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(54)发明名称

具有降低的非特异性活性的PCR反应混合物

(57)摘要

本发明提供了用于提高核酸扩增的特异性的方法，所述方法包括：将核酸分子与聚合酶-Sso7DNA结合结构域缀合物和精氨酸、亚精胺或精胺一起温育。本发明也提供了用于提高核酸扩增的特异性的反应混合物和试剂盒。

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1.一种扩增核酸分子的方法,所述方法包括:

(a)将所述核酸提供在足以进行核酸扩增的组合物中,所述组合物包含:

(i)一种或多种寡核苷酸引物;

(ii)聚合酶;和

(iii)足以提高核酸扩增的特异性的量的试剂,其中所述试剂包含游离精氨酸或其盐;和

(b)在足以扩增所述核酸的条件下温育混合物,由此扩增所述核酸,

其中所述试剂以足以使特异性扩增产物相对于非特异性扩增产物的相对产率增加至少10%的量存在,其中所述游离精氨酸或游离精氨酸盐的浓度是1mM-500mM,其中所述聚合酶与DNA结合结构域缀合,其中所述DNA结合结构域是$S_{SO}7$结构域。

2.根据权利要求1所述的方法,其中所述聚合酶没有3′-5′外切核酸酶活性。

3.根据权利要求1所述的方法,其中所述聚合酶由SEQ ID NO:1或SEQ ID NO:2的氨基酸序列表示。

4.根据权利要求1所述的方法,其中所述$S_{SO}7$结构域由SEQ ID NO:3表示,或由其中K28和/或R43位置的氨基酸被置换的SEQ ID NO:3的变体表示,其中K28位置的氨基酸被丝氨酸(S),苏氨酸(T),半胱氨酸(C),脯氨酸(P),天冬氨酸(D),谷氨酸(E),天冬酰胺(N)或谷氨酰胺(Q)置换,或R43位置的氨基酸被丙氨酸(A),半胱氨酸(C),天冬氨酸(D),谷氨酸(E),苯丙氨酸(F),甘氨酸(G),组氨酸(H),异亮氨酸(I),赖氨酸(K),亮氨酸(L),甲硫氨酸(M),天冬酰胺(N),谷氨酰胺(Q),丝氨酸(S),苏氨酸(T),缬氨酸(V),色氨酸(W)或酪氨酸(Y)置换。

5.根据权利要求1所述的方法,其中所述组合物包含缓冲液,当在0.1M浓度测量时,所述缓冲液在20℃和37℃之间时具有每摄氏度不超过0.027pH单位的变化。

6.根据权利要求5所述的方法,其中所述缓冲液选自由以下各项组成的组:HEPES、ACES、PIPES、MOPSO、BES、MOPS、TES、TAPSO、POPSO、BICINE、TAPS和AMPSO。

7.一种反应混合物,其包含:

聚合酶;和

足以提高核酸扩增的特异性的量的试剂,其中所述试剂包含游离精氨酸,或其盐,

其中所述试剂以足以使特异性扩增产物相对于非特异性扩增产物的相对产率增加至少10%的量存在,其中所述游离精氨酸或游离精氨酸盐的浓度是1mM-500mM,其中所述聚合酶与DNA结合结构域缀合,其中所述DNA结合结构域是$S_{SO}7$结构域。

8.根据权利要求7所述的反应混合物,其中所述聚合酶没有3′-5′外切核酸酶活性。

9.根据权利要求7所述的反应混合物,其中所述聚合酶由SEQ ID NO:1或SEQ ID NO:2的氨基酸序列表示。

10.根据权利要求7所述的反应混合物,其中所述$S_{SO}7$结构域由SEQ ID NO:3表示,或由其中K28和/或R43位置的氨基酸被置换的SEQ ID NO:3的变体表示,其中K28位置的氨基酸被丝氨酸(S),苏氨酸(T),半胱氨酸(C),脯氨酸(P),天冬氨酸(D),谷氨酸(E),天冬酰胺(N)或谷氨酰胺(Q)置换,或R43位置的氨基酸被丙氨酸(A),半胱氨酸(C),天冬氨酸(D),谷氨酸(E),苯丙氨酸(F),甘氨酸(G),组氨酸(H),异亮氨酸(I),赖氨酸(K),亮氨酸(L),甲硫氨酸(M),天冬酰胺(N),谷氨酰胺(Q),丝氨酸(S),苏氨酸(T),缬氨酸(V),色氨酸(W)或酪氨酸(Y)置换。

11.根据权利要求7所述的反应混合物，所述反应混合物进一步包含一种或多种寡核苷酸引物。

12.根据权利要求7所述的反应混合物，所述反应混合物进一步包含选自由以下各项组成的组的至少一个成员：缓冲液、核苷酸三磷酸、盐、稳定剂、双链DNA结合染料和无核酸酶的水。

13.根据权利要求7所述的反应混合物，其中所述反应混合物包含缓冲液，当在0.1M浓度测量时，所述缓冲液在20℃和37℃之间时具有每摄氏度不超过0.027pH单位的变化。

14.根据权利要求13所述的反应混合物，其中所述缓冲液选自由以下各项组成的组：HEPES、ACES、PIPES、MOPSO、BES、MOPS、TES、TAPSO、POPSO、BICINE、TAPS和AMPSO。

15.一种试剂盒，其包含：

聚合酶；和

足以提高核酸扩增的特异性的量的试剂，其中所述试剂包含游离精氨酸，或其盐，

其中所述试剂以足以使特异性扩增产物相对于非特异性扩增产物的相对产率增加至少10％的量存在，其中所述游离精氨酸或游离精氨酸盐的浓度是1mM-500mM，其中所述聚合酶与DNA结合结构域缀合，其中所述DNA结合结构域是$S_{SO}7$结构域。

16.根据权利要求15所述的试剂盒，其中所述聚合酶没有3′-5′外切核酸酶活性。

17.根据权利要求15所述的试剂盒，其中所述聚合酶由SEQ ID NO:1或SEQ ID NO:2的氨基酸序列表示。

18.根据权利要求15所述的试剂盒，其中所述$S_{SO}7$结构域由SEQ ID NO:3表示，或由其中K28和/或R43位置的氨基酸被置换的SEQ ID NO:3的变体表示，其中K28位置的氨基酸被丝氨酸(S)，苏氨酸(T)，半胱氨酸(C)，脯氨酸(P)，天冬氨酸(D)，谷氨酸(E)，天冬酰胺(N)或谷氨酰胺(Q)置换，或R43位置的氨基酸被丙氨酸(A)，半胱氨酸(C)，天冬氨酸(D)，谷氨酸(E)，苯丙氨酸(F)，甘氨酸(G)，组氨酸(H)，异亮氨酸(I)，赖氨酸(K)，亮氨酸(L)，甲硫氨酸(M)，天冬酰胺(N)，谷氨酰胺(Q)，丝氨酸(S)，苏氨酸(T)，缬氨酸(V)，色氨酸(W)或酪氨酸(Y)置换。

19.根据权利要求15所述的试剂盒，所述试剂盒进一步包含一种或多种寡核苷酸引物。

20.根据权利要求15所述的试剂盒，所述试剂盒进一步包含选自由以下各项组成的组的至少一个成员：缓冲液、核苷酸三磷酸、盐、稳定剂、双链DNA结合染料和无核酸酶的水。

21.根据权利要求15所述的试剂盒，其中所述聚合酶和所述试剂处于包含缓冲液的溶液中，其中当在0.1M浓度测量时，所述缓冲液在20℃和37℃之间时具有每摄氏度不超过0.027pH单位的变化。

22.根据权利要求21所述的试剂盒，其中所述缓冲液选自由以下各项组成的组：HEPES、ACES、PIPES、MOPSO、BES、MOPS、TES、TAPSO、POPSO、BICINE、TAPS和AMPSO。

具有降低的非特异性活性的PCR反应混合物

[0001] 对相关专利申请的交叉引用

[0002] 本申请要求2011年4月8日提交的美国临时专利申请号61/473,710的优先权权益，其通过引用并入。

背景技术

[0003] 核酸扩增反应，诸如聚合酶链反应(PCR)，一般是模板依赖性反应，其中所需的核酸序列通过使用过量的两种寡核苷酸引物处理靶核酸的分离的互补链来扩增。所述引物延伸形成互补引物延伸产物，所述产物起模板作用，以用于合成所需的核酸序列。在这样的过程中，选择性扩增各DNA链上的引物之间的核酸序列。但是，许多因素可以消极地影响核酸扩增反应的特异性。

发明内容

[0004] 本发明提供了扩增核酸分子的方法。在一些实施方案中，所述方法包括：

[0005] (a)将所述核酸提供在足以进行核酸扩增的组合物中，所述组合物包含：

[0006] (i)一种或多种寡核苷酸引物；

[0007] (ii)聚合酶；和

[0008] (iii)足以提高核酸扩增的特异性的量的试剂，其中所述试剂包含游离精氨酸、亚精胺或精胺，或其盐；和

[0009] (b)在足以扩增所述核酸的条件下温育所述混合物，由此扩增所述核酸，如果存在的话。

[0010] 在一些实施方案中，所述聚合酶与DNA结合结构域缀合。在一些实施方案中，所述聚合酶与Sso7结构域缀合。

[0011] 在一些实施方案中，所述试剂以足以使特异性扩增产物相对于非特异 性扩增产物的相对产率增加至少10％的量存在。在一些实施方案中，所述试剂是游离精氨酸或其盐。在一些实施方案中，所述游离精氨酸或游离精氨酸盐的浓度是约1mM至约500mM。

[0012] 在一些实施方案中，所述聚合酶基本上没有3′-5′外切核酸酶活性。在一些实施方案中，所述聚合酶具有SEQ ID NO:1或SEQ ID NO:2的氨基酸序列。在一些实施方案中，所述Sso7结构域与SEQ ID NO:3具有至少75％的氨基酸序列同一性。

[0013] 在一些实施方案中，所述组合物包含缓冲液，所述缓冲液(当在0.1M浓度测量时)当在20℃至37℃之间时具有不超过0.027pH单位/℃的变化。在一些实施方案中，所述缓冲液选自由以下各项组成的组：HEPES、ACES、PIPES、MOPSO、BES、MOPS、TES、TAPSO、POPSO、BICINE、TAPS和AMPSO。

[0014] 在一些实施方案中，所述方法包括：

[0015] (a)给所述核酸提供足以进行核酸扩增的组合物，所述组合物包含：

[0016] (i)一种或多种寡核苷酸引物；和

[0017] (ii)聚合酶，其中所述聚合酶以至少20单位/反应的浓度存在；和

[0018] (b)在足以扩增所述核酸的条件下,在所述组合物中温育所述核酸,由此扩增所述核酸。

[0019] 在一些实施方案中,所述组合物进一步包含游离精氨酸或其盐。在一些实施方案中,所述游离精氨酸或游离精氨酸盐的浓度是约1mM至约500mM。

[0020] 在一些实施方案中,所述聚合酶与DNA结合结构域缀合。在一些实施方案中,所述聚合酶与Sso7结构域缀合。

[0021] 在一些实施方案中,所述聚合酶基本上没有3′-5′外切核酸酶活性。在一些实施方案中,所述聚合酶具有SEQ ID NO:1或SEQ ID NO:2的氨基酸序列。在一些实施方案中,所述Sso7结构域与SEQ ID NO:3具有至少75%的氨基酸序列同一性。

[0022] 在一些实施方案中,所述组合物包含缓冲液,所述缓冲液(当在0.1M浓度测量时)在20℃和37℃之间时具有每摄氏度不超过0.027pH单位的变 化。在一些实施方案中,所述缓冲液选自由以下各项组成的组:HEPES、ACES、PIPES、MOPSO、BES、MOPS、TES、TAPSO、POPSO、BICINE、TAPS和AMPSO。

[0023] 在另一个方面,本发明提供了用于扩增核酸分子的反应混合物。在一些实施方案中,所述反应混合物包含:

[0024] 聚合酶;和

[0025] 足以提高核酸扩增的特异性的量的试剂,其中所述试剂包含游离精氨酸、亚精胺或精胺,或其盐。

[0026] 在另一个方面,本发明提供了用于扩增核酸分子的试剂盒。在一些实施方案中,所述试剂盒包含:

[0027] 聚合酶;和

[0028] 足以提高核酸扩增的特异性的量的试剂,其中所述试剂包含游离精氨酸、亚精胺或精胺,或其盐。

[0029] 在一些实施方案中,所述聚合酶与DNA结合结构域缀合。在一些实施方案中,所述聚合酶与Sso7结构域缀合。

[0030] 在一些实施方案中,所述试剂以足以使特异性扩增产物相对于非特异性扩增产物的相对产率增加至少10%的量存在。在一些实施方案中,所述试剂是游离精氨酸或其盐。在一些实施方案中,所述游离精氨酸或游离精氨酸盐的浓度是约1mM至约500mM。

[0031] 在一些实施方案中,所述聚合酶基本上没有3′-5′外切核酸酶活性。在一些实施方案中,所述聚合酶具有SEQ ID NO:1或SEQ ID NO:2的氨基酸序列。在一些实施方案中,所述Sso7结构域与SEQ ID NO:3具有至少75%的氨基酸序列同一性。

[0032] 在一些实施方案中,所述反应混合物和/或试剂盒进一步包含一种或多种寡核苷酸引物。在一些实施方案中,所述反应混合物和/或试剂盒进一步包含选自由以下各项组成的组的至少一个成员:缓冲液、核苷酸三磷酸、盐、稳定剂、双链DNA结合染料和无核酸酶的水。

[0033] 在一些实施方案中,所述反应混合物包含缓冲液,所述缓冲液(当在0.1M浓度测量时)在20℃和37℃之间时具有每摄氏度不超过0.027pH单位的变化。在一些实施方案中,所述缓冲液选自由以下各项组成的组:HEPES、ACES、PIPES、MOPSO、BES、MOPS、TES、TAPSO、POPSO、BICINE、TAPS和AMPSO。

[0034] 在另一个方面，本发明提供了一种反应混合物，其用于在靶核酸的实时聚合酶链反应(PCR)扩增中实现信号标准化，其中所述混合物适合用于：(a)采用高惰性参比染料浓度进行标准化的实时PCR扩增系统，和(b)采用低惰性参比染料浓度进行标准化的实时PCR扩增系统，其中所述混合物包含：多种惰性参比染料，其独立于扩增反应而产生荧光信号；和缓冲液，其中所述缓冲液(当在0.1M浓度测量时)在20℃和37℃之间时具有每摄氏度不超过0.027pH单位的变化。

[0035] 在一些实施方案中，所述缓冲液选自由以下各项组成的组：HEPES、ACES、PIPES、MOPSO、BES、MOPS、TES、TAPSO、POPSO、BICINE、TAPS和AMPSO。

[0036] 在一些实施方案中，所述反应混合物进一步包含足以提高核酸扩增的特异性的量的试剂，其中所述试剂包含游离精氨酸、亚精胺或精胺，或其盐。

[0037] 在一些实施方案中，所述混合物包含：

[0038] 具有斯托克斯位移的第一惰性参比染料，其中所述第一惰性参比染料是处于足以用于低浓度惰性参比染料标准化的浓度，其中所述第一惰性参比染料具有第一惰性参比染料激发波长最大值和第一惰性参比染料发射波长最大值；和

[0039] 具有斯托克斯位移的第二惰性参比染料，其斯托克斯位移大于所述第一惰性参比染料的斯托克斯位移，其中所述第二惰性参比染料的发射波长最大值与第一惰性参比染料发射波长最大值大致相同，且激发波长最大值显著不同于所述第一惰性参比染料激发波长最大值。

[0040] 在一些实施方案中，所述第二惰性参比染料具有至少约60nm的斯托克斯位移。在一些实施方案中，所述第一惰性参比染料包括5-和/或6-羧基-X-罗丹明，或其类似物。在一些实施方案中，所述第二惰性参比染料具有550nm或更小的激发波长最大值。在一些实施方案中，所述5-和/或6-羧基-X-罗丹明染料的浓度小于100nM。

[0041] 在一些实施方案中，所述反应混合物进一步包含以下的一种或多种：寡核苷酸引物、一种或多种脱氧核苷三磷酸；缓冲液、插入染料、逆转录酶和聚合酶。在一些实施方案中，所述反应混合物包含DNA聚合酶。在一些实施方案中，所述聚合酶与抗体复合。在一些实施方案中，所述聚合酶被化学灭活，但是通过加热来活化。

[0042] 在一些实施方案中，所述第二惰性染料是荧光点。

[0043] 在一些实施方案中，所述第二惰性参比染料与结构部分(moiety)缀合。

[0044] 还提供了一种进行实时定量聚合酶链反应的方法。在一些实施方案中，所述方法包括，用如上所述的反应混合物进行聚合酶链反应(PCR)，其中所述混合物进一步包含疑似包含靶核酸的生物样品。

[0045] 还提供了一种制备如上所述的反应混合物的方法，所述方法包括，混合所述多种惰性参比染料，由此产生所述反应混合物。

[0046] 在另一个方面，提供了一种用于进行实时定量聚合酶链反应的试剂盒。在一些实施方案中，所述试剂盒包含：具有斯托克斯位移的第一惰性参比染料，其中所述第一惰性染料具有第一惰性参比染料激发波长最大值和第一惰性参比染料发射波长最大值；具有斯托克斯位移的第二惰性参比染料，其斯托克斯位移大于所述第一惰性参比染料的斯托克斯位移，其中所述第二惰性参比染料的发射波长最大值与第一惰性参比染料发射波长最大值大致相同，且激发波长最大值显著不同于所述第一惰性参比染料激发波长最大值和缓冲液，

其中所述缓冲液(当在0.1M浓度测量时)在20℃和37℃之间时具有每摄氏度不超过0.027pH单位的变化。

[0047] 在一些实施方案中,所述缓冲液选自由以下各项组成的组:HEPES、ACES、PIPES、MOPSO、BES、MOPS、TES、TAPSO、POPSO、BICINE、TAPS和AMPSO。

[0048] 在一些实施方案中,所述试剂盒进一步包含足以提高核酸扩增的特异性的量的试剂,其中所述试剂包含游离精氨酸、亚精胺或精胺,或其盐。

[0049] 在一些实施方案中,所述第二惰性参比染料具有至少约60nm的斯托克斯位移。在一些实施方案中,所述第一惰性参比染料包括5-和/或6-羧基-X-罗丹明,或其类似物。

[0050] 在一些实施方案中,所述第一惰性参比染料包括5-和/或6-羧基-X-罗 丹明染料或其类似物,且所述第二惰性参比染料是具有至少60nm的斯托克斯位移的荧光染料,其中所述第二惰性参比染料具有约620nm的发射波长最大值。

[0051] 在一些实施方案中,所述混合物包含:5-和/或6-羧基-X-罗丹明染料;和具有至少约60nm的斯托克斯位移的第二惰性参比染料,其中所述第二惰性参比染料具有约590nm的发射波长最大值。

[0052] 在一些实施方案中,所述试剂盒进一步包含以下的一种或多种:一种或多种脱氧核苷三磷酸;一种或多种寡核苷酸引物、一种或多种脱氧核苷三磷酸;缓冲液、插入染料、逆转录酶和DNA聚合酶。

[0053] 在一些实施方案中,所述第一惰性参比染料和所述第二惰性参比染料被包含在所述试剂盒内的不同容器中。在一些实施方案中,所述第一惰性参比染料和所述第二惰性参比染料被包含在所述试剂盒内的相同容器中。

[0054] 定义

[0055] 术语“聚合酶”表示进行多核苷酸的模板指导的合成的酶。该术语包括具有聚合酶活性的全长多肽和结构域。DNA聚合酶是本领域技术人员公知的,包括但不限于,由强烈火球菌(Pyrococcus furiosus),Thermococcus litoralis,和海栖热袍菌(Thermotoga maritime),或其修饰形式分离或来源的DNA聚合酶。它们包括DNA-依赖性聚合物和RNA-依赖性聚合酶(诸如逆转录酶)二者。已知至少5个DNA-依赖性DNA聚合酶家族,尽管大部分属于家族A、B和C。不同家族之间存在很少或不存在序列相似性。大多数家族A聚合酶是单链蛋白,其可以含有多酶功能包括聚合酶、3′至5′外切核酸酶活性和5′至3′外切核酸酶活性。家族B聚合酶典型地具有聚合酶的单催化结构域和3′至5′外切核酸酶活性,以及辅助因子。家族C聚合酶典型地是多亚单元蛋白,其具有聚合和3′至5′外切核酸酶活性活性。在大肠杆菌(E.coli)中,已经发现三种类型的DNA聚合酶,即DNA聚合酶I(家族A)、II(家族B)和III(家族C)。在真核细胞中,三种不同的家族B聚合酶,即DNA聚合酶α、δ和ε,参与核复制,且家族A聚合酶,即聚合酶γ,用于线粒体DNA复制。其它类型的DNA聚合酶包括噬菌体聚合酶。类似地,RNA聚合酶典型地包括真核RNA聚合酶I、II和III, 和细菌RNA聚合酶以及噬菌体和病毒聚合酶。RNA聚合酶可以是DNA-依赖性和RNA-依赖性的。在一些实施方案中,本发明的聚合酶与SEQ ID NO:1或SEQ ID NO:2的氨基酸序列相同或基本上相同(例如,具有至少60%、65%、70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%的氨基酸序列同一性)。

[0056] 本文中使用的“热稳定的聚合酶”表示利用DNA或RNA作为模板,通过将核苷酸单元

加入至核苷酸链来催化多核苷酸合成的任何酶，并在高于45℃的温度下具有最佳活性。

[0057] 本文中使用的术语“Sso7-样蛋白”或“Sso7”表示这样的核酸和多肽多形变体、等位基因、突变体、和种间同源物：(1)优选在至少约15、25、35、50或更多个氨基酸的区域内，其氨基酸序列与SEQ ID NO:3的Sso7序列具有大于约60％的氨基酸序列同一性、65％、70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％或99％或更大的氨基酸序列同一性；(2)其结合抗体(例如，多克隆抗体)，所述抗体针对包含SEQ ID NO:3的氨基酸序列及其保守修饰的变体的免疫原而产生；(3)其在严格杂交条件下与Sso7d核酸序列特异性地杂交，所述Sso7d核酸序列编码SEQ ID NO:3的氨基酸及其保守修饰的变体；或(4)优选在至少约50、100、150或更多个核苷酸的区域内，其核酸序列与编码SEQ ID NO:3的核酸序列具有大于约90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或更高的核苷酸序列同一性。该术语包括全长Sso7多肽和所述多肽的具有序列非特异性双链结合活性的片段。Sso7-样蛋白包括Sac7d、Sac7e、Ssh7b和Sto7e。

