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(54)发明名称

用于基因组编辑的多核苷酸、组合物及方法

(57)摘要

本发明提供用于基因编辑的组合物及方法。在一些实施例中,提供一种编码Cas9的多核苷酸,其可提供改良的编辑效率、降低的免疫原性或其它益处中的一种或多种。

1.一种mRNA,其包含的开放阅读框架区为SEQ ID NO:111,113-119,146和148-154中的任一项所示的序列,其中所述开放阅读框架编码RNA引导的DNA结合剂。

2.如权利要求1所述的mRNA,其中所述mRNA包含SEQ ID NO:32、34、36、38、41或75至77中的任一项的序列的5'UTR。

3.如权利要求1所述的mRNA,其中所述mRNA包含SEQ ID NO:33、35、37、39或40中的任一项的序列的3'UTR。

4.如权利要求1所述的mRNA,其中所述mRNA包含SEQ ID NO:32、34、36、38、41或75至77中的任一项的序列的5'UTR,和SEQ ID NO:33、35、37、39或40中的任一项的序列的3'UTR。

5.如权利要求4所述的mRNA,其中所述mRNA包含来自同一来源的5'UTR及3'UTR。

6.如权利要求1-4中任一项所述的mRNA,其包含选自Cap0、Cap1及Cap2的5'帽结构。

7.如权利要求1-4中任一项所述的mRNA,其中所述RNA引导的DNA结合剂是Cas9裂解酶。

8.如权利要求1-4中任一项所述的mRNA,其中所述mRNA包含的ORF区为SEQ ID NO:111的序列。

9.如权利要求1-4中任一项所述的mRNA,其中所述RNA引导的DNA结合剂是Cas9切口酶。

10.如权利要求1-4中任一项所述的mRNA,其中所述mRNA包含的ORF区为SEQ ID NO:114的序列。

11.如权利要求1至4中任一项所述的mRNA,其中所述RNA引导的DNA结合剂包含dCas9 DNA结合域。

12.如权利要求1-4中任一项所述的mRNA,其中所述mRNA包含的ORF区为SEQ ID NO:117的序列。

13.如权利要求1-4中任一项所述的mRNA,其中所述mRNA包含编码SEQ ID NO:3、6、8或186-196中任一项所示的氨基酸序列的核酸序列。

14.如权利要求1-4中任一项所述的mRNA,其中所述RNA引导的DNA结合剂进一步包含异源功能域。

15.如权利要求14所述的mRNA,其中所述异源功能域为FokI核酸酶。

16.如权利要求14所述的mRNA,其中所述异源功能域为转录调节域。

17.如权利要求1-4中任一项所述的mRNA,其中至少10%的尿苷被经修饰的尿苷取代。

18.如权利要求17所述的mRNA,其中所述经修饰的尿苷为N1-甲基-假尿苷、假尿苷、5-甲氧基尿苷或5-碘尿苷中的一种或多种。

19.如权利要求17所述的mRNA,其中所述经修饰的尿苷为N1-甲基-假尿苷或5-甲氧基尿苷中的一种或两种。

20.如权利要求17所述的mRNA,其中所述经修饰的尿苷为N1-甲基-假尿苷。

21.如权利要求17所述的mRNA,其中所述经修饰的尿苷为5-甲氧基尿苷。

22.如权利要求17所述的mRNA,其中15%至45%的所述尿苷被经修饰的尿苷取代。

23.如权利要求17所述的mRNA,其中至少20%或至少30%的所述尿苷被经修饰的尿苷取代。

24.如权利要求23所述的mRNA,其中至少80%或至少90%的所述尿苷被经修饰的尿苷取代。

25.如权利要求24所述的mRNA,其中100%的尿苷被经修饰的尿苷取代。

26.如权利要求1-4中任一项所述的mRNA,其中所述mRNA为SEQ ID NO:177所示的序列。

27.一种表达构建体,其包含以可操作方式连接编码如权利要求1-26中任一项所述的mRNA的序列的启动子。

28.一种质粒,其包含如权利要求27所述的表达构建体。

29.一种宿主细胞,其包含如权利要求27所述的表达构建体或如权利要求28所述的质粒。

30.一种制备mRNA的方法,该方法包含使如权利要求27所述的表达构建体或如权利要求28所述的质粒与RNA聚合酶在容许进行所述mRNA的转录的条件下接触,其中在体外进行所述接触步骤。

31.一种组合物,其包含如权利要求1至26中任一项所述的mRNA及至少一种引导RNA。

32.一种脂质纳米颗粒,其包含如权利要求1至26中任一项所述的mRNA。

33.一种医药组合物,其包含如权利要求1至26中任一项所述的mRNA及医药学上可接受的载体。

34.如权利要求32所述的脂质纳米颗粒或如权利要求33所述的医药组合物,其进一步包含至少一种引导RNA。

35.如权利要求31-34中任一项所述的组合物或脂质纳米颗粒或医药组合物,其中至少一种引导RNA靶向TTR。

36.一种如权利要求1至27或31至35中任一项所述的mRNA、表达构建体、组合物、脂质纳米颗粒或医药组合物的用途,其用于制造用于基因组编辑或修饰靶基因的药剂。

37.如权利要求36所述的用途,其中所述靶基因的基因组编辑或修饰是在肝脏细胞中进行。

38.如权利要求37所述的用途,其中所述肝脏细胞为肝细胞。

39.如权利要求36至38中任一项所述的用途,其中所述靶基因的基因组编辑或修饰是在体内进行。

40.如权利要求36至38中任一项所述的用途,其中所述靶基因的基因组编辑或修饰是在经分离或培养的细胞中进行。

用于基因组编辑的多核苷酸、组合物及方法

[0001] 本申请要求基于2017年9月29日提出的美国临时申请第62/556,144号的优先权，且其全部内容以引用方式并入。

[0002] 本申请含有以ASCII格式电子提交的序列表且其全部内容以引用方式并入本文中。于2018年9月28日创建的该ASCII拷贝命名为2018-09-28_01155-0020-00PCT_ST25.txt且大小为963,200个字节。

技术领域

[0003] 本发明是关于用于基因组编辑的多核苷酸、组合物及方法，该基因组编辑涉及RNA引导的DNA结合剂，诸如CRISPR-Cas系统及其亚基。

背景技术

[0004] RNA引导的DNA结合剂，诸如CRISPR-Cas系统可用于进行靶基因组编辑，包括在真核细胞中及活体内。已显示此类编辑能够不活化某些有害等位基因或校正某些有害点突变。该结合剂可通过提供编码的mRNA来原位表达。然而，现有方法提供的编辑效率可能要比所需编辑效率低或可能具有非所期望的免疫原性，例如可能会引起细胞介素含量的升高为非所期望的。

[0005] 因此，需要经改良的用于基因组编辑的多核苷酸、组合物及方法。本发明旨在提供用于基因组编辑的组合物及方法，其提供一或多种益处，诸如经改良的编辑效率或经降低的免疫原性(例如施用后经降低的细胞介素的升高量)中的至少一者；或至少为公众提供一种有用选择。在一些实施例中，提供一种编码RNA引导的DNA结合剂的多核苷酸，其中其密码子使用、非编码序列(例如UTR)、异源域(例如NLS)及/或核苷酸含量中的一项或多项以本文所揭示的方式与现有多核苷酸不同。已发现此类特征可提供诸如上文所述的益处。在一些实施例中，经改良的编辑效率出现在哺乳动物的器官或细胞型，诸如肝脏或肝细胞中或对其具有特异性。

[0006] 发明详述

[0007] 实施例1为一种包含编码RNA引导的DNA结合剂的开放阅读框架的mRNA，其中该开放阅读框架的尿苷含量在其最小尿苷含量至最小尿苷含量的150％范围内。

[0008] 实施例2为一种包含编码RNA引导的DNA结合剂的开放阅读框架的mRNA，其中该开放阅读框架的尿苷二核苷酸含量在其最小尿苷二核苷酸含量至最小尿苷二核苷酸含量的150％范围内。

[0009] 实施例3为一种包含编码RNA引导的DNA结合剂的开放阅读框架的mRNA，其中该开放阅读框架的腺嘌呤含量在其最小腺嘌呤含量至最小腺嘌呤含量的150％范围内。

[0010] 实施例4为一种包含编码RNA引导的DNA结合剂的开放阅读框架的mRNA，其中该开放阅读框架的腺嘌呤二核苷酸含量在其最小腺嘌呤二核苷酸含量至最小腺嘌呤二核苷酸含量的150％范围内。

[0011] 实施例5为一种包含与SEQ ID NO:1、4、7、9、10、11、12、14，15、17、18、20、21、23、

24、26、27、29、30、50、52、54、65、66、或107-175任一项具有至少90％的一致性的序列的mRNA，其中该mRNA包含编码RNA引导的DNA结合剂的开放阅读框架。

[0012] 实施例6为一种包含编码RNA引导的DNA结合剂的开放阅读框架的mRNA，其中该开放阅读框架与SEQ ID NO:1、4、7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66、或107-175以下任一项在至少其前30、50、70、100、150、200、250或300个核苷酸上具有至少90％的一致性。

[0013] 实施例7为如前述技术方案中任一项的mRNA，其中开放阅读框架由一组密码子组成，其中至少75％的密码子为(i)表1、表2或表3中所列的密码子或(ii)一组表4中所列的密码子。

[0014] 实施例8为一种编码RNA引导的DNA结合剂的mRNA，其包含编码RNA引导的DNA结合剂的开放阅读框架，其中开放阅读框架由一组密码子组成，其中至少75％的密码子为表1、表2、表3中所列的密码子或(ii)一组表4中所列的密码子。

[0015] 实施例9为如技术方案7或8的mRNA，其中开放阅读框架由一组密码子组成，其中至少75％的密码子为表4中低U1组的密码子。

[0016] 实施例10为如技术方案7或8的mRNA，其中开放阅读框架由一组密码子组成，其中至少75％的密码子为表4中低A组的密码子。

[0017] 实施例11为如技术方案7或8的mRNA，其中开放阅读框架由一组密码子组成，其中至少75％的密码子为表4中低A/U组的密码子。

[0018] 实施例12为如技术方案7或8的mRNA，其中开放阅读框架由一组密码子组成，其中至少75％的密码子为表4中长半衰期组的密码子。

[0019] 实施例13为如技术方案7至12中任一项的mRNA，其中至少80％、85％、90％、95％、98％、99％或100％的密码子为(i)表1、表2或表3中所列的密码子或(ii)一组表4中所列的密码子。

[0020] 实施例14为如技术方案1至5或7至13中任一项的mRNA，其中开放阅读框架与SEQ ID NO:1、4、7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66、或107-175任一项在至少其前30、50、70、100、150、200、250或300个核苷酸上具有至少90％的一致性。

[0021] 实施例15为如前述技术方案中任一项的mRNA，其中开放阅读框架与SEQ ID NO:1、4、7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66、或107-175任一项在至少其序列之前10％、12％、15％、20％、25％、30％或35％上具有至少90％的一致性。

[0022] 实施例16为如技术方案1至4或6至15中任一项的mRNA，其中该mRNA包含与SEQ ID NO:1、4、7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66、或107-175任一项有至少90％的一致性的序列。

[0023] 实施例17为如前述技术方案中任一项的mRNA，其中开放阅读框架的尿苷二核苷酸含量在其最小尿苷二核苷酸含量至最小尿苷二核苷酸含量的101％、102％、103％、105％、110％、115％、120％、125％、130％、135％、140％、145％或150％范围内。

[0024] 实施例18为如前述技术方案中任一项的mRNA，其中开放阅读框架的尿苷含量在其最小尿苷含量至最小尿苷含量的101％、102％、103％、105％、110％、115％、120％、125％、

130%、135%、140%、145%或150%范围内。

[0025] 实施例19为如前述技术方案中任一项的mRNA,其中开放阅读框架的腺嘌呤含量在其最小腺嘌呤含量至最小腺嘌呤含量的101%、102%、103%、105%、110%、115%、120%、125%、130%、135%、140%、145%或150%范围内。

[0026] 实施例20为如前述技术方案中任一项的mRNA,其中开放阅读框架的腺嘌呤二核苷酸含量在其最小腺嘌呤二核苷酸含量至最小腺嘌呤二核苷酸含量的101%、102%、103%、105%、110%、115%、120%、125%、130%、135%、140%、145%或150%范围内。

[0027] 实施例21为如前述技术方案中任一项的mRNA,其包含与SEQ ID NO:32、34、36、38、41或75—77中的任一项有至少90%的一致性的5′UTR。

[0028] 实施例22为如前述技术方案中任一项的mRNA,其包含与SEQ ID NO:33、35、37、39或40中的任一项有至少90%的一致性的3′UTR。

[0029] 实施例23为如技术方案21或22的mRNA,其中mRNA包含来自同一来源的5′UTR及3′UTR。

[0030] 实施例24为如前述技术方案中任一项的mRNA,其包含选自Cap0、Cap1及Cap2的5′帽结构。

[0031] 实施例25为如前述技术方案中任一项的mRNA,其中开放阅读框架具有提高哺乳动物中的mRNA转译的密码子。

[0032] 实施例26为如技术方案25的mRNA,其中开放阅读框架具有提高哺乳动物的特定器官中的mRNA转译的密码子。

[0033] 实施例27为如技术方案26的mRNA,其中器官为肝脏。

[0034] 实施例28为如技术方案25至27中任一项的mRNA,其中哺乳动物为人类。

[0035] 实施例29为如技术方案25至28中任一项的mRNA,其中相对于包含具有由SEQ ID NO:5组成的序列的ORF的mRNA的转译,密码子提高哺乳动物中的mRNA的转译。

[0036] 实施例30为如前述技术方案中任一项的mRNA,其中当以医药组合物形式向哺乳动物施用该mRNA时,哺乳动物展现出比施用包含具有大于150%最小尿苷含量的编码Cas9核酸酶的ORF的mRNA的哺乳动物低至少5倍的细胞介素反应。

[0037] 实施例31为如技术方案30的mRNA,其中包含具有大于150%最小尿苷含量的编码Cas9核酸酶的ORF的mRNA具有由SEQ ID NO:5组成的序列。

[0038] 实施例32为如前述技术方案中任一项的mRNA,其中RNA引导的DNA结合剂具有双股核酸内切酶活性。

[0039] 实施例33为如技术方案32的mRNA,其中RNA引导的DNA结合剂包含Cas裂解酶。

[0040] 实施例34为如前述技术方案中任一项的mRNA,其中RNA引导的DNA结合剂具有切口酶活性。

[0041] 实施例35为如技术方案34的mRNA,其中RNA引导的DNA结合剂包含Cas切口酶。

[0042] 实施例36为如技术方案1至31中任一项的mRNA,其中RNA引导的DNA结合剂包含dCas DNA结合域。

[0043] 实施例37为如技术方案33或35至36中任一项的mRNA,其中Cas裂解酶、Cas切口酶或dCas DNA结合域为Cas9裂解酶、Cas9切口酶、或dCas9 DNA结合域。

[0044] 实施例38为如前述技术方案中任一项的mRNA,其中经编码的RNA引导的DNA结合剂

包含核定位信号(NLS)。

[0045] 实施例39为如技术方案38的mRNA,其中NLS连接于RNA引导的DNA结合剂的C端。

[0046] 实施例40为如技术方案38的mRNA,其中NLS连接于RNA引导的DNA结合剂的N端。

[0047] 实施例41为如技术方案38至40中任一项的mRNA,其中NLS包含与SEQ ID NO:78-91中的任一项具有至少80％、85％、90％或95％的一致性的序列。

[0048] 实施例42为如技术方案38至40中任一项的mRNA,其中NLS包含SEQ ID NO:78—91中的任一项的序列。

[0049] 实施例43为如技术方案38至42中任一项的mRNA,其中NLS由与SEQ ID NO:92-104中的任一项的序列具有至少80％、85％、90％、95％、98％或100％的一致性的序列编码。

[0050] 实施例44为如技术方案37至43中任一项的mRNA,其中该mRNA包含与SEQ ID NO:4、7或9有至少90％的一致性的序列。

[0051] 实施例45为如技术方案37至43中任一项的mRNA,其中该mRNA包含与SEQ ID NO:4、7或9有至少95％的一致性的序列。

[0052] 实施例46为如技术方案37至43中任一项的mRNA,其中该mRNA包含与SEQ ID NO:4、7或9有至少98％的一致性的序列。

[0053] 实施例47为如技术方案37至43中任一项的mRNA,其中该mRNA包含与SEQ ID NO:4、7或9有100％一致性的序列。

[0054] 实施例48为如技术方案37至43中任一项的mRNA,其中该mRNA包含与SEQ ID NO:111、114或117有至少90％的一致性的序列。

[0055] 实施例49为如技术方案37至43中任一项的mRNA,其中该mRNA包含与SEQ ID NO:111、114或117有至少95％的一致性的序列。

[0056] 实施例50为如技术方案37至43中任一项的mRNA,其中该mRNA包含与SEQ ID NO:111、114或117有至少98％的一致性的序列。

[0057] 实施例51为如技术方案37至43中任一项的mRNA,其中该mRNA包含与SEQ ID NO:112、122或125有100％一致性的序列。

[0058] 实施例52为如技术方案37至43中任一项的mRNA,其中该mRNA包含与SEQ ID NO:112、122或125有至少90％的一致性的序列。

[0059] 实施例53为如技术方案37至43中任一项的mRNA,其中该mRNA包含与SEQ ID NO:112、122或125有至少95％的一致性的序列。

[0060] 实施例54为如技术方案37至43中任一项的mRNA,其中该mRNA包含与SEQ ID NO:10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66、或107—175有至少90％的一致性的序列。

[0061] 实施例55为如技术方案37至43中任一项的mRNA,其中该mRNA包含与SEQ ID NO:10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66、或107-175有至少95％的一致性的序列。

[0062] 实施例56为如技术方案37至43中任一项的mRNA,其中该mRNA包含与SEQ ID NO:10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66、或107-175有至少98％的一致性的序列。

[0063] 实施例57为如技术方案37至43中任一项的mRNA,其中该mRNA包含与SEQ ID NO:

10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66、或107-175有100％一致性的序列。

[0064] 实施例58为如技术方案37至57中任一项的mRNA,其中该mRNA编码包含SEQ ID NO:3、6、8或186-196的氨基酸序列的多肽。

[0065] 实施例59为如前述技术方案中任一项的mRNA,其中RNA引导的DNA结合剂进一步包含异源功能域。

[0066] 实施例60为如技术方案59的mRNA,其中异源功能域为FokI核酸酶。

[0067] 实施例61为如技术方案59的mRNA,其中异源功能域为转录调节域。

[0068] 实施例62为如前述技术方案中任一项的mRNA,其中当以包含脂质纳米颗粒的医药组合物形式向哺乳动物施用有效量的mRNA以及靶向哺乳动物的TTR基因的引导RNA时,在得自哺乳动物肝细胞的至少50％基因组DNA中的TTR基因座处形成插入缺失标记(indel)。

[0069] 实施例63为如前述技术方案中任一项的mRNA,其中当以包含脂质纳米颗粒的医药组合物形式向哺乳动物施用有效量的mRNA以及靶向哺乳动物的TTR基因的引导RNA时,哺乳动物的血清中的TTR的浓度降低至少50％。

[0070] 实施例64为如前述技术方案中任一项的mRNA,其中至少10％的尿苷被经修饰的尿苷取代。

[0071] 实施例65为如技术方案64的mRNA,其中经修饰的尿苷为N1-甲基-假尿苷、假尿苷、5-甲氧基尿苷或5-碘尿苷中的一种或多种。

[0072] 实施例66为如技术方案64的mRNA,其中经修饰的尿苷为N1-甲基-假尿苷或5-甲氧基尿苷中的一种或两种。

[0073] 实施例67为如技术方案64的mRNA,其中经修饰的尿苷为N1-甲基-假尿苷。

[0074] 实施例68为如技术方案64的mRNA,其中经修饰的尿苷为5-甲氧基尿苷。

[0075] 实施例69为如技术方案64至68中任一项的mRNA,其中15％至45％的尿苷被经修饰的尿苷取代。

[0076] 实施例70为如技术方案64至68中任一项的mRNA,其中至少20％或至少30％的尿苷被经修饰的尿苷取代。

[0077] 实施例71为如技术方案70的mRNA,其中至少80％或至少90％的尿苷被经修饰的尿苷取代。

[0078] 实施例72为如技术方案70的mRNA,其中100％的尿苷被经修饰的尿苷取代。

[0079] 实施例73为如技术方案64至72中任一项的mRNA,其中当以包含脂质纳米颗粒的医药组合物形式向哺乳动物施用有效量的mRNA以及靶向哺乳动物的TTR基因的引导RNA时,在得自哺乳动物肝细胞的至少70％或至少90％基因组DNA中的TTR基因座处形成插入缺失标记(indel)。

[0080] 实施例74为如技术方案64至73中任一项的mRNA,其中当以包含脂质纳米颗粒的医药组合物形式向哺乳动物施用mRNA以及靶向哺乳动物的TTR基因的引导RNA时,哺乳动物的血清中的TTR的浓度降低至少70％或至少90％。

[0081] 实施例75为如技术方案62、63、71或72的mRNA,其中动物为小鼠且引导RNA具有由SEQ ID NO:42组成的序列。

[0082] 实施例76为如技术方案62、63、71或72的mRNA,其中动物为大鼠且引导RNA具有由

SEQ ID NO:69组成的序列。

[0083] 实施例77为如前述技术方案中任一项的mRNA,其中该mRNA包含与SEQ ID NO:43、44、51、53、55-61或176-185中的任一项有至少90％的一致性的序列。

[0084] 实施例78为如前述技术方案中任一项的mRNA,其中该mRNA包含与SEQ ID NO:43、44、51、53、55-61或176-185中的任一项有至少95％的一致性的序列。

[0085] 实施例79为如前述技术方案中任一项的mRNA,其中该mRNA包含与SEQ ID NO:43、44、51、53、55-61或176-185中的任一项有至少98％的一致性的序列。

[0086] 实施例80为如前述技术方案中任一项的mRNA,其中该mRNA包含与SEQ ID NO:43、44、51、53、55-61或176-185中的任一项有至少99％的一致性的序列。

[0087] 实施例81为如前述技术方案中任一项的mRNA,其中该mRNA包含与SEQ ID NO:43、44、51、53、55-61或176-185中的任一项有100％一致性的序列。

[0088] 实施例82为一种表达构建体,其包含以可操作方式连接编码如前述技术方案中任一项的mRNA的序列的启动子。

[0089] 实施例83为一种包含如技术方案82的表达构建体的质粒。

[0090] 实施例84为一种包含如技术方案82的表达构建体或如技术方案83的质粒的宿主细胞。

[0091] 实施例85为一种制备mRNA的方法,该方法包含使如技术方案82的表达构建体或如技术方案83的质粒与核糖核酸聚合酶在容许进行mRNA的转录的条件下接触。

[0092] 实施例86为如技术方案85的方法,其中活体外进行接触步骤。

[0093] 实施例87为一种组合物,其包含如技术方案1至81中任一项的mRNA及至少一种引导RNA。

[0094] 实施例88为一种脂质纳米颗粒,其包含如技术方案1至81中任一项的mRNA。

[0095] 实施例89为一种医药组合物,其包含如技术方案1至81中任一项的mRNA及医药学上可接受的载体。

[0096] 实施例90为如技术方案88的脂质纳米颗粒或如技术方案89的医药组合物,其进一步包含至少一种引导RNA。

[0097] 实施例91为如技术方案87至90中任一项的组合物或脂质纳米颗粒,其中至少一种引导RNA靶向TTR。

[0098] 实施例92为一种基因组编辑或修饰靶基因的方法,其包含使细胞与如技术方案1至83或87至91中任一项的mRNA、表达构建体、组合物或脂质纳米颗粒接触。

[0099] 实施例93为如技术方案1至83或87至91中任一项的mRNA、表达构建体、组合物或脂质纳米颗粒的用途,其用于基因组编辑或修饰靶基因。

[0100] 实施例94为如技术方案1至83或87至91中任一项的mRNA、表达构建体、组合物或脂质纳米颗粒的用途,其用于制造用于基因组编辑或修饰靶基因的药剂。

[0101] 实施例95为如技术方案92至94中任一项的方法或用途,其中基因组编辑或修饰靶基因在肝脏细胞中进行。

[0102] 实施例96为如技术方案95的方法或用途,其中肝脏细胞为肝细胞。

[0103] 实施例97为如技术方案92至96中任一项的方法或用途,其中基因组编辑或修饰靶基因在活体内进行。

[0104] 实施例98为如技术方案92至97中任一项的方法或用途,其中基因组编辑或修饰靶基因在经分离或经培养的细胞中进行。

[0105] 所揭示的序列的简要说明

[0106]

SEQ ID NO	描述
1	使用表3中所列的最小尿苷密码子的胸苷类似物的Cas9的DNA编码序列，其具有起始和终止密码子
2	使用通常在人类中高度表达的密码子的Cas9的DNA编码序列
3	具有一个核定位信号(1×NLS)作为C端7个氨基酸的Cas9的氨基酸序列
4	使用如表3中所列的最小尿苷密码子的Cas9 mRNA ORF，其具有具有起始和终止密码子
5	使用通常在人类中高度表达的密码子的Cas9 mRNA ORF，其具有具有起始和终止密码子
6	具有1×NLS作为C端7个氨基酸的Cas9切口酶的氨基酸序列
7	使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 6的，具有起始和终止密码子的Cas9切口酶mRNA ORF
8	具有1×NLS作为C端7个氨基酸的dCas9的氨基酸序列
9	使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 8的，具有起始和终止密码子的dCas9 mRNA ORF
10	使用如表3中所列的最小尿苷密码子的Cas9 mRNA编码序列(无起始或终止密码子；适于包括在融合蛋白质编码序列中)
11	使用如表3中所列的最小尿苷密码子的Cas9切口酶mRNA编码序列(无起始或终止密码子；适于包括在融合蛋白质编码序列中)
12	使用如表3中所列的最小尿苷密码子的dCas9 mRNA编码序列(无起始或终止密码子；适于包括在融合蛋白质编码序列中)
13	Cas9 (无NLS)的氨基酸序列
14	使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 13的，具有起始和终止密码子的Cas9 mRNA ORF
15	使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 13的Cas9编码序列(无起始或终止密码子；适于包括在融合蛋白质编码序列中)

[0107]

16	Cas9切口酶(无NLS)的氨基酸序列
17	使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 16的，具有起始和终止密码子的Cas9切口酶mRNA ORF
18	使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 16的Cas9切口酶编码序列(无起始或终止密码子；适于包括在融合蛋白质编码序列中)
19	dCas9 (无NLS)的氨基酸序列
20	使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 13的，具有起始和终止密码子的dCas9 mRNA ORF
21	使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 13的dCas9编码序列(无起始或终止密码子;适于包括在融合蛋白质编码序列中)
22	具有两个核定位信号(2×NLS)作为C端氨基酸的Cas9的氨基酸序列
23	使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 13的，具有起始和终止密码子的Cas9 mRNA ORF
24	使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 13的Cas9编码序列(无起始或终止密码子；适于包括在融合蛋白质编码序列中)
25	具有两个核定位信号作为C端氨基酸的Cas9切口酶的氨基酸序列
26	使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 16的，具有起始和终止密码子的Cas9切口酶mRNA ORF
27	使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 16的Cas9切口酶编码序列(无起始或终止密码子；适于包括在融合蛋白质编码序列中)
28	具有两个核定位信号作为C端氨基酸的dCas9的氨基酸序列
29	使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 13的，具有起始和终止密码子的dCas9 mRNA ORF

[0108]

30	使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 13的dCas9编码序列(无起始或终止密码子；适于包括在融合蛋白质编码序列中)
31	T7启动子
32	人类β-血球蛋白 5' UTR
33	人类β-血球蛋白 3' UTR
34	人类α-血球蛋白5' UTR
35	人类α-血球蛋白3' UTR
36	有爪蟾蜍β-血球蛋白5' UTR
37	有爪蟾蜍β-血球蛋白 3' UTR
38	牛生长激素5' UTR
39	牛生长激素3' UTR
40	小家鼠血红素α，成年链1 (Hba-a1)，3' UTR
41	HSD17B4 5' UTR
42	靶向小鼠TTR 基因的G282单一引导RNA
43	具有HSD的5' UTR、对应于SEQ ID NO: 4的ORF、Kozak序列及ALB的3' UTR的Cas9转录物
44	具有HSD的5' UTR、对应于SEQ ID NO: 4的ORF及ALB的3' UTR的Cas9转录物
45	具有19.36% U含量的替代Cas9 ORF
46	具有HSD的5' UTR、对应于SEQ ID NO: 45的ORF、Kozak序列及ALB的3' UTR的Cas9转录物
47	具有HSD的5' UTR、对应于SEQ ID NO: 45的ORF及ALB的3' UTR的Cas9转录物
48	包含使用通常在人类中高度表达的密码子的Cas9 ORF的Cas9转录物
49	包含Kozak序列以及使用通常在人类中高度表达的密码子的Cas9 ORF的Cas9转录物
50	剪接接点经移除；具有12.75% U含量的Cas9 ORF

[0109]

51	具有HSD的5' UTR、对应于SEQ ID NO: 50的ORF、Kozak序列及ALB的3' UTR的Cas9转录物
52	具有一般经常用于人类中的最小尿苷密码子；具有12.75% U含量的Cas9 ORF
53	具有HSD的5' UTR、对应于SEQ ID NO: 52的ORF、Kozak序列及ALB的3' UTR的Cas9转录物
54	具有一般不经常用于人类中的最小尿苷密码子；具有12.75% U含量的Cas9 ORF
55	具有HSD的5' UTR、对应于SEQ ID NO: 54的ORF、Kozak序列及ALB的3' UTR的Cas9转录物
56	具有AGG作为供与CleanCap™一起使用之前三个核苷酸、HSD的5' UTR、对应于SEQ ID NO: 4的ORF、Kozak序列及ALB的3' UTR的Cas9转录物
57	具有来自CMV的5' UTR、对应于SEQ ID NO: 4的ORF、Kozak序列及ALB的3' UTR的Cas9转录物
58	具有来自HBB的5' UTR、对应于SEQ ID NO: 4的ORF、Kozak序列及HBB的3' UTR的Cas9转录物
59	具有来自XBG的5' UTR、对应于SEQ ID NO: 4的ORF、Kozak序列及XBG的3' UTR的Cas9转录物
60	具有AGG作为供与CleanCap™一起使用之前三个核苷酸、来自XBG的5' UTR、对应于SEQ ID NO: 4的ORF、Kozak序列及XBG的3' UTR的Cas9转录物
61	具有AGG作为供与CleanCap™一起使用之前三个核苷酸、来自HSD的5' UTR、对应于SEQ ID NO: 4的ORF、Kozak序列及ALB的3' UTR的Cas9转录物
62	30/30/39 聚-A序列
63	聚A 100序列
64	靶向小鼠TTR 基因的G209单一引导RNA
65	使用如表3中所列的最小尿苷密码子编码脑膜炎双球菌Cas9的，

[0110]

	具有起始和终止密码子的的ORF
66	使用如表3中所列的最小尿苷密码子编码脑膜炎双球菌Cas9的ORF (无起始或终止密码子;适于包括在融合蛋白质编码序列中)
67	包含SEQ ID NO: 65 (编码脑膜炎双球菌Cas9)的转录物
68	脑膜炎双球菌Cas9的氨基酸序列
69	靶向大鼠TTR 基因的G390单一引导RNA
70	靶向食蟹猕猴TTR 基因的G502单一引导RNA
71	靶向食蟹猕猴TTR 基因的G509单一引导RNA
72	靶向大鼠TTR 基因的G534 单一引导RNA
73	eGFP的DNA编码序列
74	经修饰的 sgRNA模式
75	CMV-1 5' UTR
76	CMV-2 5' UTR
77	CMV-3 5' UTR
78	SV40 NLS
79	示例性NLS 1
80	示例性NLS 2
81	示例性NLS 3
82	示例性NLS 4
83	示例性NLS 5
84	示例性NLS 6
85	示例性NLS 7
86	示例性NLS 8
87	示例性NLS 9
88	示例性NLS 10
89	示例性NLS 11
90	替代SV40 NLS
91	核质蛋白NLS
92	SV40 NLS的示例性编码序列
93	NLS1的示例性编码序列

[0111]

94	NLS2的示例性编码序列
95	NLS3的示例性编码序列
96	NLS4的示例性编码序列
97	NLS5的示例性编码序列
98	NLS6的示例性编码序列
99	NLS7的示例性编码序列
100	NLS8的示例性编码序列
101	NLS9的示例性编码序列
102	NLS10的示例性编码序列
103	NLS11的示例性编码序列
104	替代SV40 NLS的示例性编码序列
105	示例性Kozak序列
107	使用表4的长半衰期密码子，具有起始及终止密码子的Cas9 ORF
108	使用表4的富含U的密码子，具有起始及终止密码子的Cas9 ORF
109	使用表4的低G密码子，具有起始及终止密码子的Cas9 ORF
110	使用表4的低C密码子，具有起始及终止密码子的Cas9 ORF
111	使用表4的低A密码子，具有起始及终止密码子的Cas9 ORF
112	使用表4的低A/U密码子，具有起始及终止密码子的Cas9 ORF
113	使用表4的低A密码子，具有两个C端NLS序列及起始及终止密码子的Cas9 ORF
114	使用表4的低A密码子，具有起始及终止密码子的Cas9切口酶ORF
115	使用表4的低A密码子，具有起始及终止密码子且无NLS的Cas9切口酶ORF
116	使用表4的低A密码子，具有两个C端NLS序列及起始及终止密码子的Cas9切口酶ORF
117	使用表4的低A密码子，具有起始及终止密码子的dCas9 ORF
118	使用表4的低A密码子，具有起始及终止密码子且无NLS的dCas9 ORF

[0112]

119	使用表4的低A密码子,具有两个C端NLS序列及起始及终止密码子的dCas9 ORF
120	使用表4的低A/U密码子,具有两个C端NLS序列及起始及终止密码子的Cas9 ORF
121	使用表4的低A/U密码子，具有起始及终止密码子且无NLS的Cas9 ORF
122	使用表4的低A/U密码子，具有起始及终止密码子的Cas9切口酶ORF
123	使用表4的低A/U密码子,具有两个C端NLS序列及起始及终止密码子的Cas9切口酶ORF
124	使用表4的低A/U密码子，具有起始及终止密码子且无NLS的Cas9切口酶ORF
125	使用表4的低A/U密码子，具有起始及终止密码子的dCas9 ORF
126	使用表4的低A/U密码子,具有两个C端NLS序列及起始及终止密码子的dCas9 ORF
127	使用表4的低A/U密码子，具有起始及终止密码子且无NLS的dCas9 ORF
128	使用表4的低A密码子,具有起始及终止密码子的Nme Cas9 ORF
129	使用表4的低A/U密码子，具有起始及终止密码子的Nme Cas9 ORF
130	具有NLS1的Cas9的开放阅读框架，其具有起始及终止密码子
131	具有NLS2的Cas9的开放阅读框架，其具有起始及终止密码子
132	具有NLS3的Cas9的开放阅读框架，其具有起始及终止密码子
133	具有NLS4的Cas9的开放阅读框架，其具有起始及终止密码子
134	具有NLS5的Cas9的开放阅读框架，其具有起始及终止密码子
135	具有NLS6的Cas9的开放阅读框架，其具有起始及终止密码子
136	具有NLS7的Cas9的开放阅读框架，其具有起始及终止密码子
137	具有NLS8的Cas9的开放阅读框架，其具有起始及终止密码子
138	具有NLS9的Cas9的开放阅读框架，其具有起始及终止密码子
139	具有NLS10的Cas9的开放阅读框架，其具有起始及终止密码子

[0113]

140	具有NLS11的Cas9的开放阅读框架，其具有起始及终止密码子
141	使用通常在人类中高度表达的密码子的Cas9 ORF (无起始或终止密码子；适于包括在融合蛋白质编码序列中)
142	使用表4的长半衰期密码子的Cas9 ORF (无起始或终止密码子；适于包括在融合蛋白质编码序列中)
143	使用表4的富含U的密码子的Cas9 ORF (无起始或终止密码子；适于包括在融合蛋白质编码序列中)
144	使用表4的低G密码子的Cas9 ORF (无起始或终止密码子；适于包括在融合蛋白质编码序列中)
145	使用表4的低C密码子的Cas9 ORF (无起始或终止密码子；适于包括在融合蛋白质编码序列中)
146	使用表4的低A密码子的Cas9 ORF (无起始或终止密码子；适于包括在融合蛋白质编码序列中)
147	使用表4的低A/U密码子的Cas9 ORF (无起始或终止密码子；适于包括在融合蛋白质编码序列中)
148	使用表4的低A密码子，具有两个C端NLS序列的Cas9 ORF (无起始或终止密码子；适于包括在融合蛋白质编码序列中)
149	使用表4的低A密码子的Cas9切口酶ORF (无起始或终止密码子；适于包括在融合蛋白质编码序列中)
150	使用表4的低A密码子的Cas9切口酶ORF (无NLS且无起始或终止密码子；适于包括在融合蛋白质编码序列中)
151	使用表4的低A密码子，具有两个C端NLS序列的Cas9切口酶ORF (无起始或终止密码子；适于包括在融合蛋白质编码序列中)
152	使用表4的低A密码子的dCas9 ORF (无起始或终止密码子；适于包括在融合蛋白质编码序列中)
153	使用表4的低A密码子的dCas9 ORF (无NLS且无起始或终止密码子；适于包括在融合蛋白质编码序列中)
154	使用表4的低A密码子，具有两个C端NLS序列的dCas9 ORF (无起始或终止密码子；适于包括在融合蛋白质编码序列中)

[0114]

155	使用表4的低A/U密码子，具有两个C端NLS序列的Cas9 ORF (无起始或终止密码子；适于包括在融合蛋白质编码序列中
156	使用表4的低A/U密码子的Cas9 ORF (无NLS且无起始或终止密码子；适于包括在融合蛋白质编码序列中)
157	使用表4的低A/U密码子的Cas9切口酶ORF (无起始或终止密码子；适于包括在融合蛋白质编码序列中)
158	使用表4的低A/U密码子，具有两个C端NLS序列的Cas9切口酶ORF (无起始或终止密码子;适于包括在融合蛋白质编码序列中)
159	使用表4的低A/U密码子的Cas9切口酶ORF (无NLS且无起始或终止密码子；适于包括在融合蛋白质编码序列中)
160	使用表4的低A/U密码子的dCas9 ORF (无起始或终止密码子；适于包括在融合蛋白质编码序列中)
161	使用表4的低A/U密码子，具有两个C端NLS序列的dCas9 ORF (无起始或终止密码子；适于包括在融合蛋白质编码序列中)
162	使用表4的低A/U密码子的dCas9 ORF (无NLS且无起始或终止密码子；适于包括在融合蛋白质编码序列中)
163	使用表4的低A密码子的Nme Cas9 ORF (无起始或终止密码子；适于包括在融合蛋白质编码序列中)
164	使用表4的低A/U密码子的Nme Cas9 ORF (无起始或终止密码子；适于包括在融合蛋白质编码序列中)
165	具有NLS1的Cas9的开放阅读框架(无起始或终止密码子；适于包括在融合蛋白质编码序列中)
166	具有NLS2的Cas9的开放阅读框架(无起始或终止密码子；适于包括在融合蛋白质编码序列中)
167	具有NLS3的Cas9的开放阅读框架(无起始或终止密码子；适于包括在融合蛋白质编码序列中)
168	具有NLS4的Cas9的开放阅读框架(无起始或终止密码子；适于包括在融合蛋白质编码序列中)
169	具有NLS5的Cas9的开放阅读框架(无起始或终止密码子；适于包

[0115]

