



(12)发明专利申请

(10)申请公布号 CN 106536728 A

(43)申请公布日 2017.03.22

(21)申请号 201580033818.6

(22)申请日 2015.06.25

(30)优先权数据

62/018,470 2014.06.27 US

(85)PCT国际申请进入国家阶段日

2016.12.22

(86)PCT国际申请的申请数据

PCT/US2015/037785 2015.06.25

(87)PCT国际申请的公布数据

W02015/200693 EN 2015.12.30

(71)申请人 伊鲁米那股份有限公司

地址 美国加利福尼亚州

(72)发明人 陈呈尧 艾琳·博马蒂 莫利·何

(74)专利代理机构 北京安信方达知识产权代理有限公司 11262

代理人 王玮玮 郑霞

(51)Int.Cl.

C12N 9/12(2006.01)

权利要求书4页 说明书21页
序列表70页 附图5页

(54)发明名称

用于核苷酸类似物的改进的掺入的修饰的
聚合酶

(57)摘要

本文呈现了用于核苷酸类似物,特别是在3'糖羟基处修饰的核苷酸的改进的掺入的聚合酶以及使用该聚合酶的方法和试剂盒。

1. 一种改造的聚合酶,所述改造的聚合酶在功能上等同于9°N DNA聚合酶氨基酸序列中的Lys477的位置处包含至少一个氨基酸置换突变。

2. 根据权利要求1所述的改造的聚合酶,其中所述置换突变包含突变成具有较小侧链的残基的突变。

3. 根据权利要求1所述的改造的聚合酶,其中所述置换突变包含突变成具有疏水性侧链的残基的突变。

4. 根据权利要求1所述的改造的聚合酶,其中所述置换突变包含与9°N DNA聚合酶氨基酸序列中的Lys477Met同源的突变。

5. 根据权利要求1所述的改造的聚合酶,其中所述聚合酶是DNA聚合酶。

6. 权利要求1所述的改造的聚合酶,其中所述DNA聚合酶是B家族型DNA聚合酶。

7. 根据权利要求6所述的改造的聚合酶,所述改造的聚合酶选自由以下组成的组:B家族古细菌DNA聚合酶、人DNA聚合酶- α 、T4、RB69和phi29噬菌体DNA聚合酶。

8. 根据权利要求7所述的改造的聚合酶,其中所述B家族古细菌DNA聚合酶来自选自由嗜热球菌属(Thermococcus)、火球菌属(Pyrococcus)和甲烷球菌属(Methanococcus)组成的组的属。

9. 根据权利要求7所述的B家族古细菌DNA聚合酶,所述B家族古细菌DNA聚合酶选自由Vent聚合酶、Deep Vent聚合酶、9°N聚合酶和Pfu聚合酶组成的组。

10. 根据权利要求9所述的改造的聚合酶,其中所述B家族古细菌DNA聚合酶是9°N聚合酶。

11. 根据权利要求1-10中任一项所述的改造的聚合酶,其中所述改造的聚合酶还在功能上等同于9°N DNA聚合酶氨基酸序列中的Leu408和/或Tyr409和/或Pro410的位置处包含置换突变。

12. 根据权利要求11所述的改造的聚合酶,其中所述改造的聚合酶包含与9°N DNA聚合酶氨基酸序列中的Leu408Ala和/或Tyr409Ala和/或Pro410Ile同源的置换突变。

13. 根据权利要求1-12中任一项所述的改造的聚合酶,其中所述改造的聚合酶与野生型聚合酶相比包含降低的外切核酸酶活性。

14. 根据权利要求13所述的改造的聚合酶,其中所述改造的聚合酶在功能上等同于9°N DNA聚合酶氨基酸序列中的Asp141和/或Glu143的位置处包含置换突变。

15. 根据权利要求1-14中任一项所述的改造的聚合酶,其中所述改造的聚合酶还在功能上等同于9°N DNA聚合酶氨基酸序列中的Ala485的位置处包含置换突变。

16. 根据权利要求15所述的改造的聚合酶,其中所述聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Ala485Leu或Ala485Val的置换突变。

17. 根据权利要求1-16中任一项所述的改造的聚合酶,所述改造的聚合酶还在功能上等同于9°N DNA聚合酶氨基酸序列中的Cys223的位置处包含突变成不同的氨基酸的置换突变。

18. 根据权利要求17所述的改造的聚合酶,其中所述聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Cys223Ser的置换突变。

19. 根据权利要求1-18中任一项所述的改造的聚合酶,所述改造的聚合酶还在功能上等同于9°N DNA聚合酶氨基酸序列中的Thr514的位置处包含突变成不同的氨基酸的置换突

变。

20. 根据权利要求19所述的改造的聚合酶,其中所述聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Thr514Ala或Thr514Ser的置换突变。

21. 根据权利要求1-20中任一项所述的改造的聚合酶,所述改造的聚合酶还在功能上等同于9°N DNA聚合酶氨基酸序列中的Ile521的位置处包含突变成不同的氨基酸的置换突变。

22. 根据权利要求21所述的改造的聚合酶,其中所述聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Ile521Leu的置换突变。

23. 一种改造的聚合酶,所述改造的聚合酶包含对半保守结构域的置换突变,所述半保守结构域包含SEQ ID NO:1-4中的任一个的氨基酸序列,其中所述置换突变包括选自以下的突变:在位置3处置换成除了Lys、Ile或Gln之外的任一残基。

24. 根据权利要求23所述的改造的聚合酶,其中所述突变包括在位置3处置换成Met。

25. 根据权利要求23-24中任一项所述的改造的聚合酶,其中所述改造的聚合酶还在功能上等同于9°N DNA聚合酶氨基酸序列中的Leu408和/或Tyr409和/或Pro410的位置处包含置换突变。

26. 根据权利要求25所述的改造的聚合酶,其中所述改造的聚合酶包含与9°N DNA聚合酶氨基酸序列中的Leu408Ala和/或Tyr409Ala和/或Pro410Ile同源的置换突变。

27. 根据权利要求23-26中任一项所述的改造的聚合酶,其中所述改造的聚合酶与野生型聚合酶相比包含降低的外切核酸酶活性。

28. 根据权利要求27所述的改造的聚合酶,其中所述改造的聚合酶在功能上等同于9°N DNA聚合酶氨基酸序列中的Asp141和/或Glu143的位置处包含置换突变。

29. 根据权利要求23-28中任一项所述的改造的聚合酶,其中所述改造的聚合酶还在功能上等同于9°N DNA聚合酶氨基酸序列中的Ala485的位置处包含置换突变。

30. 根据权利要求29所述的改造的聚合酶,其中所述聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Ala485Leu或Ala485Val的置换突变。

31. 根据权利要求23-30中任一项所述的改造的聚合酶,所述改造的聚合酶还在功能上等同于9°N DNA聚合酶氨基酸序列中的Cys223的位置处包含突变成不同的氨基酸的置换突变。

32. 根据权利要求31所述的改造的聚合酶,其中所述聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Cys223Ser的置换突变。

33. 一种改造的聚合酶,所述改造的聚合酶包含以下中的任一个的氨基酸序列:SEQ ID NO:6-8、10-12、14-16、18-20、22-24和26-28、30和32。

34. 一种核酸分子,所述核酸分子编码如在权利要求1-33的任一项中定义的改造的聚合酶。

35. 一种表达载体,所述表达载体包含权利要求34所述的核酸分子。

36. 一种宿主细胞,所述宿主细胞包含权利要求35所述的载体。

37. 一种用于将修饰的核苷酸掺入DNA的方法,所述方法包括允许以下组分相互作用:
(i) 根据权利要求1-33中任一项所述的改造的聚合酶, (ii) DNA模板;和 (iii) 核苷酸溶液。

38. 根据权利要求37所述的方法,其中所述DNA模板包括聚簇阵列。

39. 一种用于进行核苷酸掺入反应的试剂盒, 所述试剂盒包含: 如在权利要求1-33中任一项中定义的聚合酶和核苷酸溶液。

40. 根据权利要求39所述的试剂盒, 其中所述核苷酸溶液包含带标记的核苷酸。

41. 根据权利要求39所述的试剂盒, 其中所述核苷酸包括合成的核苷酸。

42. 根据权利要求39所述的试剂盒, 其中所述核苷酸包括修饰的核苷酸。

43. 根据权利要求42所述的试剂盒, 其中所述修饰的核苷酸已在3' 糖羟基处被修饰以使得取代基在尺寸上大于天然存在的3' 羟基。

44. 根据权利要求42所述的试剂盒, 其中修饰的核苷酸包括包含嘌呤碱基或嘧啶碱基和以下核糖或脱氧核糖部分的修饰的核苷酸或核苷分子, 所述核糖或脱氧核糖部分具有与其共价地附接的可移除的3' -OH封闭基团, 以使得3' 碳原子已附接以下结构的基团

-O-Z

其中Z是 $-C(R')_2-O-R''$ 、 $-C(R')_2-N(R'')_2$ 、 $-C(R')_2-N(H)R''$ 、 $-C(R')_2-S-R''$ 和 $-C(R')_2-F$ 中的任一个,

其中每个R''是可移除的保护基团或可移除的保护基团的一部分;

每个R' 独立地是氢原子、烷基、取代的烷基、芳基烷基、烯基、炔基、芳基、杂芳基、杂环基团、酰基、氰基、烷氧基、芳氧基、杂芳氧基或酰氨基, 或通过连接基团附接的可检测的标记物; 或(R')₂代表式 $=C(R'')_2$ 的亚烷基, 其中每个R''可以是相同的或不同的并且选自包括氢原子和卤素原子以及烷基的组; 并且

其中所述分子可以反应以产生中间体, 在所述中间体中每个R''被交换为H, 或当Z是 $-C(R')_2-F$ 时, F被交换为OH、SH或NH₂, 优选地OH, 所述中间体在水性条件下解离以提供带有游离3' OH的分子;

条件是当Z是 $-C(R')_2-S-R''$ 时, 两个R' 基团均不是H。

45. 根据权利要求44所述的试剂盒, 其中所述修饰的核苷酸或核苷的R' 是烷基或取代的烷基。

46. 根据权利要求45所述的试剂盒, 其中所述修饰的核苷酸或核苷的-Z是式 $-C(R')_2-N_3$ 。

47. 根据权利要求46所述的试剂盒, 其中Z是叠氮甲基。

48. 根据权利要求40所述的试剂盒, 其中所述修饰的核苷酸被荧光标记以允许所述修饰的核苷酸的检测。

49. 根据权利要求39所述的试剂盒, 其中所述修饰的核苷酸包括具有经由可裂解的接头与可检测的标记物附接的碱基的核苷酸或核苷。

50. 根据权利要求49所述的试剂盒, 其中所述可检测的标记物包括荧光标记物。

51. 根据权利要求39所述的试剂盒, 所述试剂盒还包含一种或更多种DNA模板分子和/或引物。

52. 一种重组DNA聚合酶, 所述重组DNA聚合酶包含与SEQ ID NO:29至少60%、70%、80%、90%、95%、99%同一的氨基酸序列, 所述重组DNA聚合酶在功能上等同于9°N DNA聚合酶氨基酸序列中的Lys477的位置处包含。

53. 一种重组DNA聚合酶, 所述重组DNA聚合酶包含与SEQ ID NO:31至少60%、70%、80%、90%、95%、99%同一的氨基酸序列, 所述重组DNA聚合酶在功能上等同于9°N DNA聚

合酶氨基酸序列中的Lys477的位置处包含。

54. 根据权利要求52-53中任一项所述的聚合酶, 其中所述聚合酶还在功能上等同于9°N DNA聚合酶氨基酸序列中的Leu408和/或Tyr409和/或Pro410的位置处包含置换突变。

55. 根据权利要求54所述的聚合酶, 其中所述聚合酶包含与9°N DNA聚合酶氨基酸序列中的Leu408Ala和/或Tyr409Ala和/或Pro410Ile同源的置换突变。

56. 根据权利要求52-55中任一项所述的聚合酶, 其中所述聚合酶与野生型聚合酶相比包含降低的外切核酸酶活性。

57. 根据权利要求56所述的聚合酶, 其中所述聚合酶在功能上等同于9°N DNA聚合酶氨基酸序列中的Asp141和/或Glu143的位置处包含置换突变。

58. 根据权利要求52-57中任一项所述的聚合酶, 其中所述聚合酶还在功能上等同于9°N DNA聚合酶氨基酸序列中的Ala485的位置处包含置换突变。

59. 根据权利要求58所述的聚合酶, 其中所述聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Ala485Leu或Ala485Val的置换突变。

60. 根据权利要求52-59中任一项所述的聚合酶, 所述聚合酶还在功能上等同于9°N DNA聚合酶氨基酸序列中的Cys223的位置处包含突变成不同的氨基酸的置换突变。

61. 根据权利要求60所述的聚合酶, 其中所述聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Cys223Ser的置换突变。

62. 根据权利要求52-61中任一项所述的聚合酶, 所述聚合酶还在功能上等同于9°N DNA聚合酶氨基酸序列中的Thr514的位置处包含突变成不同的氨基酸的置换突变。

63. 根据权利要求62所述的聚合酶, 其中所述聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Thr514Ala或Thr514Ser的置换突变。

64. 根据权利要求52-63中任一项所述的聚合酶, 所述聚合酶还在功能上等同于9°N DNA聚合酶氨基酸序列中的Ile521的位置处包含突变成不同的氨基酸的置换突变。

65. 根据权利要求64所述的聚合酶, 其中所述聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Ile521Leu的置换突变。

66. 一种重组DNA聚合酶, 所述重组DNA聚合酶包含与SEQ ID NO:5至少60%、70%、80%、90%、95%、99%同一的氨基酸序列, 所述重组聚合酶在功能上等同于9°N DNA聚合酶氨基酸序列中的Lys477的一个或更多个位置处包含至少一个氨基酸置换突变。

用于核苷酸类似物的改进的掺入的修饰的聚合酶

[0001] 背景

[0002] 所有生物体都依赖于DNA聚合酶来复制和维持其基因组。DNA聚合酶通过检测碱基间的互补性以及识别碱基的另外的结构特征允许DNA的高保真性复制。对具有核苷酸类似物,特别是在3' 糖羟基处修饰的核苷酸的改进的掺入的修饰的聚合酶存在需求。

[0003] 序列表

[0004] 本申请被连同电子格式的序列表一起提交。序列表作为标题为IP1152.TXT的文件提供,所述文件被创建于2014年5月28日,大小为186Kb。电子格式的序列表的信息通过引用以其整体被并入本文。

[0005] 简述

[0006] 本文呈现了用于核苷酸类似物的改进的掺入的聚合酶,所述核苷酸类似物特别是在3' 糖羟基处修饰以使得取代基在尺寸上大于天然存在的3' 羟基的核苷酸。本发明人已出人意外地鉴定出某些改造的聚合酶,所述改造的聚合酶表现出期望的类似物的改进的掺入并且具有许多其他相关的优势。

[0007] 在某些实施方案中,改造的聚合酶在功能上等同于9°N DNA聚合酶氨基酸序列中的Lys477的位置处包含至少一个氨基酸置换突变。野生型9°N DNA聚合酶氨基酸序列如SEQ ID NO:5所列。在某些实施方案中,置换突变包括突变成具有较小侧链的残基的突变。在某些实施方案中,置换突变包括突变成具有疏水性侧链的残基的突变。在某些实施方案中,置换突变包括突变成甲硫氨酸残基的突变。

[0008] 本文还呈现了重组的DNA聚合酶,所述重组的DNA聚合酶包含与SEQ ID NO:29至少60%、70%、80%、90%、95%、99%同一的氨基酸序列,所述重组DNA聚合酶在功能上等同于9°N DNA聚合酶氨基酸序列中的Lys477的位置处包含。

[0009] 本文还呈现了重组的DNA聚合酶,所述重组的DNA聚合酶包含与SEQ ID NO:31至少60%、70%、80%、90%、95%、99%同一的氨基酸序列,所述重组DNA聚合酶在功能上等同于9°N DNA聚合酶氨基酸序列中的Lys477的位置处包含。

[0010] 本文还呈现了重组的DNA聚合酶,所述重组的DNA聚合酶包含与SEQ ID NO:5至少60%、70%、80%、90%、95%、99%同一的氨基酸序列,所述重组的DNA聚合酶在功能上等同于9°N DNA聚合酶氨基酸序列中的Lys477的位置处包含。

[0011] 在一些实施方案中,聚合酶是DNA聚合酶。权利要求1所述的改造的聚合酶,其中DNA聚合酶是B家族型DNA聚合酶。聚合酶可以是例如B家族古细菌DNA聚合酶、人DNA聚合酶- α 、T4、RB69和phi29噬菌体DNA聚合酶。在某些实施方案中,B家族古细菌DNA聚合酶来自选自嗜热球菌属(Thermococcus)、火球菌属(Pyrococcus)和甲烷球菌属(Methanococcus)组成的组的属。例如,聚合酶可以选自Vent聚合酶、Deep Vent聚合酶、9°N聚合酶和Pfu聚合酶组成的组。在某些实施方案中,B家族古细菌DNA聚合酶是9°N聚合酶。

[0012] 在一些实施方案中,除了以上突变之外,改造的聚合酶还可以在功能上等同于9°N DNA聚合酶氨基酸序列中的Leu408和/或Tyr409和/或Pro410的位置处包含置换突变。例如,置换突变可以包括与9°N DNA聚合酶氨基酸序列中的Leu408Ala和/或Tyr409Ala和/或

Pro410Ile同源的置换突变。

[0013] 在一些实施方案中,改造的聚合酶与野生型聚合酶相比包含降低的外切核酸酶活性。例如,在某些实施方案中,改造的聚合酶在功能上等同于9°N DNA聚合酶氨基酸序列中的Asp141和/或Glu143的位置处包含置换突变。

[0014] 在某些实施方案中,改造的聚合酶还在功能上等同于9°N DNA聚合酶氨基酸序列中的Ala485的位置处包含置换突变。例如,在一些实施方案中,聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Ala485Leu或Ala485Val的置换突变。

[0015] 在某些实施方案中,改造的聚合酶还在功能上等同于9°N DNA聚合酶氨基酸序列中的Cys223的位置处包含突变成不同的氨基酸的置换突变。例如,在某些实施方案中,改造的聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Cys223Ser的置换突变。

[0016] 在某些实施方案中,至少一个置换突变包括对等同于Thr514和/或Ile521的位置的突变。例如,在某些实施方案中,改造的聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Thr514Ala、Thr514Ser和/或Ile521Leu的置换突变。

[0017] 在某些实施方案中,改造的聚合酶可以包含另外的置换突变以去除内部的甲硫氨酸。例如,在一些实施方案中,改造的聚合酶在功能上等同于9°N DNA聚合酶氨基酸序列中的Met129的位置处包含突变成不同的氨基酸的置换突变。在某些实施方案中,改造的聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Met129Ala的置换突变。