[0058] “野生型Sso7”表示天然存在的Sso7蛋白。在一些实施方案中，野生型Sso7具有SEQ ID NO:3的氨基酸序列。“野生型Sso7-样”表示天然存在的Sso7-样蛋白，例如，Sac7d、Sac7e、Ssh7b和Sto7e。

[0059] “结构域”表示蛋白或蛋白复合物的单元，其包括多肽序列、完整多肽序列、或许多多肽序列，其中所述单元具有定义的功能。理解所述功能是广泛定义的，且可以是配体结合、催化活性或可以对蛋白的结构具有稳定作用。

[0060] 术语“DNA结合结构域”表示以序列非特异性的方式结合DNA的蛋白 结构域。在一些实施方案中，所述DNA结合结构域是以显著亲和力结合DNA的蛋白结构域，对于该结构域，没有已知核酸与所述蛋白结构域的结合亲和力比具有相同核苷酸组成、但是不同核苷酸序列的另一种核酸高超过100倍。

[0061] 本文中使用的术语“聚合酶-Sso7缀合物”表示经修饰的聚合酶，其包含至少一个与聚合酶结构域或所述聚合酶结构域的催化亚基连接的Sso7DNA结合结构域。聚合酶-Sso7缀合物可以包含多个Sso7DNA结合结构域。

[0062] “连接”表示本领域中关于功能性连接蛋白结构域的任何已知方法，包括但不限于具有或不具有干扰结构域的重组融合、内含肽-介导的融合、非共价连接、和共价键结合、包括二硫键结合；氢键结合；静电结合；和构象结合，例如，抗体-抗原，和生物素-抗生物素蛋白连接。

[0063] 术语“核酸扩增”或“扩增反应”表示用于增加核酸靶序列拷贝的任意体外方法。这样的方法包括但不限于聚合酶链反应(PCR)、DNA连接酶链反应(参见美国专利号4,683,195和4,683,202；PCR Protocols:A Guide to Methods and Applications(PCR流程:方法和应用指导)(Innis等编，1990))，(LCR)、QBeta RNA复制酶、和基于RNA转录的(诸如TAS和3SR)的扩增反应以及本领域技术人员已知的其它方法。

[0064] “扩增”表示使溶液经历足以容许扩增多核苷酸的条件的步骤，条件是反应的全部组分是完整的。扩增反应的组分包括，例如，引物，多核苷酸模板、聚合酶、核苷酸等。术语“扩增”典型地表示靶核酸的“指数”增加。然而，本文中使用的“扩增”还可以表示核酸的选择靶序列数量的线性增加，诸如使用循环测序获得的。

[0065] 术语“扩增反应混合物”或“扩增反应组合物”表示包含用于扩增靶核酸的多种试

剂的水溶液。这些包括酶、水性缓冲液、盐、扩增引物、靶核酸、和核苷三磷酸。如本文中进一步讨论地，扩增反应混合物还可以进一步包括稳定剂和其它用于最优化效率和特异性的添加剂。根据上下文，所述混合物可以是完全的或不完全的扩增反应混合物。

[0066] “聚合酶链反应”或“PCR”表示这样的方法，通过该方法以几何级数扩增靶双链DNA的特定区段或子序列。PCR是本领域技术人员公知的技术；参见，例如，美国专利号4,683,195和4,683,202；和PCR Protocols:A Guide to Methods and Applications(PCR流程：方法和应用指导)，Innis等编，1990。示范性PCR反应条件典型地包括两步骤或三步骤循环。两步骤循环具有变性步骤，随后是杂交/延伸步骤。三步骤循环包括变性步骤，随后是杂交步骤，随后是单独的延伸步骤。

[0067] “寡核苷酸引物”或“引物”表示与靶核酸上的序列杂交并担当核酸合成的起始点的寡核苷酸序列。引物可以具有不同的长度，且通常长度小于50个核苷酸，例如，长度为12-30个核苷酸。用于PCR中的引物的长度和序列可以基于本领域技术人员已知的原则来设计，参见，例如，Innis等，见上文。

[0068] “模板”表示这样的多核苷酸序列，其包括侧连引物杂交位点的待扩增的多核苷酸。因此，“靶模板”包括侧连关于5’引物和3’引物的杂交位点的靶多核苷酸序列。

[0069] 关于核酸扩增使用的术语“特异性”表示，与非特异性扩增产物相比，产生特异性扩增产物的核酸扩增反应的可能性。“特异性扩增产物”表示，通过用正确配对的引物和模板(即，正确靶序列)的扩增产生的多核苷酸。“非特异性扩增产物”表示，在扩增反应过程中产生的除了特异性扩增产物以外的多核苷酸。

[0070] 关于核酸扩增使用的短语“提高的特异性”或“提高特异性”表示，与产生的非特异性扩增产物的量相比，在核酸扩增中产生的特异性扩增产物的量的可检测的增加。扩增反应的特异性可以根据任意方法来测量，包括、但不限于解链曲线分析或凝胶分析。在一些实施方案中，在下述情况下，扩增反应的特异性增加：与不含提高扩增特异性的试剂(例如，精氨酸、亚精胺或精胺，或其盐)的反应混合物中的特异性产物相对于非特异性产物的相对产率相比，包含所述试剂的反应混合物在扩增反应中具有更高的特异性产物相对于非特异性产物的相对产率(即，通过特异性产物的解链峰的高度与非特异性产物的解链峰的高度测得的产率之比)。在一些实施方案中，与其中不包括提高扩增特异性的试剂(例如，精氨酸、亚精胺或精胺，或其盐)的反应相比，提高扩增特异性的试剂(例如，精氨酸、亚精胺或精胺，或其盐)将表现出所述比率的至少10％、15％、20％、25％、30％、40％、 50％、100％、2倍(200％)、2.5倍(250％)、3倍(300％)或更大增加。

[0071] 术语“核酸”和“多核苷酸”在本文中互换地用于表示，处于单链或双链形式的脱氧核糖核苷酸或核糖核苷酸及其聚合物。该术语包括含有已知核苷酸类似物或经修饰的主链残基或键的核酸，其是合成的、天然存在的、和非天然存在的，其具有与参考核酸类似的结合性能，且其以与参考核苷酸类似的方式被代谢。这样的类似物的例子包括、但不限于，硫代磷酸酯、氨基磷酸酯、甲基膦酸酯、手性-甲基膦酸酯、2-O-甲基核糖核苷酸和肽核酸(PNA)。

[0072] 术语“多肽”、“肽”和“蛋白”在本文中互换使用，表示氨基酸残基的聚合物。该术语应用于其中一个或多个氨基酸残基是相应天然存在的氨基酸的人造化学模拟物的氨基酸聚合物，以及天然存在的氨基酸聚合物和非天然存在的氨基酸聚合物。

[0073] 术语“氨基酸”表示天然存在和合成的氨基酸，以及以与天然存在的氨基酸相似的方式起作用的氨基酸类似物和氨基酸模拟物。天然存在的氨基酸是由遗传密码编码的那些，以及稍后修饰的那些氨基酸，例如，羟基脯氨酸，γ-羧基谷氨酸，和O-磷酸丝氨酸。氨基酸类似物表示与天然存在的氨基酸具有相同基础化学结构(即，与氢、羧基、氨基、和R基结合的α碳)的化合物，例如，高丝氨酸、正亮氨酸、甲硫氨酸亚砜、甲硫氨酸甲基锍。这样的类似物具有修饰的R基(例如，正亮氨酸)或修饰肽主链，但是保持与天然存在的氨基酸相同的基础化学结构。氨基酸模拟物表示具有与氨基酸的一般化学结构不同的结构但以与天然存在的氨基酸类似的方式起作用的化学化合物。

[0074] 氨基酸在本文中可以通过其普遍已知的三字母符号或通过由IUPAC-IUB生物化学命名委员会推荐的一字母符号来提及。同样地，核苷酸可以通过其普遍接受的单字母密码来提及。

[0075] “保守修饰变体”应用于氨基酸和核酸序列二者。关于特定的核酸序列，保守修饰变体表示编码相同或基本相同氨基酸序列，或其中核酸不编码氨基酸序列的那些核酸，基本相同序列。由于遗传密码的简并，大量功能相同的核酸编码任何给定蛋白。例如，密码子GCA、GCC、GCG和GCU都编码氨基酸丙氨酸。因此，在通过密码子指定丙氨酸的各个位置处，该密码子可以改变为所述相应密码子中任一个，而不改变编码的多肽。这样的核酸变化是“沉默变化”，其是保守修饰变化的一种。本文中编码多肽的各核酸序列也描述各种可能的核酸的沉默变化。本领域技术人员应该认识到，核酸中的各密码子(除AUG(其通常仅编码甲硫氨酸)和TGG(其通常仅编码色氨酸外)可以修饰为产生功能相同的分子。因此，编码多肽的核酸的各沉默变化是各所述序列中固有的。

[0076] 关于氨基酸序列，本领域技术人员应该认识到，在编码的序列中改变、添加或缺失单氨基酸或小比例氨基酸的各置换、缺失或添加核酸、肽、多肽、或蛋白序列是“保守修饰的变体”，其中变化导致氨基酸被化学类似的氨基酸置换。提供功能相似氨基酸的保守置换表是本领域中公知的。这样的保守修饰变体是本发明多形变体、种间同源物、和等位基因以外的，但不排除这些。

[0077] 术语“启动子”表示位于转录起始上游和/或下游并参与识别和结合RNA聚合酶和其它蛋白以起始转录的区域或序列。

[0078] “载体”是用于将多核苷酸序列引入细胞中的DNA构建体。载体可以包括，例如，转录和翻译终止子，转录和翻译起始序列，和有效用于调节特定核酸表达的启动子。在一些实施方案中，载体是表达载体，其具有与多核苷酸序列可操作地连接的启动子序列，且能够实现由所述多核苷酸序列编码的多肽在合适宿主中的表达。

[0079] “重组体”表示人操作的多核苷酸或人操作的多核苷酸的拷贝或补体。例如，包括与第二多核苷酸可操作连接的启动子的重组表达盒可以包括与所述第二多核苷酸异源的启动子，这是人操作(例如，通过Sambrook等，Molecular Cloning-A Laboratory Manual(分子克隆：实验室手册)，Cold Spring Harbor Laboratory(冷泉港实验室)，Cold Spring Harbor(冷泉港)，N.Y.，(1989)或Current Protocols in Molecular Biology(当前分子生物学方案)第1-3卷，John Wiley&Sons，Inc.(1994-1998)所述的方法)包含表达盒的分离的核酸的结果。作为另一个例子，重组表达盒可以包括以这样的方式组合的多核苷酸，所述方式使得所述多核苷酸极不可能在自然界中找到。例如，人操作的限制位点或质粒载体序列

可以使启动子侧连所述第二多核苷酸或使启动子与所述第二多核苷酸分开。本领域技术人员应该认识 到,多核苷酸可以以许多方式来操作并不限于以上实例。

[0080] 术语“相同”或百分比“同一性”在两个或多个核酸或多肽序列的背景下,表示2个或多个相同的序列或子序列。在下述情况下,序列是彼此“基本上相同的”:当比较和比对以在比较窗或特定区域中获得最大对应性时,在指出的情况下在特定区域内,或者如果没有另外指出则跨整个参考序列,它们具有特定百分比的相同的核苷酸或氨基酸残基(例如,至少60%、65%、70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%同一性),其中使用一种以下序列比较算法或通过手工比对和视觉观察来测量。这些定义也表示测试序列的补体。

[0081] 为了序列比较,典型地,一个序列担当参考序列,测试序列与所述参考序列进行比较。当使用序列比较算法时,将测试序列和参考序列输入计算机,如果需要,指定子序列坐标,并指定序列算法程序参数。通常使用默认的程序参数,或可以指定备选的参数。序列比较算法然后基于程序参数,计算测试序列相对于参考序列的百分比序列同一性或相似性。

[0082] 本文中使用的“比较窗”包括对连续位置区段的提及,例如20-600连续位置,约50至约200,或约100至约150,其中当两序列最佳比对后,序列可以与相同数量的连续位置的参考序列进行比较。比对序列以进行比较的方法是本领域中公知的。用于比较的最佳序列比对可以,例如,通过Smith和Waterman(Adv.Appl.Math.(现代应用数学)2:482,1970)的局部同源比对算法,通过Needleman和Wunsch (J.Mol.Biol.(分子生物学杂志)48:443,1970)的同源比对算法,通过Pearson和Lipman(Proc.Natl.Acad.Sci.USA(美国国家科学院学报)85:2444,1988)的相似方法的搜索,通过计算机化执行这些算法(例如,GAP、BESTFIT、FASTA和TFASTA,在Wisconsin Genetics Software Package(威斯康辛州遗传学软件包)中,Genetics Computer Group,575Science Dr.,Madison,Wis.)或通过人工比对和视觉观察(参见,例如,Ausubel等Current Protocols in Molecular Biology(1995增刊))来进行。

[0083] 适合用于确定百分比序列同一性和序列相似性的算法的例子是BLAST和BLAST2.0算法,其分别记述在Altschul等(Nuc.Acids Res.25:3389-402,1977)和Altschul等(J.Mol.Biol.215:403-10,1990)中。用于进 行BLAST分析的软件可公共地获自国家生物技术信息中心(National Center for Biotechnology Information)(http://www.ncbi.nlm.nih.gov/)。该算法包括首先通过识别查询序列中长度W的短词来识别高评分序列对(HSPs),其在与数据库序列中相同长度的词比对时,匹配或满足一些正值阈值评分。T指邻近词评分阈值(Altschul等,见上文)。这些起始邻近词击中(word hits)担当开始搜索的种子,以发现包含它们的较长的HSPs。词击中以两个方向沿各序列延伸,以直至可以增加累积比对评分。词击中在各方向的延伸中以下时刻停止:累积比对评分通过量X由其最大获得值下降;累积评分变为0或以下,这归因于一个或多个负评分残基比对的累积;或达到各序列的末端。BLAST算法参数W,T,和X确定算法的灵敏性和速度。BLAST程序在默认词长(W)11时,使用BLOSUM62评分矩阵(参见Henikoff&Henikoff,Proc.Natl.Acad.Sci.USA(美国国家科学院学报)89:10915(1989))比对(B)为50,期望值(E)为10,M=5,N=-4,和两条链比较。

[0084] BLAST算法也进行两序列之间相似性的统计学分析(参见,例如,Karlin&Altschul,Proc.Nat′l.Acad.Sci.USA(美国国家科学院学报)90:5873-5787(1993))。由

BLAST算法提供的相似性的一个测量值是最小综合可能性(P(N)),其提供两核苷酸或氨基酸序列之间的匹配偶然发生的可能性指示。例如,核酸被认为与参考序列相似,条件是测试核酸与参考核酸的比较中的最小总和可能性小于约0.2,更优选小于约0.01,且最优选小于约0.001。

[0085] "惰性参比染料"表示,不会与聚合酶链反应的其它组分相互作用的荧光染料或点。例如,所述惰性参比染料不会基于核酸的存在或缺失而显著地改变它的信号,也不会在FRET相互作用中与PCR反应混合物中或实时仪器对其它染料检测中的其它染料显著相互作用。但是,在许多实施方案中,所述惰性参比染料可以不与核酸连接。所述惰性参比染料可以具有斯托克斯位移,即,使得激发波长最大值和发射最大值是不同的。

[0086] 本文中使用的"低"惰性参比染料(例如,5-或6-羧基-X-罗丹明染料诸如ROX™)浓度是,其本身适合用于低ROX浓度扩增(例如,qPCR)系统中的惰性参比染料(例如,5-或6-羧基-X-罗丹明染料或5-或6-羧基-X-罗丹明 染料类似物)的浓度。低5-或6-羧基-X-罗丹明染料浓度仪器("采用低5-或6-羧基-X-罗丹明染料浓度进行标准化的qPCR仪器")包括、但不限于:Applied Biosystems ABI7500或Applied Biosystems ViiA7或Stratagene MX系列实时PCR系统。因而,低惰性参比染料(例如,5-或6-羧基-X-罗丹明染料)浓度通常小于100nM,例如,1-10nM、10-100nM等。

[0087] 本文中使用的"高"惰性参比染料(例如,5-或6-羧基-X-罗丹明染料诸如ROX™)浓度是,适合用于高5-或6-羧基-X-罗丹明染料浓度扩增(例如,qPCR)系统中的惰性参比染料(例如,5-或6-羧基-X-罗丹明染料诸如ROX™或5-或6-羧基-X-罗丹明染料类似物)的浓度。高5-或6-羧基-X-罗丹明染料浓度仪器("采用高5-或6-羧基-X-罗丹明染料浓度进行标准化的qPCR仪器")包括、但不限于:Applied Biosystems ABI PRISM7000、7700或7900或ABI7300实时PCR系统或ABI GeneAmp5700实时PCR系统。因而,高惰性参比染料(例如,5-或6-羧基-X-罗丹明染料)浓度通常超过100nM,例如,100-300nM、300-700nM等。

[0088] "与惰性参比染料发射波长最大值大致相同的发射波长最大值"表示,在惰性参比染料发射波长最大值的30nm(且任选地,20、10、5、3或1nm)内的发射波长最大值。

[0089] 在染料激发波长的背景下,"显著不同"表示这样的波长:其充分不同,使得可以激发一种染料,而不激发具有显著不同的激发波长最大值的第二种染料。在一些实施方案中,"显著不同"是指,2种染料具有相隔至少10、20、30、40、50、60、70nm或更多的激发最大值。

具体实施方式

[0090] I.前言

[0091] 本发明提供了用于提高核酸扩增的特异性和增加扩增反应混合物对核酸扩增抑制剂的耐受性的方法和组合物。发明人已经令人惊讶地发现,将精氨酸单盐酸盐或多胺盐诸如亚精胺三盐酸盐或精胺四盐酸盐加入含有聚合酶的扩增反应中,会减少非特异性的PCR产物的形成和增加扩增试剂对PCR抑制剂的耐受性。

[0092] 因而,在一个方面,本发明提供了扩增核酸分子的方法。在一些实施 方案中,所述方法包括:(a)将所述核酸分子与足以进行核酸扩增的组合物混合,其中所述组合物包含聚合酶(例如,与DNA结合结构域缀合的聚合酶,所述DNA结合结构域包括、但不限于Sso7或Sso7-样蛋白)和足以提高核酸扩增的特异性的量的试剂,其中所述试剂包含精氨酸(即,不

是多肽的一部分的“游离”精氨酸)、亚精胺和精胺,或其盐;和(b)在足以扩增所述核酸分子的条件下,温育所述混合物。在一些实施方案中,所述试剂以足以导致特异性扩增产物相对于非特异性产物的相对产率增加至少10%的量存在。在一些实施方案中,所述聚合酶(例如,聚合酶-DNA结合结构域缀合物,例如,聚合酶-Sso7缀合物)包含缺乏3′-5′外切核酸酶活性的聚合酶结构域和/或具有一个或多个降低非特异性扩增活性的点突变的Sso7结构域。

[0093] 在一些实施方案中,所述方法包括:(a)将所述核酸分子与足以进行核酸扩增的组合物混合,其中所述组合物包含浓度为至少20单位/ml的聚合酶(例如,与DNA结合结构域缀合的聚合酶,所述DNA结合结构域包括、但不限于Sso7或Sso7-样蛋白);和(b)在足以扩增所述核酸分子的条件下,温育所述混合物。在一些实施方案中,所述组合物进一步包含足以提高核酸扩增的特异性的量的试剂,其中所述试剂包含精氨酸、亚精胺或精胺,或其盐。

[0094] 在另一个方面,本发明提供了用于进行利用聚合酶(例如,聚合酶-Sso7缀合物)的核酸扩增反应的反应混合物和试剂盒。在一些实施方案中,所述反应混合物或试剂盒包含聚合酶(例如,与DNA结合结构域缀合的聚合酶,所述DNA结合结构域包括、但不限于Sso7或Sso7-样蛋白)和足以提高核酸扩增的特异性的量的试剂,其中所述试剂包含精氨酸、亚精胺或精胺。

[0095] 还已经发现,一些缓冲液会提高惰性(即,非核酸相互作用的)荧光染料的贮存稳定性,以及提高扩增反应的特异性。基于HEPES和POPSO具有这些作用的发现,认为当在20℃至37℃之间时具有不超过0.027pH单位/℃的变化的任何缓冲液(当在0.1M浓度测量时)会提高惰性荧光染料的稳定性。尽管该发现独立于上面讨论的提高特异性的试剂的发现,但是可以将提高特异性的试剂组合在具有该特定类型的缓冲液(例如在反应混合物中)的反应混合物中。

[0096] II.提高核酸扩增的特异性的试剂

[0097] 本发明提供了当在扩增靶核酸分子之前加入扩增反应混合物中时会提高核酸扩增的特异性的试剂。在一些实施方案中,所述试剂选自游离精氨酸(例如,未与其它氨基酸连接的L-精氨酸或D-精氨酸)、亚精胺和精胺。在一些实施方案中,所述试剂是游离精氨酸。

[0098] 在一些实施方案中,所述提高核酸扩增特异性的试剂以约1mM至约500mM的浓度存在于扩增反应混合物中。在一些实施方案中,所述试剂以约1mM至约100mM、约1mM至约75mM、约1mM至约50mM、约1mM至约25mM、或约5mM至约15mM的浓度存在。

