是突变为极性氨基酸,例如His的突变。

[0072] 在一个实施方案中,改变的聚合酶包括在功能上等同于9°N聚合酶(SEQ ID NO:1)中的Val471的位置处的取代突变。在一个实施方案中,在功能上等同于Val471的位置处的取代突变是突变为极性或不带电荷的氨基酸,例如Ser的突变。

[0073] 在一个实施方案中,改变的聚合酶包括在功能上等同于9°N聚合酶(SEQ ID NO:1)中的Thr514的位置处的取代突变,如本领域中已知的并且在美国专利申请号2016/0032377中示例的。在一个实施方案中,在功能上等同于Thr514的位置处的取代突变是突变为非极性或疏水性氨基酸,例如Ala的突变。在一些实施方案中,可以与在功能上等同于Thr514的位置处的非极性或疏水性氨基酸组合使用的其他取代突变包括Phe152,Val278,M329,Val471,Lue631,Glu734或其组合。在一个实施方案中,在功能上等同于Thr514的位置处的取代突变是突变为极性或不带电荷的氨基酸,例如Ser的突变。在一些实施方案中,可以与在功能上等同于Thr514的位置上的极性或不带电荷的氨基酸组合使用的其他取代突变包括Lys476,Lys477,Ile521,Thr590或其组合。

[0074] 在一个实施方案中,改变的聚合酶包括在功能上等同于9°N聚合酶(SEQ ID NO:1)中的Leu631的位置处的取代突变。在一个实施方案中,在功能上等同于Leu631的位置处的取代突变是突变为非极性或疏水氨基酸,例如Met的突变。

[0075] 在一个实施方案中,改变的聚合酶包括在功能上等同于9°N聚合酶(SEQ ID NO:1)中的Glu734的位置处的取代突变。在一个实施方案中,在功能上等同于Glu734的位置处的取代突变是突变为极性或不带电荷的氨基酸,例如Arg的突变。

[0076] 在一个实施方案中,改变的聚合酶包括在功能上等同于9°N聚合酶(SEQ ID NO:1)中的Lys476的位置处的取代突变。在一个实施方案中,在功能上等同于Lys476的位置处的取代突变是突变为非极性或疏水氨基酸,例如Trp的突变。

[0077] 在一个实施方案中,改变的聚合酶包括在功能上等同于9°N聚合酶(SEQ ID NO:1)中的Lys477的位置处的取代突变,如本领域中已知的并且在美国专利号9,765,309中示例的。在一个实施方案中,在功能上等同于Lys477的位置处的取代突变是突变为非极性或疏水氨基酸,例如Met的突变。

[0078] 在一个实施方案中,改变的聚合酶包括在功能上等同于9°N聚合酶(SEQ ID NO:1)中的Ile521的位置处的取代突变,如本领域中已知的并且在美国专利申请号2016/0032377中示例的。在一个实施方案中,在功能上等同于Ile521的位置处的取代突变是突变为非极性氨基酸,例如Leu的突变。

[0079] 在一个实施方案中,改变的聚合酶包括在功能上等同于9°N聚合酶(SEQ ID NO:1)

中的Thr590的位置处的取代突变。在一个实施方案中,在功能上等同于Thr590的位置处的取代突变是突变为非极性或疏水氨基酸,例如Ile的突变。

[0080] 在一个实施方案中,改变的聚合酶包括在功能上等同于9°N聚合酶(SEQ ID NO:1)中的Arg247的位置处的取代突变。在一个实施方案中,在功能上等同于Arg247的位置处的取代突变是突变为非极性或不带电荷的氨基酸,例如Tyr的突变。

[0081] 在一个实施方案中,改变的聚合酶包括在功能上等同于9°N聚合酶(SEQ ID NO:1)中的Glu599的位置处的取代突变。在一个实施方案中,在功能上等同于Glu599的位置处的取代突变是突变为极性或不带电荷的氨基酸,例如Asp的突变。

[0082] 在一个实施方案中,改变的聚合酶包括在功能上等同于9°N聚合酶(SEQ ID NO:1)中的Lys620的位置处的取代突变。在一个实施方案中,在功能上等同于Lys620的位置处的取代突变是突变为极性或不带电荷的氨基酸,例如Arg的突变。

[0083] 在一个实施方案中,改变的聚合酶包括在功能上等同于9°N聚合酶(SEQ ID NO:1)中的His633的位置处的取代突变。在一个实施方案中,在功能上等同于His633的位置处的取代突变是突变为非极性、疏水性或不带电荷的氨基酸,例如Gly的突变。

[0084] 在一个实施方案中,改变的聚合酶包括在功能上等同于9°N聚合酶(SEQ ID NO:1)中的Val661的位置处的取代突变。在一个实施方案中,在功能上等同于Val661的位置处的取代突变是极性或不带电荷的氨基酸,例如Asp的突变。

[0085] 在一个实施方案中,改变的聚合酶包含在功能上等同于氨基酸Met129,Asp141,Glu143,Cys223,Lys408,Tyr409,Pro410,Ala485或其组合的位置处的至少一个取代突变。在一个实施方案中,在功能上等同于Met129,Asp141,Glu143,Lys408或Tyr409的位置处的取代突变是突变为非极性或疏水氨基酸,例如Ala的突变。在一个实施方案中,在功能上等同于Cys223的位置处的取代突变是突变为极性或不带电荷的氨基酸,例如Ser的突变。在一个实施方案中,在功能上等同于Pro410的位置处的取代突变是突变为非极性或疏水氨基酸,例如Ile的突变。在一个实施方案中,在功能上等同于Ala485的位置处的取代突变是突变为非极性或疏水氨基酸,例如Val的突变。

[0086] 在一个实施方案中,改变的聚合酶包括在功能上等同于Tyr497的位置处的氨基酸取代突变和在功能上等同于选自9°N DNA聚合酶氨基酸序列中的Phe152、Val278、Met329、Val471、Thr514、Leu631和Glu734的氨基酸的位置处的至少一个,至少两个、至少三个、至少四个、至少五个、至少六个或七个氨基酸取代突变。在一个实施方案中,改变的聚合酶还包括在功能上等同于9°N DNA聚合酶氨基酸序列中的氨基酸Met129、Asp141、Glu143、Cys223、Leu408、Tyr409、Pro410和Ala485的位置处的氨基酸取代突变。

[0087] 在一个实施方案中,改变的聚合酶包括在功能上等同于Tyr497的位置处的氨基酸取代突变,以及在功能上等同于选自9°N DNA聚合酶氨基酸序列中Lys476、Lys477、Thr514、Ile521和Thr590的氨基酸的位置处的至少一个,至少两个、至少三个、至少四个或五个氨基酸取代突变。在一个实施方案中,改变的聚合酶还包括在功能上等同于9°N DNA聚合酶氨基酸序列中的氨基酸Met129、Asp141、Glu143、Cys223、Leu408、Tyr409、Pro410和Ala485的位置处的氨基酸取代突变。

[0088] 在一个实施方案中,改变的聚合酶包括在功能上等同于Tyr497的位置处的氨基酸取代突变,以及在功能上等同于选自9°N DNA聚合酶氨基酸序列中Arg247、Glu599、Lys620、

His633和Val661的氨基酸的位置处的至少两个、至少三个、至少四个或五个氨基酸取代突变。在一个实施方案中,改变的聚合酶还包括在功能上等同于9°N DNA聚合酶氨基酸序列中的氨基酸Met129、Asp141、Glu143、Cys223、Leu408、Tyr409、Pro410和Ala485的位置处的氨基酸取代突变。

[0089] 在一个实施方案中,改变的聚合酶包括在功能上等同于Tyr497的位置处的氨基酸取代突变,功能上等同于9°N DNA聚合酶氨基酸序列中的Phe152、Val278、Met329、Val471、Thr514、Leu631或Glu734的位置处的至少一个,至少两个、至少三个、至少四个、至少五个、至少六个或七个氨基酸取代突变,以及(iii)在功能上等同于9°N DNA聚合酶氨基酸序列中的Lys476、Lys477、Thr514、Ile521或Thr590的位置处的至少一个,至少两个、至少三个、至少四个或五个氨基酸取代突变。在一个实施方案中,改变的聚合酶还包括在功能上等同于9°N DNA聚合酶氨基酸序列中的氨基酸Met129、Asp141、Glu143、Cys223、Leu408、Tyr409、Pro410和Ala485的位置处的氨基酸取代突变。

[0090] 在一个实施方案中,改变的聚合酶包括在功能上等同于Tyr497的位置处的氨基酸取代突变,在功能上等同于选自9°N DNA聚合酶氨基酸序列中Phe152、Val278、Met329、Val471、Thr514、Leu631和Glu734的氨基酸的位置处的至少一个,至少两个、至少三个、至少四个、至少五个、至少六个或七个氨基酸取代突变氨基,以及在功能上等同于选自9°N DNA聚合酶氨基酸序列中的Arg247、Glu599、Lys620、His633或Val661的氨基酸的位置处的至少一个,至少两个、至少三个、至少四个或五个氨基酸取代突变。在一个实施方案中,改变的聚合酶还包括在功能上等同于9°N DNA聚合酶氨基酸序列中的氨基酸Met129、Asp141、Glu143、Cys223、Leu408、Tyr409、Pro410和Ala485的位置处的氨基酸取代突变。

[0091] 在一个实施方案中,改变的聚合酶包括在功能上等同于Tyr497的位置处的氨基酸取代突变,在功能上等同于选自9°N DNA聚合酶氨基酸序列中的Lys476、Lys477、Thr514、Ile521或Thr590的氨基酸的位置处的至少一个,至少两个、至少三个、至少四个或五个氨基酸取代突变,以及在功能上等同于选自9°N DNA聚合酶氨基酸序列中Arg247、Glu599、Lys620、His633或Val661的氨基酸在位置处的至少一个,至少两个、至少三个、至少四个或五个氨基酸取代突变。在一个实施方案中,改变的聚合酶还包括在功能上等同于9°N DNA聚合酶氨基酸序列中的氨基酸Met129、Asp141、Glu143、Cys223、Leu408、Tyr409、Pro410和Ala485的位置处的氨基酸取代突变。

[0092] 在一个实施方案中,改变的聚合酶包括在功能上等同于Tyr497的位置处的氨基酸取代突变,在功能上等同于选自9°N DNA聚合酶氨基酸序列中的Phe152、Val278、Met329、Val471、Leu631和Glu734的氨基酸的位置处的至少一个、至少两个、至少三个、至少四个,至少五个或六个氨基酸取代突变,在功能上等同于选自9°N DNA聚合酶氨基酸序列中的Lys476、Lys477、Thr514、Ile521或Thr590的氨基酸的位置处的至少一个、至少两个、至少三个、至少四个或五个氨基酸取代突变,以及在功能上等同于选自9°N DNA聚合酶氨基酸序列中的Arg247、Glu599、Lys620、His633或Val661的氨基酸的位置处的至少一个,至少两个、至少三个、至少四个或五个氨基酸取代突变。在一个实施方案中,改变的聚合酶还包括在功能上等同于9°N DNA聚合酶氨基酸序列中的氨基酸Met129、Asp141、Glu143、Cys223、Leu408、Tyr409、Pro410和Ala485的位置处的氨基酸取代突变。

[0093] 改变的聚合酶的具体实例包括Pol 1550 (SEQ ID NO:10), Pol 1558 (SEQ ID NO:

11), Pol 1563 (SEQ ID NO:12), Pol 1565 (SEQ ID NO:13), Pol 1630 (SEQ ID NO:14), Pol 1634 (SEQ ID NO:15), Pol 1641 (SEQ ID NO:16), Pol 1573 (SEQ ID NO:17), Pol 1576 (SEQ ID NO:18), Pol 1584 (SEQ ID NO:19), Pol 1586 (SEQ ID NO:20), Pol 1601 (SEQ ID NO:21), Pol 1611 (SEQ ID NO:22), Pol 1671 (SEQ ID NO:23), Pol 1677 (SEQ ID NO:24), Pol 1682 (SEQ ID NO:25), Pol 1680 (SEQ ID NO:27), Pol 1745 (SEQ ID NO:28), Pol 1758 (SEQ ID NO:29), Pol 1761 (SEQ ID NO:30), Pol 1762 (SEQ ID NO:31), Pol 1765 (SEQ ID NO:32), Pol 1769 (SEQ ID NO:33), 和 Pol 1770 (SEQ ID NO:34)。

[0094] 本文所述的改变的聚合酶可以包括已知影响聚合酶活性的其他突变。在此类取代上,突变在功能上等同于9°N聚合酶 (SEQ ID NO:1) 中的Arg713的位置处。如本领域中已知的并且以美国专利号8,623,628例示,可以在已知导致外切核酸酶活性降低的一个或多个位置处进行多种取代突变中的任一种。在一个实施方案中,在Arg713位置处的取代突变是突变为非极性、疏水或不带电荷的氨基酸例如Gly, Met或Ala的突变。

[0095] 在一个实施方案中,如本领域已知的且以美国专利号8,623,628的公开例示,改变的聚合酶包括在功能上等同于9°N聚合酶 (SEQ ID NO:1) 中的Arg743或Lys705或其组合的位置处的取代突变。在一个实施方案中,在位置Arg743或Lys705上的取代突变是突变为非极性或疏水氨基酸例如Ala的突变。

[0096] 本公开还提供了包括本文所述的改变的聚合酶的组合物。该组合物除改变的聚合酶外还可以包括其他组分。例如,该组合物可以包括缓冲液,核苷酸溶液或其组合。核苷酸溶液可以包括核苷酸,例如标记的,合成的,修饰的核苷酸或其组合。在一个实施方案中,组合物包括靶核酸,例如靶核酸的文库。

[0097] 将聚合酶进行突变

[0098] 各种类型的诱变可任选地用于本公开中,例如,以修饰聚合酶以产生变体,例如根据如上所讨论的聚合酶模型和模型预测,或使用随机或半随机突变方法。通常,任何可用的诱变程序均可用于制备聚合酶突变体。此类诱变程序任选地包括针对一种或多种目的活性(例如,减少的焦磷酸解,增加的转换 (turnover) 率,例如对于给定的核苷酸类似物)的突变体核酸和多肽的选择。可以使用的程序包括但不限于:定点的点诱变,随机点诱变,体外或体内同源重组 (DNA改组和组合重叠PCR),使用含尿嘧啶的模板的诱变,寡核苷酸指导的诱变,硫代磷酸酯修饰的DNA诱变,使用缺口双链体DNA的诱变,点错配修复,使用修复缺陷型宿主株的诱变,限制性选择和限制性纯化,缺失诱变,通过全基因合成的诱变,简并PCR,双链断裂修复以及本领域技术人员所知的许多其他方法。用于突变的起始聚合酶可以是本文所述的任何酶,包括可用的聚合酶突变体,例如在美国专利号8,460,910和美国专利号8,623,628中鉴定的那些,其各自通过引用整体并入本文。

[0099] 任选地,诱变可以通过来自天然存在的聚合酶分子或已知改变或突变的聚合酶(例如,使用现有的突变体聚合酶)的已知信息指导,例如序列,序列比较,物理性质,晶体结构和/或上述类似内容。然而,在另一类实施方案中,修饰可以基本上是随机的(例如,如在经典或“家族”DNA改组中,参见例如Cramer et al. (1998) "DNA shuffling of a family of genes from diverse species accelerates directed evolution" Nature 391:288-291)。

[0100] 有关突变形式的其他信息参见:Sambrook et al., Molecular Cloning--A

Laboratory Manual (第3版), 第1-3卷, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 2000 ("Sambrook"); Current Protocols in Molecular Biology, F.M. Ausubel et al. 编, Current Protocols, Greene Publishing Associates, Inc. 和 John Wiley & Sons, Inc. 合资, (截至2011) ("Ausubel") 和 PCR Protocols A Guide to Methods and Applications (Innis et al. 编) Academic Press Inc. San Diego, Calif. (1990) ("Innis")。引用的以下出版物和参考文献提供了有关突变形式的更多详细信息: Arnold, Protein engineering for unusual environments, Current Opinion in Biotechnology 4:450-455 (1993); Bass et al., Mutant Trp repressors with new DNA-binding specificities, Science 242:240-245 (1988); Bordo and Argos (1991) Suggestions for "Safe" Residue Substitutions in Site-directed Mutagenesis 217:721-729; Botstein & Shortle, Strategies and applications of in vitro mutagenesis, Science 229:1193-1201 (1985); Carter et al., Improved oligonucleotide site-directed mutagenesis using M13 vectors, Nucl. Acids Res. 13:4431-4443 (1985); Carter, Site-directed mutagenesis, Biochem. J. 237:1-7 (1986); Carter, Improved oligonucleotide-directed mutagenesis using M13 vectors, Methods in Enzymol. 154:382-403 (1987); Dale et al., Oligonucleotide-directed random mutagenesis using the phosphorothioate method, Methods Mol. Biol. 57:369-374 (1996); Eghtedarzadeh & Henikoff, Use of oligonucleotides to generate large deletions, Nucl. Acids Res. 14:5115 (1986); Fritz et al., Oligonucleotide-directed construction of mutations: a gapped duplex DNA procedure without enzymatic reactions in vitro, Nucl. Acids Res. 16:6987-6999 (1988); Grundstrom et al., Oligonucleotide-directed mutagenesis by microscale "shot-gun" gene synthesis, Nucl. Acids Res. 13:3305-3316 (1985); Hayes (2002) Combining Computational and Experimental Screening for rapid Optimization of Protein Properties PNAS 99 (25) 15926-15931; Kunkel, The efficiency of oligonucleotide directed mutagenesis, in Nucleic Acids & Molecular Biology (Eckstein, F. and Lilley, D.M.J. eds., Springer Verlag, Berlin) (1987); Kunkel, Rapid and efficient site-specific mutagenesis without phenotypic selection, Proc. Natl. Acad. Sci. USA 82:488-492 (1985); Kunkel et al., Rapid and efficient site-specific mutagenesis without phenotypic selection, Methods in Enzymol. 154, 367-382 (1987); Kramer et al., The gapped duplex DNA approach to oligonucleotide-directed mutation construction, Nucl. Acids Res. 12:9441-9456 (1984); Kramer & Fritz Oligonucleotide-directed construction of mutations via gapped duplex DNA, Methods in Enzymol. 154:350-367 (1987); Kramer et al., Point Mismatch Repair, Cell 38:879-887 (1984); Kramer et al., Improved enzymatic in vitro reactions in the gapped duplex DNA approach to oligonucleotide-directed construction of mutations, Nucl. Acids Res. 16:7207 (1988); Ling et al., Approaches to DNA mutagenesis: an overview, Anal Biochem. 254(2):157-178 (1997); Lorimer and Pastan Nucleic Acids Res. 23, 3067-8 (1995); Mandecki, Oligonucleotide-directed double-strand break

repair in plasmids of *Escherichia coli*: a method for site-specific mutagenesis, *Proc. Natl. Acad. Sci. USA*, 83:7177-7181 (1986); Nakamaye&Eckstein, Inhibition of restriction endonuclease *Nci* I cleavage by phosphorothioate groups and its application to oligonucleotide-directed mutagenesis, *Nucl. Acids Res.* 14:9679-9698 (1986); Nambiar et al., Total synthesis and cloning of a gene coding for the ribonuclease S protein, *Science* 223:1299-1301 (1984); Sakamar and Khorana, Total synthesis and expression of a gene for the α -subunit of bovine rod outer segment guanine nucleotide-binding protein (transducin), *Nucl. Acids Res.* 14:6361-6372 (1988); Sayers et al., Y-T Exonucleases in phosphorothioate-based oligonucleotide-directed mutagenesis, *Nucl. Acids Res.* 16:791-802 (1988); Sayers et al., Strand specific cleavage of phosphorothioate-containing DNA by reaction with restriction endonucleases in the presence of ethidium bromide, (1988) *Nucl. Acids Res.* 16:803-814; Sieber, et al., *Nature Biotechnology*, 19:456-460 (2001); Smith, In vitro mutagenesis, *Ann. Rev. Genet.* 19:423-462 (1985); *Methods in Enzymol.* 100:468-500 (1983); *Methods in Enzymol.* 154:329-350 (1987); Stemmer, *Nature* 370, 389-91 (1994); Taylor et al., The use of phosphorothioate-modified DNA in restriction enzyme reactions to prepare nicked DNA, *Nucl. Acids Res.* 13:8749-8764 (1985); Taylor et al., The rapid generation of oligonucleotide-directed mutations at high frequency using phosphorothioate-modified DNA, *Nucl. Acids Res.* 13:8765-8787 (1985); Wells et al., Importance of hydrogen-bond formation in stabilizing the transition state of subtilisin, *Phil. Trans. R. Soc. Lond. A* 317: 415-423 (1986); Wells et al., Cassette mutagenesis: an efficient method for generation of multiple mutations at defined sites, *Gene* 34:315-323 (1985); Zoller&Smith, Oligonucleotide-directed mutagenesis using M13-derived vectors: an efficient and general procedure for the production of point mutations in any DNA fragment, *Nucleic Acids Res.* 10:6487-6500 (1982); Zoller&Smith, Oligonucleotide-directed mutagenesis of DNA fragments cloned into M13 vectors, *Methods in Enzymol.* 100:468-500 (1983); Zoller&Smith, Oligonucleotide-directed mutagenesis: a simple method using two oligonucleotide primers and a single-stranded DNA template, *Methods in Enzymol.* 154:329-350 (1987); Clackson et al. (1991) "Making antibody fragments using phage display libraries" *Nature* 352: 624-628; Gibbs et al. (2001) "Degenerate oligonucleotide gene shuffling (DOGS): a method for enhancing the frequency of recombination with family shuffling" *Gene* 271:13-20; 以及 Hiraga and Arnold (2003) "General method for sequence-independent site-directed chimeragenesis" *J. Mol. Biol.* 330:287-296. 有关上述许多方法的其他详细信息参见 *Methods in Enzymology* 第154卷, 其还描述了使用各种诱变方法解决问题的有用措施。

[0101] 制备和分离重组聚合酶

[0102] 一般而言, 可以通过克隆, 重组, 体外合成, 体外扩增和/或其他可用方法来制备编

码如本文所述的聚合酶的核酸。多种重组方法可用于表达编码如本文所述的聚合酶的表达载体。制备重组核酸,表达和分离表达产物的方法是公知的,并且是本领域中描述的。本文描述了许多示例性突变和突变的组合,以及设计期望突变的策略。在本文中以及例如在WO 2007/076057和WO 2008/051530中找到在聚合酶的活性位点中产生和选择突变的方法,包括用于修饰活性位点中或附近的区域特征以允许核苷酸类似物的改善的进入(access)。

[0103] 突变,重组和体外核酸操作方法(包括克隆,表达,PCR等)的其他有用参考文献包括Berger and Kimmel,Guide to Molecular Cloning Techniques,Methods in Enzymology第152卷Academic Press,Inc.,San Diego,Calif.(Berger);Kaufman et al.(2003)Handbook of Molecular and Cellular Methods in Biology and Medicine Second Edition Ceske(编)CRC Press(Kaufman);The Nucleic Acid Protocols Handbook Ralph Rapley(编)(2000)Cold Spring Harbor,Humana Press Inc(Rapley);Chen et al.(编)PCR Cloning Protocols,Second Edition(Methods in Molecular Biology,volume 192)Humana Press;以及Viljoen et al.(2005)Molecular Diagnostic PCR Handbook Springer,ISBN 1402034032。

[0104] 此外,用于从细胞中纯化质粒或其他相关核酸的许多试剂盒是商品化的(参见例如都来自Pharmacia Biotech的EasyPrep™和FlexiPrep™;来自Stratagene的StrataClean™;以及来自Qiagen的QIAprep™)。可以进一步操作任何分离和/或纯化的核酸以产生其他核酸,用于转染细胞,掺入相关的载体中以感染生物来进行表达等。典型的克隆载体包含转录和翻译终止剂,转录和翻译起始序列以及用于调节特定靶核酸表达的启动子。载体任选地包含通用表达盒,其包含至少一个独立的终止剂序列,允许该盒在真核生物或原核生物或两者中复制的序列(例如,穿梭载体)以及用于原核和真核系统两者的选择标志物。载体适合在原核生物,真核生物或两者中复制和整合。

[0105] 其他有用的参考文献,例如,用于细胞分离和培养的参考文献(例如,用于随后的核酸分离)包括Freshney(1994)Culture of Animal Cells,a Manual of Basic Technique,第三版,Wiley-Liss,New York及其中引用的参考文献;Payne et al.(1992)Plant Cell and Tissue Culture in Liquid Systems John Wiley&Sons,Inc.New York,N.Y.;Gamborg and Phillips(编)(1995)Plant Cell,Tissue and Organ Culture;Fundamental Methods Springer Lab Manual,Springer-Verlag(Berlin Heidelberg New York);及Atlas and Parks(编)The Handbook of Microbiological Media(1993)CRC Press,Boca Raton,Fla。

[0106] 本公开还包括编码本文公开的改变的聚合酶的核酸。特定的氨基酸可以由多个密码子编码,并且某些翻译系统(例如原核或真核细胞)通常表现出密码子偏向,例如,不同的生物通常更喜欢编码相同氨基酸的几种同义密码子之一。这样,本文提出的核酸任选地是“密码子优化的”,意指合成核酸以包括被用于表达聚合酶的特定翻译系统所优选的密码子。例如,当期望在细菌细胞(或甚至特定的细菌菌株)中表达聚合酶时,可以合成核酸以包括在该细菌细胞的基因组中最常发现的密码子,用于聚合酶的有效表达。当希望在真核细胞中表达聚合酶时可以采用类似的策略,例如,核酸可以包括该真核细胞优选的密码子。

[0107] 多种蛋白质分离和检测方法是已知的,并且可以用于例如从表达本文所述的重组聚合酶的细胞的重组培养物中分离聚合酶。多种蛋白质分离和检测方法是本领域公知的,

包括例如R.Scopes,Protein Purification,Springer-Verlag,N.Y.(1982);Deutscher,Methods in Enzymology第182卷:Guide to Protein Purification,Academic Press,Inc.N.Y.(1990);Sandana(1997)Bioseparation of Proteins,Academic Press,Inc.;Bollag et al.(1996)Protein Methods,第二版Wiley-Liss,NY;Walker(1996)The Protein Protocols Handbook Humana Press,NJ,Harris and Angal(1990)Protein Purification Applications:A Practical Approach IRL Press at Oxford,Oxford,England;Harris and Angal Protein Purification Methods:A Practical Approach IRL Press at Oxford,Oxford,England;Scopes(1993)Protein Purification:Principles and Practice第三版Springer Verlag,NY;Janson and Ryden(1998)Protein Purification:Principles,High Resolution Methods and Applications,第二版Wiley-VCH,NY;和Walker(1998)Protein Protocols on CD-ROM Humana Press,NJ;以及其中引用的参考文献。关于蛋白质纯化和检测方法的其他细节可见于Satinder Ahuja编,Handbook of Bioseparations,Academic Press(2000)。

[0108] 使用方法

[0109] 本文提出的改变的聚合酶可用于测序方法,例如合成测序(SBS)技术。简而言之,可以通过使靶核酸接触一个或多个核苷酸(例如,标记的,合成的,修饰的或其组合),DNA聚合酶等来起始SBS。使用靶核酸作为模板来延伸引物的那些特征将与可以检测的标记核苷酸整合。可以使用本文所述的改变的聚合酶显著减少测序运行中使用的掺入时间。任选地,标记的核苷酸可以进一步包括可逆的终止性质,一旦已经将核苷酸添加到引物,该可逆的终止性质终止进一步的引物延伸。例如,可以将具有可逆终止剂部分的核苷酸类似物添加至引物,使得随后的延伸直到递送解封闭剂来除去该部分时才发生。因此,对于使用可逆终止的实施方案,可以在(在发生检测之前或之后)将解封闭剂递送至流动池(flow cell)。可以在各个递送步骤之间进行洗涤。然后可以将该循环重复n次以将引物延伸n个核苷酸,从而检测长度为n的序列。可以容易地适合于与通过本公开的方法生产的阵列一起使用的示例性SBS程序,流体系统和检测平台描述于例如Bentley et al.,Nature 456:53-59(2008);WO 04/018497;WO 91/06678;WO 07/123744;美国专利号7,057,026、7,329,492、7,211,414、7,315,019、7,405,281和8,343,746。

[0110] 可以使用其他使用循环反应的测序程序,例如焦磷酸测序。焦磷酸测序检测特定的核苷酸被掺入新生核酸链中时无机焦磷酸盐(PPi)的释放(Ronaghi,et al.,Analytical Biochemistry 242(1),84-9(1996);Ronaghi,Genome Res.11(1),3-11(2001);Ronaghi et al.Science 281(5375),363(1998);美国专利号6,210,891;6,258,568和6,274,320)。在焦磷酸测序中,释放的PPi可以通过被ATP硫化酶转化为三磷酸腺苷(ATP)来检测,并且所得的ATP可以通过萤光素酶产生的光子进行检测。因此,可以通过发光检测系统监测测序反应。焦磷酸测序程序不必需用于基于荧光的检测系统的激发辐射源。可用于将焦磷酸测序应用于本公开的阵列的有用的流体系统,检测器和程序描述于例如WO 2012/058096,美国专利申请公开号2005/0191698 A1,美国专利号7,595,883和7,244,559。

[0111] 一些实施方案可以使用涉及实时监测DNA聚合酶活性的方法。例如,可以通过带有荧光团的聚合酶和 γ -磷酸酯标记的核苷酸之间的荧光共振能量转移(FRET)相互作用或使用零模波导来检测核苷酸掺入。用于基于FRET的测序的技术和试剂描述于例如Levene et

al.Science 299,682-686 (2003);Lundquist et al.Opt.Lett.33,1026-1028 (2008); Korlach et al.Proc.Natl.Acad.Sci.USA 105,1176-1181 (2008)。

[0112] 一些SBS实施方案包括检测将核苷酸掺入延伸产物后释放的质子。例如,基于释放的质子的检测的测序可以使用电检测器和可从Ion Torrent (Guilford,CT,Life Technologies子公司)商购的相关技术或美国专利号8,262,900、7,948,015、8,349,167和美国公开专利申请号2010/0137143 A1中描述的测序方法和系统。

[0113] 因此,本文呈现了将核苷酸类似物掺入DNA中的方法,包括允许以下组分相互作用:(i)根据以上实施方案中任一项的改变的聚合酶,(ii)DNA模板;和(iii)核苷酸溶液。在某些实施方案中,DNA模板包括簇状阵列(clustered array)。在某些实施方案中,核苷酸在3'糖羟基处被修饰,并且包括在3'糖羟基处的修饰,使得取代基的大小大于天然存在的3'羟基。

[0114] 编码改变的聚合酶的核酸

[0115] 本公开还包括编码本文所述的改变的聚合酶的核酸分子。对于任何给定的改变的聚合酶,可以根据分子生物学的基本原理获得编码该突变体的核苷酸序列,所述改变的聚合酶是已知氨基酸序列以及优选地还已知编码该聚合酶的野生型核苷酸序列的聚合酶的突变体形式。例如,鉴于已知编码9°N聚合酶的野生型核苷酸序列,则可以使用标准遗传密码推导编码具有一个或多个氨基酸取代的任何给定的9°N突变体形式的核苷酸序列。类似地,对于其他聚合酶,诸如例如**Vent®**聚合酶,Deep**Vent®**聚合酶,Pfu聚合酶,KOD聚合酶,Pab聚合酶等的突变体形式,可以容易地获得核苷酸序列。然后,可以使用本领域已知的标准分子生物学技术构建具有所需核苷酸序列的核酸分子。

[0116] 根据本文提出的实施方案,限定的核酸不仅包括相同的核酸,而且还包括任何次要的碱基变异,尤其包括在由于保守氨基酸取代中的简并密码而导致同义密码子(指定相同氨基酸残基的不同密码子)的情况下的取代。术语“核酸序列”还包括与关于碱基变异给出的任何单链序列的互补序列。

[0117] 本文所述的核酸分子也可有利地包含在合适的表达载体中,以在合适的宿主中表达由其编码的聚合酶蛋白。将克隆的DNA掺入合适的表达载体中用于随后转化所述细胞和随后选择转化的细胞是本领域技术人员公知的,如Sambrook et al. (1989),Molecular cloning:A Laboratory Manual,Cold Spring Harbor Laboratory所提供。

[0118] 此类表达载体包括具有根据本文提出的实施方案的核酸的载体,所述核酸可操作连接至能够实现所述DNA片段表达的调节序列,例如启动子区域。术语“可操作连接”是指并置,其中所描述的组件处于允许它们以其预期方式发挥功能的关系。可以将此类载体转化到合适的宿主细胞中以提供根据本文提出的实施方案的蛋白质的表达。

[0119] 核酸分子可以编码成熟蛋白质或具有原序列的蛋白质,包括编码前蛋白质上的前导序列的,然后所述前蛋白被宿主细胞切割以形成成熟蛋白质。例如,载体可以是质粒,病毒或噬菌体载体,其提供有复制起点,以及任选地用于表达所述核苷酸的启动子和任选地启动子的调控子。载体可以包含一种或多种选择标志物,例如抗生素抗性基因。

[0120] 表达所需的调控元件包括结合RNA聚合酶并指导适当水平的转录起始的启动子序列以及用于核糖体结合的翻译起始序列。例如,细菌表达载体可以包括启动子,例如lac启动子,并且为了翻译起始,Shine-Dalgarno序列和起始密码子AUG。类似地,真核表达载体可

以包括用于RNA聚合酶II的异源或同源启动子,下游的聚腺苷酸化信号,起始密码子AUG和用于核糖体分离的终止密码子。此类载体可商购获得或由通过本领域公知的方法所描述的序列组装。

[0121] 可以通过在载体中包含增强子序列来优化高级真核生物转录编码聚合酶的DNA。增强子是DNA的顺式作用元件,其作用于启动子以增加转录水平。除了选择标志物外,载体通常还将包括复制起点。

[0122] 本公开还提供了用于进行核苷酸掺入反应的试剂盒。该试剂盒包括至少一种本文所述的改变的聚合酶和在合适的包装材料中的核苷酸溶液,其量足以进行至少一个核苷酸掺入反应。任选地,还包括其他试剂,例如使用改变的聚合酶和核苷酸溶液所需的缓冲液和溶液。通常还包括包装组件的使用说明。

[0123] 在某些实施方案中,核苷酸溶液包括标记的核苷酸。在某些实施方案中,核苷酸是合成核苷酸。在某些实施方案中,核苷酸是修饰的核苷酸。在某些实施方案中,修饰的核苷酸已经在3' 糖羟基上被修饰,使得取代基的大小大于天然存在的3' 羟基。在某些实施方案中,修饰的核苷酸包括修饰的核苷酸或核苷分子,其包含嘌呤或嘧啶碱基和核糖或脱氧核糖糖部分,所述核糖或脱氧核糖糖部分具有与其共价连接的可除去3' -OH封闭基团,使得3' 碳原子已连接以下结构的基团

[0124] $-O-Z$

[0125] 其中Z是 $-C(R')_2-O-R''$, $-C(R')_2-N(R'')_2$, $-C(R')_2-N(H)R''$, $-C(R')_2-S-R''$ 和 $-C(R')_2-F$ 中的任何一个,

[0126] 其中各个R''是可除去保护基或是可除去保护基的一部分;

[0127] 各个R' 独立地是氢原子,烷基,取代的烷基,芳基烷基,烯基,炔基,芳基,杂芳基,杂环,酰基,氰基,烷氧基,芳氧基,杂芳氧基或酰胺基,或通过连接基团连接的可检测标记;或 $(R')_2$ 代表式 $=C(R'')_2$ 的亚烷基,其中各个R''可以相同或不同,并且选自包含氢和卤素原子以及烷基的组;和

[0128] 其中所述分子可反应产生中间体,其中各个R''被交换为H,或其中Z为 $-C(R')_2-F$,F被交换为OH,SH或 NH_2 ,优选为OH,该中间体在水性条件下解离,得到具有游离3'OH的分子;

[0129] 条件是Z为 $-C(R')_2-S-R''$ 时,两个R' 基团都不是H。

[0130] 在某些实施方案中,修饰的核苷酸或核苷的R' 是烷基或取代的烷基。在某些实施方案中,修饰的核苷酸或核苷的-Z具有式 $-C(R')_2-N_3$ 。在某些实施方案中,Z为叠氮基甲基。

[0131] 在某些实施方案中,对修饰的核苷酸进行荧光标记以允许对其进行检测。在某些实施方案中,修饰的核苷酸包括具有通过可切割的接头连接至可检测标记的碱基的核苷酸或核苷。在某些实施方案中,可检测标记包括荧光标记。