	括在融合蛋白质编码序列中)
170	具有NLS6的Cas9的开放阅读框架(无起始或终止密码子；适于包括在融合蛋白质编码序列中)
171	具有NLS7的Cas9的开放阅读框架(无起始或终止密码子；适于包括在融合蛋白质编码序列中)
172	具有NLS8的Cas9的开放阅读框架(无起始或终止密码子；适于包括在融合蛋白质编码序列中)
173	具有NLS9的Cas9的开放阅读框架(无起始或终止密码子；适于包括在融合蛋白质编码序列中)
174	具有NLS10的Cas9的开放阅读框架(无起始或终止密码子；适于包括在融合蛋白质编码序列中)
175	具有NLS11的Cas9的开放阅读框架(无起始或终止密码子；适于包括在融合蛋白质编码序列中)
176	具有XBG UTR及含有表4的低U 1密码子的Cas9 ORF的mRNA转录物
177	具有XBG UTR及含有表4的低A密码子的Cas9 ORF的mRNA转录物
178	具有XBG UTR及含有表4的低U/A密码子的Cas9 ORF的mRNA转录物
179	具有编码带有HiBiT标记标签的Cas9的ORF、HSD 5' UTR及人类ALB 3' UTR的mRNA转录物
180	具有编码带有HiBiT标签的Cas9的ORF、CMV-1 5' UTR及人类ALB 3' UTR的mRNA转录物
181	具有编码带有HiBiT标签的Cas9的ORF、CMV-2 5' UTR及人类ALB 3' UTR的mRNA转录物
182	具有编码带有HiBiT标签的Cas9的ORF、CMV-3 5' UTR及人类ALB 3' UTR的mRNA转录物
183	具有编码带有HiBiT标签的Cas9的ORF、HBA 5' UTR及人类ALB 3' UTR的mRNA转录物

[0116]

184	具有编码带有HiBiT标签的Cas9的ORF、HBB 5' UTR及人类ALB 3' UTR的mRNA转录物
185	具有编码带有HiBiT标签的Cas9的ORF、XBG 5' UTR及人类ALB 3' UTR的mRNA转录物
186	具有NLS1的Cas9的氨基酸序列
187	具有NLS2的Cas9的氨基酸序列
188	具有NLS3的Cas9的氨基酸序列
189	具有NLS4的Cas9的氨基酸序列
190	具有NLS5的Cas9的氨基酸序列
191	具有NLS6的Cas9的氨基酸序列
192	具有NLS7的Cas9的氨基酸序列
193	具有NLS8的Cas9的氨基酸序列
194	具有NLS9的Cas9的氨基酸序列
195	具有NLS10的Cas9的氨基酸序列
196	具有NLS11的Cas9的氨基酸序列
197	靶向TTR的G506引导RNA
198	靶向TTR的G510引导RNA

[0117] 对于序列本身,参见下文序列表。转录物序列一般包括GGG作为供与ARCA一起使用之前三个核苷酸,或包括AGG作为供与CleanCap™一起使用之前三个核苷酸。因此,可对前三个核苷酸进行修饰以供与其它加帽结构方法,诸如牛痘加帽结构酶一起使用。启动子及聚-A序列不包括于转录物序列中。启动子,诸如T7启动子(SEQ ID NO:31)及聚-A序列,诸如SEQ ID NO:62或63可在5′及3′端处分别附接于所揭示的转录物序列。大多数核苷酸序列以DNA形式提供,但可通过将Ts变成Us而容易转化成RNA。

[0118] 附图简述

[0119] 图1A至图1D显示以0.5或1mg/kg(mpk)施用PBS或脂质纳米颗粒(LNP)调配物LNP417至LNP421后的IFN α、IL-6、TNF α及MCP-1的含量。

[0120] 图2A至图2B显示以0.5或1mpk施用PBS或LNP调配物LNP417至LNP421后的血清TTR含量及肝脏编辑百分比。

[0121] 图3显示由Cas9 DNA构建体进行的转录的活体外转录(IVT)产量。用未经修饰的尿苷-5′-三磷酸(UTP)或单独的(横轴上,0)、用与指定比例的5-甲氧基UTP混合(横轴上,20至80)的或与100%5-甲氧基UTP混合(100)的N1-甲基-假UTP进行转录。对于各组的三个条形图,左侧条形图使用N1-甲基-假UTP及/或5-甲氧基UTP及SEQ ID NO:2;中间的条形图使用未经修饰的UTP及/或5-甲氧基UTP及SEQ ID NO:2;且右侧条形图使用未经修饰的UTP及/或5-甲氧基UTP及SEQ ID NO:1。

[0122] 图4显示根据Cas9(SEQ ID NO:2)及经最佳化的Cas9(SEQ ID NO:1)DNA构建体的

活体外转录(IVT)结果的mRNA的纯度。由SEQ ID NO:2的Cas9序列用未经修饰的尿苷-5′-三磷酸(UTP)(正方形)或用单独(0)的或与指定比例的5-甲氧基UTP混合(20至80)的或与100％5-甲氧基UTP混合(100)的N1-甲基-假UTP(黑色圆形)进行转录。由SEQ ID NO:1的Cas9序列(浅色圆形)用未经修饰的UTP(0)或与指定比例的5-甲氧基UTP混合(20至80)或与100％5-甲氧基UTP混合(100)的未经修饰的UTP进行转录。各编码序列包括一个核定位信号。

[0123] 图5A至图5D显示抗dsRNA抗体墨点分析法结果。结果是伴随双股RNA对照(A)、在UTP及/或5-甲氧基UTP存在下转录的Cas9(B)、在UTP及/或5-甲氧基UTP存在下转录的包含SEQ ID NO:4的Cas9 mRNA序列(C)及在N1-甲基-假UTP及/或5-甲氧基UTP存在下转录的Cas9(D)而产生。图(B)至图(D)是用含有0％至100％5-甲氧基UTP及100％至0％UTP或N1-甲基UTP进行。

[0124] 图6A及图6B显示用Cas9 mRNA处理的神经母细胞瘤2A细胞(Neuro 2A cell)中的mRNA的活体外编辑效率,其呈现为编辑百分比(A)或编辑EC50(B)。评定增加Cas9 mRNA中5-甲氧基-UTP的浓度的影响。由SEQ ID NO:2的Cas9序列用N1-甲基-假UTP(A中的左组;B中的深色圆形)或用单独(0)的或与指定比例的5-甲氧基UTP混合(20至80)或与100％5-甲氧基UTP混合(100)的未经修饰的尿苷-5′-三磷酸(UTP)(A中的中间组;B中的正方形)进行转录。由SEQ ID NO:1的Cas9序列(A中的右组;B中的浅色圆形)用未经修饰的UTP(0)或与指定比例的5-甲氧基UTP混合(20至80)或与100％5-甲氧基UTP混合(100)的未经修饰的UTP进行转录。各编码序列包括一个核定位信号。

[0125] 图7A至图7D展示LNP调配物LNP720至LNP724给药后4小时时的血清细胞介素含量。图7A中的星号指示至少一个独立测量小于侦测极限。

[0126] 图8A及图8B展示用LNP调配物LNP720至LNP724给药后7天时的血清TTR含量(A)及肝脏中的TTR编辑百分比(B)。图8A中的星号指示至少一个独立测量小于侦测极限。

[0127] 图9显示以1mpk用LNP调配物LNP720至LNP724给药后7天时的脾脏中的TTR编辑百分比。

[0128] 图10显示使用LNP调配物LNP720至LNP724及LNP685的初级小鼠肝细胞(PMH)中的TTR编辑百分比。

[0129] 图11A及图11B显示投配包含Cas9 mRNA的调配物之后的血清TTR含量,在该Cas9 mRNA中,ORF具有SEQ ID NO:5或4的序列。TTR数据呈现为血清含量(A)或相对于经TSS处理的动物中的TTR含量的百分比(B)。

[0130] 图12显示以5mpk或2mpk投配包含Cas9 mRNA的调配物之后的肝脏中的TTR编辑百分比,在该Cas9 mRNA中,ORF具有SEQ ID NO:5或4的序列。

[0131] 图13A至图13E显示投配指定LNP调配物之后的血清TTR含量及肝脏中的TTR编辑百分比。

[0132] 图14显示用0.3、1、3或10ng LNP815-821、823或824处理的初级小鼠肝细胞(PMH)中的TTR编辑百分比。

[0133] 图15A至图15B显示以指定引导物:Cas9比率及量投配含有Cas9 mRNA的LNP调配物之后的血清TTR含量,在该Cas9 mRNA中,ORF具有SEQ ID NO:5或4的序列。

[0134] 图16A至图16B显示以指定引导物:Cas9比率及量投配含有Cas9 mRNA的LNP调配物

之后的肝脏中的TTR编辑百分比，在该Cas9 mRNA中，ORF具有SEQ ID NO:5或4的序列。

[0135] 图17A至图17B显示以指定引导物:Cas9比率及量投配含有Cas9 mRNA的LNP调配物之后的脾脏中的TTR编辑百分比，在该Cas9 mRNA中，ORF具有SEQ ID NO:5或4的序列。

[0136] 图18显示针对以指定引导物:Cas9比率投配含有Cas9 mRNA的LNP调配物之后的肝脏中的Cas9表达的蛋白质印迹法，在该Cas9 mRNA中，ORF具有SEQ ID NO:5或4的序列。

[0137] 图19A至图19B显示以指定量投配指定LNP调配物之后的血清TTR含量。

[0138] 图20显示以指定量投配指定LNP调配物之后的肝脏中的TTR编辑百分比。

[0139] 图21A至图21C显示以指定量投配指定LNP调配物之后的肝脏编辑含量(A)及血清TTR(B以μg/ml为单位；C呈TSS对照的百分比形式)。

[0140] 图22A至22D显示以指定比率及量投配LNP调配物之后的血清TTR及编辑结果。

[0141] 图23显示用Cas9 mRNA处理之后的Hep2G细胞中的Cas9蛋白质表达，在该Cas9 mRNA中，ORF具有指定SEQ ID NO的序列。

[0142] 图24显示以指定浓度用Cas9 mRNA处理之后的Hep2G细胞中的编辑百分比，在该Cas9 mRNA中，ORF具有指定SEQ ID NO的序列。

[0143] 图25显示投配具有Cas9 mRNA的LNP调配物之后的肝脏中的Cas9表达，在该Cas9 mRNA中，ORF具有指定SEQ ID NO的序列。

[0144] 图26显示投配具有Cas9 mRNA的LNP调配物之后的TTR基因座处的活体内编辑结果，在该Cas9 mRNA中，ORF具有指定SEQ ID NO的序列。

[0145] 图27A至图27B显示投配具有Cas9 mRNA的LNP调配物之后的血清TTR(A)及血清TTR(TSS％)(B)，在该Cas9 mRNA中，ORF具有指定SEQ ID NO的序列。

[0146] 图28显示以指定量投配具有Cas9 mRNA的LNP调配物之后的活体内肝脏编辑，在该Cas9 mRNA中，ORF具有指定SEQ ID NO的序列。

[0147] 图29A至图29B显示以指定量投配具有Cas9 mRNA的LNP调配物之后的血清TTR含量(A)及血清TTR(TSS％)(B)，在该Cas9 mRNA中，ORF具有指定SEQ ID NO的序列。

[0148] 图30A至图30B显示投配具有Cas9 mRNA的LNP调配物之后的血清TTR含量(A)及肝脏中的编辑％(B)，在该Cas9 mRNA中，转录物具有指定SEQ ID NO的序列。

[0149] 图31显示以指定剂量投配经mRNA调配的LNP之后的肝脏中的TTR编辑百分比，该mRNA具有指定帽结构及转录物序列。

[0150] 图32显示以指定剂量投配经mRNA调配的LNP之后的血清TTR含量，该mRNA具有指定帽结构及转录物序列。

[0151] 图33显示投配经编码Cas9的mRNA调配的LNP之后的肝脏中的TTR编辑百分比，在该mRNA中，ORF具有指定SEQ ID NO的序列，包括如所指示的NLS。

[0152] 图34A至图34B显示投配经编码Cas9的mRNA调配的LNP之后的血清TTR含量(A)及血清TTR(TSS％)(B)，在该mRNA中，ORF具有指定SEQ ID NO的序列，包括如所指示的NLS。

[0153] 图35显示投配经编码Cas9且包括不同类别及活性含量的NLS序列的mRNA调配的LNP之后的NLS活性与编辑效率的相关性。

[0154] 图36显示HepG2细胞中来自具有指定序列及如所指示的5′UTR的mRNA转录物的Cas9蛋白质的表达水平。

[0155] 实施方式

[0156] 现将详细参考本发明的某些实施例,其实例在附图中加以说明。尽管本发明将结合所说明的实施例描述,但应理解其并不意欲将本发明限于这些实施例。相反,本发明意欲涵盖所有替代方案、修改及等效物,其可如所附权利要求所限定包括在本发明内。

[0157] 在详细描述本发明教示内容之前,应理解,本发明不限于特定组合物或方法步骤,因而可加以改变。应注意,除非上下文另外明确规定,否则如本说明书及所附权利要求中所用,单数形式"一(a/an)"及"该(the)"包括复数个所指物。因此,例如,提及"结合物"包括复数个结合物且提及"细胞"包括复数个细胞及其类似者。

[0158] 数值范围包括界定该范围的数字。考虑到有效数字及与测量相关的误差,测量及可测量值应理解为大致的。此外,"包含(comprise/comprises/comprising)"、"含有(contain/contains/containing)"及"包括(include/includes/including)"的使用并不意欲为限制性的。应理解,前述一般描述及详细描述仅为示例性及解释性的且并不限制教示内容。

[0159] 术语"约"或"大约"意谓如由一般熟习此项技术者所测定的特定值的可接受的误差,其在某种程度上视如何测量或测定该值、或变化程度不会实质上影响所述标的物的特性(例如在10%、5%、2%或1%内)而定。因此,除非有相反指示,否则以下说明书及所附权利要求中所阐述的数值参数为可视设法获得的所需特性而变化的近似值。至少,且不试图将均等论的应用限于权利要求的范畴,各数值参数至少应根据所报导的有效数字的数目且通过应用普通舍入技术来解释。

[0160] 除非以上说明书中具体指出,否则本说明书中叙述"包含"各种组分的实施例亦设想为"由所述组分组成"或"基本上由所述组分组成";本说明书中叙述"由各种组分组成"的实施例亦设想为"包含"所述组分或"基本上由所述组分组成";且本说明书中叙述"基本上由各种组分组成"的实施例亦设想为"由所述组分组成"或"包含"所述组分(此互换性并不适用于此等术语在权利要求中的使用)。

[0161] 本文所用的章节标题仅出于组织目的而不应解释为以任何方式限制所需标的物。在以引用的方式并入的任何文献与本说明书的表述内容(包括(但不限于)定义)相矛盾的情况下,以本说明书的表述内容为准。虽然本教示内容是与多个实施例结合描述,但并不意欲将本教示内容限制于该实施例。相反,如熟习此项技术者将了解,本教示内容涵盖各种替代方案、修改及等效物。

[0162] A.定义

[0163] 除非另外说明,否则如本文所用的以下术语及片语意欲具有以下含义:

[0164] 如本文所用,术语"或其组合"是指在该术语前面所列项的所有排列及组合。例如,"A、B、C或其组合"意欲包括以下中的至少一者:A、B、C、AB、AC、BC或ABC,且若在特定情况下顺序为重要的,则亦包括BA、CA、CB、ACB、CBA、BCA、BAC或CAB。继续此实例,明确地包括含有一或多个项或条项的重复的组合,诸如BB、AAA、AAB、BBC、AAABCCCC、CBBAAA、CABABB等。熟习此项技术者应理解,除非另外自上下文显而易知,否则通常不存在对任何组合中的项目或术语数目的限制。

[0165] 如本文所用,术语"试剂盒"是指相关组分,诸如一或多种多核苷酸或组合物及一或多种相关材料,诸如递送装置(例如注射器)、溶剂、溶液、缓冲剂、说明书或干燥剂的封装组。

[0166] 除非本文另有规定,否则“或”是依包括性意义使用,即,等效于“及/或”。

[0167] “多核苷酸”及“核酸”在本文中用于指代包含核苷或具有沿主链连接在一起的含氮杂环碱基或碱基类似物的核苷类似物的多聚化合物,其包括习知RNA、DNA、混合RNA-DNA及为其类似物的聚合物。核酸“主链”可由多个键组成,其包括糖-磷酸二酯键、肽-核酸键(“肽核酸”或PNA;PCT第WO 95/32305号)、硫代磷酸酯键、膦酸甲酯键或其组合中的一种或多种。核酸的糖部分可为核糖、脱氧核糖或具有取代,例如2′甲氧基或2′卤基取代的类似化合物。含氮碱基可为习知碱基(A、G、C、T、U);其类似物(例如经修饰的尿苷,诸如5-甲氧基尿苷、假尿苷或N1-甲基假尿苷或其它);肌核苷;嘌呤或嘧啶的衍生物(例如N^4-甲基脱氧鸟苷、脱氮或氮杂嘌呤、脱氮或氮杂嘧啶、在5或6位处具有取代基的嘧啶碱基(例如5-甲基胞嘧啶)、在2、6或8位处具有取代基的嘌呤碱基、2-胺基-6-甲胺基嘌呤、O^6-甲基鸟嘌呤、4-硫基-嘧啶、4-胺基-嘧啶、4-二甲基肼-嘧啶及O^4-烷基-嘧啶;美国专利第5,378,825号及PCT第WO 93/13121号)。对于一般论述,参见The Biochemistry of the Nucleic Acids 5-36,Adams等人编,第11版,1992。核酸可包括一或多个“无碱基”残基,其中主链不包括针对聚合物位置的含氮碱基(美国专利第5,585,481号)。核酸可仅包含习知RNA或DNA糖、碱基及键,或可包括习知组分及取代两者(例如具有2′甲氧基键的习知碱基或含有习知碱基及一或多个碱基类似物两者的聚合物)。核酸包括“锁核酸”(LNA),一种含有一或多种LNA核苷酸单体的类似物,该单体具有模拟糖构象的锁定于RNA中的双环呋喃糖单元,其会增强对互补RNA及DNA序列的杂交亲和性(Vester及Wengel,2004,Biochemistry 43(42):13233-41)。RNA及DNA具有不同糖部分且可通过在RNA中存在尿嘧啶或其类似物及在DNA中存在胸腺嘧啶或其类似物而有所不同。

[0168] “经修饰的尿苷”在本文中用于指代除胸苷外的具有与尿苷相同的氢键受体且与尿苷存在一或多种结构性差异的核苷。在一些实施例中,经修饰的尿苷为经取代的尿苷,即其中一或多个非质子取代(例如烷氧基,诸如甲氧基)代替质子的尿苷。在一些实施例中,经修饰的尿苷为假尿苷。在一些实施例中,经修饰的尿苷为经取代的假尿苷,即其中一或多个非质子取代(例如烷基,诸如甲基)代替质子的假尿苷。在一些实施例中,经修饰的尿苷为经取代的尿苷、假尿苷或经取代的假尿苷中的任一个。

[0169] 如本文所用,“尿苷位置”是指多核苷酸中由尿苷或经修饰的尿苷占据的位置。因此,例如,其中“100%的尿苷位置为经修饰的尿苷”的多核苷酸在相同序列的习知RNA(其中所有碱基均为标准A、U、C或G碱基)中应为尿苷的每个位置处均含有经修饰的尿苷。除非另外指明,否则本发明中或附随本发明的序列表(sequence table/sequence listing)的多核苷酸序列中的U可为尿苷或经修饰的尿苷。

[0170] 如本文所用,若第一序列与第二序列的比对显示整个第二序列的X%或大于X%的位置与第一序列相匹配,则该第一序列视为“包含与第二序列具有至少X%一致性的序列”。例如,序列AAGA包含与序列AAG具有100%一致性的序列,因为与第二序列的全部三个位置均出现匹配,所以比对结果为100%一致性。RNA与DNA之间的差异(通常而言,尿苷更换为胸苷或反之亦然)及核苷类似物(诸如经修饰的尿苷)的存在不会造成多核苷酸之间一致性或互补性的差异,只要相关核苷酸(诸如胸苷、尿苷或经修饰的尿苷)具有相同互补序列(例如针对胸苷、尿苷或经修饰的尿苷均为腺苷;另一实例为胞嘧啶及5-甲基胞嘧啶,这两者均以鸟苷作为互补序列)即可。因此,例如,序列5′-AXG(其中X为任何经修饰的尿苷,诸如假尿

苷、N1-甲基假尿苷或5-甲氧基尿苷)视为与AUG具有100％一致性,因为两者与同一序列(5′-CAU)完全互补。比对算法实例为Smith-Waterman及Needleman-Wunsch算法,其是此项技术中熟知者。熟习此项技术者应理解选择何种算法及参数设置才适合于所要比对的一对指定序列;对于具有一般类似长度及预期氨基酸有＞50％一致性或核苷酸有＞75％一致性的序列而言,由EBI于www.ebi.ac.uk网站服务器提供的Needleman-Wunsch算法接口的具有默认设置的Needleman-Wunsch算法通常为合适的。

[0171] “mRNA”在本文中用于指代非DNA且包含可转译成多肽的开放阅读框架的多核苷酸(即可作为由核糖体及氨基酰化tRNA进行转译的底物)。mRNA可包含包括核糖残基或其类似物,例如2′-甲氧基核糖残基的磷酸酯-糖主链。在一些实施例中,mRNA磷酸酯-糖主链的糖基本上由核糖残基、2′-甲氧基核糖残基或其组合组成。通常而言,mRNA不含大量胸苷残基(例如0个残基或小于30、20、10、5、4、3或2个胸苷残基;或小于10％、9％、8％、7％、6％、5％、4％、3％、2％、1％、0.5％、0.2％或0.1％的胸苷含量)。mRNA可在其一些或全部尿苷位置处含有经修饰的尿苷。

[0172] 如本文所用,“RNA引导的DNA结合剂”意谓具有RNA及DNA结合活性的多肽或多肽复合物,或此类复合物的DNA结合亚基,其中DNA结合活性具有序列特异性且视RNA的序列而定。示例性RNA引导的DNA结合剂包括Cas裂解酶/切口酶及其不活化形式(“dCas DNA结合剂”)。如本文所用,“Cas核酸酶”亦称作“Cas蛋白质”,其涵盖Cas裂解酶、Cas切口酶及dCas DNA结合剂。Cas裂解酶/切口酶及dCas DNA结合剂包括III型CRISPR系统的Csm或Cmr复合物、其Cas10、Csm1或Cmr2亚基、I型CRISPR系统的级联复合物、其Cas3亚基及2类Cas核酸酶。如本文所用,“2类Cas核酸酶”为具有经RNA引导的DNA结合活性的单链多肽,诸如Cas9核酸酶或Cpf1核酸酶。2类Cas核酸酶包括2类Cas裂解酶及2类Cas切口酶(例如H840A、D10A或N863A变异体),其进一步具有经RNA引导的DNA裂解酶或切口酶活性,及2类dCas DNA结合剂,其中裂解酶/切口酶活性未活化。2类Cas核酸酶包括例如Cas9、Cpf1、C2c1、C2c2、C2c3、HF Cas9(例如N497A、R661A、Q695A、Q926A变异体)、HypaCas9(例如N692A、M694A、Q695A、H698A变异体)、eSPCas9(1.0)(例如K810A、K1003A、R1060A变异体)及eSPCas9(1.1)(例如K848A、K1003A、R1060A变异体)蛋白质及其变体。Cpf1蛋白质(Zetsche等人,Cell,163:1-13(2015))与Cas9同源且含有类RuvC核酸酶域。Zetsche的Cpf1序列以全文引用的方式并入。参见例如Zetsche,表S1及表S3。“Cas9”涵盖Spy Cas9、本文中所列的Cas9的变异体及其等效物。参见例如,Makarova等人,Nat Rev Microbiol,13(11):722-36(2015);Shmakov等人,Molecular Cell,60:385-397(2015)。

[0173] 如本文所用,给定开放阅读框架(ORF)的“最小尿苷含量”为以下的ORF的尿苷含量:(a)每个位置处使用最小尿苷密码子及(b)编码与给定ORF相同的氨基酸序列。给定氨基酸的最小尿苷密码子为具有最少尿苷的密码子(通常0或1个,除了苯丙氨酸的密码子外,其中最小尿苷密码子具有2个尿苷)。出于评估最小尿苷含量的目的,经修饰的尿苷残基视为等效于尿苷。

[0174] 如本文所用,给定开放阅读框架(ORF)的“最小尿苷二核苷酸含量”为以下的ORF的最低可能的尿苷二核苷酸(UU)含量:(a)在每个位置处使用最小尿苷密码子(如上文所论述)及(b)编码与给定ORF相同的氨基酸序列。尿苷二核苷酸(UU)含量在绝对意义上可表示为ORF中的UU二核苷酸的计数或基于比率,表示为尿苷二核苷酸的尿苷所占据的位置的百

分比(例如,AUUAU的尿苷二核苷酸含量为40%,因为尿苷二核苷酸的尿苷占据了5个位置中的2个)。出于评估最小尿苷二核苷酸含量的目的,经修饰的尿苷残基视为等效于尿苷。

[0175] 如本文所用,给定开放阅读框架(ORF)的"最小腺嘌呤含量"为以下的ORF的腺嘌呤含量:(a)每个位置处使用最小腺嘌呤密码子及(b)编码与给定ORF相同的氨基酸序列。给定氨基酸的最小腺嘌呤密码子为具有最少腺嘌呤的密码子(通常0或1个,除了赖氨酸及天冬酰胺的密码子外,其中最小腺嘌呤密码子具有2个腺嘌呤)。出于评估最小腺嘌呤含量的目的,经修饰的腺嘌呤残基视为等效于腺嘌呤。

[0176] 如本文所用,给定开放阅读框架(ORF)的"最小腺嘌呤二核苷酸含量"为以下的ORF的最低可能的腺嘌呤二核苷酸(AA)含量:(a)在每个位置处使用最小腺嘌呤密码子(如上文所论述)及(b)编码与给定ORF相同的氨基酸序列。腺嘌呤二核苷酸(AA)含量在绝对意义上可表示为ORF中的AA二核苷酸的计数或基于比率,表示为腺嘌呤二核苷酸的腺嘌呤所占据的位置的百分比(例如,UAAUA的腺嘌呤二核苷酸含量为40%,因为腺嘌呤二核苷酸的腺嘌呤占据了5个位置中的2个)。出于评估最小腺嘌呤二核苷酸含量的目的,经修饰的腺嘌呤残基视为等效于腺嘌呤。

[0177] "引导RNA"、"gRNA"及"引导物"在本文中互换使用来指代crRNA(也称为CRISPR RNA)或crRNA与trRNA(也称为tracrRNA)的组合任一个。crRNA及trRNA可以单一RNA分子(单引导RNA,sgRNA)或以两个独立RNA分子(双引导RNA,dgRNA)形式缔合。"引导RNA"或"gRNA"是指各类型。trRNA可为天然存在的序列或与天然存在的序列相比具有修饰或变化的trRNA序列。

[0178] 如本文所用,"引导序列"是指引导RNA中与靶序列互补且通过由RNA引导的DNA结合剂将引导RNA导引至靶序列以供结合或修饰(例如裂解)的序列。"引导序列"也可称为"靶序列"或"间隔序列"。引导序列的长度可为20个碱基对,例如在化脓链球菌(Streptococcus pyogenes)(即Spy Cas9)及相关Cas9同源物/直是同源物的情况下。较短或较长序列亦可用作引导物,例如长度为15-、16-、17-、18-、19-、21-、22-、23-、24-或25-个核苷酸。在一些实施例中,靶序列处于例如基因中或染色体上,且与引导序列互补。在一些实施例中,引导序列与其相应靶序列之间的互补性或一致性的程度可为约75%、80%、85%、90%、95%、96%、97%、98%、99%或100%。在一些实施例中,引导序列与标靶区可为100%互补或一致的。在其它实施例中,引导序列与标靶区可含有至少一个错配。例如,引导序列及靶序列可含有1、2、3或4个错配,其中靶序列的总长度为至少17、18、19、20或更多个碱基对。在一些实施例中,引导序列及标靶区可含有1至4个错配,其中引导序列包含至少17、18、19、20或更多个核苷酸。在一些实施例中,引导序列及标靶区可含有1、2、3或4个错配,其中引导序列包含20个核苷酸。

[0179] 针对Cas蛋白质的靶序列包括基因组DNA的正股及负股两者(即给定序列及该序列的反向互补序列),因为Cas蛋白质的核酸底物为双股核酸。因此,在论述引导序列"与靶序列互补"的情况下,应理解,引导序列可导引引导RNA结合至靶序列的反向互补序列。因此,在一些实施例中,在引导序列结合靶序列的反向互补序列的情况下,引导序列与靶序列(例如不包括PAM的靶序列)的某些核苷酸相同,不同的处在于引导序列中U取代T。

[0180] 如本文所用,"插入缺失标记(indel)"是指由多个核苷酸组成的插入/缺失突变,该核苷酸在核酸中的双股断裂(DSB)位点处进行插入或缺失。

[0181] 如本文所用,“基因减弱(knockdown)”是指特定基因产物(例如蛋白质、mRNA或两者)的表达降低。可通过侦测由组织或细胞群(例如在血清或细胞培养基中)分泌的蛋白质或通过侦测来自相关组织或细胞群的蛋白质的总细胞量来测量蛋白质的基因减弱。用于测量mRNA的基因减弱的方法为已知的,且包括对自相关组织或细胞群分离的mRNA进行测序。在一些实施例中,“基因减弱”可指代特定基因产物表达有一些损失,例如经转录的mRNA的量下降或由细胞群(包括活体内细胞群,诸如这些出现在组织中者)表达或分泌的蛋白质的量下降。

[0182] 如本文所用,“基因剔除”是指丧失细胞中特定蛋白质的表达。可通过侦测由组织或细胞群(例如在血清或细胞培养基中)分泌的蛋白质的量或通过侦测组织或细胞群中蛋白质的总细胞量来测量基因剔除。在一些实施例中,本发明方法在一或多种细胞(例如在细胞群,包括活体内细胞群,诸如这些出现在组织中者)“基因剔除”靶蛋白。在一些实施例中,基因剔除并非例如通过插入缺失标记(indel)导致形成突变靶蛋白,而是细胞中完全丧失靶蛋白的表达。

[0183] 如本文所用,“核糖核蛋白”(RNP)或“RNP复合物”是指引导RNA以及RNA引导的DNA结合剂,诸如Cas裂解酶、切口酶或dCas DNA结合剂(例如Cas9)。在一些实施例中,引导RNA将RNA引导的DNA结合剂,诸如Cas9导引至靶序列,且该引导RNA与靶序列杂交且该结合剂结合于靶序列;在结合剂为裂解酶或切口酶的情况下,结合之后进行裂解或切口。

[0184] 如本文所用,“靶序列”是指靶基因中与gRNA的引导序列互补的核酸序列。靶序列与引导序列的相互作用导引RNA引导的DNA结合剂结合,且在靶序列中潜在地链裂或裂解(视试剂活性而定)。

[0185] 如本文所用,“治疗”是指用于个体的疾病或病症的治疗剂的任何施用或施用,且包括抑制疾病、遏制其发展、缓解疾病的一或多种症状、治愈疾病或预防疾病的一或多种症状的复发。

[0186] B.示例性多核苷酸及组合物

[0187] 1.具有低尿苷含量的mRNA及ORF

[0188] 在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷含量在其最小尿苷含量至其最小尿苷含量的约150％范围内。在一些实施例中,ORF的尿苷含量小于或等于其最小尿苷含量的约145％、140％、135％、130％、125％、120％、115％、110％、105％、104％、103％、102％或101％。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷含量等于其最小尿苷含量。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷含量小于或等于其最小尿苷含量的约150％。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷含量小于或等于其最小尿苷含量的约145％。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷含量小于或等于其最小尿苷含量的约140％。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷含量小于或等于其最小尿苷含量的约135％。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷含量小于或等于其最小尿苷含量的约130％。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的

DNA结合剂的mRNA,该ORF的尿苷含量小于或等于其最小尿苷含量的约125%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷含量小于或等于其最小尿苷含量的约120%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷含量小于或等于其最小尿苷含量的约115%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷含量小于或等于其最小尿苷含量的约110%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷含量小于或等于其最小尿苷含量的约105%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷含量小于或等于其最小尿苷含量的约104%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷含量小于或等于其最小尿苷含量的约103%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷含量小于或等于其最小尿苷含量的约102%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷含量小于或等于其最小尿苷含量的约101%。

[0189] 在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量在其最小尿苷二核苷酸含量至其最小尿苷二核苷酸含量的200%范围内。在一些实施例中,ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约195%、190%、185%、180%、175%、170%、165%、160%、155%、150%、145%、140%、135%、130%、125%、120%、115%、110%、105%、104%、103%、102%或101%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量等于其最小尿苷二核苷酸含量。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约200%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约195%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约190%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约185%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约180%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约175%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约170%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约165%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约160%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约

155%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量等于其最小尿苷二核苷酸含量。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约150%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约145%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约140%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约135%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约130%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约125%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约120%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约115%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约110%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约105%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约104%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约103%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约102%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量小于或等于其最小尿苷二核苷酸含量的约101%。

[0190] 在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷二核苷酸含量在其最小尿苷二核苷酸含量至呈编码与相关mRNA相同的蛋白质的参考序列的最大尿苷二核苷酸含量的90%或低于90%的尿苷二核苷酸含量范围内。在一些实施例中,ORF的尿苷二核苷酸含量小于或等于编码与相关mRNA相同的蛋白质的参考序列的最大尿苷二核苷酸含量的约85%、80%、75%、70%、65%、60%、55%、50%、45%、40%、35%、30%、25%、20%、15%、10%或5%。

[0191] 在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷三核苷酸含量在0个尿苷三核苷酸至1、2、3、4、5、6、7、8、9、10、20、30、40或50个尿苷三核苷酸范围内(其中一连串较长的尿苷计为其内特有三尿苷区段的数目,例如尿苷四核苷酸含有两个尿苷三核苷酸,尿苷五核苷酸含有三个尿苷三核苷酸等)。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF

的尿苷三核苷酸含量在0%尿苷三核苷酸至0.1%、0.2%、0.3%、0.4%、0.5%、0.6%、0.7%、0.8%、0.9%、1%、1.5%或2%尿苷三核苷酸范围内，其中尿苷三核苷酸的百分比含量计算为序列中由形成尿苷三核苷酸的一部分(或一连串较长尿苷)，以使得序列UUUAAA及UUUUAAAA将各具有50%的尿苷三核苷酸含量的尿苷所占据的位置的百分比。例如，在一些实施例中，ORF的尿苷三核苷酸含量小于或等于2%。例如，在一些实施例中，ORF的尿苷三核苷酸含量小于或等于1.5%。在一些实施例中，ORF的尿苷三核苷酸含量小于或等于1%。在一些实施例中，ORF的尿苷三核苷酸含量小于或等于0.9%。在一些实施例中，ORF的尿苷三核苷酸含量小于或等于0.8%。在一些实施例中，ORF的尿苷三核苷酸含量小于或等于0.7%。在一些实施例中，ORF的尿苷三核苷酸含量小于或等于0.6%。在一些实施例中，ORF的尿苷三核苷酸含量小于或等于0.5%。在一些实施例中，ORF的尿苷三核苷酸含量小于或等于0.4%。在一些实施例中，ORF的尿苷三核苷酸含量小于或等于0.3%。在一些实施例中，ORF的尿苷三核苷酸含量小于或等于0.2%。在一些实施例中，ORF的尿苷三核苷酸含量小于或等于0.1%。在一些实施例中，提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA，该ORF不合尿苷三核苷酸。

[0192] 在一些实施例中，提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA，该ORF的尿苷三核苷酸含量在其最小尿苷三核苷酸含量至呈编码与相关mRNA相同的蛋白质的参考序列的最大尿苷三核苷酸含量的90%或低于90%的尿苷三核苷酸含量范围内。在一些实施例中，ORF的尿苷三核苷酸含量小于或等于编码与相关mRNA相同的蛋白质的参考序列的最大尿苷三核苷酸含量的约85%、80%、75%、70%、65%、60%、55%、50%、45%、40%、35%、30%、25%、20%、15%、10%或5%。

[0193] 在一些实施例中，提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA，该ORF具有极少核苷酸均聚物，例如重复的相同核苷酸的串。例如，在一些实施例中，当自表1中所列的密码子选择最小尿苷密码子时，通过选择降低核苷酸均聚物的数量及长度的最小尿苷密码子(例如，对于丙氨酸，选择GCA代替GCC；或对于甘氨酸，选择GGA代替GGG；或对于赖氨酸，选择AAG代替AAA)来构筑mRNA。

[0194] 可例如通过在ORF的足够多的一部分中使用最小尿苷密码子来降低给定ORF的尿苷含量或尿苷二核苷酸含量或尿苷三核苷酸含量。例如，可通过将氨基酸转化成密码子而将RNA引导的DNA结合剂的氨基酸序列转译回ORF序列，其中ORF中的一些或全部使用以下所示的示例性最小尿苷密码子。在一些实施例中，ORF中至少约50%、55%、60%、65%、70%、75%、80%、85%、90%、95%、98%、99%或100%的密码子为表1中所列的密码子。

[0195] 表1.示例性最小尿苷密码子

[0196]

	氨基酸	最小尿苷密码子
A	丙氨酸	GCA或GCC或GCG
G	甘氨酸	GGA或GGC或GGG
V	缬氨酸	GUC或GUA或GUG
D	天冬氨酸	GAC
E	谷氨酸	GAA或GAG
I	异亮氨酸	AUC或AUA
T	苏氨酸	ACA或ACC或ACG
N	天冬酰胺	AAC
K	赖氨酸	AAG或AAA

[0197]

S	丝氨酸	AGC
R	精氨酸	AGA或AGG
L	亮氨酸	CUG或CUA或CUC
P	脯氨酸	CCG或CCA或CCC
H	组氨酸	CAC
Q	谷氨酰胺	CAG或CAA
F	苯丙氨酸	UUC
Y	酪氨酸	UAC
C	半胱氨酸	UGC
W	色氨酸	UGG
M	甲硫氨酸	AUG

[0198] 在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF由其中至少约75％、80％、85％、90％、95％、98％、99％或100％的密码子为表1中所列的密码子的一组密码子组成。

[0199] 2.具有低腺嘌呤含量的mRNA及ORF

[0200] 在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤含量在其最小腺嘌呤含量至其最小腺嘌呤含量的约150％范围内。在一些实施例中,ORF的腺嘌呤含量小于或等于其最小腺嘌呤含量的约145％、140％、135％、130％、125％、120％、115％、110％、105％、104％、103％、102％或101％。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌

呤含量等于其最小腺嘌呤含量。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤含量小于或等于其最小腺嘌呤含量的约150%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤含量小于或等于其最小腺嘌呤含量的约145%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤含量小于或等于其最小腺嘌呤含量的约140%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤含量小于或等于其最小腺嘌呤含量的约135%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤含量小于或等于其最小腺嘌呤含量的约130%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤含量小于或等于其最小腺嘌呤含量的约125%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤含量小于或等于其最小腺嘌呤含量的约120%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤含量小于或等于其最小腺嘌呤含量的约115%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤含量小于或等于其最小腺嘌呤含量的约110%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤含量小于或等于其最小腺嘌呤含量的约105%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤含量小于或等于其最小腺嘌呤含量的约104%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤含量小于或等于其最小腺嘌呤含量的约103%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤含量小于或等于其最小腺嘌呤含量的约102%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤含量小于或等于其最小腺嘌呤含量的约101%。

[0201] 在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量在其最小腺嘌呤二核苷酸含量至其最小腺嘌呤二核苷酸含量的200%范围内。在一些实施例中,ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约195%、190%、185%、180%、175%、170%、165%、160%、155%、150%、145%、140%、135%、130%、125%、120%、115%、110%、105%、104%、103%、102%或101%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量等于最小腺嘌呤二核苷酸含量。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约200%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约195%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约190%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约185%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA

引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约180%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约175%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约170%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约165%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约160%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约155%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量等于最小腺嘌呤二核苷酸含量。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约150%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约145%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约140%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约135%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约130%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约125%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约120%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约115%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约110%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约105%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约104%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约103%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约102%。在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的腺嘌呤二核苷酸含量小于或等于其最小腺嘌呤二核苷酸含量的约101%。

[0202] 在一些实施例中，提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA，该ORF的腺嘌呤二核苷酸含量在其最小腺嘌呤二核苷酸含量至呈编码与相关mRNA相同的蛋白质的参考序列的最大腺嘌呤二核苷酸含量的90％或低于90％的腺嘌呤二核苷酸含量范围内。在一些实施例中，ORF的腺嘌呤二核苷酸含量小于或等于编码与相关mRNA相同的蛋白质的参考序列的最大腺嘌呤二核苷酸含量约85％、80％、75％、70％、65％、60％、55％、50％、45％、40％、35％、30％、25％、20％、15％、10％或5％。