[0099] 本发明的精氨酸、亚精胺和/或精胺试剂可以作为盐来提供。可适用的盐形式的例子包括盐酸盐、氢溴酸盐、硫酸盐、甲磺酸盐、硝酸盐、马来酸盐、乙酸盐、柠檬酸盐、富马酸盐、酒石酸盐(例如,(+)-酒石酸盐、(-)-酒石酸盐或其混合物,包括外消旋混合物)、琥珀酸盐和苯甲酸盐。这些盐可以通过本领域技术人员已知的方法来制备。也包括碱加成盐诸如钠盐、钾盐、钙盐、铵盐、有机氨基盐、或镁盐或类似盐。当本发明的试剂含有相对碱性的官能团时,通过使这类化合物的中性形式接触足量的所需酸(净的或在合适的惰性溶剂中),可以获得酸加成盐。可接受的酸加成盐的例子包括源自无机酸的那些以及源自有机酸的那些,所述无机酸例如盐酸、氢溴酸、硝酸、碳酸、一氢碳酸、磷酸、一氢磷酸、二氢磷酸、硫酸、一氢硫酸、氢碘酸或亚磷酸等,所述有机酸例如乙酸、丙酸、异丁酸、马来酸、丙二酸、苯甲酸、琥珀酸、辛二酸、富马酸、乳酸、扁桃酸、苯二甲酸、苯磺酸、对-甲苯磺酸、柠檬酸、酒石

酸、甲磺酸等。在一些实施方案中，精氨酸、亚精胺和/或精胺盐是单盐酸盐、二盐酸盐、三盐酸盐或四盐酸盐。

[0100] 可以测量扩增反应的特异性，例如，使用解链曲线分析或凝胶分析。在一些实施方案中，如下确定核酸扩增反应的特异性：对比两种产物的相对产率，其中一种产物是具有预期的T_m的特异性产物，且另一种产物是非特异性产物，其具有比特异性产物的T_m更低或更高的T_m。与不含提高扩增特异性的试剂(例如，精氨酸、亚精胺或精胺，或其盐)的反应混合物中的特异性产物相对于非特异性产物的相对产率相比，包含所述试剂的反 应混合物将在扩增反应中具有更高的特异性产物相对于非特异性产物的相对产率(即，通过特异性产物的解链峰的高度与非特异性产物的解链峰的高度测得的产率之比)。在一些实施方案中，与其中不包括提高扩增特异性的试剂(例如，精氨酸、亚精胺或精胺，或其盐)的反应相比，提高扩增特异性的试剂(例如，精氨酸、亚精胺或精胺，或其盐)将表现出所述比率的至少10％、15％、20％、25％、30％、40％、50％、100％、2倍(200％)、2.5倍(250％)、3倍(300％)或更大增加。

[0101] 在一些实施方案中，提高扩增特异性的试剂(例如，精氨酸、亚精胺或精胺，或其盐)也会提高扩增反应混合物对扩增抑制剂的耐受性。通过在有或没有不同浓度的已知抑制剂(例如，巧克力、血液、土壤、奶或血清)存在下进行扩增反应(例如，qPCR)，可以评价抑制剂耐受性。与缺乏提高反应混合物对扩增抑制剂的耐受性的试剂(例如，精氨酸、亚精胺或精胺，或其盐)的反应混合物相比，包含所述试剂的反应混合物在有较高抑制剂浓度存在下将维持反应性能(例如，通过扩增的Ct值所测得的)。

[0102] III.聚合酶-Sso7缀合物

[0103] 在一些实施方案中，本发明的核酸扩增方法利用与DNA结合结构域或蛋白(例如，Sso7或Sso7-样结构域)缀合的聚合酶多肽或结构域。已知这样的聚合酶缀合物会表现出增加的持续合成能力。参见，例如，美国专利申请号12/683,950，其内容特此通过引用整体并入用于所有目的，尤其是关于与聚合酶、聚合酶缀合物、以及制备和使用这类聚合酶的所有方法有关的所有教导。

[0104] 在一些实施方案中，本发明的聚合酶缀合物包含缺乏3′-5′外切核酸酶活性的聚合酶结构域或多肽。本文中使用的“外切核酸酶缺陷型”是指，所述聚合酶具有大幅降低的外切核酸酶活性(即，与野生型聚合酶相比，小于10％、5％或1％的3′-5′外切核酸酶活性)或没有外切核酸酶活性)。在一些实施方案中，所述聚合酶缀合物包含在所述聚合酶结构域中的一个或多个点突变，所述点突变会提供该外切核酸酶缺陷。

[0105] 在一些实施方案中，本发明的聚合酶缀合物包含Sso7DNA结合结构域或蛋白。与缺少Sso7-样结构域的聚合酶相比，包含Sso7或Sso7-样DNA结合结构域的聚合酶缀合物会在核酸扩增中表现出增加的持续合成能力 和减少的反应时间。发明人已经令人惊讶地发现，与包含缺乏所述点突变的Sso7DNA结合结构域的聚合酶相比，在Sso7DNA结合结构域中的一个或多个残基处的点突变可以减少非特异性的PCR产物的形成。因而，在一些实施方案中，本发明的聚合酶缀合物包含在Sso7DNA结合结构域结构域中的一个或多个点突变。

[0106] 在一些实施方案中，本发明的聚合酶缀合物与SEQ ID NO:31-56的氨基酸序列基本上相同(例如，具有至少60％、65％、70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％或99％的氨基酸序列同一性)。在一些实施方案中，本发明的聚合酶

缀合物具有SEQ ID NO:31-56的氨基酸序列。

[0107] A.聚合酶

[0108] 多种聚合酶可以用作本发明的聚合酶或用作本发明的聚合酶-Sso7缀合物的聚合酶结构域的至少一部分。已知至少5个家族的DNA依赖性DNA聚合酶，尽管大部分属于家族A、B和C。不同家族之间存在很少或不存在序列相似性。大多数家族A聚合酶是单链蛋白，其可以含有多酶功能包括聚合酶、3′至5′外切核酸酶活性和5′至3′外切核酸酶活性。家族B聚合酶典型地具有单催化结构域、聚合酶和3′至5′外切核酸酶活性，以及辅助因子。家族C聚合酶典型地是多亚单元蛋白，其具有聚合和3′至5′外切核酸酶活性活性。在大肠杆菌(E.coli)中，已经发现三种类型的DNA聚合酶，即DNA聚合酶I(家族A)，II(家族B)，和III(家族C)。在真核细胞中，三种不同的家族B聚合酶，即DNA聚合酶α，δ，和ε，参与核复制，且家族A聚合酶，即聚合酶γ，用于线粒体DNA复制。其它类型的DNA聚合酶包括噬菌体聚合酶。这些聚合酶中任一种，全部或部分这些聚合酶的组合，以及两个或更多所述聚合酶之间的嵌合物或杂合物或它们的等价物可以用于形成本发明的聚合酶缀合物的一部分或全部聚合酶结构域。

[0109] 此外，在一些实施方案中，非热稳定的聚合酶也可以根据本发明使用。例如，具有突变(D355A，E357A)的大肠杆菌DNA聚合酶I(Klenow)的大片段(Klenow片段)消除3′-5′外切核酸酶活性。该酶或等价酶可以用于这样的实施方案中，其中扩增反应不在高温下进行。

[0110] 在一个示例性实施方案中，本发明的聚合酶缀合物具有源自两种亲本 聚合酶Pfu和DeepVent的聚合酶结构域。这样的聚合酶记述在例如美国申请公开号20040219558、20040214194、20040191825、20030162173中，将其每一篇特此通过引用整体并入用于所有目的，特别是用于与杂合聚合酶有关的所有教导。在一些实施方案中，所述聚合酶与SEQ ID NO:1(任选地包括接头诸如SEQ ID NO:4)或SEQ ID NO:2或SEQ ID NO:61基本上(例如，至少60、70、80、85、90或95％)相同。

[0111] 在一些实施方案中，本发明的聚合酶缀合物包括包含突变的聚合酶结构域，与包含不具有所述突变的聚合酶结构域的杂合聚合酶相比，所述突变减少或消除包含所述聚合酶结构域的任何杂合聚合酶外切核酸酶活性。可以将多种突变引入至天然聚合酶结构域中，以减少或消除3'-5'外切核酸酶活性。例如，美国专利号6,015,668、5,939,301和5,948,614描述了在Tma和Tne DNA聚合酶的3′-5′外切核酸酶结构域中金属结合的天冬氨酸向丙氨酸残基的突变。这些突变将这些酶的3′-5′外切核酸酶活性减少至低于可检测水平。类似地，美国专利号5,882,904描述了Thermococcus barossi中的类似的天冬氨酸-至-丙氨酸的突变，和美国专利号5,489,523教导了沃氏火球菌(Pyrococcus wosei)DNA聚合酶的双突变D141AE143A。这两种突变聚合酶实际上不具有可检测的3'-5'外切核酸酶活性。测定3'-5'外切核酸酶活性的方法是本领域中公知的。参见，例如，Freemont等，Proteins(蛋白)1:66(1986)；Derbyshire等，EMBO J.(EMBO杂志)16:17(1991)和Derbyshire等，Methods in Enzymology(酶学方法)262:36385(1995)。应该理解，尽管上述突变最初在一种聚合酶中识别，但是人们通常可以将该突变引入至其它聚合酶中，以减少或消除外切核酸酶活性。

[0112] 在一些实施方案中，本发明的聚合酶缀合物包含在聚合酶结构域中的与SEQ ID NO:2的D141/E143位置对应的双点突变D141A/E143A。与聚合酶氨基酸有关的术语“对应”表示，与参考聚合酶氨基酸序列(例如，SEQ ID NO:2)中的目标氨基酸(例如，D141或E143)比

对的氨基酸。序列比较可以利用任何具有默认参数的BLAST(包括BLAST2.2算法)来进行,分别记述在Altschul等,Nuc.Acids Res.(核酸研究)25:33893402(1977)和Altschul等,J.Mol.Biol.(分子生物学杂志)215:403410(1990)。在一些实施方案中,本发明的聚合酶缀合物包含与SEQ ID NO:2(任选地包括 接头诸如SEQ ID NO:4)或SEQ ID NO:61的氨基酸序列基本上相同的(例如,具有至少60%、65%、70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%的氨基酸序列同一性)的聚合酶多肽或结构域。

[0113] B.Sso7-样蛋白

[0114] Sso7d和Sac7d分别是得自超嗜热古细菌硫矿硫化叶菌(Sulfolobus solfataricus)和酸热硫化叶菌(S.acidocaldarius)的小型(约7kDa MW)碱性染色体蛋白(参见,例如,Choli等人,Biochimica et Biophysica Acta 950:193-203,1988;Baumann等人,Structural Biol.1:808-819,1994;和Gao等人,Nature Struc.Biol.5:782-786,1998)。这些蛋白以不依赖于序列的方式结合DNA,并且当结合时,会使DNA的T_m在相同条件下增加至多40℃(McAfee等,Biochemistry(生物化学)34:10063-10077,1995)。通常认为Sso7蛋白和它们的同源物参与在高温稳定基因组DNA。Sso7d、Sac7d、Sac7e和有关的序列(在本文中被称作"Sso7蛋白"或"Sso7结构域")是本领域已知的(参见,例如,UniProt数据库登录号:P39476(Sso7d);059632(Ssh7b);P13123(Sac7d);P13125(Sac7e);和Q96X56(Sto7e))。

[0115] 在一些实施方案中,所述Sso7结构域或蛋白是具有SEQ ID NO:1的氨基酸序列的野生型(即,天然存在的)Sso7结构域或蛋白。在一些实施方案中,通过在Sso7DNA结合结构域中产生一个或多个突变,从野生型Sso7修饰Sso7或Sso7-样结构域或蛋白。

[0116] 在一些实施方案中,Sso7或Sso7-样结构域具有在与SEQ ID NO:3的K28和/或R43相对应的位置处相对于天然氨基酸的氨基酸变化。应当理解,这样的位置命名不指示要求保护的分子本身的氨基酸的数目,而是指示当要求保护的分子序列与SEQ ID NO:3最大程度地比对时要求保护的分子残基所在的位置。在变体Sso7结构域的背景下,与Sso7-样蛋白序列的"对应"是基于下述惯例:根据特定序列(即,SEQ ID NO:3)的氨基酸位置编号进行编号,然后以使与SEQ ID NO:3的序列同一性百分比最大化的方式与Sso7-样蛋白序列比对。比对可以手工进行,或者使用序列比较算法进行(例如,使用具有默认参数的NCBI BLAST程序(参见,例如,Altschul等Nucl.AcidsRes.25:3389-3402,1997)。对应的序列可以总结如下:

[0117]

	与 SEQ ID NO:3 的 K28 对应的氨基酸的实际位置	与 SEQ ID NO:3 的 R43 对应的氨基酸的实际位置
Sso7	28	43
Ssh7b	28	43
Sto7e	28	42
Sac7d	28	42
Sac7e	28	42

[0118] 可以在与SEQ ID NO:3对应的K28和/或R43位置处置换任意Sso7DNA结合蛋白结构域。因而，例如，在一些实施方案中，所述变体Sso7多肽序列与例如SEQ ID NO:3、57、58、59或60中的任一个基本上(例如，至少60、70、80、85、90或95％)相同，且在与K28对应的氨基酸位置处包含除了K以外的氨基酸。在一些实施方案中，与K28对应的氨基酸位置是丝氨酸(S)、苏氨酸(T)、半胱氨酸(C)、脯氨酸(P)、天冬氨酸(D)、谷氨酸(E)、天冬酰胺(N)、谷氨酰胺(Q)、丙氨酸(A)、苯丙氨酸(F)、甘氨酸(G)、组氨酸(H)、异亮氨酸(I)、亮氨酸(L)、蛋氨酸(M)、精氨酸(R)、缬氨酸(V)、色氨酸(W)或酪氨酸(Y)。

[0119] 在一些实施方案中，所述变体Sso7多肽序列与例如SEQ ID NO:3、57、58、59或60中的任一个基本上(例如，至少60、70、80、85、90或95％)相同，且在与R43对应的氨基酸位置处包含除了R以外的氨基酸。在一些实施方案中，与R43对应的氨基酸位置是丙氨酸(A)、半胱氨酸(C)、天冬氨酸(D)、谷氨酸(E)、苯丙氨酸(F)、甘氨酸(G)、组氨酸(H)、异亮氨酸(I)、赖氨酸(K)、亮氨酸(L)、蛋氨酸(M)、天冬酰胺(N)、谷氨酰胺(Q)、丝氨酸(S)、苏氨酸(T)、缬氨酸(V)、色氨酸(W)、酪氨酸(Y)或脯氨酸(P)。

[0120] 在一些实施方案中，所述变体Sso7多肽序列与例如SEQ ID NO:3、57、58、59或60中的任一个基本上(例如，至少60、70、80、85、90或95％)相同，且包含在与K28对应的氨基酸位置处的除了K以外的氨基酸和在与R43对应的氨基酸位置处的除了R以外的氨基酸。例如，在一些实施方案中，在位置K28处的氨基酸选自由以下各项组成的组：丝氨酸(S)、 苏氨酸(T)、半胱氨酸(C)、脯氨酸(P)、天冬氨酸(D)、谷氨酸(E)、天冬酰胺(N)、谷氨酰胺(Q)、丙氨酸(A)、苯丙氨酸(F)、甘氨酸(G)、组氨酸(H)、异亮氨酸(I)、亮氨酸(L)、缬氨酸(V)、色氨酸(W)或酪氨酸(Y)，且在位置R43处的氨基酸选自由以下各项组成的组：丙氨酸(A)、半胱氨酸(C)、天冬氨酸(D)、谷氨酸(E)、苯丙氨酸(F)、甘氨酸(G)、组氨酸(H)、异亮氨酸(I)、赖氨酸(K)、亮氨酸(L)、蛋氨酸(M)、天冬酰胺(N)、谷氨酰胺(Q)、丝氨酸(S)、苏氨酸(T)、缬氨酸(V)、色氨酸(W)、酪氨酸(Y)或脯氨酸(P)。

[0121] 在一些实施方案中，所述Sso7结构域或蛋白与SEQ ID NO:5-30中的任一个的氨基酸序列相同或基本上相同(例如，具有至少60％、65％、70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％或99％的氨基酸序列同一性)。在一些实施方案中，所述Sso7结构域或蛋白具有SEQ ID NO:5-30中的任一个的氨基酸序列。

[0122] C.使Sso7-样蛋白与聚合酶缀合

[0123] 本发明的聚合酶-Sso7缀合物通常通过使用化学和/或重组方法将聚合酶结构域与Sso7或Sso7-样DNA结合结构域连接来生产。

[0124] 将DNA结合蛋白与聚合酶结构域连接的化学方法记载在，例如，Bioconjugate Techniques(生物缀合技术)，Hermanson，编，Academic Press(科学出版社)(1996)。这些包括，例如，以通过蛋白化学领域中公知的方法，直接或通过连接化合物，相互连接两种蛋白为目的的衍生化作用。例如，在一个化学缀合实施方案中，连接催化结构域和DNA结合结构域的手段包括异双功能-偶联试剂，其最终促进在两个结构部分之间形成分子间二硫键。在该能力方面有效用于本发明的其它类型的偶联试剂记述在，例如，美国专利号4,545,985中。可替换地，分子间二硫键可以方便地在各结构部分中的半胱氨酸之间形成，其天然地发生或通过遗传改造插入。连接结构部分的手段还可以使用异双功能交联试剂之间的硫醚键或特异性低pH可切割交联剂或特异性蛋白酶可切割接头或其它可切割或不可切割化学键。

[0125] 连接DNA结合结构域(例如,Sso7)和聚合酶结构域的方法还可以包括在通过标准肽合成化学或重组手段分别合成的结构部分之间形成的肽键。缀合蛋白本身也可以利用合成完整或部分氨基酸序列的化学方法来 生成。例如,肽可以通过固相技术,诸如,例如,Merrifield固相合成法来合成,其中将氨基酸顺序加入至正在增长的氨基酸链(参见,Merrifield(1963)J.Am.Chem.Soc(美国化学协会杂志),85:2149-2146)。自动化合成多肽的设备可商购自供应商诸如PE Corp.(Foster City,Calif.),并可以根据制造商的说明书来操作。合成的肽可以然后与树脂切割,并例如通过制备高效液相层析法来纯化(参见Creighton,Proteins Structures and Molecular Principles(蛋白结构与分子原理),50-60(1983))。合成的多肽或所述多肽的子片段的组成可以通过氨基酸分析或测序来验证(例如,Edman降解程序;参见Creighton,Proteins,Structures and Molecular Principles(蛋白、结构与分子原理),第34-49页(1983))。

[0126] 在一些实施方案中,DNA结合结构域和聚合酶结构域可以经由接头基团连接。所述接头基团可以是化学交联剂,包括、例如,琥珀酰亚胺基-(N-马来酰亚胺基甲基)-环己烷-1-羧酸酯(SMCC)。所述接头基团还可以是额外的氨基酸序列,包括、例如,聚丙氨酸、聚甘氨酸,或类似地,接头基团。

[0127] 在一些实施方案中,得到的融合蛋白中的每个多肽的编码序列在其氨基或羧基端通过肽键以任意顺序直接连接。可替换地,氨基酸接头序列可以用于通过足以确保各多肽折叠为二级和三级结构的距离使第一和第二多肽组分分开。利用本领域中公知的标准技术将这样的氨基酸接头序列引入至融合蛋白中。合适的肽接头序列可以基于以下因素来选择:(1)它们采取柔性延伸构象的能力;(2)它们不能采取能够与第一和第二多肽上的功能性表位相互作用的二级结构;和(3)缺乏可以与多肽功能性表位相互作用的疏水性或带电残基。典型的肽接头序列包含Gly、Ser、Val和Thr残基。其它接近中性的氨基酸(诸如Ala)也可以用于接头序列中。可以有效用作接头的氨基酸序列包括Maratea等(1985)Gene(基因)40:39-46;Murphy等(1986)Proc.Natl.Acad.Sci.USA(美国国家科学院学报)83:8258-8262;美国专利号4,935,233和4,751,180,它们各自特此通过引用整体并入用于所有目的,尤其是关于与接头有关的所有教导。接头序列通常可以是长度为1-约50个氨基酸,例如,长度为3,4,6,或10个氨基酸,但是长度可以为100或200个氨基酸。当第一和第二多肽具有非必 需N端氨基酸区时,可以不需要接头序列,所述非必需N端氨基酸区可以用于分离功能性结构域或防止位阻干扰。在一些实施方案中,接头序列包含SEQ ID NO:4的氨基酸序列。

[0128] 其它化学接头包括糖类接头、脂质接头、脂肪酸接头、聚醚接头,例如,PEG等。例如,聚乙二醇接头可获自Shearwater Polymers,Inc.Huntsville,Ala。这些接头任选地具有酰胺键、巯基键、或异双功能键。

[0129] 其它连接DNA结合结构域和聚合酶结构域的方法包括通过表达阴性和阳性尾部的离子结合和通过抗体和链霉抗生物素-生物素相互作用的间接结合。(参见,例如,Bioconjugate Techniques(生物缀合技术),见上文)。所述结构域也可以通过中间相互作用序列连接在一起。例如,DNA结合结构域-相互作用序列,即结合特定DNA结合结构域(诸如Sso7)的序列可以与聚合酶连接。由此生成的融合蛋白可以然后容许与DNA结合结构域非共价连接,以生成DNA-结合-结构域-聚合酶缀合物。

[0130] D.生产聚合酶-Sso7缀合物

[0131] 使用本领域已知的技术，可以生产本发明的聚合酶-Sso7缀合物。用于生产包含聚合酶结构域和核酸结合结构域的聚合酶的方法记载在，例如，美国专利申请公开号2006/005174、2004/0219558、2004/0214194、2004/0191825、2004/0081963、2004/0002076、2003/0162173、2003/0148330、2003/0138830和美国专利号6,627,424和7,445,898，将其每一篇通过参考整体引入以获得全部目的，特别是获得关于聚合酶、杂合/嵌合聚合酶，以及用于制备和使用所述聚合酶的所有方法的所有教导。