[0018] 本文还呈现了包含对半保守结构域的置换突变的改造的聚合酶,所述半保守结构域包含SEQ ID NO:1-4中的任一个的氨基酸序列,其中置换突变包括在位置3处突变成除了Lys、Ile或Gln之外的任一残基的突变。在某些实施方案中,改造的聚合酶在SEQ ID NO:1-4中任一个的位置3处包含突变成Met的突变。

[0019] 在一些实施方案中,除了以上突变之外,改造的聚合酶还可以在功能上等同于9°N DNA聚合酶氨基酸序列中的Leu408和/或Tyr409和/或Pro410的位置处包含置换突变。例如,置换突变可以包括与9°N DNA聚合酶氨基酸序列中的Leu408Ala和/或Tyr409Ala和/或Pro410Ile同源的置换突变。

[0020] 在一些实施方案中,改造的聚合酶与野生型聚合酶相比包含降低的外切核酸酶活性。例如,在某些实施方案中,改造的聚合酶在功能上等同于9°N DNA聚合酶氨基酸序列中的Asp141和/或Glu143的位置处包含置换突变。

[0021] 在某些实施方案中,改造的聚合酶还在功能上等同于9°N DNA聚合酶氨基酸序列中的Ala485的位置处包含置换突变。例如,在一些实施方案中,聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Ala485Leu或Ala485Val的置换突变。

[0022] 在某些实施方案中,改造的聚合酶还包含对等同于9°N DNA聚合酶氨基酸序列中的Thr514和/或Ile521的位置的突变。例如,在某些实施方案中,改造的聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Thr514Ala、Thr514Ser和/或Ile521Leu的置换突变。

[0023] 在某些实施方案中,改造的聚合酶还在功能上等同于9°N DNA聚合酶氨基酸序列中的Cys223的位置处包含突变成不同的氨基酸的置换突变。如,在某些实施方案中,改造的聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Cys223Ser的置换突变。

[0024] 在某些实施方案中,改造的聚合酶可以包含另外的置换突变以去除内部的甲硫氨酸。例如,在一些实施方案中,改造的聚合酶在功能上等同于9°N DNA聚合酶氨基酸序列中

的Met129的位置处包含突变成不同的氨基酸的置换突变。在某些实施方案中,改造的聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Met129Ala的置换突变。

[0025] 本文还呈现了包含SEQ ID NO:6-8、10-12、14-16、18-20、22-24、26-28、30和32中的任一个的氨基酸序列的改造的聚合酶。

[0026] 本文还呈现了编码如在任一以上实施方案中定义的改造的聚合酶的核酸分子。本文还呈现了包含以上描述的核酸分子的表达载体。本文还呈现了包含以上描述的载体的宿主细胞。

[0027] 本文还呈现了用于将修饰的核苷酸掺入DNA的方法,所述方法包括允许以下组分相互作用:(i)根据以上实施方案中的任一个的改造的聚合酶,(ii)DNA模板;和(iii)核苷酸溶液。在某些实施方案中,DNA模板包括聚簇阵列(clustered array)。

[0028] 本文还呈现了用于进行核苷酸掺入反应的试剂盒,所述试剂盒包含:如在以上实施方案的任一个中定义的聚合酶和核苷酸溶液。在某些实施方案中,核苷酸溶液包含带标记的核苷酸。在某些实施方案中,核苷酸包括合成的核苷酸。在某些实施方案中,核苷酸包括修饰的核苷酸。在某些实施方案中,修饰的核苷酸在3' 糖羟基处已被修饰以使得取代基在尺寸上大于天然存在的3' 羟基。在某些实施方案中,修饰的核苷酸包括包含嘌呤碱基或嘧啶碱基和以下核糖或脱氧核糖部分的修饰的核苷酸或核苷分子,所述核糖或脱氧核糖部分具有与其共价地附接的可移除的3' -OH封闭基团以使得3' 碳原子已附接以下结构

[0029] $-O-Z$

[0030] 其中Z是 $-C(R')_2-O-R''$ 、 $-C(R')_2-N(R'')_2$ 、 $-C(R')_2-N(H)R''$ 、 $-C(R')_2-S-R''$ 和 $-C(R')_2-F$ 中的任一个,

[0031] 其中每个R''是可移除的保护基团或可移除的保护基团的一部分;

[0032] 每个R' 独立地是氢原子、烷基、取代的烷基、芳基烷基、烯基、炔基、芳基、杂芳基、杂环基团、酰基、氰基、烷氧基、芳氧基、杂芳氧基或酰氨基(amido),或通过连接基团附接的可检测的标记物;或 $(R')_2$ 代表式 $=C(R'')_2$ 的亚烷基,其中每个R'' 可以是相同的或不同的并且选自包括氢原子和卤素原子以及烷基的组;并且

[0033] 其中分子可以反应以产生中间体,在所述中间体中每个R'' 被交换为H,或当Z是 $-C(R')_2-F$ 时,F被交换为OH、SH或NH₂,优选地OH,所述中间体在水性条件下解离以提供带有游离3' OH的分子;

[0034] 条件是:当Z是 $-C(R')_2-S-R''$ 时,两个R' 基团均不是H。

[0035] 在某些实施方案中,修饰的核苷酸或核苷的R' 是烷基或取代的烷基。在某些实施方案中,修饰的核苷酸或核苷的-Z是式 $-C(R')_2-N_3$ 。在某些实施方案中,Z是叠氮甲基。

[0036] 在某些实施方案中,修饰的核苷酸被荧光标记以允许其检测。在某些实施方案中,修饰的核苷酸包含具有经由可裂解的接头附接到可检测的标记物的碱基的核苷酸或核苷。在某些实施方案中,可检测的标记物包括荧光标记物。在某些实施方案中,试剂盒还包含一个或多个DNA模板分子和/或引物。

[0037] 一个或多个实施方案的细节在附图和以下说明中被描述。从说明书和附图,以及从权利要求,其他的特征、目标和优势将是明显的。

[0038] 附图简述

[0039] 图1是显示聚合酶氨基酸序列的比对的示意图,所述氨基酸序列来自嗜热球菌属

物种 *Thermococcus* sp. 9°N-7 (9°N)、9°N 聚合酶 T514S/I521L 突变体 (Po1957)、*Thermococcus gorgonarius* (TG0)、*Thermococcus kodakaraensis* (KOD1)、强烈火球菌 (*Pyrococcus furiosus*) (Pfu)、*Methanococcus maripaludis* (MMS2) 和 RB69 噬菌体 DNA 聚合酶。显示的编号代表 9°N 聚合酶中的氨基酸残基的编号。

[0040] 图2是显示图1中所示的比对的两个突出部分的示意图。

[0041] 详细描述

[0042] 本文呈现了用于核苷酸类似物的改进的掺入的聚合酶,所述核苷酸类似物特别是在3' 糖羟基处修饰以使得取代基在尺寸上大于天然存在的3' 羟基的核苷酸。本发明人已出人意外地鉴定出某些改造的聚合酶,所述改造的聚合酶表现出期望的类似物的改进的掺入并且具有许多其他相关的优势。

[0043] 如下文更详细地描述的,发明人已出人意外地发现对聚合酶中的一个或更多个残基的一个或更多个突变导致周转率的显著增大和焦磷酸解的减少。这些改造的聚合酶在 DNA 合成测序 (SBS) 中具有改善的性能,并且导致减少的 phasing 和/或 pre-phasing 错误。

[0044] 如本文使用的,术语“phasing”指在 SBS 中在给定的测序循环,由聚合酶对聚簇内的 DNA 链的一些部分中的核苷酸的不完全掺入而引起的现象。术语“pre-phasing”指在 SBS 中由掺入核苷酸而无有效的3' 终止子而引起的现象,导致掺入事件提前1个循环。Phasing 和 pre-phasing 导致对特定的循环得出的强度由当前循环的信号以及来自前面和后面的循环的噪音组成。随着循环数增加,被 phasing 影响的序列/簇的比例增加,妨碍正确碱基的识别。phasing 可以例如由进行核苷酸掺入的逆反应的聚合酶引起,所述核苷酸掺入的逆反应如已知在有益于焦磷酸解的条件下发生的。因此,降低 phasing 和/或 pre-phasing 的发生率的改造的聚合酶的发现是出人意外的,并且在 SBS 应用中提供了大的优势。例如,改造的聚合酶提供了较快的 SBS 循环时间、较低的 phasing 值和 pre-phasing 值以及较长的测序读段长度。如本文提供的改造的聚合酶的特征在以下实施例部分列出。

[0045] 在某些实施方案中,置换突变包括突变成具有较小侧链的残基的突变。氨基酸侧链的相对尺寸在本领域是熟知的,并且可以使用任何已知的度量标准比较,所述度量标准包括立体效应和/或电子密度。因此,以渐增的尺寸的顺序列出的氨基酸的一个实例将是 G、A、S、C、V、T、P、I、L、D、N、E、Q、M、K、H、F、Y、R、W。在某些实施方案中,置换突变包括突变成具有疏水性侧链的残基的突变,诸如 A、I、L、V、F、W、Y。

[0046] 本文还呈现了包含对聚合酶的半保守结构域的置换突变的改造的聚合酶。如本文使用的,术语“半保守结构域”指在多个物种中完全保守或至少部分保守的聚合酶部分。已出人意外地发现,半保守结构域中的一个或更多个残基的突变在3' 封闭的核苷酸 (3' blocked nucleotides) 的存在下影响聚合酶活性,导致周转率的显著增大和焦磷酸解的减少。这些改造的聚合酶在 DNA 合成测序中具有改善的性能,并且导致减少的 phasing 错误,如在下面的实施例部分中描述的。

[0047] 在一些实施方案中,半保守结构域包含具有 SEQ ID NO:1-4 的任一个所列的序列的氨基酸。SEQ ID NO:1-4 对应于多个物种中的半保守结构域中的残基。SEQ ID NO:4 对应于在本文中被列为 SEQ ID NO:5 的 9°N DNA 聚合酶氨基酸序列的残基 475-492。图1和2列出了显示半保守结构域在多个物种中的保守性的比对。图1和2中示出的聚合酶序列获取自 Genbank 数据库登录号 Q56366 (9°N DNA 聚合酶)、NP_577941 (Pfu)、YP_182414 (KOD1)、NP_

987500 (MMS2)、AAP75958 (RB69)、P56689 (TGo)。

[0048] 已出人意外地发现对半保守结构域中的一个或多个残基的突变导致周转率的增大和焦磷酸解的减少,导致减少的phasing错误。例如,在本文呈现的改造的聚合酶的一些实施方案中,置换突变包括在SEQ ID NO:1-4中任一个的位置3处突变成除了Lys、Ile或Gln之外的任何残基的突变。在某些实施方案中,改造的聚合酶在SEQ ID NO:1-4中任一个的位置3处包含突变成Met的突变。

[0049] 在一些实施方案中,聚合酶是DNA聚合酶。在一些实施方案中,DNA聚合酶是B家族型DNA聚合酶。聚合酶可以是例如B家族古细菌DNA聚合酶、人DNA聚合酶- α 和噬菌体聚合酶。任何噬菌体聚合酶可被用于本文呈现的实施方案,所述任何噬菌体聚合酶包括例如噬菌体聚合酶,诸如T4、RB69和phi29噬菌体DNA聚合酶。

[0050] B家族古细菌DNA聚合酶在本领域是熟知的,如由美国专利号8,283,149的公开内容示例的,其通过引用被以其整体并入。在某些实施方案中,古细菌DNA聚合酶来自超嗜热古细菌,这意指聚合酶通常是热稳定的。因此,在另外的优选的实施方案中,聚合酶选自Vent聚合酶、Deep Vent聚合酶、9 $^{\circ}$ N聚合酶和Pfu聚合酶。Vent和Deep Vent是用于从超嗜热古细菌海滨嗜热球菌(*Thermococcus litoralis*)分离的B家族DNA聚合酶的商业名。还从嗜热球菌属物种*Thermococcus* sp.鉴定出9 $^{\circ}$ N聚合酶。Pfu聚合酶分离自强烈火球菌。

[0051] 在某些实施方案中,B家族古细菌DNA聚合酶来自诸如例如嗜热球菌属(*Thermococcus*)、火球菌属(*Pyrococcus*)和甲烷球菌属(*Methanococcus*)的那些属的属。嗜热球菌属的成员在本领域是熟知的,并且包括但不限于*Thermococcus* 4557、*Thermococcus barophilus*、*Thermococcus gammatolerans*、*Thermococcus onnurineus*、*Thermococcus sibiricus*、*Thermococcus kodakarensis*、*Thermococcus gorgonarius*。火球菌属的成员在本领域是熟知的,并且包括但不限于*Pyrococcus* NA2、深海火球菌(*Pyrococcus abyssi*)、强烈火球菌、*Pyrococcus horikoshii*、*Pyrococcus yamanosii*、*Pyrococcus endeavori*、*Pyrococcus glycovorans*、沃氏火球菌(*Pyrococcus woesei*)。甲烷球菌属的成员在本领域是熟知的,并且包括但不限于*M. aeolicus*、*M. maripaludis*、万氏甲烷球菌(*M. vannieli*)、沃氏甲烷球菌(*M. voltae*)、“*M. thermolithotrophicus*”和“詹氏甲烷球菌(*M. jannaschii*)”。

[0052] 例如,聚合酶可以选自由Vent聚合酶、Deep Vent聚合酶、9 $^{\circ}$ N聚合酶和Pfu聚合酶组成的组。在某些实施方案中,B家族古细菌DNA聚合酶是9 $^{\circ}$ N聚合酶。

[0053] 序列比较、同一性和同源性

[0054] 在两个或更多个核酸或多肽序列的上下文中,术语“同一的”或“同一性百分比”指当针对最大的对应性比较和对齐时,相同或具有指定百分比的相同的氨基酸残基或核苷酸的两个或更多个序列或子序列,如利用下面描述的序列比较算法中的一个(或技术人员可得的其他算法)或通过视觉检查测量的。

[0055] 在两个核酸或多肽(例如,编码聚合酶的DNA或聚合酶的氨基酸序列)的上下文中,词组“基本上同一的”指当针对最大的对应性比较和对齐时,具有至少约60%、约80%、约90-95%、约98%、约99%或更大的核苷酸或氨基酸残基同一性的两个或更多个序列或子序列,如利用序列比较算法或通过视觉检查测量的。此类“基本上同一的”序列通常被认为是“同源的”,而不是指实际的祖先。优选地,“基本同一”存在于长度为至少约50个残基的序列

的区域上,更优选地,存在于至少约100个残基的区域上,并且最优选地,序列在至少约150个残基上或在待比较的两个序列的全长上基本上同一。

[0056] 当蛋白和/或蛋白序列天然地或人工地源自共同的祖先蛋白或蛋白序列时,所述蛋白和/或蛋白序列是“同源的”。类似的,当核酸和/或核酸序列天然地或人工地源自共同的祖先核酸或核酸序列时,所述核酸和/或核酸序列是“同源的”。同源性通常从两个或更多个核酸或蛋白(或其序列)之间的序列相似性推断出。在用于建立同源性的序列之间的相似性的精确百分比随着被讨论的核酸和蛋白而变化,但是对于50、100、150或更多个残基仅仅25%的序列相似性被常规地用于建立同源性。还可以使用较高水平的序列相似性,例如30%、40%、50%、60%、70%、80%、90%、95%或99%或更高。在本文中描述了用于确定序列相似性百分比的方法(例如,使用缺省参数的BLASTP和BLASTN),并且通常是可得的。

[0057] 对于序列比较和同源性确定,通常一个序列充当参考序列,测试序列与其比较。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中,如需要则指定子序列坐标,并指定序列算法程序参数。然后序列比较算法基于指定的程序参数计算测试序列相对于参考序列的序列同一性百分比。

[0058] 用于比较的序列的最佳对齐可以例如通过Smith&Waterman, Adv. Appl. Math. 2: 482 (1981) 的局部同源性算法,通过Needleman&Wunsch, J. Mol. Biol. 48:443 (1970) 的同源性比对算法,通过Pearson&Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988) 的相似性搜寻方法,通过这些算法的计算机化实施(Wisconsin Genetics软件包中的GAP、BESTFIT、FASTA和TFASTA, Genetics Computer Group, 575 Science Dr., Madison, Wis.)或通过视觉检查(大体参见Current Protocols in Molecular Biology, Ausubel等, 编, Current Protocols, Greene Publishing Associates, Inc. 和 John Wiley&Sons, Inc. 之间的合资企业, 2004年补充)来进行。

[0059] 适合于确定序列同一性百分比和序列相似性的算法的一个实例是BLAST算法,所述BLAST算法被描述于Altschul等, J. Mol. Biol. 215:403-410 (1990)。用于进行BLAST分析的软件是通过美国国家生物技术信息中心(National Center for Biotechnology Information)公开地可得的。该算法涉及首先通过鉴定查询序列中长度为W的短字鉴定高得分序列对(HSP),所述长度为W的短字在与数据库序列中的相同长度的字对齐时,匹配或满足一些正值的阈值得分T。T被称为相邻字得分阈值(Altschul等, 同上)。这些初始的相邻字击中充当种子,用于起始搜索以发现包含它们的更长的HSP。然后,字击中沿着每个序列在两个方向上延伸,远至累积对齐得分不可被增加。对于核苷酸序列,使用参数M(对于一对匹配的残基的奖励得分;总是>0)和N(对于不匹配的残基的罚分;总是<0)计算累积得分。对于氨基酸序列,使用得分矩阵来计算累积得分。当累积对齐得分由于一个或更多个负得分的残基对齐的累积而从其达到的最大值下降量X;累积得分到达零或零以下时,字击中在每个方向上的延伸停止。BLAST算法参数W、T和X确定算法的灵敏度和速度。BLASTN程序(对于核苷酸序列)使用字长(W) 11、期望(E) 10、截断100、M=5、N=-4和两条链的比较作为缺省。对于氨基酸序列, BLAST程序使用字长(W) 3、期望(E) 10和BLOSUM62得分矩阵(参见Henikoff&Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915)作为缺省。

[0060] 除了计算序列同一性百分比之外, BLAST算法还进行两个序列之间的相似性的统计分析(参见,例如, Karlin&Altschul, Proc. Nat'l. Acad. Sci. USA 90:5873-5787 (1993))。

由BLAST算法提供的一个相似性测量是最小总概率(P(N)),所述最小总概率提供两个核苷酸或氨基酸序列之间的匹配将偶然发生的概率的指示。例如,如果在测试核酸与参考核酸的比较中最小总概率小于约0.1、更优选地小于约0.01、并且最优选地小于约0.001,则认为核酸与参考序列相似。

[0061] 在使用完全不同的聚合酶的研究的情况下,“功能上等同”意指对照聚合酶将在酶中包含被认为发生于其它聚合酶中具有相同功能作用的氨基酸位置处的氨基酸置换。例如,在Vent DNA聚合酶中在位置412处从酪氨酸到缬氨酸的突变(Y412V)将功能上等同于在9°N聚合酶中在位置409处从酪氨酸到缬氨酸的突变(Y409V)。