[0203] 在一些实施例中，提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA，该ORF的腺嘌呤三核苷酸含量在0个腺嘌呤三核苷酸至1、2、3、4、5、6、7、8、9、10、20、30、40或50个腺嘌呤三核苷酸范围内(其中一连串较长的腺嘌呤计为其内特有三腺嘌呤区段的数目，例如腺嘌呤四核苷酸含有两个腺嘌呤三核苷酸，腺嘌呤五核苷酸含有三个腺嘌呤三核苷酸等)。在一些实施例中，提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA，该ORF的腺嘌呤三核苷酸含量在0％腺嘌呤三核苷酸至0.1％、0.2％、0.3％、0.4％、0.5％、0.6％、0.7％、0.8％、0.9％、1％、1.5％或2％腺嘌呤三核苷酸范围内，其中腺嘌呤三核苷酸的百分比含量计算为序列中由形成腺嘌呤三核苷酸的一部分(或一连串较长腺嘌呤)，以使得序列UUUAAA及UUUUAAAA将各具有50％的腺嘌呤三核苷酸含量的腺嘌呤所占据的位置的百分比。例如，在一些实施例中，ORF的腺嘌呤三核苷酸含量小于或等于2％。例如，在一些实施例中，ORF的腺嘌呤三核苷酸含量小于或等于1.5％。在一些实施例中，ORF的腺嘌呤三核苷酸含量小于或等于1％。在一些实施例中，ORF的腺嘌呤三核苷酸含量小于或等于0.9％。在一些实施例中，ORF的腺嘌呤三核苷酸含量小于或等于0.8％。在一些实施例中，ORF的腺嘌呤三核苷酸含量小于或等于0.7％。在一些实施例中，ORF的腺嘌呤三核苷酸含量小于或等于0.6％。在一些实施例中，ORF的腺嘌呤三核苷酸含量小于或等于0.5％。在一些实施例中，ORF的腺嘌呤三核苷酸含量小于或等于0.4％。在一些实施例中，ORF的腺嘌呤三核苷酸含量小于或等于0.3％。在一些实施例中，ORF的腺嘌呤三核苷酸含量小于或等于0.2％。在一些实施例中，ORF的腺嘌呤三核苷酸含量小于或等于0.1％。在一些实施例中，提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA，该ORF不含腺嘌呤三核苷酸。

[0204] 在一些实施例中，提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA，该ORF具有极少核苷酸均聚物，例如重复的相同核苷酸的串。例如，在一些实施例中，当自表1中所列的密码子选择最小腺嘌呤密码子时，通过选择降低核苷酸均聚物的数量及长度的最小腺嘌呤密码子(例如，对于丙氨酸，选择GCA代替GCC；或对于甘氨酸，选择GGA代替GGG；或对于赖氨酸，选择AAG代替AAA)来构筑mRNA。

[0205] 在一些实施例中，提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA，该ORF的腺嘌呤三核苷酸含量在其最小腺嘌呤三核苷酸含量至呈编码与相关mRNA相同的蛋白质的参考序列的最大腺嘌呤三核苷酸含量的90％或低于90％的腺嘌呤三核苷酸含量范围内。在一些实施例中，ORF的腺嘌呤三核苷酸含量小于或等于编码与相关mRNA相同的蛋白质的参考序列的最大腺嘌呤三核苷酸含量的约85％、80％、75％、70％、65％、60％、55％、50％、45％、40％、35％、30％、25％、20％、15％、10％或5％。

[0206] 可例如通过在ORF的足够多的一部分中使用最小腺嘌呤密码子来降低给定ORF的腺嘌呤含量或腺嘌呤二核苷酸含量或腺嘌呤三核苷酸含量。例如，可通过将氨基酸转化成

密码子而将RNA引导的DNA结合剂的氨基酸序列转译回ORF序列,其中ORF中的一些或全部使用以下所示的示例性最小腺嘌呤密码子。在一些实施例中,ORF中至少约50％、55％、60％、65％、70％、75％、80％、85％、90％、95％、98％、99％或100％的密码子为表2中所列的密码子。

[0207] 表2.示例性最小腺嘌呤密码子

[0208]

	氨基酸	最小腺嘌呤密码子
A	丙氨酸	GCU或GCC或GCG
G	甘氨酸	GGU或GGC或GGG
V	缬氨酸	GUC或GUU或GUG
D	天冬氨酸	GAC或GAU
E	谷氨酸	GAG
I	异亮氨酸	AUC或AUU
T	苏氨酸	ACU或ACC或ACG
N	天冬酰胺	AAC或AAU
K	赖氨酸	AAG
S	丝氨酸	UCU或UCC或UCG
R	精氨酸	CGU或CGC或CGG
L	亮氨酸	CUG或CUC或CUU
P	脯氨酸	CCG或CCU或CCC
H	组氨酸	CAC或CAU
Q	谷氨酰胺	CAG
F	苯丙氨酸	UUC或UUU
Y	酪氨酸	UAC或UAU
C	半胱氨酸	UGC或UGU
W	色氨酸	UGG
M	甲硫氨酸	AUG

[0209]

[0210] 在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF由其中至少约75％、80％、85％、90％、95％、98％、99％或100％的密码子为表2中所列的密码子的一组密码子组成。

[0211] 3.具有低腺嘌呤及低尿苷含量的mRNA及ORF

[0212] 就可行性而言,上文关于低腺嘌呤含量所述的任一特征可与上文关于低尿苷含量所述的任一特征组合。例如,可提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF的尿苷含量在其最小尿苷含量至其最小尿苷含量的约150％范围内(例如ORF的尿苷含量小于或等于其最小尿苷含量的约145％、140％、135％、130％、125％、120％、115％、110％、105％、104％、103％、102％或101％),且该ORF的腺嘌呤含量在其最小腺嘌呤含量至其最小腺嘌呤含量的约150％范围内(例如小于或等于其最小腺嘌呤含量的约145％、140％、135％、130％、125％、120％、115％、110％、105％、104％、103％、102％或101％)。对于尿苷及腺嘌呤二核苷酸亦如此。同样,ORF中的尿苷核苷酸及腺嘌呤二核苷酸的含量可如上文所阐述。同样,ORF中的尿苷二核苷酸及腺嘌呤核苷酸的含量可如上文所阐述。

[0213] 可例如通过在ORF的足够多的一部分中使用最小尿苷及腺嘌呤密码子来降低给定ORF的尿苷及腺嘌呤核苷酸及/或二核苷酸含量。例如,可通过将氨基酸转化成密码子而将RNA引导的DNA结合剂的氨基酸序列转译回ORF序列,其中ORF中的一些或全部使用以下所示的示例性最小尿苷及腺嘌呤密码子。在一些实施例中,ORF中至少约50％、55％、60％、65％、70％、75％、80％、85％、90％、95％、98％、99％或100％的密码子为表3中所列的密码子。

[0214] 表3.示例性最小尿苷及腺嘌呤密码子

[0215]

	氨基酸	最小尿苷密码子
A	丙氨酸	GCC或GCG
G	甘氨酸	GGC或GGG
V	缬氨酸	GUC或GUG
D	天冬氨酸	GAC
E	谷氨酸	GAG
I	异亮氨酸	AUC
T	苏氨酸	ACC或ACG
N	天冬酰胺	AAC
K	赖氨酸	AAG
S	丝氨酸	AGC或UCC或UCG
R	精氨酸	CGC或CGG
L	亮氨酸	CUG或CUC
P	脯氨酸	CCG或CCC
H	组氨酸	CAC
Q	谷氨酰胺	CAG
F	苯丙氨酸	UUC
Y	酪氨酸	UAC
C	半胱氨酸	UGC
W	色氨酸	UGG
M	甲硫氨酸	AUG

[0216] 在一些实施例中,提供一种编码包含开放阅读框架(ORF)的RNA引导的DNA结合剂的mRNA,该ORF由其中至少约75％、80％、85％、90％、95％、98％、99％或100％的密码子为表

3中所列的密码子的一组密码子组成。如表3中可看出,三个所列丝氨酸密码子中的每一个含有一个A或一个U。在一些实施例中,对于丝氨酸,通过使用AGC密码子来优先化进行尿苷最小化。在一些实施例中,对于丝氨酸,通过使用UCC及/或UCG密码子优先化进行腺嘌呤最小化。

[0217] 4.提高转译及/或对应于高度表达的tRNA的密码子;示例性密码子组

[0218] 在一些实施例中,mRNA包含具有增加哺乳动物,诸如人类中的转译的密码子的ORF。在其它实施例中,mRNA包含具有增加哺乳动物,例如人类的器官,诸如肝脏中的转译的密码子的ORF。在其它实施例中,mRNA包含具有增加哺乳动物,例如人类的细胞型,诸如肝细胞中的转译的密码子的ORF。哺乳动物中;哺乳动物(人类)的细胞型、器官中;人类器官等中的转译的提高可相对于ORF的野生型序列转译程度或相对于以下ORF进行测定:该ORF具有与ORF所源自的生物体或在一定氨基酸含量下含有最类似ORF的生物体,诸如化脓链球菌、金黄色葡萄球菌(S.aureus)或另一原核生物(当情况可能为来源于原核生物的Cas核酸酶,诸如来自下文所述的其它原核生物的Cas核酸酶时)的密码子分布相匹配的密码子分布。可替代地,在一些实施例中,哺乳动物中;哺乳动物(人类)的细胞型、器官中;人类器官等中的Cas9序列转译的提高是相对于具有SEQ ID NO:5,同时在所有其它方面(包括任何适用点突变、异源域及其类似者)相同的序列的ORF的转译进行测定。适用于提高人类,包括人类肝脏及人类肝细胞中的表达的密码子可为对应于人类肝脏/肝细胞中高度表达的tRNA的密码子,其论述于Dittmar KA,PLos Genetics 2(12):e221(2006)中。在一些实施例中,ORF中至少约75%、80%、85%、90%、95%、96%、97%、98%、99%或100%的密码子为对应于哺乳动物,诸如人类中的高度表达的tRNA(例如针对各氨基酸的表达最高的tRNA)的密码子。在一些实施例中,ORF中至少75%、80%、85%、90%、95%、96%、97%、98%、99%或100%的密码子为对应于哺乳动物器官,诸如人类器官中的高度表达的tRNA(例如针对各氨基酸的表达最高的tRNA)的密码子。在一些实施例中,ORF中至少75%、80%、85%、90%、95%、96%、97%、98%、99%或100%的密码子为对应于哺乳动物肝脏,诸如人类肝脏中的高度表达的tRNA(例如针对各氨基酸的表达最高的tRNA)的密码子。在一些实施例中,ORF中至少75%、80%、85%、90%、95%、96%、97%、98%、99%或100%的密码子为对应于哺乳动物肝细胞,诸如人类肝细胞中的高度表达的tRNA(例如针对各氨基酸的表达最高的tRNA)的密码子。

[0219] 可替代地,通常可使用对应于生物体(例如人类)中的高度表达的tRNA的密码子。

[0220] 前述密码子选择方法中的任一个与上文所示的最小尿苷及/或腺嘌呤密码子可例如通过以下进行组合:以表1、表2或表3的密码子开始,且随后在可利用多于一种选择的情况下,使用对应于一般生物体(例如人类)中,或相关器官或细胞型,诸如肝脏或肝细胞(例如人类肝脏或人类肝细胞)中的较高度表达的tRNA的密码子。

[0221] 在一些实施例中,ORF中至少75%、80%、85%、90%、95%、96%、97%、98%、99%或100%的密码子为来自表4中所示的密码子组(例如低U1、低A或低A/U密码子组)的密码子。低U1、低G、低C、低A及低A/U组中的密码子使用将所指定的核苷酸减至最小的密码子,同时在可利用多于一种选择的情况下,亦使用对应于高度表达的tRNA的密码子。在一些实施例中,ORF中至少75%、80%、85%、90%、95%、96%、97%、98%、99%或100%的密码子为来自表4中所示的低U1密码子组的密码子。在一些实施例中,ORF中至少75%、80%、85%、90%、95%、96%、97%、98%、99%或100%的密码子为来自表4中所示的低A密码子组的密

码子。在一些实施例中,ORF中至少75%、80%、85%、90%、95%、96%、97%、98%、99%或100%的密码子为来自表4中所示的低A/U密码子组的密码子。

[0222] 表4.示例性密码子组.

[0223]

氨基酸	低U 1	低U 2	高U	低G	低C	低A	低A/U	长半衰期
Gly	GGC	GGG	GGT	GGC	GGA	GGC	GGC	GGT
Glu	GAG	GAA	GAA	GAA	GAG	GAG	GAG	GAA
Asp	GAC	GAC	GAT	GAC	GAT	GAC	GAC	GAC
Val	GTG	GTA	GTT	GTC	GTG	GTG	GTG	GTC
Ala	GCC	GCG	GCT	GCC	GCT	GCC	GCC	GCC
Arg	AGA	CGA	CGT	AGA	AGA	CGG	CGG	AGA
Ser	AGC	AGC	TCT	TCC	AGT	TCC	AGC	TCT
Lys	AAG	AAA	AAA	AAA	AAG	AAG	AAG	AAG
Asn	AAC	AAC	AAT	AAC	AAT	AAC	AAC	AAC

[0224]

Met	ATG	ATG	ATG	ATG	AGT	ATG	ATG	ATG
Ile	ATC	ATA	ATT	ATC	ATT	ATC	ATC	ATC
Thr	ACC	ACG	ACT	ACC	ACA	ACC	ACC	ACC
Trp	TGG	TGG	TGG	TGG	TGG	TGG	TGG	TGG
Cys	TGC	TGC	TGT	TGC	TGT	TGC	TGC	TGC
Tyr	TAC	TAC	TAT	TAC	TAT	TAC	TAC	TAC
Leu	CTG	CTA	TTA	CTC	TTG	CTG	CTG	TTG
Phe	TTC	TTC	TTT	TTC	TTT	TTC	TTC	TTC
Gln	CAG	CAA	CAA	CAA	CAG	CAG	CAG	CAA
His	CAC	CAC	CAT	CAC	CAT	CAC	CAC	CAC

[0225] 5.经编码的RNA引导的DNA结合剂

[0226] 在一些实施例中,RNA引导的DNA结合剂为2类Cas核酸酶。在一些实施例中,RNA引导的DNA结合剂具有裂解酶活性,其亦可称作双股核酸内切酶活性。在一些实施例中,RNA引导的DNA结合剂包含Cas核酸酶,诸如2类Cas核酸酶(其可为例如第II型、第V型或第VI型Cas核酸酶)。2类Cas核酸酶包括例如Cas9、Cpf1、C2c1、C2c2及C2c3蛋白质及其修饰形式。Cas9核酸酶的实例包括化脓链球菌、金黄色葡萄球菌及其它原核生物(参见例如下一段落中的清单)的II型CRISPR系统的那些及其经修饰(例如经工程改造或突变型)形式。参见例如,US2016/0312198 A1;US 2016/0312199 A1。Cas核酸酶的其它实例包括III型CRISPR系统的Csm或Cmr复合物或其Cas10、Csm1或Cmr2亚基;及I型CRISPR系统的级联复合物或其Cas3亚基。在一些实施例中,Cas核酸酶可来自第IIA型、第IIB型或第IIC型系统。对于不同CRISPR系统及Cas核酸酶的论述,参见例如Makarova等人NAT.REV.MICROBIOL.9:467-477(2011);Makarova等人,NAT.REV.MICROBIOL,13:722-36(2015);Shmakov等人,MOLECULAR CELL,60:385-397(2015)。

[0227] 可衍生出Cas核酸酶的非限制性示例性物种包括化脓链球菌(Streptococcus pyogenes)、嗜热链球菌(Streptococcus thermophilus)、链球菌属(Streptococcus sp.)、

金黄色葡萄球菌(Staphylococcus aureus)、无害李氏菌(Listeria innocua)、加氏乳杆菌(Lactobacillus gasseri)、新凶手弗朗西斯氏菌(Francisella novicida)、产琥珀酸沃廉菌(Wolinella succinogenes)、华德萨特菌(Sutterella wadsworthensis)、γ-变形菌(Gammaproteobacterium)、脑膜炎双球菌(Neisseria meningitidis)、空肠弯曲杆菌(Campylobacter jejuni)、多杀性巴氏杆菌(Pasteurella multocida)、产琥珀酸丝状杆菌(Fibrobacter succinogene)、深红红螺菌(Rhodospirillum rubrum)、达松维尔拟诺卡氏菌(Nocardiopsis dassonvillei)、始旋链霉菌(Streptomyces pristinaespiralis)、产绿色链霉菌(Streptomyces viridochromogenes)、玫瑰链孢囊菌(Streptosporangium roseum)、酸热脂环杆菌(Alicyclobacillus acidocaldarius)、假蕈状芽孢杆菌(Bacillus pseudomycoides)、砷还原芽孢杆菌(Bacillus selenitireducens)、西伯利亚微小杆菌(Exiguobacterium sibiricum)、戴白氏乳杆菌(Lactobacillus delbrueckii)、唾液乳杆菌(Lactobacillus salivarius)、布氏乳杆菌(Lactobacillus buchneri)、齿垢密螺旋体(Treponema denticola)、海洋微颤菌(Microscilla marina)、伯克霍尔德氏细菌(Burkholderiales bacterium)、食萘极单胞菌(Polaromonas naphthalenivorans)、极单胞菌属(Polaromonas sp.)、瓦氏鳄球藻(Crocosphaera watsonii)、蓝丝菌属(Cyanothece sp.)、绿脓微囊藻(Microcystis aeruginosa)、聚球藻属(Synechococcus sp.)、阿拉伯糖醋杆菌(Acetohalobium arabaticum)、丹氏制氨菌(Ammonifex degensii)、热解纤维素菌(Caldicelulosiruptor becscii)、金矿菌候选种(Candidatus Desulforudis)、肉毒梭菌(Clostridium botulinum)、艰难梭菌(Clostridium difficile)、大芬戈尔德菌(Finegoldia magna)、嗜热盐碱厌氧菌(Natranaerobius thermophilus)、热丙酸盐暗色厌氧香肠状菌(Pelotomaculum thermopropionicum)、喜温嗜酸硫杆菌(Acidithiobacillus caldus)、氧化亚铁嗜酸硫杆菌(Acidithiobacillus ferrooxidans)、酒色异着色菌(Allochromatium yinosum)、海杆菌属(Marinobacter sp.)、嗜盐亚硝化球菌(Nitrosococcus halophilus)、瓦氏亚硝化球菌(Nitrosococcus watsoni)、游海假交替单胞菌(Pseudoalteromonas haloplanktis)、消旋纤线杆菌(Ktedonobacter racemifer)、调查甲烷盐菌(Methanohalobium evesrigatum)、多变念珠藻(Anabaena variabilis)、泡沫节球藻(Nodularia spumigena)、念珠藻属(Nostoc sp.)、极大节旋藻(Arthrospira maxima)、钝顶节旋藻(Arthrospira platensis)、节旋藻属(Arthrospira sp.)、鞘丝藻属(Lyngbya sp.)、原型微鞘藻(Microcoleus chthonoplastes)、颤藻属(Oscillatoria sp.)、运动石袍菌(Petrotoga mobilis)、非洲高热杆菌(Thermosipho africanus)、巴氏链球菌(Streptococcus pasteurianus)、灰色奈瑟菌(Neisseria cinerea)、红嘴鸥曲杆菌(Campylobacter lari)、食清洁剂细小棒菌(Parvibaculum lavamentivorans)、白喉棒状杆菌(Corynebacterium diphtheria)、氨基酸球菌属(Acidaminococcus sp.)、毛螺科菌ND2006(Lachnospiraceae bacterium ND2006)及海洋藻青菌(Acaryochloris marina)。

[0228] 在一些实施例中,Cas核酸酶为来自化脓链球菌(Streptococcus pyogenes)的Cas9核酸酶。在一些实施例中,Cas核酸酶为来自嗜热链球菌的Cas9核酸酶。在一些实施例中,Cas核酸酶为来自脑膜炎双球菌的Cas9核酸酶。在一些实施例中,Cas核酸酶为来自金黄色葡萄球菌的Cas9核酸酶。在一些实施例中,Cas核酸酶为来自新凶手弗朗西斯氏菌的Cpf1核酸酶。在一些实施例中,Cas核酸酶为来自氨基酸球菌属的Cpf1核酸酶。在一些实施例中,

Cas核酸酶为来自毛螺科菌ND2006的Cpf1核酸酶。在其它实施例中,Cas核酸酶为来自以下的Cpf1核酸酶:土拉文氏杆菌(Francisella tularensis)、毛螺科菌、瘤胃溶纤维丁酸弧菌(Butyrivibrio proteoclasticus)、佩氏细菌(Peregrinibacteria bacterium)、帕库氏菌(Parcubacteria bacterium)、史密斯氏菌(Smithella)、氨基酸球菌属、白蚁甲烷支原体菌候选种(Candidatus Methanoplasma termitum)、挑剔真杆菌(Eubacterium eligens)、牛眼莫拉菌(Moraxella bovoculi)、稻田钩端螺旋体(Leptospira inadai)、狗口腔卟啉单胞菌(Porphyromonas crevioricanis)、解糖胨普雷沃菌(Prevotella disiens)或猕猴卟啉单胞菌(Porphyromonas macacae)。在某些实施例中,Cas核酸酶为来自氨基酸球菌或毛螺科菌的Cpf1核酸酶。

[0229] 野生型Cas9具有两个核酸酶域:RuvC及HNH。RuvC域裂解非靶DNA股,且HNH域裂解靶DNA股。在一些实施例中,Cas9核酸酶包含多于一个RuvC域及/或多于一个HNH域。在一些实施例中,Cas9核酸酶为野生型Cas9。在一些实施例中,Cas9能够诱导靶DNA中的双股断裂。在某些实施例中,Cas核酸酶可裂解dsDNA,其可裂解dsDNA的一个股,或其可不具有DNA裂解酶或切口酶活性。一个示例性Cas9氨基酸序列提供呈SEQ ID NO:3形式。一个包括起始及终止密码子的示例性Cas9 mRNA ORF序列提供呈SEQ ID NO:4形式。一个适于包括在融合蛋白质中的示例性Cas9 mRNA编码序列提供呈SEQ ID NO:10形式。

[0230] 在一些实施例中,使用嵌合Cas核酸酶,其中该蛋白质的一个域或区经不同蛋白质的一部分置换。在一些实施例中,Cas核酸酶域可经来自诸如Fok1的不同核酸酶的域置换。在一些实施例中,Cas核酸酶可为经修饰的核酸酶。

[0231] 在其它实施例中,Cas核酸酶可来自I型CRISPR/Cas系统。在一些实施例中,Cas核酸酶可为I型CRISPR/Cas系统的级联复合物的组分。在一些实施例中,Cas核酸酶可为Cas3蛋白质。在一些实施例中,Cas核酸酶可来自III型CRISPR/Cas系统。在一些实施例中,Cas核酸酶可具有RNA裂解活性。

[0232] 在一些实施例中,RNA引导的DNA结合剂具有单股切口酶活性,即,可切割一个DNA股以产生单股断裂,亦称为“切口(nick)”。在一些实施例中,RNA引导的DNA结合剂包含Cas切口酶。切口酶为引起dsDNA中出现切口,即,切割一个股但不切割DNA双螺旋的另一股的酶。在一些实施例中,Cas切口酶为其中例如通过催化域的一或多种变化(例如点突变),使核酸内切酶活性位点不活化的Cas核酸酶的形式(例如上文所论述的Cas核酸酶)。参见例如关于Cas切口酶及示例性催化域变化的论述的美国专利第8,889,356号。在一些实施例中,Cas切口酶,诸如Cas9切口酶具有不活化的RuvC或HNH域。一个示例性Cas9切口酶氨基酸序列提供呈SEQ ID NO:6形式。一个包括起始及终止密码子的示例性Cas9切口酶mRNA ORF序列提供呈SEQ ID NO:7形式。一个适于包括在融合蛋白质中的示例性Cas9切口酶mRNA编码序列提供呈SEQ ID NO:11形式。

[0233] 在一些实施例中,RNA引导的DNA结合剂经修饰而仅含有一个功能核酸酶域。例如,该结合剂蛋白质可经修饰以使得核酸酶域中的一者经突变或完全或部分缺失以降低其核酸裂解活性。在一些实施例中,使用具有活性降低的RuvC域的切口酶。在一些实施例中,使用具有非活性RuvC域的切口酶。在一些实施例中,使用具有活性降低的HNH域的切口酶。在一些实施例中,使用具有非活性HNH域的切口酶。

[0234] 在一些实施例中,Cas蛋白质核酸酶域中的保守氨基酸经取代以降低或改变核酸

酶活性。在一些实施例中,Cas核酸酶可在RuvC或类RuvC核酸酶域中包含氨基酸取代。RuvC或类RuvC核酸酶域中的示例性氨基酸取代包括D10A(基于化脓链球菌Cas9蛋白质)。参见例如,Zetsche等人(2015)Cell Oct 22:163(3):759-771。在一些实施例中,Cas核酸酶可在HNH或类HNH核酸酶域中包含氨基酸取代。HNH或类HNH核酸酶域中的示例性氨基酸取代包括E762A、H840A、N863A、H983A及D986A(基于化脓链球菌Cas9蛋白质)。参见例如,Zetsche等人(2015)。其它示例性氨基酸取代包括D917A、E1006A及D1255A(基于新凶手弗朗西斯氏菌U112Cpf1(FnCpf1)序列(UniProtKB-A0Q7Q2(CPF1_FRATN))。

[0235] 在一些实施例中,将与一对分别与靶序列的有义股及反义股互补的引导RNA组合提供编码切口酶的mRNA。在此实施例中,引导RNA将切口酶导引至靶序列且通过在靶序列的相对股上产生切口而引入DSB(即双切口)。在一些实施例中,使用双重切口可提高特异性及减少脱靶效应。在一些实施例中,连同靶向DNA的相对股的两个独立引导RNA使用切口酶以在靶DNA中产生双重切口。在一些实施例中,连同经选择以非常接近的两个独立引导RNA使用切口酶以在靶DNA中产生双重切口。

[0236] 在一些实施例中,RNA引导的DNA结合剂缺乏裂解酶及切口酶活性。在一些实施例中,RNA引导的DNA结合剂包含dCas DNA结合多肽。dCas多肽具有DNA结合活性,而基本上缺乏催化(裂解酶/切口酶)活性。在一些实施例中,dCas多肽为dCas9多肽。在一些实施例中,缺乏裂解酶及切口酶活性的RNA引导的DNA结合剂或dCas DNA结合多肽为其中例如通过催化域的一或多种变化(例如点突变),使核酸内切酶活性位点不活化的Cas核酸酶的形式(例如上文所论述的Cas核酸酶)。参见例如US 2014/0186958 A1;US 2015/0166980 A1。示例性dCas9氨基酸序列提供呈SEQ ID NO:8形式。一个包括起始及终止密码子的示例性dCas9 mRNA ORF序列提供呈SEQ ID NO:9形式。一个适于包括在融合蛋白质中的示例性dCas9 mRNA编码序列提供呈SEQ ID NO:12形式。

[0237] 6.异源功能域;核定位信号

[0238] 在一些实施例中,RNA引导的DNA结合剂包含一或多个异源功能域(例如为或包含融合多肽)。

[0239] 在一些实施例中,异源功能域可促进将RNA引导的DNA结合剂输送至细胞核中。例如,异源功能域可为核定位信号(NLS)。在一些实施例中,RNA引导的DNA结合剂可与1至10个NLS融合。在一些实施例中,RNA引导的DNA结合剂可与1至5个NLS融合。在一些实施例中,RNA引导的DNA结合剂可与一个NLS融合。在使用一个NLS的情况下,NLS可连接在RNA引导的DNA结合剂序列的N端或C端处。在一些实施例中,RNA引导的DNA结合剂可C端融合至至少一个NLS。NLS亦可插入RNA引导的DNA结合剂序列内。在其它实施例中,RNA引导的DNA结合剂可与多于一个NLS融合。在一些实施例中,RNA引导的DNA结合剂可与2、3、4或5个NLS融合。在一些实施例中,RNA引导的DNA结合剂可与两个NLS融合。在某些情况下,两个NLS可相同(例如两个SV40 NLS)或不同。在一些实施例中,RNA引导的DNA结合剂与连接在羧基末端处的两个SV40NLS序列融合。在一些实施例中,RNA引导的DNA结合剂可与两个NLS融合,一个NLS连接在N端处且一个连接在C端处。在一些实施例中,RNA引导的DNA结合剂可与3个NLS融合。在一些实施例中,RNA引导的DNA结合剂可不与NLS融合。在一些实施例中,NLS可为单联(monopartite)序列,诸如SV40 NLS、PKKKRKV(SEQ ID NO:78)或PKKKRRV(SEQ ID NO:90)。在一些实施例中,NLS可为双联序列,诸如核质蛋白的NLS、KRPAATKKAGQAKKKK(SEQ ID NO:

91)。在一些实施例中,NLS序列可包含LAAKRSRTT(SEQ ID NO:79)、QAAKRSRTT(SEQ ID NO:80)、PAPAKRERTT(SEQ ID NO:81)、QAAKRPRTT(SEQ ID NO:82)、RAAKRPRTT(SEQ ID NO:83)、AAAKRSWSMAA(SEQ ID NO:84)、AAAKRVWSMAF(SEQ ID NO:85)、AAAKRSWSMAF(SEQ ID NO:86)、AAAKRKYFAA(SEQ ID NO:87)、RAAKRKAFAA(SEQ ID NO:88)或RAAKRKYFAV(SEQ ID NO:89)。在一具体实施例中,单一PKKKRKV(SEQ ID NO:78)NLS可连接在RNA引导的DNA结合剂的C端处。一或多个连接子视情况包括在融合位点处。在一些实施例中,根据前述实施例中任一个的一或多个NLS与一或多个额外异源功能域,诸如下文所述的异源功能域中的任一个组合存在于RNA引导的DNA结合剂中。

[0240] 在一些实施例中,异源功能域可能能够调整RNA引导的DNA结合剂的胞内半衰期。在一些实施例中,RNA引导的DNA结合剂的半衰期可得到提高。在一些实施例中,RNA引导的DNA结合剂的半衰期可得到降低。在一些实施例中,异源功能域可能够增加RNA引导的DNA结合剂的稳定性。在一些实施例中,异源功能域可能够降低RNA引导的DNA结合剂的稳定性。在一些实施例中,异源功能域可作为蛋白质降解的信号肽。在一些实施例中,蛋白质降解可由蛋白水解酶,诸如蛋白酶体、溶酶体蛋白酶或钙蛋白酶蛋白酶介导。在一些实施例中,异源功能域可包含PEST序列。在一些实施例中,RNA引导的DNA结合剂可通过添加泛素或多泛素链来修饰。在一些实施例中,泛素可为类泛素蛋白质(UBL)。类泛素蛋白质的非限制性实例包括小类泛素修饰因子(SUMO)、泛素交叉反应蛋白(UCRP,亦称为干扰素刺激基因-15(ISG15))、泛素相关修饰因子-1(URM1)、神经元-前驱体-细胞表达的发育下调蛋白-8(NEDD8,在酿酒酵母(S.cerevisiae)中亦称作Rub1)、人类白血球抗原F相关(FAT10)、自噬-8(ATG8)及自噬-12(ATG12)、Fau类泛素蛋白(FUB1)、膜锚定UBL(MUB)、泛素折叠修饰因子-1(UFM1)及类泛素蛋白-5(UBL5)。

[0241] 在一些实施例中,异源功能域可为标记域。标记域的非限制性实例包括荧光蛋白质、纯化标签、抗原决定基标签及报导基因序列。在一些实施例中,标记域可为荧光蛋白质。适合荧光蛋白质的非限制实例包括绿色荧光蛋白质(例如,GFP、GFP-2、tagGFP、turboGFP、sfGFP、EGFP、祖母绿(Emerald)、Azami绿(Azami Green)、单体Azami绿(Monomeric Azami Green)、CopGFP、AceGFP、ZsGreen1)、黄色荧光蛋白质(例如,YFP、EYFP、Citrine、Venus、YPet、PhiYFP、ZsYellow1)、蓝色荧光蛋白质(例如,EBFP、EBFP2、Azurite、mKalamal、GFPuv、蓝宝石色(Sapphire)、T-蓝宝石色(T-sapphire))、强化型蓝荧光蛋白质(例如,ECFP、Cerulean、CyPet、AmCyan1、Midoriishi强化型蓝(Midoriishi-Cyan))、红色荧光蛋白质(mKate、mKate2、mPlum、DsRed单体、mCherry、mRFP1、DsRed-Express、DsRed2、DsRed-单体、HcRed串色(HcRed-Tandem)、HcRed1、AsRed2、eqFP611、mRasberry、mStrawberry、Jred)及橙色荧光蛋白质(mOrange、mKO、Kusabira橙色(Kusabira-Orange)、单体Kusabira橙色(Monomeric Kusabira-Orange)、mTangerine、tdTomato)或任何其它适合荧光蛋白质。在其它实施例中,标记域可为纯化标签及/或抗原决定基标签。非限制性的示例性标签包括谷胱甘肽-S-转移酶(glutathione-S-transferase,GST)、壳质结合蛋白(CBP)、麦芽糖结合蛋白(MBP)、硫氧还蛋白(thioredoxin,TRX)、聚(NANP)、串联亲和纯化(tandem affinity purification,TAP)标签、myc、AcV5、AU1、AU5、E、ECS、E2、FLAG、HA、nus、Sαftag 1、Sαftag 3、Strep、SBP、Glu-Glu、HSV、KT3、S、S1、T7、V5、VSV-G、6×His、8×His、生物素羧基载体蛋白质(BCCP)、聚His及调钙蛋白。非限制性的示例性报导基因包括谷胱甘肽-S-转移酶(GST)、

辣根过氧化酶(HRP)、氯霉素乙酰基转移酶(CAT)、β-半乳糖苷酶、β-葡糖苷酸酶、荧光素酶或荧光蛋白质。

[0242] 在其它实施例中,异源功能域可将RNA引导的DNA结合剂靶向至特定细胞器、细胞型、组织或器官。在一些实施例中,异源功能域可将RNA引导的DNA结合剂靶向至线粒体。

[0243] 在其它实施例中,异源功能域可为效应子域。当RNA引导的DNA结合剂导引至其靶序列时,例如当Cas核酸酶通过gRNA导引至靶序列时,效应子域可修饰或影响靶序列。在一些实施例中,效应子域可选自核酸结合域、核酸酶域(例如非Cas核酸酶域)、表观遗传修饰域、转录活化域或转录抑制子域。在一些实施例中,异源功能域为核酸酶,诸如FokI核酸酶。参见例如美国专利第9,023,649号。在一些实施例中,异源功能域为转录活化因子或抑制子。参加例如Qi等人,“Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression”,Cell 152:1173-83(2013);Perez-Pinera等人,“RNA-guided gene activation by CRISPR-Cas9-based transcription factors”,Nat.Methods10:973-6(2013);Mali等人,“CAS9transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering”,Nat.Biotechnol.31:833-8(2013);Gilbert等人,“CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes”,Cell 154:442-51(2013)。因此,RNA引导的DNA结合剂基本上变成可使用引导RNA导引以结合所需靶序列的转录因子。在某些实施例中,DNA修饰域为甲基化域,诸如去甲基化或甲基转移酶域。在某些实施例中,效应子域为DNA修饰域,诸如碱基编辑域。在特定实施例中,DNA修饰域为将特异性修饰引入DNA中的核酸编辑域,诸如脱氨酶域。参见例如WO 2015/089406;US 2016/0304846。WO 2015/089406及US 2016/0304846中所述的核酸编辑域、脱氨酶域及Cas9变异体是以引用的方式并入本文中。

[0244] 7.UTR;Kozak序列

[0245] 在一些实施例中,mRNA包含至少一个来自羟基类固醇17-β脱氢酶4(HSD17B4或HSD)的UTR,例如来自HSD的5′UTR。在一些实施例中,mRNA包含至少一个来自血球蛋白mRNA,例如人类α血球蛋白(HBA)mRNA、人类β血球蛋白(HBB)mRNA或有爪蟾蜍β血球蛋白(XBG)mRNA的UTR。在一些实施例中,mRNA包含来自血球蛋白mRNA,诸如HBA、HBB或XBG的5′UTR、3′UTR、或5′与3′UTR。在一些实施例中,mRNA包含来自牛生长激素、细胞巨大病毒(CMV)、小鼠Hba-a1、HSD、白蛋白基因、HBA、HBB或XBG的5′UTR。在一些实施例中,mRNA包含来自牛生长激素、细胞巨大病毒(CMV)、小鼠Hba-a1、HSD、白蛋白基因、HBA、HBB或XBG的3′UTR。在一些实施例中,mRNA包含来自牛生长激素、细胞巨大病毒、小鼠Hba-a1、HSD、白蛋白基因、HBA、HBB、XBG、热休克蛋白90(Hsp90)、甘油醛3-磷酸脱氢酶(GAPDH)、β-肌动蛋白、α-微管蛋白、肿瘤蛋白质(p53)或表皮生长因子受体(EGFR)的5'与3′UTR。

[0246] 在一些实施例中,mRNA包含来自同一来源(例如组成型表达的mRNA,诸如肌动蛋白、白蛋白或血球蛋白(HBA、HBB或XBG))的5′及3′UTR。

[0247] 在一些实施例中,本文所揭示的mRNA包含与SEQ ID NO:32、34、36、38或41中的任一项有至少90%的一致性的5′UTR。在一些实施例中,本文所揭示的mRNA包含与SEQ ID NO:33、35、37、39或40中的任一项有至少90%的一致性的3′UTR。在一些实施例中,前述一致性程度中的任一项为至少95%、至少98%、至少99%或100%。在一些实施例中,本文所揭示的

mRNA包含具有SEQ ID NO:32、34、36、38或41中的任一项的序列的5'UTR。在一些实施例中，本文所揭示的mRNA包含具有SEQ ID NO:33、35、37、39或40中的任一项的序列的3'UTR。

[0248] 在一些实施例中，mRNA不包含5'UTR，例如在5'帽结构与起始密码子之间不存在额外核苷酸。在一些实施例中，mRNA在5'帽结构与起始密码子之间包含Kozak序列(下文所述)，但不具有任何额外5'UTR。在一些实施例中，mRNA不包含3'UTR，例如在终止密码子与聚-A尾之间不存在额外核苷酸。

[0249] 在一些实施例中，mRNA包含Kozak序列。Kozak序列可影响转译起始及由mRNA转译的多肽的总产量。Kozak序列包括可作为起始密码子的甲硫氨酸密码子。最小Kozak序列为NNNRUGN，其中以下至少一项成立：第一个N为A或G且第二个N为G。在核苷酸序列中，R意指嘌呤(A或G)。在一些实施例中，Kozak序列为RNNRUGN、NNNRUGG、RNNRUGG、RNNAUGN、NNNAUGG或RNNAUGG。在一些实施例中，Kozak序列为具有零错配或呈小写字母形式的位置有至多一或两个错配的rccRUGg。在一些实施例中，Kozak序列为具有零错配或呈小写字母形式的位置有至多一或两个错配的rccAUGg。在一些实施例中，Kozak序列为具有零错配或呈小写字母形式的位置有至多一个、两个或三个错配的gccRccAUGG(SEQ ID NO:105的第4核苷酸至第13核苷酸)。在一些实施例中，Kozak序列为具有零错配或呈小写字母形式的位置有至多一个、二个、三个或四个错配的gccAccAUG。在一些实施例中，Kozak序列为GCCACCAUG。在一些实施例中，Kozak序列为具有零错配或呈小写字母形式的位置有至多一个、二个、三个或四个错配的gccgccRccAUGG(SEQ ID NO:105)。