[0132] 编码聚合酶或DNA结合结构域的核酸可以利用重组遗传学领域中的常规技术来获得。公开本发明中使用的一般方法的基础课本包括Sambrook和Russell，Molecular Cloning，A Laboratory Manual(分子克隆，实验室手册)(第3版2001)；Kriegler，Gene Transfer and Expression:A Laboratory Manual(基因转移和表达:实验室手册)(1990)；和Current Protocols in Molecular Biology(当前分子生物学方案)(Ausubel等编.，1994-1999)。这样的核酸还可以通过体外扩增方法诸如本文中和Berger，Sambrook，和Ausubel，以及Mullis等，(1987)美国专利号4,683,202；PCR Protocols A Guide to Methods and Applications(PCR流程，方法和应用指导)(Innis等编)Academic Press Inc.(科学出版社)San Diego(圣地亚哥)，加利福尼亚(1990)(Innis)；Arnheim&Levinson(1990年10月1日)C&EN36-47；TheJournal OfNIH Research(NIH研究杂志)(1991)3:81-94；Kwoh等(1989)Proc.Natl.Acad.Sci.USA(美国国家科学院学报)86:1173；Guatelli等(1990)Proc.Natl.Acad.Sci.USA(美国国家科学院学报)87,1874；Lomell等(1989)J.Clin.Chem.(临床化学杂志)，35:1826；Landegren等，(1988)Science(科学)241:1077-1080；Van Brunt(1990)Biotechnology(生物技术)8:291-294；Wu和Wallace(1989)Gene(基因)4:560；和Barringer等(1990)Gene(基因)89:117中记述的那些，将其每一篇通过参考整体引入以获得全部目的，特别是关于扩增方法的全部教导。

[0133] 技术人员应该认识到可以在不消除其生物学活性的条件下，对本发明的聚合酶另外进行修饰。可以进行一些修饰，以促进结构域克隆、表达或引入融合蛋白中。这样的修饰是本领域中技术人员公知的，且包括，例如，在编码结合结构域的多核苷酸任一端添加密码子以提供，例如，在氨基端添加的甲硫氨酸以提供起始位点，或置于任一端上的另外的氨基酸(例如，聚His)以构建方便定位的限制性位点或终止密码子或纯化序列。

[0134] 本发明的聚合酶可以在多种宿主细胞，包括，大肠杆菌，其它细菌宿主，酵母，丝状真菌，和不同高等真核细胞诸如COS，CHO和HeLa细胞系和骨髓瘤细胞系中表达。在微生物体基因表达的技术记述在，例如，Smith，Gene Expression in Recombinant Microorganisms(在重组微生物中的基因表达)(Bioprocess Technology(生物处理技术)，卷22)，Marcel Dekker，1994中。有效用于表达的细菌的例子包括，但不仅限于，埃希氏菌属(Escherichia)、肠杆菌属(Enterobacter)、固氮菌属(Azotobacter)、欧文氏菌属(Erwinia)、杆菌属(Bacillus)、假单胞菌属(Pseudomonas)、克雷伯菌属(Klebsiella)、变形杆菌属(Proteus)、沙门氏菌属(Salmonella)、沙雷氏菌属(Serratia)、志贺氏菌属(Shigella)、根瘤菌属(Rhizobia)、透明颤菌属(Vitreoscilla)、和副球菌属(Paracoccus)。有效用作表达宿主的丝状真菌包括，例如，以下属:曲霉属(Aspergillus)，木霉属(Trichoderma)，链孢霉属(Neurospora)，青霉属(Penicillium)，头孢 菌属(Cephalosporium)，绵霉属(Achlya)，柄孢壳菌属(Podospora)，毛霉菌属(Mucor)，旋孢腔

菌属(Cochliobolus),和Pyricularia。参见,例如,美国专利号5,679,543和Stahl和Tudzynski编.,Molecular Biology in Filamentous Fungi(丝状真菌分子生物学),John Wiley&Sons,1992。酵母中异源蛋白的合成是公知的且记述在文献中。Methods in Yeast Genetics(酵母遗传学方法),Sherman,F.,等,Cold Spring Harbor Laboratory(冷泉港实验室),(1982)是公认的描述可用于在酵母中生成酶的不同方法的作品。

[0135] 存在许多本领域中技术人员公知的用于生成本发明的聚合酶多肽的表达系统。(参见,例如,Gene Expression Systems(基因表达系统),Fernandex和Hoeffler编.Academic Press(科学出版社),1999;Sambrook和Russell,见上文;和Ausubel等,见上文.)。典型地,将编码不同多肽的多个核酸置于启动子的控制下,所述启动子在所需宿主细胞中起作用。许多不同的启动子是本领域中技术人员可获得的并已知的,且可以用于本发明的表达载体中,这取决于具体的应用。一般地,所选的启动子取决于其中所述启动子将具有活性的细胞。还任选地包括其它表达控制序列诸如核糖体结合位点、转录终止位点等。包括这些控制序列中的一个或多个的构建体称为"表达盒"。因此,引入编码连接的多肽的核酸,以在所需宿主细胞中获得高水平表达。

[0136] 适合用于特定宿主细胞中的表达控制序列通常通过克隆在该细胞中表达的基因来获得。常用的原核控制序列,其在本文中定义为包括用于转录起始的启动子,任选地,与操纵子一起,连同核糖体结合位点序列,包括这样的常用启动子,如β-内酰胺酶(青霉素酶)和乳糖(lac)启动子系统(Change等,Nature(自然)(1977)198:1056),色氨酸(trp)启动子系统(Goeddel等,Nucleic Acids Res.(核酸研究)(1980)8:4057),tac启动子(DeBoer,等,Proc.Natl.Acad.Sci.U.S.A.(美国国家科学院学报)(1983)80:21-25);和λ-来源的PL启动子和N-基因核糖体结合位点(Shimatake等,Nature(自然)(1981)292:128)。特定的启动子系统对于本发明不是关键的,可以使用任何可获得的在原核生物中起作用的启动子。标准细菌表达载体包括质粒诸如基于pBR322的质粒,例如,pBLUESCRIPT™,pSKF,pET23D,λ-噬菌体来源的载体,和融合表达系统诸如GST和LacZ。还可 以将表位标签添加至重组蛋白,以通过方便的分离方法,例如,c-myc、HA-标签、6-His标签(SEQ ID NO:62)、麦芽糖结合蛋白、VSV-G标签、抗-DYKDDDDK(SEQ ID NO:63)标签或任何这样的标签,其中很多是本领域中技术人员公知的。

[0137] 为了在除大肠杆菌以外的原核细胞中表达,需要在特定原核物种中起作用的启动子。这样的启动子可以获自已经有该物种克隆的基因,或可以使用异源启动子。例如,除大肠杆菌外,杂合trp-lac启动子还在杆菌属物种(Bacillus sp.)中起作用。这些和其它合适的细菌启动子是本领域中公知的,且记述在,例如,Sambrook等和Ausubel等中。用于表达本发明蛋白的细菌表达系统可以在例如,大肠杆菌,杆菌属物种,和Salmonella(Palva等,Gene(基因)22:229-235(1983);Mosbach等,Nature(自然)302:543-545(1983)中获得。用于这样的表达系统的试剂盒可商购。

[0138] 用于哺乳动物细胞、酵母、和昆虫细胞的真核表达系统是本领域中公知的,且也是可商购的。在酵母中,载体包括酵母整合质粒(例如,YIp5)和酵母复制质粒(YRp系列质粒)和pGPD-2。包含两种真核病毒的调节元件的表达载体典型地用于真核表达载体,例如,SV40载体,乳头瘤病毒载体,和源自EB病毒的载体中。其它示范性真核载体包括pMSG,pAV009/A+,pMTO10/A+,pMAMneo-5,杆状病毒pDSVE,和容许在CMV启动子,SV40早期启动子,SV40晚期

启动子，金属硫蛋白启动子，鼠类乳腺肿瘤病毒启动子，劳氏肉瘤病毒启动子，多角体蛋白(polyhedrin)启动子，或显示出对在真核细胞中表达有效的其它启动子的指导下表达蛋白的任何其它载体。

[0139] 组成性或调节性启动子可以用于本发明中。调节性启动子可以是有利的，因为在诱导融合多肽表达前宿主细胞可以生长至高密度。异源蛋白的高水平表达在一些情形中延缓细胞生长。诱导型启动子是在可通过环境或发育因素诸如，例如，温度、pH、无氧或有氧条件、光、转录因子和化学制品改变表达水平的情况下指导基因表达的启动子。

[0140] 关于大肠杆菌和其它细菌宿主细胞，诱导型启动子是本领域中技术人员已知的。这些包括，例如，lac启动子，噬菌体λPL启动子，杂合trp-lac启动子(Amann等(1983)Gene(基因)25:167;de Boer等(1983)Proc.Nat′l. Acad.Sci.USA(美国国家科学院学报)80:21)，和噬菌体T7启动子(Studier等(1986)J.Mol.Biol.(分子生物学杂志);Tabor等(1985)Proc.Nat′l Acad.Sci.USA(美国国家科学院学报)82:1074-8)。这些启动子及其应用也在Sambrook等，见上文中讨论。

[0141] 翻译偶联可以用于增强表达。该策略使用源自对翻译系统天然的高度表达基因的短上游可读框，其置于启动子的下游，和核糖体结合位点，及随后相隔几个氨基酸密码子的终止密码子。紧挨着终止密码子之前是第二核糖体结合位点，且终止密码子之后是用于起始翻译的起始密码子。该系统溶解RNA中的二级结构，容许翻译的有效起始。参见Squires，等(1988)，J.Biol.Chem.(生物化学杂志)263:16297-16302。

[0142] 多核苷酸构建体的构造一般需要使用能够在细菌中复制的载体。这样的载体常用于本领域中。可商购许多试剂盒，以纯化来自细菌的质粒(例如，EasyPrep™，FlexiPrep™，来自Pharmacia Biotech(药物生物技术);StrataClean™，来自Stratagene;和，QIAexpress®表达系统，Qiagen)。分离的和纯化的质粒可以然后进一步操作，以生成其它质粒，并用于转化细胞。

[0143] 本发明的多肽可以在细胞内表达，或可以由细胞分泌。细胞内表达通常导致高产率。如果需要，可溶的活性融合多肽的量可以通过执行再折叠程序来增加(参见，例如，Sambrook等，见上文.;Marston等，Bio/Technology(生物/技术)(1984)2:800;Schoner等，Bio/Technology(生物/技术)(1985)3:151)。本发明的多肽可以在多种宿主细胞，包括大肠杆菌，其它细菌宿主、酵母和不同高等真核细胞诸如COS、CHO和HeLa细胞系和骨髓瘤细胞系中表达。所述宿主细胞可以是哺乳动物细胞、昆虫细胞、或微生物，诸如，例如，酵母细胞、细菌细胞、或真菌细胞。

[0144] 一旦表达，所述多肽可以根据本领域的标准程序，包括硫酸铵沉淀、亲和柱、柱层析、凝胶电泳等来纯化(参见，一般地，R.Scopes,Protein Purification(蛋白质纯化)，Springer-Verlag,N.Y.(1982)，Deutscher,Methods in Enzymology(酶学方法)卷182:Guide to Protein Purification.(蛋白质纯化指导)，Academic Press,Inc.(科学出版社)N.Y.(1990))。至少约90-95％的同质性的基本纯组成是优选的，且98-99％以上的同质性是最优选的。一旦部分地纯化或如果需要，纯化至同质，则可以然后使用所述多肽(例如，作为用于抗体生成的免疫原)。

[0145] 为了促进本发明的多肽的纯化，编码所述多肽的核酸还可以包括关于表位或“标签”的编码序列，关于所述表位或“标签”可以获得亲和结合试剂。合适的表位的例子包括

myc和V-5报告基因;有效用于重组生成具有这些表位的融合多肽的表达载体是可商购的(例如,Invitrogen(Carlsbad加利福尼亚)载体pcDNA3.1/Myc-His和pcDNA3.1/V5-His适合于在哺乳动物细胞中表达)。适合于使标签与本发明的融合蛋白连接的另外的表达载体和相应的检测系统是本领域中技术人员已知的,且许多是可商购的(例如,FLAG″(Kodak,Rochester NY)。合适的标签的另一个实例是聚组氨酸序列,其能够结合金属螯合的亲和配体。典型的,使用6个相邻的组氨酸,尽管人们可以使用多于或少于6个。可以担当聚组氨酸标签的结合结构部分的合适金属螯合亲和配体包括次氮基三乙酸(NTA)(Hochuli,E.(1990)″Purification of recombinant proteins with metal chelating adsorbents(使用金属螯合吸附剂纯化重组蛋白)″,在Genetic Engineering:Principles and Methods(遗传工程:原理和方法),J.K.Setlow,编,Plenum Press,N.Y.;可商购自Qiagen(Santa Clarita,加利福尼亚))。

[0146] 本领域中技术人员应该认识到在生物表达或纯化后,聚合酶缀合物可以具有与组成性多肽的天然构象基本不同的构象。在该情形中,可能必须或需要变性和还原多肽并然后导致多肽再折叠为优选的构象。还原和变性蛋白和诱导再折叠的方法是本领域中技术人员公知的(参见,Debinski等(1993)J.Biol.Chem.(生物化学杂志)268:14065-14070;Kreitman和Pastan(1993)Bioconjug.Chem.(生物缀合化学)4:581-585;和Buchner等(1992)Anal.Biochem.(分析生物化学)205:263-270)。Debinski等,例如,描述在胍-DTE中变性和还原内含体蛋白。蛋白然后在含有氧化的谷胱甘肽和L-精氨酸的氧化还原缓冲液中再折叠。

[0147] IV.用于扩增控制的通用惰性染料

[0148] 提供了通用预混合物,其可以用于任意类型的实时仪器或实时扩增方法,无需进一步加入除了要试验的样品和在一些实施方案中要使用的引物以外的试剂。在历史上,用于定量PCR(qPCR)的不同类型的扩增仪器和方法采用高或低5-或6-羧基-X-罗丹明染料浓度作为惰性参比染料进行标 准化,并且因此用于qPCR中的预混合物不是“通用的”,因为所述预混合物具有低或高浓度的5-或6-羧基-X-罗丹明染料。如果人们希望使用含有低5-或6-羧基-X-罗丹明染料浓度预混合物的高5-或6-羧基-X-罗丹明染料浓度仪器,必须向所述预混合物中加入额外的5-或6-羧基-X-罗丹明染料,由此增加额外步骤和可能的误差引入。但是,最近已经发现(参见,PCT/US2011/065617),单一混合物可以用于任一种仪器,无需加入除了试验样品本身和任选的引物以外的任何试剂。具体地,所述扩增混合物包含具有长斯托克斯位移的荧光染料(“第二惰性参比染料”)以及低浓度的第一惰性参比染料(例如,5-或6-羧基-X-罗丹明染料)。选择具有长斯托克斯位移的荧光染料,使得所述染料的激发波长显著不同于所述第一惰性参比染料的激发波长(例如,5-或6-羧基-X-罗丹明染料具有在约575nm的激发最大值),但是其发射波长最大值与惰性参比染料(5-或6-羧基-X-罗丹明染料具有约620nm的发射波长最大值)基本上相同。确定具有长斯托克斯位移的荧光染料的浓度,使得所述混合物中的具有长斯托克斯位移的荧光染料和第一惰性参比染料(其可以是、但不限于5-或6-羧基-X-罗丹明染料)的组合信号足以用于高浓度惰性参比染料实时扩增仪器中,且可以例如随后标准化数据。当用于低浓度惰性参比染料实时扩增仪器上时,具有长斯托克斯位移的荧光染料不会在惰性参比染料(例如,5-或6-羧基-X-罗丹明)通道中被激发,因而不会在该通道中产生任何额外信号,所以可以被检测/用于惰性参比染料标准

化。相反，由存在于预混合物中的低浓度惰性参比染料产生的信号用于标准化。所以，该预混合物可以用在“高惰性参比染料”和“低惰性参比染料”仪器上。

[0149] 现在已经发现，当在20℃至37℃之间时具有不超过0.027pH单位/℃的变化的POPSO缓冲液(当在0.1M浓度测量时)是一种可用于稳定惰性荧光染料的缓冲液。例如，观察到，POPSO会在低温和高温提供染料DY-510XL(Dyomics，Jena，德国)(一种具有长斯托克斯位移的荧光染料)的最佳稳定性，并且其它缓冲液Hepes、TAPS、Bicine和Tris按照该次序提供较低的稳定性。因此，提供了反应混合物，其包含具有长斯托克斯位移的荧光染料以及低浓度的第一惰性参比染料(例如，5-或6-羧基-X-罗丹明染料)和当在20℃至37℃之间时具有不超过0.027pH单位/℃的变化的缓 冲液(当在0.1M浓度测量时)。当在20℃至37℃之间时具有不超过0.027pH单位/℃的变化的缓冲液是已知的。可以确定缓冲液在不同温度的pH变化，例如，通过测量缓冲液在20℃至37℃的pKa，和确定pKa值除以度数差(17度)的商。具有不超过0.027pH单位/℃的变化的示例性缓冲液(当在0.1M浓度测量时)包括、但不限于：HEPES((4-(2-羟乙基)-1-哌嗪乙磺酸))、ACES(N-(2-乙酰氨基)-2-氨基乙磺酸)、PIPES(哌嗪-N,N′-双(2-乙磺酸)、MOPSO(3-(N-吗啉代)-2-羟基丙烷磺酸)、BES(N,N-双(2-羟乙基)-2-氨基乙磺酸)、MOPS(3-(N-吗啉代)丙烷磺酸)、TES(N-三(羟基甲基)甲基-2-氨基乙磺酸)、TAPSO(3-[N-三(羟基甲基)甲基氨基]-2-羟基丙烷磺酸)、POPSO(哌嗪-N,N′-双(2-羟基丙烷磺酸))、BICINE(N,N-双(2-羟乙基)甘氨酸)、TAPS(N-三(羟基甲基)甲基-3-氨基丙烷磺酸)和AMPSO(N-(1,1-二甲基-2-羟乙基)-3-氨基-2-羟基丙烷磺酸)。

[0150] 当在20℃至37℃之间时具有不超过0.027pH单位/℃的变化的缓冲液会促进具有长斯托克斯位移的荧光染料的特别稳定的环境的发现独立于下述发现：精氨酸和其它试剂会提高PCR特异性。但是，因为多种染料作为通用控制剂在扩增反应混合物中的应用，在一些实施方案中，所述反应混合物进一步包含提高核酸扩增的特异性的试剂，包括、但不限于，游离精氨酸、亚精胺或精胺，其以提高扩增特异性的量存在。

[0151] V.扩增方法

[0152] 如本文中所述的，本发明提供了用于核酸扩增反应中的组合物，其包括聚合酶-Sso7缀合物和选自精氨酸、亚精胺和精胺的试剂。这样的扩增反应包括但不限于聚合酶链反应(PCR)，DNA连接酶链反应(LCR)，QBeta RNA复制酶，和基于RNA转录的(诸如TAS和3SR)扩增反应以及本领域技术人员已知的其它反应。可以利用本文中所述的组合物进行的聚合酶链反应包括但不限于反转录PCR(rt-PCR)和定量PCR(qPCR)。

[0153] 使用对于扩增核酸分子而言足够的反应混合物，实现本发明的使用聚合酶(例如，聚合酶-Sso7缀合物)和选自精氨酸、亚精胺和精胺的试剂的扩增方法。在一些实施方案中，除了包含聚合酶-Sso7缀合物和精氨酸、亚精胺或精胺以外，扩增反应混合物还包含一种或多种下述组分：核苷酸三 磷酸、一种或多种寡核苷酸引物、盐、缓冲液、水、稳定剂和DNA-结合染料。

[0154] 在一些实施方案中，本发明的扩增反应混合物包含：如本文中所述的聚合酶(例如，聚合酶-Sso7缀合物)，其浓度为约1U/ml至约75U/ml(例如，约1U/ml、5U/ml、10U/ml、15U/ml、20U/ml、25U/ml、30U/ml、35U/ml、40U/ml、45U/ml、50U/ml、55U/ml、60U/ml、65U/ml、70U/ml或75U/ml)；精氨酸、亚精胺或精胺，或其盐，其浓度为约1mM至约100mM(例如，约1mM、5mM、10mM、15mM、20mM、25mM、30mM、35mM、40mM、45mM、50mM、55mM、60mM、65mM、70mM、75mM、

80mM、85mM、90mM、95mM或100mM)；dNTP，其浓度为约0.1mM至约10mM(例如，约0.1mM、0.2mM、0.3mM、0.4mM、0.5mM、0.6mM、0.7mM、0.8mM、0.9mM、1mM、2mM、3mM、4mM、5mM、6mM、7mM、8mM、9mM或10mM)；镁，例如，$MgCl_2$，其浓度为约1mM至约20mM(例如，约1mM、2mM、3mM、4mM、5mM、6mM、7mM、8mM、9mM、10mM、11mM、12mM、13mM、14mM、15mM、16mM、17mM、18mM、19mM或20mM)；$(NH_4)_2SO_4$，其浓度为约10mM至约100mM(例如，约10mM、20mM、30mM、40mM、50mM、60mM、70mM、80mM、90mM或100mM)；钾，例如，KCl，其浓度为约50mM至约200mM(例如，约50mM、60mM、70mM、80mM、90mM、100mM、110mM、120mM、130mM、140mM、150mM、160mM、170mM、180mM、190mM或200mM)；缓冲液，例如，浓度为约50mM至约200mM(例如，约50mM、60mM、70mM、80mM、90mM、100mM、110mM、120mM、130mM、140mM、150mM、160mM、170mM、180mM、190mM或200mM)的Tris pH8.5-9.5，或当在20℃至37℃之间时具有不超过0.027pH单位/℃的变化的缓冲液(当在0.1M浓度测量时)，其浓度为约5mM至约200mM(例如，约5mM、10mM、25mM、40mM、50mM、60mM、70mM、80mM、90mM、100mM、110mM、120mM、130mM、140mM、150mM、160mM、170mM、180mM、190mM或200mM)；二糖，例如，海藻糖，其浓度为约100mM至约500 mM(例如，约100mM、125mM、150mM、175mM、200mM、225mM、250mM、275mM、300mM、325mM、350mM、375mM、400mM、425mM、450mM、475mM或500mM)；一种或多种渗透剂，例如，肌氨酸、三甲胺N-氧化物(TMAO)、二甲基磺基丙酯和三甲基甘氨酸，其浓度为约50mM至约200mM(例如，约50mM、60mM、70mM、80mM、90mM、100mM、110mM、120mM、130mM、140mM、150mM、160mM、170mM、180mM、190mM或200mM)；吐温-20，其浓度为约0.1％至约0.5％(例如，约0.1％、0.2％、0.3％、0.4％或0.5％)；甘油，其浓度为约1％至约10％(例如，约1％、2％、3％、4％、5％、6％、7％、8％、9％、10％)；DMSO，其浓度为约1％至约10％(例如，约1％、1.5％、2％、2.5％、3％、3.5％、4％、4.5％、5％、5.5％、6％、6.5％、7％、7.5％、8％、8.5％、9％、9.5％或10％)；荧光素，其浓度为约0.001％至约0.01％(例如，约0.001％、0.002％、0.003％、0.004％、0.005％、0.006％、0.007％、0.008％、0.009％或0.01％)；和DNA结合染料(例如，花青染料)，其浓度为约0.5X至约5X(例如，约0.5X、0.6X、0.7X、0.8X、0.9X、1X、1.5X、2X、2.5X、3X、3.5X、4X、4.5X或5X)。