[0062] 通常,两个或更多个不同的聚合酶中的功能上等同的置换突变发生在聚合酶的氨基酸序列中的同源氨基酸位置处。因此,在本文中术语“功能上等同”的使用还包括与给定的突变“位置上等同”或“同源”的突变,不管突变的氨基酸的特定功能是否是已知的。基于序列比对和/或分子建模鉴定两个或更多个不同的聚合酶的氨基酸序列中的在位置上等同或同源的氨基酸残基是可能的。序列比对以鉴定在位置上等同和/或在功能上等同的残基的实例被列于图1和2中。因此,例如,如图2中示出的,半保守结构域中的残基被鉴定为9°N DNA聚合酶氨基酸序列的位置475-492。TGO、KOD1、Pfu、MmS2和RB69聚合酶中的相应残基在图中被鉴定为竖直地对齐的,并且被认为在位置上等同于以及功能上等同于9°N DNA聚合酶氨基酸序列的相应残基。

[0063] 上文描述的改造的聚合酶可以包含已知在3' 封闭的核苷酸的存在下和/或在DNA测序应用中增强聚合酶活性的一个或更多个方面的另外的置换突变。例如,在一些实施方案中,除了以上突变中的任一个之外,改造的聚合酶还可以在功能上等同于9°N DNA聚合酶氨基酸序列中的Leu408和/或Tyr409和/或Pro410的位置处包含置换突变。可以进行功能上等同于9°N DNA聚合酶氨基酸序列中的408-410的位置处的一个或更多个位置处的多个置换突变中的任一个,所述多个置换突变中的任一个导致封闭的核苷酸的增加的掺入,如本领域已知并由US 2006/0240439和US2006/0281109的公开内容例示的,其每一个通过引用以其整体被并入。例如,置换突变可以包括与9°N DNA聚合酶氨基酸序列中的Leu408Ala和/或Tyr409Ala和/或Pro410Ile同源的置换突变。在某些实施方案中,除了以上突变中的任一个之外,改造的聚合酶还在功能上等同于9°N DNA聚合酶氨基酸序列中的Ala485的位置处包含置换突变。例如,在一些实施方案中,聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Ala485Leu或Ala485Val的置换突变。

[0064] 在一些实施方案中,除了以上突变中的任一个之外,改造的聚合酶与野生型聚合酶相比可以包含降低的外切核酸酶活性。可以进行在已知导致降低的外切核酸酶活性的一个或更多个位置处的多个置换突变中的任一个,如在本领域已知并由US 2006/0240439和US 2006/0281109的并入材料例示的。例如,在一些实施方案中,除了以上突变之外,改造的聚合酶还可以在功能上等同于9°N DNA聚合酶氨基酸序列中的Asp141和/或Glu143的位置处包含置换突变。

[0065] 在某些实施方案中,除了以上突变中的任一个之外,改造的聚合酶还在功能上等同于9°N DNA聚合酶氨基酸序列中的Cys223的位置处包含突变成不同的氨基酸的置换突变,如在本领域已知并由US 2006/0281109的并入材料例示的。例如,在某些实施方案中,改造的聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Cys223Ser的置换突变。

[0066] 在某些实施方案中,除了以上突变中的任一个之外,改造的聚合酶可以包含对等同于9°N DNA聚合酶氨基酸序列中的Thr514和/或Ile521的位置的一个或更多个突变,如在本领域已知并由PCT/US2013/031694的公开内容例示的,其通过引用以其整体被并入。例如,在某些实施方案中,改造的聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Thr514Ala、Thr514Ser和/或Ile521Leu的置换突变。

[0067] 在某些实施方案中,除了以上突变中的任一个之外,改造的聚合酶可以包含对等同于9°N DNA聚合酶氨基酸序列中的Arg713的位置的一个或更多个突变,如在本领域已知并由美国专利号8,623,628的公开内容例示的,其通过引用以其整体被并入。例如,在某些实施方案中,改造的聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Arg713Gly、Arg713Met或Arg713Ala的置换突变。

[0068] 在某些实施方案中,除了以上突变中的任一个之外,改造的聚合酶可以包含对等同于9°N DNA聚合酶氨基酸序列中的Arg743和/或Lys705的位置的一个或更多个突变,如在本领域已知并由美国专利号8,623,628的公开内容例示的,其通过引用以其整体被并入。例如,在某些实施方案中,改造的聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Arg743Ala和/或Lys705Ala的置换突变。

[0069] 在某些实施方案中,除了以上突变中的任一个之外,改造的聚合酶可以包含一个或更多个另外的置换突变以去除内部的甲硫氨酸。例如,在一些实施方案中,改造的聚合酶在功能上等同于9°N DNA聚合酶氨基酸序列中的Met129的位置处包含突变成不同的氨基酸的置换突变。在某些实施方案中,改造的聚合酶包含功能上等同于9°N聚合酶氨基酸序列中的Met129Ala的置换突变。

[0070] 突变聚合酶

[0071] 例如根据如以上讨论的聚合酶模型和模型预测,或使用随机或半随机突变方法,多种类型的诱变被任选地用于本公开内容,例如来修饰聚合酶以产生变体。通常,任何可得的诱变程序可以被用于制备聚合酶突变体。此类诱变程序任选地包括针对一种或更多种感兴趣的活性(例如对于给定的核苷酸类似物例如降低的焦磷酸解、增加的周转)选择突变的核酸和多肽。可以使用的程序包括但不限于:定点诱变、随机点诱变、体外或体内同源重组(DNA重排和组合的重叠PCR)、使用含有尿嘧啶的模板的诱变、寡核苷酸定向诱变、硫代磷酸修饰的DNA诱变、使用有缺口的二聚体DNA的诱变、点错配修复、使用修复缺陷宿主菌株的诱变、限制性选择和限制性纯化、缺失诱变、通过全基因合成的诱变、简并PCR、双链断裂修复和技术人员已知的许多其他的程序。用于突变的起始聚合酶可以是本文提到的那些聚合酶中的任一个,包括可得的聚合酶突变体诸如例如在US 2006/0240439和US 2006/0281109中鉴定的那些聚合酶突变体,其每一个通过引用以其整体被并入。

[0072] 任选地,诱变可由来自天然存在的聚合酶分子的信息或已知的改造或突变的聚合酶(例如,使用如前面的参考中提到的现存的突变聚合酶)的信息,例如以上讨论的序列、序列比较、物理性质、晶体结构等来指导。但是,在另一类实施方案中,修饰可以是基本上随机的(例如,如在经典或“家族”DNA重排中,参见,例如Cramer等(1998)“DNA shuffling of a family of genes from diverse species accelerates directed evolution”Nature 391:288-291)。

[0073] 突变形式的另外的信息在以下中找到:Sambrook等,Molecular Cloning--A

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

mutagenesis, Proc. Natl. Acad. Sci. USA, 83:7177-7181 (1986); Nakamaye&Eckstein, Inhibition of restriction endonuclease Nci I cleavage by phosphorothioate groups and its application to oligonucleotide-directed mutagenesis, Nucl. Acids Res. 14:9679-9698 (1986); Nambiar等, Total synthesis and cloning of a gene coding for the ribonuclease S protein, Science 223:1299-1301 (1984); Sakamar和Khorana, Total synthesis and expression of a gene for the α -subunit of bovine rod outer segment guanine nucleotide-binding protein (transducin), Nucl. Acids Res. 14:6361-6372 (1988); Sayers等, Y-T Exonucleases in phosphorothioate-based oligonucleotide-directed mutagenesis, Nucl. Acids Res. 16:791-802 (1988); Sayers等, Strand specific cleavage of phosphorothioate-containing DNA by reaction with restriction endonucleases in the presence of ethidium bromide, (1988) Nucl. Acids Res. 16:803-814; Sieber, 等, Nature Biotechnology, 19:456-460 (2001); Smith, In vitro mutagenesis, Ann. Rev. Genet. 19:423-462 (1985); Methods in Enzymol. 100:468-500 (1983); Methods in Enzymol. 154:329-350 (1987); Stemmer, Nature 370, 389-91 (1994); Taylor等, The use of phosphorothioate-modified DNA in restriction enzyme reactions to prepare nicked DNA, Nucl. Acids Res. 13:8749-8764 (1985); Taylor等, The rapid generation of oligonucleotide-directed mutations at high frequency using phosphorothioate-modified DNA, Nucl. Acids Res. 13:8765-8787 (1985); Wells等, Importance of hydrogen-bond formation in stabilizing the transition state of subtilisin, Phil. Trans. R. Soc. Lond. A317: 415-423 (1986); Wells等, Cassette mutagenesis: an efficient method for generation of multiple mutations at defined sites, Gene 34:315-323 (1985); Zoller&Smith, Oligonucleotide-directed mutagenesis using M13-derived vectors: an efficient and general procedure for the production of point mutations in any DNA fragment, Nucleic Acids Res. 10:6487-6500 (1982); Zoller&Smith, Oligonucleotide-directed mutagenesis of DNA fragments cloned into M13 vectors, Methods in Enzymol. 100:468-500 (1983); Zoller&Smith, Oligonucleotide-directed mutagenesis: a simple method using two oligonucleotide primers and a single-stranded DNA template, Methods in Enzymol. 154:329-350 (1987); Clackson等 (1991) "Making antibody fragments using phage display libraries" Nature 352:624-628; Gibbs等 (2001) "Degenerate oligonucleotide gene shuffling (DOGS): a method for enhancing the frequency of recombination with family shuffling" Gene 271:13-20; 以及 Hiraga和Arnold (2003) "General method for sequence-independent site-directed chimeragenesis: J. Mol. Biol. 330:287-296. 可以在Methods in Enzymology第154卷中找到许多以上方法的另外的详细说明, 其还描述了用于对多种诱变方法解决故障的问题的有用的对照。

[0074] 制备并分离重组聚合酶

[0075] 通常, 编码如本文呈现的聚合酶的核酸可通过克隆、重组、体外合成、体外扩增和/或其他可用的方法来制备。多种重组方法可用于表达编码如本文呈现的聚合酶的表达载

体。用于制备重组核酸、表达和分离表达的产物的方法在本领域是熟知的并被充分描述。本文描述了许多示例性突变和突变组合以及用于设计期望的突变的策略。用于在聚合酶的活性位点中产生并选择突变,包括用于修饰活性位点中或活性位点附近的立体特征以允许核苷酸类似物改进地进入的方法在上文以及例如WO 2007/076057和PCT/US2007/022459中找到,其通过引用以其整体被并入。

[0076] 用于突变、重组和体外核酸操作方法(包括克隆、表达、PCR等)的另外的有用的参考文献包括:Berger和Kimmel,Guide to Molecular Cloning Techniques,Methods in Enzymology第152卷Academic Press,Inc.,San Diego,Calif.(Berger);Kaufman等(2003)Handbook of Molecular and Cellular Methods in Biology and Medicine第二版Ceske(编)CRC Press(Kaufman);和The Nucleic Acid Protocols Handbook Ralph Rapley(编)(2000)Cold Spring Harbor,Humana Press Inc(Rapley);Chen等(编)PCR Cloning Protocols,第二版(Methods in Molecular Biology,第192卷)Humana Press;和Viljoen等(2005)Molecular Diagnostic PCR Handbook Springer,ISBN1402034032。

[0077] 另外,对于从细胞纯化质粒或其他相关核酸,许多试剂盒是商业可得的(参见,例如,均来自Pharmacia Biotech的EasyPrep.TM.、FlexiPrep.TM.;来自Stratagene的StrataClean.TM.;和来自Qiagen的QIAprep.TM.)。任何分离和/或纯化的核酸可被进一步操作以产生其他核酸、用于转染细胞、掺入相关的载体以感染生物体用于表达等。通常的克隆载体含有转录和翻译终止子、转录和翻译起始序列、和用于调节特定靶核酸的表达的启动子。载体任选地包含通用表达盒,所述通用表达盒含有至少一个独立的终止子序列、允许盒在真核生物或原核生物或二者中复制的序列(例如穿梭载体)和用于原核系统和真核系统二者的选择标记物。载体适合于在原核生物、真核生物或二者中复制和整合。

[0078] 例如用于细胞分离和培养(例如,用于随后的核酸分离)的其他有用的参考文献包括Freshney(1994)Culture of Animal Cells,a Manual of Basic Technique,第三版,Wiley-Liss,New York和其中引用的参考文献;Payne等(1992)Plant Cell and Tissue Culture in Liquid Systems John Wiley&Sons,Inc.New York,N.Y.;Gamborg和Phillips(编)(1995)Plant Cell,Tissue and Organ Culture;Fundamental Methods Springer Lab Manual,Springer-Verlag(Berlin Heidelberg New York)以及Atlas和Parks(编)The Handbook of Microbiological Media(1993)CRC Press,Boca Raton,Fla。

[0079] 编码本文公开的重组聚合酶的核酸还是本文呈现的实施方案的特征。特定的氨基酸可由多个密码子编码,并且某些翻译系统(例如,原核细胞或真核细胞)往往表现出密码子偏倚,例如,不同的生物体往往优选编码同一氨基酸的若干同义密码子中的一个。因此,本文呈现的核酸任选地是“密码子优化的”,意指合成核酸以包含被采用以表达聚合酶的特定翻译系统优选的密码子。例如,当期望在细菌细胞(或甚至细菌的特定菌株)中表达聚合酶时,可以合成核酸以包含在该细菌细胞的基因组中最频繁发现的密码子,用于有效表达聚合酶。当期望在真核细胞中表达聚合酶时,可以采用类似的策略,例如,核酸可以包含该真核细胞优选的密码子。

[0080] 多种蛋白分离和检测方法是已知的,并且可用于例如从表达本文呈现的重组聚合酶的细胞的重组培养物分离聚合酶。多种蛋白分离和检测方法在本领域是熟知的,包括例如在以下参考文献中列出的那些方法:R.Scopes,Protein Purification,Springer-

Verlag, N.Y. (1982); Deutscher, Methods in Enzymology 第182卷: Guide to Protein Purification, Academic Press, Inc. N.Y. (1990); Sandana (1997) Bioseparation of Proteins, Academic Press, Inc.; Bollag等 (1996) Protein Methods, 第二增补版 Wiley-Liss, NY; Walker (1996) The Protein Protocols Handbook Humana Press, NJ; Harris和Angal (1990) Protein Purification Applications: A Practical Approach IRL Press at Oxford, Oxford, England; Harris和Angal Protein Purification Methods: A Practical Approach IRL Press at Oxford, Oxford, England; Scopes (1993) Protein Purification: Principles and Practice 第三增补版 Springer Verlag, NY; Janson and Ryden (1998) Protein Purification: Principles, High Resolution Methods and Applications, 第二版 Wiley-VCH, NY; 和 Walker (1998) Protein Protocols on CD-ROM Humana Press, NJ; 以及其中引用的参考文献。关于蛋白纯化和检测方法的另外的详细说明可以在 Satinder Ahuja 编, Handbook of Bioseparations, Academic Press (2000) 找到。

[0081] 使用方法

[0082] 本文呈现的改造的聚合酶可以用于测序程序, 诸如合成测序 (SBS) 技术。简而言之, SBS 可通过使靶核酸与一个或更多个带标记的核苷酸、DNA 聚合酶等接触起始。当使用靶核酸作为模板延伸引物时, 那些特征将掺入可被检测的带标记的核苷酸。任选地, 带标记的核苷酸还可包括可逆终止特性, 所述可逆终止特性在核苷酸已被添加至引物后终止进一步的引物延伸。例如, 具有可逆终止子部分的核苷酸类似物可被添加至引物, 以使得随后的延伸不能发生, 直到递送解封剂以去除该部分。因此, 对于使用可逆终止的实施方案, 可将解封试剂递送至流通池 (在检测发生之前或之后)。在多个递送步骤之间可进行洗涤。然后可重复循环 n 次, 以将引物延伸 n 个核苷酸, 从而检测长度 n 的序列。可容易地改编以与由本公开内容的方法产生的阵列一起使用的示例性 SBS 程序、流体系统和检测平台被描述于例如 Bentley 等, Nature 456:53-59 (2008); WO 04/018497; WO 91/06678; WO 07/123744; 美国专利号 7,057,026、7,329,492、7,211,414、7,315,019 或 7,405,281 和美国专利申请公布号 2008/0108082A1, 其每个通过引用被并入本文。

[0083] 可利用使用循环反应的其他测序程序, 诸如焦磷酸测序。焦磷酸测序检测随着特定核苷酸被掺入到初生态核酸链中的无机焦磷酸 (PPi) 的释放 (Ronaghi, 等, Analytical Biochemistry 242 (1), 84-9 (1996); Ronaghi, Genome Res. 11 (1), 3-11 (2001); Ronaghi 等 Science 281 (5375), 363 (1998); 美国专利号 6,210,891、6,258,568 和 6,274,320, 其每个被通过引用并入本文。在焦磷酸测序中, 释放的 PPi 可通过由 ATP 硫酸化酶转化成腺苷三磷酸 (ATP) 来检测, 并且得到的 ATP 可经由萤光素酶产生的光子来检测。因此, 测序反应可经由发光检测系统来监测。用于基于荧光的检测系统的激发放射源对于焦磷酸测序程序不是必需的。可用于对本公开内容的阵列应用焦磷酸测序的有用的流体系统、检测器和程序被描述在例如 WIPO 专利申请序号 PCT/US11/57111、美国专利申请公布号 2005/0191698A1、美国专利号 7,595,883 和美国专利号 7,244,559 中, 其每个通过引用被并入本文。

[0084] 一些实施方案可利用包括 DNA 聚合酶活性的实时监测的方法。例如, 核苷酸掺入可通过载有荧光团的聚合酶和 γ -磷酸标记的核苷酸之间的荧光共振能量转移 (FRET) 相互作用, 或用零模波导来检测。用于基于 FRET 的测序的技术和试剂被描述于例如 Levene 等 Science 299, 682-686 (2003); Lundquist 等 Opt. Lett. 33, 1026-1028 (2008); Korlach 等

Proc.Natl.Acad.Sci.USA 105,1176-1181 (2008),通过引用将其公开内容并入本文。

[0085] 一些SBS实施方案包括检测将核苷酸掺入延伸产物后释放的质子。例如,基于检测释放的质子的测序可使用电子检测器和从Ion Torrent (Guilford,CT,a Life Technologies subsidiary) 商业可得的相关技术,或在美国专利申请公布号2009/0026082A1、2009/0127589A1、2010/0137143A1、或2010/0282617A1中描述的测序方法和系统,通过引用将其每个并入本文。