[0250] 8.示例性序列

[0251] 在一些实施例中，mRNA包含编码RNA引导的DNA结合剂的ORF，其中该ORF包含与SEQ ID NO:1、4、7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66或107-175中的任一项有至少90％的一致性的序列。在一些实施例中，mRNA包含编码RNA引导的DNA结合剂的ORF，其中该RNA引导的DNA结合剂包含与SEQ ID NO:3、6、8、13、16、19、22、25、28、68或186-196中的任一项有至少90％的一致性的氨基酸序列:，其中ORF的尿苷含量在其最小尿苷含量至最小尿苷含量的150％范围内，及/或其尿苷二核苷酸含量在其最小尿苷二核苷酸含量至最小尿苷二核苷酸含量的150％范围内。在一些实施例中，mRNA包含编码RNA引导的DNA结合剂的ORF，其中该RNA引导的DNA结合剂包含与SEQ ID NO:3、6、8、13、16、19、22、25、28、68或186—196中的任一项有至少90％的一致性的氨基酸序列:，其中ORF的腺嘌呤含量在其最小腺嘌呤含量至最小腺嘌呤含量的150％范围内，及/或其腺嘌呤二核苷酸含量在其最小腺嘌呤二核苷酸含量至最小腺嘌呤二核苷酸含量的150％范围内。在一些此类实施例中，腺嘌呤含量及尿苷核苷酸含量两者均小于或等于其相应最小值的150％。在一些实施例中，腺嘌呤含量及尿苷二核苷酸含量两者均小于或等于其相应最小值的150％。在一些实施例中，mRNA包含与SEQ ID NO:43、44、51、53、55-61或67中的任一项有至少90％的一致性的序列，其中该序列包含编码RNA引导的DNA结合剂的ORF。在一些实施例中，mRNA包含与SEQ ID NO:43、44、51、53、55-61或67中的任一项有至少90％的一致性的序列，其中该序列包含编码RNA引导的DNA结合剂的ORF，其中省去SEQ ID NO:43、44、51、53、55-61或67的前三个核苷酸。在一些实施例中，前述一致性程度中的任一项为至少95％、至少98％、至少99％或100％。

[0252] 在一些实施例中，mRNA包含编码RNA引导的DNA结合剂的ORF，其中该ORF与SEQ ID

NO:1、4、7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66或107-175中的任一项在至少其前30、50、70、100、150、200、250或300个核苷酸上有至少90％的一致性。前30、50、70、100、150、200、250或300个核苷酸是自起始密码子(典型地,ATG)的第一个核苷酸开始测量,以使得A为第1核苷酸,T为第2核苷酸等。在一些实施例中,开放阅读框架与SEQ ID NO:1、4、7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66或107-175中的任一项在至少其序列之前10％、12％、15％、20％、25％、30％或35％上具有至少90％的一致性。ORF的序列的长度为自起始密码子开始至终止密码子结束的核苷酸的数目,且其序列之前10％、12％、15％、20％、25％、30％或35％对应于自起始密码子的第一个核苷酸开始,构成总序列的长度的指定百分比的核苷酸的数目。

[0253] 在一些实施例中,包含编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:43具有至少90％的一致性的序列,视情况地,其中SEQ ID NO:43的ORF(即SEQ ID NO:4)由SEQ ID NO:7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66或107-175中的任一项替代的ORF取代。

[0254] 在一些实施例中,包含编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:44具有至少90％的一致性的序列,视情况地,其中SEQ ID NO:44的ORF(即SEQ ID NO:4)由SEQ ID NO:7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66或107-175中的任一项替代的ORF取代。

[0255] 在一些实施例中,包含编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:56具有至少90％的一致性的序列,视情况地,其中SEQ ID NO:56的ORF(即SEQ ID NO:4)由SEQ ID NO:7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66或107-175中的任一项替代的ORF取代。

[0256] 在一些实施例中,包含编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:57具有至少90％的一致性的序列,视情况地,其中SEQ ID NO:57的ORF(即SEQ ID NO:4)由SEQ ID NO:7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66或107-175中的任一项替代的ORF取代。

[0257] 在一些实施例中,包含编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:58具有至少90％的一致性的序列,视情况地,其中SEQ ID NO:58的ORF(即SEQ ID NO:4)由SEQ ID NO:7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66或107-175中的任一项替代的ORF取代。

[0258] 在一些实施例中,包含编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:59具有至少90％的一致性的序列,视情况地,其中SEQ ID NO:59的ORF(即SEQ ID NO:4)由SEQ ID NO:7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66或107-175中的任一项替代的ORF取代。

[0259] 在一些实施例中,包含编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:60具有至少90％的一致性的序列,视情况地,其中SEQ ID NO:60的ORF(即SEQ ID NO:4)由SEQ ID NO:7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66或107-175中的任一项替代的ORF取代。

[0260] 在一些实施例中,包含编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:61具有至少90％的一致性的序列,视情况地,其中SEQ ID NO:61的ORF(即SEQ ID NO:4)由

SEQ ID NO:7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66或107-175中的任一项替代的ORF取代。

[0261] 在一些实施例中,包含编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:176具有至少90%的一致性的序列,视情况地,其中SEQ ID NO:176的ORF由SEQ ID NO:4、7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66或107—175中的任一项替代的ORF取代。

[0262] 在一些实施例中,包含编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:177具有至少90%的一致性的序列,视情况地,其中SEQ ID NO:177的ORF由SEQ ID NO:4、7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66或107—175中的任一项替代的ORF取代。

[0263] 在一些实施例中,包含编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:178具有至少90%的一致性的序列,视情况地,其中SEQ ID NO:178的ORF由SEQ ID NO:4、7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66或107-175中的任一项替代的ORF取代。

[0264] 在一些实施例中,包含编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:179具有至少90%的一致性的序列,视情况地,其中SEQ ID NO:179的ORF由SEQ ID NO:4、7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66或107-175中的任一项替代的ORF取代。

[0265] 在一些实施例中,包含编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:180具有至少90%的一致性的序列,视情况地,其中SEQ ID NO:180的ORF由SEQ ID NO:4、7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66或107-175中的任一项替代的ORF取代。

[0266] 在一些实施例中,包含编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:181具有至少90%的一致性的序列,视情况地,其中SEQ ID NO:181的ORF由SEQ ID NO:4、7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66或107-175中的任一项替代的ORF取代。

[0267] 在一些实施例中,包含编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:182具有至少90%的一致性的序列,视情况地,其中SEQ ID NO:182的ORF由SEQ ID NO:4、7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66或107-175中的任一项替代的ORF取代。

[0268] 在一些实施例中,包含编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:183具有至少90%的一致性的序列,视情况地,其中SEQ ID NO:183的ORF由SEQ ID NO:4、7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66或107-175中的任一项替代的ORF取代:。

[0269] 在一些实施例中,包含编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:184具有至少90%的一致性的序列,视情况地,其中SEQ ID NO:184的ORF由SEQ ID NO:4、7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66或107-175中的任一项替代的ORF取代。

[0270] 在一些实施例中,包含编码RNA引导的DNA结合剂的ORF的mRNA包含与SEQ ID NO:

185具有至少90%的一致性的序列,视情况地,其中SEQ ID NO:185的ORF由SEQ ID NO:4、7、9、10、11、12、14、15、17、18、20、21、23、24、26、27、29、30、50、52、54、65、66或107-175中的任一项替代的ORF取代。

[0271] 在一些实施例中,与SEQ ID NO 43、44、56-61或176-185的任选地经取代的序列的一致性程度为至少95%。在一些实施例中,与SEQ ID NO 43、44、56-61或176-185的任选地经取代的序列的一致性程度为至少98%。在一些实施例中,与SEQ ID NO 43、44、56-61或176-185的任选地经取代的序列的一致性程度为至少99%。在一些实施例中,与SEQ ID NO 43、44、56-61或176-185的任选地经取代的序列的一致性程度为100%。

[0272] 9.聚-A尾

[0273] 在一些实施例中,mRNA进一步包含聚-A尾。在一些情况下,聚-A尾在聚-A尾中的一或多个位置处经一或多个非腺嘌呤核苷酸"锚"中断。聚-A尾可包含至少8个连续腺嘌呤核苷酸,且亦包含一或多个非腺嘌呤核苷酸。如本文所用,"非腺嘌呤核苷酸"是指不包含腺嘌呤的任何天然或非天然核苷酸。鸟嘌呤、胸腺嘧啶及胞嘧啶核苷酸为示例性非腺嘌呤核苷酸。因此,本文所述的mRNA上的聚-A尾可包含位于3′至编码RNA引导的DNA结合剂或相关序列的核苷酸的连续腺嘌呤核苷酸。在一些情况下,mRNA上的聚-A尾包含位于3′至编码RNA引导的DNA结合剂或相关序列的核苷酸的非连续腺嘌呤核苷酸,其中非腺嘌呤核苷酸以规则或不规则间隔中断腺嘌呤核苷酸。

[0274] 在一些实施例中,聚-A尾编码于用于活体外转录mRNA的质粒中且变成转录物的一部分。编码于质粒中的聚-A序列,即聚-A序列中的连续腺嘌呤核苷酸的数目可能并不精确,例如质粒中的100聚-A序列可能不会在经转录的mRNA中产生恰好100聚-A序列。在一些实施例中,聚-A尾未编码于质粒中,且通过PCR加尾或酶加尾,例如使用大肠杆菌聚(A)聚合酶来添加。

[0275] 在一些实施例中,一或多个非腺嘌呤核苷酸定位成中断连续腺嘌呤核苷酸,以使得聚(A)结合蛋白可结合于连续腺嘌呤核苷酸的一段。在一些实施例中,一或多个非腺嘌呤核苷酸位于至少8、9、10、11或12个连续腺嘌呤核苷酸之后。在一些实施例中,一或多个非腺嘌呤核苷酸位于至少8至50个连续腺嘌呤核苷酸之后。在一些实施例中,一或多个非腺嘌呤核苷酸位于至少8至100个连续腺嘌呤核苷酸之后。在一些实施例中,非腺嘌呤核苷酸在一个、两个、三个、四个、五个、六个或七个腺嘌呤核苷酸之后且之后为至少8个连续腺嘌呤核苷酸。

[0276] 本发明的聚-A尾可包含一个以下顺序:连续腺嘌呤核苷酸,继之以一或多个非腺嘌呤核苷酸,视情况继之以额外腺嘌呤核苷酸。

[0277] 在一些实施例中,聚-A尾包含或含有一个非腺嘌呤核苷酸或2至10个非腺嘌呤核苷酸的一个连续段。在一些实施例中,非腺嘌呤核苷酸位于至少8、9、10、11或12个连续腺嘌呤核苷酸之后。在一些情况下,一或多个非腺嘌呤核苷酸位于至少8至50个连续腺嘌呤核苷酸之后。在一些实施例中,一或多个非腺嘌呤核苷酸位于至少8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43、44、45、46、47、48、49或50个连续腺嘌呤核苷酸之后。

[0278] 在一些实施例中,非腺嘌呤核苷酸为鸟嘌呤、胞嘧啶或胸腺嘧啶。在一些情况下,非腺嘌呤核苷酸为鸟嘌呤核苷酸。在一些实施例中,非腺嘌呤核苷酸为胞嘧啶核苷酸。在一

些实施例中,非腺嘌呤核苷酸为胸腺嘧啶核苷酸。在其中存在多于一个非腺嘌呤核苷酸的一些情况下,非腺嘌呤核苷酸可选自:a)鸟嘌呤及胸腺嘧啶核苷酸;b)鸟嘌呤及胞嘧啶核苷酸;c)胸腺嘧啶及胞嘧啶核苷酸;或d)鸟嘌呤、胸腺嘧啶及胞嘧啶核苷酸。包含非腺嘌呤核苷酸的示例性聚-A尾提供呈SEQ ID NO:62形式。

[0279] 10.经修饰的核苷酸

[0280] 在一些实施例中,mRNA在一些或所有尿苷位置处包含经修饰的尿苷。在一些实施例中,经修饰的尿苷为在5位处,例如用卤素或C1-C3烷氧基修饰的尿苷。在一些实施例中,经修饰的尿苷为在1位处,例如用C1-C3烷基修饰的假尿苷。经修饰的尿苷可为,例如假尿苷、N1-甲基-假尿苷、5-甲氧基尿苷、5-碘尿苷或其组合。在一些实施例中,经修饰的尿苷为5-甲氧基尿苷。在一些实施例中,经修饰的尿苷为5-碘尿苷。在一些实施例中,经修饰的尿苷为假尿苷。在一些实施例中,经修饰的尿苷为N1-甲基-假尿苷。在一些实施例中,经修饰的尿苷为假尿苷及N1-甲基-假尿苷的组合。在一些实施例中,经修饰的尿苷为假尿苷及5-甲氧基尿苷的组合。在一些实施例中,经修饰的尿苷为N1-甲基假尿苷及5-甲氧基尿苷的组合。在一些实施例中,经修饰的尿苷为5-碘尿苷及N1-甲基-假尿苷的组合。在一些实施例中,经修饰的尿苷为假尿苷及5-碘尿苷的组合。在一些实施例中,经修饰的尿苷为5-碘尿苷及5-甲氧基尿苷的组合。

[0281] 在一些实施例中,根据本发明的mRNA中至少10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%、98%、99%或100%的尿苷位置为经修饰的尿苷。在一些实施例中,根据本发明的mRNA中10%至25%、15%至25%、25%至35%、35%至45%、45%至55%、55%至65%、65%至75%、75%至85%、85%至95%或90%至100%的尿苷位置为经修饰的尿苷,例如5-甲氧基尿苷、5-碘尿苷、N1-甲基假尿苷、假尿苷或其组合。在一些实施例中,根据本发明的mRNA中10%至25%、15%至25%、25%至35%、35%至45%、45%至55%、55%至65%、65%至75%、75%至85%、85%至95%或90%至100%的尿苷位置为5-甲氧基尿苷。在一些实施例中,根据本发明的mRNA中10%至25%、15%至25%、25%至35%、35%至45%、45%至55%、55%至65%、65%至75%、75%至85%、85%至95%或90%至100%的尿苷位置为假尿苷。在一些实施例中,根据本发明的mRNA中10%至25%、15%至25%、25%至35%、35%至45%、45%至55%、55%至65%、65%至75%、75%至85%、85%至95%或90%至100%的尿苷位置为N1-甲基假尿苷。在一些实施例中,根据本发明的mRNA中10%至25%、15%至25%、25%至35%、35%至45%、45%至55%、55%至65%、65%至75%、75%至85%、85%至95%或90%至100%的尿苷位置为5-碘尿苷。在一些实施例中,根据本发明的mRNA中10%至25%、15%至25%、25%至35%、35%至45%、45%至55%、55%至65%、65%至75%、75%至85%、85%至95%或90%至100%的尿苷位置为5-甲氧基尿苷,且其余者为N1-甲基假尿苷。在一些实施例中,根据本发明的mRNA中10%至25%、15%至25%、25%至35%、35%至45%、45%至55%、55%至65%、65%至75%、75%至85%、85%至95%或90%至100%的尿苷位置为5-碘尿苷,且其余者为N1-甲基假尿苷。

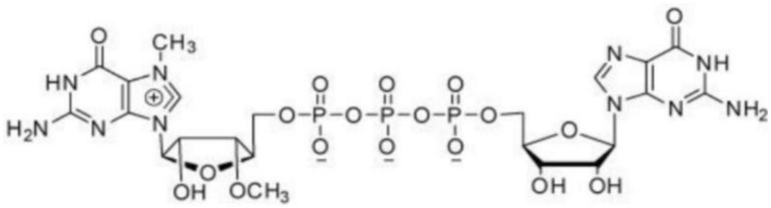
[0282] 11.5′帽结构

[0283] 在一些实施例中,本文所揭示的mRNA包含5′帽结构,诸如Cap0、Cap1或Cap2。5′帽结构通常为经由5′-三磷酸连接至mRNA的5′至3′链的第一个核苷酸,即第一个帽结构近端

核苷酸的5′位的7-甲基鸟嘌呤核糖核苷酸(其可经进一步修饰,如下文例如关于ARCA所论述)。在Cap0中,mRNA的第一及第二帽结构近端核苷酸的核糖两者均包含2′-羟基。在Cap1中,mRNA的第一及第二转录核苷酸的核糖分别包含2′-甲氧基及2′-羟基。在Cap2中,mRNA的第一及第二帽结构近端核苷酸的核糖两者均包含2′-甲氧基。参见例如Katibah等人(2014) Proc Natl Acad Sci USA 111(33):12025-30;Abbas等人(2017)Proc Natl Acad Sci USA 114(11):E2106-E2115。大多数内源高等真核生物mRNA,包括哺乳动物mRNA(诸如人类mRNA)包含Cap1或Cap2。归因于通过先天免疫系统的组分,诸如IFIT-1及IFIT-5识别为“非自体(non-self)”,Cap0及与Cap1及Cap2不同的其它帽结构在哺乳动物,诸如人类中可呈免疫原性,其可导致细胞介素,包括I型干扰素含量升高。先天免疫系统的组分,诸如IFIT-1及IFIT-5亦可与eIF4E竞争结合具有除Cap1或Cap2外的帽结构的mRNA,此可能会抑制mRNA的转译。

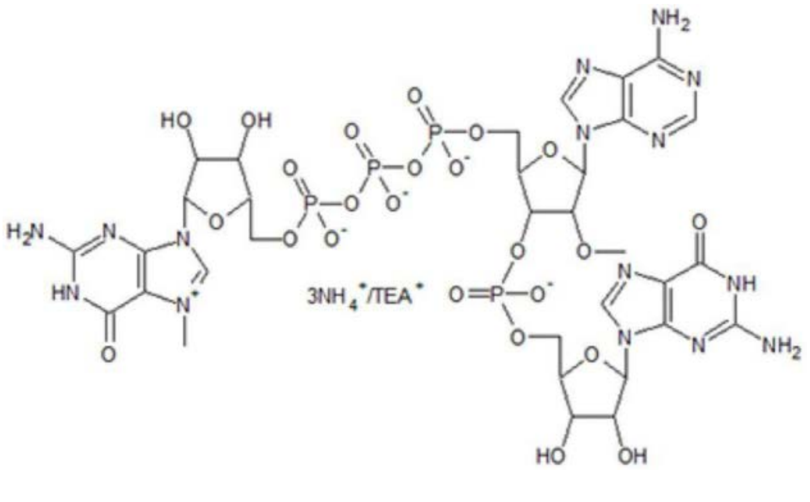
[0284]　帽结构可以共转录方式包括在内。例如,ARCA(抗反向帽结构类似物;Thermo Fisher Scientific目录号AM8045)为包含连接至鸟嘌呤核糖核苷酸的5′位的7-甲基鸟嘌呤3′-甲氧基-5′-三磷酸的帽结构类似物,其可在一开始时活体外并入转录物中。ARCA产生Cap0帽结构,其中第一个帽结构近端核苷酸的2′位为羟基。参见例如,Stepinski等人(2001)“Synthesis and properties of mRNAs containing the novel‘anti-reverse’cap analogs 7-methyl(3′-O-methyl)GpppG and 7-methyl(3′deoxy)GpppG”,RNA 7:1486-1495。ARCA结构显示如下。

[0285]



[0286]　CleanCap™ AG(m7G(5′)ppp(5′)(2′OMeA)pG;TriLink Biotechnologies目录号N-7113)或CleanCap™ GG(m7G(5′)ppp(5′)(2′OMeG)pG;TriLink Biotechnologies目录号N-7133)可用于以共转录方式提供Cap1结构。CleanCap™ AG及CleanCap™GG的3′-O-甲基化形式亦可分别以目录号N-7413及N-7433购自TriLink Biotechnologies。CleanCap™ AG结构显示如下。CleanCap™结构在本文中有时会使用上文所列的目录号的最后三个数字来指代(例如对于TriLink Biotechnologies目录号N-7113,使用“CleanCap™ 113”指代)。

[0287]



[0288] 可替代地,可以转录后方式将帽结构添加至RNA。例如,牛痘加帽结构酶可在市面上购得(New England Biolabs目录号M2080S),且具有由其D1亚基提供的RNA三磷酸酶及鸟苷酰基转移酶活性及由其D12亚基提供的鸟嘌呤甲基转移酶活性。因此,在S-腺苷甲硫氨酸及GTP存在下,可将7-甲基鸟嘌呤添加至RNA,以产生Cap0。参见例如Guo,P.及Moss,B.(1990)Proc.Natl.Acad.Sci.USA 87,4023-4027;Mao,X.及Shuman,S.(1994)J.Biol.Chem.269,24472-24479。对于帽结构及加帽结构方法的其它论述,参见例如WO2017/053297及Ishikawa等人,Nucl.Acids.Symp.Ser.(2009)第53期,129-130。

[0289] 12.引导RNA

[0290] 在一些实施例中,与本文所揭示的mRNA组合来提供至少一个引导RNA。在一些实施例中,引导RNA提供呈与mRNA分离的分子。在一些实施例中,引导RNA提供呈本文所揭示的mRNA的一部分,诸如UTR的一部分。在一些实施例中,至少一个引导RNA靶向TTR。

[0291] 在一些实施例中,引导RNA包含经修饰的sgRNA。在一些实施例中,sgRNA包含SEQ ID NO:74中所示出的修饰模式,其中N为任何天然或非天然核苷酸,且其中全部N′均包含引导序列。例如,SEQ ID NO:74涵盖在本文中,其中N′经本文所揭示的引导序列中的任一者置换。尽管N′经引导物的核苷酸取代,但修饰仍如SEQ ID NO:74中所示。即,尽管引导的核苷酸置换“N′”,但前三个核苷酸仍为经2′OMe修饰的,且在第一核苷酸与第二核苷酸、第二核苷酸与第三核苷酸及第三核苷酸与第四核苷酸之间存在硫代磷酸酯键。

[0292] 13.脂质;调配物;递送

[0293] 在一些实施例中,将本文所述的单独或伴有一或多个引导RNA的mRNA调配于脂质纳米颗粒中或经由该脂质纳米颗粒施用;参见例如2017年3月30日申请的要求2016年3月30日申请的U.S.S.N.62/315,602的优先权,且标题为“CRISPR/CAS组分的脂质纳米颗粒调配物(LIPID NANOPARTICLE FORMULATIONS FOR CRISPR/CAS COMPONENTS)”的PCT/US2017/024973,该文献的内容以全文引用的方式并入本文中。可利用熟习此项技术者已知的能够将核苷酸递送至个体的任何脂质纳米颗粒(LNP)来施用本文所述的RNA,在一些实施例中,该RNA伴有一或多个引导RNA。在一些实施例中,将本文所述的单独或伴有一或多个引导RNA的mRNA调配于脂质体、纳米颗粒、胞外体或微囊泡中或经由脂质体、纳米颗粒、胞外体或微囊泡施用。乳液、微胞及悬浮液可为用于局部及/或表面递送的适合组合物。

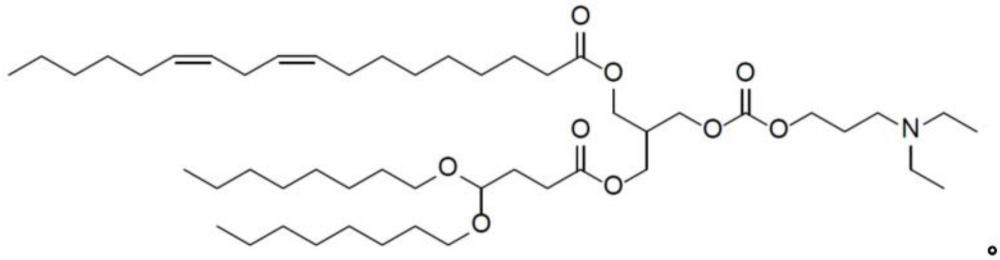
[0294] 本文揭示RNA的LNP调配物,包括CRISPR/Cas载荷(CRISPR/Cas cargo)的多个实施例。此类LNP调配物可包括(i)CCD脂质,诸如胺脂质、(ii)中性脂质、(iii)辅助脂质及(iv)隐形脂质,诸如PEG脂质。LNP调配物的一些实施例包括“胺脂质”以及辅助脂质、中性脂质及隐形脂质,诸如PEG脂质。“脂质纳米颗粒”意谓包含通过分子间力彼此物理缔合的复数个(即多于一个)脂质分子的粒子。

[0295] CCD脂质

[0296] 用于将CRISPR/Cas mRNA及引导RNA组分递送至肝脏细胞的脂质组合物包含CCD脂质。

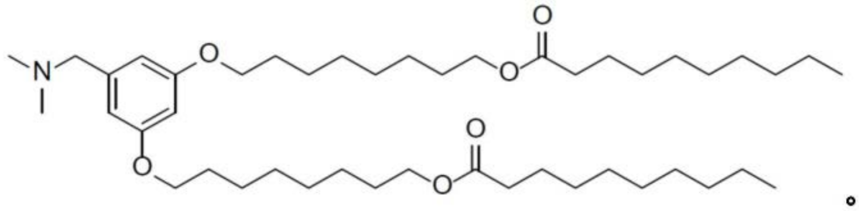
[0297] 在一些实施例中,CCD脂质为脂质A,其为十八-9,12-二烯酸(9Z,12Z)-3-((4,4-双(辛氧基)丁酰基)氧基)-2-((((3-(二乙胺基)丙氧基)羰基)氧基)甲基)丙酯,亦称作(9Z,12Z)-十八-9,12-二烯酸3-((4,4-双(辛氧基)丁酰基)氧基)-2-((((3-(二乙胺基)丙氧基)羰基)氧基)甲基)丙酯。

[0298] 脂质A可描绘为：

[0299] 

[0300] 可根据WO2015/095340(例如第84-86页)合成脂质A。

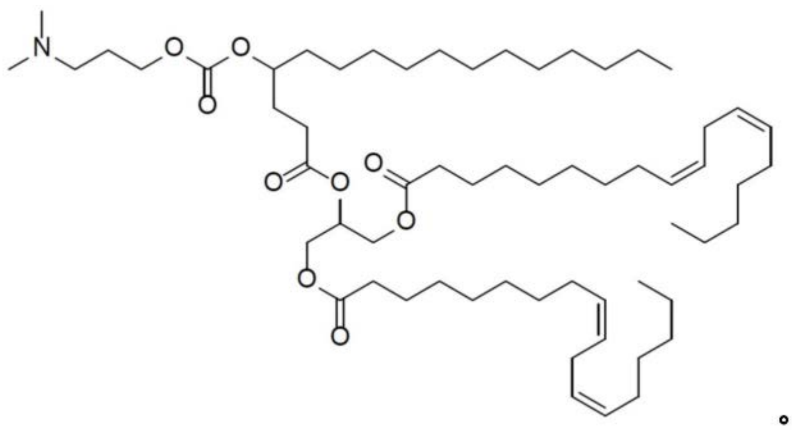
[0301] 在一些实施例中,CCD脂质为脂质B,其为((5-((二甲胺基)甲基)-1,3-伸苯基)双(氧基))双(辛烷-8,1-二基)双(癸酸酯),亦称作((5-((二甲胺基)甲基)-1,3-伸苯基)双(氧基))双(辛烷-8,1-二基)双(癸酸酯)。脂质B可描绘为：

[0302] 

[0303] 可根据WO2014/136086(例如第107-109页)合成脂质B。

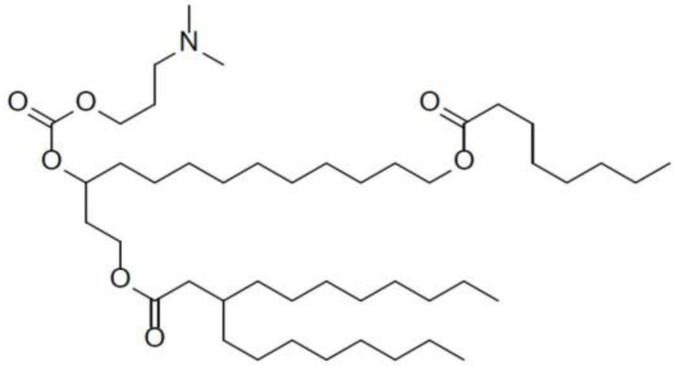
[0304] 在一些实施例中,CCD脂质为脂质C,其为2-((4-(((3-(二甲胺基)丙氧基)羰基)氧基)十六酰基)氧基)丙烷-1,3-二基(9Z,9′Z,12Z,12′Z)-双(十八-9,12-二烯酸酯)。

[0305] 脂质C可描绘为：

[0306] 

[0307] 在一些实施例中,CCD脂质为脂质D,其为3-辛基十一烷酸3-(((3-(二甲胺基)丙氧基)羰基)氧基)-13-(辛酰氧基)十三酯。

[0308] 脂质D可描绘为：

[0309] 

。

[0310] 可根据WO2015/095340合成脂质C及脂质D。

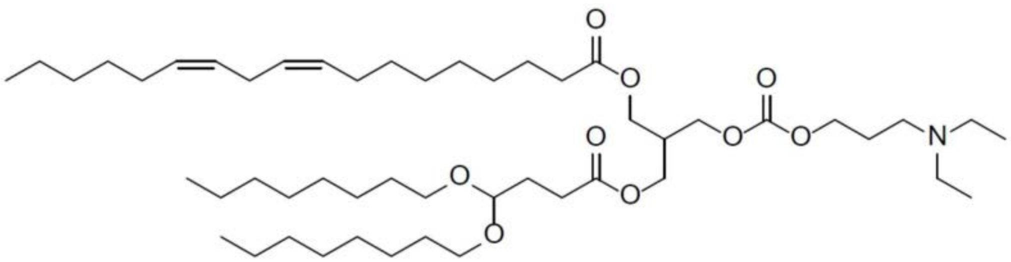
[0311] CCD脂质亦可为脂质A、脂质B、脂质C或脂质D的等效物。在某些实施例中,CCD脂质为脂质A的等效物、脂质B的等效物、脂质C的等效物或脂质D的等效物。

[0312] 胺脂质

[0313] 在一些实施例中,用于生物活性剂的递送的LNP组合物包含“胺脂质”,其定义为脂质A或其等效物,包括脂质A的缩醛类似物。

[0314] 在一些实施例中,胺脂质为脂质A,其为十八-9,12-二烯酸(9Z,12Z)-3-((4,4-双(辛氧基)丁酰基)氧基)-2-((((3-(二乙胺基)丙氧基)羰基)氧基)甲基)丙酯,亦称作(9Z,12Z)-十八-9,12-二烯酸3-((4,4-双(辛氧基)丁酰基)氧基)-2-((((3-(二乙胺基)丙氧基)羰基)氧基)甲基)丙酯。

[0315] 脂质A可描绘为:

[0316] 

。

[0317] 可根据WO2015/095340(例如第84-86页)合成脂质A。在某些实施例中,胺脂质为脂质A的等效物。

[0318] 在某些实施例中,胺脂质为脂质A的类似物。在某些实施例中,脂质A类似物为脂质A的缩醛类似物。在特定LNP组合物中,缩醛类似物为C4-C12缩醛类似物。在一些实施例中,缩醛类似物为C5-C12缩醛类似物。在其它实施例中,缩醛类似物为C5-C10缩醛类似物。在其它实施例中,缩醛类似物是选自C4、C5、C6、C7、C9、C10、C11及C12缩醛类似物。

[0319] 适用于本文所述的LNP中的胺脂质可活体内生物降解。胺脂质具有低毒性(例如以大于或等于10mg/kg的量在动物模型中得到耐受而不具有不良作用)。在某些实施例中,包含胺脂质的LNP包括其中会在8、10、12、24或48小时或3、4、5、6、7或10天内自血浆清除至少75%的胺脂质的LNP。在某些实施例中,包含胺脂质的LNP包括其中会在8、10、12、24或48小时或3、4、5、6、7或10天内自血浆清除至少50%的mRNA或gRNA的LNP。在某些实施例中,包含胺脂质的LNP包括其中例如通过测量脂质(例如,胺脂质)、RNA(例如mRNA)或其它组分,会在8、10、12、24或48小时或3、4、5、6、7或10天内自血浆清除至少50%的LNP的LNP。在某些实施例中,测量LNP的经脂质囊封组分对游离脂质组分、RNA组分或核酸组分。

[0320] 脂质清除率可如文献中所述来测量。参见Maier,M.A.等人Biodegradable Lipids Enabling Rapidly Eliminated Lipid Nanoparticles for Systemic Delivery of RNAi Therapeutics.Mol.Ther.2013,21(8),1570-78(“Maier”)。例如,在Maier中,以0.3mg/kg通过静脉内快速注射经由外侧尾部静脉向六至八周龄雄性C57BL/6小鼠施用含有靶向荧光素酶的siRNA的LNP-siRNA系统。在给药后0.083、0.25、0.5、1、2、4、8、24、48、96及168小时收集血液、肝脏及脾脏样本。在收集组织之前向小鼠灌注生理盐水且处理血液样本以获得血浆。处理且通过LC-MS分析所有样本。此外,Maier描述了一种用于评定施用LNP-siRNA调配物之后的毒性的程序。例如,以0、1、3、5及10mg/kg(5只动物/组)经由单一静脉内快速注射以5mL/kg的剂量体积向雄性史泊格-多利大鼠(Sprague-Dawley rat)施用靶向荧光素酶的siRNA。在24小时之后,由清醒的动物的颈静脉获得约1mL血液且分离血清。在给药后72小时,将所有动物安乐死以用于尸体剖检。进行临床症状、体重、血清化学、器官重量及组织病理学的评定。尽管Maier描述了用于评定siRNA-LNP调配物的方法,但此等方法亦可适用于评定本发明的LNP组合物的投药的清除率、药物动力学及毒性。

[0321] 胺脂质引起增加的清除速率。在一些实施例中,清除速率为脂质清除速率,例如自血液、血清或血浆清除胺脂质的速率。在一些实施例中,清除速率为RNA清除速率,例如自血液、血清或血浆清除mRNA或gRNA的速率。在一些实施例中,清除速率为自血液、血清或血浆清除LNP的速率。在一些实施例中,清除速率为自组织,诸如肝脏组织或脾脏组织清除LNP的速率。在某些实施例中,清除速率的高速率会产生不具有显著不良作用的安全分布。胺脂质减少循环中及组织中的LNP积聚。在一些实施例中,循环中及组织中的LNP积聚减少会产生不具有显著不良作用的安全分布。

[0322] 可视本发明的胺脂质所处的培养基的pH而定,对该胺脂质进行电离。例如,在弱酸性培养基中,胺脂质可经质子化且因此带有正电荷。相反地,在弱碱性培养基中,诸如其中pH为大约7.35的血液中,胺脂质可不经质子化且因此不带电荷。在一些实施例中,可在至少约9的pH下对本发明的胺脂质进行质子化。在一些实施例中,可在至少约10的pH下对本发明的胺脂质进行质子化。

[0323] 胺脂质带有电荷的能力与其固有pKa有关。例如,本发明的胺脂质可各自独立地具有在约5.8至约6.2范围内的pKa。例如,本发明的胺脂质可各自独立地具有在约5.8至约6.5范围内的pKa。当已发现,pKa在约5.1至约7.4范围内的阳离子型脂质对将载荷活体内递送至例如肝脏为有效的时,此可为有利的。此外,已发现,pKa在约5.3至约6.4范围内的阳离子型脂质对活体内递送至例如肿瘤为有效的。参见例如WO2014/136086。

[0324] 其它脂质

[0325] 适用于本发明的脂质组合物中的“中性脂质”包括例如多种中性、不带电荷或两性离子型脂质。适用于本发明中的中性磷脂的实例包括(但不限于)5-十七基苯-1,3-二醇(间苯二酚)、二软脂酰基磷脂酰胆碱(DPPC)、二硬脂酰基磷脂酰胆碱(DSPC)、磷酸胆碱(DOPC)、二肉豆蔻酰基磷脂酰胆碱(DMPC)、磷脂酰胆碱(PLPC)、1,2-二硬脂酰基-sn-甘油-3-磷酸胆碱(DAPC)、磷脂酰乙醇胺(PE)、卵磷脂酰胆碱(EPC)、二月桂酰基磷脂酰胆碱(DLPC)、1-肉豆蔻酰基-2-软脂酰基磷脂酰胆碱(MPPC)、1-软脂酰基-2-肉豆蔻酰基磷脂酰胆碱(PMPC)、1-软脂酰基-2-硬脂酰基磷脂酰胆碱(PSPC)、1,2-二花生酰基-sn-甘油-3-磷酸胆碱(DBPC)、1-硬脂酰基-2-软脂酰基磷脂酰胆碱(SPPC)、1,2-二十碳烯酰基-sn-甘油-3-磷酸胆碱

(DEPC)、软脂酰油酰基磷脂酰胆碱(POPC)、溶血磷脂酰基胆碱、二油酰基磷脂酰乙醇胺(DOPE)、二亚油酰基磷脂酰胆碱二硬脂酰基磷脂酰乙醇胺(DSPE)、二肉豆蔻酰基磷脂酰乙醇胺(DMPE)、二软脂酰基磷脂酰乙醇胺(DPPE)、软脂酰油酰基磷脂酰乙醇胺(POPE)、溶血磷脂酰乙醇胺及其组合。在一个实施例中,中性磷脂可选自由以下组成的群:二硬脂酰基磷脂酰胆碱(DSPC)及二肉豆蔻酰基磷脂酰乙醇胺(DMPE)。在另一实施例中,中性磷脂可为二硬脂酰基磷脂酰胆碱(DSPC)。

[0326] “辅助脂质”包括类固醇、固醇及烷基间苯二酚。适用于本发明中的辅助脂质包括(但不限于)胆固醇、5-十七基间苯二酚及胆固醇半丁二酸酯。在一个实施例中,辅助脂质可为胆固醇。在一个实施例中,辅助脂质可为胆固醇半丁二酸酯。

[0327] “隐形脂质”为改变纳米颗粒可在活体内(例如血液中)存在的时长的脂质。隐形脂质可通过例如减少粒子聚集且控制粒度辅助调配过程。本文中所用的隐形脂质可调节LNP的药物动力学特性。适用于本发明中的隐形脂质包括(但不限于)具有连接至脂质部分的亲水性头基的隐形脂质。适用于本发明脂质组合物的隐形脂质及关于此类脂质的生物化学的信息可见于Romberg等人,Pharmaceutical Research,第25卷,第1期,2008,第55-71页及Hoekstra等人,Biochimica et Biophysica Acta 1660(2004)41-52。额外适合PEG脂质揭示于例如WO 2006/007712中。

[0328] 在一个实施例中,隐形脂质的亲水性头基包含选自基于PEG的聚合物的聚合物部分。隐形脂质可包含脂质部分。在一些实施例中,隐形脂质为PEG脂质。

[0329] 在一个实施例中,隐形脂质包含选自基于以下的聚合物的聚合物部分:PEG(有时称作聚(环氧乙烷))、聚(恶唑啉)、聚(乙烯醇)、聚(甘油)、聚(N-乙烯基吡咯啶酮)、聚氨基酸及聚[N-(2-羟丙基)甲基丙烯酰胺]。

[0330] 在一个实施例中,PEG脂质包含基于PEG(有时称作聚(环氧乙烷))的聚合物部分。

[0331] PEG脂质进一步包含脂质部分。在一些实施例中,脂质部分可源自二酰基甘油或二酰基甘油酰胺(diacylglycamide),包括包含二烷基甘油基或二烷基甘油酰胺基的那些,该基团具有独立地包含约C4至约C40饱和或不饱和碳原子的烷基链长,其中该链可包含一或多个官能基,诸如酰胺基或酯基。在一些实施例中,烷基链长度包含约C10至C20。二烷基甘油或二烷基甘油酰胺基可进一步包含一或多个经取代的烷基。链长可为对称或不对称的。

[0332] 除非另外指明,否则如本文所用,术语“PEG”意谓任何聚乙二醇或其它聚伸烷醚聚合物。在一个实施例中,PEG为乙二醇或环氧乙烷的视情况经取代的直链或分支链聚合物。在一个实施例中,PEG为未经取代的。在一个实施例中,PEG经例如一或多个烷基、烷氧基、酰基、羟基、或芳基取代。在一个实施例中,该术语包括PEG共聚物,诸如PEG-聚胺基甲酸酯或PEG-聚丙烯(参见例如J.Milton Harris,Poly(ethylene glycol)chemistry:biotechnical and biomedical applications(1992));在另一实施例中,该术语不包括PEG共聚物。在一个实施例中,PEG的分子量为约130至约50,000,在一子实施例中,约150至约30,000,在一子实施例中,约150至约20,000,在一子实施例中,约150至约15,000,在一子实施例中,约150至约10,000,在一子实施例中,约150至约6,000,在一子实施例中,约150至约5,000,在一子实施例中,约150至约4,000,在一子实施例中,约150至约3,000,在一子实施例中,约300至约3,000,在一子实施例中,约1,000至约3,000且在一子实施例中,约1,500至约2,500。