[0132] 如本文所用,短语“包装材料”是指用于容纳试剂盒的内容物的一种或多种物理结构。包装材料通过已知方法构造,优选地提供无菌,无污染的环境。包装材料具有标签,该标签指示组分可以用于进行核苷酸掺入反应。另外,包装材料包含指示试剂盒中的材料如何用于进行核苷酸掺入反应的说明。如本文所用,术语“包装”是指能够将多肽保持在固定限度内的固体基质或材料,例如玻璃,塑料,纸,箔等。“使用说明”通常包括描述试剂浓度或至少一种测定方法参数的有形表达,例如要混合的试剂和样品的相对量,试剂/样品混合物的维护时间段,温度,缓冲液条件等。

[0133] 在背景技术, 示例性实施方案的详细描述以及本文其他地方引用的专利, 专利文件和出版物的完整公开内容通过引用整体并入, 就像每个都单独地并入一样。

[0134] 讨论了本发明的说明性实施方案, 并且已经提及本发明范围内的可能变化。在不脱离本发明的范围的情况下, 本发明中的这些和其他变型, 组合和修改对本领域技术人员将是显而易见的, 并且应当理解, 本发明不限于在此阐述的说明性实施方案。因此, 本发明仅由所附的权利要求书及其等同方案限制。

实施例

[0135] 通过以下实施例说明本发明。应当理解的是, 具体的实例、材料、数量和程序将根据本文所述的本发明的范围和精神来广义地解释。

[0136] 实施例1

[0137] 一般测定方法和条件

[0138] 除非另有说明, 否则这描述了本文所述实施例中使用的一般测定条件。

[0139] A. 聚合酶的克隆和表达

[0140] 用于制备重组核酸、表达和分离表达产物的方法是本领域已知和描述的。使用标准的定点诱变方法对编码9°N聚合酶 (SEQ ID NO:1) 的编码区进行诱变。使用基于PCR的方法扩增突变的编码区并添加His标签。对于每个产生的突变, 通过确定克隆的DNA的序列来确认改变的编码区的正确序列。

[0141] 将带有His标签的突变体聚合酶编码区亚克隆到pET11a载体中, 并转化到BL21 Star (DE3) 表达细胞 (Invitrogen) 中。使用来自单挑菌落的过夜培养物接种2.8L烧瓶中的表达培养物。将培养物在37°C培养直至OD600为约0.8, 然后用0.2mM IPTG诱导蛋白质表达, 并且随后再培养4小时。将培养物以7000rpm离心20分钟。将细胞沉淀物在-20°C贮存直至纯化。

[0142] 在Ready-Lyse和Omnicleave试剂 (Epicentre) 的存在下, 根据制造商的建议, 将沉淀物冷冻-融化并用5x w/v裂解缓冲液 (50mM Tris-HCl pH7.5、1mM EDTA, 0.1%BME和5%甘油) 裂解。将最终的NaCl浓度提高到500mM并将裂解物在冰上温育5分钟。离心后, 将上清液在80°C温育约70分钟。所有进一步纯化均在4°C进行。将上清液冰冻30min, 然后使用5mL Ni Sepharose HP柱 (GE) 进行离心和纯化。使用缓冲液A (50mM Tris-HCl pH 7.5、1mM EDTA, 5%甘油, 500mM NaCl和20mM咪唑) 对柱进行预平衡。使用75mL的20至500mM咪唑梯度洗脱柱。合并峰级分并用10%甘油稀释以匹配SP缓冲液A (50mM Tris-HCl pH 7.5、150mM NaCl, 1mM EDTA, 5%甘油) 的电导率, 并加载到5mL SP Sepharose柱 (GE) 上。使用从150到1000mM NaCl的100mL梯度洗脱柱。合并峰级分, 透析入贮存缓冲液 (10mM Tris-HCl pH 7.5、300mM KCL, 0.1mM EDTA和50%甘油) 中, 并在-20°C贮存。

[0143] B. 错误率和定相分析

[0144] 使用测序实验比较错误率和定相值。除非另有说明, 否则在MiniSeq™系统 (Illumina, Inc., San Diego, Calif.) 上根据制造商的说明进行实验。例如, 对于每种聚合酶, 制备单独的掺入混合物 (IMX), 并用于短运行 (读段1中为35个循环) 或长运行 (读段1中为151和读段2中为76的227个循环)。使用标准的MiniSeq Mid Output Reagent Cartridge配方, 其中标准聚合酶被测试的聚合酶替换, 浓度为90μg/mL。如本文实施例中所指出, 在流

动池上温育IMX的时间存在变化。所用的DNA文库是根据标准TruSeq™ Nano方案(Illumina, Inc.)使用大肠杆菌基因组DNA制备的,具有350bp的目标插入物大小;将PhiX DNA(Illumina, Inc)以约1:10摩尔比添加到所得文库中。使用Illumina RTA软件评估两个基因组上的错误率以及定相水平。

[0145] 实施例2

[0146] 改变的聚合酶的测序性能

[0147] 鉴定出许多改变的聚合酶,其在短掺入时间下在短运行中具有不实质上大于在标准掺入时间下在短运行中使用的对照聚合酶的错误率和定相水平。下表2比较了表1中列出的改变的聚合酶在14秒掺入时间时相对于由Pol1812 (SEQ ID NO:8)代表的对照聚合酶的测序性能。用于评估改变的聚合酶的质量度量是读段1的定相率(“R1定相”)和大肠杆菌(“大肠杆菌错误”)和噬菌体PhiX(“PhiX错误”)测序对照的累积错误率。将度量相对于标准(46sec)掺入时间(“812STD”)时对应的Pol 812定相和错误率标准化。例如,Pol 812在短掺入时间(“812Fast”)的R1定相率比其在标准掺入时间时的R1定相高6倍,而大肠杆菌和PhiX的累积错误率分别高12.5和6.4倍。类似地,Pol 963 (SEQ ID NO:9)在短掺入时间时的R1定相率比Pol 812在标准掺入时间时的R1定相高5.9倍,而Pol 963的累积大肠杆菌和PhiX错误率分别高4.6和3.6倍。

[0148] 表2:表1中列出的改变的聚合酶的性能度量

[0149]

Pol (SEQ ID NO:)	测序性能		
	R1 定相	大肠杆菌错误	PhiX 错误
812 STD (8)	1.0	1.0	1.0
812 Fast (8)	6.0	12.5	6.4
963 (9)	5.9	4.6	3.6
*1550 (10)	3.0	1.5	1.4
*1558 (11)	2.9	1.5	1.5
1563 (12)	3.5	1.7	1.6
1565 (13)	3.3	2.3	1.6
1630 (14)	3.3	1.9	1.5
1634 (15)	3.1	1.7	1.7
1641 (16)	3.1	2.0	1.5
1573 (17)	3.2	1.6	1.4

[0150]

1576 (18)	3.6	1.5	1.4
1584 (19)	3.5	2.0	1.5
1586 (20)	3.9	2.9	2.2
1601 (21)	2.9	3.1	1.9
1611 (22)	3.7	3.8	2.4
*1671 (23)	2.9	1.3	1.2
*1677 (24)	3.0	1.9	1.7
*1682 (25)	2.8	1.6	1.4
1700 (26)	3.3	1.7	1.7
*1680 (27)	2.8	1.7	1.6
*1745 (28)	2.7	1.6	1.2
*1758 (29)	2.8	1.6	1.3
*1761 (30)	2.7	1.5	1.4
*1762 (31)	2.8	1.6	1.4
*1765 (32)	2.8	1.3	1.2
*1769 (33)	2.6	1.4	1.2
*1770 (34)	2.7	2.0	1.3

[0151] 以在短掺入时间时相对定相率不大于3.0和累积错误率不大于2.0为特征的改变的聚合酶代表对于快速SBS应用而言特别有吸引力的候选物。此类聚合酶的实例在表2中以粗体和星号表示。另外的结果在图2和图3中示出。

[0152] 图2示出了当与Pol 812对照(左图)相比较时,由实施例2中鉴定的改变的聚合酶之一,Pol 1558 (SEQ ID NO:11) 证明的短掺入时间时减少的定相和累积错误率。两种酶在标准掺入时间时显示出相当的定相和错误率(右图)。

[0153] 图3A示出了当与Pol 812和Pol 963对照相比较时,由实施例2中鉴定的选择的改变的聚合酶,Pol 1558,Pol 1671 (SEQ ID NO:23),Pol 1682 (SEQ ID NO:25) 和Pol 1745 (SEQ ID NO:28) 证明的短掺入时间时减少的R1定相和大肠杆菌累积错误率。上图和下图中的虚线表示在标准掺入时间时,Pol 812所证明的累积大肠杆菌错误和R1定相率。

[0154] 图3B参照Pol 812和Pol 963对照比较了相同的改变的聚合酶的定相和预定相率,显示Pol 1558、1671、1682和1745的定相率降低。

[0155] 实施例3

[0156] 在长运行条件下改变的聚合酶的活性

[0157] 使用不同的运行长度评估实施例2中鉴定的所选的改变的聚合酶。将在标准掺入时间(46sec)时各种改变的聚合酶的累积PhiX错误率与长测序运行(读段1中的250个循环,

然后读段2中的250个循环,总共500个循环)期间在短掺入时间(22sec)时的定相和累积错误率进行比较。较长的运行条件导致每次运行产生更多的序列信息(即,确定更多核苷酸的身份),并且类似于测序平台中经常使用的条件,例如在对全基因组进行测序时。结果示于图4。

[0158] 图4比较了在长测序读段(2x250个循环)期间在标准和短掺入时间时Pol 1550 (SEQ ID NO:10)和Pol 1558 (SEQ ID NO:11)与Pol 812 (SEQ ID NO:8)对照的累积PhiX错误率。两个突变体显示在配对末端转角后错误率的明显降低。此外,在第一测序读段期间,与Pol 812相比,Pol 1550显示出错误率的显著降低。

[0159] 实施例4

[0160] 在NovaSeq™上评估测序度量

[0161] 使用S1流动池和NovaSeq™测序化学,在Illumina的NovaSeq™平台上评估了实施例2中鉴定出的改变的聚合酶之一,Pol 1671。在短和标准掺入时间时确定读段1和2的错误率、定相水平和其他测序质量度量。Pol 1671和Pol 812的结果汇总在图5中。

[0162] 图5A示出了在短掺入时间(10sec)时证明的Pol 1671 (SEQ ID NO:23)的NovaSeq™测序度量与标准(40sec)和短(10sec)掺入时间时证明的Pol 812 (SEQ ID NO:8)对照的NovaSeq™测序度量之间的比较。上图示出了通过过滤器的簇(“簇PF”)的百分比;下图示出了累积的PhiX错误率。浅色空心圆圈表示在标准掺入时间时的Pol 812度量,而深色空心圆圈表示在短掺入时间时的Pol 812度量。实心圆圈表示的所有Pol 1671度量均在短掺入时间时。结果表明,Pol 1671在短掺入时间时与标准掺入时间时的Pol 812显示出这两个读段中相当的性能。

[0163] 图5B总结了在标准(40sec)和短(22sec)掺入时间时,对于NovaSeq™读段1和2,参照Pol 812,由Pol 1671所显示的累积PhiX错误率、Q30值和定相率。使用Pol 1671时,观察到这两个读段的测序质量的显著改善。

[0164] 本文中引用的所有专利、专利申请(无论是否公布)和出版物以及电子可获得的材料(包括例如在例如GenBank和RefSeq中的核苷酸序列提交以及在例如SwissProt,PIR,PRF,PDB中的氨基酸序列提交,以及来自GenBank和RefSeq中带注释的编码区的翻译)的完整公开通过引用整体并入。出版物中引用的补充材料(例如补充表,补充图,补充材料和方法/或补充实验数据)同样通过引用完整并入。在本申请的公开与通过引用并入本文的任何文件的公开之间存在任何不一致的情况下,以本申请的公开为准。仅出于清楚理解的目的给出了前面的详细描述和示例。不应从它们理解不必要的限制。本发明不限于所示出和描述的确切细节,对于本领域技术人员显而易见的变化将包括在由权利要求书限定的本发明之内。

[0165] 除非另有说明,否则在说明书和权利要求书中使用的所有表示组分数值,分子量等的数字在所有情况下均应理解为由术语“约”修饰。因此,除非另有相反指示,否则说明书和权利要求书中列出的数字参数是近似值,其可以根据本发明试图获得的期望性质而变化。无论如何,不作为将等同原则限于权利要求范围的尝试,每个数字参数应至少根据所报告的有效数字的数目并通过应用普通的舍入技术来解释。

[0166] 尽管阐述本发明的广泛范围的数值范围和参数是近似值,然而在具体实施例中阐述的数值尽可能精确报告。然而,所有数值固有含有一定范围,该范围必然是由存在于它们

各自的测试测量中的标准偏差得出的。

[0167] 除非另有说明,否则所有标题都是为了方便读者阅读,并且不应用于限制标题后面的文本的含义。

[0001]	序列表															
[0002]	<110> 亿明达股份有限公司(ILLUMINA, INC.)															
[0003]	<120> 聚合酶,组合物及使用方法															
[0004]	<130> IP-1546-PCT/0531.001546W001															
[0005]	<140> 62/753,558															
[0006]	<141> 2018-10-31															
[0007]	<160> 34															
[0008]	<170> PatentIn version 3.5															
[0009]	<210> 1															
[0010]	<211> 775															
[0011]	<212> PRT															
[0012]	<213> 人工															
[0013]	<220>															
[0014]	<223> 工程化聚合酶															
[0015]	<400> 1															
[0016]	Met	Ile	Leu	Asp	Thr	Asp	Tyr	Ile	Thr	Glu	Asn	Gly	Lys	Pro	Val	Ile
[0017]	1				5					10					15	
[0018]	Arg	Val	Phe	Lys	Lys	Glu	Asn	Gly	Glu	Phe	Lys	Ile	Glu	Tyr	Asp	Arg
[0019]				20					25					30		
[0020]	Thr	Phe	Glu	Pro	Tyr	Phe	Tyr	Ala	Leu	Leu	Lys	Asp	Asp	Ser	Ala	Ile
[0021]			35					40					45			
[0022]	Glu	Asp	Val	Lys	Lys	Val	Thr	Ala	Lys	Arg	His	Gly	Thr	Val	Val	Lys
[0023]		50					55					60				
[0024]	Val	Lys	Arg	Ala	Glu	Lys	Val	Gln	Lys	Lys	Phe	Leu	Gly	Arg	Pro	Ile
[0025]	65					70					75				80	
[0026]	Glu	Val	Trp	Lys	Leu	Tyr	Phe	Asn	His	Pro	Gln	Asp	Val	Pro	Ala	Ile
[0027]					85					90					95	
[0028]	Arg	Asp	Arg	Ile	Arg	Ala	His	Pro	Ala	Val	Val	Asp	Ile	Tyr	Glu	Tyr
[0029]			100						105					110		
[0030]	Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro
[0031]			115						120				125			
[0032]	Met	Glu	Gly	Asp	Glu	Glu	Leu	Thr	Met	Leu	Ala	Phe	Asp	Ile	Glu	Thr
[0033]		130					135					140				
[0034]	Leu	Tyr	His	Glu	Gly	Glu	Glu	Phe	Gly	Thr	Gly	Pro	Ile	Leu	Met	Ile
[0035]	145					150					155				160	
[0036]	Ser	Tyr	Ala	Asp	Gly	Ser	Glu	Ala	Arg	Val	Ile	Thr	Trp	Lys	Lys	Ile
[0037]					165					170					175	
[0038]	Asp	Leu	Pro	Tyr	Val	Asp	Val	Val	Ser	Thr	Glu	Lys	Glu	Met	Ile	Lys

[0039]	180	185	190
[0040]	Arg Phe Leu Arg Val Val Arg Glu Lys Asp Pro Asp Val Leu Ile Thr		
[0041]	195	200	205
[0042]	Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Cys Glu		
[0043]	210	215	220
[0044]	Glu Leu Gly Ile Lys Phe Thr Leu Gly Arg Asp Gly Ser Glu Pro Lys		
[0045]	225	230	235
[0046]	Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile		
[0047]	245	250	255
[0048]	His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr		
[0049]	260	265	270
[0050]	Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu		
[0051]	275	280	285
[0052]	Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly		
[0053]	290	295	300
[0054]	Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr		
[0055]	305	310	315
[0056]	Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu		
[0057]	325	330	335
[0058]	Ile Gly Gln Ser Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu		
[0059]	340	345	350
[0060]	Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Lys Arg Asn Glu Leu Ala		
[0061]	355	360	365
[0062]	Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr		
[0063]	370	375	380
[0064]	Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile		
[0065]	385	390	395
[0066]	Val Tyr Leu Asp Phe Arg Ser Leu Tyr Pro Ser Ile Ile Ile Thr His		
[0067]	405	410	415
[0068]	Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp		
[0069]	420	425	430
[0070]	Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe		
[0071]	435	440	445
[0072]	Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys		
[0073]	450	455	460
[0074]	Arg Lys Met Lys Ala Thr Val Asp Pro Leu Glu Lys Lys Leu Leu Asp		
[0075]	465	470	475
[0076]	Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr		
[0077]	485	490	495

[0078]	Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser
[0079]	500 505 510
[0080]	Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu
[0081]	515 520 525
[0082]	Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu
[0083]	530 535 540
[0084]	His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala
[0085]	545 550 555 560
[0086]	Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu
[0087]	565 570 575
[0088]	Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys
[0089]	580 585 590
[0090]	Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu
[0091]	595 600 605
[0092]	Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala
[0093]	610 615 620
[0094]	Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val
[0095]	625 630 635 640
[0096]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro
[0097]	645 650 655
[0098]	Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp
[0099]	660 665 670
[0100]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala
[0101]	675 680 685
[0102]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu
[0103]	690 695 700
[0104]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe
[0105]	705 710 715 720
[0106]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln
[0107]	725 730 735
[0108]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys
[0109]	740 745 750
[0110]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp
[0111]	755 760 765
[0112]	Leu Lys Val Lys Gly Lys Lys
[0113]	770 775
[0114]	<210> 2
[0115]	<211> 774
[0116]	<212> PRT

[0117]	<213> 人工															
[0118]	<220>															
[0119]	<223> 工程化聚合酶															
[0120]	<400> 2															
[0121]	Met	Ile	Leu	Asp	Thr	Asp	Tyr	Ile	Thr	Lys	Asp	Gly	Lys	Pro	Ile	Ile
[0122]	1				5					10					15	
[0123]	Arg	Ile	Phe	Lys	Lys	Glu	Asn	Gly	Glu	Phe	Lys	Ile	Glu	Leu	Asp	Pro
[0124]					20					25					30	
[0125]	His	Phe	Gln	Pro	Tyr	Ile	Tyr	Ala	Leu	Leu	Lys	Asp	Asp	Ser	Ala	Ile
[0126]					35					40				45		
[0127]	Glu	Glu	Ile	Lys	Ala	Ile	Lys	Gly	Glu	Arg	His	Gly	Lys	Thr	Val	Arg
[0128]					50					55				60		
[0129]	Val	Leu	Asp	Ala	Val	Lys	Val	Arg	Lys	Lys	Phe	Leu	Gly	Arg	Glu	Val
[0130]	65					70					75				80	
[0131]	Glu	Val	Trp	Lys	Leu	Ile	Phe	Glu	His	Pro	Gln	Asp	Val	Pro	Ala	Met
[0132]					85					90					95	
[0133]	Arg	Gly	Lys	Ile	Arg	Glu	His	Pro	Ala	Val	Val	Asp	Ile	Tyr	Glu	Tyr
[0134]					100					105					110	
[0135]	Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro
[0136]					115					120				125		
[0137]	Met	Glu	Gly	Asp	Glu	Glu	Leu	Lys	Leu	Leu	Ala	Phe	Ala	Ile	Ala	Thr
[0138]	130									135				140		
[0139]	Phe	Tyr	His	Glu	Gly	Asp	Glu	Phe	Gly	Lys	Gly	Glu	Ile	Ile	Met	Ile
[0140]	145						150				155				160	
[0141]	Ser	Tyr	Ala	Asp	Glu	Glu	Glu	Ala	Arg	Val	Ile	Thr	Trp	Lys	Asn	Ile
[0142]					165					170					175	
[0143]	Asp	Leu	Pro	Tyr	Val	Asp	Val	Val	Ser	Asn	Glu	Arg	Glu	Met	Ile	Lys
[0144]					180					185				190		
[0145]	Arg	Phe	Val	Gln	Val	Val	Lys	Glu	Lys	Asp	Pro	Asp	Val	Ile	Ile	Thr
[0146]					195					200				205		
[0147]	Tyr	Asn	Gly	Asp	Asn	Phe	Asp	Leu	Pro	Tyr	Leu	Ile	Lys	Arg	Ala	Glu
[0148]					210					215				220		
[0149]	Lys	Leu	Gly	Val	Arg	Leu	Val	Leu	Gly	Arg	Asp	Lys	Glu	His	Pro	Glu
[0150]	225						230				235				240	
[0151]	Pro	Lys	Ile	Gln	Arg	Met	Gly	Asp	Ser	Phe	Ala	Val	Glu	Ile	Lys	Gly
[0152]					245					250					255	
[0153]	Arg	Ile	His	Phe	Asp	Leu	Phe	Pro	Val	Val	Arg	Arg	Thr	Ile	Asn	Leu
[0154]					260					265				270		
[0155]	Pro	Thr	Tyr	Thr	Leu	Glu	Ala	Val	Tyr	Glu	Ala	Val	Leu	Gly	Lys	Thr

[0156]	275	280	285
[0157]	Lys Ser Lys Leu Gly Ala Glu Glu Ile Ala Ala Ile Trp Glu Thr Glu		
[0158]	290	295	300
[0159]	Glu Ser Met Lys Lys Leu Ala Gln Tyr Ser Met Glu Asp Ala Arg Ala		
[0160]	305	310	315
[0161]	Thr Tyr Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Glu Leu Ala		
[0162]	325	330	335
[0163]	Lys Leu Ile Gly Gln Ser Val Trp Asp Val Ser Arg Ser Ser Thr Gly		
[0164]	340	345	350
[0165]	Asn Leu Val Glu Trp Tyr Leu Leu Arg Val Ala Tyr Ala Arg Asn Glu		
[0166]	355	360	365
[0167]	Leu Ala Pro Asn Lys Pro Asp Glu Glu Glu Tyr Lys Arg Arg Leu Arg		
[0168]	370	375	380
[0169]	Thr Thr Tyr Leu Gly Gly Tyr Val Lys Glu Pro Glu Lys Gly Leu Trp		
[0170]	385	390	395
[0171]	Glu Asn Ile Ile Tyr Leu Asp Phe Arg Ser Leu Tyr Pro Ser Ile Ile		
[0172]	405	410	415
[0173]	Val Thr His Asn Val Ser Pro Asp Thr Leu Glu Lys Glu Gly Cys Lys		
[0174]	420	425	430
[0175]	Asn Tyr Asp Val Ala Pro Ile Val Gly Tyr Arg Phe Cys Lys Asp Phe		
[0176]	435	440	445
[0177]	Pro Gly Phe Ile Pro Ser Ile Leu Gly Asp Leu Ile Ala Met Arg Gln		
[0178]	450	455	460
[0179]	Asp Ile Lys Lys Lys Met Lys Ser Thr Ile Asp Pro Ile Glu Lys Lys		
[0180]	465	470	475
[0181]	Met Leu Asp Tyr Arg Gln Arg Ala Ile Lys Leu Leu Ala Asn Ser Tyr		
[0182]	485	490	495
[0183]	Tyr Gly Tyr Met Gly Tyr Pro Lys Ala Arg Trp Tyr Ser Lys Glu Cys		
[0184]	500	505	510
[0185]	Ala Glu Ser Val Thr Ala Trp Gly Arg His Tyr Ile Glu Met Thr Ile		
[0186]	515	520	525
[0187]	Arg Glu Ile Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr		
[0188]	530	535	540
[0189]	Asp Gly Phe Tyr Ala Thr Ile Pro Gly Glu Lys Pro Glu Leu Ile Lys		
[0190]	545	550	555
[0191]	Lys Lys Ala Lys Glu Phe Leu Asn Tyr Ile Asn Ser Lys Leu Pro Gly		
[0192]	565	570	575
[0193]	Leu Leu Glu Leu Glu Tyr Glu Gly Phe Tyr Leu Arg Gly Phe Phe Val		
[0194]	580	585	590

[0195]	Thr	Lys	Lys	Arg	Tyr	Ala	Val	Ile	Asp	Glu	Glu	Gly	Arg	Ile	Thr	Thr
[0196]			595					600					605			
[0197]	Arg	Gly	Leu	Glu	Val	Val	Arg	Arg	Asp	Trp	Ser	Glu	Ile	Ala	Lys	Glu
[0198]			610					615					620			
[0199]	Thr	Gln	Ala	Lys	Val	Leu	Glu	Ala	Ile	Leu	Lys	Glu	Gly	Ser	Val	Glu
[0200]	625						630					635				640
[0201]	Lys	Ala	Val	Glu	Val	Val	Arg	Asp	Val	Val	Glu	Lys	Ile	Ala	Lys	Tyr
[0202]					645					650					655	
[0203]	Arg	Val	Pro	Leu	Glu	Lys	Leu	Val	Ile	His	Glu	Gln	Ile	Thr	Arg	Asp
[0204]				660					665						670	
[0205]	Leu	Lys	Asp	Tyr	Lys	Ala	Ile	Gly	Pro	His	Val	Ala	Ile	Ala	Lys	Arg
[0206]			675					680					685			
[0207]	Leu	Ala	Ala	Arg	Gly	Ile	Lys	Val	Lys	Pro	Gly	Thr	Ile	Ile	Ser	Tyr
[0208]		690					695					700				
[0209]	Ile	Val	Leu	Lys	Gly	Ser	Gly	Lys	Ile	Ser	Asp	Arg	Val	Ile	Leu	Leu
[0210]	705					710					715					720
[0211]	Thr	Glu	Tyr	Asp	Pro	Arg	Lys	His	Lys	Tyr	Asp	Pro	Asp	Tyr	Tyr	Ile
[0212]					725					730					735	
[0213]	Glu	Asn	Gln	Val	Leu	Pro	Ala	Val	Leu	Arg	Ile	Leu	Glu	Ala	Phe	Gly
[0214]				740					745					750		
[0215]	Tyr	Arg	Lys	Glu	Asp	Leu	Arg	Tyr	Gln	Ser	Ser	Lys	Gln	Thr	Gly	Leu
[0216]			755					760					765			
[0217]	Asp	Ala	Trp	Leu	Lys	Arg										
[0218]		770														
[0219]	<210>	3														
[0220]	<211>	777														
[0221]	<212>	PRT														
[0222]	<213>	人工														
[0223]	<220>															
[0224]	<223>	工程化聚合酶														
[0225]	<400>	3														
[0226]	Met	Ile	Leu	Asp	Ala	Asp	Tyr	Ile	Thr	Glu	Asp	Gly	Lys	Pro	Ile	Ile
[0227]	1				5					10					15	
[0228]	Arg	Ile	Phe	Lys	Lys	Glu	Asn	Gly	Glu	Phe	Lys	Val	Glu	Tyr	Asp	Arg
[0229]				20					25					30		
[0230]	Asn	Phe	Arg	Pro	Tyr	Ile	Tyr	Ala	Leu	Leu	Lys	Asp	Asp	Ser	Gln	Ile
[0231]			35					40					45			
[0232]	Asp	Glu	Val	Arg	Lys	Ile	Thr	Ala	Glu	Arg	His	Gly	Lys	Ile	Val	Arg
[0233]		50						55					60			

[0234]	Ile Ile Asp Ala Glu Lys Val Arg Lys Lys Phe Leu Gly Arg Pro Ile
[0235]	65 70 75 80
[0236]	Glu Val Trp Arg Leu Tyr Phe Glu His Pro Gln Asp Val Pro Ala Ile
[0237]	85 90 95
[0238]	Arg Asp Lys Ile Arg Glu His Ser Ala Val Ile Asp Ile Phe Glu Tyr
[0239]	100 105 110
[0240]	Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
[0241]	115 120 125
[0242]	Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Asp Ile Glu Thr
[0243]	130 135 140
[0244]	Leu Tyr His Glu Gly Glu Glu Phe Ala Lys Gly Pro Ile Ile Met Ile
[0245]	145 150 155 160
[0246]	Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
[0247]	165 170 175
[0248]	Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
[0249]	180 185 190
[0250]	Arg Phe Leu Lys Val Ile Arg Glu Lys Asp Pro Asp Val Ile Ile Thr
[0251]	195 200 205
[0252]	Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Val Lys Arg Ala Glu
[0253]	210 215 220
[0254]	Lys Leu Gly Ile Lys Leu Pro Leu Gly Arg Asp Gly Ser Glu Pro Lys
[0255]	225 230 235 240
[0256]	Met Gln Arg Leu Gly Asp Met Thr Ala Val Glu Ile Lys Gly Arg Ile
[0257]	245 250 255
[0258]	His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
[0259]	260 265 270
[0260]	Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
[0261]	275 280 285
[0262]	Lys Val Tyr Ala His Glu Ile Ala Glu Ala Trp Glu Thr Gly Lys Gly
[0263]	290 295 300
[0264]	Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr
[0265]	305 310 315 320
[0266]	Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
[0267]	325 330 335
[0268]	Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
[0269]	340 345 350
[0270]	Val Glu Trp Tyr Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
[0271]	355 360 365
[0272]	Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser

[0273]	370	375	380
[0274]	Tyr Ala Gly Gly Tyr Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Gly		
[0275]	385	390	395 400
[0276]	Leu Val Ser Leu Asp Phe Arg Ser Leu Tyr Pro Ser Ile Ile Ile Thr		
[0277]	405	410	415
[0278]	His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Glu Tyr		
[0279]	420	425	430
[0280]	Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly		
[0281]	435	440	445
[0282]	Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Glu Ile		
[0283]	450	455	460
[0284]	Lys Arg Lys Met Lys Ala Ser Lys Asp Pro Ile Glu Lys Lys Met Leu		
[0285]	465	470	475 480
[0286]	Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly		
[0287]	485	490	495
[0288]	Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu		
[0289]	500	505	510
[0290]	Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Arg Lys Glu		
[0291]	515	520	525
[0292]	Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly		
[0293]	530	535	540
[0294]	Leu Tyr Ala Thr Ile Pro Gly Ala Lys Pro Glu Glu Ile Lys Lys Lys		
[0295]	545	550	555 560
[0296]	Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu		
[0297]	565	570	575
[0298]	Glu Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys		
[0299]	580	585	590
[0300]	Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly		
[0301]	595	600	605
[0302]	Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln		
[0303]	610	615	620
[0304]	Ala Lys Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala		
[0305]	625	630	635 640
[0306]	Val Lys Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Ile		
[0307]	645	650	655
[0308]	Pro Pro Glu Lys Leu Val Ile Tyr Glu Gln Ile Thr Arg Pro Leu His		
[0309]	660	665	670
[0310]	Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala		
[0311]	675	680	685

[0312]	Ala Arg Gly Val Lys Val Arg Pro Gly Met Val Ile Gly Tyr Ile Val
[0313]	690 695 700
[0314]	Leu Arg Gly Asp Gly Pro Ile Ser Lys Arg Ala Ile Leu Ala Glu Glu
[0315]	705 710 715 720
[0316]	Phe Asp Leu Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
[0317]	725 730 735
[0318]	Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Ala Phe Gly Tyr Arg
[0319]	740 745 750
[0320]	Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ala
[0321]	755 760 765
[0322]	Trp Leu Asn Ile Lys Lys Lys Gly Ser
[0323]	770 775
[0324]	<210> 4
[0325]	<211> 775
[0326]	<212> PRT
[0327]	<213> 人工
[0328]	<220>
[0329]	<223> 工程化聚合酶
[0330]	<400> 4
[0331]	Met Ile Leu Asp Val Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
[0332]	1 5 10 15
[0333]	Arg Leu Phe Lys Lys Glu Asn Gly Lys Phe Lys Ile Glu His Asp Arg
[0334]	20 25 30
[0335]	Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Arg Asp Asp Ser Lys Ile
[0336]	35 40 45
[0337]	Glu Glu Val Lys Lys Ile Thr Gly Glu Arg His Gly Lys Ile Val Arg
[0338]	50 55 60
[0339]	Ile Val Asp Val Glu Lys Val Glu Lys Lys Phe Leu Gly Lys Pro Ile
[0340]	65 70 75 80
[0341]	Thr Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Thr Ile
[0342]	85 90 95
[0343]	Arg Glu Lys Val Arg Glu His Pro Ala Val Val Asp Ile Phe Glu Tyr
[0344]	100 105 110
[0345]	Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
[0346]	115 120 125
[0347]	Met Glu Gly Glu Glu Glu Leu Lys Ile Leu Ala Phe Ala Ile Ala Thr
[0348]	130 135 140
[0349]	Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
[0350]	145 150 155 160

[0351]	Ser Tyr Ala Asp Glu Asn Glu Ala Lys Val Ile Thr Trp Lys Asn Ile		
[0352]		165	170
[0353]	Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys		175
[0354]		180	185
[0355]	Arg Phe Leu Arg Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Val Thr		190
[0356]		195	200
[0357]	Tyr Asn Gly Asp Ser Phe Asp Phe Pro Tyr Leu Ala Lys Arg Ala Glu		205
[0358]		210	215
[0359]	Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys		220
[0360]		225	230
[0361]	Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile		235
[0362]		245	250
[0363]	His Phe Asp Leu Tyr His Val Ile Thr Arg Thr Ile Asn Leu Pro Thr		255
[0364]		260	265
[0365]	Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu		270
[0366]		275	280
[0367]	Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Ser Gly Glu Asn		285
[0368]		290	295
[0369]	Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr		300
[0370]		305	310
[0371]	Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ile Gln Leu Ser Arg Leu		315
[0372]		325	330
[0373]	Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu		335
[0374]		340	345
[0375]	Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Val Ala		350
[0376]		355	360
[0377]	Pro Asn Lys Pro Ser Glu Glu Glu Tyr Gln Arg Arg Leu Arg Glu Ser		365
[0378]		370	375
[0379]	Tyr Thr Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn		380
[0380]		385	390
[0381]	Ile Val Tyr Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr		395
[0382]		405	410
[0383]	His Asn Val Ser Pro Asp Thr Leu Asn Leu Glu Gly Cys Lys Asn Tyr		415
[0384]		420	425
[0385]	Asp Ile Ala Pro Gln Val Gly His Lys Phe Cys Lys Asp Ile Pro Gly		430
[0386]		435	440
[0387]	Phe Ile Pro Ser Leu Leu Gly His Leu Leu Glu Glu Arg Gln Lys Ile		445
[0388]		450	455
[0389]	Lys Thr Lys Met Lys Glu Thr Gln Asp Pro Ile Glu Lys Ile Leu Leu		460

[0390]	465	470	475	480
[0391]	Asp Tyr Arg Gln Lys Ala Ile Lys Leu Leu Ala Asn Ser Phe Tyr Gly			
[0392]		485	490	495
[0393]	Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu			
[0394]		500	505	510
[0395]	Ser Val Thr Ala Trp Gly Arg Lys Tyr Ile Glu Leu Val Trp Lys Glu			
[0396]		515	520	525
[0397]	Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly			
[0398]		530	535	540
[0399]	Leu Tyr Ala Thr Ile Pro Gly Gly Glu Ser Glu Glu Ile Lys Lys Lys			
[0400]	545	550	555	560
[0401]	Ala Leu Glu Phe Val Lys Tyr Ile Asn Ser Lys Leu Pro Gly Leu Leu			
[0402]		565	570	575
[0403]	Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys			
[0404]		580	585	590
[0405]	Lys Arg Tyr Ala Val Ile Asp Glu Glu Gly Lys Val Ile Thr Arg Gly			
[0406]		595	600	605
[0407]	Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln			
[0408]		610	615	620
[0409]	Ala Arg Val Leu Glu Thr Ile Leu Lys His Gly Asp Val Glu Glu Ala			
[0410]	625	630	635	640
[0411]	Val Arg Ile Val Lys Glu Val Ile Gln Lys Leu Ala Asn Tyr Glu Ile			
[0412]		645	650	655
[0413]	Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His			
[0414]		660	665	670
[0415]	Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Lys Leu Ala			
[0416]		675	680	685
[0417]	Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val			
[0418]		690	695	700
[0419]	Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu			
[0420]	705	710	715	720
[0421]	Tyr Asp Pro Lys Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn			
[0422]		725	730	735
[0423]	Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg			
[0424]		740	745	750
[0425]	Lys Glu Asp Leu Arg Tyr Gln Lys Thr Arg Gln Val Gly Leu Thr Ser			
[0426]		755	760	765
[0427]	Trp Leu Asn Ile Lys Lys Ser			
[0428]		770	775	