[0086] 相应地,本文呈现了用于将核苷酸类似物掺入DNA的方法,所述方法包括允许以下组分相互作用:(i) 根据以上实施方案中的任一个的改造的聚合酶,(ii) DNA模板;和(iii) 核苷酸溶液。在某些实施方案中,DNA模板包括聚簇阵列。在某些实施方案中,核苷酸在3' 糖羟基处被修饰,并且在3' 糖羟基处包含修饰以使得取代基在尺寸上大于天然存在的3' 羟基。

[0087] 编码改造的核苷酸的核酸

[0088] 本文还呈现了编码本文呈现的改造的聚合酶的核酸分子。对于氨基酸序列并且优选地还有编码聚合酶的野生型核苷酸序列是已知的、为突变形式的聚合酶的任何给定的改造的聚合酶,根据分子生物学的基本原理获得编码突变体的核苷酸序列是可能的。例如,给定编码9°N聚合酶的野生型核苷酸序列是已知的,则推断出使用标准遗传密码编码具有一个或更多个氨基酸置换的任何给定的突变形式的9°N的核苷酸序列是可能的。类似地,对于其他聚合酶诸如例如Vent™、Pfu、Tsp JDF-3、Taq等的突变形式,可容易地得到核苷酸序列。然后,可使用本领域已知的标准分子生物学技术构建具有需要的核苷酸序列的核酸分子。

[0089] 根据本文呈现的实施方案,定义的核酸不仅包括相同的核酸,还包括任何小的碱基变异,所述小的碱基变异特别地包括在保守氨基酸置换中由于简并密码子而导致同义密码子(指定相同氨基酸残基的不同密码子)的情况中的置换。术语“核酸序列”还包括关于碱基变异给出的任何单链序列的互补序列。

[0090] 本文描述的核酸分子还可以有利地被包含在合适的表达载体中,以在合适的宿主中从所述表达载体表达编码的聚合酶蛋白。将克隆的DNA掺入合适的表达载体是本领域技术人员熟知的,所述合适的表达载体用于随后的所述细胞的转化和随后的转化细胞的选择,如在Sambrook等(1989),Molecular cloning:A Laboratory Manual,Cold Spring Harbor Laboratory中提供的,其通过引用以其整体被并入。

[0091] 此类表达载体包括具有根据本文呈现的实施方案的核酸的载体,所述核酸可操作地连接至能够影响所述DNA片段的表达的调节序列,诸如启动子区域。术语“可操作地连接的”指其中描述的组分处于允许它们以其预期的方式发挥功能的关系的并置(juxtaposition)。此类载体可被转化入合适的宿主细胞以提供根据本文呈现的实施方案的蛋白的表达。

[0092] 核酸分子可以编码成熟蛋白或具有前序列的蛋白,所述前序列包括编码前体蛋白上的前导序列的前序列,所述前导序列然后被宿主细胞裂解以形成成熟蛋白。载体可以是例如具有复制起点、和任选地用于所述核苷酸的表达的启动子及任选地该启动子的调节物的质粒载体、病毒载体或噬菌体载体。载体可以含有一个或更多个可选择的标志物,诸如,例如,抗生素抗性基因。

[0093] 表达需要的调节元件包括:结合RNA聚合酶以及指导合适的转录起始水平的启动

子序列;以及用于核糖体结合的翻译起始序列。例如,细菌表达载体可以包括:启动子,诸如 lac 启动子;和用于翻译起始的 Shine-Dalgarno 序列;以及起始密码子 AUG。类似地,真核表达载体可以包括用于 RNA 聚合酶 II 的异源或同源启动子、下游多腺苷酸化信号、起始密码子 AUG 和用于核糖体的脱离的终止密码子。此类载体是商业可得的,或可通过本领域熟知的方法从描述的序列组装。

[0094] 通过在载体中包含增强子序列,编码聚合酶的 DNA 被高等真核生物的转录可以被优化。增强子是在启动子上起作用以增加转录的水平的 DNA 顺式作用元件。除了可选择的标志物之外,载体通常还将包含复制起点。

[0095] 实施例1

[0096] 通用测定方法和条件

[0097] 以下段落描述了在下面呈现的实施例中使用的通用测定条件。

[0098] 1. 基于凝胶的测定

[0099] 该部分描述了在下面的实施例中使用的用于监测聚合酶的焦磷酸解活性的基于凝胶的测定。

[0100] 简而言之,通过在反应缓冲液中使 300nM 酶和 100nM 双链引物-模板 DNA 分别与不同浓度 (0mM、0.125mM、0.25mM、0.5mM、1mM、2mM 和 4mM) 的焦磷酸钠混合来测量示例性修饰的聚合酶的焦磷酸解活性,所述反应缓冲液含有:50mM Tris-HCl (pH 9.0)、50mM NaCl、1mM EDTA、6mM MgSO₄ 和 0.05% (v/v) Tween-20。反应在 55℃ 进行持续 1 分钟,并且通过添加等体积的含有 0.025% 溴酚蓝、30mM EDTA 和 95% 去离子甲酰胺的 2X 猝灭溶液终止。

[0101] 使反应产物在 95℃ 变性持续 5min,并在 15% 7M 尿素-聚丙烯酰胺凝胶电泳 (尿素-PAGE) 中分辨。通过用 GE Healthcare Typhoon 8000 PhosphorImager 扫描使结果可视化。

[0102] 通过使以下寡核苷酸退火形成双链引物-模板双链体:

[0103] 引物:5'-GCTTGACAGGTGCGTTCGT*-3'

[0104] 模板:5'-CGTTAGTCCACGAACGCACCTGTGCAAGC-3'

[0105] 该引物包含与寡核苷酸的 5' 末端连接的 6-羧基四甲基罗丹明 (TAMRA) 荧光染料。最后的 "T*" 在核苷酸上含有 3' -O 叠氮甲基封闭部分。显示带标记的引物的降解的泳道指示与对照相比增强的焦磷酸解 (DNA 聚合的逆反应)。

[0106] 2. 聚合酶的克隆和表达

[0107] 该部分描述了用于克隆和表达在下面的实施例中使用的多种聚合酶突变体的方法。

[0108] 使用标准定点诱变方法在编码聚合酶的骨架基因序列的基因上进行诱变。对于进行的每个突变,通过对克隆的基因序列测序来确认突变的基因的正确序列。

[0109] 将聚合酶基因亚克隆到 pET11a 载体中,并转化到来自 Invitrogen 的 BL21 Star (DE3) 表达细胞中。在 37℃ 在 2.8L Fernback 烧瓶中培养转化细胞,直到 OD₆₀₀ 达到 0.8。然后,通过添加 1mM IPTG 诱导蛋白表达,随后另外生长 3 小时。然后将培养物以 7000rpm 离心持续 20 分钟。将细胞团块储存在 -20℃ 直至纯化。

[0110] 细菌细胞裂解通过在 10x w/v 裂解缓冲液 (Tris pH 7.5、500mM NaCl、1mM EDTA、1mM DTT) 中重悬冷冻的培养物进行。将无 EDTA 的蛋白酶抑制剂 (Roche) 添加到重悬的细胞团块中。所有的裂解和纯化步骤均在 4℃ 进行。将重悬的培养物通过微射流机

(microfluidizer)四次以完成细胞裂解。然后,使裂解物以20,000rpm离心持续20分钟以除去细胞碎片。将聚乙烯亚胺(终浓度0.5%)缓慢地添加至上清液中,搅拌持续45分钟以沉淀细菌核酸。使裂解物以20,000rpm离心持续20分钟;丢弃团块。然后,使用两体积的在无菌dH₂O中冷饱和的(NH₄)₂SO₄使裂解物硫酸铵沉淀。将沉淀的蛋白以20,000rpm离心持续20分钟。将蛋白团块重悬在250mL缓冲液A(50mM Tris pH 7.5、50mM KCl、0.1mM EDTA、1mM DTT)中。然后,使用在缓冲液A中预平衡的5mL SP FastFlow柱(GE)纯化重悬的裂解物。使用从0.1M至1M KCl的50mL梯度洗脱柱。汇集峰级分,并用缓冲液C(Tris pH 7.5、0.1mM EDTA、1mM DTT)稀释直到传导性等于缓冲液D(Tris pH 7.5、50mM KCl、0.1mM EDTA、1mM DTT)。然后,将汇集的级分装载到5mL HiTrap Heparin Fastflow柱上。然后,使用从50mM至1M KCl的100mL梯度洗脱聚合酶。将峰级分汇集,透析至储存缓冲液(20mM Tris pH 7.5、300mM KCl、0.1mM EDTA、50%甘油)中并在-80℃冷冻。

[0111] 3. Phasing/Pre-phasing分析

[0112] 该部分描述了用于分析在以下实施例中使用的聚合酶突变体在合成测序测定中的性能的方法。

[0113] 短12循环测序实验(short 12-cycle sequencing experiments)被用于产生phasing值和pre-phasing值。根据制造商的指示,在被转变成运行MiSeq Fast化学的单泳道系统的Illumina Genome Analyzer系统(Illumina, Inc., San Diego, CA)上进行实验。例如,对于每个聚合酶,产生单独的掺入混合物(IMX),并对每种IMX使用不同的位置进行4x12个循环运行。使用标准MiSeq试剂配制品并用正被测试的聚合酶替代标准聚合酶。按照标准TruSeq HT方案从PhiX基因组DNA(与Illumina试剂一起被提供为对照)制备使用的DNA文库。使用Illumina RTA软件来评价phasing水平和pre-phasing水平。

[0114] 实施例2

[0115] 针对Phasing/Pre-Phasing鉴定和筛选9°N聚合酶突变体

[0116] 进行3' 封闭袋(3' block pocket)中的残基的饱和诱变筛选。产生、克隆、表达并纯化对两个修饰的9°N聚合酶骨架序列(SEQ ID NO:29和31)的突变,如在实施例1中大体描述的。

[0117] 使用以上在实施例1中描述的基于凝胶的测定,针对爆发动力学(burst kinetics)筛选纯化的突变体聚合酶,并与具有SEQ ID NO:29和31所列的序列的对照聚合酶比较。在筛选的那些突变体中,包括以下突变体的一组突变体被进一步如在以上实施例1中描述的针对phasing/pre-phasing活性筛选。

[0118] 筛选的结果被归纳在下面的表中。如表中显示的,当与对照聚合酶Po1957或Po1955相比时,以上突变体的每一个在phasing和pre-phasing的一个或更多个方面显示出出乎意料的和显著的改进。

[0119]

突变(名称)	SEQ ID NO:	与对照相比phasing减少?
对照	29	-
K477M	30	是
对照	31	-
K477M	32	是

[0120] 实施例3[0121] 筛选9°N WT聚合酶的突变体

[0122] 如在实施例1中大体描述的产生、克隆、表达和纯化嗜热球菌属物种 *Thermococcus* sp.9°N-7 (9°N) 野生型聚合酶骨架序列 (SEQ ID NO:5) 的突变,产生了具有如SEQ ID NO:6-8列出的氨基酸序列的聚合酶。

[0123] 使用以上在实施例1中描述的基于凝胶的测定针对爆发动力学筛选所纯化的突变体聚合酶,并与具有SEQ ID NO:5所列的序列的对照聚合酶比较。在筛选的那些突变体中,一组突变体被进一步如以上在实施例1中大体描述的针对phasing/pre-phasing活性筛选。具有以下突变的那些聚合酶与对照相比显示出具有改进的phasing和/或pre-phasing活性。

突变体	SEQ ID NO:
K477M	6
K477M L408A Y409A P410I	7
K477M L408A Y409A P410V	7
K477M L408A Y409A	7
P410A	
K477M Y409A	8

[0126] 实施例4[0127] 筛选9°N Exo⁻聚合酶的突变体

[0128] 如在实施例1中大体描述的产生、克隆、表达并纯化对9°N Exo⁻聚合酶骨架序列 (SEQ ID NO:9) 的突变,产生了具有如SEQ ID NO:10-12列出的氨基酸序列的聚合酶。

[0129] 使用以上在实施例1中描述的基于凝胶的测定针对爆发动力学筛选所纯化的突变体聚合酶,并与具有SEQ ID NO:9所列的序列的对照聚合酶比较。在筛选的那些突变体中,一组突变体被进一步如以上在实施例1中大体描述的针对phasing/pre-phasing活性筛选。具有以下突变的那些聚合酶与对照相比显示出具有改进的phasing和/或pre-phasing活性。

[0130]

突变体	SEQ ID NO:
K477M	10
K477M L408A Y409A P410I	11
K477M L408A Y409A P410V	11
K477M L408A Y409A P410A	11
K477M Y409A	12

[0131] 实施例5[0132] 筛选改造的9°N聚合酶的突变体

[0133] 如在实施例1中大体描述的产生、克隆、表达并纯化改造的9°N聚合酶骨架序列 (SEQ ID NO:13) 的突变, 产生了具有如SEQ ID NO:14-16列出的氨基酸序列的聚合酶。

[0134] 使用以上在实施例1中描述的基于凝胶的测定针对爆发动力学筛选所纯化的突变体聚合酶, 并与具有SEQ ID NO:13所列的序列的对照聚合酶比较。在筛选的那些突变体中, 一组突变体被进一步如以上在实施例1中大体描述的针对phasing/pre-phasing活性筛选。具有以下突变的那些聚合酶与对照相比显示出具有改进的phasing和/或pre-phasing活性。

[0135]

突变体	SEQ ID NO:
K477M	14
K477M L408A Y409A P410I	15
K477M L408A Y409A P410V	15
K477M L408A Y409A P410A	15
K477M Y409A	16

[0136] 实施例6[0137] 筛选Pfu Exo⁻聚合酶的突变体

[0138] 基于与9°N聚合酶骨架序列的序列比对分析(参见图1),如在实施例1中大体描述的产生、克隆、表达并纯化对强烈火球菌(Pfu)Exo⁻聚合酶骨架序列(SEQ ID NO:17)的特定突变,产生了具有如SEQ ID NO:18-20列出的氨基酸序列的聚合酶。

[0139] 使用以上在实施例1中描述的基于凝胶的测定针对爆发动力学筛选所纯化的突变体聚合酶,并与具有SEQ ID NO:17所列的序列的对照聚合酶比较。在筛选的那些突变体中,一组突变体被进一步如以上在实施例1中大体描述的针对phasing/pre-phasing活性筛选。具有以下突变的那些聚合酶与对照相比显示出具有改进的phasing和/或pre-phasing活性。

[0140]

突变体	SEQ ID NO:
K477M	18
K477M L408A Y409A P410I	19
K477M L408A Y409A P410V	19
K477M L408A Y409A P410A	19
K477M Y409A	20

[0141] 实施例7

[0142] 筛选KOD1Exo⁻聚合酶的突变体

[0143] 基于与9°N聚合酶骨架序列的序列比对分析(参见图1),如在实施例1中大体描述的产生、克隆、表达并纯化*Thermococcus kodakaraensis* (KOD1) Exo⁻聚合酶骨架序列 (SEQ ID NO:21)的特定突变,产生具有如SEQ ID NO:22-24列出的氨基酸序列的聚合酶。

[0144] 使用以上在实施例1中描述的基于凝胶的测定针对爆发动力学筛选所纯化的突变体聚合酶,并与具有SEQ ID NO:21所列的序列的对照聚合酶比较。在筛选的那些突变体中,一组突变体被进一步如以上在实施例1中大体描述的针对phasing/pre-phasing活性筛选。具有以下突变的那些聚合酶与对照相比显示出具有改进的phasing和/或pre-phasing活性。

[0145]

突变体	SEQ ID NO:
K477M	22
K477M L408A Y409A P410I	23
K477M L408A	23

[0146]	Y409A P410V	
	K477M L408A Y409A P410A	23
	K477M Y409A	24

[0147] 实施例8

[0148] 筛选MMS2Exo⁻聚合酶的突变体

[0149] 基于与9°N聚合酶骨架序列的序列比对分析(参见图1),基于在与9°N聚合酶比对中的同源性(参见图2),鉴定对M.maripaludis (MMS2) Exo⁻聚合酶骨架序列(SEQ ID NO:25)的特定突变。如在实施例1中大体描述的产生、克隆、表达并纯化突变体,产生了具有如SEQ ID NO:26-28列出的氨基酸序列的聚合酶。

[0150] 使用以上在实施例1中描述的基于凝胶的测定针对爆发动力学筛选所纯化的突变体聚合酶,并与具有SEQ ID NO:25所列的序列的对照聚合酶比较。在筛选的那些突变体中,一组突变体被进一步如以上在实施例1中大体描述的针对phasing/pre-phasing活性筛选。具有以下突变的那些聚合酶与对照相比显示出具有改进的phasing和/或pre-phasing活性。

[0151]	突变体	SEQ ID NO:
	Q493M	26
	Q493M L417A Y418A P419I	27
	Q493M L417A Y418A P419V	27
	Q493M L417A Y418A P419A	27
	Q493M	28

[0152]	Y418A	
--------	-------	--

[0153] 贯穿本申请已参考多个出版物、专利和/或专利申请。这些出版物的公开内容通过引用以其整体特此并入本申请。

[0154] 在本文中术语包括意图是开放式的,不仅包括所引述的要素,而且还包括任何另

外的要素。

[0155] 已描述了很多实施方案。但是,将理解可进行多种改变。因此,其他的实施方案在以下权利要求书的范围内。

序列表

<110> 伊鲁米那股份有限公司
<120> 用于核苷酸类似物的改进的掺入的修饰的聚合酶
<130> IP-1152-PRV
<160> 32
<170> PatentIn version 3.5

<210> 1
<211> 18
<212> PRT
<213> 人工序列
<220>
<223> 多种, 包括嗜热球菌属物种 *Thermococcus* sp. 或火球菌属物种 *Pyrococcus* sp.
<220>
<221> VARIANT
<222> (2)..(2)
<223> X= 任何 AA
<220>
<221> VARIANT
<222> (3)..(3)
<223> X= 任何 AA
<220>
[0001] <221> VARIANT
<222> (4)..(4)
<223> X= 任何 AA
<220>
<221> VARIANT
<222> (8)..(8)
<223> X= 任何 AA
<220>
<221> VARIANT
<222> (10)..(10)
<223> X= 任何 AA
<220>
<221> VARIANT
<222> (11)..(11)
<223> X= 任何 AA
<220>
<221> VARIANT
<222> (12)..(12)
<223> X= 任何 AA
<220>
<221> VARIANT
<222> (14)..(14)

<223> X= 任何 AA

<400> 1

Glu Xaa Xaa Xaa Leu Asp Tyr Xaa Gln Xaa Xaa Xaa Lys Xaa Leu Ala

1

5

10

15

Asn Ser

<210> 2

<211> 18

<212> PRT

<213> 人工序列

<220>

<223> 多种, 包括嗜热球菌属物种 *Thermococcus* sp. 或火球菌属物种 *Pyrococcus* sp.