[0333] 在某些实施例中,PEG(例如与脂质部分或脂质,诸如隐形脂质结合)、为“PEG-2K”,亦称为“PEG2000”,其具有约2,000道尔顿的平均分子量。PEG-2K在本文中由下式(I)表示,其中n为45,意谓经数量平均化的聚合度包含约45个亚基。然而,亦可使用此项技术中已知的其它PEG实施例,包括例如其中经数量平均化的聚合度包含约23个亚基(n=23)及/或68个亚基(n=68)的那些。在一些实施例中,n可在约30至约60范围内。在一些实施例中,n可在约35至约55范围内。在一些实施例中,n可在约40至约50范围内。在一些实施例中,n可在约42至约48范围内。在一些实施例中,n可为45。在一些实施例中,R可选自H、经取代的烷基及未经取代的烷基。在一些实施例中,R可为未经取代的烷基。在一些实施例中,R可为甲基。

[0334] 在本文中描述的任一实施例中,PEG脂质可选自PEG-二月桂酰甘油、PEG-二肉豆蔻酰甘油(PEG-DMG)(目录号GM-020,来自日本东京的NOF)、PEG-二软脂酰甘油、PEG-二硬脂酰甘油(PEG-DSPE)(目录号DSPE-020CN,日本东京的NOF)、PEG-二月桂酰甘油酰胺、PEG-二肉豆蔻酰甘油酰胺、PEG-二软脂酰甘油酰胺、及PEG-二硬脂酰甘油酰胺、PEG-胆固醇(1-[8′-(胆甾-5-烯-3[β]-氧基)甲酰胺基-3′,6′-二氧杂辛烷]胺甲酰基-[ω]-甲基-聚(乙二醇)、PEG-DMB(3,4-双十四氧基苯甲基-[ω]-甲基-聚(乙二醇)醚)、1,2-二肉豆蔻酰基-sn-甘油-3-磷酸乙醇胺-N-[甲氧基(聚乙二醇)-2000](PEG2k-DMG)(目录号880150P,来自美国亚拉巴马州阿拉巴斯特(Alabaster,Alabama,USA)的Avanti Polar Lipids)、1,2-二硬脂酰基-sn-甘油-3-磷酸乙醇胺-N-[甲氧基(聚乙二醇)-2000](PEG2k-DSPE)(目录号880120C,来自美国亚拉巴马州阿拉巴斯特的Avanti Polar Lipids)、1,2-二硬脂酰基-sn-甘油,甲氧基聚乙二醇(PEG2k-DSG;GS-020,日本东京的NOF)、聚(乙二醇)-2000-二甲基丙烯酸酯(PEG2k-DMA)、及1,2-二硬脂氧基丙基-3-胺-N-[甲氧基(聚乙二醇)-2000](PEG2k-DSA)。在一个实施例中,PEG脂质可为PEG2k-DMG。在一些实施例中,PEG脂质可为PEG2k-DSG。在一个实施例中,PEG脂质可为PEG2k-DSPE。在一个实施例中,PEG脂质可为PEG2k-DMA。在一个实施例中,PEG脂质可为PEG2k-C-DMA。在一个实施例中,PEG脂质可为化合物S027,其揭示于WO2016/010840(第[00240]至[00244]段)中。在一个实施例中,PEG脂质可为PEG2k-DSA。在一个实施例中,PEG脂质可为PEG2k-C11。在一些实施例中,PEG脂质可为PEG2k-C14。在一些实施例中,PEG脂质可为PEG2k-C16。在一些实施例中,PEG脂质可为PEG2k-C18。

[0335] LNP可含有(i)用于囊封及用于核内体逃逸(endosomal escape)的胺脂质、(ii)用于稳定的中性脂质、(iii)亦用于稳定的辅助脂质及(iv)隐形脂质,诸如PEG脂质。

[0336] 在一些实施例中,LNP组合物可包含RNA组分,其包括以下中的一种或多种:RNA引导的DNA结合剂、Cas核酸酶mRNA、2类Cas核酸酶mRNA、Cas9 mRNA及gRNA。在一些实施例中,LNP组合物可包括2类Cas核酸酶及gRNA作为RNA组分。在某些实施例中,LNP组合物可包含RNA组分、胺脂质、辅助脂质、中性脂质及隐形脂质。在某些LNP组合物中,辅助脂质为胆固醇。在其它组合物中,中性脂质为DSPC。在其它实施例中,隐形脂质为PEG2k-DMG或PEG2k-C11。在某些实施例中,LNP组合物包含脂质A或脂质A的等效物;辅助脂质;中性脂质;隐形脂质;及引导RNA。在某些组合物中,胺脂质为脂质A。在某些组合物中,胺脂质为脂质A或其缩醛类似物;辅助脂质为胆固醇;中性脂质为DSPC且隐形脂质为PEG2k—DMG。

[0337] 在某些实施例中,根据调配物中脂质组分的相应摩尔比描述脂质组合物。本发明的实施例提供根据调配物中脂质组分的相应摩尔比描述的脂质组合物。在一个实施例中,胺脂质的mol%可为约30mol%至约60mol%。在一个实施例中,胺脂质的mol%可为约

40mol％至约60mol％。在一个实施例中，胺脂质的mol％可为约45mol％至约60mol％。在一个实施例中，胺脂质的mol％可为约50mol％至约60mol％。在一个实施例中，胺脂质的mol％可为约55mol％至约60mol％。在一个实施例中，胺脂质的mol％可为约50mol％至约55mol％。在一个实施例中，胺脂质的mol％可为约50mol％。在一个实施例中，胺脂质的mol％可为约55mol％。在一些实施例中，LNP批料的胺脂质mol％将为目标mol％的±30％、±25％、±20％、±15％、±10％、±5％或±2.5％。在一些实施例中，LNP批料的胺脂质mol％将为目标mol％的±4mol％、±3mol％、±2mol％、±1.5mol％、±1mol％、±0.5mol％或±0.25mol％。所有mol％数均以LNP组合物的脂质组分的一定分率给定。在某些实施例中，胺脂质mol％的LNP批次间变化率将低于15％、低于10％或小于5％。

[0338] 在一个实施例中，中性脂质的mol％可为约5mol％至约15mol％。在一个实施例中，中性脂质的mol％可为约7mol％至约12mol％。在一个实施例中，中性脂质的mol％可为约9mol％。在一些实施例中，LNP批料的中性脂质mol％将为目标中性脂质mol％的±30％、±25％、±20％、±15％、±10％、±5％或±2.5％。在某些实施例中，LNP批次间变化率将低于15％、低于10％或小于5％。

[0339] 在一个实施例中，辅助脂质的mol％可为约20mol％至约60mol％。在一个实施例中，辅助脂质的mol％可为约25mol％至约55mol％。在一个实施例中，辅助脂质的mol％可为约25mol％至约50mol％。在一个实施例中，辅助脂质的mol％可为约25mol％至约40mol％。在一个实施例中，辅助脂质的mol％可为约30mol％至约50mol％。在一个实施例中，辅助脂质的mol％可为约30mol％至约40mol％。在一个实施例中，基于胺脂质、中性脂质及PEG脂质浓度而调整辅助脂质的mol％以使脂质组分达100mol％。在一些实施例中，LNP批料的辅助脂质mol％将为目标mol％的±30％、±25％、±20％、±15％、±10％、±5％或±2.5％。在某些实施例中，LNP批次间变化率将低于15％、低于10％或小于5％。

[0340] 在一个实施例中，PEG脂质的mol％可为约1mol％至约10mol％。在一个实施例中，PEG脂质的mol％可为约2mol％至约10mol％。在一个实施例中，PEG脂质的mol％可为约2mol％至约8mol％。在一个实施例中，PEG脂质的mol％可为约2mol％至约4mol％。在一个实施例中，PEG脂质的mol％可为约2.5mol％至约4mol％。在一个实施例中，PEG脂质的mol％可为约3mol％。在一个实施例中，PEG脂质的mol％可为约2.5mol％。在一些实施例中，LNP批料的PEG脂质mol％将为目标PEG脂质mol％的±30％、4±25％、±20％、±15％、±10％、±5％或±2.5％。在某些实施例中，LNP批次间变化率将低于15％、低于10％或小于5％。

[0341] 在某些实施例中，载荷包括编码RNA引导的DNA结合剂(例如Cas核酸酶、2类Cas核酸酶或Cas9)的mRNA，及gRNA或编码gRNA的核酸，或mRNA及gRNA的组合。在一个实施例中，LNP组合物可包含脂质A或其等效物。在一些方面中，胺脂质为脂质A。在一些方面中，胺脂质为脂质A等效物，例如脂质A的类似物。在某些方面中，胺脂质为脂质A的缩醛类似物。在各种实施例中，LNP组合物包含胺脂质、中性脂质、辅助脂质及PEG脂质。在某些实施例中，辅助脂质为胆固醇。在某些实施例中，中性脂质为DSPC。在特定实施例中，PEG脂质为PEG2k-DMG。在一些实施例中，LNP组合物可包含脂质A、辅助脂质、中性脂质及PEG脂质。在一些实施例中，LNP组合物包含胺脂质、DSPC、胆固醇及PEG脂质。在一些实施例中，LNP组合物包含PEG脂质，该脂质包含DMG。在某些实施例中，胺脂质选自脂质A，及脂质A的等效物，包括脂质A的缩醛类似物。在其它实施例中，LNP组合物包含脂质A、胆固醇、DSPC及PEG2k-DMG。

[0342] 本发明的实施例亦提供根据待囊封的核酸的胺脂质的带正电胺基(N)与带负电磷酸基团(P)之间的摩尔比描述的脂质组合物。此可由方程式N/P数学表示。在一些实施例中,LNP组合物可包含脂质组分,其包含胺脂质、辅助脂质、中性脂质及隐形脂质;及核酸组分,其中N/P比为约3至10。在一些实施例中,LNP组合物可包含脂质组分,其包含胺脂质、辅助脂质、中性脂质及隐形脂质;及RNA组分,其中N/P比为约3至10。在一个实施例中,N/P比可为约5至7。在一个实施例中,N/P比可为约4.5至8。在一个实施例中,N/P比可为约6。在一个实施例中,N/P比可为6±1。在一个实施例中,N/P比可为6±0.5。在一些实施例中,N/P比将为目标N/P比的±30%、±25%、±20%、±15%、±10%、±5%或±2.5%。在某些实施例中,LNP批次间变化率将低于15%、低于10%或小于5%。

[0343] 在一些实施例中,RNA组分可包含mRNA,诸如本文所揭示的mRNA,例如编码Cas核酸酶的mRNA。在一个实施例中,RNA组分可包含Cas9 mRNA。在一些包含编码Cas核酸酶的mRNA的组合物中,LNP进一步包含gRNA核酸,诸如gRNA。在一些实施例中,RNA组分包含Cas核酸酶mRNA及gRNA。在一些实施例中,RNA组分包含2类Cas核酸酶mRNA及gRNA。

[0344] 在某些实施例中,LNP组合物可包含编码Cas核酸酶(诸如2类Cas核酸酶)的mRNA、胺脂质、辅助脂质、中性脂质及PEG脂质。在某些包含编码Cas核酸酶(诸如2类Cas核酸酶)的mRNA的LNP组合物中,辅助脂质为胆固醇。在其它包含编码Cas核酸酶(诸如2类Cas核酸酶)的mRNA的组合物中,中性脂质为DSPC。在其它包含编码Cas核酸酶(诸如2类Cas核酸酶)的mRNA的实施例中,PEG脂质为PEG2k-DMG或PEG2k-C11。在包含编码Cas核酸酶(诸如2类Cas核酸酶)的mRNA的特定组合物中,胺脂质选自脂质A及其等效物,诸如脂质A的缩醛类似物。

[0345] 在一些实施例中,LNP组合物可包含gRNA。在某些实施例中,LNP组合物可包含胺脂质、gRNA、辅助脂质、中性脂质及PEG脂质。在某些包含gRNA的LNP组合物中,辅助脂质为胆固醇。在一些包含gRNA的组合物中,中性脂质为DSPC。在其它包含gRNA的实施例中,PEG脂质为PEG2k-DMG或PEG2k-C11。在某些实施例中,胺脂质选自脂质A及其等效物,诸如脂质A的缩醛类似物。

[0346] 在一个实施例中,LNP组合物可包含sgRNA。在一个实施例中,LNP组合物可包含Cas9 sgRNA。在一个实施例中,LNP组合物可包含Cpf1 sgRNA。在一些包含sgRNA的组合物中,LNP包括胺脂质、辅助脂质、中性脂质及PEG脂质。在某些包含sgRNA的组合物中,辅助脂质为胆固醇。在其它包含sgRNA的组合物中,中性脂质为DSPC。在其它包含sgRNA的实施例中,PEG脂质为PEG2k-DMG或PEG2k-C11。在某些实施例中,胺脂质选自脂质A及其等效物,诸如脂质A的缩醛类似物。

[0347] 在某些实施例中,LNP组合物包含本文所揭示的mRNA,例如编码Cas核酸酶的mRNA,及gRNA,其可为sgRNA。在一个实施例中,LNP组合物可包含胺脂质、编码Cas核酸酶的mRNA、gRNA、辅助脂质、中性脂质及PEG脂质。在某些包含编码Cas核酸酶的mRNA及gRNA的组合物中,辅助脂质为胆固醇。在一些包含编码Cas核酸酶的mRNA及gRNA的组合物中,中性脂质为DSPC。在其它包含编码Cas核酸酶的mRNA及gRNA的实施例中,PEG脂质为PEG2k-DMG或PEG2k-C11。在某些实施例中,胺脂质选自脂质A及其等效物,诸如脂质A的缩醛类似物。

[0348] 在某些实施例中,LNP组合物包括Cas核酸酶mRNA,诸如2类Cas mRNA,及至少一个gRNA。在某些实施例中,LNP组合物包括比率为约25:1至约1:25的gRNA与Cas核酸酶mRNA,诸如2类Cas核酸酶mRNA。在某些实施例中,LNP调配物包括比率为约10:1至约1:10的gRNA与

Cas核酸酶mRNA,诸如2类Cas核酸酶mRNA。在某些实施例中,LNP调配物包括比率为约8:1至约1:8的gRNA与Cas核酸酶mRNA,诸如2类Cas核酸酶mRNA。如本文中所测量,比率是以重量计。在一些实施例中,LNP调配物包括比率为约5:1至约1:5的gRNA与Cas核酸酶mRNA,诸如2类Cas mRNA。在一些实施例中,比率范围为约3:1至1:3、约2:1至1:2、约5:1至1:2、约5:1至1:1、约3:1至1:2、约3:1至1:1、约3:1、约2:1至1:1。在一些实施例中,gRNA与mRNA的比为约3:1或约2:1。在一些实施例中,gRNA与Cas核酸酶mRNA,诸如2类Cas核酸酶的比为约1:1。比率可为约25:1、10:1、5:1、3:1、1:1、1:3、1:5、1:10或1:25。

[0349] 本文中所揭示的LNP组合物可包括模板核酸。模板核酸可经编码Cas核酸酶的mRNA,诸如2类Cas核酸酶mRNA共调配。在一些实施例中,模板核酸可经引导RNA共调配。在一些实施例中,模板核酸可经编码Cas核酸酶的mRNA及引导RNA两者共调配。在一些实施例中,模板核酸可经编码Cas核酸酶的mRNA及引导RNA分开调配。模板核酸可与LNP组合物一起或与其分开递送。在一些实施例中,模板核酸可为单股或双股的,其视所需修复机制而定。模板可具有与靶DNA或与邻近于靶DNA的序列同源的区域。

[0350] 本文所述的LNP及LNP调配物中的任一者适用于递送单独或与一或多个引导RNA一起的编码RNA引导的DNA结合剂,诸如Cas核酸酶的mRNA。在一些实施例中,涵盖一种包含以下的LNP组合物:RNA组分及脂质组分,其中脂质组分包含胺脂质、中性脂质、辅助脂质及隐形脂质;且其中N/P比为约1至10。

[0351] 在一些情况下,脂质组分包含脂质A或其缩醛类似物、胆固醇、DSPC及PEG-DMG;且其中N/P比为约1至10。在一些实施例中,脂质组分包含:约40至60mol%胺脂质;约5至15mol%中性脂质;及约1.5%至10mol%PEG脂质,其中脂质组分的其余部分为辅助脂质,且其中LNP组合物的N/P比为约3至10。在一些实施例中,脂质组分包含:约50至60mol%胺脂质;约8至10mol%中性脂质;及约2.5%至4mol%PEG脂质,其中脂质组分的其余部分为辅助脂质,且其中LNP组合物的N/P比为约3至8。在一些情况下,脂质组分包含:约50至60mol%胺脂质;约5至15mol%DSPC;及约2.5%至4mol%PEG脂质,其中脂质组分的其余部分为胆固醇,且其中LNP组合物的N/P比为约3至8。在一些情况下,脂质组分包含:48至53mol%脂质A;约8至10mol%DSPC;及约1.5%至10mol%PEG脂质,其中脂质组分的其余部分为胆固醇,且其中LNP组合物的N/P比为3至8±0.2。

[0352] 在一些实施例中,LNP是通过混合RNA水溶液与有机溶剂基脂质溶液,例如100%乙醇而形成。适合溶液或溶剂包括或可含有:水、PBS、Tris缓冲液、NaCl、柠檬酸盐缓冲液、乙醇、氯仿、二乙醚、环己烷、四氢呋喃、甲醇、异丙醇。可将医药学上可接受的缓冲液用于例如LNP的活体内投药。在某些实施例中,缓冲液用于将包含LNP的组合物的pH维持处于或高于pH 6.5。在某些实施例中,缓冲液用于将包含LNP的组合物的pH维持处于或高于pH 7.0。在某些实施例中,组合物的pH在约7.2至约7.7范围内。在其它实施例中,组合物的pH在约7.3至约7.7范围内或约7.4至约7.6范围内。在其它实施例中,组合物的pH为约7.2、7.3、7.4、7.5、7.6或7.7。组合物的pH可用微型pH探针进行测量。在某些实施例中,组合物中包括低温保护剂。低温保护剂的非限制性实例包括蔗糖、海藻糖、甘油、DMSO及乙二醇。示例性组合物可包括至多10%低温保护剂,诸如蔗糖。在某些实施例中,LNP组合物可包括约1%、2%、3%、4%、5%、6%、7%、8%、9%或10%低温保护剂。在某些实施例中,LNP组合物可包括约1%、2%、3%、4%、5%、6%、7%、8%、9%或10%蔗糖。在一些实施例中,LNP组合物可包括

缓冲液。在一些实施例中,缓冲液可包含磷酸酯缓冲液(PBS)、Tris缓冲液、柠檬酸盐缓冲液及其混合物。在某些示例性实施例中,缓冲液包含NaCl。在某些实施例中,省去NaCl。NaCl的示例性量可在约20mM至约45mM范围内。NaCl的示例性量可在约40mM至约50mM范围内。在一些实施例中,NaCl的量为约45mM。在一些实施例中,缓冲液为Tris缓冲液。Tris的示例性量可在约20mM至约60mM范围内。Tris的示例性量可在约40mM至约60mM范围内。在一些实施例中,Tris的量为约50mM。在一些实施例中,缓冲液包含NaCl及Tris。LNP组合物的某些示例性实施例含有5%蔗糖及45mM NaCl的Tris缓冲液。在其它示例性实施例中,组合物含有呈约5%w/v的量的蔗糖、约45mM NaCl及pH 7.5下的约50mM Tris。盐、缓冲液及低温保护剂量可有所变化以使总体调配物的重量莫耳渗透浓度得到维持。例如,最终重量莫耳渗透浓度可维持低于450mOsm/L。在其它实施例中,重量莫耳渗透浓度在350与250mOsm/L之间。某些实施例的最终重量莫耳渗透浓度为300+/-20mOsm/L。

[0353] 在一些实施例中,使用微流混合、T型混合或交错混合。在某些方面中,流动速率、接头大小、接头几何结构、接头形状、管径、溶液及/或RNA及脂质浓度可有所变化。LNP或LNP组合物可例如经由渗析、切向流过滤或层析得到浓缩或纯化。LNP可以例如悬浮液、乳液或冻干粉末形式储存。在一些实施例中,LNP组合物储存于2至8℃下,在某些方面中,LNP组合物储存于室温下。在其它实施例中,冷冻储存,例如在-20°℃或-80℃下储存LNP组合物。在其它实施例中,将LNP组合物储存于约0℃至约-80℃范围内的温度下。可在使用之前例如在冰上,在4℃下、在室温下或在25℃下融化冷冻LNP组合物。可将冷冻LNP组合物维持在不同温度下,例如在冰上,在4℃下、在室温下、在25℃下或在37℃下。

[0354] 在一些实施例中,LNP组合物具有大于约80%的囊封率。在一些实施例中,LNP组合物的粒度小于约120nm。在一些实施例中,LNP组合物的pdi小于约0.2。在一些实施例中,存在此等特征中的至少两者。在一些实施例中,存在此等三个特征中的每一者。用于测定此等参数的分析方法论述于下文通用试剂及方法章节中。

[0355] 在一些实施例中,与本文所揭示的mRNA缔合的LNP供用于制备药剂。

[0356] 电致孔为递送载荷的熟知手段,且任何电致孔方法可用于递送本文所揭示的gRNA中的任一者。在一些实施例中,电致孔可用于递送本文所揭示的mRNA及一或多个引导RNA。

[0357] 在一些实施例中,提供一种用于将本文所揭示的mRNA递送至离体细胞的方法,其中mRNA与LNP缔合或不与LNP缔合。在一些实施例中,mRNA/LNP或mRNA亦与一或多个引导RNA缔合。

[0358] 在一些实施例中,当以医药组合物形式向哺乳动物施用本文所揭示的mRNA时,该哺乳动物展现出比施用最小尿苷含量大于150%的编码Cas9核酸酶的mRNA的哺乳动物要低至少1.5、2、2.5、3、3.5、4、4.5、5、5.5、6、7、7.5、8、8.5、9、9.5或10倍的细胞介素反应。可如实例中所述测定细胞介素反应。细胞介素反应之间的差异可以一组细胞介素,诸如以下细胞介素中的至少一种、两种、三种或四种的平均变化的形式来测量:IFN α、IL-6、TNF α及MCP-1。在一些实施例中,当以医药组合物形式向哺乳动物施用本文所揭示的mRNA时,该哺乳动物展现出比施用具有编码Cas9核酸酶的ORF的mRNA的哺乳动物要低至少1.5、2、2.5、3、3.5、4、4.5、5、5.5、6、7、7.5、8、8.5、9、9.5或10倍的细胞介素反应,其中ORF的序列由SEQ ID NO:5组成。在一些实施例中,具有由SEQ ID NO:5组成的序列的ORF中的尿苷为未经修饰的。据通常理解,除mRNA外的比较组合物的特征应保持恒定,包括剂量,且剂量应处于适当范

围,诸如0.1至5mpk或本文所述的其它范围内(例如,如mRNA功效的测定章节所论述)。

[0359] 在一些实施例中,编码引导RNA的核苷酸序列可位于相同载体、转录物或包含编码RNA引导的DNA结合剂的核苷酸序列的mRNA上。在一些实施例中,引导RNA的表达及RNA引导的DNA结合剂的表达可通过其自身对应启动子来驱动。在一些实施例中,引导RNA的表达可由驱动RNA引导的DNA结合剂的表达的相同启动子来驱动。在一些实施例中,引导RNA及编码RNA引导的DNA结合剂的ORF可包含于单一转录物中。例如,引导RNA可处于RNA引导的DNA结合剂转录物的非转译区(UTR)中。在一些实施例中,引导RNA可处于RNA引导的DNA结合剂转录物的5′UTR中。在一些实施例中,引导RNA可处于RNA引导的DNA结合剂转录物的3′UTR中。在一些实施例中,RNA引导的DNA结合剂转录物的胞内半衰期可通过将引导RNA包含在该转录物的3′UTR中且藉此缩短其3′UTR的长度而减小。在其它实施例中,引导RNA可处于RNA引导的DNA结合剂转录物的内含子中。在一些实施例中,可在引导RNA所处的内含子处添加适合剪接位点以使得自转录物恰当地剪接掉引导RNA。在一些实施例中,相同载体上紧邻的RNA引导的DNA结合剂及引导RNA的表达可促进较高效形成RNA引导的DNA结合剂与引导RNA的核糖核蛋白复合物。

[0360] 在一些实施例中,提供一种包含本发明的mRNA的医药调配物。在一些实施例中,提供一种包含至少一种脂质,例如包含本发明的mRNA的LNP的医药调配物。可使用适用于递送RNA的任何LNP,诸如上文所述的LNP;其它示例性LNP描述于3月30,2017日申请的PCT/US2017/024973中。医药调配物可进一步包含医药学上可接受的载体,例如水或缓冲液。医药调配物可进一步包含一或多种医药学上可接受的赋形剂,诸如稳定剂、防腐剂、膨化剂或其类似者。医药调配物可进一步包含一或多种医药学上可接受的盐,诸如氯化钠。在一些实施例中,医药调配物经调配以用于静脉内施用。在一些实施例中,医药调配物经调配以用于递送至肝循环中。

[0361] C.mRNA功效的测定

[0362] 在一些实施例中,当与RNP的其它组分,例如至少一个gRNA,诸如靶向TTR的gRNA一起表达时,测定mRNA的功效。

[0363] 具有裂解酶活性的RNA引导的DNA结合剂可引起DNA中发生双股断裂。非同源末端连接(NHEJ)为一种以下方法,通过该方法,DNA中的双股断裂(DSB)经由重新连接断裂末端而得到修补,其可能会产生呈插入/缺失(插入缺失标记(indel))突变形式的错误。在重新连接末端之前,DSB的DNA末端常常会经历酶处理,其引起在一或两个股处添加或移除核苷酸。重新连接之前的此等添加或移除引起DNA序列中NHEJ修复位点处存在插入或缺失(插入缺失标记(indel))突变。归因于插入缺失标记(indel)的多种突变会改变阅读框架或过早地引入终止密码子,且因此产生非功能性蛋白质。

[0364] 在一些实施例中,基于活体外模型而测定编码核酸酶的mRNA的功效。在一些实施例中,活体外模型为HEK293细胞。在一些实施例中,活体外模型为HUH7人类肝癌细胞。在一些实施例中,活体外模型为初级肝细胞,诸如初级人类或小鼠肝细胞。

[0365] 在一些实施例中,通过TTR的编辑百分比测量RNA的功效。用于测定编辑百分比的示例性程序在以下实例中给出。在一些实施例中,将TTR的编辑百分比与当mRNA包含具有未经修饰的尿苷的SEQ ID NO:5的ORF且所有其它者为相同的时所获得的编辑百分比进行比较。

[0366] 在一些实施例中，使用施用包含mRNA及靶向TTR的gRNA，例如SEQ ID NO:42的LNP后的小鼠中的血清TTR浓度来测定mRNA的功效。在一些实施例中，使用施用包含mRNA及靶向TTR的gRNA，例如SEQ ID NO:69的LNP后的大鼠中的血清TTR浓度来测定mRNA的功效。血清TTR浓度可以绝对术语或以相对于假处理对照的基因减弱％来表示。在一些实施例中，使用施用包含mRNA及靶向TTR的gRNA，例如SEQ ID NO:42的LNP后的小鼠中的肝脏中的编辑百分比来测定mRNA的功效。在一些实施例中，有效量能够达成血清TTR的至少50的编辑％或50的基因减弱％。示例性有效量在0.1至10mg/kg(mpk)，例如0.1至0.3mpk、0.3至0.5mpk、0.5至1mpk、1至2mpk、2至3mpk、3至5mpk、5至10mpk范围内，或为0.1、0.2、0.3、0.5、1、2、3、5、或10mpk。

[0367] 在一些实施例中，侦测基因编辑事件，诸如靶DNA中的插入/缺失(“插入缺失标记(indel)”)突变的形成及同源定向修复(HDR)事件会利用伴随加标签的引物且分离加标签的扩增产物的线性扩增(后文称为“LAM-PCR”或“线性扩增(LA)”法)。

[0368] 在一些实施例中，该方法包含自已经诱导而具有双股断裂(DSB)且视情况地，已具有HDR模板以修复DSB的细胞分离细胞DNA；用加标签的引物进行至少一轮DNA的线性扩增；分离包含标签的线性扩增产物，藉此舍弃用非加标签的引物扩增的任何扩增产物；视情况进一步扩增分离产物；且分析线性扩增产物或经进一步扩增的产物以测定靶DNA中是否存在编辑事件，诸如双股断裂、插入、缺失或HDR模板序列。在一些情况下，编辑事件可经定量。如本文所用(包括在HDR及非HDR编辑事件，诸如插入缺失标记(indel)的情形下)，定量及其类似者包括侦测总体中编辑事件的次数及/或类型。

[0369] 在一些实施例中，仅进行一轮线性扩增。

[0370] 在一些情况下，加标签的引物包含分子条形码。在一些实施例中，加标签的引物包含分子条形码，且仅进行一轮线性扩增。

[0371] 在一些实施例中，分析步骤包含对线性扩增产物或经进一步扩增的产物进行测序。测序可包含熟习此项技术者已知的任何方法，包括次世代测序(next generation sequencing)及将线性扩增产物或经进一步扩增的产物克隆于质粒中且对质粒或质粒的一部分进行测序。在其它方面中，分析步骤包含对线性扩增产物或经进一步扩增的产物进行数字PCR(dPCR)或液滴式数字PCR(ddPCR)。在其它情况下，分析步骤包含使线性扩增产物或经进一步扩增的产物与经设计以鉴别包含HDR模板序列的DNA的核酸探针接触，且侦测已结合至线性扩增产物或经进一步扩增的产物的探针。在一些实施例中，该方法进一步包含测定靶DNA中HDR模板的位置。

[0372] 在某些实施例中，该方法进一步包含测定靶DNA中插入位点的序列，其中插入位点为HDR模板并入靶DNA中所处的位置，且其中插入位点可包括部分靶DNA序列及部分HDR模板序列。

[0373] 在一些实施例中，使用加标签的引物的靶DNA的线性扩增进行1至50轮、1至60轮、1至70轮、1至80轮、1至90轮或1至100轮。

[0374] 在一些实施例中，使用加标签的引物的靶DNA的线性扩增包含分离DNA双螺旋体的变性步骤、使引物结合的黏接步骤及延伸步骤。在一些实施例中，线性扩增为等温的(不需要温度变化)。在一些实施例中，等温线性扩增为环介导等温扩增(LAMP)、股置换扩增(SDA)、解螺旋酶依赖性扩增或切口酶扩增反应。

[0375] 在一些实施例中,加标签的引物黏接至与期望编辑事件位置,例如插入、缺失或模板插入位点相距至少50、至少60、至少70、至少80、至少90、至少100、至少110、至少120、至少130、至少140、至少150、至少160、至少170、至少180、至少190、至少200、至少210、至少220、至少230、至少240、至少250、至少260、至少270、至少280、至少290、至少300、至少1,000、至少5,000或至少10,000个核苷酸的靶DNA。

[0376] 在一些实施例中,加标签的引物包含分子条形码。在一些实施例中,分子条形码包含与靶DNA不互补的序列。在一些实施例中,分子条形码包含6、8、10或12个核苷酸。

[0377] 在一些实施例中,引物上的标签为生物素、抗生蛋白链菌素、地高辛(digoxigenin)、DNA序列或荧光异硫氰酸盐(FITC)。

[0378] 在一些实施例中,使用对引物上的标签具有特异性的捕捉试剂分离线性扩增产物。在一些实施例中,捕捉试剂处于珠粒、固体载体、基质或管柱上。在一些实施例中,分离步骤包含使线性扩增产物与对引物上的标签具有特异性的捕捉试剂接触。在一些实施例中,捕捉试剂为生物素、抗生蛋白链菌素、地高辛、DNA序列或荧光异硫氰酸盐(FITC)。

[0379] 在一些实施例中,标签为生物素且捕捉试剂为抗生蛋白链菌素。在一些实施例中,标签为抗生蛋白链菌素且捕捉试剂为生物素。在一些实施例中,标签处于引物的5′端、引物的3′端上或处于引物内部。在一些实施例中,在分离步骤之后移除标签及/或捕捉试剂。在一些实施例中,不移除标签及/或捕捉试剂,且在标签及/或捕捉试剂存在下进行进一步扩增及分析步骤。

[0380] 在一些实施例中,进一步扩增为非线性的。在一些实施例中,进一步扩增为数字PCR、qPCR或RT-PCR。在一些实施例中,测序为次世代测序(NGS)。

[0381] 在一些实施例中,靶DNA为基因组或线粒体。在一些实施例中,靶DNA为原核或真核细胞的基因组DNA。在一些实施例中,靶DNA为哺乳动物DNA。靶DNA可来自非分裂细胞或分裂细胞。在一些实施例中,靶DNA可来自初级细胞。在一些实施例中,靶DNA来自复制细胞。

[0382] 在一些情况下,在线性扩增之前剪切细胞DNA。在一些实施例中,经剪切的DNA的平均尺寸在0.5kb与20kb之间。在一些情况下,细胞DNA经剪切而呈0.5、0.75、1.0、1.25、1.5、1.75、2.0、2.25、2.5、2.75、3.0、3.25、3.5、3.75、4.0、4.25、4.5、4.75、5.0、5.25、5.5、5.75、6.0、6.25、6.5、6.75、7.0、7.25、7.5、7.75、8.0、8.25、8.5、8.75、9.0、9.25、9.5、9.75、10.0、10.25、10.5、10.75、11.0、11.25、11.5、11.75、12.0、12.25、12.5、12.75、13.0、13.25、13.5、13.75、14.0、14.25、14.5、14.75、15.0、15.25、15.5、15.75、16.0、16.25、16.5、16.75、17.0、17.25、17.5、17.75、18.0、18.25、18.5、18.75、19.0、19.25、19.5、19.75或20.0kb的平均尺寸。在一些情况下,细胞DNA经剪切而呈约1.5kb的平均尺寸。

[0383] D.示例性用途、方法及治疗

[0384] 在一些实施例中,mRNA、LNP或医药组合物供用于基因组编辑,例如编辑靶基因中。在一些实施例中,mRNA、LNP或医药组合物供用于修饰靶基因,例如改变其序列或后生状态(epigenetic status)中。在一些实施例中,mRNA、LNP或医药组合物供用于在靶基因中诱导双股断裂(DSB)中。在一些实施例中,mRNA、LNP或医药组合物供用于在靶基因中诱导插入缺失标记(indel)中。在一些实施例中,提供本文所揭示的mRNA、LNP或医药组合物的用途,其用于制备用于基因组编辑,例如编辑靶基因的药剂。在一些实施例中,提供本文所揭示的mRNA、LNP或医药组合物的用途,其用于制备用于修饰靶基因,例如改变其序列或后生状态

的药剂。在一些实施例中,提供本文所揭示的mRNA、LNP或医药组合物的用途,其用于制备用于在靶基因中诱导双股断裂(DSB)的药剂。在一些实施例中,提供本文所揭示的mRNA、LNP或医药组合物的用途,其用于制备用于在靶基因中诱导插入缺失标记(indel)的药剂。在一些实施例中,靶基因处于个体,诸如哺乳动物,诸如人类中。在一些实施例中,靶基因处于器官,诸如肝脏,诸如哺乳动物肝脏,诸如人类肝脏中。在一些实施例中,靶基因处于肝脏细胞,诸如哺乳动物肝脏细胞,诸如人类肝脏细胞中。在一些实施例中,靶基因处于肝细胞,诸如哺乳动物肝细胞,诸如人类肝细胞中。在一些实施例中,肝脏细胞或肝细胞处于原位。在一些实施例中,肝脏细胞或肝细胞分离于例如培养物中,诸如原代培养物中。亦提供对应于本文所揭示的用途的方法,其包含向个体施用本文所揭示的mRNA、LNP或医药组合物或使细胞,诸如上文所述的细胞与本文所揭示的mRNA、LNP或医药组合物接触。

[0385] 在一些实施例中,mRNA、LNP或医药组合物供用于疗法中或治疗疾病,例如与TTR相关的淀粉样变性(ATTR)中。在一些实施例中,提供本文所揭示的mRNA(例如以本文所提供的组合物形式)的用途,其用于制备例如用于治疗患有与TTR相关的淀粉样变性(ATTR)的个体的药剂。

[0386] 在一些实施例中,出于上文关于生物体、器官或原位细胞所论述的用途中的任一者静脉内施用mRNA、LNP或医药组合物。在一些实施例中,以在0.01至10mg/kg(mpk),例如0.01至0.1mpk、0.1至0.3mpk、0.3至0.5mpk、0.5至1mpk、1至2mpk、2至3mpk、3至5mpk、5至10mpk范围内或0.1、0.2、0.3、0.5、1、2、3、5或10mpk的剂量施用mRNA、LNP或医药组合物。

[0387] 在涉及个体之前述实施例中的任一者中,个体可为哺乳动物。在涉及个体之前述实施例中的任一者中,个体可为人类。在涉及个体之前述实施例中的任一者中,个体可为牛、猪、猴、绵羊、狗、猫、鱼或家禽。

[0388] 在一些实施例中,静脉内施用本文所揭示的mRNA、LNP或医药组合物或将其用于静脉内投药。在一些实施例中,将引导RNA、组合物及调配物施用至肝循环中或用于肝循环中的投药。

[0389] 在一些实施例中,单次施用本文所揭示的mRNA、LNP或医药组合物足以使靶基因产物的表达发生基因减弱。在一些实施例中,单次施用本文所揭示的mRNA、LNP或医药组合物足以使靶基因产物的表达发生基因剔除。在其它实施例中,不止一次施用本文所揭示的mRNA、LNP或医药组合物对于使编辑、修饰、插入缺失标记(indel)形成、DSB形成或其类似者经由累积效应达至最大可为有益的。

[0390] 在一些实施例中,会在递送之后1年、2年、3年、4年、5年或10年看到本文所揭示的mRNA、LNP或医药组合物的治疗功效。

[0391] 在一些实施例中,治疗会减缓或中断疾病进展。

[0392] 在一些实施例中,治疗会引起器官功能或器官,诸如肝脏的疾病的症状得到改善、稳定或减缓其变化。

[0393] 在一些实施例中,通过增加个体的存活时间来测量治疗功效。

[0394] E.示例性DNA分子、载体、表达构建体、宿主细胞及制造方法

[0395] 在某些实施例中,本发明提供一种DNA分子,其包含编码mRNA中的任一者的序列,该mRNA编码本文所述的RNA引导的DNA结合剂。在一些实施例中,除了RNA引导的DNA结合剂序列之外,DNA分子进一步包含不编码RNA引导的DNA结合剂的核酸。不编码RNA引导的DNA结

合剂的核酸包括(但不限于)启动子、强化子、调节序列及编码引导RNA的核酸。

[0396] 在一些实施例中,DNA分子进一步包含编码crRNA、trRNA或crRNA及trRNA的核苷酸序列。在一些实施例中,编码crRNA、trRNA或crRNA及trRNA的核苷酸序列包含或由以下组成:通过来自天然存在的CRISPR/Cas系统的重复序列的全部或一部分侧接的引导序列。包含或由crRNA、trRNA或crRNA及trRNA组成的核酸可进一步构成载体序列,其中该载体序列包含或由以下组成:不会与crRNA、trRNA或crRNA及trRNA一起经天然发现的核酸。在一些实施例中,crRNA及trRNA由一个载体中的非连续核酸编码。在其它实施例中,crRNA及trRNA可由连续核酸编码。在一些实施例中,crRNA及trRNA由单一核酸的相对股编码。在其它实施例中,crRNA及trRNA由单一核酸的相同股编码。

[0397] 在一些实施例中,DNA分子进一步包含以可操作方式连接编码mRNA中的任一者的序列的启动子,该mRNA编码本文所述的RNA引导的DNA结合剂。在一些实施例中,DNA分子为适用于在哺乳动物细胞,例如人类细胞或小鼠细胞,诸如人类肝细胞或啮齿动物(例如小鼠)肝细胞中表达的表达构建体。在一些实施例中,DNA分子为适用于在哺乳动物器官,例如人类肝脏或啮齿动物(例如小鼠)肝脏的细胞中表达的表达构建体。在一些实施例中,DNA分子为质粒或游离基因组。在一些实施例中,DNA分子包含于宿主细胞,诸如细菌或经培养的真核细胞中。示例性细菌包括变形菌门,诸如大肠杆菌。示例性经培养的真核细胞包括初级肝细胞,包括啮齿动物(例如小鼠)或人源的肝细胞;肝细胞细胞株,包括啮齿动物(例如小鼠)或人源的肝细胞;人类细胞株;啮齿动物(例如小鼠)细胞株;CHO细胞;微生物真菌,诸如裂殖或出芽酵母,例如酵母菌,诸如酿酒酵母;及昆虫细胞。