40

[0468]	His	Phe	Asp	Leu	Tyr	Pro	Val	Ile	Arg	Arg	Thr	Ile	Asn	Leu	Pro	Thr
[0469]				260					265					270		
[0470]	Tyr	Thr	Leu	Glu	Ala	Val	Tyr	Glu	Ala	Val	Phe	Gly	Gln	Pro	Lys	Glu
[0471]				275					280					285		
[0472]	Lys	Val	Tyr	Ala	Glu	Glu	Ile	Thr	Thr	Ala	Trp	Glu	Thr	Gly	Glu	Asn
[0473]				290					295					300		
[0474]	Leu	Glu	Arg	Val	Ala	Arg	Tyr	Ser	Met	Glu	Asp	Ala	Lys	Val	Thr	Tyr
[0475]	305						310					315				320
[0476]	Glu	Leu	Gly	Lys	Glu	Phe	Leu	Pro	Met	Glu	Ala	Gln	Leu	Ser	Arg	Leu
[0477]					325					330					335	
[0478]	Ile	Gly	Gln	Ser	Leu	Trp	Asp	Val	Ser	Arg	Ser	Ser	Thr	Gly	Asn	Leu
[0479]					340					345					350	
[0480]	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Glu	Arg	Asn	Glu	Leu	Ala
[0481]					355				360					365		
[0482]	Pro	Asn	Lys	Pro	Asp	Glu	Lys	Glu	Leu	Ala	Arg	Arg	Arg	Gln	Ser	Tyr
[0483]					370				375					380		
[0484]	Glu	Gly	Gly	Tyr	Val	Lys	Glu	Pro	Glu	Arg	Gly	Leu	Trp	Glu	Asn	Ile
[0485]	385					390					395					400
[0486]	Val	Tyr	Leu	Asp	Phe	Arg	Ser	Leu	Tyr	Pro	Ser	Ile	Ile	Ile	Thr	His
[0487]					405					410						415
[0488]	Asn	Val	Ser	Pro	Asp	Thr	Leu	Asn	Arg	Glu	Gly	Cys	Lys	Glu	Tyr	Asp
[0489]					420					425					430	
[0490]	Val	Ala	Pro	Gln	Val	Gly	His	Arg	Phe	Cys	Lys	Asp	Phe	Pro	Gly	Phe
[0491]					435				440					445		
[0492]	Ile	Pro	Ser	Leu	Leu	Gly	Asp	Leu	Leu	Glu	Glu	Arg	Gln	Lys	Ile	Lys
[0493]					450					455				460		
[0494]	Lys	Lys	Met	Lys	Ala	Thr	Ile	Asp	Pro	Ile	Glu	Arg	Lys	Leu	Leu	Asp
[0495]	465						470					475				480
[0496]	Tyr	Arg	Gln	Arg	Ala	Ile	Lys	Ile	Leu	Ala	Asn	Ser	Tyr	Tyr	Gly	Tyr
[0497]					485					490					495	
[0498]	Tyr	Gly	Tyr	Ala	Arg	Ala	Arg	Trp	Tyr	Cys	Lys	Glu	Cys	Ala	Glu	Ser
[0499]					500					505					510	
[0500]	Val	Thr	Ala	Trp	Gly	Arg	Glu	Tyr	Ile	Thr	Met	Thr	Ile	Lys	Glu	Ile
[0501]					515					520				525		
[0502]	Glu	Glu	Lys	Tyr	Gly	Phe	Lys	Val	Ile	Tyr	Ser	Asp	Thr	Asp	Gly	Phe
[0503]					530					535				540		
[0504]	Phe	Ala	Thr	Ile	Pro	Gly	Ala	Asp	Ala	Glu	Thr	Val	Lys	Lys	Lys	Ala
[0505]	545						550				555					560
[0506]	Met	Glu	Phe	Leu	Lys	Tyr	Ile	Asn	Ala	Lys	Leu	Pro	Gly	Ala	Leu	Glu

[0507]	565	570	575
[0508]	Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys Lys		
[0509]	580	585	590
[0510]	Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu		
[0511]	595	600	605
[0512]	Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala		
[0513]	610	615	620
[0514]	Arg Val Leu Glu Ala Leu Leu Lys Asp Gly Asp Val Glu Lys Ala Val		
[0515]	625	630	635
[0516]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro		
[0517]	645	650	655
[0518]	Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Lys Asp		
[0519]	660	665	670
[0520]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala		
[0521]	675	680	685
[0522]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu		
[0523]	690	695	700
[0524]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Phe Asp Glu Phe		
[0525]	705	710	715
[0526]	Asp Pro Thr Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln		
[0527]	725	730	735
[0528]	Val Leu Pro Ala Val Glu Arg Ile Leu Arg Ala Phe Gly Tyr Arg Lys		
[0529]	740	745	750
[0530]	Glu Asp Leu Arg Tyr Gln Lys Thr Arg Gln Val Gly Leu Ser Ala Trp		
[0531]	755	760	765
[0532]	Leu Lys Pro Lys Gly Thr		
[0533]	770		
[0534]	<210> 6		
[0535]	<211> 773		
[0536]	<212> PRT		
[0537]	<213> 人工		
[0538]	<220>		
[0539]	<223> 工程化聚合酶		
[0540]	<400> 6		
[0541]	Met Ile Ile Asp Ala Asp Tyr Ile Thr Glu Asp Gly Lys Pro Ile Ile		
[0542]	1	5	10
[0543]	Arg Ile Phe Lys Lys Glu Lys Gly Glu Phe Lys Val Glu Tyr Asp Arg		
[0544]	20	25	30
[0545]	Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile		

[0546]	35	40	45
[0547]	Asp Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Lys Ile Val Arg		
[0548]	50	55	60
[0549]	Ile Thr Glu Val Glu Lys Val Gln Lys Lys Phe Leu Gly Arg Pro Ile		
[0550]	65	70	75
[0551]	Glu Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Ala Ile		
[0552]	85	90	95
[0553]	Arg Glu Lys Ile Arg Glu His Pro Ala Val Val Asp Ile Phe Glu Tyr		
[0554]	100	105	110
[0555]	Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Thr Pro		
[0556]	115	120	125
[0557]	Met Glu Gly Asn Glu Glu Leu Thr Phe Leu Ala Val Asp Ile Glu Thr		
[0558]	130	135	140
[0559]	Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile		
[0560]	145	150	155
[0561]	Ser Tyr Ala Asp Glu Glu Gly Ala Lys Val Ile Thr Trp Lys Ser Ile		
[0562]	165	170	175
[0563]	Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys		
[0564]	180	185	190
[0565]	Arg Leu Val Lys Val Ile Arg Glu Lys Asp Pro Asp Val Ile Ile Thr		
[0566]	195	200	205
[0567]	Tyr Asn Gly Asp Asn Phe Asp Phe Pro Tyr Leu Leu Lys Arg Ala Glu		
[0568]	210	215	220
[0569]	Lys Leu Gly Ile Lys Leu Pro Leu Gly Arg Asp Asn Ser Glu Pro Lys		
[0570]	225	230	235
[0571]	Met Gln Arg Met Gly Asp Ser Leu Ala Val Glu Ile Lys Gly Arg Ile		
[0572]	245	250	255
[0573]	His Phe Asp Leu Phe Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr		
[0574]	260	265	270
[0575]	Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Ser Lys Glu		
[0576]	275	280	285
[0577]	Lys Val Tyr Ala His Glu Ile Ala Glu Ala Trp Glu Thr Gly Lys Gly		
[0578]	290	295	300
[0579]	Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Val Thr Phe		
[0580]	305	310	315
[0581]	Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ala Arg Leu		
[0582]	325	330	335
[0583]	Val Gly Gln Pro Val Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu		
[0584]	340	345	350

[0585]	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Glu	Arg	Asn	Glu	Leu	Ala
[0586]				355					360					365		
[0587]	Pro	Asn	Lys	Pro	Asp	Glu	Arg	Glu	Tyr	Glu	Arg	Arg	Leu	Arg	Glu	Ser
[0588]			370					375					380			
[0589]	Tyr	Glu	Gly	Gly	Tyr	Val	Lys	Glu	Pro	Glu	Lys	Gly	Leu	Trp	Glu	Gly
[0590]	385					390					395				400	
[0591]	Ile	Val	Ser	Leu	Asp	Phe	Arg	Ser	Leu	Tyr	Pro	Ser	Ile	Ile	Ile	Thr
[0592]					405					410					415	
[0593]	His	Asn	Val	Ser	Pro	Asp	Thr	Leu	Asn	Arg	Glu	Asn	Cys	Lys	Glu	Tyr
[0594]				420						425				430		
[0595]	Asp	Val	Ala	Pro	Gln	Val	Gly	His	Arg	Phe	Cys	Lys	Asp	Phe	Pro	Gly
[0596]			435					440					445			
[0597]	Phe	Ile	Pro	Ser	Leu	Leu	Gly	Asn	Leu	Leu	Glu	Glu	Arg	Gln	Lys	Ile
[0598]		450					455					460				
[0599]	Lys	Lys	Arg	Met	Lys	Glu	Ser	Lys	Asp	Pro	Val	Glu	Lys	Lys	Leu	Leu
[0600]	465					470					475				480	
[0601]	Asp	Tyr	Arg	Gln	Arg	Ala	Ile	Lys	Ile	Leu	Ala	Asn	Ser	Tyr	Tyr	Gly
[0602]				485						490				495		
[0603]	Tyr	Tyr	Gly	Tyr	Ala	Lys	Ala	Arg	Trp	Tyr	Cys	Lys	Glu	Cys	Ala	Glu
[0604]				500					505					510		
[0605]	Ser	Val	Thr	Ala	Trp	Gly	Arg	Gln	Tyr	Ile	Asp	Leu	Val	Arg	Arg	Glu
[0606]			515					520					525			
[0607]	Leu	Glu	Ser	Arg	Gly	Phe	Lys	Val	Leu	Tyr	Ile	Asp	Thr	Asp	Gly	Leu
[0608]		530					535					540				
[0609]	Tyr	Ala	Thr	Ile	Pro	Gly	Ala	Lys	His	Glu	Glu	Ile	Lys	Glu	Lys	Ala
[0610]	545					550					555				560	
[0611]	Leu	Lys	Phe	Val	Glu	Tyr	Ile	Asn	Ser	Lys	Leu	Pro	Gly	Leu	Leu	Glu
[0612]				565					570				575			
[0613]	Leu	Glu	Tyr	Glu	Gly	Phe	Tyr	Ala	Arg	Gly	Phe	Phe	Val	Thr	Lys	Lys
[0614]				580					585				590			
[0615]	Lys	Tyr	Ala	Leu	Ile	Asp	Glu	Glu	Gly	Lys	Ile	Val	Thr	Arg	Gly	Leu
[0616]			595					600					605			
[0617]	Glu	Ile	Val	Arg	Arg	Asp	Trp	Ser	Glu	Ile	Ala	Lys	Glu	Thr	Gln	Ala
[0618]		610					615					620				
[0619]	Lys	Val	Leu	Glu	Ala	Ile	Leu	Lys	His	Gly	Asn	Val	Asp	Glu	Ala	Val
[0620]	625					630					635				640	
[0621]	Lys	Ile	Val	Lys	Glu	Val	Thr	Glu	Lys	Leu	Ser	Lys	Tyr	Glu	Ile	Pro
[0622]				645					650				655			
[0623]	Pro	Glu	Lys	Leu	Val	Ile	Tyr	Glu	Gln	Ile	Thr	Arg	Pro	Leu	Ser	Glu

[0624]	660	665	670
[0625]	Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala		
[0626]	675	680	685
[0627]	Lys Gly Val Lys Val Lys Pro Gly Met Val Ile Gly Tyr Ile Val Leu		
[0628]	690	695	700
[0629]	Arg Gly Asp Gly Pro Ile Ser Lys Arg Ala Ile Ala Ile Glu Glu Phe		
[0630]	705	710	715
[0631]	Asp Pro Lys Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln		
[0632]	725	730	735
[0633]	Val Leu Pro Ala Val Glu Arg Ile Leu Arg Ala Phe Gly Tyr Arg Lys		
[0634]	740	745	750
[0635]	Glu Asp Leu Lys Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp		
[0636]	755	760	765
[0637]	Leu Lys Phe Gly Ser		
[0638]	770		
[0639]	<210> 7		
[0640]	<211> 775		
[0641]	<212> PRT		
[0642]	<213> 人工		
[0643]	<220>		
[0644]	<223> 工程化聚合酶		
[0645]	<400> 7		
[0646]	Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Asp Gly Lys Pro Val Ile		
[0647]	1	5	10
[0648]	Arg Val Phe Lys Lys Glu Lys Gly Glu Phe Lys Ile Asn Tyr Asp Arg		15
[0649]	20	25	30
[0650]	Asp Phe Glu Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile		
[0651]	35	40	45
[0652]	Glu Asp Ile Lys Lys Ile Thr Ala Glu Arg His Gly Thr Thr Val Arg		
[0653]	50	55	60
[0654]	Val Thr Arg Ala Glu Arg Val Lys Lys Lys Phe Leu Gly Arg Pro Val		
[0655]	65	70	75
[0656]	Glu Val Trp Lys Leu Tyr Phe Thr His Pro Gln Asp Val Pro Ala Ile		
[0657]	85	90	95
[0658]	Arg Asp Lys Ile Arg Glu His Pro Ala Val Val Asp Ile Tyr Glu Tyr		
[0659]	100	105	110
[0660]	Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro		
[0661]	115	120	125
[0662]	Met Glu Gly Asn Glu Glu Leu Arg Met Leu Ala Phe Asp Ile Glu Thr		

[0663]	130	135	140
[0664]	Leu Tyr His Glu Gly Glu Glu Phe Gly Glu Gly Pro Ile Leu Met Ile		
[0665]	145	150	155
[0666]	Ser Tyr Ala Asp Glu Glu Gly Ala Arg Val Ile Thr Trp Lys Asn Ile		
[0667]	165	170	175
[0668]	Asp Leu Pro Tyr Val Glu Ser Val Ser Thr Glu Lys Glu Met Ile Lys		
[0669]	180	185	190
[0670]	Arg Phe Leu Lys Val Ile Gln Glu Lys Asp Pro Asp Val Leu Ile Thr		
[0671]	195	200	205
[0672]	Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Ser Glu		
[0673]	210	215	220
[0674]	Thr Leu Gly Val Lys Phe Ile Leu Gly Arg Asp Gly Ser Glu Pro Lys		
[0675]	225	230	235
[0676]	Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile		
[0677]	245	250	255
[0678]	His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr		
[0679]	260	265	270
[0680]	Tyr Thr Leu Glu Thr Val Tyr Glu Ala Ile Phe Gly Gln Pro Lys Glu		
[0681]	275	280	285
[0682]	Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly		
[0683]	290	295	300
[0684]	Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr		
[0685]	305	310	315
[0686]	Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu		
[0687]	325	330	335
[0688]	Val Gly Gln Ser Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu		
[0689]	340	345	350
[0690]	Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala		
[0691]	355	360	365
[0692]	Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Ala Glu Ser Tyr		
[0693]	370	375	380
[0694]	Ala Gly Gly Tyr Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn Ile		
[0695]	385	390	395
[0696]	Val Tyr Leu Asp Tyr Lys Ser Leu Tyr Pro Ser Ile Ile Ile Thr His		
[0697]	405	410	415
[0698]	Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Glu Tyr Asp		
[0699]	420	425	430
[0700]	Val Ala Pro Gln Val Gly His Arg Phe Cys Lys Asp Phe Pro Gly Phe		
[0701]	435	440	445

[0702]	Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Val Lys		
[0703]	450	455	460
[0704]	Lys Lys Met Lys Ala Thr Val Asp Pro Ile Glu Arg Lys Leu Leu Asp		
[0705]	465	470	475 480
[0706]	Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly Tyr		
[0707]	485	490	495
[0708]	Tyr Gly Tyr Ala Asn Ala Arg Trp Tyr Cys Arg Glu Cys Ala Glu Ser		
[0709]	500	505	510
[0710]	Val Thr Ala Trp Gly Arg Gln Tyr Ile Glu Thr Thr Met Arg Glu Ile		
[0711]	515	520	525
[0712]	Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Phe		
[0713]	530	535	540
[0714]	Phe Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Thr		
[0715]	545	550	555 560
[0716]	Lys Glu Phe Leu Asn Tyr Ile Asn Pro Arg Leu Pro Gly Leu Leu Glu		
[0717]	565	570	575
[0718]	Leu Glu Tyr Glu Gly Phe Tyr Arg Arg Gly Phe Phe Val Thr Lys Lys		
[0719]	580	585	590
[0720]	Lys Tyr Ala Val Ile Asp Glu Glu Asp Lys Ile Thr Thr Arg Gly Leu		
[0721]	595	600	605
[0722]	Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala		
[0723]	610	615	620
[0724]	Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val		
[0725]	625	630	635 640
[0726]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Arg Tyr Glu Val Pro		
[0727]	645	650	655
[0728]	Pro Glu Lys Leu Val Ile Tyr Glu Gln Ile Thr Arg Asn Leu Arg Asp		
[0729]	660	665	670
[0730]	Tyr Arg Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala		
[0731]	675	680	685
[0732]	Arg Gly Ile Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu		
[0733]	690	695	700
[0734]	Lys Gly Pro Gly Arg Val Gly Asp Arg Ala Ile Pro Phe Asp Glu Phe		
[0735]	705	710	715 720
[0736]	Asp Pro Ala Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln		
[0737]	725	730	735
[0738]	Val Leu Pro Ala Val Glu Arg Ile Leu Arg Ala Phe Gly Tyr Arg Lys		
[0739]	740	745	750
[0740]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Ala Gly Leu Gly Ala Trp		

[0741]	755	760	765
[0742]	Leu Lys Pro Lys Thr Gly Ser		
[0743]	770	775	
[0744]	<210> 8		
[0745]	<211> 775		
[0746]	<212> PRT		
[0747]	<213> 人工		
[0748]	<220>		
[0749]	<223> 工程化聚合酶		
[0750]	<400> 8		
[0751]	Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile		
[0752]	1 5 10 15		
[0753]	Arg Val Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg		
[0754]	20 25 30		
[0755]	Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile		
[0756]	35 40 45		
[0757]	Glu Asp Val Lys Lys Val Thr Ala Lys Arg His Gly Thr Val Val Lys		
[0758]	50 55 60		
[0759]	Val Lys Arg Ala Glu Lys Val Gln Lys Lys Phe Leu Gly Arg Pro Ile		
[0760]	65 70 75 80		
[0761]	Glu Val Trp Lys Leu Tyr Phe Asn His Pro Gln Asp Val Pro Ala Ile		
[0762]	85 90 95		
[0763]	Arg Asp Arg Ile Arg Ala His Pro Ala Val Val Asp Ile Tyr Glu Tyr		
[0764]	100 105 110		
[0765]	Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro		
[0766]	115 120 125		
[0767]	Ala Glu Gly Asp Glu Glu Leu Thr Met Leu Ala Phe Ala Ile Ala Thr		
[0768]	130 135 140		
[0769]	Leu Tyr His Glu Gly Glu Glu Phe Gly Thr Gly Pro Ile Leu Met Ile		
[0770]	145 150 155 160		
[0771]	Ser Tyr Ala Asp Gly Ser Glu Ala Arg Val Ile Thr Trp Lys Lys Ile		
[0772]	165 170 175		
[0773]	Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Lys Glu Met Ile Lys		
[0774]	180 185 190		
[0775]	Arg Phe Leu Arg Val Val Arg Glu Lys Asp Pro Asp Val Leu Ile Thr		
[0776]	195 200 205		
[0777]	Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Ser Glu		
[0778]	210 215 220		
[0779]	Glu Leu Gly Ile Lys Phe Thr Leu Gly Arg Asp Gly Ser Glu Pro Lys		

[0780]	225	230	235	240
[0781]	Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile			
[0782]		245	250	255
[0783]	His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr			
[0784]		260	265	270
[0785]	Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu			
[0786]		275	280	285
[0787]	Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly			
[0788]		290	295	300
[0789]	Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr			
[0790]	305	310	315	320
[0791]	Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu			
[0792]		325	330	335
[0793]	Ile Gly Gln Ser Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu			
[0794]		340	345	350
[0795]	Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Lys Arg Asn Glu Leu Ala			
[0796]		355	360	365
[0797]	Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr			
[0798]		370	375	380
[0799]	Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile			
[0800]	385	390	395	400
[0801]	Val Tyr Leu Asp Phe Arg Ser Ala Ala Ile Ser Ile Ile Ile Thr His			
[0802]		405	410	415
[0803]	Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp			
[0804]		420	425	430
[0805]	Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe			
[0806]		435	440	445
[0807]	Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys			
[0808]		450	455	460
[0809]	Arg Lys Met Lys Ala Thr Val Asp Pro Leu Glu Lys Lys Leu Leu Asp			
[0810]	465	470	475	480
[0811]	Tyr Arg Gln Arg Val Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr			
[0812]		485	490	495
[0813]	Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser			
[0814]		500	505	510
[0815]	Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu			
[0816]		515	520	525
[0817]	Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu			
[0818]		530	535	540

[0819]	His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala		
[0820]	545	550	555 560
[0821]	Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu		
[0822]		565	570 575
[0823]	Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys		
[0824]		580	585 590
[0825]	Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu		
[0826]		595	600 605
[0827]	Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala		
[0828]		610	615 620
[0829]	Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val		
[0830]		625	630 635 640
[0831]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro		
[0832]		645	650 655
[0833]	Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp		
[0834]		660	665 670
[0835]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala		
[0836]		675	680 685
[0837]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu		
[0838]		690	695 700
[0839]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe		
[0840]		705	710 715 720
[0841]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln		
[0842]		725	730 735
[0843]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys		
[0844]		740	745 750
[0845]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp		
[0846]		755	760 765
[0847]	Leu Lys Val Lys Gly Lys Lys		
[0848]		770	775
[0849]	<210> 9		
[0850]	<211> 775		
[0851]	<212> PRT		
[0852]	<213> 人工		
[0853]	<220>		
[0854]	<223> 工程化聚合酶		
[0855]	<400> 9		
[0856]	Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile		
[0857]	1	5	10 15

[0858]	Arg	Val	Phe	Lys	Lys	Glu	Asn	Gly	Glu	Phe	Lys	Ile	Glu	Tyr	Asp	Arg
[0859]				20					25					30		
[0860]	Thr	Phe	Glu	Pro	Tyr	Phe	Tyr	Ala	Leu	Leu	Lys	Asp	Asp	Ser	Ala	Ile
[0861]				35					40					45		
[0862]	Glu	Asp	Val	Lys	Lys	Val	Thr	Ala	Lys	Arg	His	Gly	Thr	Val	Val	Lys
[0863]				50					55					60		
[0864]	Val	Lys	Arg	Ala	Glu	Lys	Val	Gln	Lys	Lys	Phe	Leu	Gly	Arg	Pro	Ile
[0865]	65						70					75				80
[0866]	Glu	Val	Trp	Lys	Leu	Tyr	Phe	Asn	His	Pro	Gln	Asp	Val	Pro	Ala	Ile
[0867]					85					90					95	
[0868]	Arg	Asp	Arg	Ile	Arg	Ala	His	Pro	Ala	Val	Val	Asp	Ile	Tyr	Glu	Tyr
[0869]				100						105					110	
[0870]	Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro
[0871]				115						120					125	
[0872]	Ala	Glu	Gly	Asp	Glu	Glu	Leu	Thr	Met	Leu	Ala	Phe	Ala	Ile	Ala	Thr
[0873]				130						135					140	
[0874]	Leu	Tyr	His	Glu	Gly	Glu	Glu	Phe	Gly	Thr	Gly	Pro	Ile	Leu	Met	Ile
[0875]	145						150					155				160
[0876]	Ser	Tyr	Ala	Asp	Gly	Ser	Glu	Ala	Arg	Val	Ile	Thr	Trp	Lys	Lys	Ile
[0877]					165					170					175	
[0878]	Asp	Leu	Pro	Tyr	Val	Asp	Val	Val	Ser	Thr	Glu	Lys	Glu	Met	Ile	Lys
[0879]				180						185					190	
[0880]	Arg	Phe	Leu	Arg	Val	Val	Arg	Glu	Lys	Asp	Pro	Asp	Val	Leu	Ile	Thr
[0881]				195						200					205	
[0882]	Tyr	Asn	Gly	Asp	Asn	Phe	Asp	Phe	Ala	Tyr	Leu	Lys	Lys	Arg	Ser	Glu
[0883]			210						215					220		
[0884]	Glu	Leu	Gly	Ile	Lys	Phe	Thr	Leu	Gly	Arg	Asp	Gly	Ser	Glu	Pro	Lys
[0885]	225						230					235				240
[0886]	Ile	Gln	Arg	Met	Gly	Asp	Arg	Phe	Ala	Val	Glu	Val	Lys	Gly	Arg	Ile
[0887]					245					250					255	
[0888]	His	Phe	Asp	Leu	Tyr	Pro	Val	Ile	Arg	Arg	Thr	Ile	Asn	Leu	Pro	Thr
[0889]				260						265					270	
[0890]	Tyr	Thr	Leu	Glu	Ala	Val	Tyr	Glu	Ala	Val	Phe	Gly	Lys	Pro	Lys	Glu
[0891]				275						280					285	
[0892]	Lys	Val	Tyr	Ala	Glu	Glu	Ile	Ala	Gln	Ala	Trp	Glu	Ser	Gly	Glu	Gly
[0893]			290							295				300		
[0894]	Leu	Glu	Arg	Val	Ala	Arg	Tyr	Ser	Met	Glu	Asp	Ala	Lys	Val	Thr	Tyr
[0895]	305						310					315				320
[0896]	Glu	Leu	Gly	Arg	Glu	Phe	Phe	Pro	Met	Glu	Ala	Gln	Leu	Ser	Arg	Leu

[0897]		325		330		335
[0898]	Ile Gly Gln Ser Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu					
[0899]		340		345		350
[0900]	Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Lys Arg Asn Glu Leu Ala					
[0901]		355		360		365
[0902]	Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr					
[0903]		370		375		380
[0904]	Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile					
[0905]		385		390		395
[0906]	Val Tyr Leu Asp Phe Arg Ser Ala Ala Ile Ser Ile Ile Ile Thr His					
[0907]		405		410		415
[0908]	Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp					
[0909]		420		425		430
[0910]	Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe					
[0911]		435		440		445
[0912]	Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys					
[0913]		450		455		460
[0914]	Arg Lys Met Lys Ala Thr Val Asp Pro Leu Glu Lys Met Leu Leu Asp					
[0915]		465		470		475
[0916]	Tyr Arg Gln Arg Val Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr					
[0917]		485		490		495
[0918]	Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser					
[0919]		500		505		510
[0920]	Val Ser Ala Trp Gly Arg Glu Tyr Leu Glu Met Val Ile Arg Glu Leu					
[0921]		515		520		525
[0922]	Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu					
[0923]		530		535		540
[0924]	His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala					
[0925]		545		550		555
[0926]	Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu					
[0927]		565		570		575
[0928]	Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys					
[0929]		580		585		590
[0930]	Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu					
[0931]		595		600		605
[0932]	Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala					
[0933]		610		615		620
[0934]	Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val					
[0935]		625		630		635
						640

[0936]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro		
[0937]	645	650	655
[0938]	Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp		
[0939]	660	665	670
[0940]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala		
[0941]	675	680	685
[0942]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu		
[0943]	690	695	700
[0944]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe		
[0945]	705	710	715
[0946]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln		
[0947]	725	730	735
[0948]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys		
[0949]	740	745	750
[0950]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp		
[0951]	755	760	765
[0952]	Leu Lys Val Lys Gly Lys Lys		
[0953]	770	775	
[0954]	<210> 10		
[0955]	<211> 775		
[0956]	<212> PRT		
[0957]	<213> 人工		
[0958]	<220>		
[0959]	<223> 工程化聚合酶		
[0960]	<400> 10		
[0961]	Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile		
[0962]	1	5	10
[0963]	Arg Val Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg		
[0964]	20	25	30
[0965]	Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile		
[0966]	35	40	45
[0967]	Glu Asp Val Lys Lys Val Thr Ala Lys Arg His Gly Thr Val Val Lys		
[0968]	50	55	60
[0969]	Val Lys Arg Ala Glu Lys Val Gln Lys Lys Phe Leu Gly Arg Pro Ile		
[0970]	65	70	75
[0971]	Glu Val Trp Lys Leu Tyr Phe Asn His Pro Gln Asp Val Pro Ala Ile		
[0972]	85	90	95
[0973]	Arg Asp Arg Ile Arg Ala His Pro Ala Val Val Asp Ile Tyr Glu Tyr		
[0974]	100	105	110

[0975]	Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro
[0976]			115					120					125			
[0977]	Ala	Glu	Gly	Asp	Glu	Glu	Leu	Thr	Met	Leu	Ala	Phe	Ala	Ile	Ala	Thr
[0978]			130					135					140			
[0979]	Leu	Tyr	His	Glu	Gly	Glu	Glu	Gly	Gly	Thr	Gly	Pro	Ile	Leu	Met	Ile
[0980]	145						150					155				160
[0981]	Ser	Tyr	Ala	Asp	Gly	Ser	Glu	Ala	Arg	Val	Ile	Thr	Trp	Lys	Lys	Ile
[0982]					165					170					175	
[0983]	Asp	Leu	Pro	Tyr	Val	Asp	Val	Val	Ser	Thr	Glu	Lys	Glu	Met	Ile	Lys
[0984]					180					185					190	
[0985]	Arg	Phe	Leu	Arg	Val	Val	Arg	Glu	Lys	Asp	Pro	Asp	Val	Leu	Ile	Thr
[0986]					195					200					205	
[0987]	Tyr	Asn	Gly	Asp	Asn	Phe	Asp	Phe	Ala	Tyr	Leu	Lys	Lys	Arg	Ser	Glu
[0988]					210					215					220	
[0989]	Glu	Leu	Gly	Ile	Lys	Phe	Thr	Leu	Gly	Arg	Asp	Gly	Ser	Glu	Pro	Lys
[0990]	225					230					235					240
[0991]	Ile	Gln	Arg	Met	Gly	Asp	Arg	Phe	Ala	Val	Glu	Val	Lys	Gly	Arg	Ile
[0992]					245						250					255
[0993]	His	Phe	Asp	Leu	Tyr	Pro	Val	Ile	Arg	Arg	Thr	Ile	Asn	Leu	Pro	Thr
[0994]					260						265					270
[0995]	Tyr	Thr	Leu	Glu	Ala	Leu	Tyr	Glu	Ala	Val	Phe	Gly	Lys	Pro	Lys	Glu
[0996]					275						280					285
[0997]	Lys	Val	Tyr	Ala	Glu	Glu	Ile	Ala	Gln	Ala	Trp	Glu	Ser	Gly	Glu	Gly
[0998]					290						295					300
[0999]	Leu	Glu	Arg	Val	Ala	Arg	Tyr	Ser	Met	Glu	Asp	Ala	Lys	Val	Thr	Tyr
[1000]	305						310					315				320
[1001]	Glu	Leu	Gly	Arg	Glu	Phe	Phe	Pro	His	Glu	Ala	Gln	Leu	Ser	Arg	Leu
[1002]					325						330					335
[1003]	Ile	Gly	Gln	Ser	Leu	Trp	Asp	Val	Ser	Arg	Ser	Ser	Thr	Gly	Asn	Leu
[1004]					340						345					350
[1005]	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Lys	Arg	Asn	Glu	Leu	Ala
[1006]					355						360					365
[1007]	Pro	Asn	Lys	Pro	Asp	Glu	Arg	Glu	Leu	Ala	Arg	Arg	Arg	Gly	Gly	Tyr
[1008]					370						375					380
[1009]	Ala	Gly	Gly	Tyr	Val	Lys	Glu	Pro	Glu	Arg	Gly	Leu	Trp	Asp	Asn	Ile
[1010]	385						390					395				400
[1011]	Val	Tyr	Leu	Asp	Phe	Arg	Ser	Ala	Ala	Ile	Ser	Ile	Ile	Ile	Thr	His
[1012]					405						410					415
[1013]	Asn	Val	Ser	Pro	Asp	Thr	Leu	Asn	Arg	Glu	Gly	Cys	Lys	Glu	Tyr	Asp

[1014]	420	425	430
[1015]	Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe		
[1016]	435	440	445
[1017]	Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys		
[1018]	450	455	460
[1019]	Arg Lys Met Lys Ala Thr Ser Asp Pro Leu Glu Lys Lys Leu Leu Asp		
[1020]	465	470	475
[1021]	Tyr Arg Gln Arg Val Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr		
[1022]	485	490	495
[1023]	Gly Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser		
[1024]	500	505	510
[1025]	Val Ala Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu		
[1026]	515	520	525
[1027]	Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu		
[1028]	530	535	540
[1029]	His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala		
[1030]	545	550	555
[1031]	Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu		
[1032]	565	570	575
[1033]	Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys		
[1034]	580	585	590
[1035]	Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu		
[1036]	595	600	605
[1037]	Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala		
[1038]	610	615	620
[1039]	Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val		
[1040]	625	630	635
[1041]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro		
[1042]	645	650	655
[1043]	Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp		
[1044]	660	665	670
[1045]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala		
[1046]	675	680	685
[1047]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu		
[1048]	690	695	700
[1049]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe		
[1050]	705	710	715
[1051]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln		
[1052]	725	730	735

[1053]	Val	Leu	Pro	Ala	Val	Glu	Arg	Ile	Leu	Lys	Ala	Phe	Gly	Tyr	Arg	Lys
[1054]				740					745						750	
[1055]	Glu	Asp	Leu	Arg	Tyr	Gln	Lys	Thr	Lys	Gln	Val	Gly	Leu	Gly	Ala	Trp
[1056]				755					760						765	
[1057]	Leu	Lys	Val	Lys	Gly	Lys	Lys									
[1058]				770					775							
[1059]	<210>	11														
[1060]	<211>	775														
[1061]	<212>	PRT														
[1062]	<213>	人工														
[1063]	<220>															
[1064]	<223>	工程化聚合酶														
[1065]	<400>	11														
[1066]	Met	Ile	Leu	Asp	Thr	Asp	Tyr	Ile	Thr	Glu	Asn	Gly	Lys	Pro	Val	Ile
[1067]	1				5					10					15	
[1068]	Arg	Val	Phe	Lys	Lys	Glu	Asn	Gly	Glu	Phe	Lys	Ile	Glu	Tyr	Asp	Arg
[1069]				20						25					30	
[1070]	Thr	Phe	Glu	Pro	Tyr	Phe	Tyr	Ala	Leu	Leu	Lys	Asp	Asp	Ser	Ala	Ile
[1071]				35						40					45	
[1072]	Glu	Asp	Val	Lys	Lys	Val	Thr	Ala	Lys	Arg	His	Gly	Thr	Val	Val	Lys
[1073]				50						55					60	
[1074]	Val	Lys	Arg	Ala	Glu	Lys	Val	Gln	Lys	Lys	Phe	Leu	Gly	Arg	Pro	Ile
[1075]	65					70					75					80
[1076]	Glu	Val	Trp	Lys	Leu	Tyr	Phe	Asn	His	Pro	Gln	Asp	Val	Pro	Ala	Ile
[1077]					85						90					95
[1078]	Arg	Asp	Arg	Ile	Arg	Ala	His	Pro	Ala	Val	Val	Asp	Ile	Tyr	Glu	Tyr
[1079]				100						105					110	
[1080]	Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro
[1081]				115						120					125	
[1082]	Ala	Glu	Gly	Asp	Glu	Glu	Leu	Thr	Met	Leu	Ala	Phe	Ala	Ile	Ala	Thr
[1083]				130						135					140	
[1084]	Leu	Tyr	His	Glu	Gly	Glu	Glu	Phe	Gly	Thr	Gly	Pro	Ile	Leu	Met	Ile
[1085]	145					150					155					160
[1086]	Ser	Tyr	Ala	Asp	Gly	Ser	Glu	Ala	Arg	Val	Ile	Thr	Trp	Lys	Lys	Ile
[1087]					165						170					175
[1088]	Asp	Leu	Pro	Tyr	Val	Asp	Val	Val	Ser	Thr	Glu	Lys	Glu	Met	Ile	Lys
[1089]				180							185					190
[1090]	Arg	Phe	Leu	Arg	Val	Val	Arg	Glu	Lys	Asp	Pro	Asp	Val	Leu	Ile	Thr
[1091]				195						200						205