<220>

<221> VARIANT

<222> (2)..(2)

<223> X=K, R, Y

<220>

<221> VARIANT

<222> (3)..(3)

<223> X=K, I, Q

<220>

[0002]

<221> VARIANT

<222> (4)..(4)

<223> X=L, I, M

<220>

<221> VARIANT

<222> (8)..(8)

<223> X=R, E

<220>

<221> VARIANT

<222> (10)..(10)

<223> X=R, K, I

<220>

<221> VARIANT

<222> (11)..(11)

<223> X=A, K, L, V, S

<220>

<221> VARIANT

<222> (12)..(12)

<223> X=I, V, L, N

<220>

<221> VARIANT

<222> (14)..(14)

[0003]

<223> X=I, V, L

<400> 2

Glu Xaa Xaa Xaa Leu Asp Tyr Xaa Gln Xaa Xaa Xaa Lys Xaa Leu Ala

1 5 10 15

Asn Ser

<210> 3

<211> 18

<212> PRT

<213> 人工序列

<220>

<223> 多种, 包括嗜热球菌属物种 *Thermococcus* sp. 或火球菌属物种 *Pyrococcus* sp.

<220>

<221> VARIANT

<222> (2)..(2)

<223> X=R, K

<220>

<221> VARIANT

<222> (3)..(3)

<223> X=K, I

<220>

<221> VARIANT

<222> (10)..(10)

<223> X=R, K

<220>

<221> VARIANT

<222> (14)..(14)

<223> X=I, L

<400> 3

Glu Xaa Xaa Leu Leu Asp Tyr Arg Gln Xaa Ala Ile Lys Xaa Leu Ala

1 5 10 15

Asn Ser

<210> 4

<211> 18

<212> PRT

<213> 人工序列

<220>

<223> 多种, 包括嗜热球菌属物种 *Thermococcus* sp. 或火球菌属物种 *Pyrococcus* sp.

<400> 4

Glu Lys Lys Leu Leu Asp Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala

1 5 10 15

Asn Ser

<210> 5

<211> 775

<212> PRT

<213> 嗜热球菌属物种 Thermococcus sp. 9N-7

<400> 5

```

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

```

[0004]

[0005]

Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly		
290	295	300
Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr		
305	310	315 320
Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu		
325	330	335
Ile Gly Gln Ser 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 Lys Arg Asn Glu Leu Ala		
355	360	365
Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr		
370	375	380
Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile		
385	390	395 400
Val Tyr Leu Asp Phe Arg Ser Leu Tyr Pro Ser Ile Ile Ile Thr His		
405	410	415
Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp		
420	425	430
Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe		
435	440	445
Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys		
450	455	460
Arg Lys Met Lys Ala Thr Val Asp Pro Leu Glu Lys Lys Leu Leu Asp		
465	470	475 480
Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr		
485	490	495
Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser		
500	505	510
Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu		
515	520	525
Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu		
530	535	540
His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala		
545	550	555 560
Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu		
565	570	575
Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys		
580	585	590
Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu		
595	600	605
Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala		
610	615	620
Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val		
625	630	635 640

Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro
 645 650 655
 Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp
 660 665 670
 Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala
 675 680 685
 Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu
 690 695 700
 Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe
 705 710 715 720
 Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln
 725 730 735
 Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys
 740 745 750
 Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp
 755 760 765
 Leu Lys Val Lys Gly Lys Lys
 770 775

<210> 6

<211> 775

<212> PRT

[0006]

<213> 嗜热球菌属物种 *Thermococcus* sp. 9N-7

<400> 6

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

[0007]

```

Ser Tyr Ala Asp Gly Ser Glu Ala Arg Val Ile Thr Trp Lys Lys Ile
      165              170              175
Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Lys Glu Met Ile Lys
      180              185              190
Arg Phe Leu Arg Val Val Arg Glu Lys Asp Pro Asp Val Leu Ile Thr
      195              200              205
Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Cys Glu
      210              215              220
Glu Leu Gly Ile Lys Phe Thr Leu Gly Arg Asp Gly Ser Glu Pro Lys
      225              230              235              240
Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile
      245              250              255
His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
      260              265              270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu
      275              280              285
Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly
      290              295              300
Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr
      305              310              315              320
Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
      325              330              335
Ile Gly Gln Ser 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 Lys Arg Asn Glu Leu Ala
      355              360              365
Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr
      370              375              380
Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile
      385              390              395              400
Val Tyr Leu Asp Phe Arg Ser Leu Tyr Pro Ser Ile Ile Ile Thr His
      405              410              415
Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp
      420              425              430
Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe
      435              440              445
Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys
      450              455              460
Arg Lys Met Lys Ala Thr Val Asp Pro Leu Glu Lys Met Leu Leu Asp
      465              470              475              480
Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr
      485              490              495
Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser
      500              505              510

```

[0008]

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

<210> 7

<211> 775

<212> PRT

<213> 嗜热球菌属物种 Thermococcus sp. 9N-7

<220>

<221> VARIANT

<222> (410)..(410)

<223> X=L, V, A

<400> 7

[0009]

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

[0010]

Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Lys Arg Asn Glu Leu Ala		
355	360	365
Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr		
370	375	380
Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile		
385	390	395
Val Tyr Leu Asp Phe Arg Ser Ala Ala Xaa Ser Ile Ile Ile Thr His		
405	410	415
Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp		
420	425	430
Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe		
435	440	445
Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys		
450	455	460
Arg Lys Met Lys Ala Thr Val Asp Pro Leu Glu Lys Met Leu Leu Asp		
465	470	475
Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr		
485	490	495
Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser		
500	505	510
Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu		
515	520	525
Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu		
530	535	540
His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala		
545	550	555
Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu		
565	570	575
Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys		
580	585	590
Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu		
595	600	605
Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala		
610	615	620
Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val		
625	630	635
Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro		
645	650	655
Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp		
660	665	670
Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala		
675	680	685
Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu		
690	695	700

Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe
 705 710 715 720
 Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln
 725 730 735
 Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys
 740 745 750
 Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp
 755 760 765
 Leu Lys Val Lys Gly Lys Lys
 770 775

<210> 8

<211> 775

<212> PRT

<213> 嗜热球菌属物种 Thermococcus sp. 9N-7

<400> 8

[0011]

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

[0012]

Glu Leu Gly Ile Lys Phe Thr Leu Gly Arg Asp Gly Ser Glu Pro Lys			
225	230	235	240
Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile			
245	250	255	
His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr			
260	265	270	
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu			
275	280	285	
Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly			
290	295	300	
Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr			
305	310	315	320
Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu			
325	330	335	
Ile Gly Gln Ser 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 Lys Arg Asn Glu Leu Ala			
355	360	365	
Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr			
370	375	380	
Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile			
385	390	395	400
Val Tyr Leu Asp Phe Arg Ser Leu Ala Pro Ser Ile Ile Ile Thr His			
405	410	415	
Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp			
420	425	430	
Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe			
435	440	445	
Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys			
450	455	460	
Arg Lys Met Lys Ala Thr Val Asp Pro Leu Glu Lys Met Leu Leu Asp			
465	470	475	480
Tyr Arg Glu Arg Ala Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr			
485	490	495	
Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser			
500	505	510	
Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu			
515	520	525	
Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu			
530	535	540	
His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala			
545	550	555	560
Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu			
565	570	575	

[0013]

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

<210> 9

<211> 775

<212> PRT

<213> 嗜热球菌属物种 *Thermococcus* sp. 9N-7

<400> 9

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

[0014]

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

[0015]

```

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

```

<210> 10

<211> 775
 <212> PRT
 <213> 嗜热球菌属物种 *Thermococcus* sp. 9N-7
 <400> 10
 Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile
 1 5 10 15
 Arg Val Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg
 20 25 30
 Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile
 35 40 45
 Glu Asp Val Lys Lys Val Thr Ala Lys Arg His Gly Thr Val Val Lys
 50 55 60
 Val Lys Arg Ala Glu Lys Val Gln Lys Lys Phe Leu Gly Arg Pro Ile
 65 70 75 80
 Glu Val Trp Lys Leu Tyr Phe Asn His Pro Gln Asp Val Pro Ala Ile
 85 90 95
 Arg Asp Arg Ile Arg Ala His Pro Ala Val Val Asp Ile Tyr 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 Thr Met Leu Ala Phe Ala Ile Ala Thr
 130 135 140
 [0016] Leu Tyr His Glu Gly Glu Glu Phe Gly Thr Gly Pro Ile Leu Met Ile
 145 150 155 160
 Ser Tyr Ala Asp Gly Ser Glu Ala Arg Val Ile Thr Trp Lys Lys Ile
 165 170 175
 Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Lys Glu Met Ile Lys
 180 185 190
 Arg Phe Leu Arg Val Val Arg Glu Lys Asp Pro Asp Val Leu Ile Thr
 195 200 205
 Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Cys Glu
 210 215 220
 Glu Leu Gly Ile Lys Phe Thr Leu Gly Arg Asp Gly Ser Glu Pro Lys
 225 230 235 240
 Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile
 245 250 255
 His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
 260 265 270
 Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu
 275 280 285
 Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly
 290 295 300
 Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr
 305 310 315 320

[0017]

Glu Leu Gly Arg	Glu Phe Phe Pro Met	Glu Ala Gln Leu Ser Arg Leu
325	330	335
Ile Gly Gln Ser 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 Lys Arg Asn Glu Leu Ala		
355	360	365
Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr		
370	375	380
Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile		
385	390	395
Val Tyr Leu Asp Phe Arg Ser Leu Tyr Pro Ser Ile Ile Ile Thr His		
405	410	415
Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp		
420	425	430
Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe		
435	440	445
Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys		
450	455	460
Arg Lys Met Lys Ala Thr Val Asp Pro Leu Glu Lys Met Leu Leu Asp		
465	470	475
Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr		
485	490	495
Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser		
500	505	510
Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu		
515	520	525
Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu		
530	535	540
His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala		
545	550	555
Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu		
565	570	575
Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys		
580	585	590
Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu		
595	600	605
Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala		
610	615	620
Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val		
625	630	635
Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro		
645	650	655
Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp		
660	665	670

Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala
 675 680 685
 Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu
 690 695 700
 Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe
 705 710 715 720
 Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln
 725 730 735
 Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys
 740 745 750
 Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp
 755 760 765
 Leu Lys Val Lys Gly Lys Lys
 770 775

<210> 11
 <211> 775
 <212> PRT
 <213> 嗜热球菌属物种 Thermococcus sp. 9N-7
 <220>
 <221> VARIANT
 <222> (410)..(410)
 <223> X=L, V, A
 <400> 11

[0018]

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

[0019]

```

Ser Tyr Ala Asp Gly Ser Glu Ala Arg Val Ile Thr Trp Lys Lys Ile
      165                      170                      175
Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Lys Glu Met Ile Lys
      180                      185                      190
Arg Phe Leu Arg Val Val Arg Glu Lys Asp Pro Asp Val Leu Ile Thr
      195                      200                      205
Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Cys Glu
      210                      215                      220
Glu Leu Gly Ile Lys Phe Thr Leu Gly Arg Asp Gly Ser Glu Pro Lys
      225                      230                      235                      240
Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile
      245                      250                      255
His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
      260                      265                      270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu
      275                      280                      285
Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly
      290                      295                      300
Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr
      305                      310                      315                      320
Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
      325                      330                      335
Ile Gly Gln Ser 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 Lys Arg Asn Glu Leu Ala
      355                      360                      365
Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr
      370                      375                      380
Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile
      385                      390                      395                      400
Val Tyr Leu Asp Phe Arg Ser Ala Ala Xaa Ser Ile Ile Ile Thr His
      405                      410                      415
Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp
      420                      425                      430
Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe
      435                      440                      445
Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys
      450                      455                      460
Arg Lys Met Lys Ala Thr Val Asp Pro Leu Glu Lys Met Leu Leu Asp
      465                      470                      475                      480
Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr
      485                      490                      495
Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser
      500                      505                      510

```

[0020]

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

<210> 12

<211> 775

<212> PRT

<213> 嗜热球菌属物种 Thermococcus sp. 9N-7

<400> 12

Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile
 1 5 10 15
 Arg Val Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg
 20 25 30

[0021]

```

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

```

[0022]

Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile			
385	390	395	400
Val Tyr Leu Asp Phe Arg Ser Leu Ala Pro Ser Ile Ile Ile Thr His			
405	410	415	
Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp			
420	425	430	
Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe			
435	440	445	
Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys			
450	455	460	
Arg Lys Met Lys Ala Thr Val Asp Pro Leu Glu Lys Met Leu Leu Asp			
465	470	475	480
Tyr Arg Gln Arg Ala Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr			
485	490	495	
Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser			
500	505	510	
Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu			
515	520	525	
Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu			
530	535	540	
His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala			
545	550	555	560
Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu			
565	570	575	
Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys			
580	585	590	
Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu			
595	600	605	
Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala			
610	615	620	
Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val			
625	630	635	640
Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro			
645	650	655	
Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp			
660	665	670	
Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala			
675	680	685	
Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu			
690	695	700	
Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe			
705	710	715	720
Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln			
725	730	735	

Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys
 740 745 750
 Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp
 755 760 765
 Leu Lys Val Lys Gly Lys Lys
 770 775

<210> 13

<211> 775

<212> PRT

<213> 嗜热球菌属物种 Thermococcus sp. 9N-7

<400> 13

[0023]

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

[0024]

His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr		
260	265	270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu		
275	280	285
Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly		
290	295	300
Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr		
305	310	315
Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu		
325	330	335
Ile Gly Gln Ser 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 Lys Arg Asn Glu Leu Ala		
355	360	365
Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr		
370	375	380
Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile		
385	390	395
Val Tyr Leu Asp Phe Arg Ser Ala Ala Ala Ser Ile Ile Ile Thr His		
405	410	415
Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp		
420	425	430
Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe		
435	440	445
Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys		
450	455	460
Arg Lys Met Lys Ala Thr Val Asp Pro Leu Glu Lys Lys Leu Leu Asp		
465	470	475
Tyr Arg Gln Arg Leu Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr		
485	490	495
Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser		
500	505	510
Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu		
515	520	525
Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu		
530	535	540
His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala		
545	550	555
Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu		
565	570	575
Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys		
580	585	590
Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu		
595	600	605

Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala
 610 615 620
 Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val
 625 630 635 640
 Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro
 645 650 655
 Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp
 660 665 670
 Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala
 675 680 685
 Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu
 690 695 700
 Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe
 705 710 715 720
 Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln
 725 730 735
 Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys
 740 745 750
 Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp
 755 760 765
 Leu Lys Val Lys Gly Lys Lys
 770 775

[0025]

<210> 14
 <211> 775
 <212> PRT
 <213> 嗜热球菌属物种 Thermococcus sp. 9N-7
 <400> 14
 Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile
 1 5 10 15
 Arg Val Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg
 20 25 30
 Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile
 35 40 45
 Glu Asp Val Lys Lys Val Thr Ala Lys Arg His Gly Thr Val Val Lys
 50 55 60
 Val Lys Arg Ala Glu Lys Val Gln Lys Lys Phe Leu Gly Arg Pro Ile
 65 70 75 80
 Glu Val Trp Lys Leu Tyr Phe Asn His Pro Gln Asp Val Pro Ala Ile
 85 90 95
 Arg Asp Arg Ile Arg Ala His Pro Ala Val Val Asp Ile Tyr Glu Tyr
 100 105 110
 Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro
 115 120 125

[0026]

```

Met Glu Gly Asp Glu Glu Leu Thr Met Leu Ala Phe Ala Ile Ala Thr
 130                      135                      140
Leu Tyr His Glu Gly Glu Glu Phe Gly Thr Gly Pro Ile Leu Met Ile
 145                      150                      155                      160
Ser Tyr Ala Asp Gly Ser Glu Ala Arg Val Ile Thr Trp Lys Lys Ile
                      165                      170                      175
Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Lys Glu Met Ile Lys
                      180                      185                      190
Arg Phe Leu Arg Val Val Arg Glu Lys Asp Pro Asp Val Leu Ile Thr
                      195                      200                      205
Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Ser Glu
 210                      215                      220
Glu Leu Gly Ile Lys Phe Thr Leu Gly Arg Asp Gly Ser Glu Pro Lys
 225                      230                      235                      240
Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile
                      245                      250                      255
His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
                      260                      265                      270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu
                      275                      280                      285
Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly
 290                      295                      300
Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr
 305                      310                      315                      320
Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
                      325                      330                      335
Ile Gly Gln Ser 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 Lys Arg Asn Glu Leu Ala
                      355                      360                      365
Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr
 370                      375                      380
Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile
 385                      390                      395                      400
Val Tyr Leu Asp Phe Arg Ser Ala Ala Ala Ser Ile Ile Ile Thr His
                      405                      410                      415
Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp
                      420                      425                      430
Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe
                      435                      440                      445
Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys
 450                      455                      460
Arg Lys Met Lys Ala Thr Val Asp Pro Leu Glu Lys Met Leu Leu Asp
 465                      470                      475                      480

```

[0027]

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

<210> 15

<211> 775

<212> PRT

<213> 嗜热球菌属物种 Thermococcus sp. 9N-7

<220>

<221> VARIANT
 <222> (410).. (410)
 <223> X=L, V, A
 <400> 15
 Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asn Gly Lys Pro Val Ile
 1 5 10 15
 Arg Val Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg
 20 25 30
 Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile
 35 40 45
 Glu Asp Val Lys Lys Val Thr Ala Lys Arg His Gly Thr Val Val Lys
 50 55 60
 Val Lys Arg Ala Glu Lys Val Gln Lys Lys Phe Leu Gly Arg Pro Ile
 65 70 75 80
 Glu Val Trp Lys Leu Tyr Phe Asn His Pro Gln Asp Val Pro Ala Ile
 85 90 95
 Arg Asp Arg Ile Arg Ala His Pro Ala Val Val Asp Ile Tyr 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 Thr Met Leu Ala Phe Ala Ile Ala Thr
 130 135 140
 [0028] Leu Tyr His Glu Gly Glu Glu Phe Gly Thr Gly Pro Ile Leu Met Ile
 145 150 155 160
 Ser Tyr Ala Asp Gly Ser Glu Ala Arg Val Ile Thr Trp Lys Lys Ile
 165 170 175
 Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Lys Glu Met Ile Lys
 180 185 190
 Arg Phe Leu Arg Val Val Arg Glu Lys Asp Pro Asp Val Leu Ile Thr
 195 200 205
 Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Ser Glu
 210 215 220
 Glu Leu Gly Ile Lys Phe Thr Leu Gly Arg Asp Gly Ser Glu Pro Lys
 225 230 235 240
 Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile
 245 250 255
 His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
 260 265 270
 Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu
 275 280 285
 Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly
 290 295 300
 Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr
 305 310 315 320

[0029]