[0398] 在一些实施例中,提供一种制造本文所揭示的mRNA的方法。在一些实施例中,此类方法包含使本文所述的DNA分子与核糖核酸聚合酶在容许转录的条件下接触。在一些实施例中,活体外,例如在无细胞系统中进行接触。在一些实施例中,核糖核酸聚合酶为噬菌体源的核糖核酸聚合酶,诸如T7核糖核酸聚合酶。在一些实施例中,提供包括至少一种如上文所论述的经修饰的核苷酸的NTP。在一些实施例中,NTP包括至少一种如上文所论述的经修饰的核苷酸且不包含UTP。

[0399] 在一些实施例中,单独或与一或多个引导RNA一起的本文所揭示的mRNA可包含在一或多个载体的载体系统中或通过其递送。在一些实施例中,载体中的一种或多种或所有载体可为DNA载体。在一些实施例中,载体中的一种或多种或所有载体可为RNA载体。在一些实施例中,载体中的一种或多种或所有载体可为环状的。在一些实施例中,载体中的一种或多种或所有载体可为线性的。在一些实施例中,载体中的一种或多种或所有载体可包封在脂质纳米颗粒、脂质体、非脂质纳米颗粒或病毒衣壳中。非限制性示例性载体包括质粒、噬菌粒、黏质粒、人工染色体、袖珍染色体、转座子、病毒载体及表达载体。

[0400] 非限制性示例性病毒载体包括腺相关病毒(AAV)载体、慢病毒载体、腺病毒载体、辅助依赖型腺病毒载体(HDAd)、单纯疱疹病毒(HSV-1)载体、噬菌体T4、杆状病毒载体及反转录病毒载体。在一些实施例中,病毒载体可为AAV载体。在其它实施例中,病毒载体可为慢病毒载体。在一些实施例中,慢病毒可为非整合性的。在一些实施例中,病毒载体可为腺病毒载体。在一些实施例中,腺病毒可为高克隆容量或“无肠(gutless)”腺病毒,其中除5′及3′倒转末端重复序列(ITR)及包装信号(‘I’)之外的所有病毒编码区自病毒中删除以增加其包装容量。在又其它实施例中,病毒载体可为HSV-1载体。在一些实施例中,HSV-1类载体

为辅助依赖型,且在其它实施例中,其为非辅助依赖型。例如,仅保留包装序列的扩增子载体需要具有结构性组分的辅助病毒用于包装,而移除非必需病毒功能的缺失30kb的HSV-1载体不需要辅助病毒。在其它实施例中,病毒载体可为噬菌体T4。在一些实施例中,当清空病毒头时,噬菌体T4可能能够包装任何线性或环状DNA或RNA分子。在其它实施例中,病毒载体可为杆状病毒载体。在又其它实施例中,病毒载体可为反转录病毒载体。在使用具有较小克隆容量的AAV或慢病毒载体的实施例中,可能需要使用多于一个载体以递送如本文所揭示的载体系统的全部组分。例如,一个AAV载体可含有编码Cas蛋白的序列,而第二AAV载体可含有一或多个引导序列。

[0401] 在一些实施例中,载体可能能够驱动细胞中一或多个编码序列,诸如本文所揭示的mRNA的编码序列的表达。在一些实施例中,细胞可为原核细胞,诸如细菌细胞。在一些实施例中,细胞可为真核细胞,诸如酵母、植物、昆虫或哺乳动物细胞。在一些实施例中,真核细胞可为哺乳动物细胞。在一些实施例中,真核细胞可为啮齿动物细胞。在一些实施例中,真核细胞可为人类细胞。驱动不同类型细胞中的表达的适合启动子为此项技术中已知的。在一些实施例中,启动子可为野生型。在其它实施例中,启动子可经修饰以供较高效或较有效表达。在又其它实施例中,启动子可经截短但仍保留其功能。例如,启动子可具有正常尺寸或适用于将载体适当包装于病毒中的减小的尺寸。

[0402] 在一些实施例中,载体系统可包含一个编码RNA引导的DNA结合剂的核苷酸序列的复本。在其它实施例中,载体系统可包含多于一个编码RNA引导的DNA结合剂的核苷酸序列的复本。在一些实施例中,编码RNA引导的DNA结合剂的核苷酸序列可以可操作方式连接至少一个转录或转译控制序列。在一些实施例中,编码核酸酶的核苷酸序列可以可操作方式连接至少一个启动子。

[0403] 在一些实施例中,启动子可为组成性、诱导性或组织特异性的。在一些实施例中,启动子可为组成性启动子。非限制性示例性的组成性启动子包括细胞巨大病毒即刻早期启动子(CMV)、猴病毒(SV40)启动子、腺病毒主要晚期启动子(MLP)、劳斯肉瘤病毒(RSV)启动子、小鼠乳腺肿瘤病毒(MMTV)启动子、磷酸甘油酸激酶(PGK)启动子、延长因子-α(EF1a)启动子、泛素启动子、肌动蛋白启动子、微管蛋白启动子、免疫球蛋白启动子、其功能性片段或前述中的任一者的组合。在一些实施例中,启动子可为CMV启动子。在一些实施例中,启动子可为经截短的CMV启动子。在其它实施例中,启动子可为EF1a启动子。在一些实施例中,启动子可为诱导性启动子。非限制性示例性的诱导性启动子包括可通过热休克、光、化学物质、肽、金属、类固醇、抗生素或醇诱导的诱导性启动子。在一些实施例中,诱导性启动子可为具有低基础(非经诱导)表达程度的诱导性启动子,诸如Tet-On®启动子(Clontech)。

[0404] 在一些实施例中,启动子可为组织特异性启动子,例如对肝脏中的表达具有特异性的启动子。

[0405] 载体可进一步包含编码至少一个引导RNA的核苷酸序列。在一些实施例中,载体包含一个引导RNA的复本。在其它实施例中,载体包含多于一个引导RNA的复本。在具有多于一个引导RNA的实施例中,引导RNA可不同以使得其靶向不同靶序列,或可相同以便其靶向相同靶序列。在其中载体包含多于一个引导RNA的一些实施例中,各引导RNA可具有其它不同特性,诸如在与RNA引导的DNA结合剂的核糖核蛋白复合物中的活性或稳定性。在一些实施例中,编码引导RNA的核苷酸序列可以可操作方式连接至少一个转录或转译控制序列,诸如

启动子、3'UTR或5'UTR。在一个实施例中,启动子可为tRNA启动子,例如tRNA[Lys3],或tRNA嵌合体。参见Mefferd等人,RNA.201521:1683-9;Scherer等人,Nucleic Acids Res.2007 35:2620-2628。在一些实施例中,启动子可通过RNA聚合酶III(Pol III)识别。Pol III启动子的非限制性实例包括U6及H1启动子。在一些实施例中,编码引导RNA的核苷酸序列可以可操作方式连接小鼠或人类U6启动子。在其它实施例中,编码引导RNA的核苷酸序列可以可操作方式连接小鼠或人类H1启动子。在具有多于一个引导RNA的实施例中,用于驱动表达的启动子可相同或不同。在一些实施例中,编码引导RNA的crRNA的核苷酸及编码引导RNA的trRNA的核苷酸可提供于相同载体上。在一些实施例中,编码crRNA的核苷酸及编码trRNA的核苷酸可通过同一启动子驱动。在一些实施例中,crRNA及trRNA可转录为单一转录物。例如,crRNA及trRNA可由单一转录物进行处理以形成双分子引导RNA。或者,crRNA及trRNA可转录为单分子引导RNA。在其它实施例中,crRNA及trRNA可通过其处于相同载体上的相应启动子来驱动。在又其它实施例中,crRNA及trRNA可通过不同载体编码。

[0406]　在一些实施例中,组合物包含载体系统,其中该系统包含多于一个载体。在一些实施例中,载体系统可包含一个单一载体。在其它实施例中,载体系统可包含两个载体。在其它实施例中,载体系统可包含三个载体。当将不同引导RNA用于多任务(multiplexing)时或当使用引导RNA的多个复本时,载体系统可包含多于三个载体。

[0407]　在一些实施例中,载体系统可包含诱导性启动子以仅在其递送至靶细胞之后开始表达。非限制性示例性的诱导性启动子包括可通过热休克、光、化学物质、肽、金属、类固醇、抗生素或醇诱导的诱导性启动子。在一些实施例中,诱导性启动子可为具有低基础(非经诱导)表达程度的诱导性启动子,诸如Tet-On®启动子(Clontech)。

[0408]　在其它实施例中,载体系统可包含组织特异性启动子以仅在其递送至特定组织中之后开始表达。

[0409]　实例

[0410]　提供以下实例以说明某些所揭示的实施例且不应理解为以任何方式限制本发明的范畴。

[0411]　通用试剂及方法.除非另外指明,否则通过活体外转录(IVT)使用线性化质粒DNA模板及T7核糖核酸聚合酶来合成mRNA。转录通常由以下来进行:包含T7启动子的构建体;本文所揭示的转录物序列,诸如SEQ ID NO:43(其包含SEQ ID NO:1且编码SEQ ID NO:4的RNA ORF)或SEQ ID NO:48(其包含SEQ ID NO:2且编码SEQ ID NO:5的RNA ORF)及编码于质粒中的聚-A尾(SEQ ID NO:63)。其中测试多个UTR的实验使用相似构建体,不同的处在于使用转录物序列,诸如SEQ ID NO:58及SEQ ID NO:59。通过与XbaI利用以下条件在37℃下培育2小时来线性化含有T7启动子及100nt聚(A/T)区的质粒DNA:200ng/μL质粒、2U/μL XbaI(NEB)及1×反应缓冲液。通过在65℃下加热反应20分钟来灭活XbaI。使用二氧化硅最大自旋管柱(Epoch Life Sciences)自酶及缓冲盐纯化线性化质粒且通过琼脂糖凝胶加以分析以证实线性化。用于产生经Cas9修饰的mRNA的IVT反应物在37℃下在以下条件下培育4小时:50ng/μL线性化质粒;2mM的GTP、ATP、CTP及UTP(或若指示,代替CTP或UTP的经修饰的三磷酸核苷酸(例如N1-甲基假UTP(Trilink))中的每一者;10mM ARCA(Trilink);5U/μL T7 RNA聚合酶(NEB);1U/μL鼠类RNA酶抑制剂(NEB);0.004U/μL无机大肠杆菌焦磷酸酶(NEB);及1×反应缓冲液。在4小时培育之后,添加TURBO脱氧核糖核酸酶(ThermoFisher)至0.01U/μL的最终

浓度，且再培育反应物30分钟以移除DNA模板。使用MegaClear转录清除试剂盒根据制造商方案(ThermoFisher)自酶及核苷酸纯化Cas9 mRNA。经由沉淀方案(在一些情况下，其继之以基于HPLC的纯化)来纯化mRNA。简言之，在DNA酶消化之后，通过添加0.21×体积的7.5M LiCl溶液且加以混合来沉淀mRNA，且通过离心来集结经沉淀的mRNA。移除上清液后，将mRNA复水。使用乙酸铵及乙醇再次沉淀mRNA。将5M乙酸铵连同2×体积的100％EtOH一起添加至mRNA溶液中，使最终浓度为2M。混合溶液且在-20℃下将其培育15分钟。再次通过离心集结经沉淀的mRNA，移除上清液且将mRNA复水。使用乙酸钠及乙醇沉淀mRNA作为最终步骤。将1/10体积的3M乙酸钠(pH 5.5)连同2×体积的100％EtOH一起添加至溶液中。混合溶液且在-20℃下将其培育15分钟。再次通过离心集结经沉淀的mRNA，移除上清液，用70％低温乙醇洗涤集结粒且使其风干。将mRNA复水。对于经HPLC纯化的mRNA，在LiCl沉淀及复水之后，通过RP-IP HPLC纯化mRNA(参见例如Kariko等人Nucleic Acids Research，2011，第39卷，第21期e142)。合并选择用于汇集的溶离份且通过如上文所述的乙酸钠/乙醇沉淀来去盐。

[0412] 对于所有方法，通过测量260nm处的吸光度(Nanodrop)测定转录物浓度，且通过利用Bioanlayzer(Agilent)进行毛细电泳法来分析转录物。

[0413] 除非另外指明，否则用来自Charles River Laboratories的CD-1雌性小鼠及史泊格-多利大鼠进行活体内编辑实验。除非另外指明，否则小鼠中的血清TTR含量的分析如下进行。收集血液且如所指示地分离血清。

[0414] 在适用实例中指明的情况下，亦测量经处理的小鼠中的细胞介素诱导。对于此分析，通过尾静脉切口收集大约50至100μL血液用于血清细胞介素测量。使血液在室温下凝结持续大约2小时，且随后在1000×g下离心10分钟，随后收集血清。测量IL-6、TNF-α、IFN-α及MCP-1的基于Luminex的磁珠多任务分析(Affymetrix ProcartaPlus，目录号Exp040-00000-801)用于收集样本中的细胞介素分析。如制造商方案中所指导地制备试剂盒试剂及标准品。使用所提供的样本稀释剂对小鼠血清进行4倍稀释，且将50μL添加至含有经50μL稀释抗体涂布的磁珠的孔中。将板在室温下培育2小时且随后加以洗涤。稀释生物素抗体(50μL)添加至珠粒且在室温下培育1小时。再次洗涤珠粒，之后将50μL稀释抗生蛋白链菌素-PE添加至各孔中，接着培育30分钟。再次洗涤珠粒且随后悬浮于100μL洗涤缓冲液中且在Bio-Plex 200仪器(Bio-Rad)上读取。使用Bioplex Manager 6.1版分析软件包，通过使用五参数逻辑曲线拟合脱离标准曲线计算的细胞介素浓度分析数据。

[0415] 除非另外指明，否则使用未经修饰的ATP、GTP、CTP及UTP。除非另外指明，否则所有mRNA均编码有一个核定位信号。

[0416] 通过使用Precision Nanosystems NanoAssemblr™ Benchtop仪器，根据制造商方案，将脂质及RNA溶液微流混合或错流混合而形成LNP，如下文所述。除非另外指明，否则LNP含有45％脂质A、9％DSPC、44％胆固醇及2％PEG2k-DMG及4.5的N:P比。

[0417] LNP调配-NanoAssemblr

[0418] 通常，以不同脂质组分摩尔比将脂质纳米颗粒组分溶解于100％乙醇中。将RNA载荷溶解于25mM柠檬酸盐、100mM NaCl(pH 5.0)中，产生大约0.45mg/mL的RNA载荷浓度。用约4.5或约6的脂质胺与RNA磷酸(N:P)摩尔比，及以重量计呈1:1的mRNA与gRNA的比调配LNP。

[0419] 通过使用Precision Nanosystems NanoAssemblr™ Benchtop仪器，根据制造商的方案，将脂质及RNA溶液微流混合而形成LNP。使用差分流动速率在混合期间维持水相与有

机溶剂的2:1比率。混合之后,收集LNP,在水中稀释(大约1:1v/v),在室温下保持1小时,且进一步用水稀释(大约1:1v/v),之后进行最终缓冲液更换。用PD-10去盐管柱(GE)来完成将最终缓冲液更换为50mM Tris、45mM NaCl、5%(w/v)蔗糖,pH 7.5(TSS)。必要时,用Amicon 100kDa离心过滤器(Millipore)通过离心浓缩调配物。所得混合物随后使用0.2μm无菌过滤器过滤。在进一步使用之前,将最终LNP储存于-80℃下。

[0420] LNP调配-错流

[0421] 对于使用错流技术制备的LNP,通过脂质的乙醇溶液与两个体积的RNA溶液及一个体积的水进行撞击喷流混合来形成LNP。经由混合十字管使脂质的乙醇溶液与两个体积的RNA溶液混合。经由沿线T形管将第四水流与十字管的引出流混合(参见WO2016010840图2.)。将LNP在室温下保持1小时且进一步用水稀释(大约1:1v/v)。使用切向流过滤在平板滤筒(Sartorius,100kD MWCO)上浓缩经稀释的LNP,且随后通过透滤,将其缓冲液更换为50mM Tris、45mM NaCl、5%(w/v)蔗糖,pH 7.5(TSS)。可替代地,用PD-10去盐管柱(GE)来完成将最终缓冲液更换为TSS。必要时,用Amicon 100kDa离心过滤器(Millipore)通过离心浓缩调配物。所得混合物随后使用0.2μm无菌过滤器过滤。在进一步使用之前,将最终LNP储存于4℃或-80℃下。

[0422] 调配物分析

[0423] 使用动态光散射("DLS")表征本发明的LNP的多分散性指数("pdi")及尺寸。DLS测量由将样本置于光源下而产生的光的散射。如根据DLS测量所测定,PDI表示总体中(平均粒度周围)粒度的分布,其中完全均一总体的PDI为零。平均粒径及多分散性是通过动态光散射(DLS)使用Malvern Zetasizer仪器来测量。在通过DLS测量之前,在PBS中将LNP样本稀释30×。连同数目平均直径及pdi一起报导Z-平均直径,其为平均粒径的基于强度的量度。Malvern Zetasizer仪器亦用于测量LNP的ζ电位。测量之前,将样本在0.1× PBS,pH 7.4中以1:17(50μL于800μL中)稀释。

[0424] 使用基于荧光的分析(Ribogreen®,ThermoFisher Scientific)来测定总RNA浓度及游离RNA。囊封效率计算为(总RNA-游离RNA)/总RNA。用含有0.2%Triton-X 100的1×TE缓冲液以适当方式稀释LNP样本以测定总RNA或用1×TE缓冲液稀释以测定游离RNA。通过利用用于制得调配物的起始RNA溶液制备标准曲线,且稀释于1×TE缓冲液+/-0.2% Triton-X 100中。随后将经稀释的RiboGreen®染料(根据制造商的说明书)添加至标准品及样本中的每一者中,且使其在不存在光下在室温下培育大约10分钟。使用SpectraMax M5微定量盘式读取器(Molecular Devices),伴随分别设定成488nm、515nm及525nm的激发、自动截止及发射波长来读取样本。根据合适标准曲线测定总RNA及游离RNA。

[0425] 囊封效率计算为(总RNA-游离RNA)/总RNA。可使用相同程序测定基于DNA的载荷组分的囊封效率。对于单股DNA,可使用Oligreen染料,且对于双股DNA,可使用Picogreen染料。

[0426] 典型地,当制备LNP时,囊封效率>80%,粒度<120nm且pdi为<0.2。

[0427] LNP活体内递送

[0428] 除非另外指出,否则各研究中使用在6至10周龄范围内的CD-1雌性小鼠。对动物称重且根据体重分组以基于组平均体重制备投配溶液。经由侧尾静脉以每只动物0.2mL(每公

斤体重大约10mL)的体积投配LNP。在给药后大约6小时观测动物副作用。在投药后二十四小时测量体重,且动物通过在异氟烷麻醉下经由心脏穿刺放血而在各个时间点安乐死。将血液收集至血清分离管中或含有用于如本文所述的血浆的缓冲柠檬酸钠的管中。对于涉及活体内编辑的研究,自来自各动物的中叶或三个独立叶片(例如右中、左中及左外侧叶片)收集肝脏组织用于DNA提取及分析。

[0429] 针对肝脏编辑通过次世代测序(NGS)及血清TTR含量(资料未示)测量小鼠群组。

[0430] 甲状腺素转运蛋白(TTR)ELISA分析

[0431] 收集血液且如所指示地分离血清。使用小鼠前白蛋白(甲状腺素转运蛋白)ELISA试剂盒(Aviva Systems Biology,目录号OKIA00111)测定总小鼠TTR血清含量。根据制造商方案,使用大鼠特异性ELISA试剂盒(Aviva Systems Biology目录号OKIA00159)来测量大鼠TTR血清含量。简言之,用试剂盒样本稀释剂将血清连续稀释至最终稀释度为10,000倍。随后将此稀释样本添加至ELISA板且随后根据指示进行分析。

[0432] NGS测序

[0433] 简言之,为了定量地测定基因组中靶位置处的编辑效率,分离基因组DNA且利用深度测序鉴别存在通过基因编辑造成的插入及缺失。

[0434] 在靶位点(例如TTR)周围设计PCR引物,且扩增相关基因组区。引物序列提供于下文中。根据制造商的方案(Illumina)进行额外PCR以对于测序添加所需化学性质。在Illumina MiSeq仪器上对扩增子测序。在消除具有低质量评分的读段之后,将读段与人类参考基因组(例如hg38)进行比对。将含有读段的所得档案映像至参考基因组(BAM档案),其中选择与相关目标区重迭的读段且计算野生型读段的数目相对于含有插入、取代或缺失的读段的数目。

[0435] 编辑百分比(例如“编辑效率”或“编辑%”)定义为具有插入或缺失的序列读段的总数相比于包括野生型的序列读段的总数。

[0436] 1.具有经修饰的核苷酸的Cas9 mRNA的活体内表征

[0437] 用不同的如下表5中所示的经修饰的核苷酸内含物制备包含如SEQ ID NO:5中所阐述的ORF的mRNA。将mRNA与靶向甲状腺素转运蛋白基因(TTR)的引导RNA(G282;SEQ ID NO:42)合并且并入至LNP中。未经修饰的胞苷用于所有LNP中,除了LNP420。

[0438] 表5.用于活体内研究的LNP417至LNP421

[0439]

LNP	Cas9 SEQ ID NO	经修饰的核苷酸
LNP417	5	N1-甲基-假尿苷
LNP418	5	无
LNP419	5	假尿苷
LNP420	5	假尿苷及5-甲基胞苷
LNP421	5	60% N1-甲基-假尿苷(40%未经修饰的尿苷)

[0440] 以0.5mg/kg(mpk)或1mpk剂量向小鼠施用LNP417至LNP421。给药后4小时(hpd)测量细胞介素(IFN α、IL-6、TNF α及MCP-1)诱导。结果示于图1A至图1D中。

[0441] 在投药之后7天进行尸体剖检时,收集血清及肝脏分别用于血清TTR测量及编辑功效的分析。结果示于图2A至图2B中。

[0442] 观测到,使用假尿苷及5-甲基CTP几乎完全消除细胞介素诱导。使用呈60%(LNP421)或100%(LNP417)的N1-甲基假尿苷亦会引起比未经修饰的Cas9 mRNA要少的细胞介素诱导,且60%N1-甲基假尿苷下的减少程度与100%类似。

[0443] 所有经修饰的Cas9构建体在降低血清TTR方面有相似效应,且可能归因于增加的稳定性,其比未经修饰的构建体更有效。根据肝脏编辑数据,使用假尿苷及N1-甲基假尿苷的构建体同样有效。具有假尿苷及5-甲基胞苷的构建体的效应明显要比用单独假尿苷的构建体低。具有60%N1-甲基假尿苷的构建体的效应可能要比具有100%N1-甲基假尿苷的构建体略低。

[0444] 2.编码Cas9的经修饰的mRNA的研发及活体外表征

[0445] Cas9序列(SEQ ID NO:1)经设计以改善肝脏表达且将尿苷减至最少。基于具有最少可能的尿苷含量及肝脏中对应tRNA的最大表达来选择密码子。对于肝脏tRNA表达,参见Dittmar KA,PLos Genetics 2(12):e221(2006)。降低Cas9 mRNA的尿苷含量意欲降低对mRNA的先天性免疫反应及/或提供其它益处。表6显示基于tRNA含量的最佳肝脏密码子及具有最少可能数目的尿苷的密码子。最小尿苷密码子与最佳肝脏密码子不同的情况呈粗斜体形式。表格亦显示化脓链球菌Cas9的氨基酸序列(SEQ ID NO:3)中的各氨基酸的数目。

[0446] 表6:密码子最佳化参数

[0447]

	氨基酸	最佳肝脏密码子	最小尿苷密码子	Cas9 频率
A	丙氨酸	GCA	GCA	73
G	甘氨酸	GGA	GGA	73
V	缬氨酸	GTC	GTC	74
D	天冬氨酸	GAT	***GAC***	100
E	谷氨酸	GAA	GAA	111
I	异亮氨酸	ATC	ATC	93
T	苏氨酸	ACA	ACA	66
N	天冬酰胺	AAC	AAC	70
K	赖氨酸	AAG	AAG	155

[0448]

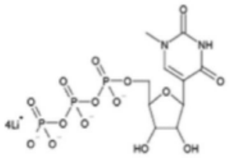
S	丝氨酸	TCG	***AGC***	79
R	精氨酸	AGA	AGA	79
L	亮氨酸	CTG	CTG	148
P	脯氨酸	CCG	CCG	36
H	组氨酸	CAC	CAC	32
Q	谷氨酰胺	CAG	CAG	52
F	苯丙氨酸	TTC	TTC	64
Y	酪氨酸	TAC	TAC	55
C	半胱氨酸	TGC	TGC	2
W	色氨酸	TGG	TGG	7
M	甲硫氨酸	ATG	ATG	22

[0449] 在天冬氨酸及丝氨酸的情况下,对应于表达最高的tRNA的肝脏密码子包含胸苷,其将在对应mRNA中转录为尿苷。针对天冬氨酸及丝氨酸选择最小尿苷密码子(分别为GAC及AGC)。Cas9 ORF序列呈4140nt长,含有528个U(12.8%尿苷含量),且在ORF中避免具有3个或多于3个连续尿苷的任何连接。序列中存在63种UU二核苷酸的情况(126/4140=3%尿苷二核苷酸含量)。SEQ ID NO:2提供含有19.6%尿苷作为RNA ORF的替代Cas9序列。

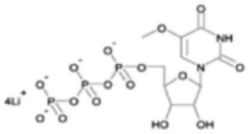
[0450] SEQ ID NO:3提供由SEQ ID NO:1及SEQ ID NO:2两者编码的Cas9的氨基酸序列作为未改变经编码的氨基酸序列的Cas9 ORF的新型设计。SEQ ID NO:4为SEQ ID NO:1的ORF的RNA形式。SEQ ID NO:5为SEQ ID NO:2的ORF的RNA形式。

[0451] 亦评估经修饰的核苷酸的作用。用于转录Cas9转录的经修饰的UTP包括N1-甲基-假UTP及5-甲氧基-UTP。

[0452] N1-甲基-假UTP的结构为:

[0453] 

[0454] 5-甲氧基-UTP的结构为:

[0455] 

[0456] 针对包含SEQ ID NO:4及SEQ ID NO:5的ORF的mRNA,测定活体外转录(IVT)产量。两者均编码核定位信号(NLS)。在未经修饰的UTP或N1-甲基-假UTP任一者存在下,转录包含SEQ ID NO:5的序列。在未经修饰的UTP存在下,转录包含SEQ ID NO:4的序列。亦在渐增的5-甲氧基-UTP百分比下进行IVT,如图3的X轴上所示,其显示以分光光度法测定的此等构建

体中的每一者的产量。

[0457]　此等结果显示,当mRNA的5-甲氧基尿苷含量增加时,产量略微降低,但mRNA产量在所有条件下为可接受的。因此,可针对两个Cas9序列,经由所测试的条件产生具有可接受产量的Cas9 mRNA。

[0458]　使用曲线下面积(AUC)分析法,依利用Agilent Bioanalyzer 2100获得的mRNA毛细电泳法(CE)迹线,计算活体外转录的mRNA的纯度(图4)。使用未经修饰的UTP所生成SEQ ID NO:5Cas9 mRNA的纯度通常会随5-甲氧基-UTP取代增加而提高,而用N1-甲基-假UTP制得的相同构建体则受增加的5-甲氧基-UTP取代的影响较小。

[0459]　用未经修饰的UTP制得的SEQ ID NO:4Cas9似乎相对不受5-甲氧基-UTP取代的影响,在0％与20％之间的5-甲氧基-UTP取代度下,略微提高纯度。

[0460]　不同mRNA的免疫原性的评估法是采用墨点分析法,使用抗dsRNA抗体作为双股(ds)mRNA特征的量度,其是潜在免疫原性的指标(图5A至图5D)。图5B及图5D使用包含SEQ ID NO:5的Cas9 mRNA序列且图5C使用包含SEQ ID NO:4的Cas9 mRNA序列。对于使用未经修饰的UTP所生成的构建体(图5B至图5C),随着5-甲氧基-UTP含量增加,双股型通常会明显降低。使用N1-甲基-假UTP所生成的mRNA(图5D)显示与抗dsRNA抗体的结合性下降,但与抗体的结合性亦呈现随5-甲氧基-UTP含量增加而降低。

[0461]　随后由mRNA与靶向甲状腺素转运蛋白(TTR)的引导序列(G209;SEQ ID NO:64)一起转染至Neuro 2A细胞中,且测量编辑百分比,于活体外评估编辑效率。

[0462]　如图6A中所示,评估由包含具有N1-甲基-假UTP及2个核定位序列及HA标签的SEQ ID NO:2的构建体转录的Cas9 mRNA(由最左边括号指示的群组)、由包含具有UTP及2个核定位序列及HA标签转录的SEQ ID NO:2的构建体转录的Cas9 mRNA(通过中间括号指示的组)及由包含具有UTP的SEQ ID NO:1的构建体转录的Cas9 mRNA(由最右边括号指示的群组)。对于各组,针对呈如X轴上所指示的0％至100％的增加量的5-甲氧基-UTP的转录来评定0.1ng至100ng的mRNA的不同浓度。未处理细胞未显示可测量的编辑。图6B显示表示为EC50值(ng)的编辑效率数据。

[0463]　转录期间增加的5-甲氧基-UTP含量呈现对两个SEQ ID NO:5条件下的编辑效率具有不利作用,亦含有N1-甲基-假UTP的转录物要比含UTP的转录物稳健(例如在60％及80％5-甲氧基-UTP下)。相比之下,包含SEQ ID NO:4的Cas9 mRNA序列的编辑效率对增加的5-甲氧基-UTP含量显示很小(若存在)效应。因此,根据此系统,包含SEQ ID NO:4mRNA的Cas9 mRNA序列可伴随至多100％5-甲氧基-尿苷提供与含有未经修饰的尿苷的形式类似的编辑效率。

[0464]　3.编码Cas9的mRNA的活体内表征

[0465]　评估包含SEQ ID NO:4的Cas9 mRNA序列对包含SEQ ID NO:5的Cas9 mRNA序列的活体内功效及在未经修饰的UTP、N1-甲基-假UTP、40％5-甲氧基-UTP+60％未经修饰的UTP或100％5-甲氧基-UTP存在下包含SEQ ID NO:4的Cas9 mRNA序列的转录的效应。表7提供关于此等活体内研究组的信息。以脂质纳米颗粒(LNP)调配物形式施用各mRNA。

[0466]　表7.用于活体内研究的LNP720至LNP724

[0467]

LNP	Cas9 ORF SEQ ID NO	经修饰的核苷酸
LNP720	5	N1-甲基-假尿苷

[0468]

LNP721	4	N1-甲基-假尿苷
LNP722	4	未经修饰
LNP723	4	40% 5-甲氧基尿苷/60%未经修饰
LNP724	4	5-甲氧基尿苷

[0469] 活体内研究设计如下。CD-1雌性小鼠是来自Charles River(每组n＝5)。以每公斤1mg(mpk)或0.5mpk连同针对甲状腺素转运蛋白(TTR)的单一引导RNA(SEQ ID NO:42)一起对动物进行静脉内(i.v.)投配。在给药后4小时(hpd)针对MCP-1、IL-6、IFN-α及TNF-α的细胞介素分析对接受1mpk剂量的动物进行抽血。24hpd时针对总体健康对动物加以评定。在给药后7天进行尸体剖检,同时针对血清TTR分析收集血液,且针对次世代测序(NGS)编辑分析收集肝脏。

[0470] 4hpd时收集来自以1mpk投配的动物的血清,且制备血清且根据制造商说明书运作ProcartaPlex®小鼠4丛分析(Thermo Fisher)。针对MCP-1、IL-6、IFN-α及TNF-α的血清含量的结果展现于图7A至图7D中。此等结果表明,用经修饰的UTP制备的包含SEQ ID NO:4的Cas9 mRNA序列(LNP721、LNP723或LNP724)显示相对较低含量的细胞介素产量。

[0471] 亦在给药后7天评定血清中的TTR含量,如图8A及表8中所示。TSS(即5％蔗糖、45mM NaCl、50mM Tris(pH 7.5))样本指示未进行LNP处理的TTR含量。所有LNP调配物均描述于表7中。

[0472] 表8:投配LNP720至LNP724之后的血清TTR含量的结果

[0473]

LNP	Cas9 ORF SEQ ID NO	经修饰的核苷酸	TTR (μg/ml), 0.5 mpk	TTR (μg/ml), 1 mpk
TSS	不适用	不适用	1019.0	
LNP720	5	N1-甲基-假尿苷	559.4	287.2
LNP721	4	N1-甲基-假尿苷	160.1	35.3
LNP722	4	未经修饰	483.4	247.0
LNP723	4	40% 5-甲氧基尿苷/	525.8	170.1

[0474]

		60%未经修饰		
LNP724	4	5-甲氧基尿苷	774.0	505.4

[0475] 表9及图8B提供关于如由次世代测序(NGS)测量的肝脏中TTR的编辑百分比的结果。

[0476] 表9:呈投配LNP720至LNP724之后的肝脏中TTR的编辑百分比的结果

[0477]

LNP	Cas9 SEQ ID NO	经修饰的核苷酸	编辑%,0.5 mpk	编辑%, 1 mpk
TSS	不适用	不适用	0.16	
LNP720	5	N1-甲基-假尿苷	34.9	50.3
LNP721	4	N1-甲基-假尿苷	63.3	74.8
LNP722	4	未经修饰	43.6	53.7
LNP723	4	40% 5-甲氧基尿苷/60%未经修饰	31.8	63.2
LNP724	4	5-甲氧基尿苷	15.9	35.2

[0478] 与TSS对照样本相比,包含Cas9的所有LNP均显示血清TTR含量降低及高于基线的编辑。在比较均用N1-甲基-假UTP转录的标准Cas9 mRNA(SEQ ID NO:5,LNP720)与包含SEQ ID NO:4mRNA的Cas9 mRNA序列(SEQ ID NO:4,LNP721)时,包含SEQ ID NO:4的Cas9 mRNA序列显示经改良的活性(较低TTR及较高编辑%)。对于包含SEQ ID NO:4的Cas9 mRNA序列,活性在N1-甲基-假UTP情况下为最高的,且伴随40%5-甲氧基-UTP+60%未经修饰的UTP的转录(LNP723)产生的活性要比100%5-甲氧基-UTP(LNP724)高。

[0479] 作为脱靶效应的量度,亦测量用1mpk上文所述的LNP调配物投配的动物的脾脏中的编辑,如图7及表10中所示。对于所有LNP调配物,无论在Cas9或经最佳化的Cas9下,均在肝脏中看到大于20倍的较高编辑(图6A)。

[0480] 表10:关于投配1mpk包含sgRNA及不同Cas9的LNP之后的脾脏中的TTR的编辑百分比的结果

[0481]

LNP	Cas9 SEQ ID NO	经修饰的核苷酸	编辑%, 1 mpk
TSS	不适用	不适用	0.1
LNP720	5	N1-甲基-假尿苷	0.66
LNP721	4	N1-甲基-假尿苷	2.42
LNP722	4	未经修饰	0.68
LNP723	4	40% 5-甲氧基尿苷/ 60%未经修饰	1.12
LNP724	4	5-甲氧基尿苷	0.34

[0482] 4.编码Cas9的mRNA在初级小鼠肝细胞中的功效的表征

[0483] 活体外评估不同LNP在初级小鼠肝细胞(PMH)中的功效。

[0484] 在100ng下,表5中所述的所有LNP均支持TTR的编辑,如图10中所示。如所预期,未处理的细胞未显示可测量的TTR的编辑。

[0485] 表11显示基于图10中展现的资料计算的各LNP的EC50值。

[0486] 表11:针对PMH中TTR的基因编辑的经估算的EC50值(ng)

[0487]

LNP	EC50
LNP720	45.65
LNP721	23.04
LNP722	54.00
LNP723	52.40
LNP724	164.1
LNP685	59.88

[0488] 5.含Cas9 mRNA的LNP在大鼠中的活体内表征

[0489] 在大鼠中评估包含SEQ ID NO:4的Cas9 mRNA序列对包含SEQ ID NO:5的Cas9 mRNA序列的活体内功效。表12提供关于此等活体内研究组的信息。标准Cas9 mRNA是指SEQ ID NO:5,而缺乏U(U-dep)的mRNA是指SEQ ID NO:4。各mRNA是以脂质纳米颗粒(LNP)调配物形式施用。

[0490] LNP716(标准Cas9)及LNP738(缺乏U)LNP调配物的详情显示于表12中。

[0491] 表12:LNP调配物表征

[0492]

LNP ID	RNA制备及方法	N:P	RNA 浓度(mg/mL)	囊封率(%)	粒度(nm)	粒子PDI
716	柠檬酸盐-NaCl;X-flow_TFF	4.5	2.00	98	88.42	0.056
738	柠檬酸盐-NaCl;X-flow_TFF	4.5	2.22	97	92.80	0.044

[0493] PDI＝多分散性指数

[0494] N:P＝N:P比,如上文所述

[0495] 如先前所述测量血清TTR。

[0496] 在大鼠中在2mpk及5mpk的剂量下将具有SEQ ID NO:5的ORF的Cas9 mRNA与具有SEQ ID NO:4的ORF的Cas9 mRNA(图11A至图11B)进行比较,如图11A及表13中所示。此等资料表明,在2mpk及5mpk两者下,与SEQ ID NO:5的Cas9 ORF相比,SEQ ID NO:4的Cas9 ORF引起血清TTR较显著降低。图11B及表13展示呈相对于经TSS处理的对照的值的百分比形式的此等结果。5mpk剂量的U-dep Cas9 LNP引起血清TTR含量降低大于90％。

[0497] 表13:用LNP716及LNP738 Cas9调配物投配之后的血清TTR含量

[0498]

LNP	Cas9	剂量(MPK)	血清TTR (μg/ml)	血清TTR (KD%)
TSS	-	-	1954.40	-

[0499]

716	标准	5	950.36	51.37
		2	1474.58	24.55
738	U-Dep	5	153.30	92.16
		2	824.93	57.79

[0500] KD％＝与TSS样本的平均血清浓度相比的基因减弱％

[0501] 图10及表14显示用LNP716(标准)及LNP738(U-dep)调配物以2mpk及5mpk投配之后的TTR的肝脏编辑。尽管TSS显示可忽略的编辑,但LNP716及LNP738调配物两者均引起TTR的肝脏编辑。在比较调配物中,包含U缺乏的LNP738调配物引起的编辑为包含标准Cas9的LNP716调配物的两倍。

[0502] 表14:用缺乏U及标准Cas9调配物投配之后的TTR的肝脏编辑

[0503]

LNP	Cas9	剂量(MPK)	肝脏编辑(%)
TSS	-	-	0.10
716	标准	5	32.14
		2	8.04
738	U-Dep	5	66.02
		2	31.60

[0504] 此等数据表明,缺乏U的Cas9 mRNA显著地改良肝脏中TTR的编辑程度。

[0505] 6.具有不同UTR的mRNA的表征

[0506] 将如表15中所指示的具有UTR及+/-血球凝集素(HA)标签的编码Cas9的mRNA与靶向TTR的引导RNA(G282;SEQ ID NO:42)调配为LNP。使用Nano Assemblr™组配LNP,其含有45％脂质A、9％DSPC、44％胆固醇及2％PEG2k-DMG,且使用Amicon PD 10过滤器纯化,且以0.5mg/ml的浓度(LNP浓度)使用。以0.5或1.0mpk静脉内投配CD-1雌性小鼠(每组n＝5)。在给药后7天,将动物处死,收集血液及肝脏,且测量血清TTR及肝脏编辑。

[0507] 表15.LNP662至LNP669 mRNA描述及血清TTR及肝脏编辑分析的结果

[0508]

LNP	mRNA描述	剂 量	血 清 TTR	血 清 TTR	肝 脏 编 辑

[0509]