[1092]	Tyr	Asn	Gly	Asp	Asn	Phe	Asp	Phe	Ala	Tyr	Leu	Lys	Lys	Arg	Ser	Glu
[1093]		210					215				220					
[1094]	Glu	Leu	Gly	Ile	Lys	Phe	Thr	Leu	Gly	Arg	Asp	Gly	Ser	Glu	Pro	Lys
[1095]	225					230				235					240	
[1096]	Ile	Gln	Arg	Met	Gly	Asp	Arg	Phe	Ala	Val	Glu	Val	Lys	Gly	Arg	Ile
[1097]					245					250					255	
[1098]	His	Phe	Asp	Leu	Tyr	Pro	Val	Ile	Arg	Arg	Thr	Ile	Asn	Leu	Pro	Thr
[1099]			260						265					270		
[1100]	Tyr	Thr	Leu	Glu	Ala	Val	Tyr	Glu	Ala	Val	Phe	Gly	Lys	Pro	Lys	Glu
[1101]			275					280					285			
[1102]	Lys	Val	Tyr	Ala	Glu	Glu	Ile	Ala	Gln	Ala	Trp	Glu	Ser	Gly	Glu	Gly
[1103]		290					295					300				
[1104]	Leu	Glu	Arg	Val	Ala	Arg	Tyr	Ser	Met	Glu	Asp	Ala	Lys	Val	Thr	Tyr
[1105]	305					310					315					320
[1106]	Glu	Leu	Gly	Arg	Glu	Phe	Phe	Pro	His	Glu	Ala	Gln	Leu	Ser	Arg	Leu
[1107]				325						330					335	
[1108]	Ile	Gly	Gln	Ser	Leu	Trp	Asp	Val	Ser	Arg	Ser	Ser	Thr	Gly	Asn	Leu
[1109]				340					345					350		
[1110]	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Lys	Arg	Asn	Glu	Leu	Ala
[1111]			355					360					365			
[1112]	Pro	Asn	Lys	Pro	Asp	Glu	Arg	Glu	Leu	Ala	Arg	Arg	Arg	Gly	Gly	Tyr
[1113]		370					375					380				
[1114]	Ala	Gly	Gly	Tyr	Val	Lys	Glu	Pro	Glu	Arg	Gly	Leu	Trp	Asp	Asn	Ile
[1115]	385					390					395					400
[1116]	Val	Tyr	Leu	Asp	Phe	Arg	Ser	Ala	Ala	Ile	Ser	Ile	Ile	Ile	Thr	His
[1117]				405						410					415	
[1118]	Asn	Val	Ser	Pro	Asp	Thr	Leu	Asn	Arg	Glu	Gly	Cys	Lys	Glu	Tyr	Asp
[1119]			420						425					430		
[1120]	Val	Ala	Pro	Glu	Val	Gly	His	Lys	Phe	Cys	Lys	Asp	Phe	Pro	Gly	Phe
[1121]			435				440						445			
[1122]	Ile	Pro	Ser	Leu	Leu	Gly	Asp	Leu	Leu	Glu	Glu	Arg	Gln	Lys	Ile	Lys
[1123]		450					455					460				
[1124]	Arg	Lys	Met	Lys	Ala	Thr	Ser	Asp	Pro	Leu	Glu	Lys	Lys	Leu	Leu	Asp
[1125]	465					470					475					480
[1126]	Tyr	Arg	Gln	Arg	Val	Ile	Lys	Ile	Leu	Ala	Asn	Ser	Phe	Tyr	Gly	Tyr
[1127]				485						490					495	
[1128]	Gly	Gly	Tyr	Ala	Lys	Ala	Arg	Trp	Tyr	Cys	Lys	Glu	Cys	Ala	Glu	Ser
[1129]				500					505					510		
[1130]	Val	Ala	Ala	Trp	Gly	Arg	Glu	Tyr	Ile	Glu	Met	Val	Ile	Arg	Glu	Leu

[1131]	515	520	525
[1132]	Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu		
[1133]	530	535	540
[1134]	His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala		
[1135]	545	550	555
[1136]	Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu		
[1137]	565	570	575
[1138]	Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys		
[1139]	580	585	590
[1140]	Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu		
[1141]	595	600	605
[1142]	Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala		
[1143]	610	615	620
[1144]	Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val		
[1145]	625	630	635
[1146]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro		
[1147]	645	650	655
[1148]	Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp		
[1149]	660	665	670
[1150]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala		
[1151]	675	680	685
[1152]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu		
[1153]	690	695	700
[1154]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe		
[1155]	705	710	715
[1156]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln		
[1157]	725	730	735
[1158]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys		
[1159]	740	745	750
[1160]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp		
[1161]	755	760	765
[1162]	Leu Lys Val Lys Gly Lys Lys		
[1163]	770	775	
[1164]	<210> 12		
[1165]	<211> 775		
[1166]	<212> PRT		
[1167]	<213> 人工		
[1168]	<220>		
[1169]	<223> 工程化聚合酶		

[1170]	<400> 12
[1171]	Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile
[1172]	1 5 10 15
[1173]	Arg Val Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg
[1174]	20 25 30
[1175]	Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile
[1176]	35 40 45
[1177]	Glu Asp Val Lys Lys Val Thr Ala Lys Arg His Gly Thr Val Val Lys
[1178]	50 55 60
[1179]	Val Lys Arg Ala Glu Lys Val Gln Lys Lys Phe Leu Gly Arg Pro Ile
[1180]	65 70 75 80
[1181]	Glu Val Trp Lys Leu Tyr Phe Asn His Pro Gln Asp Val Pro Ala Ile
[1182]	85 90 95
[1183]	Arg Asp Arg Ile Arg Ala His Pro Ala Val Val Asp Ile Tyr Glu Tyr
[1184]	100 105 110
[1185]	Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
[1186]	115 120 125
[1187]	Ala Glu Gly Asp Glu Glu Leu Thr Met Leu Ala Phe Ala Ile Ala Thr
[1188]	130 135 140
[1189]	Leu Tyr His Glu Gly Glu Glu Phe Gly Thr Gly Pro Ile Leu Met Ile
[1190]	145 150 155 160
[1191]	Ser Tyr Ala Asp Gly Ser Glu Ala Arg Val Ile Thr Trp Lys Lys Ile
[1192]	165 170 175
[1193]	Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Lys Glu Met Ile Lys
[1194]	180 185 190
[1195]	Arg Phe Leu Arg Val Val Arg Glu Lys Asp Pro Asp Val Leu Ile Thr
[1196]	195 200 205
[1197]	Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Ser Glu
[1198]	210 215 220
[1199]	Glu Leu Gly Ile Lys Phe Thr Leu Gly Arg Asp Gly Ser Glu Pro Lys
[1200]	225 230 235 240
[1201]	Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile
[1202]	245 250 255
[1203]	His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
[1204]	260 265 270
[1205]	Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu
[1206]	275 280 285
[1207]	Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly
[1208]	290 295 300

[1209]	Leu	Glu	Arg	Val	Ala	Arg	Tyr	Ser	Met	Glu	Asp	Ala	Lys	Val	Thr	Tyr
[1210]	305					310					315					320
[1211]	Glu	Leu	Gly	Arg	Glu	Phe	Phe	Pro	His	Glu	Ala	Gln	Leu	Ser	Arg	Leu
[1212]					325					330					335	
[1213]	Ile	Gly	Gln	Ser	Leu	Trp	Asp	Val	Ser	Arg	Ser	Ser	Thr	Gly	Asn	Leu
[1214]				340					345					350		
[1215]	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Lys	Arg	Asn	Glu	Leu	Ala
[1216]			355					360					365			
[1217]	Pro	Asn	Lys	Pro	Asp	Glu	Arg	Glu	Leu	Ala	Arg	Arg	Arg	Gly	Gly	Tyr
[1218]		370					375					380				
[1219]	Ala	Gly	Gly	Tyr	Val	Lys	Glu	Pro	Glu	Arg	Gly	Leu	Trp	Asp	Asn	Ile
[1220]	385					390					395					400
[1221]	Val	Tyr	Leu	Asp	Phe	Arg	Ser	Ala	Ala	Ile	Ser	Ile	Ile	Ile	Thr	His
[1222]				405						410					415	
[1223]	Asn	Val	Ser	Pro	Asp	Thr	Leu	Asn	Arg	Glu	Gly	Cys	Lys	Glu	Tyr	Asp
[1224]				420					425					430		
[1225]	Val	Ala	Pro	Glu	Val	Gly	His	Lys	Phe	Cys	Lys	Asp	Phe	Pro	Gly	Phe
[1226]			435					440					445			
[1227]	Ile	Pro	Ser	Leu	Leu	Gly	Asp	Leu	Leu	Glu	Glu	Arg	Gln	Lys	Ile	Lys
[1228]		450					455					460				
[1229]	Arg	Lys	Met	Lys	Ala	Thr	Ser	Asp	Pro	Leu	Glu	Lys	Lys	Leu	Leu	Asp
[1230]	465					470					475					480
[1231]	Tyr	Arg	Gln	Arg	Val	Ile	Lys	Ile	Leu	Ala	Asn	Ser	Phe	Tyr	Gly	Tyr
[1232]				485						490					495	
[1233]	Gly	Gly	Tyr	Ala	Lys	Ala	Arg	Trp	Tyr	Cys	Lys	Glu	Cys	Ala	Glu	Ser
[1234]				500					505					510		
[1235]	Val	Ala	Ala	Trp	Gly	Arg	Glu	Tyr	Ile	Glu	Met	Val	Ile	Arg	Glu	Leu
[1236]			515					520					525			
[1237]	Glu	Glu	Lys	Phe	Gly	Phe	Lys	Val	Leu	Tyr	Ala	Asp	Thr	Asp	Gly	Leu
[1238]		530					535					540				
[1239]	His	Ala	Thr	Ile	Pro	Gly	Ala	Asp	Ala	Glu	Thr	Val	Lys	Lys	Lys	Ala
[1240]	545					550					555					560
[1241]	Lys	Glu	Phe	Leu	Lys	Tyr	Ile	Asn	Pro	Lys	Leu	Pro	Gly	Leu	Leu	Glu
[1242]				565						570					575	
[1243]	Leu	Glu	Tyr	Glu	Gly	Phe	Tyr	Val	Arg	Gly	Phe	Phe	Val	Thr	Lys	Lys
[1244]				580					585					590		
[1245]	Lys	Tyr	Ala	Val	Ile	Asp	Glu	Glu	Gly	Lys	Ile	Thr	Thr	Arg	Gly	Leu
[1246]			595					600					605			
[1247]	Glu	Ile	Val	Arg	Arg	Asp	Trp	Ser	Glu	Ile	Ala	Lys	Glu	Thr	Gln	Ala

[1248]	610	615	620
[1249]	Arg Val Leu Glu Ala Ile Met Lys His Gly Asp Val Glu Glu Ala Val		
[1250]	625	630	635
[1251]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro		
[1252]	645	650	655
[1253]	Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp		
[1254]	660	665	670
[1255]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala		
[1256]	675	680	685
[1257]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu		
[1258]	690	695	700
[1259]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe		
[1260]	705	710	715
[1261]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln		
[1262]	725	730	735
[1263]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys		
[1264]	740	745	750
[1265]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp		
[1266]	755	760	765
[1267]	Leu Lys Val Lys Gly Lys Lys		
[1268]	770	775	
[1269]	<210> 13		
[1270]	<211> 775		
[1271]	<212> PRT		
[1272]	<213> 人工		
[1273]	<220>		
[1274]	<223> 工程化聚合酶		
[1275]	<400> 13		
[1276]	Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile		
[1277]	1	5	10
[1278]	Arg Val Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg		
[1279]	20	25	30
[1280]	Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile		
[1281]	35	40	45
[1282]	Glu Asp Val Lys Lys Val Thr Ala Lys Arg His Gly Thr Val Val Lys		
[1283]	50	55	60
[1284]	Val Lys Arg Ala Glu Lys Val Gln Lys Lys Phe Leu Gly Arg Pro Ile		
[1285]	65	70	75
[1286]	Glu Val Trp Lys Leu Tyr Phe Asn His Pro Gln Asp Val Pro Ala Ile		

[1287]					85				90				95			
[1288]	Arg	Asp	Arg	Ile	Arg	Ala	His	Pro	Ala	Val	Val	Asp	Ile	Tyr	Glu	Tyr
[1289]					100				105				110			
[1290]	Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro
[1291]					115				120				125			
[1292]	Ala	Glu	Gly	Asp	Glu	Glu	Leu	Thr	Met	Leu	Ala	Phe	Ala	Ile	Ala	Thr
[1293]					130				135				140			
[1294]	Leu	Tyr	His	Glu	Gly	Glu	Glu	Phe	Gly	Thr	Gly	Pro	Ile	Leu	Met	Ile
[1295]	145				150				155				160			
[1296]	Ser	Tyr	Ala	Asp	Gly	Ser	Glu	Ala	Arg	Val	Ile	Thr	Trp	Lys	Lys	Ile
[1297]					165				170				175			
[1298]	Asp	Leu	Pro	Tyr	Val	Asp	Val	Val	Ser	Thr	Glu	Lys	Glu	Met	Ile	Lys
[1299]					180				185				190			
[1300]	Arg	Phe	Leu	Arg	Val	Val	Arg	Glu	Lys	Asp	Pro	Asp	Val	Leu	Ile	Thr
[1301]					195				200				205			
[1302]	Tyr	Asn	Gly	Asp	Asn	Phe	Asp	Phe	Ala	Tyr	Leu	Lys	Lys	Arg	Ser	Glu
[1303]	210				215				220							
[1304]	Glu	Leu	Gly	Ile	Lys	Phe	Thr	Leu	Gly	Arg	Asp	Gly	Ser	Glu	Pro	Lys
[1305]	225				230				235				240			
[1306]	Ile	Gln	Arg	Met	Gly	Asp	Arg	Phe	Ala	Val	Glu	Val	Lys	Gly	Arg	Ile
[1307]					245				250				255			
[1308]	His	Phe	Asp	Leu	Tyr	Pro	Val	Ile	Arg	Arg	Thr	Ile	Asn	Leu	Pro	Thr
[1309]					260				265				270			
[1310]	Tyr	Thr	Leu	Glu	Ala	Leu	Tyr	Glu	Ala	Val	Phe	Gly	Lys	Pro	Lys	Glu
[1311]					275				280				285			
[1312]	Lys	Val	Tyr	Ala	Glu	Glu	Ile	Ala	Gln	Ala	Trp	Glu	Ser	Gly	Glu	Gly
[1313]	290				295				300							
[1314]	Leu	Glu	Arg	Val	Ala	Arg	Tyr	Ser	Met	Glu	Asp	Ala	Lys	Val	Thr	Tyr
[1315]	305				310				315				320			
[1316]	Glu	Leu	Gly	Arg	Glu	Phe	Phe	Pro	His	Glu	Ala	Gln	Leu	Ser	Arg	Leu
[1317]					325				330				335			
[1318]	Ile	Gly	Gln	Ser	Leu	Trp	Asp	Val	Ser	Arg	Ser	Ser	Thr	Gly	Asn	Leu
[1319]					340				345				350			
[1320]	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Lys	Arg	Asn	Glu	Leu	Ala
[1321]					355				360				365			
[1322]	Pro	Asn	Lys	Pro	Asp	Glu	Arg	Glu	Leu	Ala	Arg	Arg	Arg	Gly	Gly	Tyr
[1323]	370				375				380							
[1324]	Ala	Gly	Gly	Tyr	Val	Lys	Glu	Pro	Glu	Arg	Gly	Leu	Trp	Asp	Asn	Ile
[1325]	385				390				395				400			

[1326]	Val	Tyr	Leu	Asp	Phe	Arg	Ser	Ala	Ala	Ile	Ser	Ile	Ile	Ile	Thr	His
[1327]					405					410					415	
[1328]	Asn	Val	Ser	Pro	Asp	Thr	Leu	Asn	Arg	Glu	Gly	Cys	Lys	Glu	Tyr	Asp
[1329]					420					425					430	
[1330]	Val	Ala	Pro	Glu	Val	Gly	His	Lys	Phe	Cys	Lys	Asp	Phe	Pro	Gly	Phe
[1331]					435					440					445	
[1332]	Ile	Pro	Ser	Leu	Leu	Gly	Asp	Leu	Leu	Glu	Glu	Arg	Gln	Lys	Ile	Lys
[1333]					450					455					460	
[1334]	Arg	Lys	Met	Lys	Ala	Thr	Ser	Asp	Pro	Leu	Glu	Lys	Lys	Leu	Leu	Asp
[1335]					465					470					475	
[1336]	Tyr	Arg	Gln	Arg	Val	Ile	Lys	Ile	Leu	Ala	Asn	Ser	Phe	Tyr	Gly	Tyr
[1337]					485					490					495	
[1338]	Gly	Gly	Tyr	Ala	Lys	Ala	Arg	Trp	Tyr	Cys	Lys	Glu	Cys	Ala	Glu	Ser
[1339]					500					505					510	
[1340]	Val	Thr	Ala	Trp	Gly	Arg	Glu	Tyr	Ile	Glu	Met	Val	Ile	Arg	Glu	Leu
[1341]					515					520					525	
[1342]	Glu	Glu	Lys	Phe	Gly	Phe	Lys	Val	Leu	Tyr	Ala	Asp	Thr	Asp	Gly	Leu
[1343]					530					535					540	
[1344]	His	Ala	Thr	Ile	Pro	Gly	Ala	Asp	Ala	Glu	Thr	Val	Lys	Lys	Lys	Ala
[1345]					545					550					555	
[1346]	Lys	Glu	Phe	Leu	Lys	Tyr	Ile	Asn	Pro	Lys	Leu	Pro	Gly	Leu	Leu	Glu
[1347]					565					570					575	
[1348]	Leu	Glu	Tyr	Glu	Gly	Phe	Tyr	Val	Arg	Gly	Phe	Phe	Val	Thr	Lys	Lys
[1349]					580					585					590	
[1350]	Lys	Tyr	Ala	Val	Ile	Asp	Glu	Glu	Gly	Lys	Ile	Thr	Thr	Arg	Gly	Leu
[1351]					595					600					605	
[1352]	Glu	Ile	Val	Arg	Arg	Asp	Trp	Ser	Glu	Ile	Ala	Lys	Glu	Thr	Gln	Ala
[1353]					610					615					620	
[1354]	Arg	Val	Leu	Glu	Ala	Ile	Leu	Lys	His	Gly	Asp	Val	Glu	Glu	Ala	Val
[1355]					625					630					635	
[1356]	Arg	Ile	Val	Lys	Glu	Val	Thr	Glu	Lys	Leu	Ser	Lys	Tyr	Glu	Val	Pro
[1357]					645					650					655	
[1358]	Pro	Glu	Lys	Leu	Val	Ile	His	Glu	Gln	Ile	Thr	Arg	Asp	Leu	Arg	Asp
[1359]					660					665					670	
[1360]	Tyr	Lys	Ala	Thr	Gly	Pro	His	Val	Ala	Val	Ala	Lys	Arg	Leu	Ala	Ala
[1361]					675					680					685	
[1362]	Arg	Gly	Val	Lys	Ile	Arg	Pro	Gly	Thr	Val	Ile	Ser	Tyr	Ile	Val	Leu
[1363]					690					695					700	
[1364]	Lys	Gly	Ser	Gly	Arg	Ile	Gly	Asp	Arg	Ala	Ile	Pro	Ala	Asp	Glu	Phe

[1365]	705	710	715	720
[1366]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Arg Asn Gln			
[1367]		725	730	735
[1368]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys			
[1369]		740	745	750
[1370]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp			
[1371]		755	760	765
[1372]	Leu Lys Val Lys Gly Lys Lys			
[1373]		770	775	
[1374]	<210> 14			
[1375]	<211> 775			
[1376]	<212> PRT			
[1377]	<213> 人工			
[1378]	<220>			
[1379]	<223> 工程化聚合酶			
[1380]	<400> 14			
[1381]	Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile			
[1382]	1	5	10	15
[1383]	Arg Val Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg			
[1384]		20	25	30
[1385]	Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile			
[1386]		35	40	45
[1387]	Glu Asp Val Lys Lys Val Thr Ala Lys Arg His Gly Thr Val Val Lys			
[1388]		50	55	60
[1389]	Val Lys Arg Ala Glu Lys Val Gln Lys Lys Phe Leu Gly Arg Pro Ile			
[1390]	65	70	75	80
[1391]	Glu Val Trp Lys Leu Tyr Phe Asn His Pro Gln Asp Val Pro Ala Ile			
[1392]		85	90	95
[1393]	Arg Asp Arg Ile Arg Ala His Pro Ala Val Val Asp Ile Tyr Glu Tyr			
[1394]		100	105	110
[1395]	Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro			
[1396]		115	120	125
[1397]	Ala Glu Gly Asp Glu Glu Leu Thr Met Leu Ala Phe Ala Ile Ala Thr			
[1398]		130	135	140
[1399]	Leu Tyr His Glu Gly Glu Glu Phe Gly Thr Gly Pro Ile Leu Met Ile			
[1400]	145	150	155	160
[1401]	Ser Tyr Ala Asp Gly Ser Glu Ala Arg Val Ile Thr Trp Lys Lys Ile			
[1402]		165	170	175
[1403]	Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Lys Glu Met Ile Lys			

[1404]		180		185		190
[1405]	Arg Phe Leu Arg Val Val Arg Glu Lys Asp Pro Asp Val Leu Ile Thr					
[1406]		195		200		205
[1407]	Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Ser Glu					
[1408]		210		215		220
[1409]	Glu Leu Gly Ile Lys Phe Thr Leu Gly Arg Asp Gly Ser Glu Pro Lys					
[1410]	225		230		235	240
[1411]	Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile					
[1412]		245		250		255
[1413]	His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr					
[1414]		260		265		270
[1415]	Tyr Thr Leu Glu Ala Leu Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu					
[1416]		275		280		285
[1417]	Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly					
[1418]		290		295		300
[1419]	Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr					
[1420]	305		310		315	320
[1421]	Glu Leu Gly Arg Glu Phe Phe Pro His Glu Ala Gln Leu Ser Arg Leu					
[1422]		325		330		335
[1423]	Ile Gly Gln Ser Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu					
[1424]		340		345		350
[1425]	Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Lys Arg Asn Glu Leu Ala					
[1426]		355		360		365
[1427]	Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr					
[1428]		370		375		380
[1429]	Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile					
[1430]	385		390		395	400
[1431]	Val Tyr Leu Asp Phe Arg Ser Ala Ala Ile Ser Ile Ile Ile Thr His					
[1432]		405		410		415
[1433]	Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp					
[1434]		420		425		430
[1435]	Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe					
[1436]		435		440		445
[1437]	Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys					
[1438]		450		455		460
[1439]	Arg Lys Met Lys Ala Thr Ser Asp Pro Leu Glu Lys Lys Leu Leu Asp					
[1440]	465		470		475	480
[1441]	Tyr Arg Gln Arg Val Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr					
[1442]		485		490		495

[1443]	Gly Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser
[1444]	500 505 510
[1445]	Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu
[1446]	515 520 525
[1447]	Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu
[1448]	530 535 540
[1449]	His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala
[1450]	545 550 555 560
[1451]	Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu
[1452]	565 570 575
[1453]	Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys
[1454]	580 585 590
[1455]	Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu
[1456]	595 600 605
[1457]	Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala
[1458]	610 615 620
[1459]	Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val
[1460]	625 630 635 640
[1461]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro
[1462]	645 650 655
[1463]	Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp
[1464]	660 665 670
[1465]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala
[1466]	675 680 685
[1467]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu
[1468]	690 695 700
[1469]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe
[1470]	705 710 715 720
[1471]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln
[1472]	725 730 735
[1473]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys
[1474]	740 745 750
[1475]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp
[1476]	755 760 765
[1477]	Leu Lys Val Lys Gly Lys Lys
[1478]	770 775
[1479]	<210> 15
[1480]	<211> 775
[1481]	<212> PRT

[1482]	<213> 人工															
[1483]	<220>															
[1484]	<223> 工程化聚合酶															
[1485]	<400> 15															
[1486]	Met	Ile	Leu	Asp	Thr	Asp	Tyr	Ile	Thr	Glu	Asn	Gly	Lys	Pro	Val	Ile
[1487]	1				5					10					15	
[1488]	Arg	Val	Phe	Lys	Lys	Glu	Asn	Gly	Glu	Phe	Lys	Ile	Glu	Tyr	Asp	Arg
[1489]					20					25					30	
[1490]	Thr	Phe	Glu	Pro	Tyr	Phe	Tyr	Ala	Leu	Leu	Lys	Asp	Asp	Ser	Ala	Ile
[1491]					35					40					45	
[1492]	Glu	Asp	Val	Lys	Lys	Val	Thr	Ala	Lys	Arg	His	Gly	Thr	Val	Val	Lys
[1493]					50					55					60	
[1494]	Val	Lys	Arg	Ala	Glu	Lys	Val	Gln	Lys	Lys	Phe	Leu	Gly	Arg	Pro	Ile
[1495]	65									70					75	
[1496]	Glu	Val	Trp	Lys	Leu	Tyr	Phe	Asn	His	Pro	Gln	Asp	Val	Pro	Ala	Ile
[1497]										85					90	
[1498]	Arg	Asp	Arg	Ile	Arg	Ala	His	Pro	Ala	Val	Val	Asp	Ile	Tyr	Glu	Tyr
[1499]										100					105	
[1500]	Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro
[1501]										115					120	
[1502]	Ala	Glu	Gly	Asp	Glu	Glu	Leu	Thr	Met	Leu	Ala	Phe	Ala	Ile	Ala	Thr
[1503]										130					135	
[1504]	Leu	Tyr	His	Glu	Gly	Glu	Glu	Phe	Gly	Thr	Gly	Pro	Ile	Leu	Met	Ile
[1505]	145									150					155	
[1506]	Ser	Tyr	Ala	Asp	Gly	Ser	Glu	Ala	Arg	Val	Ile	Thr	Trp	Lys	Lys	Ile
[1507]										165					170	
[1508]	Asp	Leu	Pro	Tyr	Val	Asp	Val	Val	Ser	Thr	Glu	Lys	Glu	Met	Ile	Lys
[1509]										180					185	
[1510]	Arg	Phe	Leu	Arg	Val	Val	Arg	Glu	Lys	Asp	Pro	Asp	Val	Leu	Ile	Thr
[1511]										195					200	
[1512]	Tyr	Asn	Gly	Asp	Asn	Phe	Asp	Phe	Ala	Tyr	Leu	Lys	Lys	Arg	Ser	Glu
[1513]										210					215	
[1514]	Glu	Leu	Gly	Ile	Lys	Phe	Thr	Leu	Gly	Arg	Asp	Gly	Ser	Glu	Pro	Lys
[1515]										225					230	
[1516]	Ile	Gln	Arg	Met	Gly	Asp	Arg	Phe	Ala	Val	Glu	Val	Lys	Gly	Arg	Ile
[1517]										245					250	
[1518]	His	Phe	Asp	Leu	Tyr	Pro	Val	Ile	Arg	Arg	Thr	Ile	Asn	Leu	Pro	Thr
[1519]										260					265	
[1520]	Tyr	Thr	Leu	Glu	Ala	Val	Tyr	Glu	Ala	Val	Phe	Gly	Lys	Pro	Lys	Glu

[1521]	275	280	285
[1522]	Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly		
[1523]	290	295	300
[1524]	Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr		
[1525]	305	310	315
[1526]	Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu		
[1527]	325	330	335
[1528]	Ile Gly Gln Ser Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu		
[1529]	340	345	350
[1530]	Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Lys Arg Asn Glu Leu Ala		
[1531]	355	360	365
[1532]	Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr		
[1533]	370	375	380
[1534]	Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile		
[1535]	385	390	395
[1536]	Val Tyr Leu Asp Phe Arg Ser Ala Ala Ile Ser Ile Ile Ile Thr His		
[1537]	405	410	415
[1538]	Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp		
[1539]	420	425	430
[1540]	Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe		
[1541]	435	440	445
[1542]	Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys		
[1543]	450	455	460
[1544]	Arg Lys Met Lys Ala Thr Ser Asp Pro Leu Glu Lys Lys Leu Leu Asp		
[1545]	465	470	475
[1546]	Tyr Arg Gln Arg Val Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr		
[1547]	485	490	495
[1548]	Gly Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser		
[1549]	500	505	510
[1550]	Val Ala Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu		
[1551]	515	520	525
[1552]	Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu		
[1553]	530	535	540
[1554]	His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala		
[1555]	545	550	555
[1556]	Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu		
[1557]	565	570	575
[1558]	Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys		
[1559]	580	585	590

[1560]	Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu
[1561]	595 600 605
[1562]	Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala
[1563]	610 615 620
[1564]	Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val
[1565]	625 630 635 640
[1566]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro
[1567]	645 650 655
[1568]	Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp
[1569]	660 665 670
[1570]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala
[1571]	675 680 685
[1572]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu
[1573]	690 695 700
[1574]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe
[1575]	705 710 715 720
[1576]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln
[1577]	725 730 735
[1578]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys
[1579]	740 745 750
[1580]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp
[1581]	755 760 765
[1582]	Leu Lys Val Lys Gly Lys Lys
[1583]	770 775
[1584]	<210> 16
[1585]	<211> 775
[1586]	<212> PRT
[1587]	<213> 人工
[1588]	<220>
[1589]	<223> 工程化聚合酶
[1590]	<400> 16
[1591]	Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile
[1592]	1 5 10 15
[1593]	Arg Val Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg
[1594]	20 25 30
[1595]	Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile
[1596]	35 40 45
[1597]	Glu Asp Val Lys Lys Val Thr Ala Lys Arg His Gly Thr Val Val Lys
[1598]	50 55 60

[1599]	Val	Lys	Arg	Ala	Glu	Lys	Val	Gln	Lys	Lys	Phe	Leu	Gly	Arg	Pro	Ile
[1600]	65					70					75					80
[1601]	Glu	Val	Trp	Lys	Leu	Tyr	Phe	Asn	His	Pro	Gln	Asp	Val	Pro	Ala	Ile
[1602]					85					90					95	
[1603]	Arg	Asp	Arg	Ile	Arg	Ala	His	Pro	Ala	Val	Val	Asp	Ile	Tyr	Glu	Tyr
[1604]					100					105					110	
[1605]	Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro
[1606]					115					120				125		
[1607]	Ala	Glu	Gly	Asp	Glu	Glu	Leu	Thr	Met	Leu	Ala	Phe	Ala	Ile	Ala	Thr
[1608]					130					135				140		
[1609]	Leu	Tyr	His	Glu	Gly	Glu	Glu	Gly	Gly	Thr	Gly	Pro	Ile	Leu	Met	Ile
[1610]	145					150					155					160
[1611]	Ser	Tyr	Ala	Asp	Gly	Ser	Glu	Ala	Arg	Val	Ile	Thr	Trp	Lys	Lys	Ile
[1612]					165					170					175	
[1613]	Asp	Leu	Pro	Tyr	Val	Asp	Val	Val	Ser	Thr	Glu	Lys	Glu	Met	Ile	Lys
[1614]					180					185					190	
[1615]	Arg	Phe	Leu	Arg	Val	Val	Arg	Glu	Lys	Asp	Pro	Asp	Val	Leu	Ile	Thr
[1616]					195					200				205		
[1617]	Tyr	Asn	Gly	Asp	Asn	Phe	Asp	Phe	Ala	Tyr	Leu	Lys	Lys	Arg	Ser	Glu
[1618]					210					215				220		
[1619]	Glu	Leu	Gly	Ile	Lys	Phe	Thr	Leu	Gly	Arg	Asp	Gly	Ser	Glu	Pro	Lys
[1620]	225					230					235					240
[1621]	Ile	Gln	Arg	Met	Gly	Asp	Arg	Phe	Ala	Val	Glu	Val	Lys	Gly	Arg	Ile
[1622]					245					250					255	
[1623]	His	Phe	Asp	Leu	Tyr	Pro	Val	Ile	Arg	Arg	Thr	Ile	Asn	Leu	Pro	Thr
[1624]					260					265					270	
[1625]	Tyr	Thr	Leu	Glu	Ala	Leu	Tyr	Glu	Ala	Val	Phe	Gly	Lys	Pro	Lys	Glu
[1626]					275					280				285		
[1627]	Lys	Val	Tyr	Ala	Glu	Glu	Ile	Ala	Gln	Ala	Trp	Glu	Ser	Gly	Glu	Gly
[1628]					290					295				300		
[1629]	Leu	Glu	Arg	Val	Ala	Arg	Tyr	Ser	Met	Glu	Asp	Ala	Lys	Val	Thr	Tyr
[1630]	305					310					315					320
[1631]	Glu	Leu	Gly	Arg	Glu	Phe	Phe	Pro	Met	Glu	Ala	Gln	Leu	Ser	Arg	Leu
[1632]					325					330					335	
[1633]	Ile	Gly	Gln	Ser	Leu	Trp	Asp	Val	Ser	Arg	Ser	Ser	Thr	Gly	Asn	Leu
[1634]					340					345				350		
[1635]	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Lys	Arg	Asn	Glu	Leu	Ala
[1636]					355					360				365		
[1637]	Pro	Asn	Lys	Pro	Asp	Glu	Arg	Glu	Leu	Ala	Arg	Arg	Arg	Gly	Gly	Tyr

[1638]	370	375	380
[1639]	Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile		
[1640]	385	390	395 400
[1641]	Val Tyr Leu Asp Phe Arg Ser Ala Ala Ile Ser Ile Ile Ile Thr His		
[1642]	405	410	415
[1643]	Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp		
[1644]	420	425	430
[1645]	Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe		
[1646]	435	440	445
[1647]	Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys		
[1648]	450	455	460
[1649]	Arg Lys Met Lys Ala Thr Ser Asp Pro Leu Glu Lys Lys Leu Leu Asp		
[1650]	465	470	475 480
[1651]	Tyr Arg Gln Arg Val Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr		
[1652]	485	490	495
[1653]	Gly Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser		
[1654]	500	505	510
[1655]	Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu		
[1656]	515	520	525
[1657]	Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu		
[1658]	530	535	540
[1659]	His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala		
[1660]	545	550	555 560
[1661]	Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu		
[1662]	565	570	575
[1663]	Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys		
[1664]	580	585	590
[1665]	Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu		
[1666]	595	600	605
[1667]	Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala		
[1668]	610	615	620
[1669]	Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val		
[1670]	625	630	635 640
[1671]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro		
[1672]	645	650	655
[1673]	Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp		
[1674]	660	665	670
[1675]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala		
[1676]	675	680	685

[1677]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu
[1678]	690 695 700
[1679]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe
[1680]	705 710 715 720
[1681]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln
[1682]	725 730 735
[1683]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys
[1684]	740 745 750
[1685]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp
[1686]	755 760 765
[1687]	Leu Lys Val Lys Gly Lys Lys
[1688]	770 775
[1689]	<210> 17
[1690]	<211> 775
[1691]	<212> PRT
[1692]	<213> 人工
[1693]	<220>
[1694]	<223> 工程化聚合酶
[1695]	<400> 17
[1696]	Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile
[1697]	1 5 10 15
[1698]	Arg Val Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg
[1699]	20 25 30
[1700]	Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile
[1701]	35 40 45
[1702]	Glu Asp Val Lys Lys Val Thr Ala Lys Arg His Gly Thr Val Val Lys
[1703]	50 55 60
[1704]	Val Lys Arg Ala Glu Lys Val Gln Lys Lys Phe Leu Gly Arg Pro Ile
[1705]	65 70 75 80
[1706]	Glu Val Trp Lys Leu Tyr Phe Asn His Pro Gln Asp Val Pro Ala Ile
[1707]	85 90 95
[1708]	Arg Asp Arg Ile Arg Ala His Pro Ala Val Val Asp Ile Tyr Glu Tyr
[1709]	100 105 110
[1710]	Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
[1711]	115 120 125
[1712]	Ala Glu Gly Asp Glu Glu Leu Thr Met Leu Ala Phe Ala Ile Ala Thr
[1713]	130 135 140
[1714]	Leu Tyr His Glu Gly Glu Glu Phe Gly Thr Gly Pro Ile Leu Met Ile
[1715]	145 150 155 160