Glu Leu Gly Arg	Glu Phe Phe Pro Met	Glu Ala Gln Leu Ser Arg Leu
325	330	335
Ile Gly Gln Ser 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 Lys Arg Asn Glu Leu Ala		
355	360	365
Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr		
370	375	380
Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile		
385	390	395
Val Tyr Leu Asp Phe Arg Ser Ala Ala Xaa Ser Ile Ile Ile Thr His		
405	410	415
Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp		
420	425	430
Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe		
435	440	445
Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys		
450	455	460
Arg Lys Met Lys Ala Thr Val Asp Pro Leu Glu Lys Met Leu Leu Asp		
465	470	475
Tyr Arg Gln Arg Leu Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr		
485	490	495
Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser		
500	505	510
Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu		
515	520	525
Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu		
530	535	540
His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala		
545	550	555
Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu		
565	570	575
Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys		
580	585	590
Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu		
595	600	605
Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala		
610	615	620
Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val		
625	630	635
Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro		
645	650	655
Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp		
660	665	670

Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala
 675 680 685
 Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu
 690 695 700
 Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe
 705 710 715 720
 Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln
 725 730 735
 Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys
 740 745 750
 Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp
 755 760 765
 Leu Lys Val Lys Gly Lys Lys
 770 775

<210> 16

<211> 775

<212> PRT

<213> 嗜热球菌属物种 Thermococcus sp. 9N-7

<400> 16

[0030]

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

[0031]

Arg Phe Leu Arg Val Val Arg Glu Lys Asp Pro Asp Val Leu Ile Thr		
195	200	205
Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Ser Glu		
210	215	220
Glu Leu Gly Ile Lys Phe Thr Leu Gly Arg Asp Gly Ser Glu Pro Lys		
225	230	235
Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile		
245	250	255
His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr		
260	265	270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu		
275	280	285
Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly		
290	295	300
Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr		
305	310	315
Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu		
325	330	335
Ile Gly Gln Ser 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 Lys Arg Asn Glu Leu Ala		
355	360	365
Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr		
370	375	380
Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile		
385	390	395
Val Tyr Leu Asp Phe Arg Ser Leu Ala Pro Ser Ile Ile Ile Thr His		
405	410	415
Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp		
420	425	430
Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe		
435	440	445
Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys		
450	455	460
Arg Lys Met Lys Ala Thr Val Asp Pro Leu Glu Lys Met Leu Leu Asp		
465	470	475
Tyr Arg Gln Arg Leu Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr		
485	490	495
Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser		
500	505	510
Val Thr Ala Trp Gly Arg Glu Tyr Ile Glu Met Val Ile Arg Glu Leu		
515	520	525
Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu		
530	535	540

[0032]

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

<210> 17

<211> 775

<212> PRT

<213> 强烈火球菌 (Pyrococcus furiosus)

<400> 17

Met Ile Leu Asp Val 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 Arg Asp Asp Ser Lys Ile
 35 40 45
 Glu Glu Val Lys Lys Ile Thr Gly Glu Arg His Gly Lys Ile Val Arg
 50 55 60

[0033]

Ile Val Asp Val Glu Lys Val Glu Lys Lys Phe Leu Gly Lys Pro Ile			
65	70	75	80
Thr Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Thr Ile			
	85	90	95
Arg Glu 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 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
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 Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Val Thr			
	195	200	205
Tyr Asn Gly Asp Ser Phe Asp Phe 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
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 Thr 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 Ser Gly Glu Asn			
	290	295	300
Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr			
	305	310	315
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 Gln Arg Arg Leu Arg Glu Ser			
	370	375	380
Tyr Thr Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn			
	385	390	395
Ile Val Tyr Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr			
	405	410	415

[0034]

His Asn Val Ser Pro Asp Thr Leu Asn Leu Glu Gly Cys Lys Asn Tyr		
420	425	430
Asp Ile Ala Pro Gln Val Gly His Lys Phe Cys Lys Asp Ile Pro Gly		
435	440	445
Phe Ile Pro Ser Leu Leu Gly His Leu Leu Glu Glu Arg Gln Lys Ile		
450	455	460
Lys Thr Lys Met Lys Glu Thr Gln Asp Pro Ile Glu Lys Ile Leu Leu		
465	470	475
Asp Tyr Arg Gln Lys Leu Ile Lys Leu 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 Leu 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 Ser Glu Glu Ile Lys Lys Lys		
545	550	555
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 Lys 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 Asp Val Glu Glu Ala		
625	630	635
Val Arg Ile Val Lys Glu Val Ile Gln Lys Leu Ala Asn 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 Lys 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
Tyr Asp Pro Lys 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 Tyr Gln Lys Thr Arg Gln Val Gly Leu Thr Ser		
755	760	765

Trp Leu Asn Ile Lys Lys Ser
 770 775

 <210> 18
 <211> 775
 <212> PRT
 <213> 强烈火球菌(Pyrococcus furiosus)
 <400> 18
 Met Ile Leu Asp Val 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 Arg Asp Asp Ser Lys Ile
 35 40 45
 Glu Glu Val Lys Lys Ile Thr Gly Glu Arg His Gly Lys Ile Val Arg
 50 55 60
 Ile Val Asp Val Glu Lys Val Glu Lys Lys Phe Leu Gly Lys Pro Ile
 65 70 75 80
 Thr Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Thr Ile
 85 90 95
 Arg Glu Lys Val Arg Glu His Pro Ala Val Val Asp Ile Phe Glu Tyr
 100 105 110
 [0035] 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 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 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 Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Val Thr
 195 200 205
 Tyr Asn Gly Asp Ser Phe Asp Phe 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 Thr 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

[0036]

Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Ser Gly Glu Asn		
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 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 Gln Arg Arg Leu Arg Glu Ser		
370	375	380
Tyr Thr Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn		
385	390	395 400
Ile Val Tyr 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 Leu Glu Gly Cys Lys Asn Tyr		
420	425	430
Asp Ile Ala Pro Gln Val Gly His Lys Phe Cys Lys Asp Ile Pro Gly		
435	440	445
Phe Ile Pro Ser Leu Leu Gly His Leu Leu Glu Glu Arg Gln Lys Ile		
450	455	460
Lys Thr Lys Met Lys Glu Thr Gln Asp Pro Ile Glu Lys Met Leu Leu		
465	470	475 480
Asp Tyr Arg Gln Lys Leu Ile Lys Leu 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 Leu 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 Ser 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 Lys 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 Asp Val Glu Glu Ala		
625	630	635 640

Val Arg Ile Val Lys Glu Val Ile Gln Lys Leu Ala Asn 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 Lys 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 Lys 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 Tyr Gln Lys Thr Arg Gln Val Gly Leu Thr Ser
755 760 765
Trp Leu Asn Ile Lys Lys Ser
770 775

<210> 19
<211> 775
<212> PRT
[0037] <213> 强烈火球菌 (Pyrococcus furiosus)
<220>
<221> VARIANT
<222> (411)..(411)
<223> X-I, V, A
<400> 19

Met Ile Leu Asp Val 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 Arg Asp Asp Ser Lys Ile
35 40 45
Glu Glu Val Lys Lys Ile Thr Gly Glu Arg His Gly Lys Ile Val Arg
50 55 60
Ile Val Asp Val Glu Lys Val Glu Lys Lys Phe Leu Gly Lys Pro Ile
65 70 75 80
Thr Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Thr Ile
85 90 95
Arg Glu 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

[0038]

Met	Glu	Gly	Glu	Glu	Glu	Leu	Lys	Ile	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	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	Ile	Ile	Arg	Glu	Lys	Asp	Pro	Asp	Ile	Ile	Val	Thr
				195				200						205	
Tyr	Asn	Gly	Asp	Ser	Phe	Asp	Phe	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	Thr	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	Ser	Gly	Glu	Asn
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	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	Gln	Arg	Arg	Leu	Arg	Glu	Ser
370						375								380	
Tyr	Thr	Gly	Gly	Phe	Val	Lys	Glu	Pro	Glu	Lys	Gly	Leu	Trp	Glu	Asn
385						390						395		400	
Ile	Val	Tyr	Leu	Asp	Phe	Arg	Ala	Ala	Ala	Xaa	Ser	Ile	Ile	Ile	Thr
				405						410				415	
His	Asn	Val	Ser	Pro	Asp	Thr	Leu	Asn	Leu	Glu	Gly	Cys	Lys	Asn	Tyr
				420					425					430	
Asp	Ile	Ala	Pro	Gln	Val	Gly	His	Lys	Phe	Cys	Lys	Asp	Ile	Pro	Gly
				435					440					445	
Phe	Ile	Pro	Ser	Leu	Leu	Gly	His	Leu	Leu	Glu	Glu	Arg	Gln	Lys	Ile
450						455								460	
Lys	Thr	Lys	Met	Lys	Glu	Thr	Gln	Asp	Pro	Ile	Glu	Lys	Met	Leu	Leu
465						470								475	
															480

[0039]

Asp Tyr Arg Gln Lys Leu Ile Lys Leu 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 Leu 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 Ser 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 Lys 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 Asp Val Glu Glu Ala
 625 630 635 640
 Val Arg Ile Val Lys Glu Val Ile Gln Lys Leu Ala Asn 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 Lys 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 Lys 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 Tyr Gln Lys Thr Arg Gln Val Gly Leu Thr Ser
 755 760 765
 Trp Leu Asn Ile Lys Lys Ser
 770 775

<210> 20

<211> 775

<212> PRT

<213> 强烈火球菌(Pyrococcus furiosus)

<400> 20

[0040]

```

Met Ile Leu Asp Val 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 Arg Asp Asp Ser Lys Ile
          35          40          45
Glu Glu Val Lys Lys Ile Thr Gly Glu Arg His Gly Lys Ile Val Arg
          50          55          60
Ile Val Asp Val Glu Lys Val Glu Lys Lys Phe Leu Gly Lys Pro Ile
65          70          75          80
Thr Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Thr Ile
          85          90          95
Arg Glu 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 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 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 Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Val Thr
          195         200         205
Tyr Asn Gly Asp Ser Phe Asp Phe 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 Thr 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 Ser Gly Glu Asn
          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 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

```

[0041]

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 Gln Arg Arg Leu Arg Glu Ser		
370	375	380
Tyr Thr Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn		
385	390	395
Ile Val Tyr Leu Asp Phe Arg Ala Leu Ala Pro Ser Ile Ile Ile Thr		
405	410	415
His Asn Val Ser Pro Asp Thr Leu Asn Leu Glu Gly Cys Lys Asn Tyr		
420	425	430
Asp Ile Ala Pro Gln Val Gly His Lys Phe Cys Lys Asp Ile Pro Gly		
435	440	445
Phe Ile Pro Ser Leu Leu Gly His Leu Leu Glu Glu Arg Gln Lys Ile		
450	455	460
Lys Thr Lys Met Lys Glu Thr Gln Asp Pro Ile Glu Lys Met Leu Leu		
465	470	475
Asp Tyr Arg Gln Lys Leu Ile Lys Leu 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 Leu 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 Ser Glu Glu Ile Lys Lys Lys		
545	550	555
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 Lys 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 Asp Val Glu Glu Ala		
625	630	635
Val Arg Ile Val Lys Glu Val Ile Gln Lys Leu Ala Asn 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 Lys 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 Lys 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 Tyr Gln Lys Thr Arg Gln Val Gly Leu Thr Ser
 755 760 765
 Trp Leu Asn Ile Lys Lys Ser
 770 775

<210> 21

<211> 774

<212> PRT

<213> Thermococcus kodakaraensis

<400> 21

[0042]

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

[0043]

Lys	Leu	Gly	Ile	Asn	Phe	Ala	Leu	Gly	Arg	Asp	Gly	Ser	Glu	Pro	Lys
225				230					235					240	
Ile	Gln	Arg	Met	Gly	Asp	Arg	Phe	Ala	Val	Glu	Val	Lys	Gly	Arg	Ile
			245						250					255	
His	Phe	Asp	Leu	Tyr	Pro	Val	Ile	Arg	Arg	Thr	Ile	Asn	Leu	Pro	Thr
			260						265					270	
Tyr	Thr	Leu	Glu	Ala	Val	Tyr	Glu	Ala	Val	Phe	Gly	Gln	Pro	Lys	Glu
			275						280					285	
Lys	Val	Tyr	Ala	Glu	Glu	Ile	Thr	Thr	Ala	Trp	Glu	Thr	Gly	Glu	Asn
			290						295					300	
Leu	Glu	Arg	Val	Ala	Arg	Tyr	Ser	Met	Glu	Asp	Ala	Lys	Val	Thr	Tyr
305						310					315				320
Glu	Leu	Gly	Lys	Glu	Phe	Leu	Pro	Met	Glu	Ala	Gln	Leu	Ser	Arg	Leu
						325					330				335
Ile	Gly	Gln	Ser	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	Lys	Glu	Leu	Ala	Arg	Arg	Arg	Gln	Ser	Tyr
			370						375					380	
Glu	Gly	Gly	Tyr	Val	Lys	Glu	Pro	Glu	Arg	Gly	Leu	Trp	Glu	Asn	Ile
385						390					395				400
Val	Tyr	Leu	Asp	Phe	Arg	Ser	Leu	Tyr	Pro	Ser	Ile	Ile	Ile	Thr	His
				405							410				415
Asn	Val	Ser	Pro	Asp	Thr	Leu	Asn	Arg	Glu	Gly	Cys	Lys	Glu	Tyr	Asp
				420							425				430
Val	Ala	Pro	Gln	Val	Gly	His	Arg	Phe	Cys	Lys	Asp	Phe	Pro	Gly	Phe
				435							440				445
Ile	Pro	Ser	Leu	Leu	Gly	Asp	Leu	Leu	Glu	Glu	Arg	Gln	Lys	Ile	Lys
				450							455				460
Lys	Lys	Met	Lys	Ala	Thr	Ile	Asp	Pro	Ile	Glu	Arg	Lys	Leu	Leu	Asp
465						470					475				480
Tyr	Arg	Gln	Arg	Leu	Ile	Lys	Ile	Leu	Ala	Asn	Ser	Tyr	Tyr	Gly	Tyr
				485							490				495
Tyr	Gly	Tyr	Ala	Arg	Ala	Arg	Trp	Tyr	Cys	Lys	Glu	Cys	Ala	Glu	Ser
				500							505				510
Val	Thr	Ala	Trp	Gly	Arg	Glu	Tyr	Ile	Thr	Met	Thr	Ile	Lys	Glu	Ile
				515							520				525
Glu	Glu	Lys	Tyr	Gly	Phe	Lys	Val	Ile	Tyr	Ser	Asp	Thr	Asp	Gly	Phe
				530							535				540
Phe	Ala	Thr	Ile	Pro	Gly	Ala	Asp	Ala	Glu	Thr	Val	Lys	Lys	Lys	Ala
545						550					555				560
Met	Glu	Phe	Leu	Lys	Tyr	Ile	Asn	Ala	Lys	Leu	Pro	Gly	Ala	Leu	Glu
				565							570				575

[0044]

Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys Lys
 580 585 590
 Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu
 595 600 605
 Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala
 610 615 620
 Arg Val Leu Glu Ala Leu Leu Lys Asp Gly Asp Val Glu Lys Ala Val
 625 630 635 640
 Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro
 645 650 655
 Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Lys Asp
 660 665 670
 Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala
 675 680 685
 Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu
 690 695 700
 Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Phe Asp Glu Phe
 705 710 715 720
 Asp Pro Thr Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln
 725 730 735
 Val Leu Pro Ala Val Glu Arg Ile Leu Arg Ala Phe Gly Tyr Arg Lys
 740 745 750
 Glu Asp Leu Arg Tyr Gln Lys Thr Arg Gln Val Gly Leu Ser Ala Trp
 755 760 765
 Leu Lys Pro Lys Gly Thr
 770

<210> 22

<211> 774

<212> PRT

<213> Thermococcus kodakaraensis

<400> 22

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

[0045]

Arg Asp Lys Ile Arg Glu His Pro Ala Val Ile Asp Ile Tyr Glu Tyr		
100	105	110
Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Val Pro		
115	120	125
Met Glu Gly Asp Glu Glu Leu Lys Met Leu Ala Phe Ala Ile Ala Thr		
130	135	140
Leu Tyr His Glu Gly Glu Glu Phe Ala Glu Gly Pro Ile Leu Met Ile		
145	150	155
Ser Tyr Ala Asp Glu Glu Gly Ala Arg Val Ile Thr Trp Lys Asn Val		
165	170	175
Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Arg Glu Met Ile Lys		
180	185	190
Arg Phe Leu Arg Val Val Lys Glu Lys Asp Pro Asp Val Leu Ile Thr		
195	200	205
Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Cys Glu		
210	215	220
Lys Leu Gly Ile Asn Phe Ala Leu Gly Arg Asp Gly Ser Glu Pro Lys		
225	230	235
Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile		
245	250	255
His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr		
260	265	270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Gln Pro Lys Glu		
275	280	285
Lys Val Tyr Ala Glu Glu Ile Thr Thr Ala Trp Glu Thr Gly Glu Asn		
290	295	300
Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr		
305	310	315
Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ala Gln Leu Ser Arg Leu		
325	330	335
Ile Gly Gln Ser 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 Lys Glu Leu Ala Arg Arg Arg Gln Ser Tyr		
370	375	380
Glu Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Glu Asn Ile		
385	390	395
Val Tyr Leu Asp Phe Arg Ser Leu Tyr Pro Ser Ile Ile Ile Thr His		
405	410	415
Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp		
420	425	430
Val Ala Pro Gln Val Gly His Arg Phe Cys Lys Asp Phe Pro Gly Phe		
435	440	445

[0046]

```

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

```

<210> 23

<211> 774
 <212> PRT
 <213> Thermococcus kodakaraensis
 <220>
 <221> VARIANT
 <222> (410)..(410)
 <223> x=L, V, A
 <400> 23
 Met Ile Leu Asp Thr Asp Tyr Ile Thr Glu Asp Gly Lys Pro Val Ile
 1 5 10 15
 Arg Ile Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Tyr Asp Arg
 20 25 30
 Thr Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile
 35 40 45
 Glu Glu Val Lys Lys Ile Thr Ala Glu Arg His Gly Thr Val Val Thr
 50 55 60
 Val Lys Arg Val Glu Lys Val Gln Lys Lys Phe Leu Gly Arg Pro Val
 65 70 75 80
 Glu Val Trp Lys Leu Tyr Phe Thr His Pro Gln Asp Val Pro Ala Ile
 85 90 95
 Arg Asp Lys Ile Arg Glu His Pro Ala Val Ile Asp Ile Tyr Glu Tyr
 100 105 110
 Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Val Pro
 115 120 125
 Met Glu Gly Asp Glu Glu Leu Lys Met Leu Ala Phe Ala Ile Ala Thr
 130 135 140
 Leu Tyr His Glu Gly Glu Glu Phe Ala Glu Gly Pro Ile Leu Met Ile
 145 150 155 160
 Ser Tyr Ala Asp Glu Glu Gly Ala Arg Val Ile Thr Trp Lys Asn Val
 165 170 175
 Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Arg Glu Met Ile Lys
 180 185 190
 Arg Phe Leu Arg Val Val Lys Glu Lys Asp Pro Asp Val Leu Ile Thr
 195 200 205
 Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Cys Glu
 210 215 220
 Lys Leu Gly Ile Asn Phe Ala Leu Gly Arg Asp Gly Ser Glu Pro Lys
 225 230 235 240
 Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile
 245 250 255
 His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
 260 265 270
 Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Gln Pro Lys Glu
 275 280 285