		(mpk)	(平均μg/mL)	(平均KD%)	(%)
TSS	-	-	944.52		0.06
LNP662	ORF：SEQ ID NO: 5;无HA标签	1	729.56	22.76	20.08
		0.5	988.75	-4.68	8.26
LNP663	ORF：具有HA标签的SEQ ID NO: 45	1	488.62	48.27	39.12
		0.5	842.88	10.76	20.18
LNP664	ORF：具有HA标签;HBA UTR的SEQ ID NO: 45	1	628.35	33.47	32.68
		0.5	1087.10	-15.10	14.68
LNP665	ORF：具有HA标签;HBB UTR的SEQ ID NO: 45	1	524.43	44.48	42.70
		0.5	797.37	15.58	18.72
LNP666	ORF：具有HA标签;XBG UTR的SEQ ID NO: 45	1	233.46	75.28	54.28
		0.5	1011.22	-7.06	17.96
LNP667	ORF：SEQ ID NO: 4;无HA标签	1	197.58	79.08	58.64
		0.5	689.24	27.03	31.26
LNP668	ORF：SEQ ID NO: 4;无HA标签;未经修饰的NTP	1	622.42	34.10	34.44
		0.5	811.94	14.04	21.30
LNP669	ORF：SEQ ID NO: 5;无HA标签;未经修饰的NTP	1	1050.68	-11.24	9.82
		0.5	1189.70	-25.96	4.04

[0510] 除非另外指明,否则mRNA中的UTR为HSD/Alb。HBA:人类α血球蛋白;HBB:人类β血球蛋白(HBB);XBG:爪蟾β血球蛋白(XBG)。除非另外指明,否则mRNA含有100％N1-甲基假尿苷代替尿苷。

[0511] 图13A至图13E显示血清TTR(在图13A中显示为μg/ml,且在图13B中显示为TSS的％);所有LNP662至LNP669的肝脏编辑(图13C);其中仅UTR变化的LNP663至LNP666的肝脏编辑(图13D);及其中仅mRNA序列及UTP修饰变化的LNP662及LNP667至LNP669的肝脏编辑(图13E)。

[0512] 人类白蛋白、人类α血球蛋白、人类β血球蛋白及爪蟾β血球蛋白UTR大致同样有效;人类α血球蛋白的值可略微较低但并不明确差值是否明显。

[0513] 含有较少尿苷的SEQ ID NO:4的ORF增加肝脏中的编辑的量。用N1-甲基假尿苷制得的Cas9 mRNA要比用未经修饰的尿苷制得的Cas9 mRNA有效。

[0514] 7.使用不同引导物:Cas9比的活体外及活体内编辑

[0515] 将包含根据SEQ ID NO:4或SEQ ID NO:5的ORF的mRNA与靶向TTR的引导RNA以如表16中所示的不同引导物:Cas9 mRNA重量比调配为LNP。通过如上文所指示的IVT合成用代替尿苷三磷酸的N1-甲基假尿苷三磷酸、HSD 5′UTR、人类白蛋白3′UTR及聚-A尾制得Cas9 mRNA。

[0516] 表16.用于活体外及活体内研究的LNP815至LNP824

[0517]

LNP	Cas9 SEQ ID NO	RNA比(引导物:Cas9)
LNP815	5	2:1
LNP816	5	1:1
LNP817	5	1:2
LNP818	5	1:4
LNP819	5	1:8
LNP820	4	2:1
LNP821	4	1:1
LNP822	4	1:2
LNP823	4	1:4
LNP824	4	1:8

[0518] 将初级小鼠肝细胞(PMH)涂铺于补充有3％食蟹猕猴血清的培养基中持续24小时,且随后用表16中所示的0.3、1、3或10ng LNP处理。48小时之后裂解细胞,且通过NGS测定编辑％。结果显示在图14及表17中。

[0519] 表17.PMH中的活体外编辑

[0520]

LNP	mRNA	引导物:mRNA比	10 ng	3 ng	1 ng	0.3 ng
LNP815	5	2:1	75.0	41.7	9.3	1.3
LNP816	5	1:1	80.9	51.5	15.5	2.6
LNP817	5	1:2	79.1	49.8	16.3	2.2
LNP819	5	1:8	90.7	67.2	27.8	5.2
LNP820	4	2:1	78.8	44.3	9.8	0.9
LNP821	4	1:1	81.9	49.9	12.3	2.1
LNP823	4	1:4	85.5	58.3	17.8	2.0
LNP824	4	1:8	84.9	47.4	13.1	1.6

[0521] 对于活体内表征,以0.2、0.5或1mpk向小鼠施用LNP(每组n＝5)。在给药后8天,将动物处死,收集血液及肝脏以及脾脏,且测量血清TTR、肝脏编辑及脾脏编辑。血清TTR结果显示在图15A至图15B及表18中。肝脏编辑结果显示在图16A至图16B及表19中。脾脏编辑结

果显示在图17A至图17B及表20中。用媒剂(转化及储存溶液;“TSS”)投配阴性对照小鼠。使用LNP815至LNP819的实验及使用LNP820至LNP824的实验运作独立对照。

[0522] 表18.用LNP815至LNP824投配之后的血清TTR含量

[0523]

LNP	引导物:Cas9比	剂量(mpk)	血清TTR(μg/mL)	血清TTR(KD%)
TSS			974.23	-
LNP815	2:1	1	300.32	69.17
		0.5	539.37	44.64
		0.2	800.85	17.80
LNP816	1:1	1	183.61	81.15
		0.5	466.63	52.10
		0.2	859.05	11.82
LNP817	1:2	1	117.86	87.90
		0.5	487.26	49.99
		0.2	715.35	26.57
LNP818	1:4	1	168.44	82.71
		0.5	428.89	55.98
		0.2	935.14	4.01
LNP819	1:8	1	323.87	72.29
		0.5	664.80	31.76
		0.2	1039.66	-6.72
TSS			1104.27	-
LNP820	2:1	1	38.12	96.55
		0.5	122.59	88.90
		0.2	358.88	67.50
LNP821	1:1	1	38.53	96.51
		0.5	190.30	82.77
		0.2	501.05	54.63
LNP822	1:2	1	25.76	97.67
		0.5	123.34	88.83
		0.2	520.73	52.84
LNP823	1:4	1	28.00	97.46
		0.5	98.99	91.04
		0.2	529.35	52.06
LNP824	1:8	1	93.65	91.52
		0.5	174.43	84.20
		0.2	731.43	33.76

[0524]

[0525] KD%提供相对于TSS对照的TTR含量的基因减弱%。

[0526] 表19.用LNP815至LNP824投配之后的肝脏编辑

[0527]

LNP	引导物:Cas9比	剂 量(MPK)	编辑%
TSS			0.78
LNP815	2:1	1	57.52
		0.5	38.76
		0.2	12.28
LNP816	1:1	1	63.46
		0.5	40.26
		0.2	14.12
LNP817	1:2	1	68.18
		0.5	38.38
		0.2	17.58
LNP818	1:4	1	61.8
		0.5	41.58
		0.2	9.44
LNP819	1:8	1	55.88
		0.5	31.26

[0528]

		0.2	6.4
TSS			0.22
LNP820	2:1	1	67
		0.5	69.58
		0.2	48.78
LNP821	1:1	1	75.82
		0.5	64.02
		0.2	41.2
LNP822	1:2	1	73.26
		0.5	69.74
		0.2	44.16
LNP823	1:4	1	75.48
		0.5	66.7
		0.2	38.7
LNP824	1:8	1	69.14
		0.5	63.16
		0.2	20.78

[0529] LNP820至LNP824通常产生大于或约等于相同比率下的其LNP815至LNP819对应物的肝脏编辑结果。LNP820至LNP824在0.5及1mpk下测试的比率范围内及0.2mpk下2∶1至1∶4

的比率下显示恒定效能。

[0530] 表20.用LNP815至LNP824投配之后的脾脏编辑

[0531]

LNP	引导物:Cas9比	剂 量 (MPK)	编辑%
TSS			0.12
LNP815	2:1	1	0.6
		0.5	0.62
		0.2	0.28
LNP816	1:1	1	0.74
		0.5	1
		0.2	0.28
LNP817	1:2	1	0.74
		0.5	0.58
		0.2	0.22
LNP818	1:4	1	1.22
		0.5	0.44
		0.2	0.3

[0532]

LNP819	1:8	1	0.9
		0.5	0.64
		0.2	0.36
TSS			0.225
LNP820	2:1	1	0.83
		0.5	0.825
		0.2	0.525
LNP821	1:1	1	1.425
		0.5	0.9
		0.2	0.425
LNP822	1:2	1	1.85
		0.5	0.625
		0.2	1.74
LNP823	1:4	1	1.475
		0.5	0.8
		0.2	0.32
LNP824	1:8	1	1.14
		0.5	1.34
		0.2	0.56

[0533] 用各调配物以3mpk投配额外小鼠组(n＝2),且在6hpd处死以用于测定肝脏中的蛋白质表达。来自用3mpk 1∶1及1∶4比率调配物(LNP816、LNP818、LNP821及LNP823)处理的小鼠的肝脏蛋白质的蛋白质印迹法展示在图18中。针对蛋白质印迹法的初级Ab为呈1∶5,000

的Immunoprecise™兔抗Cas9,且二级Ab为呈1∶12,500的Dylight™山羊抗兔。Cas9蛋白质表达在使用具有SEQ ID NO:4的ORF的mRNA的LNP中明显较高。

[0534] 8.经修饰的核苷酸的影响的表征

[0535] 将编码Cas9且含有如表21中所指示的经修饰的核苷酸的mRNA与靶向TTR的引导RNA(G282;SEQ ID NO:42)调配为LNP。LNP1034含有自Trilink Biotechnologies有限责任公司市售获得的Cas9 mRNA且包括CleanCap™(其中7-甲基鸟嘌呤帽结构之后的第一核苷酸经2′-O-甲基化的Cap1结构)。LNP1027至LNP1033含有包含根据SEQ ID NO:4的ORF的mRNA及ARCA(抗反向帽结构类似物)Cap0。使用Nano Assemblr™组配LNP,其含有45％脂质A、9％DSPC、44％胆固醇及2％PEG2k-DMG,使用Amicon PD10过滤器纯化,且悬浮于TSS缓冲液中。LNP中的N∶P(氮与磷酸)比为4.5且调配物的RNA浓度为0.4mg/ml。以0.1或0.3mpk静脉内投配CD-1雌性小鼠(每组n＝5)。在给药后7天,将动物处死,收集血液及肝脏,且测量血清TTR及肝脏编辑。

[0536] 表21.用于活体内研究的LNP1027至LNP1034

[0537]

LNP ID	Cas9 ORF	帽结构	经修饰的核苷酸
LNP1027	SEQ ID NO: 4	ARCA	N1-甲基-假尿苷
LNP1028	SEQ ID NO: 4	ARCA	25% 5-碘尿苷
LNP1029	SEQ ID NO: 4	ARCA	50% 5-碘尿苷
LNP1030	SEQ ID NO: 4	ARCA	25% 5-碘胞苷
LNP1031	SEQ ID NO: 4	ARCA	25% 5-碘尿苷及25% 5-碘胞苷
LNP1032	SEQ ID NO: 4	ARCA	假尿苷
LNP1033	SEQ ID NO: 4	ARCA	假尿苷及5-甲基胞苷
LNP1034	Trilink Cas9 mRNA	CleanCap™	5-甲氧基尿苷

[0538] 对于其中列出经修饰的尿苷及/或胞苷核苷酸呈25％或50％的LNP,尿苷及/或胞苷的剩余部分分别为未经修饰的。

[0539] 血清TTR结果展示于图19A至图19B(血清TTR结果分别以μg/mL及TSS对照的％表示);图20(肝脏编辑);及表22中。

[0540] 表22.LNP1027至LNP1034的血清TTR及肝脏编辑结果

[0541]

LNP ID	剂量(mpk)	经修饰的核苷酸	血清TTR(μg/mL)	血清TTR(KD%)	平均编辑%
TSS	-		1438.438	-	0.20
LNP1027	0.3	N1-甲基-假	381.474	73.48	51.08

[0542]

	0.1	尿苷	979.404	31.91	15.76
LNP 1028	0.3	25% 5-碘尿苷	311.738	78.33	54.96
	0.1		758.41	47.28	18.82
LNP1029	0.3	50% 5-碘尿苷	714.748	50.31	31.94
	0.1		1034.69	28.07	8.26
LNP1030	0.3	25% 5-碘胞苷	676.164	52.99	26.28
	0.1		973.836	32.30	6.58
LNP1031	0.3	25% 5-碘尿苷及25% 5-碘胞苷	546.946	61.98	30.30
	0.1		969.92	32.57	6.12
LNP1032	0.3	假尿苷	448.582	68.81	42.68
	0.1		947.602	34.12	9.60
LNP1033	0.3	假尿苷及5-甲基胞苷	979.284	31.92	11.36
	0.1		1031.33	28.30	2.22
LNP1034	0.3	5-甲氧基尿苷	1133.826	21.18	4.82
	0.1		1339.304	6.89	0.78

[0543] LNP1027的含N1-甲基假尿苷的mRNA具有比LNP1032的含假尿苷的mRNA略微较高的编辑效率。含有假尿苷及5-甲基胞嘧啶核苷两者的mRNA(LNP1033)的效能显著降低。含有25％5-碘尿苷的mRNA显示与含N1-甲基假尿苷的mRNA相等的编辑效率。在50％5-碘尿苷下，效能有所降低。来自Trilink的5-甲氧基尿苷mRNA显示较低活性。

[0544] 9.大鼠中具有不同UTR的mRNA的影响的表征

[0545] 此研究评估经ARCA加帽结构的具有HBB(人类β-血球蛋白)5′及3′UTR;XBG(爪蟾β-血球蛋白)5′及3′UTR;或具有人类HSD17B4(HSD)5′UTR及白蛋白(ALB)3′UTR的Cas9 mRNA在

大鼠中的活体内功效。

[0546] 使用上文所述的错流方法制备含有在LNP中呈1:1摩尔比的靶向大鼠TTR基因的引导RNA(G534;SEQ ID NO:72)及Cas9 mRNA的调配物且将其在VivaFlow™ 50膜上过滤。LNP含有呈45:9:43:3摩尔比的阳离子型脂质(脂质A)、胆固醇、DSPC及PEG2k-DMG且具有6.0的N:P比。以1mpk及0.3mpk投配调配物。所有大鼠均为来自Charles River的史泊格多利雌性,每组n=5。尸体剖检(给药后7天)时,针对TTR分析收集血清且针对编辑分析收集肝脏。在LNP1058中,mRNA含有HBB UTR。在LNP1059中,mRNA含有XBG UTR。在LNP1060中,mRNA分别含有HSD及ALB 5'及3'UTR。在所有情况下,mRNA编码序列是根据SEQ ID NO:4。

[0547] 肝脏编辑及血清TTR结果展示于图21A至图21C及表23中。

[0548] 表23.大鼠中使用LNP1058至LNP1060的肝脏编辑及血清TTR结果.

[0549]

LNP	UTR	剂量(mpk)	肝脏编辑(%)	血清TTR(μg/ml)	血清TTR(KD %)
TSS			0.0	1366.9	
1058	HBB (3'及5')	1	66.3	84.4	93.8
		0.3	27.6	881.1	35.5
1059	XBG (3'及5')	1	69.1	63.0	95.4
		0.3	31.6	748.7	45.2
1060	HSD (5') 及 ALB (3')	1	62.6	115.6	91.5
		0.3	20.9	896.0	34.4

[0550] 结果表明,LNP1058至LNP1060中所有经测试的mRNA均能够支持编辑。伴随LNP1059中含有XBG UTR的mRNA看到最高编辑程度及血清TTR的最显著降低。

[0551] 10.RNA载荷:mRNA及gRNA共调配

[0552] 此研究评估不同gRNA与mRNA比在小鼠中的活体内功效。通过如实例1中所指示的IVT合成用代替尿苷三磷酸的N1-甲基假尿苷三磷酸来制得经CleanCap™加帽结构的具有SEQ ID NO:4的ORF、HSD 5'UTR、人类白蛋白3'UTR、Kozak序列及聚-A尾的Cas9 mRNA。

[0553] 由所述mRNA及如实例2中所述的sg282(SEQ ID NO:42;G282)与脂质A、胆固醇、DSPC及PEG2k-DMG以55:33:9:3摩尔比且以6的N:P比制备LNP调配物。调配物的gRNA:Cas9 mRNA重量比如表24中所示。

[0554] 表24.LNP1110至LNP1116的表征.

[0555]

LNP ID	RNA浓度(mg/mL)	EE (%)	粒度(nm)	粒子PDI	数量平均(nm)
1110	0.92	99	69.52	0.022	56.47
1111	0.86	97	76.65	0.065	57.36
1112	0.90	99	76.58	0.036	63.11
1113	0.97	99	76.60	0.071	58.92
1114	1.05	99	76.34	0.018	62.82
1115	0.65	99	82.64	0.018	66.63
1116	0.75	100	82.01	0.039	65.05

[0556] 对于活体内表征,以每公斤0.1mg总RNA(引导RNA毫克数+mRNA毫克数)向小鼠施用

以上LNP(每组n＝5)。在给药后7至9天,将动物处死,收集血液及肝脏,且如上文所述测量血清TTR及肝脏编辑。血清TTR及肝脏编辑结果展示于图22A及图22B中。用TSS媒剂投配阴性对照小鼠。

[0557] 此外,以每公斤0.05mg mRNA的恒定mRNA剂量,同时将gRNA剂量自0.06mg/kg变为0.4mg/kg来向小鼠施用以上LNP(每组n＝5)。在给药后7至9天,将动物处死,收集血液及肝脏,且测量血清TTR及肝脏编辑。血清TTR及肝脏编辑结果展示于图22C及图22D中。用TSS媒剂投配阴性对照小鼠。

[0558] 11.密码子方案的表征

[0559] 设计使用不同密码子方案的Cas9序列以针对经改良的蛋白质表达进行测试。各序列经设计以使用独特密码子组编码SEQ ID No:3的Cas9氨基酸。在各开放阅读框架序列中,使用单一密码子编码各氨基酸。基于密码子在基于NCBI-GenBank Flat File Release 160.0(Nakamura等人(2000)Nucl.Acids Res.28,292;Benson等人(2006)Nucleic Acids Res.34(数据库期),D16-20)的智人中的整个蛋白质编码基因中的出现频率及特定核苷酸在密码子中的丰度来改变序列。基于Table 4中所示的密码子方案,构筑编码SEQ ID NO:3的Cas9蛋白质的七个不同Cas9开放阅读框架(SEQ ID NO:52、SEQ ID NO:54及SEQ ID NO:108至SEQ ID NO:112)。将此等并入至亦含有HSD 5′UTR(SEQ ID NO:41)、白蛋白3′UTR、T7启动子及聚A尾的构建体中。含有白蛋白3′UTR及聚A尾的示例性序列为SEQ ID NO:53,其中3′UTR及聚A尾在HSD 5′UTR及SEQ ID NO:52的ORF之后。使用如由Presnyak及同事(2015)所述的基于用于改良的mRNA半衰期的最佳密码子的密码子流程编码SEQ ID NO:3的Cas9蛋白质的以类似方式构成的构建体(SEQ ID NO:107,使用表4的长半衰期密码子组)亦包括于此等评估中。

[0560] 通过IVT产生各构建体的信使RNA。用800ng各Cas9 mRNA使用Lipofectamine™ MessengerMAX™转染剂(ThermoFisher)转染HepG2细胞。转染后六小时,通过冻融裂解细胞且通过离心清除。通过ELISA分析测定Cas9蛋白质含量。简言之,通过二辛可宁酸(蛋白)含量测定法测定总蛋白质浓度。根据制造商方案使用Cas9小鼠抗体(Origene,目录号CF811179)作为捕捉抗体且使用Cas9(7A9-3A3)小鼠mAb(Cell Signaling Technology,目录号14697)作为侦测抗体来制备MSD GOLD 96孔抗生蛋白链菌素SECTOR板(Meso Scale Diagnostics,目录号L15SA-1)。在具有无EDTA的1×Halt™蛋白酶抑制剂混合液(ThermoFisher,目录号78437)的稀释剂39中的呈0、0.12、0.49、1.95、7.81、31.25、125及500ng/mL的Cas9蛋白质用作校正标准。使用Meso Quickplex SQ120仪器(Meso Scale Discovery)读取ELISA板且用Discovery Workbench 4.0软件包(Meso Scale Discovery)分析资料。

[0561] 通过将mRNA以及靶向甲状腺素转运蛋白(TTR)的引导物(G502;SEQ ID NO:70)转染于HepG2细胞中且测量编辑百分比来活体外评定编辑效率。在3ng至100ng的mRNA浓度下评定包含表25中所指示的SEQ ID No的Cas9 mRNA。未处理细胞未显示可测量的编辑。图23至图24及表25显示不同密码子组对Cas9蛋白质表达及活体外编辑的影响。

[0562] 表25.具有不同密码子组的ORF的活体外编辑及表达.

[0563]

ORF (密码子组)	每毫克总蛋白质的Cas9纳克数	每毫克总蛋白质的Cas9纳克数标准差	编辑%(经转染的30 ng mRNA)	编辑标准差
SEQ ID No: 50 (表6最小尿苷密码子,剪接接点经移除)	10.99	1.04	35.6	2.11
SEQ ID No: 107 (表4长半衰期)	18.78	2.83	36.5	3.27
SEQ ID No: 52 (表4低U 1)	31.23	4.47	22.2	2.83
SEQ ID No: 54 (表4低U 2)	1.54	0.16	14.7	0.40
SEQ ID No: 108 (表4高U)	1.41	0.12	14.0	2.95
SEQ ID No: 109 (表4低G)	4.95	0.70	19.6	2.29
SEQ ID No: 110 (表4低C)	2.26	0.16	23.1	4.07
SEQ ID No: 111 (表4低A)	74.62	15.53	41.3	3.56
SEQ ID No: 112 (表4低A/U)	77.32	10.60	34.8	7.32
SEQ ID No: 4 (表6最小尿苷密码子)	17.16	1.54	34.7	1.15

[0564] 为了测定密码子方案的活体内有效性,当使用表4中所述的密码子方案自编码Cas9的mRNA活体内表达时,测量Cas9蛋白质表达。将如表26中所指示的信使RNA与靶向TTR的引导RNA(G282;SEQ ID NO:42)调配为LNP。使用错流程序组配LNP,且其含有分别呈50:38:9:3摩尔比的50％脂质A、9％DSPC、38％胆固醇及3％PEG2k-DMG,且具有6.0的N:P比。使用Amicon PD-10过滤器(GE Healthcare)纯化LNP,且以0.32mg/ml的浓度(LNP浓度)使用。以1mpk静脉内投配CD-1雌性小鼠(每组n＝5)。在给药后3小时,将动物处死,收集肝脏且测量肝脏中的Cas9表达。使用上文所述的Meso Scale Discovery ELISA分析测量肝脏中的Cas9蛋白质表达。通过珠粒研磨机在具有1×Complete蛋白酶抑制剂片剂(Roche,目录号11836170001)的RIPA缓冲液(Boston Bioproducts BP-115)中将大约40mg肝脏组织均质化。图25及表26显示肝脏中的Cas9表达结果。低A及low A/U密码子方案(SEQ ID NO:111及SEQ ID NO:112的ORF)的mRNA显示所测试的ORF的最高Cas9表达。阴性对照及SEQ ID NO:54

的ORF的Cas9蛋白质表达小于定量下限(LLOQ)。

[0565]　表26

[0566]

ORF	平均Cas9(每公克肝脏的纳克数)	标准差
TSS	＜LLOQ	0.0
SEQ ID No:4	1644	1172
SEQ ID NO:52	1562	951
SEQ ID NO:54	＜LLOQ	0.0
SEQ ID NO:111	2630	730
SEQ ID NO:112	2134	362

[0567]　为了测定密码子方案的活体内有效性,自使用不同密码子方案的编码Cas9的mRNA活体内测量基因组编辑。将如表27中所指示的信使RNA与靶向TTR的引导RNA(G282;SEQ ID NO:42)调配为LNP。使用错流程序组配LNP,且其含有分别呈50:38:9:3摩尔比的50％脂质A、9％DSPC、38％胆固醇及3％PEG2k-DMG,且具有6.0的N:P比。使用Amicon PD-10过滤器(GE Healthcare)纯化LNP,且以0.05mg/ml的浓度(LNP浓度)使用。以0.1mpk静脉内投配CD-1雌性小鼠(每组n＝5,不同的处在于用SEQ ID NO:52处理的组,n＝4)。在给药后6天,将动物处死,收集血液及肝脏,且测量血清TTR及肝脏编辑。表27及图26显示活体内编辑结果。表27及图27A至图27B显示血清TTR含量。

[0568]　表27

[0569]

ORF	平均编辑%	编辑标准差	血清TTR (μg/ml)	血清TTR标准差	n
TSS	0.06	0.05	856	68	5
SEQ ID No: 4	40.96	8.41	329	143	5
SEQ ID No: 107	44.28	11.45	255	97	5
SEQ ID No: 52	60.10	8.07	143	78	4
SEQ ID No: 54	1.50	0.66	822	161	5
SEQ ID No: 108	0.74	0.36	914	182	5
SEQ ID No: 111	57.26	4.15	216	62	5
SEQ ID No: 112	61.44	4.50	100	79	5

[0570]　为了测定不同mRNA浓度下的密码子方案的功效,进行活体内剂量反应实验。将如表28中所指示的信使RNA与靶向TTR的引导RNA(G282;SEQ ID NO:42)调配为LNP。使用错流方法组配LNP且其含有50％脂质A、9％DSPC、38％胆固醇及3％PEG2k-DMG。使用Amicon PD-10过滤器(GE Healthcare)纯化LNP,且以0.7mg/ml的浓度(LNP浓度)使用。以0.03、0.1或0.3mpk静脉内投配CD-1雌性小鼠(每组n＝5)。在给药后7天,将动物处死,收集血液及肝脏,且测量血清TTR及肝脏编辑。表28及图28显示活体内编辑结果。表28及图29A至图29B显示血清TTR含量。

[0571]　表28

[0572]

ORF	剂量(mpk)	肝脏编辑(%)	血清TTR(μg/mL)	血清TTR(KD%)
TSS	不适用	0.1	576.8	0.0
SEQ ID No: 4	0.3	51.3	165.6	71.3
	0.1	17.3	540.7	6.3
	0.03	1.9	761.4	-32.0
SEQ ID No: 52	0.3	57.0	100.8	82.5
	0.1	29.6	336.1	41.7
	0.03	5.0	636.4	-10.3
SEQ ID NO: 111	0.3	59.4	93.8	83.7
	0.1	30.6	373.5	35.2
	0.03	5.9	559.6	3.0
SEQ ID	0.3	60.6	92.0	87.2

[0573]

NO: 112	0.1	25.5	397.5	31.1
	0.03	7.8	555.3	3.7

[0574] 为了测定具有不同UTR的密码子方案的有效性，在施用编码Cas9的mRNA后活体内测量基因组编辑。将如表29中所指示的信使RNA与靶向TTR的引导RNA(G282;SEQ ID NO:42)调配为LNP。使用错流程序组配LNP，且其含有分别呈50:38:9:3摩尔比的50％脂质A、9％DSPC、38％胆固醇及3％PEG2k-DMG，且具有6.0的N:P比。使用Amicon PD-10过滤器(GE Healthcare)纯化LNP，且以0.05mg/ml的浓度(LNP浓度)使用。以0.1mpk静脉内投配CD-1雌性小鼠(每组n＝5；针对SEQ ID NO:43编辑，n＝4)。在给药后6天，将动物处死，收集血液及肝脏，且测量血清TTR及肝脏编辑。表29及图30A至图30B显示活体内编辑(B)及血清TTR结果(A)。

[0575] 表29

[0576]

mRNA构建体	编辑％	标准差	血清TTR(μg/ml)	标准差
TSS	0	0	1274	214
SEQ ID No:43	28	4	630	152
SEQ ID No:176	35	8	482	138
SEQ ID No:177	37	9	316	143
SEQ ID No:178	42	6	524	192

[0577] 12.加帽结构的影响的表征

[0578] 将编码Cas9且含有如表30中所指示的帽结构、UTR及聚A尾的mRNA与靶向TTR的引导RNA(G282;SEQ ID NO:42)调配为LNP。使用错流程序组配LNP，其含有分别呈50:38:9:3摩尔比的50％脂质A、9％DSPC、38％胆固醇及3％PEG2k-DMG，且具有6.0的N:P比。使用Amicon PD-10过滤器(GE Healthcare)纯化LNP，且以0.06mg/ml的浓度(LNP浓度)使用。以0.1或0.3mpk静脉内投配CD-1雌性小鼠(每组n＝5)。在给药后7天，将动物处死，收集血液及肝脏，

且测量血清TTR及肝脏编辑。

[0579] 图31及表30显示0.1mpk剂量下，具有Cap 1的mRNA具有比具有Cap 0的mRNA高约10％的平均编辑。在0.3mpk剂量下，具有XBG UTR的mRNA具有比具有HSD UTR的mRNA略微较高的平均编辑，除酶cap 0外。血清TTR结果显示于图32(血清TTR结果分别以μg/mL及TSS对照的％表示)；图31(肝脏编辑)；及表30中。

[0580] 表30.针对活体内加帽结构研究的血清TTR及肝脏编辑结果

[0581]

mRNA构建体	帽结构类型	帽结构	5' UTR	剂量	平均编辑(%)	标准差
SEQ ID No. 43	Cap 0	ARCA	HSD	0.1 mpk	21.76	11.61
SEQ ID No. 59	Cap 0	ARCA	XBG	0.1 mpk	22.9	5.53
SEQ ID No. 59	Cap 0	酶Cap 0	XBG	0.1 mpk	17.98	7.04
SEQ ID No. 59	Cap 1	酶Cap 1	XBG	0.1 mpk	31.03	6.4
SEQ ID No. 60	Cap 1	Clean Cap 113	XBG	0.1 mpk	31.08	8.67
SEQ ID No. 60	Cap 1	Clean Cap 413	XBG	0.1 mpk	32.78	2.05
SEQ ID No. 43	Cap 0	ARCA	HSD	0.3 mpk	52.28	5.14
SEQ ID No. 59	Cap 0	ARCA	XBG	0.3 mpk	59.56	4.57
SEQ ID No. 59	Cap 0	酶Cap 0	XBG	0.3 mpk	54.93	10.22
SEQ ID No. 59	Cap 1	酶Cap 1	XBG	0.3 mpk	63.2	0.28
SEQ ID No. 60	Cap 1	Clean Cap 113	XBG	0.3 mpk	61.28	4.76
SEQ ID No. 60	Cap 1	Clean Cap 413	XBG	0.3 mpk	60.56	3.97

[0582] 13.核定位信号的表征

[0583] 设计且测试使用若干核定位信号(NLS)的Cas9序列以测定功效。十一种不同强度的非典型NLS是选自由Kosugi等人(2009)Journal of Biological Chemistry,284(1),478-485所鉴别的NLS，如表31中所示。将此等氨基酸序列添加至Cas9氨基酸序列(SEQ ID NO:13)的羧基端。对照序列编码SEQ ID No.4。

[0584] 表31

[0585]

NLS名称	NLS氨基酸序列	NLS编码序列(CDS)	SEQ ID No (NLS 氨基酸, NLS CDS, ORF CDS)
SV40	PKKKRKV	CCGAAGAAGAAGAGAAA GGTC	78, 92, 4
NLSl	LAAKRSRTT	CTGGCAGCAAAGAGAAGC AGA ACAACA	79, 93, 130
NLS2	QAAKRSRTT	CAGGCAGCAAAGAGAAG CAGAACAACA	80, 94, 131
NLS3	PAPAKRERTT	CCGGCACCGGCAAAGAGA GAAAGAACAACA	81, 95, 132
NLS4	QAAKRPRTT	CAGGCAGCAAAGAGACCG AGAACAACA	82, 96, 133
NLS5	RAAKRPRTT	AGAGCAGCAAAGAGACC GAGAACAACA	83, 97, 134
NLS6	AAAKRSWSM AA	GCAGCAGCAAAGAGAAG CTGG AGCATGGCAGCA	84, 98, 135
NLS7	AAAKRVWSM AF	GCAGCAGCAAAGAGAGTC TGGAGCATGGCATTC	85, 99, 136
NLS8	AAAKRSWSM AF	GCAGCAGCAAAGAGAAG CTGGAGCATGGCATTC	86, 100, 137
NLS9	AAAKRKYFAA	GCAGCAGCAAAGAGAAA GTACTTCGCAGCA	87, 101, 138
NLS10	RAAKRKAFAA	AGAGCAGCAAAGAGAAA GGCATTCGCAGCA	88, 102, 139
NLS11	RAAKRKYFAV	AGAGCAGCAAAGAGAAA GTACTTCGCAGTC	89, 103, 140

[0586] 将具有如表31中所指示的NLS的编码Cas9的mRNA与与靶向TTR的引导RNA(G282;SEQ ID NO:42)调配为LNP。使用错流程序组配LNP,且其含有分别呈50∶38∶9∶3摩尔比的50%脂质A、9%DSPC、38%胆固醇及3%PEG2k-DMG,且具有6.0的N∶P比。使用Amicon PD-10过滤器(GE Healthcare)纯化LNP,且以0.07mg/ml的浓度(LNP浓度)使用。以0.1mpk静脉内投配CD-1雌性小鼠(每组n=5)。在给药后7天,将动物处死,收集血液及肝脏,且测量血清TTR及肝脏编辑。结果展示在表32及图33中。对于对应于表32中列出的NLS的SEQ ID NO,参见表31。

[0587] 表32-伴随不同核定位信号的肝脏编辑

[0588]

NLS	NLS 类别	NLS 强度	0.1 MPK 编辑%	STDEV
SV40	不适用	不适用	14.67	4.17
NLS1	2	3	3.76	1.61
NLS2	2	4	5.86	1.69
NLS3	2	5	2.50	1.82
NLS4	2	6	27.38	11.98
NLS5	2	9	27.80	2.37
NLS6	3	1	2.20	0.82
NLS7	3	6	7.90	0.42
NLS8	3	10	25.52	15.75
NLS9	4	2	3.26	1.65
NLS10	4	5	0.23	0.04
NLS11	4	8	21.02	4.9

[0589] NLS5显示相比于SV40 NLS的统计学上显著的增加(单向ANOVA,p＝0.006)。NLS4及NLS8各展现与SV40 NLS相比编辑增加的可能趋势,但在此实验中差距在统计学上并不显著。图34A至图34B显示施用核定位信号变异体后的血清TTR含量。Kosugi等人(2009)(见上文),针对核定位程度的NLS的评级活性(表32中的”“NLS强度”),其中10为完全在核内,且1为弥漫在整个细胞中。如此文献中评级的NLS活性与编辑效率正相关,如图35中所示。

[0590] 14.UTR的影响的活体外表征

[0591] 表33及图36显示来自具有不同5′UTR的转录物的Cas9表达。所有构建体均使用3′人类白蛋白UTR。通过IVT产生各构建体的信使RNA。使用线性化质粒产生SEQ ID No:179的信使RNA,且使用PCR产物作为模板生成所有其它者。用100ng各Cas9 mRNA及25nM最终浓度的靶向甲状腺素转运蛋白(TTR)的引导物(G502;SEQ ID NO:70)使用Lipofectamine™ MessengerMAX™转染剂(ThermoFisher)转染HepG2细胞。转染后六小时通过Nano-Glo® HiBiT裂解分析(Promega)裂解细胞。通过使用Nano-Glo® Nano-Glo HiBiT胞外侦测系统(Promega,目录号N2420)测定Cas9蛋白质含量。表33及图36显示来自具有不同5′UTR的转录物的Cas9表达。

[0592] 表33:Cas9表达

[0593]

mRNA构建体SEQ ID NO	5' UTR	分子Cas9 (10^7)	标准差(10^7)
179	HSD	447	61
180	CMV-1	723	39
181	CMV-2	672	158
182	CMV-3	662	117
183	HBA	488	101
184	HBB	595	124
185	XBG	813	62

[0594] 15.至非人类灵长类动物的LNP递送

[0595] 用如上文所述使用X-flow/TFF方法制备的LNP调配物进行三项研究。特定莫耳量及载荷提供于表34至表36中。含有Cas9 mRNA及引导RNA(gRNA)的各调配物具有以重量计1:1的mRNA:gRNA比。在表格中指示LNP的剂量(以mg/kg为单位,总RNA含量)、给药途径及动物是否接受地塞米松的预处理。对于接受地塞米松(Dex)预处理的动物,在施用LNP或媒剂之前1小时通过IV快速注射以2mg/kg施用Dex。

[0596] 对于血液化学分析,针对所测量的各因素在如表格中所指示的时间对动物进行抽血。在处理前及处理后的NHP中测量细胞介素诱导。自受约束的清醒动物的外周静脉将最少0.5mL的全血收集于4ml血清分离管中。在室温下使血液结块最少30分钟,之后以2000×g离心15分钟。将血清等分于2个120μL聚丙烯微管中,且在分析之前各储存于-60℃至-86℃下。使用来自Meso Scale Discovery(MSD)的非人类灵长类动物U-Plex细胞介素定制试剂盒来加以分析。分析中包括以下参数:INF-g、IL-1b、IL-2、IL-4、IL-6、IL-8、IL-10、IL-12p40、MCP-1及TNF-α,其中聚焦于IL-6及MCP-1。如制造商方案中所指导地制备试剂盒试剂及标准品。以纯形式使用NHP血清。将板运作于MSD Sector成像仪6000,伴随用MSD Discovery work bench软件版本4012进行分析。

[0597] 在处理前及处理后动物中通过酶免疫分析测量补体含量。自受约束的清醒动物的外周静脉将0.5mL体积的全血收集于0.5mL k_2EDTA管中。以2000×g离心血液15分钟。将血浆等分于2个120μL聚丙烯微管中,且在分析之前各储存于-60℃至-86℃下。使用Quidel MicroVue Complement Plus EIA试剂盒(C3a-Cat号A031)或(Bb-Cat号A027)来加以分析。如制造商方案中所指导地制备试剂盒试剂及标准品。将板运作于450nm下的光密度下的MSD Sector成像仪6000上。使用4参数曲线拟合分析结果。

[0598] 细胞介素诱导及补体活化的数据提供于下表中。“BLQ”意谓小于定量限值。

[0599] 表34,研究1

[0600]

处理组	分子比(分别呈脂质A、胆固醇、DSPC及PEG2k-DMG)	N:P	载荷	样本大小(n)	途径	剂量程度，总RNA含量(mg/kg)	Dex
(1) TSS (媒剂)	不适用	不适用	不适用	3	IV输注	不适用	否
(2) LNP699 G502	45/44/9/2	4.5	Cas9 mRNA (SEQ ID NO:48)；G000502	3	IV输注	3	否
(3) LNP688 G506	45/44/9/2	4.5	Cas9 mRNA (SEQ ID NO:48)；G000506	3	IV输注	3	否
(4) LNP689 G509	45/44/9/2	4.5	Cas9 mRNA (SEQ ID NO:48)；G000509	3	IV输注	3	否
(5) LNP690 G510	45/44/9/2	4.5	Cas9 mRNA (SEQ ID NO:48)；G000510	3	IV输注	3	否

[0601]

[0602] 表35,研究2

[0603]

处理组	分子比(分别呈脂质A、胆固醇、DSPC 及 PEG2k-DMG)	N:P	载荷	样本大小(n)	途径	剂量程度，总RNA含量(mg/kg)	Dex
(1) TSS (媒剂)	不适用	不适用		1	IV 快速注射	不适用	是
(2) TSS (媒剂)	不适用	不适用		1	IV 快速注射	不适用	否
(3) LNP898 G502	45/44/9/2	4.5	Cas9 mRNA (SEQ ID NO:48); G000502	1	IV 输注	3	是
(4) LNP898 G502	45/44/9/2	4.5	Cas9 mRNA (SEQ ID	1	IV 输	3	否

[0604]

			NO:48);G000502		注		
(5) LNP897 G502	45/43/9/3	4.5	Cas9 mRNA (SEQ ID NO:48);G000502	1	IV快速注射	3	是
(6) LNP897 G502	45/43/9/3	4.5	Cas9 mRNA (SEQ ID NO:48);G000502	1	IV快速注射	3	否
(7) LNP897 G502	45/43/9/3	4.5	Cas9 mRNA (SEQ ID NO:48);G000502	1	IV输注	3	是
(8) LNP897 G502	45/43/9/3	4.5	Cas9 mRNA (SEQ ID NO:48);G000502	1	IV输注	3	否
(9) LNP916 GFP	45/43/9/3	4.5	eGFP mRNA (SEQ ID NO:73)	1	IV输注	6	是
(10) LNP916 GFP	45/43/9/3	4.5	eGFP mRNA (SEQ ID NO:73	1	IV输注	6	否