[1716]	Ser	Tyr	Ala	Asp	Gly	Ser	Glu	Ala	Arg	Val	Ile	Thr	Trp	Lys	Lys	Ile
[1717]					165					170					175	
[1718]	Asp	Leu	Pro	Tyr	Val	Asp	Val	Val	Ser	Thr	Glu	Lys	Glu	Met	Ile	Lys
[1719]					180					185					190	
[1720]	Arg	Phe	Leu	Arg	Val	Val	Arg	Glu	Lys	Asp	Pro	Asp	Val	Leu	Ile	Thr
[1721]					195					200					205	
[1722]	Tyr	Asn	Gly	Asp	Asn	Phe	Asp	Phe	Ala	Tyr	Leu	Lys	Lys	Arg	Ser	Glu
[1723]			210						215					220		
[1724]	Glu	Leu	Gly	Ile	Lys	Phe	Thr	Leu	Gly	Arg	Asp	Gly	Ser	Glu	Pro	Lys
[1725]		225							230					235		240
[1726]	Ile	Gln	Arg	Met	Gly	Asp	Arg	Phe	Ala	Val	Glu	Val	Lys	Gly	Arg	Ile
[1727]					245					250					255	
[1728]	His	Phe	Asp	Leu	Tyr	Pro	Val	Ile	Arg	Arg	Thr	Ile	Asn	Leu	Pro	Thr
[1729]					260					265					270	
[1730]	Tyr	Thr	Leu	Glu	Ala	Val	Tyr	Glu	Ala	Val	Phe	Gly	Lys	Pro	Lys	Glu
[1731]					275					280					285	
[1732]	Lys	Val	Tyr	Ala	Glu	Glu	Ile	Ala	Gln	Ala	Trp	Glu	Ser	Gly	Glu	Gly
[1733]					290					295					300	
[1734]	Leu	Glu	Arg	Val	Ala	Arg	Tyr	Ser	Met	Glu	Asp	Ala	Lys	Val	Thr	Tyr
[1735]		305							310						315	320
[1736]	Glu	Leu	Gly	Arg	Glu	Phe	Phe	Pro	Met	Glu	Ala	Gln	Leu	Ser	Arg	Leu
[1737]					325					330					335	
[1738]	Ile	Gly	Gln	Ser	Leu	Trp	Asp	Val	Ser	Arg	Ser	Ser	Thr	Gly	Asn	Leu
[1739]					340					345					350	
[1740]	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Lys	Arg	Asn	Glu	Leu	Ala
[1741]					355					360					365	
[1742]	Pro	Asn	Lys	Pro	Asp	Glu	Arg	Glu	Leu	Ala	Arg	Arg	Arg	Gly	Gly	Tyr
[1743]					370					375					380	
[1744]	Ala	Gly	Gly	Tyr	Val	Lys	Glu	Pro	Glu	Arg	Gly	Leu	Trp	Asp	Asn	Ile
[1745]		385							390					395		400
[1746]	Val	Tyr	Leu	Asp	Phe	Arg	Ser	Ala	Ala	Ile	Ser	Ile	Ile	Ile	Thr	His
[1747]					405					410					415	
[1748]	Asn	Val	Ser	Pro	Asp	Thr	Leu	Asn	Arg	Glu	Gly	Cys	Lys	Glu	Tyr	Asp
[1749]					420					425					430	
[1750]	Val	Ala	Pro	Glu	Val	Gly	His	Lys	Phe	Cys	Lys	Asp	Phe	Pro	Gly	Phe
[1751]					435					440					445	
[1752]	Ile	Pro	Ser	Leu	Leu	Gly	Asp	Leu	Leu	Glu	Glu	Arg	Gln	Lys	Ile	Lys
[1753]					450					455					460	
[1754]	Arg	Lys	Met	Lys	Ala	Thr	Val	Asp	Pro	Leu	Glu	Trp	Lys	Leu	Leu	Asp

[1755]	465	470	475	480
[1756]	Tyr Arg Gln Arg Val Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr			
[1757]	485	490	495	
[1758]	Gly Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser			
[1759]	500	505	510	
[1760]	Val Ala Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu			
[1761]	515	520	525	
[1762]	Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu			
[1763]	530	535	540	
[1764]	His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala			
[1765]	545	550	555	560
[1766]	Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu			
[1767]	565	570	575	
[1768]	Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys			
[1769]	580	585	590	
[1770]	Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu			
[1771]	595	600	605	
[1772]	Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala			
[1773]	610	615	620	
[1774]	Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val			
[1775]	625	630	635	640
[1776]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro			
[1777]	645	650	655	
[1778]	Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp			
[1779]	660	665	670	
[1780]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala			
[1781]	675	680	685	
[1782]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu			
[1783]	690	695	700	
[1784]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe			
[1785]	705	710	715	720
[1786]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln			
[1787]	725	730	735	
[1788]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys			
[1789]	740	745	750	
[1790]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp			
[1791]	755	760	765	
[1792]	Leu Lys Val Lys Gly Lys Lys			
[1793]	770	775		

[1794]	<210> 18															
[1795]	<211> 775															
[1796]	<212> PRT															
[1797]	<213> 人工															
[1798]	<220>															
[1799]	<223> 工程化聚合酶															
[1800]	<400> 18															
[1801]	Met	Ile	Leu	Asp	Thr	Asp	Tyr	Ile	Thr	Glu	Asn	Gly	Lys	Pro	Val	Ile
[1802]	1				5					10					15	
[1803]	Arg	Val	Phe	Lys	Lys	Glu	Asn	Gly	Glu	Phe	Lys	Ile	Glu	Tyr	Asp	Arg
[1804]				20					25					30		
[1805]	Thr	Phe	Glu	Pro	Tyr	Phe	Tyr	Ala	Leu	Leu	Lys	Asp	Asp	Ser	Ala	Ile
[1806]			35					40					45			
[1807]	Glu	Asp	Val	Lys	Lys	Val	Thr	Ala	Lys	Arg	His	Gly	Thr	Val	Val	Lys
[1808]		50					55					60				
[1809]	Val	Lys	Arg	Ala	Glu	Lys	Val	Gln	Lys	Lys	Phe	Leu	Gly	Arg	Pro	Ile
[1810]	65					70					75					80
[1811]	Glu	Val	Trp	Lys	Leu	Tyr	Phe	Asn	His	Pro	Gln	Asp	Val	Pro	Ala	Ile
[1812]				85						90					95	
[1813]	Arg	Asp	Arg	Ile	Arg	Ala	His	Pro	Ala	Val	Val	Asp	Ile	Tyr	Glu	Tyr
[1814]				100					105					110		
[1815]	Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro
[1816]			115					120					125			
[1817]	Ala	Glu	Gly	Asp	Glu	Glu	Leu	Thr	Met	Leu	Ala	Phe	Ala	Ile	Ala	Thr
[1818]		130					135					140				
[1819]	Leu	Tyr	His	Glu	Gly	Glu	Glu	Phe	Gly	Thr	Gly	Pro	Ile	Leu	Met	Ile
[1820]	145					150					155					160
[1821]	Ser	Tyr	Ala	Asp	Gly	Ser	Glu	Ala	Arg	Val	Ile	Thr	Trp	Lys	Lys	Ile
[1822]				165						170					175	
[1823]	Asp	Leu	Pro	Tyr	Val	Asp	Val	Val	Ser	Thr	Glu	Lys	Glu	Met	Ile	Lys
[1824]				180					185					190		
[1825]	Arg	Phe	Leu	Arg	Val	Val	Arg	Glu	Lys	Asp	Pro	Asp	Val	Leu	Ile	Thr
[1826]			195					200					205			
[1827]	Tyr	Asn	Gly	Asp	Asn	Phe	Asp	Phe	Ala	Tyr	Leu	Lys	Lys	Arg	Ser	Glu
[1828]		210					215					220				
[1829]	Glu	Leu	Gly	Ile	Lys	Phe	Thr	Leu	Gly	Arg	Asp	Gly	Ser	Glu	Pro	Lys
[1830]	225					230					235					240
[1831]	Ile	Gln	Arg	Met	Gly	Asp	Arg	Phe	Ala	Val	Glu	Val	Lys	Gly	Arg	Ile
[1832]				245						250					255	

[1833]	His	Phe	Asp	Leu	Tyr	Pro	Val	Ile	Arg	Arg	Thr	Ile	Asn	Leu	Pro	Thr
[1834]				260					265					270		
[1835]	Tyr	Thr	Leu	Glu	Ala	Val	Tyr	Glu	Ala	Val	Phe	Gly	Lys	Pro	Lys	Glu
[1836]				275					280					285		
[1837]	Lys	Val	Tyr	Ala	Glu	Glu	Ile	Ala	Gln	Ala	Trp	Glu	Ser	Gly	Glu	Gly
[1838]				290					295					300		
[1839]	Leu	Glu	Arg	Val	Ala	Arg	Tyr	Ser	Met	Glu	Asp	Ala	Lys	Val	Thr	Tyr
[1840]	305							310				315				320
[1841]	Glu	Leu	Gly	Arg	Glu	Phe	Phe	Pro	Met	Glu	Ala	Gln	Leu	Ser	Arg	Leu
[1842]					325						330					335
[1843]	Ile	Gly	Gln	Ser	Leu	Trp	Asp	Val	Ser	Arg	Ser	Ser	Thr	Gly	Asn	Leu
[1844]					340					345					350	
[1845]	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Lys	Arg	Asn	Glu	Leu	Ala
[1846]					355					360					365	
[1847]	Pro	Asn	Lys	Pro	Asp	Glu	Arg	Glu	Leu	Ala	Arg	Arg	Arg	Gly	Gly	Tyr
[1848]					370					375				380		
[1849]	Ala	Gly	Gly	Tyr	Val	Lys	Glu	Pro	Glu	Arg	Gly	Leu	Trp	Asp	Asn	Ile
[1850]	385						390					395				400
[1851]	Val	Tyr	Leu	Asp	Phe	Arg	Ser	Ala	Ala	Ile	Ser	Ile	Ile	Ile	Thr	His
[1852]					405						410					415
[1853]	Asn	Val	Ser	Pro	Asp	Thr	Leu	Asn	Arg	Glu	Gly	Cys	Lys	Glu	Tyr	Asp
[1854]					420						425				430	
[1855]	Val	Ala	Pro	Glu	Val	Gly	His	Lys	Phe	Cys	Lys	Asp	Phe	Pro	Gly	Phe
[1856]					435						440				445	
[1857]	Ile	Pro	Ser	Leu	Leu	Gly	Asp	Leu	Leu	Glu	Glu	Arg	Gln	Lys	Ile	Lys
[1858]					450							455			460	
[1859]	Arg	Lys	Met	Lys	Ala	Thr	Val	Asp	Pro	Leu	Glu	Lys	Lys	Leu	Leu	Asp
[1860]	465							470				475				480
[1861]	Tyr	Arg	Gln	Arg	Val	Ile	Lys	Ile	Leu	Ala	Asn	Ser	Phe	Tyr	Gly	Tyr
[1862]					485							490				495
[1863]	Gly	Gly	Tyr	Ala	Lys	Ala	Arg	Trp	Tyr	Cys	Lys	Glu	Cys	Ala	Glu	Ser
[1864]					500							505			510	
[1865]	Val	Ser	Ala	Trp	Gly	Arg	Glu	Tyr	Leu	Glu	Met	Val	Ile	Arg	Glu	Leu
[1866]					515							520			525	
[1867]	Glu	Glu	Lys	Phe	Gly	Phe	Lys	Val	Leu	Tyr	Ala	Asp	Thr	Asp	Gly	Leu
[1868]					530							535			540	
[1869]	His	Ala	Thr	Ile	Pro	Gly	Ala	Asp	Ala	Glu	Thr	Val	Lys	Lys	Lys	Ala
[1870]	545							550				555				560
[1871]	Lys	Glu	Phe	Leu	Lys	Tyr	Ile	Asn	Pro	Lys	Leu	Pro	Gly	Leu	Leu	Glu

[1872]		565		570		575
[1873]	Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Ile Lys Lys					
[1874]		580		585		590
[1875]	Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu					
[1876]		595		600		605
[1877]	Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala					
[1878]		610		615		620
[1879]	Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val					
[1880]	625		630		635	640
[1881]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro					
[1882]		645		650		655
[1883]	Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp					
[1884]		660		665		670
[1885]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala					
[1886]		675		680		685
[1887]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu					
[1888]		690		695		700
[1889]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe					
[1890]	705		710		715	720
[1891]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln					
[1892]		725		730		735
[1893]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys					
[1894]		740		745		750
[1895]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp					
[1896]		755		760		765
[1897]	Leu Lys Val Lys Gly Lys Lys					
[1898]		770		775		
[1899]	<210> 19					
[1900]	<211> 775					
[1901]	<212> PRT					
[1902]	<213> 人工					
[1903]	<220>					
[1904]	<223> 工程化聚合酶					
[1905]	<400> 19					
[1906]	Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile					
[1907]	1	5		10		15
[1908]	Arg Val Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg					
[1909]		20		25		30
[1910]	Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile					

[1911]	35	40	45
[1912]	Glu Asp Val Lys Lys Val Thr Ala Lys Arg His Gly Thr Val Val Lys		
[1913]	50	55	60
[1914]	Val Lys Arg Ala Glu Lys Val Gln Lys Lys Phe Leu Gly Arg Pro Ile		
[1915]	65	70	75
[1916]	Glu Val Trp Lys Leu Tyr Phe Asn His Pro Gln Asp Val Pro Ala Ile		
[1917]	85	90	95
[1918]	Arg Asp Arg Ile Arg Ala His Pro Ala Val Val Asp Ile Tyr Glu Tyr		
[1919]	100	105	110
[1920]	Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro		
[1921]	115	120	125
[1922]	Ala Glu Gly Asp Glu Glu Leu Thr Met Leu Ala Phe Ala Ile Ala Thr		
[1923]	130	135	140
[1924]	Leu Tyr His Glu Gly Glu Glu Phe Gly Thr Gly Pro Ile Leu Met Ile		
[1925]	145	150	155
[1926]	Ser Tyr Ala Asp Gly Ser Glu Ala Arg Val Ile Thr Trp Lys Lys Ile		
[1927]	165	170	175
[1928]	Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Lys Glu Met Ile Lys		
[1929]	180	185	190
[1930]	Arg Phe Leu Arg Val Val Arg Glu Lys Asp Pro Asp Val Leu Ile Thr		
[1931]	195	200	205
[1932]	Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Ser Glu		
[1933]	210	215	220
[1934]	Glu Leu Gly Ile Lys Phe Thr Leu Gly Arg Asp Gly Ser Glu Pro Lys		
[1935]	225	230	235
[1936]	Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile		
[1937]	245	250	255
[1938]	His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr		
[1939]	260	265	270
[1940]	Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu		
[1941]	275	280	285
[1942]	Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly		
[1943]	290	295	300
[1944]	Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr		
[1945]	305	310	315
[1946]	Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu		
[1947]	325	330	335
[1948]	Ile Gly Gln Ser Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu		
[1949]	340	345	350

[1950]	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Lys	Arg	Asn	Glu	Leu	Ala
[1951]			355					360					365			
[1952]	Pro	Asn	Lys	Pro	Asp	Glu	Arg	Glu	Leu	Ala	Arg	Arg	Arg	Gly	Gly	Tyr
[1953]			370					375					380			
[1954]	Ala	Gly	Gly	Tyr	Val	Lys	Glu	Pro	Glu	Arg	Gly	Leu	Trp	Asp	Asn	Ile
[1955]	385						390					395				400
[1956]	Val	Tyr	Leu	Asp	Phe	Arg	Ser	Ala	Ala	Ile	Ser	Ile	Ile	Ile	Thr	His
[1957]					405					410						415
[1958]	Asn	Val	Ser	Pro	Asp	Thr	Leu	Asn	Arg	Glu	Gly	Cys	Lys	Glu	Tyr	Asp
[1959]				420					425						430	
[1960]	Val	Ala	Pro	Glu	Val	Gly	His	Lys	Phe	Cys	Lys	Asp	Phe	Pro	Gly	Phe
[1961]				435					440						445	
[1962]	Ile	Pro	Ser	Leu	Leu	Gly	Asp	Leu	Leu	Glu	Glu	Arg	Gln	Lys	Ile	Lys
[1963]				450					455						460	
[1964]	Arg	Lys	Met	Lys	Ala	Thr	Val	Asp	Pro	Leu	Glu	Trp	Met	Leu	Leu	Asp
[1965]	465						470					475				480
[1966]	Tyr	Arg	Gln	Arg	Val	Ile	Lys	Ile	Leu	Ala	Asn	Ser	Phe	Tyr	Gly	Tyr
[1967]					485					490						495
[1968]	Gly	Gly	Tyr	Ala	Lys	Ala	Arg	Trp	Tyr	Cys	Lys	Glu	Cys	Ala	Glu	Ser
[1969]					500					505					510	
[1970]	Val	Ser	Ala	Trp	Gly	Arg	Glu	Tyr	Leu	Glu	Met	Val	Ile	Arg	Glu	Leu
[1971]					515					520					525	
[1972]	Glu	Glu	Lys	Phe	Gly	Phe	Lys	Val	Leu	Tyr	Ala	Asp	Thr	Asp	Gly	Leu
[1973]					530					535					540	
[1974]	His	Ala	Thr	Ile	Pro	Gly	Ala	Asp	Ala	Glu	Thr	Val	Lys	Lys	Lys	Ala
[1975]	545						550					555				560
[1976]	Lys	Glu	Phe	Leu	Lys	Tyr	Ile	Asn	Pro	Lys	Leu	Pro	Gly	Leu	Leu	Glu
[1977]					565					570						575
[1978]	Leu	Glu	Tyr	Glu	Gly	Phe	Tyr	Val	Arg	Gly	Phe	Phe	Val	Thr	Lys	Lys
[1979]					580					585					590	
[1980]	Lys	Tyr	Ala	Val	Ile	Asp	Glu	Glu	Gly	Lys	Ile	Thr	Thr	Arg	Gly	Leu
[1981]					595					600					605	
[1982]	Glu	Ile	Val	Arg	Arg	Asp	Trp	Ser	Glu	Ile	Ala	Lys	Glu	Thr	Gln	Ala
[1983]					610					615					620	
[1984]	Arg	Val	Leu	Glu	Ala	Ile	Leu	Lys	His	Gly	Asp	Val	Glu	Glu	Ala	Val
[1985]	625						630					635				640
[1986]	Arg	Ile	Val	Lys	Glu	Val	Thr	Glu	Lys	Leu	Ser	Lys	Tyr	Glu	Val	Pro
[1987]					645					650						655
[1988]	Pro	Glu	Lys	Leu	Val	Ile	His	Glu	Gln	Ile	Thr	Arg	Asp	Leu	Arg	Asp

[1989]	660							665							670													
[1990]	Tyr	Lys	Ala	Thr	Gly	Pro	His	Val	Ala	Val	Ala	Lys	Arg	Leu	Ala	Ala												
[1991]	675							680							685													
[1992]	Arg	Gly	Val	Lys	Ile	Arg	Pro	Gly	Thr	Val	Ile	Ser	Tyr	Ile	Val	Leu												
[1993]	690							695							700													
[1994]	Lys	Gly	Ser	Gly	Arg	Ile	Gly	Asp	Arg	Ala	Ile	Pro	Ala	Asp	Glu	Phe												
[1995]	705							710							715							720						
[1996]	Asp	Pro	Thr	Lys	His	Arg	Tyr	Asp	Ala	Glu	Tyr	Tyr	Ile	Glu	Asn	Gln												
[1997]	725							730							735													
[1998]	Val	Leu	Pro	Ala	Val	Glu	Arg	Ile	Leu	Lys	Ala	Phe	Gly	Tyr	Arg	Lys												
[1999]	740							745							750													
[2000]	Glu	Asp	Leu	Arg	Tyr	Gln	Lys	Thr	Lys	Gln	Val	Gly	Leu	Gly	Ala	Trp												
[2001]	755							760							765													
[2002]	Leu	Lys	Val	Lys	Gly	Lys	Lys																					
[2003]	770							775																				
[2004]	<210> 20																											
[2005]	<211> 775																											
[2006]	<212> PRT																											
[2007]	<213> 人工																											
[2008]	<220>																											
[2009]	<223> 工程化聚合酶																											
[2010]	<400> 20																											
[2011]	Met	Ile	Leu	Asp	Thr	Asp	Tyr	Ile	Thr	Glu	Asn	Gly	Lys	Pro	Val	Ile												
[2012]	1	5				10				15																		
[2013]	Arg	Val	Phe	Lys	Lys	Glu	Asn	Gly	Glu	Phe	Lys	Ile	Glu	Tyr	Asp	Arg												
[2014]	20				25				30																			
[2015]	Thr	Phe	Glu	Pro	Tyr	Phe	Tyr	Ala	Leu	Leu	Lys	Asp	Asp	Ser	Ala	Ile												
[2016]	35				40				45																			
[2017]	Glu	Asp	Val	Lys	Lys	Val	Thr	Ala	Lys	Arg	His	Gly	Thr	Val	Val	Lys												
[2018]	50				55				60																			
[2019]	Val	Lys	Arg	Ala	Glu	Lys	Val	Gln	Lys	Lys	Phe	Leu	Gly	Arg	Pro	Ile												
[2020]	65				70				75				80															
[2021]	Glu	Val	Trp	Lys	Leu	Tyr	Phe	Asn	His	Pro	Gln	Asp	Val	Pro	Ala	Ile												
[2022]	85				90				95																			
[2023]	Arg	Asp	Arg	Ile	Arg	Ala	His	Pro	Ala	Val	Val	Asp	Ile	Tyr	Glu	Tyr												
[2024]	100				105				110																			
[2025]	Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro												
[2026]	115				120				125																			
[2027]	Ala	Glu	Gly	Asp	Glu	Glu	Leu	Thr	Met	Leu	Ala	Phe	Ala	Ile	Ala	Thr												

[2028]	130	135	140
[2029]	Leu Tyr His Glu Gly Glu Glu Phe Gly Thr Gly Pro Ile Leu Met Ile		
[2030]	145	150	155
[2031]	Ser Tyr Ala Asp Gly Ser Glu Ala Arg Val Ile Thr Trp Lys Lys Ile		
[2032]	165	170	175
[2033]	Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Lys Glu Met Ile Lys		
[2034]	180	185	190
[2035]	Arg Phe Leu Arg Val Val Arg Glu Lys Asp Pro Asp Val Leu Ile Thr		
[2036]	195	200	205
[2037]	Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Ser Glu		
[2038]	210	215	220
[2039]	Glu Leu Gly Ile Lys Phe Thr Leu Gly Arg Asp Gly Ser Glu Pro Lys		
[2040]	225	230	235
[2041]	Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile		
[2042]	245	250	255
[2043]	His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr		
[2044]	260	265	270
[2045]	Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu		
[2046]	275	280	285
[2047]	Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly		
[2048]	290	295	300
[2049]	Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr		
[2050]	305	310	315
[2051]	Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu		
[2052]	325	330	335
[2053]	Ile Gly Gln Ser Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu		
[2054]	340	345	350
[2055]	Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Lys Arg Asn Glu Leu Ala		
[2056]	355	360	365
[2057]	Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr		
[2058]	370	375	380
[2059]	Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile		
[2060]	385	390	395
[2061]	Val Tyr Leu Asp Phe Arg Ser Ala Ala Ile Ser Ile Ile Ile Thr His		
[2062]	405	410	415
[2063]	Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp		
[2064]	420	425	430
[2065]	Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe		
[2066]	435	440	445

[2067]	Ile	Pro	Ser	Leu	Leu	Gly	Asp	Leu	Leu	Glu	Glu	Arg	Gln	Lys	Ile	Lys
[2068]	450						455					460				
[2069]	Arg	Lys	Met	Lys	Ala	Thr	Val	Asp	Pro	Leu	Glu	Lys	Met	Leu	Leu	Asp
[2070]	465						470					475				480
[2071]	Tyr	Arg	Gln	Arg	Val	Ile	Lys	Ile	Leu	Ala	Asn	Ser	Phe	Tyr	Gly	Tyr
[2072]							485					490				495
[2073]	Gly	Gly	Tyr	Ala	Lys	Ala	Arg	Trp	Tyr	Cys	Lys	Glu	Cys	Ala	Glu	Ser
[2074]							500					505			510	
[2075]	Val	Thr	Ala	Trp	Gly	Arg	Glu	Tyr	Ile	Glu	Met	Val	Ile	Arg	Glu	Leu
[2076]							515					520			525	
[2077]	Glu	Glu	Lys	Phe	Gly	Phe	Lys	Val	Leu	Tyr	Ala	Asp	Thr	Asp	Gly	Leu
[2078]							530					535			540	
[2079]	His	Ala	Thr	Ile	Pro	Gly	Ala	Asp	Ala	Glu	Thr	Val	Lys	Lys	Lys	Ala
[2080]	545						550					555				560
[2081]	Lys	Glu	Phe	Leu	Lys	Tyr	Ile	Asn	Pro	Lys	Leu	Pro	Gly	Leu	Leu	Glu
[2082]							565					570				575
[2083]	Leu	Glu	Tyr	Glu	Gly	Phe	Tyr	Val	Arg	Gly	Phe	Phe	Val	Thr	Lys	Lys
[2084]							580					585			590	
[2085]	Lys	Tyr	Ala	Val	Ile	Asp	Glu	Glu	Gly	Lys	Ile	Thr	Thr	Arg	Gly	Leu
[2086]							595					600			605	
[2087]	Glu	Ile	Val	Arg	Arg	Asp	Trp	Ser	Glu	Ile	Ala	Lys	Glu	Thr	Gln	Ala
[2088]							610					615			620	
[2089]	Arg	Val	Leu	Glu	Ala	Ile	Leu	Lys	His	Gly	Asp	Val	Glu	Glu	Ala	Val
[2090]	625						630					635				640
[2091]	Arg	Ile	Val	Lys	Glu	Val	Thr	Glu	Lys	Leu	Ser	Lys	Tyr	Glu	Val	Pro
[2092]							645					650				655
[2093]	Pro	Glu	Lys	Leu	Val	Ile	His	Glu	Gln	Ile	Thr	Arg	Asp	Leu	Arg	Asp
[2094]							660					665			670	
[2095]	Tyr	Lys	Ala	Thr	Gly	Pro	His	Val	Ala	Val	Ala	Lys	Arg	Leu	Ala	Ala
[2096]							675					680			685	
[2097]	Arg	Gly	Val	Lys	Ile	Arg	Pro	Gly	Thr	Val	Ile	Ser	Tyr	Ile	Val	Leu
[2098]							690					695			700	
[2099]	Lys	Gly	Ser	Gly	Arg	Ile	Gly	Asp	Arg	Ala	Ile	Pro	Ala	Asp	Glu	Phe
[2100]	705						710					715				720
[2101]	Asp	Pro	Thr	Lys	His	Arg	Tyr	Asp	Ala	Glu	Tyr	Tyr	Ile	Glu	Asn	Gln
[2102]							725					730			735	
[2103]	Val	Leu	Pro	Ala	Val	Glu	Arg	Ile	Leu	Lys	Ala	Phe	Gly	Tyr	Arg	Lys
[2104]							740					745			750	
[2105]	Glu	Asp	Leu	Arg	Tyr	Gln	Lys	Thr	Lys	Gln	Val	Gly	Leu	Gly	Ala	Trp

[2106]	755	760	765
[2107]	Leu Lys Val Lys Gly Lys Lys		
[2108]	770	775	
[2109]	<210> 21		
[2110]	<211> 775		
[2111]	<212> PRT		
[2112]	<213> 人工		
[2113]	<220>		
[2114]	<223> 工程化聚合酶		
[2115]	<400> 21		
[2116]	Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile		
[2117]	1 5 10 15		
[2118]	Arg Val Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg		
[2119]	20 25 30		
[2120]	Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile		
[2121]	35 40 45		
[2122]	Glu Asp Val Lys Lys Val Thr Ala Lys Arg His Gly Thr Val Val Lys		
[2123]	50 55 60		
[2124]	Val Lys Arg Ala Glu Lys Val Gln Lys Lys Phe Leu Gly Arg Pro Ile		
[2125]	65 70 75 80		
[2126]	Glu Val Trp Lys Leu Tyr Phe Asn His Pro Gln Asp Val Pro Ala Ile		
[2127]	85 90 95		
[2128]	Arg Asp Arg Ile Arg Ala His Pro Ala Val Val Asp Ile Tyr Glu Tyr		
[2129]	100 105 110		
[2130]	Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro		
[2131]	115 120 125		
[2132]	Ala Glu Gly Asp Glu Glu Leu Thr Met Leu Ala Phe Ala Ile Ala Thr		
[2133]	130 135 140		
[2134]	Leu Tyr His Glu Gly Glu Glu Phe Gly Thr Gly Pro Ile Leu Met Ile		
[2135]	145 150 155 160		
[2136]	Ser Tyr Ala Asp Gly Ser Glu Ala Arg Val Ile Thr Trp Lys Lys Ile		
[2137]	165 170 175		
[2138]	Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Lys Glu Met Ile Lys		
[2139]	180 185 190		
[2140]	Arg Phe Leu Arg Val Val Arg Glu Lys Asp Pro Asp Val Leu Ile Thr		
[2141]	195 200 205		
[2142]	Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Ser Glu		
[2143]	210 215 220		
[2144]	Glu Leu Gly Ile Lys Phe Thr Leu Gly Arg Asp Gly Ser Glu Pro Lys		

[2145]	225	230	235	240
[2146]	Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile			
[2147]	245	250	255	
[2148]	His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr			
[2149]	260	265	270	
[2150]	Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu			
[2151]	275	280	285	
[2152]	Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly			
[2153]	290	295	300	
[2154]	Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr			
[2155]	305	310	315	320
[2156]	Glu Leu Gly Arg Glu Phe Phe Pro His Glu Ala Gln Leu Ser Arg Leu			
[2157]	325	330	335	
[2158]	Ile Gly Gln Ser Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu			
[2159]	340	345	350	
[2160]	Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Lys Arg Asn Glu Leu Ala			
[2161]	355	360	365	
[2162]	Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr			
[2163]	370	375	380	
[2164]	Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile			
[2165]	385	390	395	400
[2166]	Val Tyr Leu Asp Phe Arg Ser Ala Ala Ile Ser Ile Ile Ile Thr His			
[2167]	405	410	415	
[2168]	Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp			
[2169]	420	425	430	
[2170]	Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe			
[2171]	435	440	445	
[2172]	Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys			
[2173]	450	455	460	
[2174]	Arg Lys Met Lys Ala Thr Val Asp Pro Leu Glu Trp Met Leu Leu Asp			
[2175]	465	470	475	480
[2176]	Tyr Arg Gln Arg Val Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr			
[2177]	485	490	495	
[2178]	Gly Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser			
[2179]	500	505	510	
[2180]	Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu			
[2181]	515	520	525	
[2182]	Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu			
[2183]	530	535	540	

[2184]	His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala
[2185]	545 550 555 560
[2186]	Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu
[2187]	565 570 575
[2188]	Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys
[2189]	580 585 590
[2190]	Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu
[2191]	595 600 605
[2192]	Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala
[2193]	610 615 620
[2194]	Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val
[2195]	625 630 635 640
[2196]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro
[2197]	645 650 655
[2198]	Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp
[2199]	660 665 670
[2200]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala
[2201]	675 680 685
[2202]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu
[2203]	690 695 700
[2204]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe
[2205]	705 710 715 720
[2206]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln
[2207]	725 730 735
[2208]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys
[2209]	740 745 750
[2210]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp
[2211]	755 760 765
[2212]	Leu Lys Val Lys Gly Lys Lys
[2213]	770 775
[2214]	<210> 22
[2215]	<211> 775
[2216]	<212> PRT
[2217]	<213> 人工
[2218]	<220>
[2219]	<223> 工程化聚合酶
[2220]	<400> 22
[2221]	Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile
[2222]	1 5 10 15

[2223]	Arg	Val	Phe	Lys	Lys	Glu	Asn	Gly	Glu	Phe	Lys	Ile	Glu	Tyr	Asp	Arg
[2224]				20					25					30		
[2225]	Thr	Phe	Glu	Pro	Tyr	Phe	Tyr	Ala	Leu	Leu	Lys	Asp	Asp	Ser	Ala	Ile
[2226]				35					40					45		
[2227]	Glu	Asp	Val	Lys	Lys	Val	Thr	Ala	Lys	Arg	His	Gly	Thr	Val	Val	Lys
[2228]				50					55					60		
[2229]	Val	Lys	Arg	Ala	Glu	Lys	Val	Gln	Lys	Lys	Phe	Leu	Gly	Arg	Pro	Ile
[2230]	65						70				75					80
[2231]	Glu	Val	Trp	Lys	Leu	Tyr	Phe	Asn	His	Pro	Gln	Asp	Val	Pro	Ala	Ile
[2232]					85					90					95	
[2233]	Arg	Asp	Arg	Ile	Arg	Ala	His	Pro	Ala	Val	Val	Asp	Ile	Tyr	Glu	Tyr
[2234]					100					105					110	
[2235]	Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro
[2236]					115					120				125		
[2237]	Ala	Glu	Gly	Asp	Glu	Glu	Leu	Thr	Met	Leu	Ala	Phe	Ala	Ile	Ala	Thr
[2238]							130			135				140		
[2239]	Leu	Tyr	His	Glu	Gly	Glu	Glu	Phe	Gly	Thr	Gly	Pro	Ile	Leu	Met	Ile
[2240]	145						150				155					160
[2241]	Ser	Tyr	Ala	Asp	Gly	Ser	Glu	Ala	Arg	Val	Ile	Thr	Trp	Lys	Lys	Ile
[2242]					165					170					175	
[2243]	Asp	Leu	Pro	Tyr	Val	Asp	Val	Val	Ser	Thr	Glu	Lys	Glu	Met	Ile	Lys
[2244]					180					185					190	
[2245]	Arg	Phe	Leu	Arg	Val	Val	Arg	Glu	Lys	Asp	Pro	Asp	Val	Leu	Ile	Thr
[2246]					195					200					205	
[2247]	Tyr	Asn	Gly	Asp	Asn	Phe	Asp	Phe	Ala	Tyr	Leu	Lys	Lys	Arg	Ser	Glu
[2248]					210					215				220		
[2249]	Glu	Leu	Gly	Ile	Lys	Phe	Thr	Leu	Gly	Arg	Asp	Gly	Ser	Glu	Pro	Lys
[2250]	225						230				235					240
[2251]	Ile	Gln	Arg	Met	Gly	Asp	Arg	Phe	Ala	Val	Glu	Val	Lys	Gly	Arg	Ile
[2252]					245					250					255	
[2253]	His	Phe	Asp	Leu	Tyr	Pro	Val	Ile	Arg	Arg	Thr	Ile	Asn	Leu	Pro	Thr
[2254]					260					265					270	
[2255]	Tyr	Thr	Leu	Glu	Ala	Val	Tyr	Glu	Ala	Val	Phe	Gly	Lys	Pro	Lys	Glu
[2256]					275					280				285		
[2257]	Lys	Val	Tyr	Ala	Glu	Glu	Ile	Ala	Gln	Ala	Trp	Glu	Ser	Gly	Glu	Gly
[2258]					290					295				300		
[2259]	Leu	Glu	Arg	Val	Ala	Arg	Tyr	Ser	Met	Glu	Asp	Ala	Lys	Val	Thr	Tyr
[2260]	305						310				315					320
[2261]	Glu	Leu	Gly	Arg	Glu	Phe	Phe	Pro	Met	Glu	Ala	Gln	Leu	Ser	Arg	Leu

[2262]					325					330						335
[2263]	Ile	Gly	Gln	Ser	Leu	Trp	Asp	Val	Ser	Arg	Ser	Ser	Thr	Gly	Asn	Leu
[2264]					340					345						350
[2265]	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Lys	Arg	Asn	Glu	Leu	Ala
[2266]					355					360						365
[2267]	Pro	Asn	Lys	Pro	Asp	Glu	Arg	Glu	Leu	Ala	Arg	Arg	Arg	Gly	Gly	Tyr
[2268]					370					375						380
[2269]	Ala	Gly	Gly	Tyr	Val	Lys	Glu	Pro	Glu	Arg	Gly	Leu	Trp	Asp	Asn	Ile
[2270]	385						390				395					400
[2271]	Val	Tyr	Leu	Asp	Phe	Arg	Ser	Ala	Ala	Ile	Ser	Ile	Ile	Ile	Thr	His
[2272]					405					410						415
[2273]	Asn	Val	Ser	Pro	Asp	Thr	Leu	Asn	Arg	Glu	Gly	Cys	Lys	Glu	Tyr	Asp
[2274]					420					425						430
[2275]	Val	Ala	Pro	Glu	Val	Gly	His	Lys	Phe	Cys	Lys	Asp	Phe	Pro	Gly	Phe
[2276]					435					440						445
[2277]	Ile	Pro	Ser	Leu	Leu	Gly	Asp	Leu	Leu	Glu	Glu	Arg	Gln	Lys	Ile	Lys
[2278]					450					455						460
[2279]	Arg	Lys	Met	Lys	Ala	Thr	Val	Asp	Pro	Leu	Glu	Trp	Met	Leu	Leu	Asp
[2280]	465						470				475					480
[2281]	Tyr	Arg	Gln	Arg	Val	Ile	Lys	Ile	Leu	Ala	Asn	Ser	Phe	Tyr	Gly	Tyr
[2282]					485					490						495
[2283]	Gly	Gly	Tyr	Ala	Lys	Ala	Arg	Trp	Tyr	Cys	Lys	Glu	Cys	Ala	Glu	Ser
[2284]					500					505						510
[2285]	Val	Thr	Ala	Trp	Gly	Arg	Glu	Tyr	Ile	Glu	Met	Val	Ile	Arg	Glu	Leu
[2286]					515					520						525
[2287]	Glu	Glu	Lys	Phe	Gly	Phe	Lys	Val	Leu	Tyr	Ala	Asp	Thr	Asp	Gly	Leu
[2288]					530					535						540
[2289]	His	Ala	Thr	Ile	Pro	Gly	Ala	Asp	Ala	Glu	Thr	Val	Lys	Lys	Lys	Ala
[2290]	545						550				555					560
[2291]	Lys	Glu	Phe	Leu	Lys	Tyr	Ile	Asn	Pro	Lys	Leu	Pro	Gly	Leu	Leu	Glu
[2292]					565					570						575
[2293]	Leu	Glu	Tyr	Glu	Gly	Phe	Tyr	Val	Arg	Gly	Phe	Phe	Val	Thr	Lys	Lys
[2294]					580					585						590
[2295]	Lys	Tyr	Ala	Val	Ile	Asp	Glu	Glu	Gly	Lys	Ile	Thr	Thr	Arg	Gly	Leu
[2296]					595					600						605
[2297]	Glu	Ile	Val	Arg	Arg	Asp	Trp	Ser	Glu	Ile	Ala	Lys	Glu	Thr	Gln	Ala
[2298]					610					615						620
[2299]	Arg	Val	Leu	Glu	Ala	Ile	Leu	Lys	His	Gly	Asp	Val	Glu	Glu	Ala	Val
[2300]	625						630				635					640