[0155] 已经发现，在扩增反应中包括POPSO可以提高扩增特异性，并且基于该发现，认为，当在20℃至37℃之间时具有不超过0.027pH单位/℃的变化的任意缓冲液(例如，POPSO)将具有相同的作用。因而，上面描述的反应混合物可以包括，例如，HEPES((4-(2-羟乙基)-1-哌嗪乙磺酸))、ACES(N-(2-乙酰氨基)-2-氨基乙磺酸)、PIPES(哌嗪-N,N′-双(2-乙磺酸)、MOPSO(3-(N-吗啉代)-2-羟基丙烷磺酸)、BES(N,N-双(2-羟乙基)-2-氨基乙磺酸)、MOPS(3-(N-吗啉代)丙烷磺酸)、TES(N-三(羟基甲基)甲基-2-氨基乙磺酸)、TAPSO(3-[N-三(羟基甲基)甲基氨基]-2-羟基丙烷磺酸)、POPSO(哌嗪-N,N′-双(2-羟基丙烷磺酸))、BICINE(N,N-双(2-羟乙基)甘氨酸)、TAPS(N-三(羟基甲基)甲基-3-氨基丙烷磺酸)或AMPSO(N-(1,1-二甲基-2-羟乙基)-3-氨基-2-羟基丙烷磺酸)。

[0156] 此外，已经发现了使用具有不良作用的增加量的聚合酶的方法。在以前，由于高浓度的聚合酶可以引起非特异性结合的有害作用，本领域技术 人员不会向扩增反应混合物中加入超过约20U/ml的浓度的聚合酶。令人惊奇地，发明人已经发现，即使在高聚合酶浓度，选自精氨酸、亚精胺和精胺，或其盐的试剂的加入，会增加聚合酶-Sso7缀合物的特异性。因而，在一些实施方案中，本发明的反应混合物包含：浓度为约1mM至约100mM的选自精氨酸、亚精胺和精胺，或其盐的试剂，和浓度为超过50U/ml、超过55U/ml、超过60U/ml、超过

65U/ml、超过70U/ml或超过75U/ml的聚合酶-Sso7缀合物。

[0157] 使用本领域已知的和在下面更详细地描述的测定，可以鉴别和定量由本发明的一些方面引起的效率和特异性的提高。

[0158] 在一些实施方案中，可以使用基于染料的qPCR检测方法来监测利用本发明组分的扩增反应。这样的检测方法一般依赖于监测由于DNA结合染料与扩增的DNA的结合而引起的荧光信号增加。例如，SYBR Green I，即一种常用的荧光DNA结合染料，结合所有双链DNA并且通过测量整个扩增循环中荧光增加来监测检测。SYBR Green I具有分别在494nm和521nm的激发和发射最大值。

[0159] 在其它实施方案中，使用基于探针的qPCR检测方法来监测利用本发明组分的扩增反应。这样的检测方法一般依赖于所需PCR产物的序列特异性检测。与检测全部双链DNA的基于染料的qPCR方法不同，基于探针的qPCR利用荧光标记的靶标特异性探针，其检测扩增DNA中的特定序列。

[0160] 用于提高效率的添加剂

[0161] 在一些方面，可能需要包括另外的化合物作为添加剂来提高扩增反应，包括但不仅限于qPCR中的效率。在一些实施方案中，与缺乏添加剂的对照混合物相比，包含添加剂足以使聚合酶缀合物的效率增加至少5、10、15、20、25、35、40或50％或更多。

[0162] 在一些实施方案中，本发明的聚合酶缀合物在用于包括一些结合染料(诸如，EvaGreen DNA结合染料)的制剂中时，表现出对于一些靶标的低效率。所述低效率中一些实施方案中，可以导致与低输入DNA浓度相关的Ct值延迟。用于测量特定反应的效率的方法是本领域中已知的并在下文中更详细地描述。

[0163] 在一些实施方案中，所述添加剂是包括在本发明的扩增反应中的渗透剂(osmolyte)，以提高效率。渗透剂家族的成员已经显示出提高蛋白的热稳定性(Santoro，Biochemistry(生物化学)，1992)以及降低DNA双螺旋稳定性(Chadalavada，FEBS Letters(FEBS书信)，1997)。在一些实施方案中，渗透剂是小分子或化合物，其通过有生命的生物体应答环境压力诸如极端温度、脱水、或盐度而产生并且保护它们的细胞成分并帮助维持最佳胞质条件。用于本发明中的渗透剂可以包括但不仅限于肌氨酸，三甲胺N-氧化物(TMAO)，二甲基磺基丙酯，和三甲基甘氨酸。肌氨酸化学上类似甜菜碱，即一种已经显示出改善常规PCR的化学制品(Henke，Nucleic Acids Research(核酸研究)，1997)。

[0164] 在渗透剂的常规应用中，该化合物的稳定作用一般在相对高浓度(＞1M)下观察到。然而，在本发明的方法中，已经发现毫摩尔浓度的渗透剂有效提高扩增反应诸如qPCR的反应效率。在不受作用机制束缚的条件下，效率的提高可能是提高含有低浓度输入DNA样品的反应中的DNA聚合酶与DNA模板靶向区域的易接近性的结果。在一些实施方案中，浓度为约100-约1000mM的渗透剂用于本发明的方法和试剂盒中。在其它实施方案中，浓度为约50-约700、约100-约600、约150-约500、约200-约400mM和约300-约350mM的渗透剂用于本发明的方法和试剂盒中。在一些实施方案中，本发明的方法、反应混合物、和试剂盒中使用的渗透剂是肌氨酸(任选地，处于以上列出的浓度)。

[0165] VI.反应混合物

[0166] 在另一个方面，本发明提供了反应混合物，其包含聚合酶(例如，聚合酶-Sso7缀合物)和足以提高核酸扩增的特异性的量的试剂，其中所述试剂选自精氨酸、亚精胺和精胺，

或其盐。所述反应混合物可以任选地包含生物样品，所述生物样品包含靶核酸、一种或多种寡核苷酸、缓冲液、核苷酸三磷酸、盐、稳定剂、一种或多种用于提高效率的添加剂、无核酸酶的水和/或双链DNA结合染料。在一些实施方案中，所述反应混合物包含当在20℃至37℃之间时具有不超过0.027pH单位/℃的变化的缓冲液(例如，POPSO)。在一些实施方案中，所述反应混合物包含具有长斯托克斯位移的荧光染料以及低浓度的第一惰性参比染料(例如，5-或6-羧基-X-罗丹明 染料)。

[0167] 在一些实施方案中，本发明的反应混合物包含：浓度为约1U/ml至约40U/ml的聚合酶-Sso7缀合物，和浓度为约1mM至约100mM的选自精氨酸、亚精胺和精胺，或其盐的试剂。在一些实施方案中，本发明的反应混合物包含：浓度为约5U/ml至约40U/ml的聚合酶-Sso7缀合物，和浓度为约1mM至约50mM的选自精氨酸、亚精胺和精胺，或其盐的试剂。在一些实施方案中，本发明的反应混合物包含：浓度为约10U/ml至约50U/ml的聚合酶-Sso7缀合物，和浓度为约3mM至约30mM的选自精氨酸、亚精胺和精胺，或其盐的试剂。

[0168] VII.试剂盒

[0169] 在另一个方面，本发明提供了用于进行核酸扩增反应的试剂盒。在一些实施方案中，所述试剂盒包括聚合酶(例如，聚合酶-Sso7缀合物)和足以提高核酸扩增的特异性的量的试剂，其中所述试剂选自精氨酸、亚精胺和精胺，或其盐。在一些实施方案中，本发明的试剂盒包括具有聚合酶结构域的聚合酶缀合物，所述聚合酶结构域与SEQ ID NO:1或SEQ ID NO:2基本上相同或相同。在一些实施方案中，本发明的试剂盒包括包含Sso7结构域的聚合酶缀合物，所述Sso7结构域与SEQ ID NO:3或5-30中的任一个基本上相同或相同。在一些实施方案中，本发明的试剂盒包括聚合酶缀合物，所述聚合酶缀合物与SEQ ID NO:31-56中的任一个基本上相同或相同。

[0170] 任选地，所述试剂盒包含一种或多种dNTP、至少一种缓冲液(例如，当在20℃至37℃之间时具有不超过0.027pH单位/℃的变化的缓冲液(例如，POPSO))和/或双链DNA结合染料。在一些实施方案中，所述试剂盒包含具有长斯托克斯位移的荧光染料以及低浓度的第一惰性参比染料(例如，5-或6-羧基-X-罗丹明染料)。这样的试剂盒还可以包括稳定剂和其它添加剂(例如，肌氨酸)，以增加扩增反应的效率。这样的试剂盒还可以包括一种或多种引物以及关于使用所述试剂盒的组分进行核酸扩增反应的说明书。

[0171] 实施例

[0172] 下述实施例意图解释、而不是限制要求保护的发明。

[0173] 实施例1

[0174] 基于SEQ ID NO:2(作为聚合酶结构域)和SEQ ID NO:3(作为Sso7d结构域)，制备了一系列Sso7d-聚合酶缀合物。所述Sso7d结构域在位置28(野生型K)或43(野生型R)处发生突变。如下试验得到的缀合物：扩增模板DNA分子，并使用解链温度分析用SYBR GREEN检测得到的产物。使用1ng HeLa细胞衍生出的cDNA作为模板，进行两步PCR以扩增18s扩增子(18s68)。使用以下的常规2-步PCR方案，在Bio-Rad CFX96qPCR仪器上进行qPCR：在每个扩增循环中，在95℃保持5s的变性步骤，随后在61℃保持30s的退火-延伸步骤。进行40个PCR扩增循环，并随后使用0.5℃温度增量进行解链曲线分析，在每个步骤中保持5s。使用解链曲线分析来评价PCR特异性，这是基于它们的解离(解链)行为来表征双链DNA(dsDNA)的技术，因为它们随着温度升高(Tm)而从dsDNA转变为单链DNA(ssDNA)。一般而言，在解链曲线

分析之前，通过PCR来扩增靶序列。根据核苷酸序列、长度、GC含量和链互补性，PCR产物的解链将产生特异性的解链温度的单个峰。因此，单个解链峰指示一种特异性的PCR产物。多个峰指示除了特异性产物以外还有非特异性产物存在。

[0175] 尽管野生型缀合物产生了非常非特异性的产物(即，在解链曲线分析中的2个峰)，许多在与K28对应的位置处的置换会导致提高的活性，即，降低的非特异性聚合酶活性。其中R28改变为S、T、C、P、D、E、N或Q中的任一个的突变体具有比R28野生型提高的活性(特异性)。R28M和R28R没有显著优于野生型。试验的K28变体Sso7d-聚合酶缀合物包括具有根据SEQ ID NO:31-38的序列的那些缀合物。

[0176] 然后使用1ng HeLa细胞衍生的cDNA作为模板，并在如上所述的相同条件下，进行两步PCR以扩增β-肌动蛋白扩增子(ActB86)，从而评价R43变体Sso7d-聚合酶缀合物的PCR特异性。许多置换导致降低的非特异性聚合酶活性。例如，其中R43改变为G、A、S、T、C、V、L、I、M、F、Y、D、E、N、Q、H、K或W中的任一个的突变体具有比R43野生型提高的活性(特异性)。R43M具有轻微非特异性的肩，但是仍然比野生型显著提高。试验的R43变体Sso7-聚合酶缀合物包括具有根据SEQ ID NO:39-56的序列的那些缀合物。

[0177] 另外，发现通过向PCR反应混合物中加入试剂也可以降低非特异性聚合酶活性。使用10ng、1ng、100pg和10pg HeLa细胞衍生的cDNA作为模板，进行PCR以扩增18s扩增子(18s68)。使用以下的常规2-步PCR方案进行qPCR：在每个扩增循环中，在98℃保持5s的变性步骤，随后在60℃保持30s的退火-延伸步骤。进行40个PCR扩增，并随后如上所述进行解链曲线分析。L-精氨酸单盐酸盐(10mM)、亚精胺三盐酸盐(5mM)或精胺四盐酸盐(5或10mM)的加入会进一步降低试验的Sso7d-聚合酶缀合物变体的非特异性活性。这在使用含有10mM L-精氨酸单盐酸盐的、具有不同模板浓度(从10ng至10pg)的反应混合物的qPCR结果中得到进一步证实。与缺乏游离精氨酸的对照组相比，含有游离精氨酸的对应反应在每种模板浓度具有更多特异性的产物和显著更低的非特异性产物。

[0178] 接着，试验了精氨酸的增强qPCR试剂混合物的抑制剂耐受性的作用。扩增反应混合物含有终浓度为约24U/ml的、与突变的Sso7d结构域(SEQ ID NO:34)缀合的外切核酸酶缺陷型聚合酶(SEQ ID NO:2)和聚合酶抑制剂(2种不同巧克力(Enlveonet或Tanzanie)之一，常见的PCR抑制剂)。从一组样品中省略精氨酸，并以10mM的浓度加入另一组样品中。巧克力在qPCR反应物中的最终百分比浓度在0-2％范围内。使用1ng HeLa细胞衍生的cDNA作为模板，进行PCR以扩增ADAR扩增子(ADAR_162)。使用以下的常规2-步PCR方案进行qPCR：在每个扩增循环中，在95℃保持5s的变性步骤，随后在60℃保持30s的退火-延伸步骤。进行40个PCR扩增，并随后如上所述进行解链曲线分析。在有不同浓度的抑制剂存在下的成功PCR扩增(由扩增反映，并且也通过Ct值评价)指示反应混合物对PCR抑制剂的耐受程度。对于两种巧克力，在有较高浓度的抑制剂存在下，针对在没有精氨酸存在下的试剂混合物观察到差的扩增或没有扩增。相比而言，即使在有2％抑制剂存在下，补充了精氨酸的试剂混合物仍然表现出良好PCR扩增，从而提示了更高的抑制剂耐受性。

[0179] 实施例2

[0180] 确定了POPSO缓冲液用于提高通用qPCR超混合物在检测miRNA中的特异性的方面的作用。如下确定微RNA(miR223)和对照snoRNA (RNU48)的检测和定量：首先将mi/snoRNA转化成cDNA，然后使用杂合融合聚合酶通过实时qPCR定量，所述融合聚合酶具有突变体

Sso7d结构域且不具有外切核酸酶活性。在该实验中，首先通过通用聚腺苷酰化和反转录过程将混合的全人RNA(购自Ambion)转化成cDNA。然后使用预设计的靶标特异性的引物对和用Tris-缓冲液或POPSO-缓冲液构建的qPCR超混合物(所述qPCR超混合物的其它组分是相同的)，使用合成的cDNA作为模板，进行mi/snoRNA检测和定量。如在miR233测定中证实的，基于Tris的制剂在解链曲线分析中产生非特异性的扩增和多个峰。相反，基于POPSO的制剂产生具有干净的单个解链峰的PCR产物，这表明发生了特异性的扩增。除了特异性提高以外，在基于POPSO的制剂和基于Tris的制剂之间没有显著性能差异。两种制剂都在对照测定RNU48snoRNA上表现出可比较的Cq值和干净的解链峰。

[0181] 应当理解，本文所述的实施例和实施方案仅用于例证目的，并且提示本领域技术人员可以以此为依据进行各种变型或改变，且它们包括在本申请的精神和范围和所附权利要求范围内。本文引述的所有公开文献、专利和专利申请通过参考引用为所有目的整体并入本文。

[0182] 非正式序列表

[0183] SEQ ID NO:1

[0184] 具有3'-5'外切核酸酶活性的DNA聚合酶的氨基酸序列

[0185]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
RPITVWRLYFEHPQDVPTIREKIREHSAVVDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFDIETLYHEGEEFGK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVLY
IDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDW
SEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSWL
NIKKS

[0186] SEQ ID NO:2

[0187] 缺乏3'-5'外切核酸酶活性的DNA聚合酶的氨基酸序列

[0188]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
RPITVWRLYFEHPQDVPTIREKIREHSAVVDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFAIATLYHEGEEFGK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVLY
IDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDW
SEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVK

IKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSWL
NIKKS

[0189] SEQ ID NO:3

[0190] Sso7d/Ssh7A/SsoP2

[0191] ＞gi|3891427|pdb|1BNZ|A链A,超嗜热蛋白DNA复合物

[0192] MATVKFKYKGEEKEVDISKIKKVWRVGKMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK

[0193] SEQ ID NO:4

[0194] 接头序列

[0195] GTGGGG

[0196] SEQ ID NO:5

[0197] Sso7d-K28S

[0198] MATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK

[0199] SEQ ID NO:6

[0200] Sso7d-K28T

[0201] MATVKFKYKGEEKEVDISKIKKVWRVGTMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK

[0202] SEQ ID NO:7

[0203] Sso7d-K28C

[0204] MATVKFKYKGEEKEVDISKIKKVWRVGCMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK

[0205] SEQ ID NO:8

[0206] Sso7d-K28P

[0207] MATVKFKYKGEEKEVDISKIKKVWRVGPMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK

[0208] SEQ ID NO:9

[0209] Sso7d-K28D

[0210] MATVKFKYKGEEKEVDISKIKKVWRVGDMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK

[0211] SEQ ID NO:10

[0212] Sso7d-K28E

[0213] MATVKFKYKGEEKEVDISKIKKVWRVGEMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK

[0214] SEQ ID NO:11

[0215] Sso7d-K28N

[0216] MATVKFKYKGEEKEVDISKIKKVWRVGNMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK

[0217] SEQ ID NO:12

[0218] Sso7d-K28Q

[0219] MATVKFKYKGEEKEVDISKIKKVWRVGQMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK

[0220] SEQ ID NO:13

[0221] Sso7d-R43G

[0222] MATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGGGAVSEKDAPKELLQMLEKQKK

[0223] SEQ ID NO:14

[0224] Sso7d-R43A

[0225] MATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGAGAVSEKDAPKELLQMLEKQKK

[0226] SEQ ID NO:15

[0227] Sso7d-R43S

[0228] MATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGSGAVSEKDAPKELLQMLEKQKK

[0229] SEQ ID NO:16

[0230] Sso7d-R43T

[0231] MATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGTGAVSEKDAPKELLQMLEKQKK

[0232] SEQ ID NO:17

[0233] Sso7d-R43C

[0234] MATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGCGAVSEKDAPKELLQMLEKQKK

[0235] SEQ ID NO:18

[0236] Sso7d-R43V

[0237] MATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGVGAVSEKDAPKELLQMLEKQKK

[0238] SEQ ID NO:19

[0239] Sso7d-R43L

[0240] MATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGLGAVSEKDAPKELLQMLEKQKK

[0241] SEQ ID NO:20

[0242] Sso7d-R43I

[0243] MATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGIGAVSEKDAPKELLQMLEKQKK

[0244] SEQ ID NO:21

[0245] Sso7d-R43M

[0246] MATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGMGAVSEKDAPKELLQMLEKQKK

[0247] SEQ ID NO:22

[0248] Sso7d-R43F

[0249] MATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGFGAVSEKDAPKELLQMLEKQKK

[0250] SEQ ID NO:23

[0251] Sso7d-R43Y

[0252] MATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGYGAVSEKDAPKELLQMLEKQKK

[0253] SEQ ID NO:24

[0254] Sso7d-R43W

[0255] MATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGWGAVSEKDAPKELLQMLEKQKK

[0256] SEQ ID NO:25

[0257] Sso7d-R43D

[0258] MATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGDGAVSEKDAPKELLQMLEKQKK

[0259] SEQ ID NO:26

[0260] Sso7d-R43E

[0261] MATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGEGAVSEKDAPKELLQMLEKQKK

[0262] SEQ ID NO:27

[0263] Sso7d-R43N

[0264] MATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGNGAVSEKDAPKELLQMLEKQKK

[0265] SEQ ID NO:28

[0266] Sso7d-R43Q

[0267] MATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGQGAVSEKDAPKELLQMLEKQKK

[0268] SEQ ID NO:29

[0269] Sso7d-R43H

[0270] MATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGHGAVSEKDAPKELLQMLEKQKK

[0271] SEQ ID NO:30

[0272] Sso7d-R43K

[0273] MATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGKGAVSEKDAPKELLQMLEKQKK

[0274] SEQ ID NO:31

[0275] 外-聚合酶+接头+Sso7d-K28S

[0276]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
RPITVWRLYFEHPQDVPTIREKIREHSAVVDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFAIATLYHEGEEFGK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVLY
IDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDW
SEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSWL
NIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK

[0277] SEQ ID NO:32

[0278] 外-聚合酶+接头+Sso7d-K28T

[0279]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
RPITVWRLYFEHPQDVPTIREKIREHSAVVDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFAIATLYHEGEEFGK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKT KMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVL
YIDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRD
WSEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGV
KIKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSW
LNIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGTMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK

[0280] SEQ ID NO:33

[0281] 外-聚合酶+接头+Sso7d-K28C

[0282]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
RPITVWRLYFEHPQDVPTIREKIREHSAVVDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFAIATLYHEGEEFGK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVLY
IDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDW
SEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSWL
NIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGCMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK

[0283] SEQ ID NO:34

[0284] 外-聚合酶+接头+Sso7d-K28P

[0285]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
RPITVWRLYFEHPQDVPTIREKIREHSAVVDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFAIATLYHEGEEFGK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVLY
IDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDW
SEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSWL
NIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGPMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK

[0286] SEQ ID NO:35

[0287] 外-聚合酶+接头+Sso7d-K28D

[0288]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
RPITVWRLYFEHPQDVPTIREKIREHSAVVDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFAIATLYHEGEEFGK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
A K Y S M E D A K A T Y E L G K E F F P M E A Q L S R L V G Q P L W D V S R S S T G
NLVEWFLLRKAYERNELAPNKPDEREYERRLRESYAGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNRE
GCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQKIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKAR
WYCKECAESVTAWGREYIEFVWKELEEKFGFKVLYIDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYE
GFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDWSEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPP
EKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVKIKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIE

NQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSWLNIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGDMISF
TYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK

[0289] SEQ ID NO:36

[0290] 外-聚合酶+接头+Sso7d-K28E

[0291]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
RPITVWRLYFEHPQDVPTIREKIREHSAVVDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFAIATLYHEGEEFGK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVLY
IDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDW
SEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSWL
NIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGEMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK

[0292] SEQ ID NO:37

[0293] 外-聚合酶+接头+Sso7d-K28N

[0294]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
RPITVWRLYFEHPQDVPTIREKIREHSAVVDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFAIATLYHEGEEFGK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVLY
IDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDW
SEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSWL
NIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGNMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK

[0295] SEQ ID NO:38

[0296] 外-聚合酶+接头+Sso7d-K28Q

[0297]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
RPITVWRLYFEHPQDVPTIREKIREHSAVVDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFAIATLYHEGEEFGK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGS EPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLER
VAKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRES
YAGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDER

QKIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVL
YIDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRD
WSEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGV
KIKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSW
LNIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGQMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK

[0298] SEQ ID NO:39

[0299] 外-聚合酶+接头+Sso7d-R43G

[0300]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
RPITVWRLYFEHPQDVPTIREKIREHSAVVDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFAIATLYHEGEEFGK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVLY
IDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDW
SEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSWL
NIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK

[0301] SEQ ID NO:40

[0302] 外-聚合酶+接头+Sso7d-R43A

[0303]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
RPITVWRLYFEHPQDVPTIREKIREHSAVVDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFAIATLYHEGEEFGK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVLY
IDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDW
SEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSWL
NIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK

[0304] SEQ ID NO:41

[0305] 外-聚合酶+接头+Sso7d-R43S

[0306]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
R P I T V W R L Y F E H P Q D V P T I R E K I R E H S A V V D I F E Y D I P F A K
RYLIDKGLIPMEGDEELKLLAFAIATLYHEGEEFGKGPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFL

KIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLTIGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPT
YTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERVAKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSST
GNLVEWFLLRKAYERNELAPNKPDEREYERRLRESYAGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNR
EGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQKIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKA
RWYCKECAESVTAWGREYIEFVWKELEEKFGFKVLYIDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEY
EGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDWSEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIP
PEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVKIKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYI
ENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSWLNIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGSMIS
FTYDEGGGKTG[illegible]GAVSEKDAPKELLQMLEKQKK

[0307] SEQ ID NO:42

[0308] 外-聚合酶+接头+Sso7d-R43T

[0309]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
RPITVWRLYFEHPQDVPTIREKIREHSAVVDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFAIATLYHEGEEFGK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVLY
IDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDW
SEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSWL
NIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTG[illegible]GAVSEKDAPKELLQMLEKQKK

[0310] SEQ ID NO:43

[0311] 外-聚合酶+接头+Sso7d-R43C

[0312]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
RPITVWRLYFEHPQDVPTIREKIREHSAVVDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFAIATLYHEGEEFGK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKTKMKAS QDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVL
YIDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRD
WSEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGV
KIKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSW
LNIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTG[illegible]GAVSEKDAPKELLQMLEKQKK

[0313] SEQ ID NO:44

[0314] 外-聚合酶+接头+Sso7d-R43V

[0315]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
RPITVWRLYFEHPQDVPTIREKIREHSAVVDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFAIATLYHEGEEFGK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVLY
IDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDW
SEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSWL
NIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTG[redacted]GAVSEKDAPKELLQMLEKQKK

[0316] SEQ ID NO:45

[0317] 外-聚合酶+接头+Sso7d-R43L

[0318]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
RPITVWRLYFEHPQDVPTIREKIREHSAVVDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFAIATLYHEGEEFGK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVLY
IDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDW
SEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSWL
NIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTG[redacted]GAVSEKDAPKELLQMLEKQKK

[0319] SEQ ID NO:46

[0320] 外-聚合酶+接头+Sso7d-R43I

[0321]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
RPITVWRLYFEHPQDVPTIREKIREHSAVVDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFAIATLYHEGEEFGK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVLY
IDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDW
SEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSWL

NIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTG[illegible]GAVSEKDAPKELLQMLEKQKK

[0322] SEQ ID NO:47

[0323] 外-聚合酶+接头+Sso7d-R43M

[0324]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
RPITVWRLYFEHPQDVPTIREKIREHSAVVDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFAIATLYHEGEEFGK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVLY
IDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDW
SEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSWL
NIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTG[illegible]GAVSEKDAPKELLQMLEKQKK

[0325] SEQ ID NO:48

[0326] 外-聚合酶+接头+Sso7d-R43F

[0327]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
RPITVWRLYFEHPQDVPTIREKIREHSAVVDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFAIATLYHEGEEFGK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVLY
IDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDW
SEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSWL
NIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTG[illegible]GAVSEKDAPKELLQMLEKQKK

[0328] SEQ ID NO:49

[0329] 外-聚合酶+接头+Sso7d-R43Y

[0330]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
RPITVWRLYFEHPQDVPTIREKIREHSAVVDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFAIATLYHEGEEFGK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVLY

IDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDW
SEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKE
DLRWQKTKQTGLTSWLNIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTG[illegible]
GAVSEKDAPKELLQMLEKQKK

[0331] SEQ ID NO:50

[0332] 外-聚合酶+接头+Sso7d-R43W

[0333]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
RPITVWRLYFEHPQDVPTIREKIREHSAVVDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFAIATLYHEGEEFGK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVLY
IDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDW
SEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSWL
NIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTG[illegible]GAVSEKDAPKELLQMLEKQKK

[0334] SEQ ID NO:51

[0335] 外-聚合酶+接头+Sso7d-R43D

[0336]

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GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVLY
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SEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSWL
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[0337] SEQ ID NO:52

[0338] 外-聚合酶+接头+Sso7d-R43E

[0339]

MILDADYITEEGKPVIRLFKKENGEFKIEHDRTFRPYIYALLKDDSKIEEVKKITAERHGKIVRIVDAEKVEKKFLG
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GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT

IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
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AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVLY
IDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDW
SEIAKETQARVLEAILKHGNVE EAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGV
KIKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSW
LNIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTG[illegible]GAVSEKDAPKELLQMLEKQKK

[0340] SEQ ID NO:53

[0341] 外-聚合酶+接头+Sso7d-R43N

[0342]

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GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
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IDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDW
SEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSWL
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[0343] SEQ ID NO:54

[0344] 外-聚合酶+接头+Sso7d-R43Q

[0345]

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GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
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AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
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SEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVK
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NIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTG[illegible]GAVSEKDAPKELLQMLEKQKK

[0346] SEQ ID NO:55

[0347] 外-聚合酶+接头+Sso7d-R43H

[0348]

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GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
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AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
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KIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYI
EFVWKELEEKFGFKVLYIDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALID
EEGKIITRGLEIVRRDWSEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKA
IGPHVAVAKRLAAKGVKIKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRK
EDLRWQKTKQTGLTSWLNIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTG[illegible]
GAVSEKDAPKELLQMLEKQKK

[0349] SEQ ID NO:56

[0350] 外-聚合酶+接头+Sso7d-R43K

[0351]

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GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKIIREKDPDIIITYNGDSFDLPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWETGEGLERV
AKYSMEDAKATYELGKEFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESY
AGGFVKEPEKGLWENIVSLDFRALYPSIIITHNVSPDTLNREGCRNYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
KIKTKMKASQDPIEKIMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVWKELEEKFGFKVLY
IDTDGLYATIPGGKSEEIKKKALEFVDYINAKLPGLLELEYEGFYKRGFFVTKKKYALIDEEGKIITRGLEIVRRDW
SEIAKETQARVLEAILKHGNVEEAVRIVKEVTQKLSKYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPRKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRWQKTKQTGLTSWL
NIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGSMISFTYDEGGGKTG[illegible]GAVSEKDAPKELLQMLEKQKK

[0352] SEQ ID NO:57

[0353] Ssh7b

[0354] >gi|3138797|dbj|BAA28275.1|[芝田硫化叶菌(Sulfolobus shibatae)]

[0355] MVTVKFKYKGEEKEVDTSKIKKVWRVGKMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK

[0356] SEQ ID NO:58

[0357] Sac7d

[0358] >gi|152933|gb|AAA80315.1|DNA结合蛋白[硫化叶菌属物种(Sulfolobus sp.)]

[0359] MVKVKFKYKGEEKEVDTSKIKKVWRVGKMVSFTYDDNGKTGRGAVSEKDAPKELLDMLARAEREKK

[0360] SEQ ID NO:59

[0361] Sac7e

[0362] >gi|70606201|ref|YP_255071.1|DNA结合蛋白7e

[0363] [酸热硫化叶菌(Sulfolobus acidocaldarius)DSM639]

[0364] MAKVRFKYKGEEKEVDTSKIKKVWRVGKMVSFTYDDNGKTGRGAVSEKDAPKELMDMLARAEKKK

[0365] SEQ ID NO:60

[0366] Sto7e

[0367] ＞gi|15920860|ref|NP_376529.1|DNA结合蛋白7e[Sulfolobus tokodaii菌株7]

[0368] MVTVKFKYKGEEKEVDISKIKKVWRVGKMISFTYDDNGKTGRGAVSEKDAPKELLQMLEKSGKK

[0369] SEQ ID NO:61(聚合酶序列)

[0370]

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GPIIMISYADENEAKVITWKNIDLPYVEVVSSEREMIKRFLRVIREKDPDVIITYNGDSFDFPYLVKRAEKLGIKLT
IGRDGSEPKMQRLGDMTAVEIKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYADEIAEAWESGEGLERV
AKYSMEDAKVTYELGKEFLPMEIQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNEVAPNKPSEEEYERRLRESY
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SEIAKETQARVLETILKHGNVEEAVKIVKEVTQKLANYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKKLAARGVK
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NIKKS

序列表

<110> 程曼
伯乐实验室公司

<120> 具有降低的非特异性活性的PCR反应混合物

<130> 65654-831499

<140> WO尚未指定
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<150> US 61/473,710
<151> 2011-04-08

<160> 63

<170> FastSEQ for Windows Version 4.0

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<211> 775
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<220>
<223> 具有3'-5'外切核酸酶活性的合成的DNA聚合酶

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Arg Leu Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu His Asp Arg
20 25 30
Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Lys Ile
35 40 45
Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Lys Ile Val Arg
50 55 60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65 70 75 80
Thr Val Trp Arg Leu Tyr Phe Glu His Pro Gln Asp Val Pro Thr Ile
85 90 95
Arg Glu Lys Ile Arg Glu His Ser Ala Val Val Asp Ile Phe Glu Tyr
100 105 110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
115 120 125
Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Asp Ile Glu Thr
130 135 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145 150 155 160
Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
165 170 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
180 185 190
Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
195 200 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
210 215 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225 230 235 240
Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
245 250 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
260 265 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
275 280 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
290 295 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305 310 315 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
325 330 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
340 345 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
355 360 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
370 375 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn

[0002]

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385                 390                 395                 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
                405                 410                 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
            420                 425                 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
        435                 440                 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
    450                 455                 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465                 470                 475                 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
                485                 490                 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
            500                 505                 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
        515                 520                 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
    530                 535                 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545                 550                 555                 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
                565                 570                 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
            580                 585                 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
        595                 600                 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
    610                 615                 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625                 630                 635                 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
                645                 650                 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
            660                 665                 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
        675                 680                 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
    690                 695                 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705                 710                 715                 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
                725                 730                 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
            740                 745                 750
Lys Glu Asp Leu Arg Trp Glu Lys Thr Lys Gln Thr Gly Leu Thr Ser
        755                 760                 765
Trp Leu Asn Ile Lys Lys Ser
    770                 775
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<210> 2
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<220>
<223> 缺乏3’—5’外切核酸酶活性的合成的DNA聚合酶

<400> 2

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            20                  25                  30
Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Lys Ile
        35                  40                  45
Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Lys Ile Val Arg
    50                  55                  60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65                  70                  75                  80
Thr Val Trp Arg Leu Tyr Phe Glu His Pro Glu Asp Val Pro Thr Ile
                85                  90                  95
Arg Glu Lys Ile Arg Glu His Ser Ala Val Val Asp Ile Phe Glu Tyr
            100                 105                 110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
        115                 120                 125
Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Ala Ile Ala Thr
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[0003]

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Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145                 150                 155                 160
Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
                165                 170                 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
            180                 185                 190
Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
        195                 200                 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
    210                 215                 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225                 230                 235                 240
Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
                245                 250                 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
            260                 265                 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
        275                 280                 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
    290                 295                 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305                 310                 315                 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
                325                 330                 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
            340                 345                 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
        355                 360                 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
    370                 375                 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385                 390                 395                 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
                405                 410                 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
            420                 425                 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
        435                 440                 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
    450                 455                 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465                 470                 475                 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
                485                 490                 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
            500                 505                 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
        515                 520                 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
    530                 535                 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545                 550                 555                 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
                565                 570                 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
            580                 585                 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
        595                 600                 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
    610                 615                 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625                 630                 635                 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
                645                 650                 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
            660                 665                 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
        675                 680                 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
    690                 695                 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705                 710                 715                 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
                725                 730                 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
            740                 745                 750
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Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
        755                 760                 765
Trp Leu Asn Ile Lys Lys Ser
    770                 775


<210> 3
<211> 64
<212> PRT
<213> 酸热硫化叶菌(Sulfolobus acidocaldarius)

<220>
<223> 酸热硫化叶菌 Sso7d, Sso7d/Ssh7A/SsoP2,
      DNA结合蛋白7d, 链A, 超嗜热蛋白DNA复合物; Sulfolobus solfataricus菌株P2 Sso7d

<400> 3
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 1               5                  10                  15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Lys Met Ile Ser Phe
            20                  25                  30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Arg Gly Ala Val Ser Glu
        35                  40                  45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
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<210> 4
<211> 6
<212> PRT
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<220>
<223> 合成的杂合聚合酶或聚合酶缀合接头

<400> 4
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[0004]

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<220>
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Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe
            20                  25                  30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Arg Gly Ala Val Ser Glu
        35                  40                  45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
    50                  55                  60


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<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-K28T

<400> 6
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
 1               5                  10                  15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Thr Met Ile Ser Phe
            20                  25                  30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Arg Gly Ala Val Ser Glu
        35                  40                  45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
    50                  55                  60
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<210> 7
<211> 64
<212> PRT
<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-K28C

<400> 7
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Cys Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Arg Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 8
<211> 64
<212> PRT
<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-K28P

<400> 8
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Pro Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Arg Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

[0005] <210> 9
<211> 64
<212> PRT
<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-K28D

<400> 9
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Asp Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Arg Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 10
<211> 64
<212> PRT
<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-K28E

<400> 10
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Glu Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Arg Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 11

<211> 64
<212> PRT
<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-K28N

<400> 11
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Asn Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Arg Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 12
<211> 64
<212> PRT
<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-K28Q

<400> 12
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Gln Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Arg Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

[0006]

<210> 13
<211> 64
<212> PRT
<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-R43G

<400> 13
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Gly Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 14
<211> 64
<212> PRT
<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-R43A

<400> 14
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Ala Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 15
<211> 64

<212> PRT
<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-R43S

<400> 15
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Ser Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 16
<211> 64
<212> PRT
<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-R43T

<400> 16
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Thr Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 17
<211> 64
[0007] <212> PRT
<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-R43C

<400> 17
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Cys Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 18
<211> 64
<212> PRT
<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-R43V

<400> 18
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Val Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 19
<211> 64
<212> PRT

<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-R43L

<400> 19
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Leu Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 20
<211> 64
<212> PRT
<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-R43I

<400> 20
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Ile Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 21
<211> 64
<212> PRT
[0008] <213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-R43M

<400> 21
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Met Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 22
<211> 64
<212> PRT
<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-R43F

<400> 22
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Phe Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 23
<211> 64
<212> PRT
<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-R43Y

<400> 23
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Tyr Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 24
<211> 64
<212> PRT
<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-R43W

<400> 24
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Trp Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 25
<211> 64
<212> PRT
<213> 人工序列

[0009]

<220>
<223> 合成的Sso7结构域Sso7d-R43D

<400> 25
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Asp Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 26
<211> 64
<212> PRT
<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-R43E

<400> 26
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Glu Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 27
<211> 64
<212> PRT
<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-R43N

<400> 27
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Asn Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 28
<211> 64
<212> PRT
<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-R43Q

<400> 28
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Gln Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 29
<211> 64
<212> PRT
<213> 人工序列

[0010] <220>
<223> 合成的Sso7结构域Sso7d-R43H

<400> 29
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly His Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 30
<211> 64
<212> PRT
<213> 人工序列

<220>
<223> 合成的Sso7结构域Sso7d-R43K

<400> 30
Met Ala Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Lys Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 31
<211> 844
<212> PRT
<213> 人工序列

<220>

<223> 合成的外-聚合酶 + 接头 + Sso7d-K28S
聚合酶缀合物

<400> 31

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Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
 1               5                  10                  15
Arg Leu Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu His Asp Arg
            20                  25                  30
Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Lys Ile
        35                  40                  45
Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Lys Ile Val Arg
    50                  55                  60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65                  70                  75                  80
Thr Val Trp Arg Leu Tyr Phe Glu His Pro Gln Asp Val Pro Thr Ile
                85                  90                  95
Arg Glu Lys Ile Arg Glu His Ser Ala Val Val Asp Ile Phe Glu Tyr
            100                 105                 110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
        115                 120                 125
Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Ala Ile Ala Thr
    130                 135                 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145                 150                 155                 160
Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
                165                 170                 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
            180                 185                 190
Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
        195                 200                 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
    210                 215                 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225                 230                 235                 240
Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
                245                 250                 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
            260                 265                 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
        275                 280                 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
    290                 295                 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305                 310                 315                 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
                325                 330                 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
            340                 345                 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
        355                 360                 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
    370                 375                 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385                 390                 395                 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
                405                 410                 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
            420                 425                 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
        435                 440                 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
    450                 455                 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465                 470                 475                 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
                485                 490                 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
            500                 505                 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
        515                 520                 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
    530                 535                 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545                 550                 555                 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
                565                 570                 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
```

[0011]

580 585 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
595 600 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
610 615 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625 630 635 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
645 650 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
660 665 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
675 680 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
690 695 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705 710 715 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
725 730 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
740 745 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
755 760 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
770 775 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785 790 795 800
Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe Thr Tyr Asp Glu
805 810 815
Gly Gly Gly Lys Thr Gly Arg Gly Ala Val Ser Glu Lys Asp Ala Pro
820 825 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
835 840

<210> 32
<211> 844
<212> PRT
[0012] <213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-K28T
聚合酶缀合物

<400> 32
Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
1 5 10 15
Arg Leu Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu His Asp Arg
20 25 30
Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Lys Ile
35 40 45
Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Lys Ile Val Arg
50 55 60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65 70 75 80
Thr Val Trp Arg Leu Tyr Phe Glu His Pro Gln Asp Val Pro Thr Ile
85 90 95
Arg Glu Lys Ile Arg Glu His Ser Ala Val Val Asp Ile Phe Glu Tyr
100 105 110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
115 120 125
Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Ala Ile Ala Thr
130 135 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145 150 155 160
Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
165 170 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
180 185 190
Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
195 200 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
210 215 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225 230 235 240
Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
245 250 255

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

[0013] Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
565 570 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
580 585 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
595 600 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
610 615 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625 630 635 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
645 650 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
660 665 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
675 680 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
690 695 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705 710 715 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
725 730 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
740 745 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
755 760 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
770 775 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785 790 795 800
Lys Lys Val Trp Arg Val Gly Thr Met Ile Ser Phe Thr Tyr Asp Glu
805 810 815
Gly Gly Gly Lys Thr Gly Arg Gly Ala Val Ser Glu Lys Asp Ala Pro
820 825 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
835 840

<210> 33

<211> 844
<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-K28C
聚合酶缀合物

<400> 33

[0014]

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Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
 1               5                  10                  15
Arg Leu Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu His Asp Arg
            20                  25                  30
Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Lys Ile
        35                  40                  45
Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Lys Ile Val Arg
    50                  55                  60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65                  70                  75                  80
Thr Val Trp Arg Leu Tyr Phe Glu His Pro Gln Asp Val Pro Thr Ile
                85                  90                  95
Arg Glu Lys Ile Arg Glu His Ser Ala Val Val Asp Ile Phe Glu Tyr
            100                 105                 110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
        115                 120                 125
Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Ala Ile Ala Thr
    130                 135                 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145                 150                 155                 160
Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
                165                 170                 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
            180                 185                 190
Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
        195                 200                 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
    210                 215                 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225                 230                 235                 240
Met Glu Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
                245                 250                 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
            260                 265                 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
        275                 280                 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
    290                 295                 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305                 310                 315                 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
                325                 330                 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
            340                 345                 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
        355                 360                 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
    370                 375                 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385                 390                 395                 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
                405                 410                 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
            420                 425                 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
        435                 440                 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Glu Lys Ile
    450                 455                 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465                 470                 475                 480
Asp Tyr Arg Glu Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
                485                 490                 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
            500                 505                 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
        515                 520                 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
    530                 535                 540
```

```
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545                 550                 555                 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
                565                 570                 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
            580                 585                 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
        595                 600                 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
    610                 615                 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625                 630                 635                 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
                645                 650                 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Glu Ile Thr Arg Pro Leu His
            660                 665                 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
        675                 680                 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
    690                 695                 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705                 710                 715                 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
                725                 730                 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
            740                 745                 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
        755                 760                 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
    770                 775                 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785                 790                 795                 800
Lys Lys Val Trp Arg Val Gly Cys Met Ile Ser Phe Thr Tyr Asp Glu
                805                 810                 815
Gly Gly Gly Lys Thr Gly Arg Gly Ala Val Ser Glu Lys Asp Ala Pro
            820                 825                 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
        835                 840
```