[0047]

[0048]

Lys Val Tyr Ala Glu Glu Ile Thr Thr Ala Trp Glu Thr Gly Glu Asn		
290	295	300
Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr		
305	310	315 320
Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ala Gln Leu Ser Arg Leu		
325	330	335
Ile Gly Gln Ser 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 Lys Glu Leu Ala Arg Arg Arg Gln Ser Tyr		
370	375	380
Glu Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Glu Asn Ile		
385	390	395 400
Val Tyr Leu Asp Phe Arg Ser Ala Ala Xaa Ser Ile Ile Ile Thr His		
405	410	415
Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp		
420	425	430
Val Ala Pro Gln Val Gly His Arg Phe Cys Lys Asp Phe Pro Gly Phe		
435	440	445
Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys		
450	455	460
Lys Lys Met Lys Ala Thr Ile Asp Pro Ile Glu Arg Met Leu Leu Asp		
465	470	475 480
Tyr Arg Gln Arg Leu Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly Tyr		
485	490	495
Tyr Gly Tyr Ala Arg Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser		
500	505	510
Val Thr Ala Trp Gly Arg Glu Tyr Ile Thr Met Thr Ile Lys Glu Ile		
515	520	525
Glu Glu Lys Tyr Gly Phe Lys Val Ile Tyr Ser Asp Thr Asp Gly Phe		
530	535	540
Phe Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala		
545	550	555 560
Met Glu Phe Leu Lys Tyr Ile Asn Ala Lys Leu Pro Gly Ala Leu Glu		
565	570	575
Leu Glu Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val Thr Lys Lys		
580	585	590
Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu		
595	600	605
Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala		
610	615	620
Arg Val Leu Glu Ala Leu Leu Lys Asp Gly Asp Val Glu Lys Ala Val		
625	630	635 640

Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro
 645 650 655
 Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Lys Asp
 660 665 670
 Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala
 675 680 685
 Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu
 690 695 700
 Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Phe Asp Glu Phe
 705 710 715 720
 Asp Pro Thr Lys His Lys Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln
 725 730 735
 Val Leu Pro Ala Val Glu Arg Ile Leu Arg Ala Phe Gly Tyr Arg Lys
 740 745 750
 Glu Asp Leu Arg Tyr Gln Lys Thr Arg Gln Val Gly Leu Ser Ala Trp
 755 760 765
 Leu Lys Pro Lys Gly Thr
 770

<210> 24

<211> 774

<212> PRT

<213> Thermococcus kodakaraensis

<400> 24

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

[0049]

[0050]

```

Ser Tyr Ala Asp Glu Glu Gly Ala Arg Val Ile Thr Trp Lys Asn Val
      165              170              175
Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Arg Glu Met Ile Lys
      180              185              190
Arg Phe Leu Arg Val Val Lys Glu Lys Asp Pro Asp Val Leu Ile Thr
      195              200              205
Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Cys Glu
      210              215              220
Lys Leu Gly Ile Asn Phe Ala Leu Gly Arg Asp Gly Ser Glu Pro Lys
      225              230              235              240
Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile
      245              250              255
His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
      260              265              270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Gln Pro Lys Glu
      275              280              285
Lys Val Tyr Ala Glu Glu Ile Thr Thr Ala Trp Glu Thr Gly Glu Asn
      290              295              300
Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr
      305              310              315              320
Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ala Gln Leu Ser Arg Leu
      325              330              335
Ile Gly Gln Ser 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 Lys Glu Leu Ala Arg Arg Arg Gln Ser Tyr
      370              375              380
Glu Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Glu Asn Ile
      385              390              395              400
Val Tyr Leu Asp Phe Arg Ser Leu Ala Pro Ser Ile Ile Ile Thr His
      405              410              415
Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp
      420              425              430
Val Ala Pro Gln Val Gly His Arg Phe Cys Lys Asp Phe Pro Gly Phe
      435              440              445
Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys
      450              455              460
Lys Lys Met Lys Ala Thr Ile Asp Pro Ile Glu Arg Met Leu Leu Asp
      465              470              475              480
Tyr Arg Gln Arg Leu Ile Lys Ile Leu Ala Asn Ser Tyr Tyr Gly Tyr
      485              490              495
Tyr Gly Tyr Ala Arg Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser
      500              505              510

```

[0051]

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

<210> 25

<211> 784

<212> PRT

<213> *Methanococcus maripaludis*

<400> 25

Met Glu Ser Leu Ile Asp Leu Asp Tyr Asn Ser Asp Asp Leu Cys Ile
 1 5 10 15
 Tyr Leu Tyr Leu Ile Asn Ser Ile Ile Lys Glu Lys Asp Phe Lys Pro
 20 25 30

[0052]

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

[0053]

Tyr Gln Lys Arg Ile Arg Ser Ser Tyr Glu Gly Gly Tyr Val Lys Glu			
385	390	395	400
Pro Leu Lys Gly Ile His Glu Asp Ile Val Ser Met Asp Phe Leu Ser			
405	410	415	
Leu Tyr Pro Ser Ile Ile Met Ser His Asn Leu Ser Pro Glu Thr Ile			
420	425	430	
Asp Cys Thr Cys Cys Ser Asp Glu Glu Asn Gly Glu Asn Glu Glu Ile			
435	440	445	
Leu Gly His Lys Phe Cys Lys Lys Ser Ile Gly Ile Ile Pro Lys Thr			
450	455	460	
Leu Met Asp Leu Ile Asn Arg Arg Lys Lys Val Lys Lys Val Leu Arg			
465	470	475	480
Glu Lys Ala Glu Lys Gly Glu Phe Asp Glu Glu Tyr Gln Ile Leu Asp			
485	490	495	
Tyr Glu Gln Arg Leu Ile Lys Val Leu Ala Asn Ser His Tyr Gly Tyr			
500	505	510	
Leu Ala Phe Pro Met Ala Arg Trp Tyr Ser Arg Asp Cys Ala Glu Ile			
515	520	525	
Thr Thr His Leu Gly Arg Gln Tyr Ile Gln Lys Thr Ile Glu Glu Ala			
530	535	540	
Glu Asn Phe Gly Phe Lys Val Ile Tyr Ala Asp Thr Asp Gly Phe Tyr			
545	550	555	560
Ser Lys Trp Ala Asp Asp Lys Glu Lys Leu Ser Lys Tyr Glu Leu Leu			
565	570	575	
Glu Lys Thr Arg Glu Phe Leu Lys Asn Ile Asn Asn Thr Leu Pro Gly			
580	585	590	
Glu Met Glu Leu Glu Phe Glu Gly Tyr Phe Lys Arg Gly Ile Phe Val			
595	600	605	
Thr Lys Lys Lys Tyr Ala Leu Ile Asp Glu Asn Glu Lys Ile Thr Val			
610	615	620	
Lys Gly Leu Glu Val Val Arg Arg Asp Trp Ser Asn Val Ser Lys Asn			
625	630	635	640
Thr Gln Lys Asn Val Leu Asn Ala Leu Leu Lys Glu Gly Ser Val Glu			
645	650	655	
Asn Ala Lys Lys Val Ile Gln Asp Thr Ile Lys Glu Leu Lys Asp Gly			
660	665	670	
Lys Val Asn Asn Glu Asp Leu Leu Ile His Thr Gln Leu Thr Lys Arg			
675	680	685	
Ile Glu Asp Tyr Lys Thr Thr Ala Pro His Val Glu Val Ala Lys Lys			
690	695	700	
Ile Leu Lys Ser Gly Asn Arg Val Asn Thr Gly Asp Val Ile Ser Tyr			
705	710	715	720
Ile Ile Thr Ser Gly Asn Lys Ser Ile Ser Glu Arg Ala Glu Ile Leu			
725	730	735	

Glu Asn Ala Lys Asn Tyr Asp Thr Asn Tyr Tyr Ile Glu Asn Gln Ile
 740 745 750
 Leu Pro Pro Val Ile Arg Leu Met Glu Ala Leu Gly Ile Thr Lys Asp
 755 760 765
 Glu Leu Lys Asp Ser Lys Lys Gln Tyr Thr Leu His His Phe Leu Lys
 770 775 780

<210> 26

<211> 784

<212> PRT

<213> Methanococcus maripaludis

<400> 26

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

[0054]

[0055]

Asn Ser Lys Ser Tyr Ile Pro Gly Arg Val His Ile Asp Leu Tyr Pro		
260	265	270
Ile Ala Arg Arg Leu Leu Asn Leu Thr Lys Tyr Arg Leu Glu Asn Val		
275	280	285
Thr Glu Ala Leu Phe Asp Val Lys Lys Val Asp Val Gly His Glu Asn		
290	295	300
Ile Pro Lys Met Trp Asp Asn Leu Asp Glu Thr Leu Val Glu Tyr Ser		
305	310	315
His Gln Asp Ala Tyr Tyr Thr Gln Arg Ile Gly Glu Gln Phe Leu Pro		
325	330	335
Leu Glu Ile Met Phe Ser Arg Val Val Asn Gln Ser Leu Tyr Asp Ile		
340	345	350
Asn Arg Met Ser Ser Ser Gln Met Val Glu Tyr Leu Leu Leu Lys Asn		
355	360	365
Ser Tyr Lys Met Gly Val Ile Ala Pro Asn Arg Pro Ser Gly Lys Glu		
370	375	380
Tyr Gln Lys Arg Ile Arg Ser Ser Tyr Glu Gly Gly Tyr Val Lys Glu		
385	390	395
Pro Leu Lys Gly Ile His Glu Asp Ile Val Ser Met Asp Phe Leu Ser		
405	410	415
Leu Tyr Pro Ser Ile Ile Met Ser His Asn Leu Ser Pro Glu Thr Ile		
420	425	430
Asp Cys Thr Cys Cys Ser Asp Glu Glu Asn Gly Glu Asn Glu Glu Ile		
435	440	445
Leu Gly His Lys Phe Cys Lys Lys Ser Ile Gly Ile Ile Pro Lys Thr		
450	455	460
Leu Met Asp Leu Ile Asn Arg Arg Lys Lys Val Lys Lys Val Leu Arg		
465	470	475
Glu Lys Ala Glu Lys Gly Glu Phe Asp Glu Glu Tyr Met Ile Leu Asp		
485	490	495
Tyr Glu Gln Arg Leu Ile Lys Val Leu Ala Asn Ser His Tyr Gly Tyr		
500	505	510
Leu Ala Phe Pro Met Ala Arg Trp Tyr Ser Arg Asp Cys Ala Glu Ile		
515	520	525
Thr Thr His Leu Gly Arg Gln Tyr Ile Gln Lys Thr Ile Glu Glu Ala		
530	535	540
Glu Asn Phe Gly Phe Lys Val Ile Tyr Ala Asp Thr Asp Gly Phe Tyr		
545	550	555
Ser Lys Trp Ala Asp Asp Lys Glu Lys Leu Ser Lys Tyr Glu Leu Leu		
565	570	575
Glu Lys Thr Arg Glu Phe Leu Lys Asn Ile Asn Asn Thr Leu Pro Gly		
580	585	590
Glu Met Glu Leu Glu Phe Glu Gly Tyr Phe Lys Arg Gly Ile Phe Val		
595	600	605

Thr Lys Lys Lys Tyr Ala Leu Ile Asp Glu Asn Glu Lys Ile Thr Val
 610 615 620
 Lys Gly Leu Glu Val Val Arg Arg Asp Trp Ser Asn Val Ser Lys Asn
 625 630 635 640
 Thr Gln Lys Asn Val Leu Asn Ala Leu Leu Lys Glu Gly Ser Val Glu
 645 650 655
 Asn Ala Lys Lys Val Ile Gln Asp Thr Ile Lys Glu Leu Lys Asp Gly
 660 665 670
 Lys Val Asn Asn Glu Asp Leu Leu Ile His Thr Gln Leu Thr Lys Arg
 675 680 685
 Ile Glu Asp Tyr Lys Thr Thr Ala Pro His Val Glu Val Ala Lys Lys
 690 695 700
 Ile Leu Lys Ser Gly Asn Arg Val Asn Thr Gly Asp Val Ile Ser Tyr
 705 710 715 720
 Ile Ile Thr Ser Gly Asn Lys Ser Ile Ser Glu Arg Ala Glu Ile Leu
 725 730 735
 Glu Asn Ala Lys Asn Tyr Asp Thr Asn Tyr Tyr Ile Glu Asn Gln Ile
 740 745 750
 Leu Pro Pro Val Ile Arg Leu Met Glu Ala Leu Gly Ile Thr Lys Asp
 755 760 765
 Glu Leu Lys Asp Ser Lys Lys Gln Tyr Thr Leu His His Phe Leu Lys
 770 775 780

[0056]

<210> 27
 <211> 784
 <212> PRT
 <213> Methanococcus maripaludis
 <220>
 <221> VARIANT
 <222> (419)..(419)
 <223> X=L, V, A
 <400> 27
 Met Glu Ser Leu Ile Asp Leu Asp Tyr Asn Ser Asp Asp Leu Cys Ile
 1 5 10 15
 Tyr Leu Tyr Leu Ile Asn Ser Ile Ile Lys Glu Lys Asp Phe Lys Pro
 20 25 30
 Tyr Phe Tyr Val Asn Ser Thr Asp Lys Glu Gln Ile Leu Glu Phe Leu
 35 40 45
 Lys Asp Tyr Glu Lys Lys His Lys Leu Asp Ser Glu Ile Ser Lys Met
 50 55 60
 Ile Glu Asn Ile Glu Thr Val Lys Lys Ile Val Phe Asp Glu Asn Tyr
 65 70 75 80
 Gln Glu Lys Glu Leu Ser Lys Val Thr Val Lys Tyr Pro Asn Asn Val
 85 90 95

[0057]

Lys Thr Val Arg Glu Ile Leu Met Glu Phe Glu Arg Leu Tyr Glu Tyr		
100	105	110
Asp Ile Pro Phe Val Arg Arg Tyr Leu Ile Asp Asn Ser Val Ile Pro		
115	120	125
Thr Ser Thr Trp Asp Phe Glu Asn Asn Lys Lys Ile Asp Asn Lys Ile		
130	135	140
Pro Asp Phe Lys Thr Val Ser Phe Ala Ile Ala Val Tyr Cys Asn Lys		
145	150	155
Glu Pro Asn Pro Lys Lys Asp Pro Ile Ile Met Ala Ser Phe Ser Ser		
165	170	175
Lys Asp Phe Asn Thr Val Val Ser Thr Lys Lys Phe Asp His Glu Lys		
180	185	190
Leu Glu Tyr Val Lys Asp Glu Lys Glu Leu Ile Lys Arg Ile Ile Glu		
195	200	205
Ile Leu Lys Glu Tyr Asp Ile Ile Tyr Thr Tyr Asn Gly Asp Asn Phe		
210	215	220
Asp Phe Pro Tyr Leu Lys Lys Arg Ala Glu Ser Phe Gly Leu Glu Leu		
225	230	235
Lys Leu Gly Lys Asn Asp Glu Lys Ile Lys Ile Thr Lys Gly Gly Met		
245	250	255
Asn Ser Lys Ser Tyr Ile Pro Gly Arg Val His Ile Asp Leu Tyr Pro		
260	265	270
Ile Ala Arg Arg Leu Leu Asn Leu Thr Lys Tyr Arg Leu Glu Asn Val		
275	280	285
Thr Glu Ala Leu Phe Asp Val Lys Lys Val Asp Val Gly His Glu Asn		
290	295	300
Ile Pro Lys Met Trp Asp Asn Leu Asp Glu Thr Leu Val Glu Tyr Ser		
305	310	315
His Gln Asp Ala Tyr Tyr Thr Gln Arg Ile Gly Glu Gln Phe Leu Pro		
325	330	335
Leu Glu Ile Met Phe Ser Arg Val Val Asn Gln Ser Leu Tyr Asp Ile		
340	345	350
Asn Arg Met Ser Ser Ser Gln Met Val Glu Tyr Leu Leu Leu Lys Asn		
355	360	365
Ser Tyr Lys Met Gly Val Ile Ala Pro Asn Arg Pro Ser Gly Lys Glu		
370	375	380
Tyr Gln Lys Arg Ile Arg Ser Ser Tyr Glu Gly Gly Tyr Val Lys Glu		
385	390	395
Pro Leu Lys Gly Ile His Glu Asp Ile Val Ser Met Asp Phe Leu Ser		
405	410	415
Ala Ala Xaa Ser Ile Ile Met Ser His Asn Leu Ser Pro Glu Thr Ile		
420	425	430
Asp Cys Thr Cys Cys Ser Asp Glu Glu Asn Gly Glu Asn Glu Glu Ile		
435	440	445

[0058]

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

<210> 28

<211> 784
 <212> PRT
 <213> *Methanococcus maripaludis*
 <400> 28
 Met Glu Ser Leu Ile Asp Leu Asp Tyr Asn Ser Asp Asp Leu Cys Ile
 1 5 10 15
 Tyr Leu Tyr Leu Ile Asn Ser Ile Ile Lys Glu Lys Asp Phe Lys Pro
 20 25 30
 Tyr Phe Tyr Val Asn Ser Thr Asp Lys Glu Gln Ile Leu Glu Phe Leu
 35 40 45
 Lys Asp Tyr Glu Lys Lys His Lys Leu Asp Ser Glu Ile Ser Lys Met
 50 55 60
 Ile Glu Asn Ile Glu Thr Val Lys Lys Ile Val Phe Asp Glu Asn Tyr
 65 70 75 80
 Gln Glu Lys Glu Leu Ser Lys Val Thr Val Lys Tyr Pro Asn Asn Val
 85 90 95
 Lys Thr Val Arg Glu Ile Leu Met Glu Phe Glu Arg Leu Tyr Glu Tyr
 100 105 110
 Asp Ile Pro Phe Val Arg Arg Tyr Leu Ile Asp Asn Ser Val Ile Pro
 115 120 125
 Thr Ser Thr Trp Asp Phe Glu Asn Asn Lys Lys Ile Asp Asn Lys Ile
 130 135 140
 Pro Asp Phe Lys Thr Val Ser Phe Ala Ile Ala Val Tyr Cys Asn Lys
 145 150 155 160
 Glu Pro Asn Pro Lys Lys Asp Pro Ile Ile Met Ala Ser Phe Ser Ser
 165 170 175
 Lys Asp Phe Asn Thr Val Val Ser Thr Lys Lys Phe Asp His Glu Lys
 180 185 190
 Leu Glu Tyr Val Lys Asp Glu Lys Glu Leu Ile Lys Arg Ile Ile Glu
 195 200 205
 Ile Leu Lys Glu Tyr Asp Ile Ile Tyr Thr Tyr Asn Gly Asp Asn Phe
 210 215 220
 Asp Phe Pro Tyr Leu Lys Lys Arg Ala Glu Ser Phe Gly Leu Glu Leu
 225 230 235 240
 Lys Leu Gly Lys Asn Asp Glu Lys Ile Lys Ile Thr Lys Gly Gly Met
 245 250 255
 Asn Ser Lys Ser Tyr Ile Pro Gly Arg Val His Ile Asp Leu Tyr Pro
 260 265 270
 Ile Ala Arg Arg Leu Leu Asn Leu Thr Lys Tyr Arg Leu Glu Asn Val
 275 280 285
 Thr Glu Ala Leu Phe Asp Val Lys Lys Val Asp Val Gly His Glu Asn
 290 295 300
 Ile Pro Lys Met Trp Asp Asn Leu Asp Glu Thr Leu Val Glu Tyr Ser
 305 310 315 320