[2301]	Arg	Ile	Val	Lys	Glu	Val	Thr	Glu	Lys	Leu	Ser	Lys	Tyr	Glu	Val	Pro
[2302]					645					650					655	
[2303]	Pro	Glu	Lys	Leu	Val	Ile	His	Glu	Gln	Ile	Thr	Arg	Asp	Leu	Arg	Asp
[2304]					660					665					670	
[2305]	Tyr	Lys	Ala	Thr	Gly	Pro	His	Val	Ala	Val	Ala	Lys	Arg	Leu	Ala	Ala
[2306]					675					680					685	
[2307]	Arg	Gly	Val	Lys	Ile	Arg	Pro	Gly	Thr	Val	Ile	Ser	Tyr	Ile	Val	Leu
[2308]					690					695					700	
[2309]	Lys	Gly	Ser	Gly	Arg	Ile	Gly	Asp	Arg	Ala	Ile	Pro	Ala	Asp	Glu	Phe
[2310]	705					710					715					720
[2311]	Asp	Pro	Thr	Lys	His	Arg	Tyr	Asp	Ala	Glu	Tyr	Tyr	Ile	Arg	Asn	Gln
[2312]					725						730					735
[2313]	Val	Leu	Pro	Ala	Val	Glu	Arg	Ile	Leu	Lys	Ala	Phe	Gly	Tyr	Arg	Lys
[2314]					740						745					750
[2315]	Glu	Asp	Leu	Arg	Tyr	Gln	Lys	Thr	Lys	Gln	Val	Gly	Leu	Gly	Ala	Trp
[2316]					755						760					765
[2317]	Leu	Lys	Val	Lys	Gly	Lys	Lys									
[2318]					770											775
[2319]	<210> 23															
[2320]	<211> 775															
[2321]	<212> PRT															
[2322]	<213> 人工															
[2323]	<220>															
[2324]	<223> 工程化聚合酶															
[2325]	<400> 23															
[2326]	Met	Ile	Leu	Asp	Thr	Asp	Tyr	Ile	Thr	Glu	Asn	Gly	Lys	Pro	Val	Ile
[2327]	1				5					10					15	
[2328]	Arg	Val	Phe	Lys	Lys	Glu	Asn	Gly	Glu	Phe	Lys	Ile	Glu	Tyr	Asp	Arg
[2329]					20					25					30	
[2330]	Thr	Phe	Glu	Pro	Tyr	Phe	Tyr	Ala	Leu	Leu	Lys	Asp	Asp	Ser	Ala	Ile
[2331]					35					40					45	
[2332]	Glu	Asp	Val	Lys	Lys	Val	Thr	Ala	Lys	Arg	His	Gly	Thr	Val	Val	Lys
[2333]					50					55					60	
[2334]	Val	Lys	Arg	Ala	Glu	Lys	Val	Gln	Lys	Lys	Phe	Leu	Gly	Arg	Pro	Ile
[2335]	65					70					75					80
[2336]	Glu	Val	Trp	Lys	Leu	Tyr	Phe	Asn	His	Pro	Gln	Asp	Val	Pro	Ala	Ile
[2337]					85						90					95
[2338]	Arg	Asp	Arg	Ile	Arg	Ala	His	Pro	Ala	Val	Val	Asp	Ile	Tyr	Glu	Tyr
[2339]					100						105					110

[2340]	Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro
[2341]			115					120					125			
[2342]	Ala	Glu	Gly	Asp	Glu	Glu	Leu	Thr	Met	Leu	Ala	Phe	Ala	Ile	Ala	Thr
[2343]			130					135					140			
[2344]	Leu	Tyr	His	Glu	Gly	Glu	Glu	Phe	Gly	Thr	Gly	Pro	Ile	Leu	Met	Ile
[2345]	145						150					155				160
[2346]	Ser	Tyr	Ala	Asp	Gly	Ser	Glu	Ala	Arg	Val	Ile	Thr	Trp	Lys	Lys	Ile
[2347]					165					170					175	
[2348]	Asp	Leu	Pro	Tyr	Val	Asp	Val	Val	Ser	Thr	Glu	Lys	Glu	Met	Ile	Lys
[2349]				180						185					190	
[2350]	Arg	Phe	Leu	Arg	Val	Val	Arg	Glu	Lys	Asp	Pro	Asp	Val	Leu	Ile	Thr
[2351]				195					200					205		
[2352]	Tyr	Asn	Gly	Asp	Asn	Phe	Asp	Phe	Ala	Tyr	Leu	Lys	Lys	Arg	Ser	Glu
[2353]			210					215					220			
[2354]	Glu	Leu	Gly	Ile	Lys	Phe	Thr	Leu	Gly	Arg	Asp	Gly	Ser	Glu	Pro	Lys
[2355]	225					230					235					240
[2356]	Ile	Gln	Arg	Met	Gly	Asp	Tyr	Phe	Ala	Val	Glu	Val	Lys	Gly	Arg	Ile
[2357]					245					250					255	
[2358]	His	Phe	Asp	Leu	Tyr	Pro	Val	Ile	Arg	Arg	Thr	Ile	Asn	Leu	Pro	Thr
[2359]				260						265					270	
[2360]	Tyr	Thr	Leu	Glu	Ala	Val	Tyr	Glu	Ala	Val	Phe	Gly	Lys	Pro	Lys	Glu
[2361]				275					280					285		
[2362]	Lys	Val	Tyr	Ala	Glu	Glu	Ile	Ala	Gln	Ala	Trp	Glu	Ser	Gly	Glu	Gly
[2363]			290						295				300			
[2364]	Leu	Glu	Arg	Val	Ala	Arg	Tyr	Ser	Met	Glu	Asp	Ala	Lys	Val	Thr	Tyr
[2365]	305					310					315					320
[2366]	Glu	Leu	Gly	Arg	Glu	Phe	Phe	Pro	Met	Glu	Ala	Gln	Leu	Ser	Arg	Leu
[2367]					325					330					335	
[2368]	Ile	Gly	Gln	Ser	Leu	Trp	Asp	Val	Ser	Arg	Ser	Ser	Thr	Gly	Asn	Leu
[2369]				340						345				350		
[2370]	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Lys	Arg	Asn	Glu	Leu	Ala
[2371]				355					360					365		
[2372]	Pro	Asn	Lys	Pro	Asp	Glu	Arg	Glu	Leu	Ala	Arg	Arg	Arg	Gly	Gly	Tyr
[2373]			370					375					380			
[2374]	Ala	Gly	Gly	Tyr	Val	Lys	Glu	Pro	Glu	Arg	Gly	Leu	Trp	Asp	Asn	Ile
[2375]	385					390					395					400
[2376]	Val	Tyr	Leu	Asp	Phe	Arg	Ser	Ala	Ala	Ile	Ser	Ile	Ile	Ile	Thr	His
[2377]					405					410					415	
[2378]	Asn	Val	Ser	Pro	Asp	Thr	Leu	Asn	Arg	Glu	Gly	Cys	Lys	Glu	Tyr	Asp

[2379]	420							425							430													
[2380]	Val	Ala	Pro	Glu	Val	Gly	His	Lys	Phe	Cys	Lys	Asp	Phe	Pro	Gly	Phe												
[2381]	435							440							445													
[2382]	Ile	Pro	Ser	Leu	Leu	Gly	Asp	Leu	Leu	Glu	Glu	Arg	Gln	Lys	Ile	Lys												
[2383]	450							455							460													
[2384]	Arg	Lys	Met	Lys	Ala	Thr	Val	Asp	Pro	Leu	Glu	Lys	Lys	Leu	Leu	Asp												
[2385]	465							470							475							480						
[2386]	Tyr	Arg	Gln	Arg	Val	Ile	Lys	Ile	Leu	Ala	Asn	Ser	Phe	Tyr	Gly	Tyr												
[2387]	485							490							495													
[2388]	Gly	Gly	Tyr	Ala	Lys	Ala	Arg	Trp	Tyr	Cys	Lys	Glu	Cys	Ala	Glu	Ser												
[2389]	500							505							510													
[2390]	Val	Thr	Ala	Trp	Gly	Arg	Glu	Tyr	Ile	Glu	Met	Val	Ile	Arg	Glu	Leu												
[2391]	515							520							525													
[2392]	Glu	Glu	Lys	Phe	Gly	Phe	Lys	Val	Leu	Tyr	Ala	Asp	Thr	Asp	Gly	Leu												
[2393]	530							535							540													
[2394]	His	Ala	Thr	Ile	Pro	Gly	Ala	Asp	Ala	Glu	Thr	Val	Lys	Lys	Lys	Ala												
[2395]	545							550							555							560						
[2396]	Lys	Glu	Phe	Leu	Lys	Tyr	Ile	Asn	Pro	Lys	Leu	Pro	Gly	Leu	Leu	Glu												
[2397]	565							570							575													
[2398]	Leu	Glu	Tyr	Glu	Gly	Phe	Tyr	Val	Arg	Gly	Phe	Phe	Val	Thr	Lys	Lys												
[2399]	580							585							590													
[2400]	Lys	Tyr	Ala	Val	Ile	Asp	Asp	Glu	Gly	Lys	Ile	Thr	Thr	Arg	Gly	Leu												
[2401]	595							600							605													
[2402]	Glu	Ile	Val	Arg	Arg	Asp	Trp	Ser	Glu	Ile	Ala	Lys	Glu	Thr	Gln	Ala												
[2403]	610							615							620													
[2404]	Arg	Val	Leu	Glu	Ala	Ile	Leu	Lys	Gly	Gly	Asp	Val	Glu	Glu	Ala	Val												
[2405]	625							630							635							640						
[2406]	Arg	Ile	Val	Lys	Glu	Val	Thr	Glu	Lys	Leu	Ser	Lys	Tyr	Glu	Val	Pro												
[2407]	645							650							655													
[2408]	Pro	Glu	Lys	Leu	Val	Ile	His	Glu	Gln	Ile	Thr	Arg	Asp	Leu	Arg	Asp												
[2409]	660							665							670													
[2410]	Tyr	Lys	Ala	Thr	Gly	Pro	His	Val	Ala	Val	Ala	Lys	Arg	Leu	Ala	Ala												
[2411]	675							680							685													
[2412]	Arg	Gly	Val	Lys	Ile	Arg	Pro	Gly	Thr	Val	Ile	Ser	Tyr	Ile	Val	Leu												
[2413]	690							695							700													
[2414]	Lys	Gly	Ser	Gly	Arg	Ile	Gly	Asp	Arg	Ala	Ile	Pro	Ala	Asp	Glu	Phe												
[2415]	705							710							715							720						
[2416]	Asp	Pro	Thr	Lys	His	Arg	Tyr	Asp	Ala	Glu	Tyr	Tyr	Ile	Glu	Asn	Gln												
[2417]	725							730							735													

[2418]	Val	Leu	Pro	Ala	Val	Glu	Arg	Ile	Leu	Lys	Ala	Phe	Gly	Tyr	Arg	Lys
[2419]				740					745						750	
[2420]	Glu	Asp	Leu	Arg	Tyr	Gln	Lys	Thr	Lys	Gln	Val	Gly	Leu	Gly	Ala	Trp
[2421]				755					760						765	
[2422]	Leu	Lys	Val	Lys	Gly	Lys	Lys									
[2423]				770					775							
[2424]	<210>	24														
[2425]	<211>	775														
[2426]	<212>	PRT														
[2427]	<213>	人工														
[2428]	<220>															
[2429]	<223>	工程化聚合酶														
[2430]	<400>	24														
[2431]	Met	Ile	Leu	Asp	Thr	Asp	Tyr	Ile	Thr	Glu	Asn	Gly	Lys	Pro	Val	Ile
[2432]	1				5					10					15	
[2433]	Arg	Val	Phe	Lys	Lys	Glu	Asn	Gly	Glu	Phe	Lys	Ile	Glu	Tyr	Asp	Arg
[2434]				20					25					30		
[2435]	Thr	Phe	Glu	Pro	Tyr	Phe	Tyr	Ala	Leu	Leu	Lys	Asp	Asp	Ser	Ala	Ile
[2436]				35					40					45		
[2437]	Glu	Asp	Val	Lys	Lys	Val	Thr	Ala	Lys	Arg	His	Gly	Thr	Val	Val	Lys
[2438]				50					55					60		
[2439]	Val	Lys	Arg	Ala	Glu	Lys	Val	Gln	Lys	Lys	Phe	Leu	Gly	Arg	Pro	Ile
[2440]	65					70					75				80	
[2441]	Glu	Val	Trp	Lys	Leu	Tyr	Phe	Asn	His	Pro	Gln	Asp	Val	Pro	Ala	Ile
[2442]					85					90					95	
[2443]	Arg	Asp	Arg	Ile	Arg	Ala	His	Pro	Ala	Val	Val	Asp	Ile	Tyr	Glu	Tyr
[2444]				100					105						110	
[2445]	Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro
[2446]				115					120					125		
[2447]	Ala	Glu	Gly	Asp	Glu	Glu	Leu	Thr	Met	Leu	Ala	Phe	Ala	Ile	Ala	Thr
[2448]				130					135					140		
[2449]	Leu	Tyr	His	Glu	Gly	Glu	Glu	Phe	Gly	Thr	Gly	Pro	Ile	Leu	Met	Ile
[2450]	145					150					155				160	
[2451]	Ser	Tyr	Ala	Asp	Gly	Ser	Glu	Ala	Arg	Val	Ile	Thr	Trp	Lys	Lys	Ile
[2452]					165					170					175	
[2453]	Asp	Leu	Pro	Tyr	Val	Asp	Val	Val	Ser	Thr	Glu	Lys	Glu	Met	Ile	Lys
[2454]				180						185					190	
[2455]	Arg	Phe	Leu	Arg	Val	Val	Arg	Glu	Lys	Asp	Pro	Asp	Val	Leu	Ile	Thr
[2456]				195					200					205		

[2457]	Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Ser Glu
[2458]	210 215 220
[2459]	Glu Leu Gly Ile Lys Phe Thr Leu Gly Arg Asp Gly Ser Glu Pro Lys
[2460]	225 230 235 240
[2461]	Ile Gln Arg Met Gly Asp Tyr Phe Ala Val Glu Val Lys Gly Arg Ile
[2462]	245 250 255
[2463]	His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
[2464]	260 265 270
[2465]	Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu
[2466]	275 280 285
[2467]	Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly
[2468]	290 295 300
[2469]	Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr
[2470]	305 310 315 320
[2471]	Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
[2472]	325 330 335
[2473]	Ile Gly Gln Ser Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
[2474]	340 345 350
[2475]	Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Lys Arg Asn Glu Leu Ala
[2476]	355 360 365
[2477]	Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr
[2478]	370 375 380
[2479]	Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile
[2480]	385 390 395 400
[2481]	Val Tyr Leu Asp Phe Arg Ser Ala Ala Ile Ser Ile Ile Ile Thr His
[2482]	405 410 415
[2483]	Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp
[2484]	420 425 430
[2485]	Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe
[2486]	435 440 445
[2487]	Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys
[2488]	450 455 460
[2489]	Arg Lys Met Lys Ala Thr Val Asp Pro Leu Glu Lys Lys Leu Leu Asp
[2490]	465 470 475 480
[2491]	Tyr Arg Gln Arg Val Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr
[2492]	485 490 495
[2493]	Gly Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser
[2494]	500 505 510
[2495]	Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu

[2496]	515	520	525
[2497]	Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu		
[2498]	530	535	540
[2499]	His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala		
[2500]	545	550	555
[2501]	Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu		
[2502]	565	570	575
[2503]	Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys		
[2504]	580	585	590
[2505]	Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu		
[2506]	595	600	605
[2507]	Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala		
[2508]	610	615	620
[2509]	Arg Val Leu Glu Ala Ile Leu Lys Gly Gly Asp Val Glu Glu Ala Val		
[2510]	625	630	635
[2511]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro		
[2512]	645	650	655
[2513]	Pro Glu Lys Leu Asp Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp		
[2514]	660	665	670
[2515]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala		
[2516]	675	680	685
[2517]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu		
[2518]	690	695	700
[2519]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe		
[2520]	705	710	715
[2521]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln		
[2522]	725	730	735
[2523]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys		
[2524]	740	745	750
[2525]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp		
[2526]	755	760	765
[2527]	Leu Lys Val Lys Gly Lys Lys		
[2528]	770	775	
[2529]	<210> 25		
[2530]	<211> 775		
[2531]	<212> PRT		
[2532]	<213> 人工		
[2533]	<220>		
[2534]	<223> 工程化聚合酶		

[2535]	<400> 25																
[2536]	Met	Ile	Leu	Asp	Thr	Asp	Tyr	Ile	Thr	Glu	Asn	Gly	Lys	Pro	Val	Ile	
[2537]	1			5			10			15							
[2538]	Arg	Val	Phe	Lys	Lys	Glu	Asn	Gly	Glu	Phe	Lys	Ile	Glu	Tyr	Asp	Arg	
[2539]	20				25				30								
[2540]	Thr	Phe	Glu	Pro	Tyr	Phe	Tyr	Ala	Leu	Leu	Lys	Asp	Asp	Ser	Ala	Ile	
[2541]	35				40				45								
[2542]	Glu	Asp	Val	Lys	Lys	Val	Thr	Ala	Lys	Arg	His	Gly	Thr	Val	Val	Lys	
[2543]	50			55			60										
[2544]	Val	Lys	Arg	Ala	Glu	Lys	Val	Gln	Lys	Lys	Phe	Leu	Gly	Arg	Pro	Ile	
[2545]	65			70			75			80							
[2546]	Glu	Val	Trp	Lys	Leu	Tyr	Phe	Asn	His	Pro	Gln	Asp	Val	Pro	Ala	Ile	
[2547]	85				90				95								
[2548]	Arg	Asp	Arg	Ile	Arg	Ala	His	Pro	Ala	Val	Val	Asp	Ile	Tyr	Glu	Tyr	
[2549]	100				105				110								
[2550]	Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro	
[2551]	115			120			125										
[2552]	Ala	Glu	Gly	Asp	Glu	Glu	Leu	Thr	Met	Leu	Ala	Phe	Ala	Ile	Ala	Thr	
[2553]	130			135			140										
[2554]	Leu	Tyr	His	Glu	Gly	Glu	Glu	Phe	Gly	Thr	Gly	Pro	Ile	Leu	Met	Ile	
[2555]	145			150			155			160							
[2556]	Ser	Tyr	Ala	Asp	Gly	Ser	Glu	Ala	Arg	Val	Ile	Thr	Trp	Lys	Lys	Ile	
[2557]	165				170				175								
[2558]	Asp	Leu	Pro	Tyr	Val	Asp	Val	Val	Ser	Thr	Glu	Lys	Glu	Met	Ile	Lys	
[2559]	180				185				190								
[2560]	Arg	Phe	Leu	Arg	Val	Val	Arg	Glu	Lys	Asp	Pro	Asp	Val	Leu	Ile	Thr	
[2561]	195			200			205										
[2562]	Tyr	Asn	Gly	Asp	Asn	Phe	Asp	Phe	Ala	Tyr	Leu	Lys	Lys	Arg	Ser	Glu	
[2563]	210			215			220										
[2564]	Glu	Leu	Gly	Ile	Lys	Phe	Thr	Leu	Gly	Arg	Asp	Gly	Ser	Glu	Pro	Lys	
[2565]	225			230			235			240							
[2566]	Ile	Gln	Arg	Met	Gly	Asp	Tyr	Phe	Ala	Val	Glu	Val	Lys	Gly	Arg	Ile	
[2567]	245				250				255								
[2568]	His	Phe	Asp	Leu	Tyr	Pro	Val	Ile	Arg	Arg	Thr	Ile	Asn	Leu	Pro	Thr	
[2569]	260				265				270								
[2570]	Tyr	Thr	Leu	Glu	Ala	Val	Tyr	Glu	Ala	Val	Phe	Gly	Lys	Pro	Lys	Glu	
[2571]	275			280			285										
[2572]	Lys	Val	Tyr	Ala	Glu	Glu	Ile	Ala	Gln	Ala	Trp	Glu	Ser	Gly	Glu	Gly	
[2573]	290			295			300										

[2574]	Leu	Glu	Arg	Val	Ala	Arg	Tyr	Ser	Met	Glu	Asp	Ala	Lys	Val	Thr	Tyr
[2575]	305					310					315					320
[2576]	Glu	Leu	Gly	Arg	Glu	Phe	Phe	Pro	Met	Glu	Ala	Gln	Leu	Ser	Arg	Leu
[2577]					325					330						335
[2578]	Ile	Gly	Gln	Ser	Leu	Trp	Asp	Val	Ser	Arg	Ser	Ser	Thr	Gly	Asn	Leu
[2579]				340					345					350		
[2580]	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Lys	Arg	Asn	Glu	Leu	Ala
[2581]			355					360					365			
[2582]	Pro	Asn	Lys	Pro	Asp	Glu	Arg	Glu	Leu	Ala	Arg	Arg	Arg	Gly	Gly	Tyr
[2583]	370						375					380				
[2584]	Ala	Gly	Gly	Tyr	Val	Lys	Glu	Pro	Glu	Arg	Gly	Leu	Trp	Asp	Asn	Ile
[2585]	385					390					395					400
[2586]	Val	Tyr	Leu	Asp	Phe	Arg	Ser	Ala	Ala	Ile	Ser	Ile	Ile	Ile	Thr	His
[2587]				405						410						415
[2588]	Asn	Val	Ser	Pro	Asp	Thr	Leu	Asn	Arg	Glu	Gly	Cys	Lys	Glu	Tyr	Asp
[2589]				420					425					430		
[2590]	Val	Ala	Pro	Glu	Val	Gly	His	Lys	Phe	Cys	Lys	Asp	Phe	Pro	Gly	Phe
[2591]			435					440					445			
[2592]	Ile	Pro	Ser	Leu	Leu	Gly	Asp	Leu	Leu	Glu	Glu	Arg	Gln	Lys	Ile	Lys
[2593]	450						455					460				
[2594]	Arg	Lys	Met	Lys	Ala	Thr	Val	Asp	Pro	Leu	Glu	Lys	Lys	Leu	Leu	Asp
[2595]	465					470					475					480
[2596]	Tyr	Arg	Gln	Arg	Val	Ile	Lys	Ile	Leu	Ala	Asn	Ser	Phe	Tyr	Gly	Tyr
[2597]				485						490						495
[2598]	Gly	Gly	Tyr	Ala	Lys	Ala	Arg	Trp	Tyr	Cys	Lys	Glu	Cys	Ala	Glu	Ser
[2599]				500					505					510		
[2600]	Val	Thr	Ala	Trp	Gly	Arg	Glu	Tyr	Ile	Glu	Met	Val	Ile	Arg	Glu	Leu
[2601]			515					520					525			
[2602]	Glu	Glu	Lys	Phe	Gly	Phe	Lys	Val	Leu	Tyr	Ala	Asp	Thr	Asp	Gly	Leu
[2603]		530					535					540				
[2604]	His	Ala	Thr	Ile	Pro	Gly	Ala	Asp	Ala	Glu	Thr	Val	Lys	Lys	Lys	Ala
[2605]	545					550					555					560
[2606]	Lys	Glu	Phe	Leu	Lys	Tyr	Ile	Asn	Pro	Lys	Leu	Pro	Gly	Leu	Leu	Glu
[2607]				565						570						575
[2608]	Leu	Glu	Tyr	Glu	Gly	Phe	Tyr	Val	Arg	Gly	Phe	Phe	Val	Thr	Lys	Lys
[2609]				580					585					590		
[2610]	Lys	Tyr	Ala	Val	Ile	Asp	Asp	Glu	Gly	Lys	Ile	Thr	Thr	Arg	Gly	Leu
[2611]			595					600					605			
[2612]	Glu	Ile	Val	Arg	Arg	Asp	Trp	Ser	Glu	Ile	Ala	Arg	Glu	Thr	Gln	Ala

[2613]	610	615	620
[2614]	Arg Val Leu Glu Ala Ile Leu Lys Gly Gly Asp Val Glu Glu Ala Val		
[2615]	625	630	635
[2616]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro		
[2617]	645	650	655
[2618]	Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp		
[2619]	660	665	670
[2620]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala		
[2621]	675	680	685
[2622]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu		
[2623]	690	695	700
[2624]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe		
[2625]	705	710	715
[2626]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln		
[2627]	725	730	735
[2628]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys		
[2629]	740	745	750
[2630]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp		
[2631]	755	760	765
[2632]	Leu Lys Val Lys Gly Lys Lys		
[2633]	770	775	
[2634]	<210> 26		
[2635]	<211> 775		
[2636]	<212> PRT		
[2637]	<213> 人工		
[2638]	<220>		
[2639]	<223> 工程化聚合酶		
[2640]	<400> 26		
[2641]	Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile		
[2642]	1	5	10
[2643]	Arg Val Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg		
[2644]	20	25	30
[2645]	Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile		
[2646]	35	40	45
[2647]	Glu Asp Val Lys Lys Val Thr Ala Lys Arg His Gly Thr Val Val Lys		
[2648]	50	55	60
[2649]	Val Lys Arg Ala Glu Lys Val Gln Lys Lys Phe Leu Gly Arg Pro Ile		
[2650]	65	70	75
[2651]	Glu Val Trp Lys Leu Tyr Phe Asn His Pro Gln Asp Val Pro Ala Ile		

[2652]	85															90					95									
[2653]	Arg	Asp	Arg	Ile	Arg	Ala	His	Pro	Ala	Val	Val	Asp	Ile	Tyr	Glu	Tyr														
[2654]	100															105					110									
[2655]	Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro														
[2656]	115															120					125									
[2657]	Ala	Glu	Gly	Asp	Glu	Glu	Leu	Thr	Met	Leu	Ala	Phe	Ala	Ile	Ala	Thr														
[2658]	130															135					140									
[2659]	Leu	Tyr	His	Glu	Gly	Glu	Glu	Phe	Gly	Thr	Gly	Pro	Ile	Leu	Met	Ile														
[2660]	145															150					155					160				
[2661]	Ser	Tyr	Ala	Asp	Gly	Ser	Glu	Ala	Arg	Val	Ile	Thr	Trp	Lys	Lys	Ile														
[2662]	165															170					175									
[2663]	Asp	Leu	Pro	Tyr	Val	Asp	Val	Val	Ser	Thr	Glu	Lys	Glu	Met	Ile	Lys														
[2664]	180															185					190									
[2665]	Arg	Phe	Leu	Arg	Val	Val	Arg	Glu	Lys	Asp	Pro	Asp	Val	Leu	Ile	Thr														
[2666]	195															200					205									
[2667]	Tyr	Asn	Gly	Asp	Asn	Phe	Asp	Phe	Ala	Tyr	Leu	Lys	Lys	Arg	Ser	Glu														
[2668]	210															215					220									
[2669]	Glu	Leu	Gly	Ile	Lys	Phe	Thr	Leu	Gly	Arg	Asp	Gly	Ser	Glu	Pro	Lys														
[2670]	225															230					235					240				
[2671]	Ile	Gln	Arg	Met	Gly	Asp	Tyr	Phe	Ala	Val	Glu	Val	Lys	Gly	Arg	Ile														
[2672]	245															250					255									
[2673]	His	Phe	Asp	Leu	Tyr	Pro	Val	Ile	Arg	Arg	Thr	Ile	Asn	Leu	Pro	Thr														
[2674]	260															265					270									
[2675]	Tyr	Thr	Leu	Glu	Ala	Val	Tyr	Glu	Ala	Val	Phe	Gly	Lys	Pro	Lys	Glu														
[2676]	275															280					285									
[2677]	Lys	Val	Tyr	Ala	Glu	Glu	Ile	Ala	Gln	Ala	Trp	Glu	Ser	Gly	Glu	Gly														
[2678]	290															295					300									
[2679]	Leu	Glu	Arg	Val	Ala	Arg	Tyr	Ser	Met	Glu	Asp	Ala	Lys	Val	Thr	Tyr														
[2680]	305															310					315					320				
[2681]	Glu	Leu	Gly	Arg	Glu	Phe	Phe	Pro	Met	Glu	Ala	Gln	Leu	Ser	Arg	Leu														
[2682]	325															330					335									
[2683]	Ile	Gly	Gln	Ser	Leu	Trp	Asp	Val	Ser	Arg	Ser	Ser	Thr	Gly	Asn	Leu														
[2684]	340															345					350									
[2685]	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Lys	Arg	Asn	Glu	Leu	Ala														
[2686]	355															360					365									
[2687]	Pro	Asn	Lys	Pro	Asp	Glu	Arg	Glu	Leu	Ala	Arg	Arg	Arg	Gly	Gly	Tyr														
[2688]	370															375					380									
[2689]	Ala	Gly	Gly	Tyr	Val	Lys	Glu	Pro	Glu	Arg	Gly	Leu	Trp	Asp	Asn	Ile														
[2690]	385															390					395					400				

[2691]	Val	Tyr	Leu	Asp	Phe	Arg	Ser	Ala	Ala	Ile	Ser	Ile	Ile	Ile	Thr	His
[2692]					405					410					415	
[2693]	Asn	Val	Ser	Pro	Asp	Thr	Leu	Asn	Arg	Glu	Gly	Cys	Lys	Glu	Tyr	Asp
[2694]					420					425					430	
[2695]	Val	Ala	Pro	Glu	Val	Gly	His	Lys	Phe	Cys	Lys	Asp	Phe	Pro	Gly	Phe
[2696]					435					440					445	
[2697]	Ile	Pro	Ser	Leu	Leu	Gly	Asp	Leu	Leu	Glu	Glu	Arg	Gln	Lys	Ile	Lys
[2698]					450					455					460	
[2699]	Arg	Lys	Met	Lys	Ala	Thr	Val	Asp	Pro	Leu	Glu	Lys	Lys	Leu	Leu	Asp
[2700]	465									470				475		480
[2701]	Tyr	Arg	Gln	Arg	Val	Ile	Lys	Ile	Leu	Ala	Asn	Ser	Phe	Tyr	Gly	Tyr
[2702]					485					490					495	
[2703]	Gly	Gly	Tyr	Ala	Lys	Ala	Arg	Trp	Tyr	Cys	Lys	Glu	Cys	Ala	Glu	Ser
[2704]					500					505					510	
[2705]	Val	Thr	Ala	Trp	Gly	Arg	Glu	Tyr	Ile	Glu	Met	Val	Ile	Arg	Glu	Leu
[2706]					515					520					525	
[2707]	Glu	Glu	Lys	Phe	Gly	Phe	Lys	Val	Leu	Tyr	Ala	Asp	Thr	Asp	Gly	Leu
[2708]					530					535					540	
[2709]	His	Ala	Thr	Ile	Pro	Gly	Ala	Asp	Ala	Glu	Thr	Val	Lys	Lys	Lys	Ala
[2710]	545					550				555						560
[2711]	Lys	Glu	Phe	Leu	Lys	Tyr	Ile	Asn	Pro	Lys	Leu	Pro	Gly	Leu	Leu	Glu
[2712]					565					570					575	
[2713]	Leu	Glu	Tyr	Glu	Gly	Phe	Tyr	Val	Arg	Gly	Phe	Phe	Val	Thr	Lys	Lys
[2714]					580					585					590	
[2715]	Lys	Tyr	Ala	Val	Ile	Asp	Glu	Glu	Gly	Lys	Ile	Thr	Thr	Arg	Gly	Leu
[2716]					595					600					605	
[2717]	Glu	Ile	Val	Arg	Arg	Asp	Trp	Ser	Glu	Ile	Ala	Lys	Glu	Thr	Gln	Ala
[2718]					610					615					620	
[2719]	Arg	Val	Leu	Glu	Ala	Ile	Leu	Lys	Gly	Gly	Asp	Val	Glu	Glu	Ala	Val
[2720]	625					630				635						640
[2721]	Arg	Ile	Val	Lys	Glu	Val	Thr	Glu	Lys	Leu	Ser	Lys	Tyr	Glu	Val	Pro
[2722]					645					650					655	
[2723]	Pro	Glu	Lys	Leu	Val	Ile	His	Glu	Gln	Ile	Thr	Arg	Asp	Leu	Arg	Asp
[2724]					660					665					670	
[2725]	Tyr	Lys	Ala	Thr	Gly	Pro	His	Val	Ala	Val	Ala	Lys	Arg	Leu	Ala	Ala
[2726]					675					680					685	
[2727]	Arg	Gly	Val	Lys	Ile	Arg	Pro	Gly	Thr	Val	Ile	Ser	Tyr	Ile	Val	Leu
[2728]					690					695					700	
[2729]	Lys	Gly	Ser	Gly	Arg	Ile	Gly	Asp	Arg	Ala	Ile	Pro	Ala	Asp	Glu	Phe

[2730]	705	710	715	720
[2731]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln			
[2732]		725	730	735
[2733]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys			
[2734]		740	745	750
[2735]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp			
[2736]		755	760	765
[2737]	Leu Lys Val Lys Gly Lys Lys			
[2738]		770	775	
[2739]	<210> 27			
[2740]	<211> 775			
[2741]	<212> PRT			
[2742]	<213> 人工			
[2743]	<220>			
[2744]	<223> 工程化聚合酶			
[2745]	<400> 27			
[2746]	Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile			
[2747]	1	5	10	15
[2748]	Arg Val Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg			
[2749]		20	25	30
[2750]	Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile			
[2751]		35	40	45
[2752]	Glu Asp Val Lys Lys Val Thr Ala Lys Arg His Gly Thr Val Val Lys			
[2753]		50	55	60
[2754]	Val Lys Arg Ala Glu Lys Val Gln Lys Lys Phe Leu Gly Arg Pro Ile			
[2755]	65	70	75	80
[2756]	Glu Val Trp Lys Leu Tyr Phe Asn His Pro Gln Asp Val Pro Ala Ile			
[2757]		85	90	95
[2758]	Arg Asp Arg Ile Arg Ala His Pro Ala Val Val Asp Ile Tyr Glu Tyr			
[2759]		100	105	110
[2760]	Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro			
[2761]		115	120	125
[2762]	Ala Glu Gly Asp Glu Glu Leu Thr Met Leu Ala Phe Ala Ile Ala Thr			
[2763]		130	135	140
[2764]	Leu Tyr His Glu Gly Glu Glu Phe Gly Thr Gly Pro Ile Leu Met Ile			
[2765]	145	150	155	160
[2766]	Ser Tyr Ala Asp Gly Ser Glu Ala Arg Val Ile Thr Trp Lys Lys Ile			
[2767]		165	170	175
[2768]	Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Lys Glu Met Ile Lys			