[0015]

<210> 34
<211> 844
<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-K28P
聚合酶缀合物

<400> 34

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

[0016]

```
     210                 215                 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225                 230                 235                 240
Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
                245                 250                 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
            260                 265                 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
        275                 280                 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
    290                 295                 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305                 310                 315                 320
Glu Leu Gly Lys Gln Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
                325                 330                 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
            340                 345                 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
        355                 360                 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
    370                 375                 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385                 390                 395                 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
                405                 410                 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
            420                 425                 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
        435                 440                 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
    450                 455                 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465                 470                 475                 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
                485                 490                 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
            500                 505                 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
        515                 520                 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
    530                 535                 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545                 550                 555                 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
                565                 570                 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
            580                 585                 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
        595                 600                 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
    610                 615                 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625                 630                 635                 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
                645                 650                 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
            660                 665                 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
        675                 680                 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
    690                 695                 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705                 710                 715                 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
                725                 730                 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
            740                 745                 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
        755                 760                 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
    770                 775                 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785                 790                 795                 800
Lys Lys Val Trp Arg Val Gly Pro Met Ile Ser Phe Thr Tyr Asp Glu
                805                 810                 815
Gly Gly Gly Lys Thr Gly Arg Gly Ala Val Ser Glu Lys Asp Ala Pro
            820                 825                 830
```

Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
835 840

<210> 35
<211> 844
<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-K28D
聚合酶缀合物

<400> 35
Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
1 5 10 15
Arg Leu Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu His Asp Arg
20 25 30
Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Lys Ile
35 40 45
Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Lys Ile Val Arg
50 55 60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65 70 75 80
Thr Val Trp Arg Leu Tyr Phe Glu His Pro Gln Asp Val Pro Thr Ile
85 90 95
Arg Glu Lys Ile Arg Glu His Ser Ala Val Val Asp Ile Phe Glu Tyr
100 105 110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
115 120 125
Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Ala Ile Ala Thr
130 135 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145 150 155 160
Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
165 170 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
180 185 190
[0017] Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
195 200 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
210 215 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225 230 235 240
Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
245 250 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
260 265 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
275 280 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
290 295 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305 310 315 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
325 330 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
340 345 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
355 360 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
370 375 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385 390 395 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
405 410 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
420 425 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
435 440 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
450 455 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465 470 475 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
485 490 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu

[0018]

```
                500                 505                 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
        515                 520                 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
    530                 535                 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545                 550                 555                 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
                565                 570                 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
            580                 585                 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
        595                 600                 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
    610                 615                 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625                 630                 635                 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
                645                 650                 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
            660                 665                 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
        675                 680                 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
    690                 695                 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705                 710                 715                 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
                725                 730                 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
            740                 745                 750
Lys Glu Asp Leu Arg Trp Glu Lys Thr Lys Gln Thr Gly Leu Thr Ser
        755                 760                 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
    770                 775                 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785                 790                 795                 800
Lys Lys Val Trp Arg Val Gly Asp Met Ile Ser Phe Thr Tyr Asp Glu
                805                 810                 815
Gly Gly Gly Lys Thr Gly Arg Gly Ala Val Ser Glu Lys Asp Ala Pro
            820                 825                 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
        835                 840
```

<210> 36
<211> 844
<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-K28E
聚合酶缀合物

<400> 36

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

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

[0019]

Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
485 490 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
500 505 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
515 520 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
530 535 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545 550 555 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
565 570 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
580 585 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
595 600 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
610 615 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625 630 635 640
Val Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Ile
645 650 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
660 665 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
675 680 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
690 695 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705 710 715 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
725 730 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
740 745 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
755 760 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
770 775 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile

785 790 795 800
Lys Lys Val Trp Arg Val Gly Glu Met Ile Ser Phe Thr Tyr Asp Glu
805 810 815
Gly Gly Gly Lys Thr Gly Arg Gly Ala Val Ser Glu Lys Asp Ala Pro
820 825 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
835 840

<210> 37
<211> 844
<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-K28N
聚合酶缀合物

<400> 37
Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
1 5 10 15
Arg Leu Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu His Asp Arg
20 25 30
Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Lys Ile
35 40 45
Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Lys Ile Val Arg
50 55 60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65 70 75 80
Thr Val Trp Arg Leu Tyr Phe Glu His Pro Gln Asp Val Pro Thr Ile
85 90 95
Arg Glu Lys Ile Arg Glu His Ser Ala Val Val Asp Ile Phe Glu Tyr
100 105 110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
115 120 125
Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Ala Ile Ala Thr
130 135 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145 150 155 160
Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
165 170 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
180 185 190
Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
195 200 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
210 215 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225 230 235 240
Met Glu Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
245 250 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
260 265 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
275 280 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
290 295 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305 310 315 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
325 330 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
340 345 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
355 360 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
370 375 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385 390 395 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
405 410 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
420 425 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
435 440 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
450 455 460

[0020]

```
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465                 470                 475                 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
                485                 490                 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
            500                 505                 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
        515                 520                 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
    530                 535                 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545                 550                 555                 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
                565                 570                 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
            580                 585                 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
        595                 600                 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
    610                 615                 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625                 630                 635                 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
                645                 650                 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
            660                 665                 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
        675                 680                 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
    690                 695                 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705                 710                 715                 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
                725                 730                 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
            740                 745                 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
        755                 760                 765
```

[0021]

```
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
    770                 775                 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785                 790                 795                 800
Lys Lys Val Trp Arg Val Gly Asn Met Ile Ser Phe Thr Tyr Asp Glu
                805                 810                 815
Gly Gly Gly Lys Thr Gly Arg Gly Ala Val Ser Glu Lys Asp Ala Pro
            820                 825                 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
        835                 840
```

<210> 38
<211> 844
<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-K28Q
聚合酶缀合物

<400> 38

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

[0022]

```
    130                 135                 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145                 150                 155                 160
Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
                165                 170                 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
            180                 185                 190
Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
        195                 200                 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
    210                 215                 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225                 230                 235                 240
Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
                245                 250                 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
            260                 265                 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
        275                 280                 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
    290                 295                 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305                 310                 315                 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
                325                 330                 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
            340                 345                 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
        355                 360                 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
    370                 375                 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385                 390                 395                 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
                405                 410                 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
            420                 425                 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
        435                 440                 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
    450                 455                 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465                 470                 475                 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
                485                 490                 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
            500                 505                 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
        515                 520                 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
    530                 535                 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545                 550                 555                 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
                565                 570                 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
            580                 585                 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
        595                 600                 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
    610                 615                 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625                 630                 635                 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
                645                 650                 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
            660                 665                 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
        675                 680                 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
    690                 695                 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705                 710                 715                 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
                725                 730                 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
            740                 745                 750
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Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
755 760 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
770 775 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785 790 795 800
Lys Lys Val Trp Arg Val Gly Gln Met Ile Ser Phe Thr Tyr Asp Glu
805 810 815
Gly Gly Gly Lys Thr Gly Arg Gly Ala Val Ser Glu Lys Asp Ala Pro
820 825 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
835 840

<210> 39
<211> 844
<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-R43G
聚合酶缀合物

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Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
1 5 10 15
Arg Leu Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu His Asp Arg
20 25 30
Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Lys Ile
35 40 45
Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Lys Ile Val Arg
50 55 60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65 70 75 80
Thr Val Trp Arg Leu Tyr Phe Glu His Pro Gln Asp Val Pro Thr Ile
85 90 95
Arg Glu Lys Ile Arg Glu His Ser Ala Val Val Asp Ile Phe Glu Tyr
100 105 110
[0023] Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
115 120 125
Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Ala Ile Ala Thr
130 135 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145 150 155 160
Ser Tyr Ala Asp Glu Glu Gln Ala Lys Val Ile Thr Trp Lys Lys Ile
165 170 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
180 185 190
Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
195 200 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
210 215 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225 230 235 240
Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
245 250 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
260 265 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
275 280 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
290 295 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305 310 315 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
325 330 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
340 345 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
355 360 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
370 375 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385 390 395 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
405 410 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr

[0024]

```
                420                 425                 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
        435                 440                 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
    450                 455                 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465                 470                 475                 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
                485                 490                 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
            500                 505                 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
        515                 520                 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
    530                 535                 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545                 550                 555                 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
                565                 570                 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
            580                 585                 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
        595                 600                 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
    610                 615                 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625                 630                 635                 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
                645                 650                 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
            660                 665                 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
        675                 680                 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
    690                 695                 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705                 710                 715                 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
                725                 730                 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
            740                 745                 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
        755                 760                 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
    770                 775                 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785                 790                 795                 800
Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe Thr Tyr Asp Glu
                805                 810                 815
Gly Gly Gly Lys Thr Gly Gly Gly Ala Val Ser Glu Lys Asp Ala Pro
            820                 825                 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
        835                 840
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<210> 40
<211> 844
<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-R43A
聚合酶缀合物

<400> 40

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Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
 1                   5                  10                  15
Arg Leu Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu His Asp Arg
            20                  25                  30
Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Lys Ile
        35                  40                  45
Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Lys Ile Val Arg
    50                  55                  60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65                  70                  75                  80
Thr Val Trp Arg Leu Tyr Phe Glu His Pro Gln Asp Val Pro Thr Ile
                85                  90                  95
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Arg Glu Lys Ile Arg Glu His Ser Ala Val Val Asp Ile Phe Glu Tyr
100 105 110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
115 120 125
Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Ala Ile Ala Thr
130 135 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145 150 155 160
Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
165 170 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
180 185 190
Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
195 200 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
210 215 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225 230 235 240
Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
245 250 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
260 265 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
275 280 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
290 295 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305 310 315 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
325 330 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
340 345 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
355 360 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
370 375 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385 390 395 400
[0025] Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
405 410 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
420 425 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
435 440 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
450 455 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465 470 475 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
485 490 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
500 505 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
515 520 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
530 535 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545 550 555 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
565 570 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
580 585 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
595 600 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
610 615 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625 630 635 640
Val Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Ile
645 650 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Glu Ile Thr Arg Pro Leu His
660 665 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
675 680 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
690 695 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu

705 710 715 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
725 730 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
740 745 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
755 760 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
770 775 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785 790 795 800
Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe Thr Tyr Asp Glu
805 810 815
Gly Gly Gly Lys Thr Gly Ala Gly Ala Val Ser Glu Lys Asp Ala Pro
820 825 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
835 840

<210> 41
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聚合酶缀合物

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Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
1 5 10 15
Arg Leu Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu His Asp Arg
20 25 30
Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Lys Ile
35 40 45
Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Lys Ile Val Arg
50 55 60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65 70 75 80
Thr Val Trp Arg Leu Tyr Phe Glu His Pro Gln Asp Val Pro Thr Ile
85 90 95
Arg Glu Lys Ile Arg Glu His Ser Ala Val Val Asp Ile Phe Glu Tyr
100 105 110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
115 120 125
Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Ala Ile Ala Thr
130 135 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145 150 155 160
Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
165 170 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
180 185 190
Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
195 200 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
210 215 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225 230 235 240
Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
245 250 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
260 265 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
275 280 285
Lys Val Tyr Ala Asp Gln Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
290 295 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305 310 315 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
325 330 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
340 345 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
355 360 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
370 375 380

[0026]

Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385 390 395 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
405 410 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
420 425 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
435 440 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
450 455 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465 470 475 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
485 490 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
500 505 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
515 520 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
530 535 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545 550 555 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
565 570 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
580 585 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
595 600 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
610 615 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625 630 635 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
645 650 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
660 665 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
675 680 685

[0027]

Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
690 695 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705 710 715 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
725 730 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
740 745 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
755 760 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
770 775 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785 790 795 800
Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe Thr Tyr Asp Glu
805 810 815
Gly Gly Gly Lys Thr Gly Ser Gly Ala Val Ser Glu Lys Asp Ala Pro
820 825 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
835 840

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<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-R43T
聚合酶缀合物

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

[0028]

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     50                  55                  60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65                  70                  75                  80
Thr Val Trp Arg Leu Tyr Phe Glu His Pro Gln Asp Val Pro Thr Ile
                85                  90                  95
Arg Glu Lys Ile Arg Glu His Ser Ala Val Val Asp Ile Phe Glu Tyr
            100                 105                 110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
        115                 120                 125
Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Ala Ile Ala Thr
    130                 135                 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145                 150                 155                 160
Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
                165                 170                 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
            180                 185                 190
Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
        195                 200                 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
    210                 215                 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225                 230                 235                 240
Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
                245                 250                 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
            260                 265                 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
        275                 280                 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
    290                 295                 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305                 310                 315                 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
                325                 330                 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
            340                 345                 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
        355                 360                 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
    370                 375                 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385                 390                 395                 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
                405                 410                 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
            420                 425                 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
        435                 440                 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
    450                 455                 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465                 470                 475                 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
                485                 490                 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
            500                 505                 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
        515                 520                 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
    530                 535                 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545                 550                 555                 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
                565                 570                 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
            580                 585                 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
        595                 600                 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
    610                 615                 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625                 630                 635                 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
                645                 650                 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
            660                 665                 670
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Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
            675                 680                 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
        690                 695                 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705                 710                 715                 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
                725                 730                 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
            740                 745                 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
            755                 760                 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
        770                 775                 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785                 790                 795                 800
Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe Thr Tyr Asp Glu
                805                 810                 815
Gly Gly Gly Lys Thr Gly Thr Gly Ala Val Ser Glu Lys Asp Ala Pro
            820                 825                 830
Lys Glu Leu Leu Glu Met Leu Glu Lys Gln Lys Lys
            835                 840
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<210> 43
<211> 844
<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-R43C
聚合酶缀合物

<400> 43

[0029]

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Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
 1               5                  10                  15
Arg Leu Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu His Asp Arg
            20                  25                  30
Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Lys Ile
            35                  40                  45
Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Lys Ile Val Arg
        50                  55                  60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65                  70                  75                  80
Thr Val Trp Arg Leu Tyr Phe Glu His Pro Gln Asp Val Pro Thr Ile
                85                  90                  95
Arg Glu Lys Ile Arg Glu His Ser Ala Val Val Asp Ile Phe Glu Tyr
            100                 105                 110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
            115                 120                 125
Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Ala Ile Ala Thr
        130                 135                 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145                 150                 155                 160
Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
                165                 170                 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
            180                 185                 190
Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
            195                 200                 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
        210                 215                 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225                 230                 235                 240
Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
                245                 250                 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
            260                 265                 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
            275                 280                 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
        290                 295                 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305                 310                 315                 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
                325                 330                 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
```

[0030]

```
                340                 345                 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
        355                 360                 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
    370                 375                 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385                 390                 395                 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
                405                 410                 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
            420                 425                 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
        435                 440                 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
    450                 455                 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465                 470                 475                 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
                485                 490                 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
            500                 505                 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
        515                 520                 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
    530                 535                 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545                 550                 555                 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
                565                 570                 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
            580                 585                 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
        595                 600                 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
    610                 615                 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625                 630                 635                 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
                645                 650                 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Glu Ile Thr Arg Pro Leu His
            660                 665                 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
        675                 680                 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
    690                 695                 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705                 710                 715                 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
                725                 730                 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
            740                 745                 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
        755                 760                 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
    770                 775                 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785                 790                 795                 800
Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe Thr Tyr Asp Glu
                805                 810                 815
Gly Gly Gly Lys Thr Gly Cys Gly Ala Val Ser Glu Lys Asp Ala Pro
            820                 825                 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
        835                 840
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<210> 44
<211> 844
<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-R43V
聚合酶缀合物

<400> 44

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

[0031] Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
325 330 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
340 345 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
355 360 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
370 375 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385 390 395 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
405 410 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
420 425 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
435 440 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
450 455 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465 470 475 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
485 490 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
500 505 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
515 520 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
530 535 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545 550 555 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
565 570 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
580 585 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
595 600 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
610 615 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala

625 630 635 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
645 650 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
660 665 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
675 680 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
690 695 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705 710 715 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
725 730 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
740 745 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
755 760 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
770 775 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785 790 795 800
Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe Thr Tyr Asp Glu
805 810 815
Gly Gly Gly Lys Thr Gly Val Gly Ala Val Ser Glu Lys Asp Ala Pro
820 825 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
835 840

<210> 45
<211> 844
<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-R43L
聚合酶缀合物

[0032] <400> 45
Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
1 5 10 15
Arg Leu Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu His Asp Arg
20 25 30
Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Lys Ile
35 40 45
Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Lys Ile Val Arg
50 55 60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65 70 75 80
Thr Val Trp Arg Leu Tyr Phe Glu His Pro Gln Asp Val Pro Thr Ile
85 90 95
Arg Glu Lys Ile Arg Glu His Ser Ala Val Val Asp Ile Phe Glu Tyr
100 105 110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
115 120 125
Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Ala Ile Ala Thr
130 135 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145 150 155 160
Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
165 170 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
180 185 190
Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
195 200 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
210 215 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225 230 235 240
Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
245 250 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
260 265 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
275 280 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
290 295 300

Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305 310 315 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
325 330 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
340 345 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
355 360 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Gln Arg Arg Leu Arg Glu Ser
370 375 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385 390 395 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
405 410 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
420 425 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
435 440 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
450 455 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465 470 475 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
485 490 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
500 505 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
515 520 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
530 535 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545 550 555 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
565 570 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
580 585 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
595 600 605

[0033] Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
610 615 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625 630 635 640
Val Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Ile
645 650 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Glu Ile Thr Arg Pro Leu His
660 665 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
675 680 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
690 695 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705 710 715 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
725 730 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
740 745 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
755 760 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
770 775 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785 790 795 800
Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe Thr Tyr Asp Glu
805 810 815
Gly Gly Gly Lys Thr Gly Leu Gly Ala Val Ser Glu Lys Asp Ala Pro
820 825 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
835 840

<210> 46
<211> 844
<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-R43I

聚合酶缀合物

<400> 46

[0034]

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Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
 1               5                  10                  15
Arg Leu Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu His Asp Arg
            20                  25                  30
Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Lys Ile
        35                  40                  45
Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Lys Ile Val Arg
    50                  55                  60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65                  70                  75                  80
Thr Val Trp Arg Leu Tyr Phe Glu His Pro Gln Asp Val Pro Thr Ile
                85                  90                  95
Arg Glu Lys Ile Arg Glu His Ser Ala Val Val Asp Ile Phe Glu Tyr
            100                 105                 110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
        115                 120                 125
Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Ala Ile Ala Thr
    130                 135                 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145                 150                 155                 160
Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
                165                 170                 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
            180                 185                 190
Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
        195                 200                 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
    210                 215                 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225                 230                 235                 240
Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
                245                 250                 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
            260                 265                 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
        275                 280                 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
    290                 295                 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305                 310                 315                 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
                325                 330                 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
            340                 345                 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
        355                 360                 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
    370                 375                 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385                 390                 395                 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
                405                 410                 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
            420                 425                 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
        435                 440                 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
    450                 455                 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465                 470                 475                 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
                485                 490                 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
            500                 505                 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
        515                 520                 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
    530                 535                 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545                 550                 555                 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
                565                 570                 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
            580                 585                 590
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Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
            595                 600                 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
        610                 615                 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625                 630                 635                 640
Val Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Ile
                645                 650                 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
            660                 665                 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
        675                 680                 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
    690                 695                 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705                 710                 715                 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
                725                 730                 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
            740                 745                 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
        755                 760                 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
    770                 775                 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785                 790                 795                 800
Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe Thr Tyr Asp Glu
                805                 810                 815
Gly Gly Gly Lys Thr Gly Ile Gly Ala Val Ser Glu Lys Asp Ala Pro
            820                 825                 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
        835                 840
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<210> 47
<211> 844
<212> PRT
<213> 人工序列

[0035]

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-R43M
聚合酶缀合物

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

[0036]

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                260                 265                 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
        275                 280                 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
    290                 295                 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305                 310                 315                 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
                325                 330                 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
            340                 345                 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
        355                 360                 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
    370                 375                 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385                 390                 395                 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
                405                 410                 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
            420                 425                 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
        435                 440                 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
    450                 455                 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465                 470                 475                 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
                485                 490                 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
            500                 505                 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
        515                 520                 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
    530                 535                 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545                 550                 555                 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
                565                 570                 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
            580                 585                 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
        595                 600                 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
    610                 615                 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625                 630                 635                 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
                645                 650                 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
            660                 665                 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
        675                 680                 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
    690                 695                 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705                 710                 715                 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
                725                 730                 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
            740                 745                 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Glu Thr Gly Leu Thr Ser
        755                 760                 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
    770                 775                 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785                 790                 795                 800
Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe Thr Tyr Asp Glu
                805                 810                 815
Gly Gly Gly Lys Thr Gly Met Gly Ala Val Ser Glu Lys Asp Ala Pro
            820                 825                 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
        835                 840
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<210> 48
<211> 844

<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-R43F
聚合酶缀合物

<400> 48

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

[0037] Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
245 250 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
260 265 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
275 280 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
290 295 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305 310 315 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
325 330 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
340 345 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
355 360 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
370 375 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385 390 395 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
405 410 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
420 425 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
435 440 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
450 455 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465 470 475 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
485 490 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
500 505 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
515 520 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
530 535 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys

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545                 550                 555                 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
                565                 570                 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
            580                 585                 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
        595                 600                 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
    610                 615                 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625                 630                 635                 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
                645                 650                 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
            660                 665                 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
        675                 680                 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
    690                 695                 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705                 710                 715                 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
                725                 730                 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
            740                 745                 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
        755                 760                 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
    770                 775                 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785                 790                 795                 800
Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe Thr Tyr Asp Glu
                805                 810                 815
Gly Gly Gly Lys Thr Gly Phe Gly Ala Val Ser Glu Lys Asp Ala Pro
            820                 825                 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
        835                 840
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[0038]

<210> 49
<211> 844
<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-R43Y
聚合酶缀合物

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Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
 1                  5                  10                  15
Arg Leu Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu His Asp Arg
            20                  25                  30
Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Lys Ile
        35                  40                  45
Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Lys Ile Val Arg
    50                  55                  60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65                  70                  75                  80
Thr Val Trp Arg Leu Tyr Phe Glu His Pro Gln Asp Val Pro Thr Ile
                85                  90                  95
Arg Glu Lys Ile Arg Glu His Ser Ala Val Val Asp Ile Phe Glu Tyr
            100                 105                 110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
        115                 120                 125
Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Ala Ile Ala Thr
    130                 135                 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145                 150                 155                 160
Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
                165                 170                 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
            180                 185                 190
Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
        195                 200                 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
    210                 215                 220
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Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225 230 235 240
Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
245 250 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
260 265 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
275 280 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
290 295 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305 310 315 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
325 330 335
Val Gly Glu Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
340 345 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
355 360 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
370 375 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385 390 395 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
405 410 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
420 425 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
435 440 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
450 455 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465 470 475 480
Asp Tyr Arg Glu Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
485 490 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
500 505 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
515 520 525

[0039] Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
530 535 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545 550 555 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
565 570 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
580 585 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
595 600 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
610 615 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625 630 635 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
645 650 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
660 665 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
675 680 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
690 695 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705 710 715 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
725 730 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
740 745 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
755 760 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
770 775 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785 790 795 800
Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe Thr Tyr Asp Glu
805 810 815
Gly Gly Gly Lys Thr Gly Tyr Gly Ala Val Ser Glu Lys Asp Ala Pro
820 825 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys

835 840

<210> 50
<211> 844
<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-R43W
聚合酶缀合物

<400> 50

[0040]

Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
1 5 10 15
Arg Leu Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu His Asp Arg
20 25 30
Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Lys Ile
35 40 45
Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Lys Ile Val Arg
50 55 60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65 70 75 80
Thr Val Trp Arg Leu Tyr Phe Glu His Pro Glu Asp Val Pro Thr Ile
85 90 95
Arg Glu Lys Ile Arg Glu His Ser Ala Val Val Asp Ile Phe Glu Tyr
100 105 110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
115 120 125
Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Ala Ile Ala Thr
130 135 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145 150 155 160
Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
165 170 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
180 185 190
Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
195 200 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
210 215 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225 230 235 240
Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
245 250 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
260 265 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
275 280 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
290 295 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305 310 315 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
325 330 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
340 345 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
355 360 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
370 375 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385 390 395 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
405 410 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
420 425 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
435 440 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
450 455 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465 470 475 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
485 490 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
500 505 510

Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
515 520 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
530 535 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545 550 555 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
565 570 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
580 585 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
595 600 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
610 615 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625 630 635 640
Val Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Ile
645 650 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
660 665 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
675 680 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
690 695 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705 710 715 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
725 730 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
740 745 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
755 760 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
770 775 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785 790 795 800
Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe Thr Tyr Asp Glu
805 810 815
[0041] Gly Gly Gly Lys Thr Gly Trp Gly Ala Val Ser Glu Lys Asp Ala Pro
820 825 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
835 840

<210> 51
<211> 844
<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-R43D
聚合酶缀合物

<400> 51
Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
1 5 10 15
Arg Leu Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu His Asp Arg
20 25 30
Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Lys Ile
35 40 45
Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Lys Ile Val Arg
50 55 60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65 70 75 80
Thr Val Trp Arg Leu Tyr Phe Glu His Pro Glu Asp Val Pro Thr Ile
85 90 95
Arg Glu Lys Ile Arg Glu His Ser Ala Val Val Asp Ile Phe Glu Tyr
100 105 110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
115 120 125
Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Ala Ile Ala Thr
130 135 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145 150 155 160
Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
165 170 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys

[0042]

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                180                 185                 190
Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
        195                 200                 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
    210                 215                 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225                 230                 235                 240
Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
                245                 250                 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
            260                 265                 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
        275                 280                 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
    290                 295                 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305                 310                 315                 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
                325                 330                 335
Val Gly Glu Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
            340                 345                 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
        355                 360                 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
    370                 375                 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385                 390                 395                 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
                405                 410                 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
            420                 425                 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
        435                 440                 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
    450                 455                 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465                 470                 475                 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
                485                 490                 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
            500                 505                 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
        515                 520                 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
    530                 535                 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545                 550                 555                 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
                565                 570                 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
            580                 585                 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
        595                 600                 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
    610                 615                 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625                 630                 635                 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
                645                 650                 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
            660                 665                 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
        675                 680                 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
    690                 695                 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705                 710                 715                 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
                725                 730                 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
            740                 745                 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
        755                 760                 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
    770                 775                 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785                 790                 795                 800
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Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe Thr Tyr Asp Glu
                805                 810                 815
Gly Gly Gly Lys Thr Gly Asp Gly Ala Val Ser Glu Lys Asp Ala Pro
            820                 825                 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
        835                 840
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<210> 52
<211> 844
<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-R43E
聚合酶缀合物

<400> 52

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Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
 1               5                  10                  15
Arg Leu Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu His Asp Arg
            20                  25                  30
Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Lys Ile
        35                  40                  45
Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Lys Ile Val Arg
    50                  55                  60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65                  70                  75                  80
Thr Val Trp Arg Leu Tyr Phe Glu His Pro Gln Asp Val Pro Thr Ile
                85                  90                  95
Arg Glu Lys Ile Arg Glu His Ser Ala Val Val Asp Ile Phe Glu Tyr
            100                 105                 110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
        115                 120                 125
Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Ala Ile Ala Thr
    130                 135                 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145                 150                 155                 160
Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
                165                 170                 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
            180                 185                 190
Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
        195                 200                 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
    210                 215                 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225                 230                 235                 240
Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
                245                 250                 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
            260                 265                 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
        275                 280                 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
    290                 295                 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305                 310                 315                 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
                325                 330                 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
            340                 345                 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
        355                 360                 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
    370                 375                 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385                 390                 395                 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
                405                 410                 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
            420                 425                 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
        435                 440                 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
    450                 455                 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
```

[0043]

[0044]

```
465                 470                 475                 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
                485                 490                 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
            500                 505                 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
        515                 520                 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
    530                 535                 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545                 550                 555                 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
                565                 570                 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
            580                 585                 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
        595                 600                 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
    610                 615                 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625                 630                 635                 640
Val Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Ile
                645                 650                 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Glu Ile Thr Arg Pro Leu His
            660                 665                 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
        675                 680                 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
    690                 695                 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705                 710                 715                 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
                725                 730                 735
Glu Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
            740                 745                 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
        755                 760                 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
    770                 775                 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785                 790                 795                 800
Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe Thr Tyr Asp Glu
                805                 810                 815
Gly Gly Gly Lys Thr Gly Glu Gly Ala Val Ser Glu Lys Asp Ala Pro
            820                 825                 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
        835                 840
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<210> 53
<211> 844
<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-R43N
聚合酶嵌合物

<400> 53

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

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

[0045]

Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
450 455 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465 470 475 480
Asp Tyr Arg Glu Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
485 490 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
500 505 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
515 520 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
530 535 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545 550 555 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
565 570 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
580 585 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
595 600 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
610 615 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625 630 635 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
645 650 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
660 665 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
675 680 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
690 695 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705 710 715 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
725 730 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
740 745 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser

```
          755                 760                 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
    770                 775                 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785                 790                 795                 800
Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe Thr Tyr Asp Glu
                805                 810                 815
Gly Gly Gly Lys Thr Gly Asn Gly Ala Val Ser Glu Lys Asp Ala Pro
            820                 825                 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
        835                 840
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<210> 54
<211> 844
<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-R43Q
聚合酶缀合物

<400> 54

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Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
 1               5                  10                  15
Arg Leu Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu His Asp Arg
            20                  25                  30
Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Lys Ile
        35                  40                  45
Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Lys Ile Val Arg
    50                  55                  60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65                  70                  75                  80
Thr Val Trp Arg Leu Tyr Phe Glu His Pro Gln Asp Val Pro Thr Ile
                85                  90                  95
Arg Glu Lys Ile Arg Glu His Ser Ala Val Val Asp Ile Phe Glu Tyr
            100                 105                 110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
        115                 120                 125
Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Ala Ile Ala Thr
    130                 135                 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145                 150                 155                 160
Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
                165                 170                 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
            180                 185                 190
Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
        195                 200                 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
    210                 215                 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225                 230                 235                 240
Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
                245                 250                 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
            260                 265                 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
        275                 280                 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
    290                 295                 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305                 310                 315                 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
                325                 330                 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
            340                 345                 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
        355                 360                 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
    370                 375                 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385                 390                 395                 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
                405                 410                 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
            420                 425                 430
```

[0046]

Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
435 440 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
450 455 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465 470 475 480
Asp Tyr Arg Glu Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
485 490 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
500 505 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
515 520 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
530 535 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545 550 555 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
565 570 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
580 585 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
595 600 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
610 615 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625 630 635 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
645 650 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
660 665 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
675 680 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
690 695 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705 710 715 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
725 730 735
[0047] Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
740 745 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
755 760 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
770 775 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785 790 795 800
Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe Thr Tyr Asp Glu
805 810 815
Gly Gly Gly Lys Thr Gly Gln Gly Ala Val Ser Glu Lys Asp Ala Pro
820 825 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
835 840

<210> 55
<211> 844
<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-R43H
聚合酶缀合物

<400> 55
Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
1 5 10 15
Arg Leu Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu His Asp Arg
20 25 30
Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Lys Ile
35 40 45
Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Lys Ile Val Arg
50 55 60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65 70 75 80
Thr Val Trp Arg Leu Tyr Phe Glu His Pro Gln Asp Val Pro Thr Ile
85 90 95
Arg Glu Lys Ile Arg Glu His Ser Ala Val Val Asp Ile Phe Glu Tyr

[0048]

```
                100                 105                 110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
            115                 120                 125
Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Ala Ile Ala Thr
        130                 135                 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145                 150                 155                 160
Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
                165                 170                 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
            180                 185                 190
Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
        195                 200                 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
    210                 215                 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225                 230                 235                 240
Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
                245                 250                 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
            260                 265                 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
        275                 280                 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
    290                 295                 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305                 310                 315                 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
                325                 330                 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
            340                 345                 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
        355                 360                 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
    370                 375                 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385                 390                 395                 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
                405                 410                 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
            420                 425                 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
        435                 440                 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
    450                 455                 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465                 470                 475                 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
                485                 490                 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
            500                 505                 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
        515                 520                 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
    530                 535                 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545                 550                 555                 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
                565                 570                 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
            580                 585                 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
        595                 600                 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
    610                 615                 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625                 630                 635                 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
                645                 650                 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
            660                 665                 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
        675                 680                 685
Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
    690                 695                 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705                 710                 715                 720
```

Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
725 730 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
740 745 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
755 760 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
770 775 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785 790 795 800
Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe Thr Tyr Asp Glu
805 810 815
Gly Gly Gly Lys Thr Gly His Gly Ala Val Ser Glu Lys Asp Ala Pro
820 825 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
835 840

<210> 56
<211> 844
<212> PRT
<213> 人工序列

<220>
<223> 合成的外-聚合酶 + 接头 + Sso7d-R43K
聚合酶缀合物

<400> 56
Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
1 5 10 15
Arg Leu Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu His Asp Arg
20 25 30
Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Lys Ile
35 40 45
Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Lys Ile Val Arg
50 55 60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65 70 75 80
[0049] Thr Val Trp Arg Leu Tyr Phe Glu His Pro Gln Asp Val Pro Thr Ile
85 90 95
Arg Glu Lys Ile Arg Glu His Ser Ala Val Val Asp Ile Phe Glu Tyr
100 105 110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
115 120 125
Met Glu Gly Asp Glu Glu Leu Lys Leu Leu Ala Phe Ala Ile Ala Thr
130 135 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145 150 155 160
Ser Tyr Ala Asp Glu Glu Glu Ala Lys Val Ile Thr Trp Lys Lys Ile
165 170 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
180 185 190
Arg Phe Leu Lys Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Ile Thr
195 200 205
Tyr Asn Gly Asp Ser Phe Asp Leu Pro Tyr Leu Ala Lys Arg Ala Glu
210 215 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225 230 235 240
Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile
245 250 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
260 265 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
275 280 285
Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly
290 295 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr
305 310 315 320
Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
325 330 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
340 345 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala
355 360 365
Pro Asn Lys Pro Asp Glu Arg Glu Tyr Glu Arg Arg Leu Arg Glu Ser
370 375 380
Tyr Ala Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn

385 390 395 400
Ile Val Ser Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr
405 410 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Asn Tyr
420 425 430
Asp Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
435 440 445
Phe Ile Pro Ser Leu Leu Lys Arg Leu Leu Asp Glu Arg Gln Lys Ile
450 455 460
Lys Thr Lys Met Lys Ala Ser Gln Asp Pro Ile Glu Lys Ile Met Leu
465 470 475 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly
485 490 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
500 505 510
Ser Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Phe Val Trp Lys Glu
515 520 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
530 535 540
Leu Tyr Ala Thr Ile Pro Gly Gly Lys Ser Glu Glu Ile Lys Lys Lys
545 550 555 560
Ala Leu Glu Phe Val Asp Tyr Ile Asn Ala Lys Leu Pro Gly Leu Leu
565 570 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys
580 585 590
Lys Lys Tyr Ala Leu Ile Asp Glu Glu Gly Lys Ile Ile Thr Arg Gly
595 600 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
610 615 620
Ala Arg Val Leu Glu Ala Ile Leu Lys His Gly Asn Val Glu Glu Ala
625 630 635 640
Val Arg Ile Val Lys Glu Val Thr Gln Lys Leu Ser Lys Tyr Glu Ile
645 650 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Gln Ile Thr Arg Pro Leu His
660 665 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Arg Leu Ala
675 680 685
[0050] Ala Lys Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
690 695 700
Leu Arg Gly Asp Gly Pro Ile Ser Asn Arg Ala Ile Leu Ala Glu Glu
705 710 715 720
Tyr Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
725 730 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
740 745 750
Lys Glu Asp Leu Arg Trp Gln Lys Thr Lys Gln Thr Gly Leu Thr Ser
755 760 765
Trp Leu Asn Ile Lys Lys Ser Gly Thr Gly Gly Gly Gly Ala Thr Val
770 775 780
Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp Ile Ser Lys Ile
785 790 795 800
Lys Lys Val Trp Arg Val Gly Ser Met Ile Ser Phe Thr Tyr Asp Glu
805 810 815
Gly Gly Gly Lys Thr Gly Lys Gly Ala Val Ser Glu Lys Asp Ala Pro
820 825 830
Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
835 840

<210> 57
<211> 64
<212> PRT
<213> 芝田硫化叶菌(Sulfolobus shibatae)

<220>
<223> Ssh7b, DNA结合蛋白7b

<400> 57
Met Val Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Thr Ser Lys Ile Lys Lys Val Trp Arg Val Gly Lys Met Ile Ser Phe
20 25 30
Thr Tyr Asp Glu Gly Gly Gly Lys Thr Gly Arg Gly Ala Val Ser Glu
35 40 45
Lys Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Gln Lys Lys
50 55 60

<210> 58
<211> 66
<212> PRT
<213> 硫化叶菌属物种(Sulfolobus sp.)

<220>
<223> 硫化叶菌属物种(Sulfolobus sp.) Sac7d, DNA结合蛋白7d, 螺旋稳定蛋白; 酸热硫化叶菌(Sulfolobus acidocaldarius) 菌株DSM 639 Sac7d, DNA结合蛋白7d, Saci_0064

<400> 58
Met Val Lys Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Thr Ser Lys Ile Lys Lys Val Trp Arg Val Gly Lys Met Val Ser Phe
20 25 30
Thr Tyr Asp Asp Asn Gly Lys Thr Gly Arg Gly Ala Val Ser Glu Lys
35 40 45
Asp Ala Pro Lys Glu Leu Leu Asp Met Leu Ala Arg Ala Glu Arg Glu
50 55 60
Lys Lys
65

<210> 59
<211> 65
<212> PRT
<213> 酸热硫化叶菌(Sulfolobus acidocaldarius)

<220>
<223> 酸热硫化叶菌(Sulfolobus acidocaldarius)菌株DSM 639 Sac7e, DNA结合蛋白7e, Saci_0362

<400> 59
Met Ala Lys Val Arg Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Thr Ser Lys Ile Lys Lys Val Trp Arg Val Gly Lys Met Val Ser Phe
20 25 30
[0051] Thr Tyr Asp Asp Asn Gly Lys Thr Gly Arg Gly Ala Val Ser Glu Lys
35 40 45
Asp Ala Pro Lys Glu Leu Met Asp Met Leu Ala Arg Ala Glu Lys Lys
50 55 60
Lys
65

<210> 60
<211> 64
<212> PRT
<213> Sulfolobus tokodaii

<220>
<223> Sulfolobus tokodaii菌株7 Sto7e, DNA-结合蛋白7, STS077, STK_06395, STS226, STK_20955

<400> 60
Met Val Thr Val Lys Phe Lys Tyr Lys Gly Glu Glu Lys Glu Val Asp
1 5 10 15
Ile Ser Lys Ile Lys Lys Val Trp Arg Val Gly Lys Met Ile Ser Phe
20 25 30
Thr Tyr Asp Asp Asn Gly Lys Thr Gly Arg Gly Ala Val Ser Glu Lys
35 40 45
Asp Ala Pro Lys Glu Leu Leu Gln Met Leu Glu Lys Ser Gly Lys Lys
50 55 60

<210> 61
<211> 775
<212> PRT
<213> 人工序列

<220>
<223> 合成的杂合聚合酶, 聚合酶缀合物

<400> 61
Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile
1 5 10 15

Arg Leu Phe Lys Lys Glu Asn Gly Lys Phe Lys Ile Glu His Asp Arg
20 25 30
Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Lys Ile
35 40 45
Asp Glu Val Arg Lys Ile Thr Gly Glu Arg His Gly Lys Ile Val Arg
50 55 60
Ile Val Asp Ala Glu Lys Val Glu Lys Lys Phe Leu Gly Arg Pro Ile
65 70 75 80
Glu Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Ala Ile
85 90 95
Arg Asp Lys Val Arg Glu His Pro Ala Val Val Asp Ile Phe Glu Tyr
100 105 110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
115 120 125
Met Glu Gly Glu Glu Glu Leu Lys Ile Leu Ala Phe Asp Ile Glu Thr
130 135 140
Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile
145 150 155 160
Ser Tyr Ala Asp Glu Asn Glu Ala Lys Val Ile Thr Trp Lys Asn Ile
165 170 175
Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys
180 185 190
Arg Phe Leu Arg Val Ile Arg Glu Lys Asp Pro Asp Val Ile Ile Thr
195 200 205
Tyr Asn Gly Asp Ser Phe Asp Phe Pro Tyr Leu Val Lys Arg Ala Glu
210 215 220
Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys
225 230 235 240
Met Gln Arg Leu Gly Asp Met Thr Ala Val Glu Ile Lys Gly Arg Ile
245 250 255
His Phe Asp Leu Tyr His Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
260 265 270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu
275 280 285
Lys Val Tyr Ala Asp Glu Ile Ala Glu Ala Trp Glu Ser Gly Glu Gly
290 295 300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr
305 310 315 320
[0052] Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ile Gln Leu Ser Arg Leu
325 330 335
Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu
340 345 350
Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Val Ala
355 360 365
Pro Asn Lys Pro Ser Glu Glu Glu Tyr Glu Arg Arg Leu Arg Glu Ser
370 375 380
Tyr Ala Gly Gly Tyr Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn
385 390 395 400
Ile Val Ser Leu Asp Phe Arg Ser Leu Tyr Pro Ser Ile Ile Ile Thr
405 410 415
His Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Arg Glu Tyr
420 425 430
Asp Ile Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly
435 440 445
Phe Ile Pro Ser Leu Leu Lys His Leu Leu Glu Glu Arg Gln Lys Ile
450 455 460
Lys Thr Lys Met Lys Glu Ser Gln Asp Pro Ile Glu Lys Lys Met Leu
465 470 475 480
Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly
485 490 495
Tyr Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu
500 505 510
Ser Val Thr Ala Trp Gly Arg Lys Tyr Ile Glu Phe Val Trp Lys Glu
515 520 525
Leu Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ile Asp Thr Asp Gly
530 535 540
Leu Tyr Ala Thr Ile Pro Gly Gly Glu Pro Glu Glu Ile Lys Lys Lys
545 550 555 560
Ala Leu Glu Phe Val Lys Tyr Ile Asn Ser Lys Leu Pro Gly Leu Leu
565 570 575
Glu Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys
580 585 590
Lys Arg Tyr Ala Val Ile Asp Glu Glu Gly Lys Val Ile Thr Arg Gly
595 600 605
Leu Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln
610 615 620
Ala Arg Val Leu Glu Thr Ile Leu Lys His Gly Asn Val Glu Glu Ala

625 630 635 640
Val Lys Ile Val Lys Glu Val Thr Gln Lys Leu Ala Asn Tyr Glu Ile
645 650 655
Pro Pro Glu Lys Leu Ala Ile Tyr Glu Glu Ile Thr Arg Pro Leu His
660 665 670
Glu Tyr Lys Ala Ile Gly Pro His Val Ala Val Ala Lys Lys Leu Ala
675 680 685
Ala Arg Gly Val Lys Ile Lys Pro Gly Met Val Ile Gly Tyr Ile Val
690 695 700
Leu Arg Gly Asp Gly Pro Ile Ser Lys Arg Ala Ile Leu Ala Glu Glu
705 710 715 720
Phe Asp Pro Arg Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn
725 730 735
Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Gly Phe Gly Tyr Arg
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六邻接组氨酸

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