[0059]

[0060]

His Gln Asp Ala Tyr Tyr Thr Gln Arg Ile Gly Glu Gln Phe Leu Pro		
325	330	335
Leu Glu Ile Met Phe Ser Arg Val Val Asn Gln Ser Leu Tyr Asp Ile		
340	345	350
Asn Arg Met Ser Ser Ser Gln Met Val Glu Tyr Leu Leu Leu Lys Asn		
355	360	365
Ser Tyr Lys Met Gly Val Ile Ala Pro Asn Arg Pro Ser Gly Lys Glu		
370	375	380
Tyr Gln Lys Arg Ile Arg Ser Ser Tyr Glu Gly Gly Tyr Val Lys Glu		
385	390	395
Pro Leu Lys Gly Ile His Glu Asp Ile Val Ser Met Asp Phe Leu Ser		
405	410	415
Leu Ala Pro Ser Ile Ile Met Ser His Asn Leu Ser Pro Glu Thr Ile		
420	425	430
Asp Cys Thr Cys Cys Ser Asp Glu Glu Asn Gly Glu Asn Glu Glu Ile		
435	440	445
Leu Gly His Lys Phe Cys Lys Lys Ser Ile Gly Ile Ile Pro Lys Thr		
450	455	460
Leu Met Asp Leu Ile Asn Arg Arg Lys Lys Val Lys Lys Val Leu Arg		
465	470	475
Glu Lys Ala Glu Lys Gly Glu Phe Asp Glu Glu Tyr Met Ile Leu Asp		
485	490	495
Tyr Glu Gln Arg Leu Ile Lys Val Leu Ala Asn Ser His Tyr Gly Tyr		
500	505	510
Leu Ala Phe Pro Met Ala Arg Trp Tyr Ser Arg Asp Cys Ala Glu Ile		
515	520	525
Thr Thr His Leu Gly Arg Gln Tyr Ile Gln Lys Thr Ile Glu Glu Ala		
530	535	540
Glu Asn Phe Gly Phe Lys Val Ile Tyr Ala Asp Thr Asp Gly Phe Tyr		
545	550	555
Ser Lys Trp Ala Asp Asp Lys Glu Lys Leu Ser Lys Tyr Glu Leu Leu		
565	570	575
Glu Lys Thr Arg Glu Phe Leu Lys Asn Ile Asn Asn Thr Leu Pro Gly		
580	585	590
Glu Met Glu Leu Glu Phe Glu Gly Tyr Phe Lys Arg Gly Ile Phe Val		
595	600	605
Thr Lys Lys Lys Tyr Ala Leu Ile Asp Glu Asn Glu Lys Ile Thr Val		
610	615	620
Lys Gly Leu Glu Val Val Arg Arg Asp Trp Ser Asn Val Ser Lys Asn		
625	630	635
Thr Gln Lys Asn Val Leu Asn Ala Leu Leu Lys Glu Gly Ser Val Glu		
645	650	655
Asn Ala Lys Lys Val Ile Gln Asp Thr Ile Lys Glu Leu Lys Asp Gly		
660	665	670

Lys Val Asn Asn Glu Asp Leu Leu Ile His Thr Gln Leu Thr Lys Arg
 675 680 685
 Ile Glu Asp Tyr Lys Thr Thr Ala Pro His Val Glu Val Ala Lys Lys
 690 695 700
 Ile Leu Lys Ser Gly Asn Arg Val Asn Thr Gly Asp Val Ile Ser Tyr
 705 710 715 720
 Ile Ile Thr Ser Gly Asn Lys Ser Ile Ser Glu Arg Ala Glu Ile Leu
 725 730 735
 Glu Asn Ala Lys Asn Tyr Asp Thr Asn Tyr Tyr Ile Glu Asn Gln Ile
 740 745 750
 Leu Pro Pro Val Ile Arg Leu Met Glu Ala Leu Gly Ile Thr Lys Asp
 755 760 765
 Glu Leu Lys Asp Ser Lys Lys Gln Tyr Thr Leu His His Phe Leu Lys
 770 775 780

<210> 29

<211> 775

<212> PRT

<213> 嗜热球菌属物种 Thermococcus sp. 9N-7

<400> 29

[0061]

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

[0062]

Arg Phe Leu Arg Val Val Arg Glu Lys Asp Pro Asp Val Leu Ile Thr		
195	200	205
Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Ser Glu		
210	215	220
Glu Leu Gly Ile Lys Phe Thr Leu Gly Arg Asp Gly Ser Glu Pro Lys		
225	230	235
Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile		
245	250	255
His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr		
260	265	270
Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu		
275	280	285
Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly		
290	295	300
Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr		
305	310	315
Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu		
325	330	335
Ile Gly Gln Ser 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 Lys Arg Asn Glu Leu Ala		
355	360	365
Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr		
370	375	380
Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile		
385	390	395
Val Tyr Leu Asp Phe Arg Ser Ala Ala Ile Ser Ile Ile Ile Thr His		
405	410	415
Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp		
420	425	430
Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe		
435	440	445
Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys		
450	455	460
Arg Lys Met Lys Ala Thr Val Asp Pro Leu Glu Lys Lys Leu Leu Asp		
465	470	475
Tyr Arg Gln Arg Val Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr		
485	490	495
Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser		
500	505	510
Val Ser Ala Trp Gly Arg Glu Tyr Leu Glu Met Val Ile Arg Glu Leu		
515	520	525
Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu		
530	535	540

[0063]

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

<210> 30

<211> 775

<212> PRT

<213> 嗜热球菌属物种 *Thermococcus* sp. 9N-7

<400> 30

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

[0064]

Val	Lys	Arg	Ala	Glu	Lys	Val	Gln	Lys	Lys	Phe	Leu	Gly	Arg	Pro	Ile
65					70					75					80
Glu	Val	Trp	Lys	Leu	Tyr	Phe	Asn	His	Pro	Gln	Asp	Val	Pro	Ala	Ile
				85					90						95
Arg	Asp	Arg	Ile	Arg	Ala	His	Pro	Ala	Val	Val	Asp	Ile	Tyr	Glu	Tyr
				100				105							110
Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro
				115				120							125
Ala	Glu	Gly	Asp	Glu	Glu	Leu	Thr	Met	Leu	Ala	Phe	Ala	Ile	Ala	Thr
				130				135							140
Leu	Tyr	His	Glu	Gly	Glu	Glu	Phe	Gly	Thr	Gly	Pro	Ile	Leu	Met	Ile
				145				150							160
Ser	Tyr	Ala	Asp	Gly	Ser	Glu	Ala	Arg	Val	Ile	Thr	Trp	Lys	Lys	Ile
				165				170							175
Asp	Leu	Pro	Tyr	Val	Asp	Val	Val	Ser	Thr	Glu	Lys	Glu	Met	Ile	Lys
				180				185							190
Arg	Phe	Leu	Arg	Val	Val	Arg	Glu	Lys	Asp	Pro	Asp	Val	Leu	Ile	Thr
				195				200							205
Tyr	Asn	Gly	Asp	Asn	Phe	Asp	Phe	Ala	Tyr	Leu	Lys	Lys	Arg	Ser	Glu
				210				215							220
Glu	Leu	Gly	Ile	Lys	Phe	Thr	Leu	Gly	Arg	Asp	Gly	Ser	Glu	Pro	Lys
				225				230							240
Ile	Gln	Arg	Met	Gly	Asp	Arg	Phe	Ala	Val	Glu	Val	Lys	Gly	Arg	Ile
				245				250							255
His	Phe	Asp	Leu	Tyr	Pro	Val	Ile	Arg	Arg	Thr	Ile	Asn	Leu	Pro	Thr
				260				265							270
Tyr	Thr	Leu	Glu	Ala	Val	Tyr	Glu	Ala	Val	Phe	Gly	Lys	Pro	Lys	Glu
				275				280							285
Lys	Val	Tyr	Ala	Glu	Glu	Ile	Ala	Gln	Ala	Trp	Glu	Ser	Gly	Glu	Gly
				290				295							300
Leu	Glu	Arg	Val	Ala	Arg	Tyr	Ser	Met	Glu	Asp	Ala	Lys	Val	Thr	Tyr
				305				310							320
Glu	Leu	Gly	Arg	Glu	Phe	Phe	Pro	Met	Glu	Ala	Gln	Leu	Ser	Arg	Leu
				325				330							335
Ile	Gly	Gln	Ser	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	Lys	Arg	Asn	Glu	Leu	Ala
				355				360							365
Pro	Asn	Lys	Pro	Asp	Glu	Arg	Glu	Leu	Ala	Arg	Arg	Arg	Gly	Gly	Tyr
				370				375							380
Ala	Gly	Gly	Tyr	Val	Lys	Glu	Pro	Glu	Arg	Gly	Leu	Trp	Asp	Asn	Ile
				385				390							400
Val	Tyr	Leu	Asp	Phe	Arg	Ser	Ala	Ala	Ile	Ser	Ile	Ile	Ile	Thr	His
				405				410							415

[0065]

Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp		
420	425	430
Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe		
435	440	445
Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys		
450	455	460
Arg Lys Met Lys Ala Thr Val Asp Pro Leu Glu Lys Met Leu Leu Asp		
465	470	475
Tyr Arg Gln Arg Val Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr		
485	490	495
Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser		
500	505	510
Val Ser Ala Trp Gly Arg Glu Tyr Leu Glu Met Val Ile Arg Glu Leu		
515	520	525
Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu		
530	535	540
His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala		
545	550	555
Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu		
565	570	575
Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys		
580	585	590
Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu		
595	600	605
Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala		
610	615	620
Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val		
625	630	635
Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro		
645	650	655
Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp		
660	665	670
Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala		
675	680	685
Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu		
690	695	700
Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe		
705	710	715
Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln		
725	730	735
Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys		
740	745	750
Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp		
755	760	765

Leu Lys Val Lys Gly Lys Lys
770 775

<210> 31

<211> 775

<212> PRT

<213> 嗜热球菌属物种 Thermococcus sp. 9N-7

<400> 31

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

[0066]

[0067]

Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly		
290	295	300
Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr		
305	310	315 320
Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu		
325	330	335
Ile Gly Gln Ser 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 Lys Arg Asn Glu Leu Ala		
355	360	365
Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr		
370	375	380
Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile		
385	390	395 400
Val Tyr Leu Asp Phe Arg Ser Ala Ala Ile Ser Ile Ile Ile Thr His		
405	410	415
Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp		
420	425	430
Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe		
435	440	445
Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys		
450	455	460
Arg Lys Met Lys Ala Thr Val Asp Pro Leu Glu Lys Lys Leu Leu Asp		
465	470	475 480
Tyr Arg Gln Arg Val Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr		
485	490	495
Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser		
500	505	510
Val Ala Ala Trp Gly Arg Glu Tyr Leu Glu Met Val Ile Arg Glu Leu		
515	520	525
Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr Asp Gly Leu		
530	535	540
His Ala Thr Ile Pro Gly Ala Asp Ala Glu Thr Val Lys Lys Lys Ala		
545	550	555 560
Lys Glu Phe Leu Lys Tyr Ile Asn Pro Lys Leu Pro Gly Leu Leu Glu		
565	570	575
Leu Glu Tyr Glu Gly Phe Tyr Val Arg Gly Phe Phe Val Thr Lys Lys		
580	585	590
Lys Tyr Ala Val Ile Asp Glu Glu Gly Lys Ile Thr Thr Arg Gly Leu		
595	600	605
Glu Ile Val Arg Arg Asp Trp Ser Glu Ile Ala Lys Glu Thr Gln Ala		
610	615	620
Arg Val Leu Glu Ala Ile Leu Lys His Gly Asp Val Glu Glu Ala Val		
625	630	635 640

Arg Ile Val Lys Glu Val Thr Glu Lys Leu Ser Lys Tyr Glu Val Pro
 645 650 655
 Pro Glu Lys Leu Val Ile His Glu Gln Ile Thr Arg Asp Leu Arg Asp
 660 665 670
 Tyr Lys Ala Thr Gly Pro His Val Ala Val Ala Lys Arg Leu Ala Ala
 675 680 685
 Arg Gly Val Lys Ile Arg Pro Gly Thr Val Ile Ser Tyr Ile Val Leu
 690 695 700
 Lys Gly Ser Gly Arg Ile Gly Asp Arg Ala Ile Pro Ala Asp Glu Phe
 705 710 715 720
 Asp Pro Thr Lys His Arg Tyr Asp Ala Glu Tyr Tyr Ile Glu Asn Gln
 725 730 735
 Val Leu Pro Ala Val Glu Arg Ile Leu Lys Ala Phe Gly Tyr Arg Lys
 740 745 750
 Glu Asp Leu Arg Tyr Gln Lys Thr Lys Gln Val Gly Leu Gly Ala Trp
 755 760 765
 Leu Lys Val Lys Gly Lys Lys
 770 775

<210> 32

<211> 775

<212> PRT

[0068]

<213> 嗜热球菌属物种 *Thermococcus* sp. 9N-7

<400> 32

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

[0069]

Ser Tyr Ala Asp Gly Ser Glu Ala Arg Val Ile Thr Trp Lys Lys Ile
 165 170 175
 Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Lys Glu Met Ile Lys
 180 185 190
 Arg Phe Leu Arg Val Val Arg Glu Lys Asp Pro Asp Val Leu Ile Thr
 195 200 205
 Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Ser Glu
 210 215 220
 Glu Leu Gly Ile Lys Phe Thr Leu Gly Arg Asp Gly Ser Glu Pro Lys
 225 230 235 240
 Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile
 245 250 255
 His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr
 260 265 270
 Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Phe Gly Lys Pro Lys Glu
 275 280 285
 Lys Val Tyr Ala Glu Glu Ile Ala Gln Ala Trp Glu Ser Gly Glu Gly
 290 295 300
 Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr
 305 310 315 320
 Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu
 325 330 335
 Ile Gly Gln Ser 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 Lys Arg Asn Glu Leu Ala
 355 360 365
 Pro Asn Lys Pro Asp Glu Arg Glu Leu Ala Arg Arg Arg Gly Gly Tyr
 370 375 380
 Ala Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp Asp Asn Ile
 385 390 395 400
 Val Tyr Leu Asp Phe Arg Ser Ala Ala Ile Ser Ile Ile Ile Thr His
 405 410 415
 Asn Val Ser Pro Asp Thr Leu Asn Arg Glu Gly Cys Lys Glu Tyr Asp
 420 425 430
 Val Ala Pro Glu Val Gly His Lys Phe Cys Lys Asp Phe Pro Gly Phe
 435 440 445
 Ile Pro Ser Leu Leu Gly Asp Leu Leu Glu Glu Arg Gln Lys Ile Lys
 450 455 460
 Arg Lys Met Lys Ala Thr Val Asp Pro Leu Glu Lys Met Leu Leu Asp
 465 470 475 480
 Tyr Arg Gln Arg Val Ile Lys Ile Leu Ala Asn Ser Phe Tyr Gly Tyr
 485 490 495
 Tyr Gly Tyr Ala Lys Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ser
 500 505 510

[0070]

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

图 1

图1 (续)

[illegible]

图1 (续)

[illegible]

图1 (续)

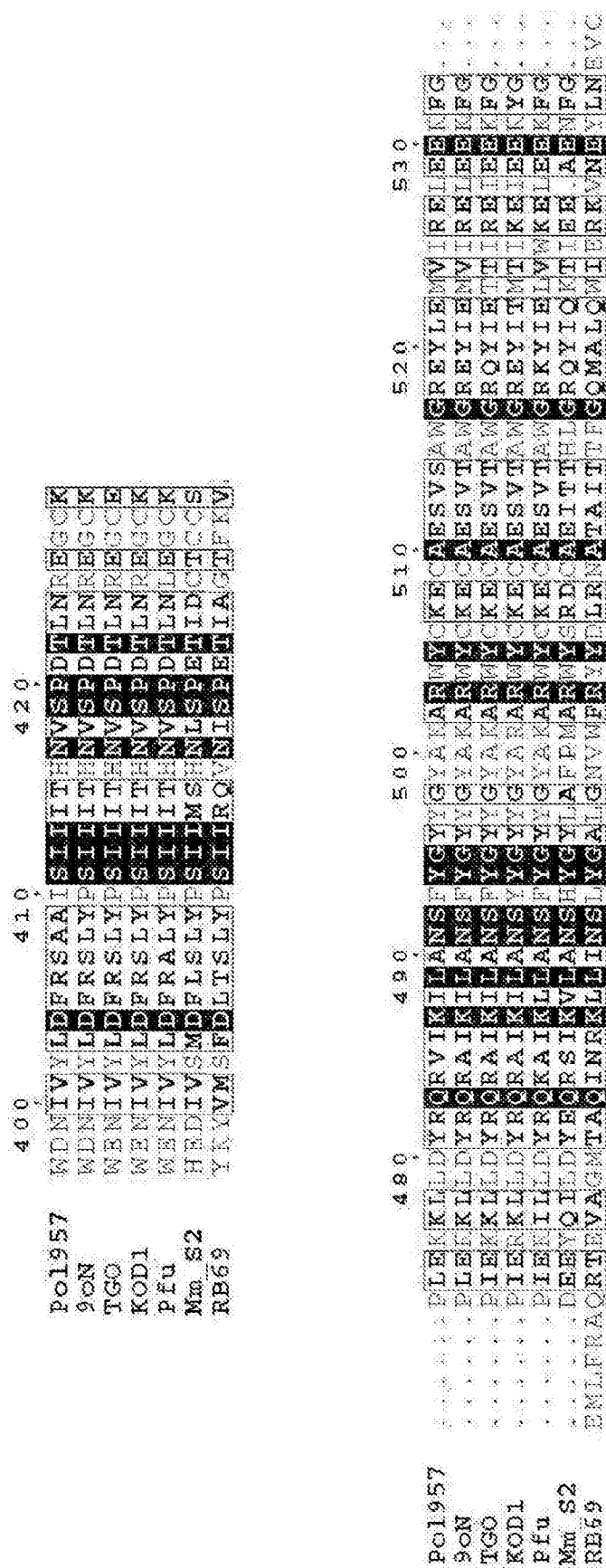


图 2