[2769]		180		185		190
[2770]	Arg Phe Leu Arg Val Val Arg Glu Lys Asp Pro Asp Val Leu Ile Thr					
[2771]		195		200		205
[2772]	Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Ser Glu					
[2773]		210		215		220
[2774]	Glu Leu Gly Ile Lys Phe Thr Leu Gly Arg Asp Gly Ser Glu Pro Lys					
[2775]		225		230		235
[2776]	Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile					
[2777]		245		250		255
[2778]	His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr					
[2779]		260		265		270
[2780]	Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu					
[2781]		275		280		285
[2782]	Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly					
[2783]		290		295		300
[2784]	Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr					
[2785]		305		310		315
[2786]	Glu Leu Gly Arg Glu Phe Phe Pro His Glu Ala Gln Leu Ser Arg Leu					
[2787]		325		330		335
[2788]	Ile Gly Gln Ser Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu					
[2789]		340		345		350
[2790]	Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Lys Arg Asn Glu Leu Ala					
[2791]		355		360		365
[2792]	Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr					
[2793]		370		375		380
[2794]	Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile					
[2795]		385		390		395
[2796]	Val Tyr Leu Asp Phe Arg Ser Ala Ala Ile Ser Ile Ile Ile Thr His					
[2797]		405		410		415
[2798]	Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp					
[2799]		420		425		430
[2800]	Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe					
[2801]		435		440		445
[2802]	Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys					
[2803]		450		455		460
[2804]	Arg Lys Met Lys Ala Thr Val Asp Pro Leu Glu Lys Lys Leu Leu Asp					
[2805]		465		470		475
[2806]	Tyr Arg Gln Arg Val Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr					
[2807]		485		490		495

[2808]	Gly Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser
[2809]	500 505 510
[2810]	Val Ala Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu
[2811]	515 520 525
[2812]	Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu
[2813]	530 535 540
[2814]	His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala
[2815]	545 550 555 560
[2816]	Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu
[2817]	565 570 575
[2818]	Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys
[2819]	580 585 590
[2820]	Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu
[2821]	595 600 605
[2822]	Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Arg Glu Thr Gln Ala
[2823]	610 615 620
[2824]	Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val
[2825]	625 630 635 640
[2826]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro
[2827]	645 650 655
[2828]	Pro Glu Lys Leu Asp Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp
[2829]	660 665 670
[2830]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala
[2831]	675 680 685
[2832]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu
[2833]	690 695 700
[2834]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe
[2835]	705 710 715 720
[2836]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln
[2837]	725 730 735
[2838]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys
[2839]	740 745 750
[2840]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp
[2841]	755 760 765
[2842]	Leu Lys Val Lys Gly Lys Lys
[2843]	770 775
[2844]	<210> 28
[2845]	<211> 775
[2846]	<212> PRT

[2847]	<213> 人工															
[2848]	<220>															
[2849]	<223> 工程化聚合酶															
[2850]	<400> 28															
[2851]	Met	Ile	Leu	Asp	Thr	Asp	Tyr	Ile	Thr	Glu	Asn	Gly	Lys	Pro	Val	Ile
[2852]	1				5					10					15	
[2853]	Arg	Val	Phe	Lys	Lys	Glu	Asn	Gly	Glu	Phe	Lys	Ile	Glu	Tyr	Asp	Arg
[2854]					20					25					30	
[2855]	Thr	Phe	Glu	Pro	Tyr	Phe	Tyr	Ala	Leu	Leu	Lys	Asp	Asp	Ser	Ala	Ile
[2856]					35					40					45	
[2857]	Glu	Asp	Val	Lys	Lys	Val	Thr	Ala	Lys	Arg	His	Gly	Thr	Val	Val	Lys
[2858]					50					55					60	
[2859]	Val	Lys	Arg	Ala	Glu	Lys	Val	Gln	Lys	Lys	Phe	Leu	Gly	Arg	Pro	Ile
[2860]	65									70					75	
[2861]	Glu	Val	Trp	Lys	Leu	Tyr	Phe	Asn	His	Pro	Gln	Asp	Val	Pro	Ala	Ile
[2862]										85					90	
[2863]	Arg	Asp	Arg	Ile	Arg	Ala	His	Pro	Ala	Val	Val	Asp	Ile	Tyr	Glu	Tyr
[2864]										100					105	
[2865]	Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro
[2866]										115					120	
[2867]	Ala	Glu	Gly	Asp	Glu	Glu	Leu	Thr	Met	Leu	Ala	Phe	Ala	Ile	Ala	Thr
[2868]										130					135	
[2869]	Leu	Tyr	His	Glu	Gly	Glu	Glu	Phe	Gly	Thr	Gly	Pro	Ile	Leu	Met	Ile
[2870]	145									150					155	
[2871]	Ser	Tyr	Ala	Asp	Gly	Ser	Glu	Ala	Arg	Val	Ile	Thr	Trp	Lys	Lys	Ile
[2872]										165					170	
[2873]	Asp	Leu	Pro	Tyr	Val	Asp	Val	Val	Ser	Thr	Glu	Lys	Glu	Met	Ile	Lys
[2874]										180					185	
[2875]	Arg	Phe	Leu	Arg	Val	Val	Arg	Glu	Lys	Asp	Pro	Asp	Val	Leu	Ile	Thr
[2876]										195					200	
[2877]	Tyr	Asn	Gly	Asp	Asn	Phe	Asp	Phe	Ala	Tyr	Leu	Lys	Lys	Arg	Ser	Glu
[2878]										210					215	
[2879]	Glu	Leu	Gly	Ile	Lys	Phe	Thr	Leu	Gly	Arg	Asp	Gly	Ser	Glu	Pro	Lys
[2880]	225									230					235	
[2881]	Ile	Gln	Arg	Met	Gly	Asp	Tyr	Phe	Ala	Val	Glu	Val	Lys	Gly	Arg	Ile
[2882]										245					250	
[2883]	His	Phe	Asp	Leu	Tyr	Pro	Val	Ile	Arg	Arg	Thr	Ile	Asn	Leu	Pro	Thr
[2884]										260					265	
[2885]	Tyr	Thr	Leu	Glu	Ala	Leu	Tyr	Glu	Ala	Val	Phe	Gly	Lys	Pro	Lys	Glu

[2886]	275	280	285
[2887]	Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly		
[2888]	290	295	300
[2889]	Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr		
[2890]	305	310	315
[2891]	Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu		
[2892]	325	330	335
[2893]	Ile Gly Gln Ser Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu		
[2894]	340	345	350
[2895]	Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Lys Arg Asn Glu Leu Ala		
[2896]	355	360	365
[2897]	Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr		
[2898]	370	375	380
[2899]	Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile		
[2900]	385	390	395
[2901]	Val Tyr Leu Asp Phe Arg Ser Ala Ala Ile Ser Ile Ile Ile Thr His		
[2902]	405	410	415
[2903]	Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp		
[2904]	420	425	430
[2905]	Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe		
[2906]	435	440	445
[2907]	Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys		
[2908]	450	455	460
[2909]	Arg Lys Met Lys Ala Thr Ser Asp Pro Leu Glu Lys Lys Leu Leu Asp		
[2910]	465	470	475
[2911]	Tyr Arg Gln Arg Val Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr		
[2912]	485	490	495
[2913]	Gly Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser		
[2914]	500	505	510
[2915]	Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu		
[2916]	515	520	525
[2917]	Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu		
[2918]	530	535	540
[2919]	His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala		
[2920]	545	550	555
[2921]	Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu		
[2922]	565	570	575
[2923]	Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys		
[2924]	580	585	590

[2925]	Lys Tyr Ala Val Ile Asp Asp Glu Gly Lys Ile Thr Thr Arg Gly Leu
[2926]	595 600 605
[2927]	Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala
[2928]	610 615 620
[2929]	Arg Val Leu Glu Ala Ile Leu Lys Gly Gly Asp Val Glu Glu Ala Val
[2930]	625 630 635 640
[2931]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro
[2932]	645 650 655
[2933]	Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp
[2934]	660 665 670
[2935]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala
[2936]	675 680 685
[2937]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu
[2938]	690 695 700
[2939]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe
[2940]	705 710 715 720
[2941]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln
[2942]	725 730 735
[2943]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys
[2944]	740 745 750
[2945]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp
[2946]	755 760 765
[2947]	Leu Lys Val Lys Gly Lys Lys
[2948]	770 775
[2949]	<210> 29
[2950]	<211> 775
[2951]	<212> PRT
[2952]	<213> 人工
[2953]	<220>
[2954]	<223> 工程化聚合酶
[2955]	<400> 29
[2956]	Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile
[2957]	1 5 10 15
[2958]	Arg Val Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg
[2959]	20 25 30
[2960]	Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile
[2961]	35 40 45
[2962]	Glu Asp Val Lys Lys Val Thr Ala Lys Arg His Gly Thr Val Val Lys
[2963]	50 55 60

[2964]	Val	Lys	Arg	Ala	Glu	Lys	Val	Gln	Lys	Lys	Phe	Leu	Gly	Arg	Pro	Ile
[2965]	65					70					75					80
[2966]	Glu	Val	Trp	Lys	Leu	Tyr	Phe	Asn	His	Pro	Gln	Asp	Val	Pro	Ala	Ile
[2967]					85					90					95	
[2968]	Arg	Asp	Arg	Ile	Arg	Ala	His	Pro	Ala	Val	Val	Asp	Ile	Tyr	Glu	Tyr
[2969]				100					105					110		
[2970]	Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro
[2971]			115					120					125			
[2972]	Ala	Glu	Gly	Asp	Glu	Glu	Leu	Thr	Met	Leu	Ala	Phe	Ala	Ile	Ala	Thr
[2973]		130					135					140				
[2974]	Leu	Tyr	His	Glu	Gly	Glu	Glu	Gly	Gly	Thr	Gly	Pro	Ile	Leu	Met	Ile
[2975]	145					150					155					160
[2976]	Ser	Tyr	Ala	Asp	Gly	Ser	Glu	Ala	Arg	Val	Ile	Thr	Trp	Lys	Lys	Ile
[2977]				165					170					175		
[2978]	Asp	Leu	Pro	Tyr	Val	Asp	Val	Val	Ser	Thr	Glu	Lys	Glu	Met	Ile	Lys
[2979]			180						185					190		
[2980]	Arg	Phe	Leu	Arg	Val	Val	Arg	Glu	Lys	Asp	Pro	Asp	Val	Leu	Ile	Thr
[2981]		195						200					205			
[2982]	Tyr	Asn	Gly	Asp	Asn	Phe	Asp	Phe	Ala	Tyr	Leu	Lys	Lys	Arg	Ser	Glu
[2983]		210						215					220			
[2984]	Glu	Leu	Gly	Ile	Lys	Phe	Thr	Leu	Gly	Arg	Asp	Gly	Ser	Glu	Pro	Lys
[2985]	225					230					235					240
[2986]	Ile	Gln	Arg	Met	Gly	Asp	Tyr	Phe	Ala	Val	Glu	Val	Lys	Gly	Arg	Ile
[2987]				245					250					255		
[2988]	His	Phe	Asp	Leu	Tyr	Pro	Val	Ile	Arg	Arg	Thr	Ile	Asn	Leu	Pro	Thr
[2989]			260						265					270		
[2990]	Tyr	Thr	Leu	Glu	Ala	Leu	Tyr	Glu	Ala	Val	Phe	Gly	Lys	Pro	Lys	Glu
[2991]		275						280					285			
[2992]	Lys	Val	Tyr	Ala	Glu	Glu	Ile	Ala	Gln	Ala	Trp	Glu	Ser	Gly	Glu	Gly
[2993]		290						295				300				
[2994]	Leu	Glu	Arg	Val	Ala	Arg	Tyr	Ser	Met	Glu	Asp	Ala	Lys	Val	Thr	Tyr
[2995]	305					310					315					320
[2996]	Glu	Leu	Gly	Arg	Glu	Phe	Phe	Pro	Met	Glu	Ala	Gln	Leu	Ser	Arg	Leu
[2997]				325					330					335		
[2998]	Ile	Gly	Gln	Ser	Leu	Trp	Asp	Val	Ser	Arg	Ser	Ser	Thr	Gly	Asn	Leu
[2999]			340						345					350		
[3000]	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Lys	Arg	Asn	Glu	Leu	Ala
[3001]		355						360					365			
[3002]	Pro	Asn	Lys	Pro	Asp	Glu	Arg	Glu	Leu	Ala	Arg	Arg	Arg	Gly	Gly	Tyr

[3003]	370	375	380
[3004]	Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile		
[3005]	385	390	395 400
[3006]	Val Tyr Leu Asp Phe Arg Ser Ala Ala Ile Ser Ile Ile Ile Thr His		
[3007]	405	410	415
[3008]	Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp		
[3009]	420	425	430
[3010]	Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe		
[3011]	435	440	445
[3012]	Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys		
[3013]	450	455	460
[3014]	Arg Lys Met Lys Ala Thr Ser Asp Pro Leu Glu Lys Lys Leu Leu Asp		
[3015]	465	470	475 480
[3016]	Tyr Arg Gln Arg Val Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr		
[3017]	485	490	495
[3018]	Gly Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser		
[3019]	500	505	510
[3020]	Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu		
[3021]	515	520	525
[3022]	Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu		
[3023]	530	535	540
[3024]	His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala		
[3025]	545	550	555 560
[3026]	Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu		
[3027]	565	570	575
[3028]	Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys		
[3029]	580	585	590
[3030]	Lys Tyr Ala Val Ile Asp Asp Glu Gly Lys Ile Thr Thr Arg Gly Leu		
[3031]	595	600	605
[3032]	Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Arg Glu Thr Gln Ala		
[3033]	610	615	620
[3034]	Arg Val Leu Glu Ala Ile Leu Lys Gly Gly Asp Val Glu Glu Ala Val		
[3035]	625	630	635 640
[3036]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro		
[3037]	645	650	655
[3038]	Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp		
[3039]	660	665	670
[3040]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala		
[3041]	675	680	685

[3042]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu
[3043]	690 695 700
[3044]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe
[3045]	705 710 715 720
[3046]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln
[3047]	725 730 735
[3048]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys
[3049]	740 745 750
[3050]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp
[3051]	755 760 765
[3052]	Leu Lys Val Lys Gly Lys Lys
[3053]	770 775
[3054]	<210> 30
[3055]	<211> 775
[3056]	<212> PRT
[3057]	<213> 人工
[3058]	<220>
[3059]	<223> 工程化聚合酶
[3060]	<400> 30
[3061]	Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile
[3062]	1 5 10 15
[3063]	Arg Val Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg
[3064]	20 25 30
[3065]	Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile
[3066]	35 40 45
[3067]	Glu Asp Val Lys Lys Val Thr Ala Lys Arg His Gly Thr Val Val Lys
[3068]	50 55 60
[3069]	Val Lys Arg Ala Glu Lys Val Gln Lys Lys Phe Leu Gly Arg Pro Ile
[3070]	65 70 75 80
[3071]	Glu Val Trp Lys Leu Tyr Phe Asn His Pro Gln Asp Val Pro Ala Ile
[3072]	85 90 95
[3073]	Arg Asp Arg Ile Arg Ala His Pro Ala Val Val Asp Ile Tyr Glu Tyr
[3074]	100 105 110
[3075]	Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
[3076]	115 120 125
[3077]	Ala Glu Gly Asp Glu Glu Leu Thr Met Leu Ala Phe Ala Ile Ala Thr
[3078]	130 135 140
[3079]	Leu Tyr His Glu Gly Glu Glu Phe Gly Thr Gly Pro Ile Leu Met Ile
[3080]	145 150 155 160

[3081]	Ser	Tyr	Ala	Asp	Gly	Ser	Glu	Ala	Arg	Val	Ile	Thr	Trp	Lys	Lys	Ile
[3082]					165					170					175	
[3083]	Asp	Leu	Pro	Tyr	Val	Asp	Val	Val	Ser	Thr	Glu	Lys	Glu	Met	Ile	Lys
[3084]					180					185					190	
[3085]	Arg	Phe	Leu	Arg	Val	Val	Arg	Glu	Lys	Asp	Pro	Asp	Val	Leu	Ile	Thr
[3086]					195					200					205	
[3087]	Tyr	Asn	Gly	Asp	Asn	Phe	Asp	Phe	Ala	Tyr	Leu	Lys	Lys	Arg	Ser	Glu
[3088]			210						215					220		
[3089]	Glu	Leu	Gly	Ile	Lys	Phe	Thr	Leu	Gly	Arg	Asp	Gly	Ser	Glu	Pro	Lys
[3090]	225						230					235				240
[3091]	Ile	Gln	Arg	Met	Gly	Asp	Tyr	Phe	Ala	Val	Glu	Val	Lys	Gly	Arg	Ile
[3092]					245						250					255
[3093]	His	Phe	Asp	Leu	Tyr	Pro	Val	Ile	Arg	Arg	Thr	Ile	Asn	Leu	Pro	Thr
[3094]					260						265					270
[3095]	Tyr	Thr	Leu	Glu	Ala	Val	Tyr	Glu	Ala	Val	Phe	Gly	Lys	Pro	Lys	Glu
[3096]					275						280					285
[3097]	Lys	Val	Tyr	Ala	Glu	Glu	Ile	Ala	Gln	Ala	Trp	Glu	Ser	Gly	Glu	Gly
[3098]					290										300	
[3099]	Leu	Glu	Arg	Val	Ala	Arg	Tyr	Ser	Met	Glu	Asp	Ala	Lys	Val	Thr	Tyr
[3100]	305						310					315				320
[3101]	Glu	Leu	Gly	Arg	Glu	Phe	Phe	Pro	Met	Glu	Ala	Gln	Leu	Ser	Arg	Leu
[3102]					325							330				335
[3103]	Ile	Gly	Gln	Ser	Leu	Trp	Asp	Val	Ser	Arg	Ser	Ser	Thr	Gly	Asn	Leu
[3104]					340							345				350
[3105]	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Lys	Arg	Asn	Glu	Leu	Ala
[3106]					355							360				365
[3107]	Pro	Asn	Lys	Pro	Asp	Glu	Arg	Glu	Leu	Ala	Arg	Arg	Arg	Gly	Gly	Tyr
[3108]					370							375				380
[3109]	Ala	Gly	Gly	Tyr	Val	Lys	Glu	Pro	Glu	Arg	Gly	Leu	Trp	Asp	Asn	Ile
[3110]	385						390					395				400
[3111]	Val	Tyr	Leu	Asp	Phe	Arg	Ser	Ala	Ala	Ile	Ser	Ile	Ile	Ile	Thr	His
[3112]					405							410				415
[3113]	Asn	Val	Ser	Pro	Asp	Thr	Leu	Asn	Arg	Glu	Gly	Cys	Lys	Glu	Tyr	Asp
[3114]					420							425				430
[3115]	Val	Ala	Pro	Glu	Val	Gly	His	Lys	Phe	Cys	Lys	Asp	Phe	Pro	Gly	Phe
[3116]					435							440				445
[3117]	Ile	Pro	Ser	Leu	Leu	Gly	Asp	Leu	Leu	Glu	Glu	Arg	Gln	Lys	Ile	Lys
[3118]					450							455				460
[3119]	Arg	Lys	Met	Lys	Ala	Thr	Ser	Asp	Pro	Leu	Glu	Lys	Lys	Leu	Leu	Asp

[3120]	465	470	475	480
[3121]	Tyr Arg Gln Arg Val Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr			
[3122]	485	490	495	
[3123]	Gly Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser			
[3124]	500	505	510	
[3125]	Val Ala Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu			
[3126]	515	520	525	
[3127]	Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu			
[3128]	530	535	540	
[3129]	His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala			
[3130]	545	550	555	560
[3131]	Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu			
[3132]	565	570	575	
[3133]	Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys			
[3134]	580	585	590	
[3135]	Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu			
[3136]	595	600	605	
[3137]	Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Arg Glu Thr Gln Ala			
[3138]	610	615	620	
[3139]	Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val			
[3140]	625	630	635	640
[3141]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro			
[3142]	645	650	655	
[3143]	Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp			
[3144]	660	665	670	
[3145]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala			
[3146]	675	680	685	
[3147]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu			
[3148]	690	695	700	
[3149]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe			
[3150]	705	710	715	720
[3151]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln			
[3152]	725	730	735	
[3153]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys			
[3154]	740	745	750	
[3155]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp			
[3156]	755	760	765	
[3157]	Leu Lys Val Lys Gly Lys Lys			
[3158]	770	775		

[3198]	His	Phe	Asp	Leu	Tyr	Pro	Val	Ile	Arg	Arg	Thr	Ile	Asn	Leu	Pro	Thr
[3199]				260					265					270		
[3200]	Tyr	Thr	Leu	Glu	Ala	Val	Tyr	Glu	Ala	Val	Phe	Gly	Lys	Pro	Lys	Glu
[3201]				275					280					285		
[3202]	Lys	Val	Tyr	Ala	Glu	Glu	Ile	Ala	Gln	Ala	Trp	Glu	Ser	Gly	Glu	Gly
[3203]				290					295					300		
[3204]	Leu	Glu	Arg	Val	Ala	Arg	Tyr	Ser	Met	Glu	Asp	Ala	Lys	Val	Thr	Tyr
[3205]	305						310				315					320
[3206]	Glu	Leu	Gly	Arg	Glu	Phe	Phe	Pro	His	Glu	Ala	Gln	Leu	Ser	Arg	Leu
[3207]					325					330					335	
[3208]	Ile	Gly	Gln	Ser	Leu	Trp	Asp	Val	Ser	Arg	Ser	Ser	Thr	Gly	Asn	Leu
[3209]				340						345				350		
[3210]	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Lys	Arg	Asn	Glu	Leu	Ala
[3211]				355					360					365		
[3212]	Pro	Asn	Lys	Pro	Asp	Glu	Arg	Glu	Leu	Ala	Arg	Arg	Arg	Gly	Gly	Tyr
[3213]				370					375					380		
[3214]	Ala	Gly	Gly	Tyr	Val	Lys	Glu	Pro	Glu	Arg	Gly	Leu	Trp	Asp	Asn	Ile
[3215]	385					390					395					400
[3216]	Val	Tyr	Leu	Asp	Phe	Arg	Ser	Ala	Ala	Ile	Ser	Ile	Ile	Ile	Thr	His
[3217]					405					410					415	
[3218]	Asn	Val	Ser	Pro	Asp	Thr	Leu	Asn	Arg	Glu	Gly	Cys	Lys	Glu	Tyr	Asp
[3219]				420						425				430		
[3220]	Val	Ala	Pro	Glu	Val	Gly	His	Lys	Phe	Cys	Lys	Asp	Phe	Pro	Gly	Phe
[3221]				435					440					445		
[3222]	Ile	Pro	Ser	Leu	Leu	Gly	Asp	Leu	Leu	Glu	Glu	Arg	Gln	Lys	Ile	Lys
[3223]				450					455					460		
[3224]	Arg	Lys	Met	Lys	Ala	Thr	Ser	Asp	Pro	Leu	Glu	Lys	Lys	Leu	Leu	Asp
[3225]	465					470					475					480
[3226]	Tyr	Arg	Gln	Arg	Val	Ile	Lys	Ile	Leu	Ala	Asn	Ser	Phe	Tyr	Gly	Tyr
[3227]					485					490					495	
[3228]	Gly	Gly	Tyr	Ala	Lys	Ala	Arg	Trp	Tyr	Cys	Lys	Glu	Cys	Ala	Glu	Ser
[3229]				500					505					510		
[3230]	Val	Ala	Ala	Trp	Gly	Arg	Glu	Tyr	Ile	Glu	Met	Val	Ile	Arg	Glu	Leu
[3231]				515					520					525		
[3232]	Glu	Glu	Lys	Phe	Gly	Phe	Lys	Val	Leu	Tyr	Ala	Asp	Thr	Asp	Gly	Leu
[3233]				530					535					540		
[3234]	His	Ala	Thr	Ile	Pro	Gly	Ala	Asp	Ala	Glu	Thr	Val	Lys	Lys	Lys	Ala
[3235]	545					550					555					560
[3236]	Lys	Glu	Phe	Leu	Lys	Tyr	Ile	Asn	Pro	Lys	Leu	Pro	Gly	Leu	Leu	Glu

[3237]		565		570		575
[3238]	Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys					
[3239]		580		585		590
[3240]	Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu					
[3241]		595		600		605
[3242]	Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala					
[3243]		610		615		620
[3244]	Arg Val Leu Glu Ala Ile Leu Lys Gly Gly Asp Val Glu Glu Ala Val					
[3245]	625		630		635	640
[3246]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro					
[3247]		645		650		655
[3248]	Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp					
[3249]		660		665		670
[3250]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala					
[3251]		675		680		685
[3252]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu					
[3253]		690		695		700
[3254]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe					
[3255]	705		710		715	720
[3256]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln					
[3257]		725		730		735
[3258]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys					
[3259]		740		745		750
[3260]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp					
[3261]		755		760		765
[3262]	Leu Lys Val Lys Gly Lys Lys					
[3263]		770		775		
[3264]	<210> 32					
[3265]	<211> 775					
[3266]	<212> PRT					
[3267]	<213> 人工					
[3268]	<220>					
[3269]	<223> 工程化聚合酶					
[3270]	<400> 32					
[3271]	Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile					
[3272]	1	5		10		15
[3273]	Arg Val Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg					
[3274]		20		25		30
[3275]	Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile					

[3276]	35	40	45
[3277]	Glu Asp Val Lys Lys Val Thr Ala Lys Arg His Gly Thr Val Val Lys		
[3278]	50	55	60
[3279]	Val Lys Arg Ala Glu Lys Val Gln Lys Lys Phe Leu Gly Arg Pro Ile		
[3280]	65	70	75
[3281]	Glu Val Trp Lys Leu Tyr Phe Asn His Pro Gln Asp Val Pro Ala Ile		
[3282]	85	90	95
[3283]	Arg Asp Arg Ile Arg Ala His Pro Ala Val Val Asp Ile Tyr Glu Tyr		
[3284]	100	105	110
[3285]	Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro		
[3286]	115	120	125
[3287]	Ala Glu Gly Asp Glu Glu Leu Thr Met Leu Ala Phe Ala Ile Ala Thr		
[3288]	130	135	140
[3289]	Leu Tyr His Glu Gly Glu Glu Phe Gly Thr Gly Pro Ile Leu Met Ile		
[3290]	145	150	155
[3291]	Ser Tyr Ala Asp Gly Ser Glu Ala Arg Val Ile Thr Trp Lys Lys Ile		
[3292]	165	170	175
[3293]	Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Lys Glu Met Ile Lys		
[3294]	180	185	190
[3295]	Arg Phe Leu Arg Val Val Arg Glu Lys Asp Pro Asp Val Leu Ile Thr		
[3296]	195	200	205
[3297]	Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Ser Glu		
[3298]	210	215	220
[3299]	Glu Leu Gly Ile Lys Phe Thr Leu Gly Arg Asp Gly Ser Glu Pro Lys		
[3300]	225	230	235
[3301]	Ile Gln Arg Met Gly Asp Tyr Phe Ala Val Glu Val Lys Gly Arg Ile		
[3302]	245	250	255
[3303]	His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr		
[3304]	260	265	270
[3305]	Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu		
[3306]	275	280	285
[3307]	Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly		
[3308]	290	295	300
[3309]	Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr		
[3310]	305	310	315
[3311]	Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu		
[3312]	325	330	335
[3313]	Ile Gly Gln Ser Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu		
[3314]	340	345	350

[3315]	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Lys	Arg	Asn	Glu	Leu	Ala
[3316]			355					360					365			
[3317]	Pro	Asn	Lys	Pro	Asp	Glu	Arg	Glu	Leu	Ala	Arg	Arg	Arg	Gly	Gly	Tyr
[3318]			370					375					380			
[3319]	Ala	Gly	Gly	Tyr	Val	Lys	Glu	Pro	Glu	Arg	Gly	Leu	Trp	Asp	Asn	Ile
[3320]	385						390					395				400
[3321]	Val	Tyr	Leu	Asp	Phe	Arg	Ser	Ala	Ala	Ile	Ser	Ile	Ile	Ile	Thr	His
[3322]					405					410						415
[3323]	Asn	Val	Ser	Pro	Asp	Thr	Leu	Asn	Arg	Glu	Gly	Cys	Lys	Glu	Tyr	Asp
[3324]				420					425						430	
[3325]	Val	Ala	Pro	Glu	Val	Gly	His	Lys	Phe	Cys	Lys	Asp	Phe	Pro	Gly	Phe
[3326]			435					440					445			
[3327]	Ile	Pro	Ser	Leu	Leu	Gly	Asp	Leu	Leu	Glu	Glu	Arg	Gln	Lys	Ile	Lys
[3328]		450					455					460				
[3329]	Arg	Lys	Met	Lys	Ala	Thr	Ser	Asp	Pro	Leu	Glu	Lys	Lys	Leu	Leu	Asp
[3330]	465					470					475					480
[3331]	Tyr	Arg	Gln	Arg	Val	Ile	Lys	Ile	Leu	Ala	Asn	Ser	Phe	Tyr	Gly	Tyr
[3332]				485						490						495
[3333]	Gly	Gly	Tyr	Ala	Lys	Ala	Arg	Trp	Tyr	Cys	Lys	Glu	Cys	Ala	Glu	Ser
[3334]				500					505					510		
[3335]	Val	Ala	Ala	Trp	Gly	Arg	Glu	Tyr	Ile	Glu	Met	Val	Ile	Arg	Glu	Leu
[3336]			515					520					525			
[3337]	Glu	Glu	Lys	Phe	Gly	Phe	Lys	Val	Leu	Tyr	Ala	Asp	Thr	Asp	Gly	Leu
[3338]		530					535					540				
[3339]	His	Ala	Thr	Ile	Pro	Gly	Ala	Asp	Ala	Glu	Thr	Val	Lys	Lys	Lys	Ala
[3340]	545					550					555					560
[3341]	Lys	Glu	Phe	Leu	Lys	Tyr	Ile	Asn	Pro	Lys	Leu	Pro	Gly	Leu	Leu	Glu
[3342]				565					570					575		
[3343]	Leu	Glu	Tyr	Glu	Gly	Phe	Tyr	Val	Arg	Gly	Phe	Phe	Val	Thr	Lys	Lys
[3344]				580					585					590		
[3345]	Lys	Tyr	Ala	Val	Ile	Asp	Asp	Glu	Gly	Lys	Ile	Thr	Thr	Arg	Gly	Leu
[3346]			595					600					605			
[3347]	Glu	Ile	Val	Arg	Arg	Asp	Trp	Ser	Glu	Ile	Ala	Arg	Glu	Thr	Gln	Ala
[3348]		610					615					620				
[3349]	Arg	Val	Leu	Glu	Ala	Ile	Leu	Lys	His	Gly	Asp	Val	Glu	Glu	Ala	Val
[3350]	625					630					635					640
[3351]	Arg	Ile	Val	Lys	Glu	Val	Thr	Glu	Lys	Leu	Ser	Lys	Tyr	Glu	Val	Pro
[3352]				645					650					655		
[3353]	Pro	Glu	Lys	Leu	Val	Ile	His	Glu	Gln	Ile	Thr	Arg	Asp	Leu	Arg	Asp

[3354]	660	665	670
[3355]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala		
[3356]	675	680	685
[3357]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu		
[3358]	690	695	700
[3359]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe		
[3360]	705	710	715
[3361]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln		
[3362]	725	730	735
[3363]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys		
[3364]	740	745	750
[3365]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp		
[3366]	755	760	765
[3367]	Leu Lys Val Lys Gly Lys Lys		
[3368]	770	775	
[3369]	<210> 33		
[3370]	<211> 775		
[3371]	<212> PRT		
[3372]	<213> 人工		
[3373]	<220>		
[3374]	<223> 工程化聚合酶		
[3375]	<400> 33		
[3376]	Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile		
[3377]	1	5	10
[3378]	Arg Val Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg		15
[3379]	20	25	30
[3380]	Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile		
[3381]	35	40	45
[3382]	Glu Asp Val Lys Lys Val Thr Ala Lys Arg His Gly Thr Val Val Lys		
[3383]	50	55	60
[3384]	Val Lys Arg Ala Glu Lys Val Gln Lys Lys Phe Leu Gly Arg Pro Ile		
[3385]	65	70	75
[3386]	Glu Val Trp Lys Leu Tyr Phe Asn His Pro Gln Asp Val Pro Ala Ile		
[3387]	85	90	95
[3388]	Arg Asp Arg Ile Arg Ala His Pro Ala Val Val Asp Ile Tyr Glu Tyr		
[3389]	100	105	110
[3390]	Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro		
[3391]	115	120	125
[3392]	Ala Glu Gly Asp Glu Glu Leu Thr Met Leu Ala Phe Ala Ile Ala Thr		

[3393]	130	135	140
[3394]	Leu Tyr His Glu Gly Glu Glu Phe Gly Thr Gly Pro Ile Leu Met Ile		
[3395]	145	150	155
[3396]	Ser Tyr Ala Asp Gly Ser Glu Ala Arg Val Ile Thr Trp Lys Lys Ile		
[3397]	165	170	175
[3398]	Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Lys Glu Met Ile Lys		
[3399]	180	185	190
[3400]	Arg Phe Leu Arg Val Val Arg Glu Lys Asp Pro Asp Val Leu Ile Thr		
[3401]	195	200	205
[3402]	Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Ser Glu		
[3403]	210	215	220
[3404]	Glu Leu Gly Ile Lys Phe Thr Leu Gly Arg Asp Gly Ser Glu Pro Lys		
[3405]	225	230	235
[3406]	Ile Gln Arg Met Gly Asp Tyr Phe Ala Val Glu Val Lys Gly Arg Ile		
[3407]	245	250	255
[3408]	His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr		
[3409]	260	265	270
[3410]	Tyr Thr Leu Glu Ala Leu Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu		
[3411]	275	280	285
[3412]	Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly		
[3413]	290	295	300
[3414]	Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr		
[3415]	305	310	315
[3416]	Glu Leu Gly Arg Glu Phe Phe Pro His Glu Ala Gln Leu Ser Arg Leu		
[3417]	325	330	335
[3418]	Ile Gly Gln Ser Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu		
[3419]	340	345	350
[3420]	Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Lys Arg Asn Glu Leu Ala		
[3421]	355	360	365
[3422]	Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr		
[3423]	370	375	380
[3424]	Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile		
[3425]	385	390	395
[3426]	Val Tyr Leu Asp Phe Arg Ser Ala Ala Ile Ser Ile Ile Ile Thr His		
[3427]	405	410	415
[3428]	Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp		
[3429]	420	425	430
[3430]	Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe		
[3431]	435	440	445

[3432]	Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys
[3433]	450 455 460
[3434]	Arg Lys Met Lys Ala Thr Ser Asp Pro Leu Glu Lys Lys Leu Leu Asp
[3435]	465 470 475 480
[3436]	Tyr Arg Gln Arg Val Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr
[3437]	485 490 495
[3438]	Gly Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser
[3439]	500 505 510
[3440]	Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu
[3441]	515 520 525
[3442]	Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu
[3443]	530 535 540
[3444]	His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala
[3445]	545 550 555 560
[3446]	Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu
[3447]	565 570 575
[3448]	Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys
[3449]	580 585 590
[3450]	Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu
[3451]	595 600 605
[3452]	Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala
[3453]	610 615 620
[3454]	Arg Val Leu Glu Ala Ile Leu Lys Gly Gly Asp Val Glu Glu Ala Val
[3455]	625 630 635 640
[3456]	Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro
[3457]	645 650 655
[3458]	Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp
[3459]	660 665 670
[3460]	Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala
[3461]	675 680 685
[3462]	Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu
[3463]	690 695 700
[3464]	Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe
[3465]	705 710 715 720
[3466]	Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln
[3467]	725 730 735
[3468]	Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys
[3469]	740 745 750
[3470]	Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp

[3471]	755	760	765
[3472]	Leu Lys Val Lys Gly Lys Lys		
[3473]	770	775	
[3474]	<210> 34		
[3475]	<211> 775		
[3476]	<212> PRT		
[3477]	<213> 人工		
[3478]	<220>		
[3479]	<223> 工程化聚合酶		
[3480]	<400> 34		
[3481]	Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile		
[3482]	1 5 10 15		
[3483]	Arg Val Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg		
[3484]	20 25 30		
[3485]	Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile		
[3486]	35 40 45		
[3487]	Glu Asp Val Lys Lys Val Thr Ala Lys Arg His Gly Thr Val Val Lys		
[3488]	50 55 60		
[3489]	Val Lys Arg Ala Glu Lys Val Gln Lys Lys Phe Leu Gly Arg Pro Ile		
[3490]	65 70 75 80		
[3491]	Glu Val Trp Lys Leu Tyr Phe Asn His Pro Gln Asp Val Pro Ala Ile		
[3492]	85 90 95		
[3493]	Arg Asp Arg Ile Arg Ala His Pro Ala Val Val Asp Ile Tyr Glu Tyr		
[3494]	100 105 110		
[3495]	Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro		
[3496]	115 120 125		
[3497]	Ala Glu Gly Asp Glu Glu Leu Thr Met Leu Ala Phe Ala Ile Ala Thr		
[3498]	130 135 140		
[3499]	Leu Tyr His Glu Gly Glu Glu Phe Gly Thr Gly Pro Ile Leu Met Ile		
[3500]	145 150 155 160		
[3501]	Ser Tyr Ala Asp Gly Ser Glu Ala Arg Val Ile Thr Trp Lys Lys Ile		
[3502]	165 170 175		
[3503]	Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Lys Glu Met Ile Lys		
[3504]	180 185 190		
[3505]	Arg Phe Leu Arg Val Val Arg Glu Lys Asp Pro Asp Val Leu Ile Thr		
[3506]	195 200 205		
[3507]	Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Ser Glu		
[3508]	210 215 220		
[3509]	Glu Leu Gly Ile Lys Phe Thr Leu Gly Arg Asp Gly Ser Glu Pro Lys		

[3510]	225	230	235	240
[3511]	Ile Gln Arg Met Gly Asp Tyr Phe Ala Val Glu Val Lys Gly Arg Ile			
[3512]		245	250	255
[3513]	His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr			
[3514]		260	265	270
[3515]	Tyr Thr Leu Glu Ala Leu Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu			
[3516]		275	280	285
[3517]	Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly			
[3518]		290	295	300
[3519]	Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr			
[3520]	305	310	315	320
[3521]	Glu Leu Gly Arg Glu Phe Phe Pro His Glu Ala Gln Leu Ser Arg Leu			
[3522]		325	330	335
[3523]	Ile Gly Gln Ser Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu			
[3524]		340	345	350
[3525]	Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Lys Arg Asn Glu Leu Ala			
[3526]		355	360	365
[3527]	Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr			
[3528]		370	375	380
[3529]	Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile			
[3530]	385	390	395	400
[3531]	Val Tyr Leu Asp Phe Arg Ser Ala Ala Ile Ser Ile Ile Ile Thr His			
[3532]		405	410	415
[3533]	Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp			
[3534]		420	425	430
[3535]	Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe			
[3536]		435	440	445
[3537]	Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys			
[3538]		450	455	460
[3539]	Arg Lys Met Lys Ala Thr Ser Asp Pro Leu Glu Lys Lys Leu Leu Asp			
[3540]	465	470	475	480
[3541]	Tyr Arg Gln Arg Val Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr			
[3542]		485	490	495
[3543]	Gly Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser			
[3544]		500	505	510
[3545]	Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu			
[3546]		515	520	525
[3547]	Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu			
[3548]		530	535	540

[3549]	His	Ala	Thr	Ile	Pro	Gly	Ala	Asp	Ala	Glu	Thr	Val	Lys	Lys	Lys	Ala
[3550]	545					550				555						560
[3551]	Lys	Glu	Phe	Leu	Lys	Tyr	Ile	Asn	Pro	Lys	Leu	Pro	Gly	Leu	Leu	Glu
[3552]					565					570						575
[3553]	Leu	Glu	Tyr	Glu	Gly	Phe	Tyr	Val	Arg	Gly	Phe	Phe	Val	Thr	Lys	Lys
[3554]				580					585					590		
[3555]	Lys	Tyr	Ala	Val	Ile	Asp	Asp	Glu	Gly	Lys	Ile	Thr	Thr	Arg	Gly	Leu
[3556]			595					600					605			
[3557]	Glu	Ile	Val	Arg	Arg	Asp	Trp	Ser	Glu	Ile	Ala	Arg	Glu	Thr	Gln	Ala
[3558]		610					615					620				
[3559]	Arg	Val	Leu	Glu	Ala	Ile	Leu	Lys	Gly	Gly	Asp	Val	Glu	Glu	Ala	Val
[3560]	625					630					635					640
[3561]	Arg	Ile	Val	Lys	Glu	Val	Thr	Glu	Lys	Leu	Ser	Lys	Tyr	Glu	Val	Pro
[3562]				645						650						655
[3563]	Pro	Glu	Lys	Leu	Val	Ile	His	Glu	Gln	Ile	Thr	Arg	Asp	Leu	Arg	Asp
[3564]				660						665						670
[3565]	Tyr	Lys	Ala	Thr	Gly	Pro	His	Val	Ala	Val	Ala	Lys	Arg	Leu	Ala	Ala
[3566]			675							680				685		
[3567]	Arg	Gly	Val	Lys	Ile	Arg	Pro	Gly	Thr	Val	Ile	Ser	Tyr	Ile	Val	Leu
[3568]		690					695					700				
[3569]	Lys	Gly	Ser	Gly	Arg	Ile	Gly	Asp	Arg	Ala	Ile	Pro	Ala	Asp	Glu	Phe
[3570]	705					710					715					720
[3571]	Asp	Pro	Thr	Lys	His	Arg	Tyr	Asp	Ala	Glu	Tyr	Tyr	Ile	Glu	Asn	Gln
[3572]				725						730					735	
[3573]	Val	Leu	Pro	Ala	Val	Glu	Arg	Ile	Leu	Lys	Ala	Phe	Gly	Tyr	Arg	Lys
[3574]				740						745				750		
[3575]	Glu	Asp	Leu	Arg	Tyr	Gln	Lys	Thr	Lys	Gln	Val	Gly	Leu	Gly	Ala	Trp
[3576]			755						760				765			
[3577]	Leu	Lys	Val	Lys	Gly	Lys	Lys									
[3578]		770														775

SEQ	ID NO:1	MILDTDYITENGKPVIRVFKKENGEFKEYDRTFEPYFYALLKKDDSAIEDVKKVTAKRHG	60
SEQ	ID NO:2	MILDTDYITKDGGKIIRIFKKENGEFKIELDPHFQPIYIALLKKDDSAIEEIKAIKERHG	60
SEQ	ID NO:7	MILDADYITEDGKPVIRVFKKEKGFEKINYDRDFEPIYIALLKKDDSAIEDIKKITAERHG	60
SEQ	ID NO:5	MILDTDYITEDGKPVIRIFKKENGEFKIEYDRTFEPYFYALLKKDDSAIEEVKKITAERHG	60
SEQ	ID NO:4	MILDVDYITEEGKPVIRLFKKENKGFKIEHRTFRPYIYALLRDDRDSKIEEVKKITGERHG	60
SEQ	ID NO:3	MILDADYITEDGKPIIRIFKKENGEFKVEYDRNFRPYIYALLKKDDSQIDEVRKITAERHG	60
SEQ	ID NO:6	MIIDADYITEDGKPIIRIFKKKEGFEKYDRTFRPYIYALLKKDDSAIDEVKKITAERHG	60
		:*.*.*::****:**::**::*.:*****:*.*.*.*.*.*.*.*.*..*.*.	
SEQ	ID NO:1	TVVVKVRAEKVQKKFLGRPIEVWKLIFYFNHPQDVPAIRDRIRAHPAVVDIYEYDIPFAKRY	120
SEQ	ID NO:2	KTVRVLDADVRRKKFLGREVEVWKLI FEHPQDV PAMRGKIREHPAVVDIYEYDIPFAKRY	120
SEQ	ID NO:7	TTVRVTTRAEVVKKKFLGRPVEVWKLYFTTHPQDVPAIRDKIREHPAVVDIYEYDIPFAKRY	120
SEQ	ID NO:5	TVVTVKRVEKVQKKFLGRPVEVWKLYFTTHPQDVPAIRDKIREHPAVIDIYEYDIPFAKRY	120
SEQ	ID NO:4	KIVRIDVVEKVEKKFLGKPITVWVKLYLEHPQDVPTIREKVREHPAVVDIFEYDIPFAKRY	120
SEQ	ID NO:3	KIVRIIDAEKVRRKKFLGRPIEVWRPLYFEHPQDVPAIRDKIREHSAAVIDIFEYDIPFAKRY	120
SEQ	ID NO:6	KIVRITEVEKVQKKFLGRPIEVWVKLYLEHPQDVPAIRDKIREHPAVVDIFEYDIPFAKRY	120
		. *: . :*.*****: ; **:* : *****;*: * :* * **;*.*.*.*.*.*.*.*.	
SEQ	ID NO:1	LIDKGLIPMEGDDEELTMLAFDIETLYHEGEFFGTGPILMISYADGSEARVITWKKIDL PY	180
SEQ	ID NO:2	LIDKGLIPMEGDDEELKLLAFIAATFYHEGDEFKGGEIMISYADEEEEARVITWKNIDL PY	180
SEQ	ID NO:7	LIDKGLIPMEGNHEELRLAFDIETLYHEGEFFGEGPILMISYADEEGARVITWKNIDL PY	180
SEQ	ID NO:5	LIDKGLVPMEGDDEELKMLAFIAATLYHEGEFFAEAGPILMISYADEEGARVITWKNVDL PY	180
SEQ	ID NO:4	LIDKGLIPMEGEELKILAADIETLYHEGEFFKGKGPIMISYADENEAKVITWKNIDL PY	180
SEQ	ID NO:3	LIDKGLIPMEGDDEELKLLAFIAATLYHEGEFFAKGPIMISYADEEEAKVITWKKIDL PY	180
SEQ	ID NO:6	LIDKGLTPMEGNEELTFLAVAIAATLYHEGEFFKGKGPIMISYADEEGAKVITWKSIDL PY	180
		***** *****:***: **. *. *.:*****;.**. * :*****. *.:*****:*****.	

图 1

[illegible]

图1续

```

SEQ ID NO:1 RKAYKRNELAPNKPDERELARR-RGGYAGGYVKEPERGLWDNIIVLDFRSLYPSIIITHN 417
SEQ ID NO:2 RVAYARNELAPNKPDEEYKRRRLRTTYLGYYVKEPEKGLWENIIVLDFRSLYPSIIIVTHN 420
SEQ ID NO:7 RKAYERNELAPNKPDERELARR-AESYAGGYVKEPEKGLWENIIVLDYKSLYPSIIITHN 417
SEQ ID NO:5 RKAYERNELAPNKPDEKELARR-RQSYEGGYVKEPERGLWENIIVLDFRSLYPSIIITHN 417
SEQ ID NO:4 RKAYERNEVAPNKPSEEEYQRRLRESYTGGFVKEPEKGLWENIIVLDFRALYPSIIITHN 418
SEQ ID NO:3 RKAYERNELAPNKPDEREYERRLRRESYAGGYVKEPEKGLWGLVSLDFRSLYPSIIITHN 418
SEQ ID NO:6 RKAYERNELAPNKPDEREYERRLRRESYEGGYVKEPEKGLWEGIVSLDFRSLYPSIIITHN 418
      * ** ***:*****.* ** * ***:*****;****;.: **::*****:***
SEQ ID NO:1 VSPDTLNREGCKEYDVAPEVGHKFKCFKDFPGFIPSLGLDLLEERQKIKRKMKA TVDPLEKK 477
SEQ ID NO:2 VSPDTLEKEGCKNYDVAPIGVYRFCKDFPGFIPSILGDLIAMRQDIKKMKSTIDPIEKK 480
SEQ ID NO:7 VSPDTLNREGCREYDVAPQVGHFRFCDFPGFIPSLGLDLLEERQKVKKMKA TVDPIERK 477
SEQ ID NO:5 VSPDTLNREGCKEYDVAPQVGHFRFCDFPGFIPSLGLDLLEERQKIKRKMKA TIDPIERK 477
SEQ ID NO:4 VSPDTLNLEGCKNYDIAPQVGHKFKCDIPGFIPSLGLHLLEERQKIKTKMKETQDPIEKI 478
SEQ ID NO:3 VSPDTLNREGCREYDVAPEVGHKFKCFKDFPGFIPSLLRLLDERQEIKRMMKASKDPIEKK 478
SEQ ID NO:6 VSPDTLNRENCKEYDVAPQVGHFRFCDFKDFPGFIPSLNLGNLLEERQKIKRMMKESKD PVEKK 478
      *****;*.**:***:**:* ***:*****;* *: **::* **: ** : **::**
SEQ ID NO:1 LLDYRQRAIKILANSFYGGYGAKARWYCKECAESVTAWGREYIEMVIRELEEKF GKVL 537
SEQ ID NO:2 MLDYRQRAIKILLANSYGYGMGP K ARWYSKECAESVTAWGRHYIEMTIRIEEEKFGFVKVL 540
SEQ ID NO:7 LLDYRQRAIKILANSYGGYG YANARWYCRECAESVTAWGRQYIETTMR IEEEKFGFVKVL 537
SEQ ID NO:5 LLDYRQRAIKILANSYGGYG YARARWYCKECAESVTAWGREYITMTIKEIEEKYGFVKVI 537
SEQ ID NO:4 LLDYRQRAIKILLANSFYGGYG AKARWYCKECAESVTAWGRKYIELVWKELEEEKFGFVKVL 538
SEQ ID NO:3 MLDYRQRAIKILANSYGGYG AKARWYCKECAESVTAWGREYIEFVRKELEEEKFGFVKVL 538
SEQ ID NO:6 LLDYRQRAIKILANSYGGYG AKARWYCKECAESVTAWGRQYIDLVRRELES-RGFVKVL 537
      :*****:*****:*** ** .*****.:*****:*** . **::*****:

```

图1续

SEQ	ID NO:1	YADTDGLHATIPGADAETVKKKKAKEFLKYINPKLPGLLELEYEGFYVRGFFVTWKKKYAVI	597
SEQ	ID NO:2	YADTDGFYATIPGEKPELIKKAKEFLNYINSKLPGLLLELEYEGFYLGRGFFVTWKRYAVI	600
SEQ	ID NO:7	YADTDGFFATIPGADAETVKKKTKEFLNYINPRLPGLLELEYEGFYRRGFFVTWKKKYAVI	597
SEQ	ID NO:5	YSDTDGFFATIPGADAETVKKKAMEFLKYINAKLPGALELEYEGFYKRGGFFVTWKKKYAVI	597
SEQ	ID NO:4	YIDTDGLYATIPGGESEEEKKALEFVKYINSKLPGLLLELEYEGFYKRGGFFVTWKRYAVI	598
SEQ	ID NO:3	YIDTDGLYATIPGAKPEEIKKALEFVDYINAKLPGLLELEYEGFYVRGFFVTWKKKYALI	598
SEQ	ID NO:6	YIDTDGLYATIPGAKHEEIKEALKFVEYINSKLPGLLLELEYEGFYARGFFVTWKKKYALI	597
		* **** . * :*: *:*: ** :*** ***** ***** ***** *	
SEQ	ID NO:1	DEEGKITTRGLEIVRRDWEIAKETQARVLEAILKHGDVEEA VRIVKEVTEKLSKYEVP	657
SEQ	ID NO:2	DEEGRITTRGLEIVRRDWEIAKETQAKVLEAILKEGSVEKAVEVRDVVEKIAKYRVPL	660
SEQ	ID NO:7	DEEDKITTRGLEIVRRDWEIAKETQARVLEAILKHGDVEEA VRIVKEVTEKLSRYEVPP	657
SEQ	ID NO:5	DEEGKITTRGLEIVRRDWEIAKETQARVLEALLKDGDVEKAVRIVKEVTEKLSKYEVP	657
SEQ	ID NO:4	DEEGKVITTRGLEIVRRDWEIAKETQARVLETILKHGDVEEA VRIVKEVIQKLANYEIPP	658
SEQ	ID NO:3	DEEGKIITTRGLEIVRRDWEIAKETQAKVLEAILKHGNVEEA VKIVKEVTEKLSKYEIPP	658
SEQ	ID NO:6	DEEGKIVTRGLEIVRRDWEIAKETQAKVLEAILKHGNVDEA VKIVKEVTEKLSKYEIPP	657
		:: **:*****:****:*. *. *. *. *. *. *. *. *. *. *. *	
SEQ	ID NO:1	EKLVIHEQITRDLRDYKATGPHVA VAKRLAARGVKIRPGTVISYVLKSGRIGDRAIPA	717
SEQ	ID NO:2	EKLVIHEQITRDLKD YKAIGPHVAIAKRLAARGIKVKPGTII SYIVLKGSGKISDRVILL	720
SEQ	ID NO:7	EKLVIYEQITRNLRDY RATGPHVA VAKRLAARGIKIRPGTVISYIVLKGPRVGDR AIPF	717
SEQ	ID NO:5	EKLVIHEQITRDLKD YKATGPHVA VAKRLAARGVKIRPGTVISYIVLKGSGRIGDRAIPF	717
SEQ	ID NO:4	EKLAIYEQITRPLHEYKAIGPHVA VAKKLAAGVKIKPGMVG IYVLRGDGPISNR AILA	718
SEQ	ID NO:3	EKLVIYEQITRPLHEYKAIGPHVA VAKRLAARGVKVRPGMVG IYVLRGDGPISKRAILA	718
SEQ	ID NO:6	EKLVIYEQITRPLSEYKAIGPHVA VAKRLAAGVKVKPGMVG IYVLRGDGPISKRAIAI	717
		.*:** * :*: * *****:***:****:*. *. *. *. *. *. *. *. *. *	

图1续

SEQ ID NO: 1	DEFDPTKHKRYDAEYYIENQVLP	PAVERILKAFGYRKEDLRYQTKQVGLGAWLKVKGKK-	775
SEQ ID NO: 2	TEYDPRKKHKYDPDYIENQVLP	AVLRILEAFGYRKEDLRYQSSKQTGLDAWLKR-----	774
SEQ ID NO: 7	DEFDPAKHRYDAEYYIENQVLP	PAVERILRAFGYRKEDLRYQTKQAGLGAWLKPKTGS-	775
SEQ ID NO: 5	DEFDPTKHKYDAEYYIENQVLP	PAVERILRAFGYRKEDLRYQTKRQVGLSAWLKPKGT--	774
SEQ ID NO: 4	EEYDPKKHKYDAEYYIENQVLP	AVLRILEGFGYRKEDLRYQTKRQVGLTSWLNIKKS--	775
SEQ ID NO: 3	EEFDLRKKHKYDAEYYIENQVLP	AVLRILEAFGYRKEDLRWQTKQTGLTAWLNIKKKS	777
SEQ ID NO: 6	EEFDPKKKHKYDAEYYIENQVLP	PAVERILRAFGYRKEDLKYQTKQVGLGAWLKFGS---	773
	: **:** :***** ***.*****::**.* **:		

图1续

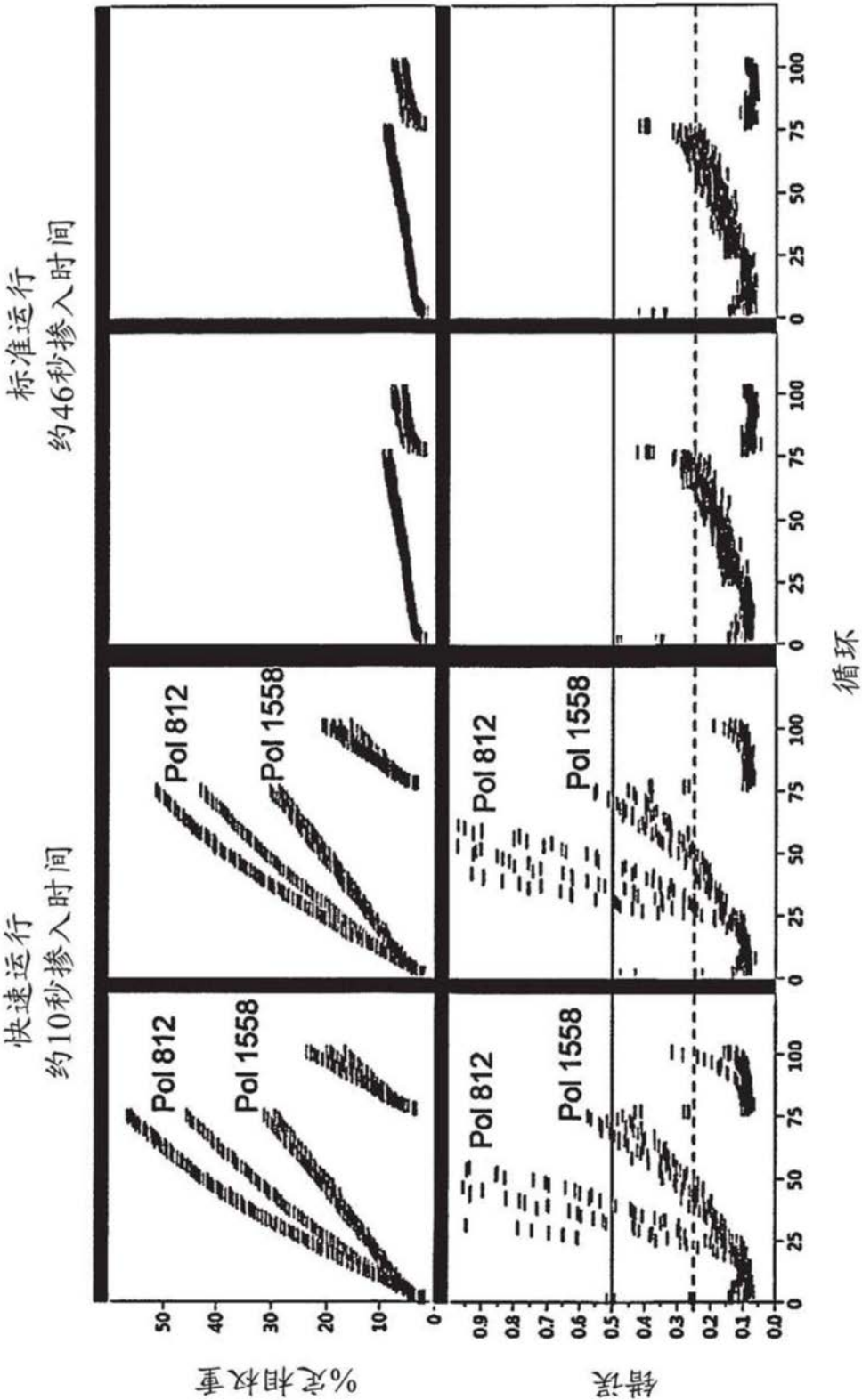


图2

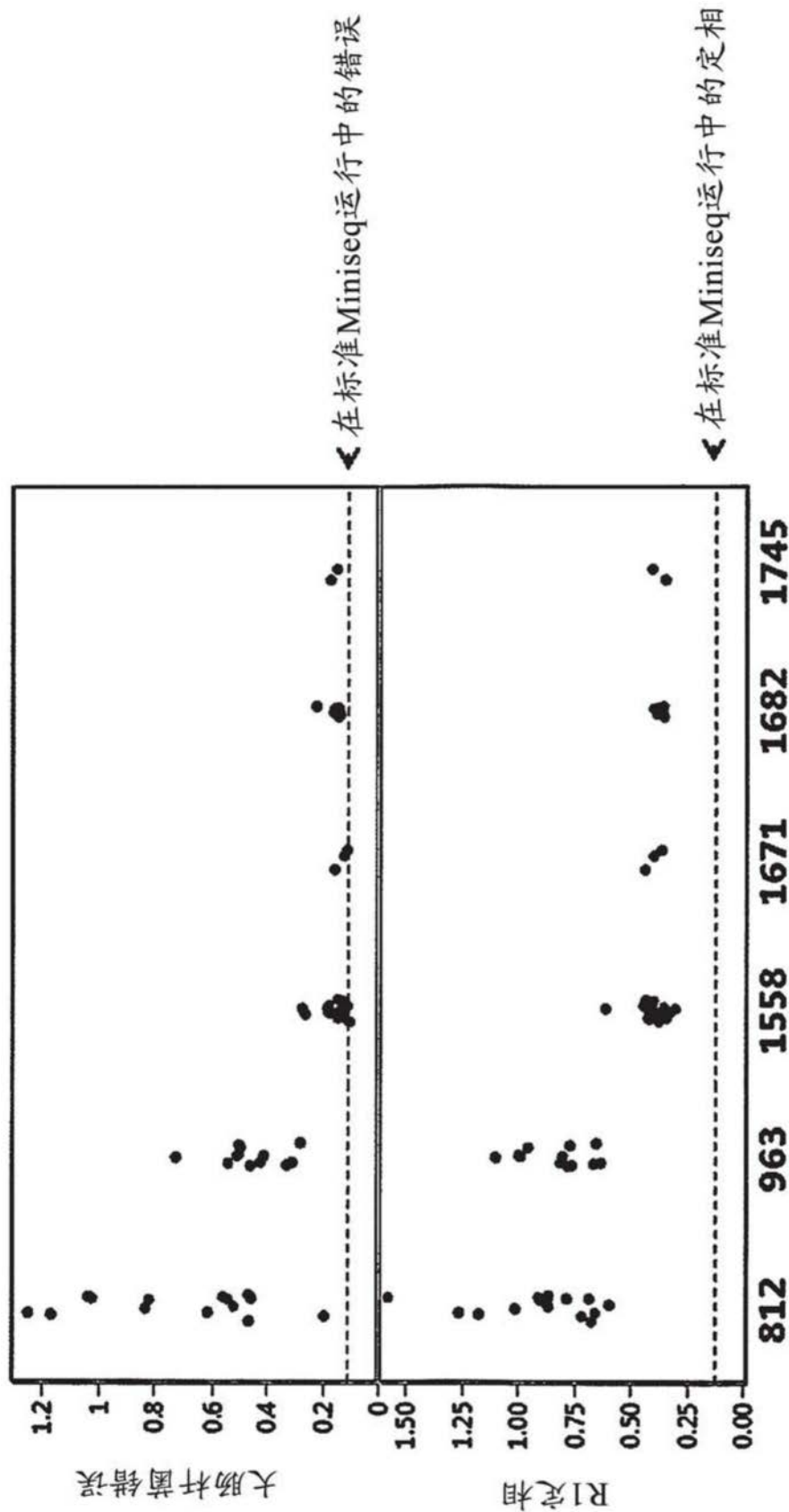


图3A

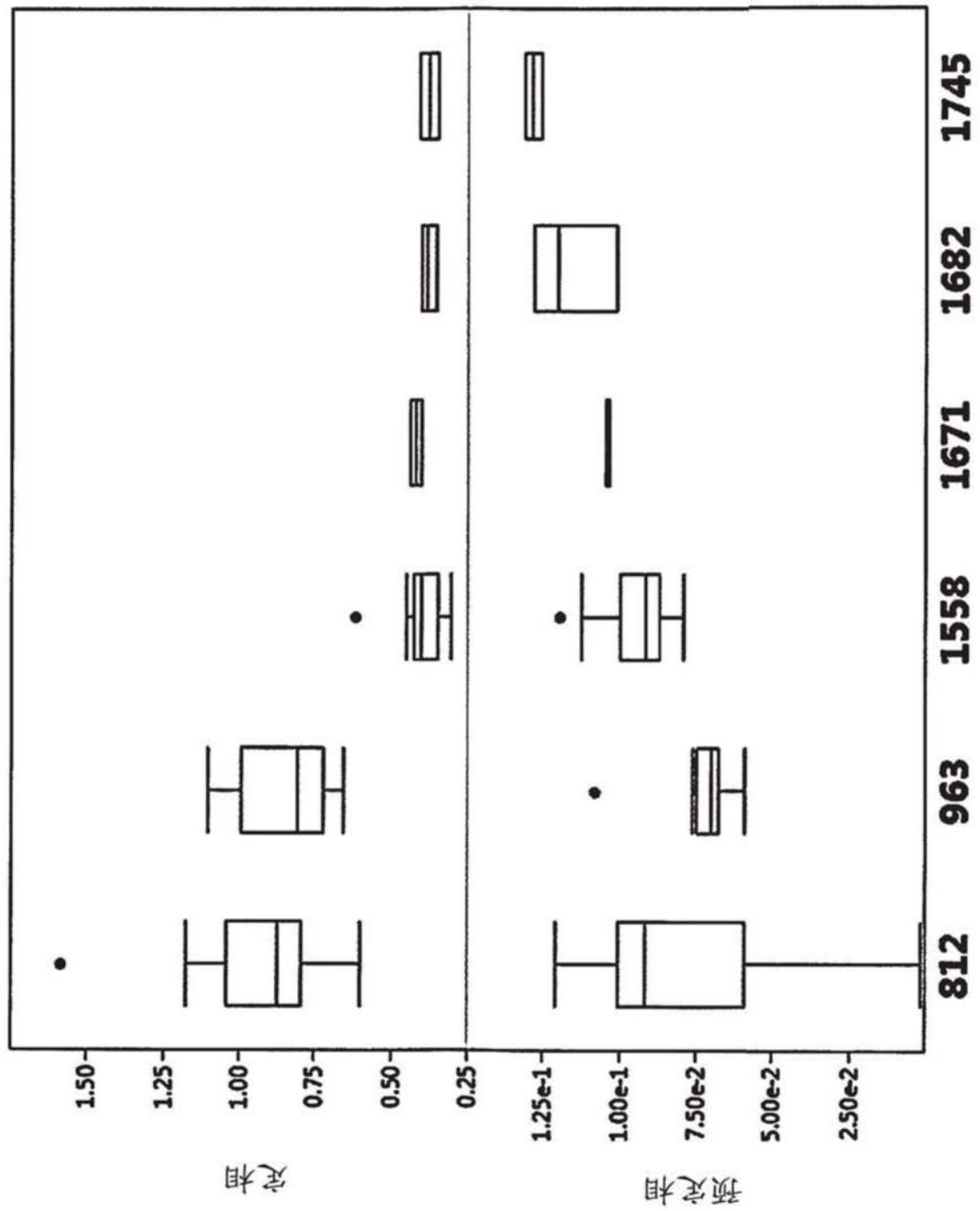


图3B

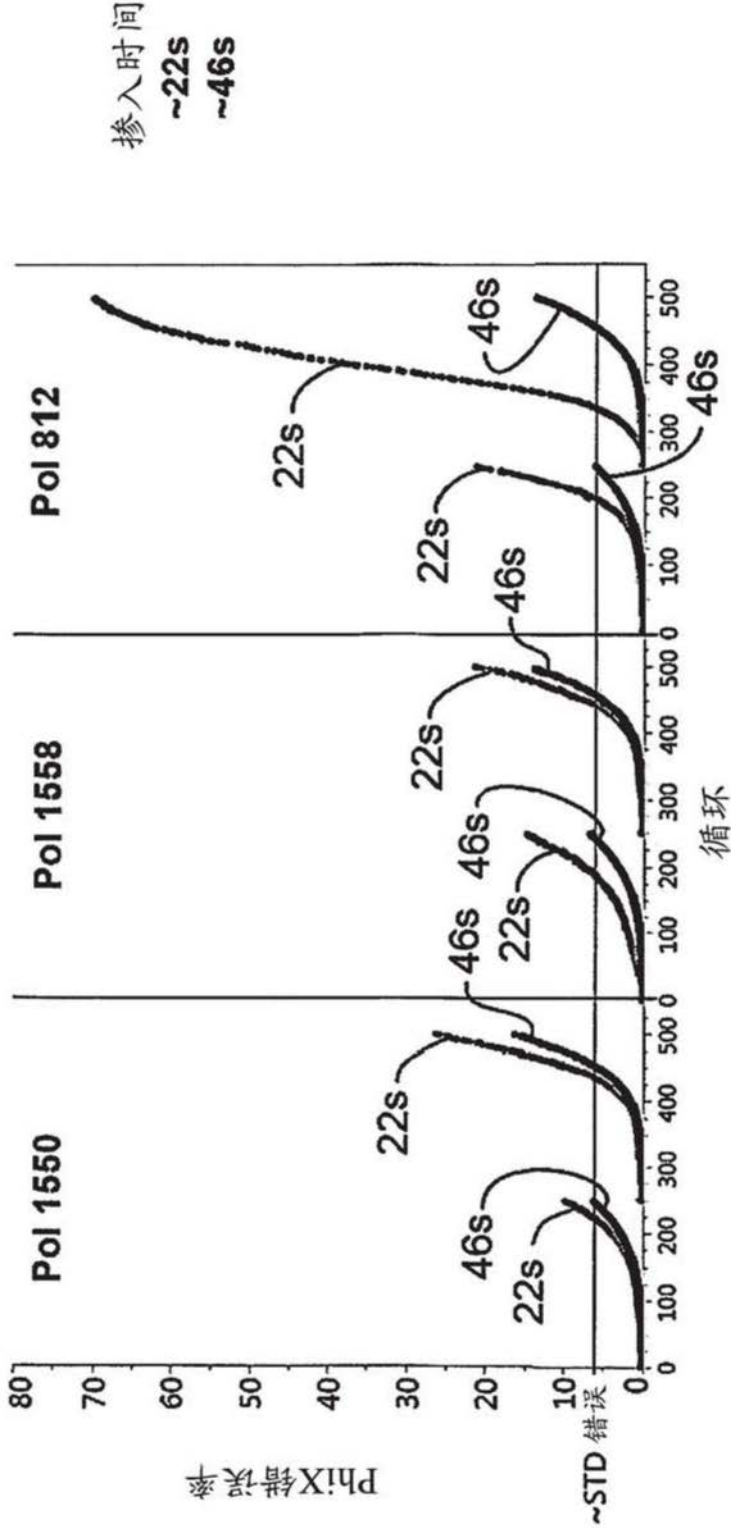


图4

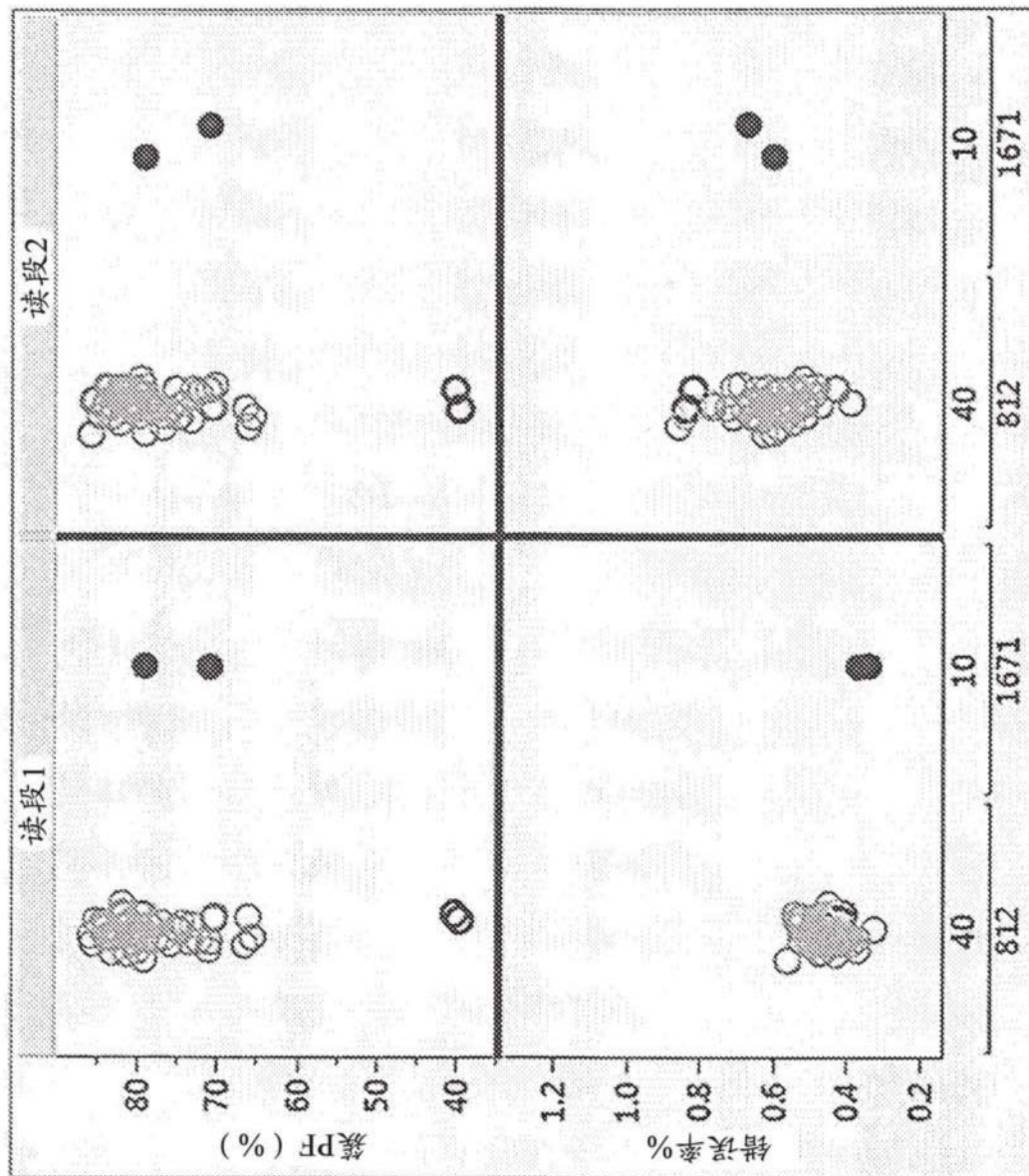


图5A

		S1基线	S1, 快	
			Pol 812	Pol 1671
PhiX ER	R1	0.47	0.49	0.4
PhiX ER	R2	0.6	1.11	0.65
Q30	R1	93.1	92.1	94.4
Q30	R2	89.3	84.7	88.4
定相	R1	0.094	0.189	0.116
定相	R2	0.101	0.231	0.144
掺入时间		40s	22s	

图5B