[0605] 表36,研究3

[0606]

处理组	分子比(分别呈脂质A、胆固醇、DSPC及PEG2k-DMG)	N:P	载荷	样本大小(n)	途径	剂量程度，总RNA含量(mg/kg)	Dex
(1) TSS	不适用	不适用	不适用	3	IV快速注射	不适用	否
(2) LNP1021 G502	50/38/9/3	6	Cas9 mRNA (SEQ ID NO:43)；G000502	3	IV快速注射	1	否
(3) LNP1021 G502	50/38/9/4	6	Cas9 mRNA (SEQ ID NO:43)；G000502	1	IV快速注射	1	是
(4) LNP1022 G502	55/33/9/3	6	Cas9 mRNA (SEQ ID NO:43)；G000502	3	IV快速注射	1	否
(5) LNP1023 G502	45/43/9/3	4.5	Cas9 mRNA (SEQ ID NO:43)；G000502	3	IV快速注射	3	否
(6) LNP1024 G509	50/38/9/3	6	Cas9 mRNA (SEQ ID NO:43)；G000509	3	IV快速注射	1	否
(7) LNP1024 G509	50/38/9/4	6	Cas9 mRNA (SEQ ID NO:43)；G000509	1	IV快速注射	1	是
(8) LNP1025 G509	55/33/9/3	6	Cas9 mRNA (SEQ ID NO:43)；	3	IV快速注	1	否

[0607]

			G000509		射		
(9) LNP1021 G502	50/38/9/3	6	Cas9 mRNA (SEQ ID NO:43); G000502	1	IV快速注射	3	否
(10) LNP1022 G502	50/38/9/3	6	Cas9 mRNA (SEQ ID NO:43); G000502	1	IV快速注射	3	否

[0608] 表37.根据研究1的IL-6测量

[0609]

处理组	采血前	6小时	24小时
(1)TSS(媒剂)	5.71±2.70	29.1±20.37	7.05±3.49
(2)LNP699G502	9.73±8.34	1296.41±664.71	5.43±7.68
(3)LNP688G506	16.83±4.08	1749.47±1727.22	38.57±39.39
(4)LNP689G509	18.11±11.51	1353.49±766.66	32.42±18.40
(5)LNP690G510	13.95±1.85	11838±17161.74	90.07±96.02

[0610] 表38.根据研究1的MCP-1测量

[0611]

处理组	采血前	6小时	24小时
(1)TSS(媒剂)	810.49±178.27	1351.16±397.31	745.25±56.49
(2)LNP699G502	842.31±350.65	19298.49±11981.14	2092.89±171.21
(3)LNP688G506	1190.79±383.64	13500.17±12691.60	1414.71±422.43
(4)LNP689G509	838.63±284.42	14427.7±8715.48	1590±813.23
(5)LNP690G510	785.32±108.97	52557.24±48034.68	6319.77±983.37

[0612] 表39.根据研究1的补体C3a测量

[0613]

处理组	采血前	6小时	第7天
(1)TSS(媒剂)	23.9±11.95	25.51±14.79	30.67±18.36
(2)LNP699 G502	32.36±11.29	94.33±58.45	38.50±12.69
(3)LNP688 G506	22.30±1.73	127.00±22.34	37.80±6.86
(4)LNP689 G509	35.83±21.94	174.00±44.51	50.83±21.92
(5)LNP690 G510	36.30±8.21	163.00±40.60	42.50±12.44

[0614] 表40.根据研究1的补体bb测量

[0615]

处理组	04-bb	采血前	6小时	第7天
(1) TSS (媒剂)	对照	1.53±0.19	3.37±2.13	1.43±0.71
(2) LNP699 G502	G502	1.45±0.39	9.01±5.28	1.57±0.54

[0616]

(3) LNP688 G506	G506	1.45±0.78	11.78±2.33	1.78±0.84
(4) LNP689 G509	G509	1.95±0.99	15.73±2.23	2.83±0.88
(5) LNP690 G510	G510	2.12±0.44	13.57±1.23	2.21±0.72

[0617] 表41.根据研究2的IL-6测量

[0618]

处理组	采血前	90分钟	6小时	24小时	第7天
(1)TSS(媒剂)	1.77	11.46	4.2	2.76	3.01
(2)TSS(媒剂)	5.23	18.11	20.36	13.2	6.36
(3)LNP898G502	2.02	1305.75	1138.22	383.32	16.02
(4)LNP898G502	2.34	37.19	91.59	14.11	3.07
(5)LNP897G502	2.1	55.79	6.89	2.26	2.01
(6)LNP897G502	6.8	10.1	44.72	5.4	2.01
(7)LNP897G502	1.97	44.87	32.61	2.97	1.11
(8)LNP897G502	3.14	37.68	73.41	8.58	2.22
(9)LNP916GFP	1.6	BLQ	95.32	27.58	BLQ
(10)LNP916GFP	2.43	BLQ	883.01	66.71	BLQ

[0619] 表42.根据研究2的MCP-1测量

[0620]

处理组	采血前	90分钟	6小时	24小时	第7天
(1) TSS (媒剂)	312.12	197.24	145.36	177.02	403.82
(2) TSS (媒剂)	232.44	175.08	187.72	136.64	325.69
(3) LNP898 G502	249.1	2183.5	1814.64	1887.41	372.38
(4) LNP898 G502	349.51	430.49	5635.55	953.05	236.6
(5) LNP897 G502	492.3	989.98	409.08	302.97	506.82
(6) LNP897 G502	283.79	225.1	1141.08	484.59	259.46
(7) LNP897 G502	223.16	349.79	398.57	172.67	287.09
(8) LNP897 G502	584.42	853.51	3880.81	1588.46	692.99
(9) LNP916 GFP	325.84	BLQ	1189.97	2279.82	BLQ
(10) LNP916 GFP	175.47	BLQ	3284.16	2023.53	BLQ

[0621] 表43.根据研究2的补体C3a测量

[0622]

处理组	采血前	90分钟	6小时	24小时	第7天

[0623]

(1) TSS (媒剂)	0.087	0.096	0.048	0.033	0.038
(2) TSS (媒剂)	0.369	0.311	0.146	0.1	0.106
(3) LNP898 G502	0.087	0.953	0.647	0.277	0.065
(4) LNP898 G502	0.099	0.262	0.123	0.049	0.044
(5) LNP897 G502	0.067	0.479	0.209	0.036	0.036
(6) LNP897 G502	0.141	0.433	0.34	0.11	0.074
(7) LNP897 G502	0.1	0.345	0.396	0.096	0.127
(8) LNP897 G502	0.261	0.458	0.409	0.244	0.313
(9) LNP916 GFP	0.149	BLQ	0.714	0.382	BLQ
(10) LNP916 GFP	0.117	BLQ	0.752	0.723	BLQ

[0624] 表44.根据研究2的补体bb测量

[0625]

处理组	采血前	90分钟	6小时	24小时	第7天
(1)TSS(媒剂)	0.087	0.096	0.048	0.033	0.038
(2)TSS(媒剂)	0.369	0.311	0.146	0.1	0.106
(3)LNP898G502	0.087	0.953	0.647	0.277	0.065
(4)LNP898G502	0.099	0.262	0.123	0.049	0.044
(5)LNP897G502	0.067	0.479	0.209	0.036	0.036
(6)LNP897G502	0.141	0.433	0.34	0.11	0.074
(7)LNP897G502	0.1	0.345	0.396	0.096	0.127
(8)LNP897G502	0.261	0.458	0.409	0.244	0.313
(9)LNP916GFP	0.149	BLQ	0.714	0.382	BLQ
(10)LNP916GFP	0.117	BLQ	0.752	0.723	BLQ

[0626] 表45.根据研究3的IL-6测量

[0627]

处理组	采血前	90分钟	6小时	24小时	第7天
(1)TSS	1.89±0.97	2.56±1.41	0.90±0.71	BLQ	0.08
(2)LNP1021G502	210±0.35	7.44±5.16	6.94±8.45	1.07±1.11	1.76±0.98
(3)LNP1021G502	0.79	2.96	4.25	0.67	0.27
(4)LNP1022G502	1.54±1.32	20.42±31.60	13.94±10.10	0.98±0.41	2.04±0.65
(5)LNP1023G502	2.92±1.68	6.28±7.18	6.06±2.31	3.62±4.68	2.00±1.21
(6)LNP1024G509	1.43±0.62	2.64±1.92	7.72±11.96	0.45±0.19	0.88±0.79
(7)LNP1024G509	1.35±0.74	2.64±2.35	1.71±0.41	0.36±0.58	0.51±0.32
(8)LNP1025G509	1.64	2.68	25.65	0.58	2.00
(9)LNP1021G502	0.56	6.15	28.80	0.85	0.61
(10)LNP1022G502	1.76	8.66	2907.86	11.26	1.72

[0628] 表46.根据研究2的MCP-1测量

[0629]

处理组	采血前	90分钟	6小时	24小时	第7天
(1) TSS	204.01±46.39	197.62±19.54	310.84±45.87	179.07±20.77	234.61±71.79

[0630]

(2) LNP1021 G502	303.67±36.37	337.63±195.18	755.20±581.45	339.75±206.20	214.82±40.81
(3) LNP1021 G502	229.30	358.10	3182.00	413.56	178.30
(4) LNP1022 G502	393.63±187.81	467.72±221.61	1852.94±2199.66	497.12±412.30	382.19±67.27
(5) LNP1023 G502	213.72±8.85	196.18±62.81	1722.18±1413.90	197.83±74.01	156.16±18.87
(6) LNP1024 G509	237.76±96.36	210.37±95.17	468.53±250.42	22.32±69.06	141.20±71.90
(7) LNP1024 G509	207.36	183.07	1885.66	235.70	163.11
(8) LNP1025 G509	259.57±112.98	299.21±304.89	1193.10±974.04	258.82±88.53	219.86±219.86
(9) LNP1021 G502	199.29	286.04	2001.23	197.57	196.44
(10) LNP1022 G502	305.81	970.65	7039.06	8379.05	203.47

[0631] 表47.根据研究3的补体C3a测量

[0632]

处理组	采血前	90分钟	6小时	24小时	第7天
(1) TSS	42.47±10.30	55.40±13.58	29.30±14.46	41.70±23.65	27.43±12.43
(2) LNP1021 G502	34.37±0.50	86.50±3.66	90.07±4.85	56.60±2.25	32.53±0.93
(3) LNP1021 G502	34.30	128.00	93.30	33.40	28.20
(4) LNP1022 G502	41.55±13.51	151.37±109.98	82.00±31.82	45.57±18.58	32.77±6.45
(5) LNP1023 G502	31.67±3.19	74.40±22.08	74.13±48.61	33.83±9.75	27.70±8.05

[0633]

(6) LNP1024 G509	56.60±25.61	100.37±77.95	74.73±70.15	55.20±48.34	49.97±39.94
(7) LNP1024 G509	33.80	33.90	33.70	26.10	20.90
(8) LNP1025 G509	39.90±13.01	75.73±1.38	46.13±30.56	25.00±3.80	23.90±7.18
(9) LNP1021 G502	34	85.70	133.00	62.00	25.50
(10) LNP1022 G502	29.8	68.10	113.00	71.70	23.30

[0634] 表48.根据研究3的补体bb测量

[0635]

处理组	采血前	90分钟	6小时	24小时	第7天
(1)TSS	1.46±0.70	2.18±0.78	1.96±0.64	0.945±0.15	1.34±0.50
(2)LNP1021 G502	1.77±0.60	6.51±3.66	11.00±4.85	3.59±2.25	2.07±0.93
(3)LNP1021 G502	1.24	2.90	11.50	2.97	1.24
(4)LNP1022 G502	1.52±0.34	5.67±2.28	10.2±3.36	3.66±1.68	1.84±0.24
(5)LNP1023 G502	1.65±0.94	4.4±1	7.68±4.67	2.64±1.18	2.08±1.32
(6)LNP1024 G509	1.61±0.13	4.52±1.81	4.50±3.22	1.63±0.84	1.63±0.32
(7)LNP1024 G509	0.96	2.99	2.64	1.13	1.07
(8)LNP1025 G509	1.37±0.17	4.9±4.51	3.79±3.84	1.66±1.43	1.35±0.44
(9)LNP1021 G502	1.41	5.67	11.50	4.64	1.38
(10)LNP1022 G502	1.28	5.22	14.10	5.64	1.87

[0636] 16.小鼠肝脏中不同mRNA的Cas9表达的比较

[0637] 在施用编码Cas9的不同mRNA后活体内测量Cas9表达。将如表49中所指示的信使RNA与靶向小鼠TTR基因的小鼠sgRNA(1:2的sgRNA:mRNA重量比)调配为LNP。使用错流程序组配具有50％脂质A、9％DSPC、38％胆固醇及3％PEG2k-DMG及6.0的N:P比的LNP。使用Sartocon Slice 200(Sartorius)纯化LNP,且以1.53mg/ml的浓度(RNA浓度)使用。针对如上文所述的RNA的平均粒径、多分散性(pdi)、总RNA含量及囊封效率分析LNP调配物(数据未示)。

[0638] 以0.3mpk静脉内投配CD-1雌性小鼠(每组n＝5)。在给药后1小时、3小时及6小时,将动物处死,收集肝脏组织,且如实例11中所述通过MSD ELISA测量Cas9蛋白质含量。表49显示Cas9蛋白质含量。在各时间点,在用SEQ ID NO:177处理的动物中侦测到比用SEQ ID NO:43处理的动物要多的Cas9蛋白质。

[0639] 表49

[0640]

mRNA	时间点(小时)	每公克肝脏的Cas9纳克数	标准差	样本大小(n)
TSS		0	28	5
SEQ ID NO: 43	1	429	164	5
SEQ ID NO: 177	1	1872	907	5
SEQ ID NO: 43	3	1167	814	5
SEQ ID NO: 177	3	2233	929	5
SEQ ID NO: 43	6	535	297	5
SEQ ID NO: 177	6	1663	443	5

[0641] 17.不同mRNA的剂量反应的比较

[0642] 比较编码Cas9的不同mRNA的活体内剂量反应曲线。用SEQ ID No.43及SEQ ID No.177的mRNA及sg502(SEQ ID NO:70;G502)制备LNP调配物,如实例16中所述进行调配。将脂质纳米颗粒组分溶解于100％乙醇中,其中脂质组分摩尔比为50/9/38/3(LP01/DSPC/胆固醇/PEG-DMG)。以约6的脂质胺与RNA磷酸(N:P)摩尔比及以重量计1:2的gRNA与mRNA比调配LNP。针对如上文所述的RNA的平均粒径、多分散性(pdi)、总RNA含量及囊封效率分析LNP调配物(数据未示)。

[0643] 对于活体内表征,以每公斤(每组n＝5)0.03、0.1或0.3mg总RNA(引导RNA毫克数+mRNA毫克数)静脉内投配CD-1雌性小鼠(每组n＝5)。在给药后七天,将动物处死,收集血液及肝脏,且如实例1中所述测量血清TTR及肝脏编辑。用TSS媒剂投配阴性对照动物。编辑数据提供于下表50中。对于SEQ ID NO:43,提供平均8项活体内实验,各伴随5只动物。对于SEQ ID NO:177,提供活体内实验的平均值,各剂量下伴随5只动物。在各剂量下,用SEQ ID NO:177处理的动物中的编辑％要高于用SEQ ID NO:43处理的动物。

[0644] 表50

[0645]

mRNA	编辑%		
	0.3 mg/kg剂量 平均值 (范围)	0.1 mg/kg 平均值 (范围)	0.03 mg/kg 平均值 (范围)
SEQ ID NO: 43	65.8% (62.2-71.2%)	40.6% (29.2-55.6%)	11.4% (6.2-20.1%)
SEQ ID NO: 177	71.2%	58.9%	29.3%

[0646] 序列表

[0647] 以下序列表提供本文所揭示的序列的清单。应理解,若关于RNA提及DNA序列(包含Ts),则Ts应由Us替代(其可视情况而为经修饰或未经修饰的),且反之亦然。

[0648]

描述	序列	SEQ ID No.
Cas9 DNA 编码序列2	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGG CAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAA CACAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGC GGAGAAACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACAC AAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCA AAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAG ACAAGAAGCACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATA CCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACA GACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCA GAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAA GCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCA ACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCA GAAGACTGGAAAACCTGATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGT TCGGAAACCTGATCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTT CGACCTGGCAGAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGA CCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACCTGTTCCTGGCA GCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAG AAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCA CCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTAC AAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAG GAGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGAAAAGATGGA CGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCA GAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCAC GCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAAGGACAACAGAGAAA AGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGA GGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGT GGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTTCATCGAAA GAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACA GCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTC ACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATC GTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAA GACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAAG ACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGA CAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTG ACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATACG CACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGG ATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGG AAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATG CAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGG TCAGCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCC CGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAA GGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAAC CAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGA AGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAA CACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGAC ATGTACGTCGACCAGGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACC ACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGAC AAGAAGCGACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGT CAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATCACACAG AGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAGGACTGAGCGAACTGGAC AAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACAGATCACAAAGCACG TCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAACGACAAGCT GATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGA AAGGACTTCCAGTTCTACAAGGTCAGAGAAATCAACAACTACCACCACGCACACG ACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCT	1

[0649]

	GGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATC GCAAAGAGCGAACAGGAAATCGGAAAGGCAACAGCAAAGTACTTCTTCTACAGCA ACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAA GAGACCGCTGATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGG AAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTC AAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAG AGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTAC GGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAAGGTCG AAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAA TCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTGGAAGCAAAGGG ATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTC GAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAG GGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCC ACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCG TCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAG CAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACAAC AAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCA CACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGA CAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAG AGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACG GAGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCTAG	
Cas9 DNA 编码序列1	ATGGATAAGAAGTACTCAATCGGGCTGGATATCGGAACTAATTCCGTGGGTTGGGC AGTGATCACGGATGAATACAAAGTGCCGTCCAAGAAGTTCAAGGTCCTGGGGAAC ACCGATAGACACAGCATCAAGAAAAATCTCATCGGAGCCCTGCTGTTTGACTCCGG CGAAACCGCAGAAGCGACCCGGCTCAAACGTACCGCGAGGCGACGCTACACCCGG CGGAAGAATCGCATCTGCTATCTGCAAGAGATCTTTTCGAACGAAATGGCAAAGGT CGACGACAGCTTCTTCCACCGCCTGGAAGAATCTTTCCTGGTGGAGGAGGACAAG AAGCATGAACGGCATCCTATCTTTGGAAACATCGTCGACGAAGTGGCGTACCACGA AAAGTACCCGACCATCTACCATCTGCGGAAGAAGTTGGTTGACTCAACTGACAAGG CCGACCTCAGATTGATCTACTTGGCCCTCGCCCATATGATCAAATTCCGCGGACACT TCCTGATCGAAGGCGATCTGAACCCTGATAACTCCGACGTGGATAAGCTTTTCATTC AACTGGTGCAGACCTACAACCAACTGTTCGAAGAAAACCCAATCAATGCTAGCGG CGTCGATGCCAAGGCCATCCTGTCCGCCCGGCTGTCGAAGTCGCGGCGCCTCGAAA ACCTGATCGCACAGCTGCCGGGAGAGAAAAAGAACGGACTTTTCGGCAACTTGAT CGCTCTCTCACTGGGACTCACTCCCAATTTCAAGTCCAATTTTGACCTGGCCGAGG ACGCGAAGCTGCAACTCTCAAAGGACACCTACGACGACGACTTGGACAATTTGCT GGCACAAATTGGCGATCAGTACGCGGATCTGTTCCTTGCCGCTAAGAACCTTTCGG ACGCAATCTTGCTGTCCGATATCCTGCGCGTGAACACCGAAATAACCAAAGCGCCG CTTAGCGCCTCGATGATTAAGCGGTACGACGAGCATCACCAGGATCTCACGCTGCT CAAAGCGCTCGTGAGACAGCAACTGCCTGAAAAGTACAAGGAGATCTTCTTCGAC CAGTCCAAGAATGGGTACGCAGGGTACATCGATGGAGGCGCTAGCCAGGAAGAGT TCTATAAGTTCATCAAGCCAATCCTGGAAAAGATGGACGGAACCGAAGAACTGCTG GTCAAGCTGAACAGGGAGGATCTGCTCCGGAAACAGAGAACCTTTGACAACGGAT CCATTCCCCACCAGATCCATCTGGGTGAGCTGCACGCCATCTTGCGGCGCCAGGAG GACTTTTACCCATTCCTCAAGGACAACCGGGAAAAGATCGAGAAAATTCTGACGTT CCGCATCCCGTATTACGTGGGCCCACTGGCGCGCGGCAATTCGCGCTTCGCGTGGA TGACTAGAAAATCAGAGGAAACCATCACTCCTTGGAATTTCGAGGAAGTTGTGGAT AAGGGAGCTTCGGCACAAAGCTTCATCGAACGAATGACCAACTTCGACAAGAATC TCCCAAACGAGAAGGTGCTTCCTAAGCACAGCCTCCTTTACGAATACTTCACTGTC TACAACGAACTGACTAAAGTGAAATACGTTACTGAAGGAATGAGGAAGCCGGCCT TTCTGTCCGGAGAACAGAAGAAAGCAATTGTCGATCTGCTGTTCAAGACCAACCG CAAGGTGACCGTCAAGCAGCTTAAAGAGGACTACTTCAAGAAGATCGAGTGTTTC GACTCAGTGGAAATCAGCGGGGTGGAGGACAGATTCAACGCTTCGCTGGGAACCT ATCATGATCTCCTGAAGATCATCAAGGACAAGGACTTCCTTGACAACGAGGAGAAC GAGGACATCCTGGAAGATATCGTCCTGACCTTGACCCTTTTCGAGGATCGCGAGAT GATCGAGGAGAGGCTTAAGACCTACGCTCATCTCTTCGACGATAAGGTCATGAAAC AACTCAAGCGCCGCCGGTACACTGGTTGGGGCCGCCTCTCCCGCAAGCTGATCAA CGGTATTCGCGATAAACAGAGCGGTAAAACTATCCTGGATTTCCTCAAATCGGATGG CTTCGCTAATCGTAACTTCATGCAATTGATCCACGACGACAGCCTGACCTTTAAGGA GGACATCCAAAAAGCACAAGTGTCCGGACAGGGAGACTCACTCCATGAACACATC GCGAATCTGGCCGGTTCGCCGGCGATTAAGAAGGGAATTCTGCAAACTGTGAAGG TGGTCGACGAGCTGGTGAAGGTCATGGGACGGCACAAACCGGAGAATATCGTGAT TGAAATGGCCCGAGAAAACCAGACTACCCAGAAGGGCCAGAAAAACTCCCGCGA AAGGATGAAGCGGATCGAAGAAGGAATCAAGGAGCTGGGCAGCCAGATCCTGAA AGAGCACCCGGTGGAAAACACGCAGCTGCAGAACGAGAAGCTCTACCTGTACTAT TTGCAAAATGGACGGGACATGTACGTGGACCAAGAGCTGGACATCAATCGGTTGTC TGATTACGACGTGGACCACATCGTTCCACAGTCCTTTCTGAAGGATGACTCGATCGA	2

	TAACAAGGTGTTGACTCGCAGCGACAAGAACAGAGGGAAGTCAGATAATGTGCCATCGGAGGAGGTCGTGAAGAAGATGAAGAATTACTGGCGGCAGCTCCTGAATGCGAAGCTGATTACCCAGAGAAAGTTTGACAATCTCACTAAAGCCGAGCGCGGCGGACTCTCAGAGCTGGATAAGGCTGGATTCATCAAACGGCAGCTGGTCGAGACTCGGCAGATTACCAAGCACGTGGCGCAGATCTTGGACTCCCGCATGAACACTAAATACGACGAGAACGATAAGCTCATCCGGGAAGTGAAGGTGATTACCCTGAAAAGCAAACTTGTGTCGGACTTTCGGAAGGACTTTCAGTTTTACAAAGTGAGAGAAATCAACAACTACCATCACGCGCATGACGCATACCTCAACGCTGTGGTCGGTACCGCCCTGATCAAAAAGTACCCTAAACTTGAATCGGAGTTTGTGTACGGAGACTACAAGGTCTACGACGTGAGGAAGATGATAGCCAAGTCCGAACAGGAAATCGGGAAAGCAACTGCGAAATACTTCTTTTACTCAAACATCATGAACTTTTTCAAGACTGAAATTACGCTGGCCAATGGAGAAATCAGGAAGAGGCCACTGATCGAAACTAACGGAGAAACGGGCGAAATCGTGTGGGACAAGGGCAGGGACTTCGCAACTGTTCGCAAAGTGCTCTCTATGCCGCAAGTCAATATTGTGAAGAAAACCGAAGTGCAAACCGGCGGATTTTCAAAGGAATCGATCCTCCCAAAGAGAAATAGCGACAAGCTCATTGCACGCAAGAAAGACTGGGACCCGAAGAAGTACGGAGGATTCGATTCGCCGACTGTCGCATACTCCGTCCTCGTGGTGGCCAAGGTGGAGAAGGGAAAGAGCAAAAAGCTCAAATCCGTCAAAGAGCTGCTGGGGATTACCATCATGGAACGATCCTCGTTCGAGAAGAACCCGATTGATTTCCTCGAGGCGAAGGGTTACAAGGAGGTGAAGAAGGATCTGATCATCAAACTCCCCAAGTACTCACTGTTCGAACTGGAAAATGGTCGGAAGCGCATGCTGGCTTCGGCCGGAGAACTCCAAAAAGGAAATGAGCTGGCCTTGCCTAGCAAGTACGTCAACTTCCTCTATCTTGCTTCGCACTACGAAAAACTCAAAGGGTCACCGGAAGATAACGAACAGAAGCAGCTTTTCGTGGAGCAGCACAAGCATTATCTGGATGAAATCATCGAACAAATCTCCGAGTTTTCAAAGCGCGTGATCCTCGCCGACGCCAACCTCGACAAAGTCCTGTCGGCCTACAATAAGCATAGAGATAAGCCGATCAGAGAACAGGCCGAGAACATTATCCACTTGTTCACCCTGACTAACCTGGGAGCCCCAGCCGCCTTCAAGTACTTCGATACTACTATCGATCGCAAAAGATACACGTCCACCAAGGAAGTTCTGGACGCGACCCTGATCCACCAAAGCATCACTGGACTCTACGAAACTAGGATCGATCTGTCGCAGCTGGGTGGCGATGGCGGTGGATCTCCGAAAAAGAAGAGAAAGGTGTAATGA	
[0650] Cas9氨基酸序列	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSPKKKRKV	3
Cas9 mRNA开放阅读框架(ORF) 2	AUGGACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGAUGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGA	4

[0651]

	GCGCAAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGCCG GGAGAAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACU GACACCGAACUUCAAGAGCAACUUCGACCUGGCAGAAGACGCAAAGCUGCAGC UGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGA GACCAGUACGCAGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUG CUGAGCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGC AAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGG CACUGGUCAGACAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAG AGCAAGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUU CUACAAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGC UGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAAC GGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAU CCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGCA GAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUC GAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUGAC AAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCU GUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAG AAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAGAAGGCAAUCGUC GACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGA CUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAG ACAGAUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAG GACAAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGU CCUGACACUGACACUGUUCGAAGACAGAGAAAUGAUCGAAGAAAGACUGAAGA CAUACGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGA UACACAGGAUGGGGAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAA GCAGAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCAAACA GAAACUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUC CAGAAGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACACAUCGCAAA CCUGGCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCG UCGACGAACUGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUC GAAAUGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGA AAGAAUGAAGAGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGA AGGAACACCCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUAC UACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAG ACUGAGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGA CAGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCG ACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGC AGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGACAGC UGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAGCAGA AUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUCAAGGUCAU CACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACA AGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAG UCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUC UACGGAGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACA GGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACU UCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCUG AUCGAAACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUU CGCAACAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAACAUCGUCAAGAAGA CAGAAGUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAAC AGCGACAAGCUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGG AUUCGACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAA AGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUC AUGGAAAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGG AUACAAGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGU UCGAACUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAG AAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGC AAGCCACUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGC UGUUCGUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGC GAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAG CGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCA UCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUCG ACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCA ACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAG CCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGUCUAG	

[0652]

Cas9 mRNA ORF 1	AUGGAUAAGAAGUACUCAAUCGGGCUGGAUAUCGGAACUAAUUCCGUGGGUUG GGCAGUGAUCACGGAUGAAUACAAAGUGCCGUCCAAGAAGUUCAAGGUCCUGG GGAACACCGAUAGACACAGCAUCAAGAAAAAUCUCAUCGGAGCCCUGCUGUUU GACUCCGGCGAAACCGCAGAAGCGACCCGGCUCAAACGUACCGCGAGGCGACGC UACACCCGGCGGAAGAAUCGCAUCUGCUAUCUGCAAGAGAUCUUUUCGAACGA AAUGGCAAAGGUCGACGACAGCUUCUUCCACCGCCUGGAAGAAUCUUUCCUGG UGGAGGAGGACAAGAAGCAUGAACGGCAUCCUAUCUUUGGAAACAUCGUCGAC GAAGUGGCGUACCACGAAAAGUACCCGACCAUCUACCAUCUGCGGAAGAAGUU GGUUGACUCAACUGACAAGGCCGACCUCAGAUUGAUCUACUUGGCCCUCGCCCA UAUGAUCAAAUUCCGCGGACACUUCCUGAUCGAAGGCGAUCUGAACCCUGAUA ACUCCGACGUGGAUAAGCUUUUCAUUCAACUGGUGCAGACCUACAACCAACUG UUCGAAGAAAACCCAAUCAAUGCUAGCGGCGUCGAUGCCAAGGCCAUCCUGUCC GCCCGGCUGUCGAAGUCGCGGCGCCUCGAAAACCUGAUCGCACAGCUGCCGGGA GAGAAAAAGAACGGACUUUUCGGCAACUUGAUCGCUCUCUCACUGGGACUCAC UCCCAAUUUCAAGUCCAAUUUUGACCUGGCCGAGGACGCGAAGCUGCAACUCUC AAAGGACACCUACGACGACGACUUGGACAAUUUGCUGGCACAAAUUGGCGAUC AGUACGCGGAUCUGUUCCUUGCCGCUAAGAACCUUUCGGACGCAAUCUUGCUG UCCGAUAUCCUGCGCGUGAACACCGAAAUAACCAAAGCGCCGCUUAGCGCCUCG AUGAUUAAGCGGUACGACGAGCAUCACCAGGAUCUCACGCUGCUCAAAGCGCU CGUGAGACAGCAACUGCCUGAAAAGUACAAGGAGAUCUUCUUCGACCAGUCCA AGAAUGGGUACGCAGGGUACAUCGAUGGAGGCGCUAGCCAGGAAGAGUUCUAU AAGUUCAUCAAGCCAAUCCUGGAAAAGAUGGACGGAACCGAAGAACUGCUGGU CAAGCUGAACAGGGAGGAUCUGCUCCGGAAACAGAGAACCUUUGACAACGGAU CCAUUCCCCACCAGAUCCAUCUGGGUGAGCUGCACGCCAUCUUGCGGCGCCAGG AGGACUUUUACCCAUUCCUCAAGGACAACCGGGAAAAGAUCGAGAAAAUUCUG ACGUUCCGCAUCCCGUAUUACGUGGGCCCACUGGCGCGCGGCAAUUCGCGCUUC GCGUGGAUGACUAGAAAAUCAGAGGAAACCAUCACUCCUUGGAAUUUCGAGGA AGUUGUGGAUAAGGGAGCUUCGGCACAAAGCUUCAUCGAACGAAUGACCAACU UCGACAAGAAUCUCCCAAACGAGAAGGUGCUUCCUAAGCACAGCCUCCUUUACG AAUACUUCACUGUCUACAACGAACUGACUAAAGUGAAAUACGUUACUGAAGGA AUGAGGAAGCCGGCCUUUCUGUCCGGAGAACAGAAGAAAGCAAUUGUCGAUCU GCUGUUCAAGACCAACCGCAAGGUGACCGUCAAGCAGCUUAAAGAGGACUACU UCAAGAAGAUCGAGUGUUUCGACUCAGUGGAAAUCAGCGGGGUGGAGGACAGA UUCAACGCUUCGCUGGGAACCUAUCAUGAUCUCCUGAAGAUCAUCAAGGACAA GGACUUCCUUGACAACGAGGAGAACGAGGACAUCCUGGAAGAUAUCGUCCUGA CCUUGACCCUUUUCGAGGAUCGCGAGAUGAUCGAGGAGAGGCUUAAGACCUAC GCUCAUCUCUUCGACGAUAAGGUCAUGAAACAACUCAAGCGCCGCCGGUACACU GGUUGGGGCCGCCUCUCCCGCAAGCUGAUCAACGGUAUUCGCGAUAAACAGAG CGGUAAAACUAUCCUGGAUUUCCUCAAAUCGGAUGGCUUCGCUAAUCGUAACU UCAUGCAAUUGAUCCACGACGACAGCCUGACCUUUAAGGAGGACAUCCAAAAA GCACAAGUGUCCGGACAGGGAGACUCACUCCAUGAACACAUCGCGAAUCUGGCC GGUUCGCCGGCGAUUAAGAAGGGAAUUCUGCAAACUGUGAAGGUGGUCGACGA GCUGGUGAAGGUCAUGGGACGGCACAAACCGGAGAAUAUCGUGAUUGAAAUGG CCCGAGAAAACCAGACUACCCAGAAGGGCCAGAAAAACUCCCGCGAAAGGAUG AAGCGGAUCGAAGAAGGAAUCAAGGAGCUGGGCAGCCAGAUCCUGAAAGAGCA CCCGGUGGAAAACACGCAGCUGCAGAACGAGAAGCUCUACCUGUACUAUUUGC AAAAUGGACGGGACAUGUACGUGGACCAAGAGCUGGACAUCAAUCGGUUGUCU GAUUACGACGUGGACCACAUCGUUCCACAGUCCUUUCUGAAGGAUGACUCGAU CGAUAACAAGGUGUUGACUCGCAGCGACAAGAACAGAGGGAAGUCAGAUAAUG UGCCAUCGGAGGAGGUCGUGAAGAAGAUGAAGAAUUACUGGCGGCAGCUCCUG AAUGCGAAGCUGAUUACCCAGAGAAAGUUUGACAAUCUCACUAAAGCCGAGCG CGGCGGACUCUCAGAGCUGGAUAAGGCUGGAUUCAUCAAACGGCAGCUGGUCG AGACUCGGCAGAUUACCAAGCACGUGGCGCAGAUCUUGGACUCCCGCAUGAAC ACUAAAUACGACGAGAACGAUAAGCUCAUCCGGGAAGUGAAGGUGAUUACCCU GAAAAGCAAACUUGUGUCGGACUUUCGGAAGGACUUUCAGUUUUACAAAGUGA GAGAAAUCAACAACUACCAUCACGCGCAUGACGCAUACCUCAACGCUGUGGUCG GUACCGCCCUGAUCAAAAAGUACCCUAAACUUGAAUCGGAGUUUGUGUACGGA GACUACAAGGUCUACGACGUGAGGAAGAUGAUAGCCAAGUCCGAACAGGAAAU CGGGAAAGCAACUGCGAAAUACUUCUUUUACUCAAACAUCAUGAACUUUUUCA AGACUGAAAUUACGCUGGCCAAUGGAGAAAUCAGGAAGAGGCCACUGAUCGAA ACUAACGGAGAAACGGGCGAAAUCGUGUGGGACAAGGGCAGGGACUUCGCAAC UGUUCGCAAAGUGCUCUCUAUGCCGCAAGUCAAUAUUGUGAAGAAAACCGAAG UGCAAACCGGCGGAUUUUCAAAGGAAUCGAUCCUCCCAAAGAGAAAUAGCGAC AAGCUCAUUGCACGCAAGAAAGACUGGGACCCGAAGAAGUACGGAGGAUUCGA UUCGCCGACUGUCGCAUACUCCGUCCUCGUGGUGGCCAAGGUGGAGAAGGGAA AGAGCAAAAAGCUCAAAUCCGUCAAAGAGCUGCUGGGGAUUACCAUCAUGGAA CGAUCCUCGUUCGAGAAGAACCCGAUUGAUUUCCUCGAGGCGAAGGGUUACAA	5

[0653]

	GGAGGUGAAGAAGGAUCUGAUCAUCAAACUCCCCAAGUACUCACUGUUCGAAC UGGAAAAUGGUCGGAAGCGCAUGCUGGCUUCGGCCGGAGAACUCCAAAAAGGA AAUGAGCUGGCCUUGCCUAGCAAGUACGUCAACUUCCUCUAUCUUGCUUCGCAC UACGAAAAACUCAAAGGGUCACCGGAAGAUAACGAACAGAAGCAGCUUUUCGU GGAGCAGCACAAGCAUUAUCUGGAUGAAAUCAUCGAACAAAUCUCCGAGUUUU CAAAGCGCGUGAUCCUCGCCGACGCCAACCUCGACAAAGUCCUGUCGGCCUACA AUAAGCAUAGAGAUAAGCCGAUCAGAGAACAGGCCGAGAACAUUAUCCACUUG UUCACCCUGACUAACCUGGGAGCCCCAGCCGCCUUCAAGUACUUCGAUACUACU AUCGAUCGCAAAAGAUACACGUCCACCAAGGAAGUUCUGGACGCGACCCUGAU CCACCAAAGCAUCACUGGACUCUACGAAACUAGGAUCGAUCUGUCGCAGCUGG GUGGCGAUGGCGGUGGAUCUCCGAAAAAGAAGAGAAAGGUGUAAUGA	
Cas9 切口酶 (D10A) 氨基酸序列	MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGET AEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHP IFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNP DNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKN GLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAA KNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFD QSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPH QIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEET ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYV TEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFN ASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFK EDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEM ARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRD MYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKK MKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILD SRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVV GTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITL ANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESIL PKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIM ERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANL DKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDAT LIHQSITGLYETRIDLSQLGGDGGSPKKKRKV	6
Cas9 切口酶 (D10A) mRNA ORF	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUG GGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUGG GAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUC GACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAAG AUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGCAACG AAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUG GUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGAAACAUCGUCGA CGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGC UGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCA CACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGACCUGAACCCGGA CAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCU GUUCGAAGAAAACCCGAUCAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGA GCGCAAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGCCG GGAGAAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACU GACACCGAACUUCAAGAGCAACUUCGACCUGGCAGAAGACGCAAAGCUGCAGC UGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGA GACCAGUACGCAGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUG CUGAGCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGC AAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGG CACUGGUCAGACAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAG AGCAAGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUU CUACAAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGC UGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAAC GGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAU CCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGCA GAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUC GAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUGAC AAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCU GUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAG AAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAGAAGGCAAUCGUC	7

[0654]

	GACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGA CUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAG ACAGAUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAG GACAAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGU CCUGACACUGACACUGUUCGAAGACAGAGAAAUGAUCGAAGAAAGACUGAAGA CAUACGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGA UACACAGGAUGGGGAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAA GCAGAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCAAACA GAAACUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUC CAGAAGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACACAUCGCAAA CCUGGCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCG UCGACGAACUGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUC GAAAUGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGA AAGAAUGAAGAGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGA AGGAACACCCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUAC UACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAG ACUGAGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGA CAGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCG ACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGC AGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGACAGC UGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAGCAGA AUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUCAAGGUCAU CACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACA AGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAG UCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUC UACGGAGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACA GGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACU UCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCUG AUCGAAACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUU CGCAACAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAACAUCGUCAAGAAGA CAGAAGUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAAC AGCGACAAGCUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGG AUUCGACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAA AGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUC AUGGAAAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGG AUACAAGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGU UCGAACUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAG AAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGC AAGCCACUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGC UGUUCGUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGC GAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAG CGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCA UCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUCG ACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCA ACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAG CCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGUCUAG	
dCas9 (D10A H840A) 氨基酸序列	MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGET AEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHP IFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNP DNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKN GLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAA KNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFD QSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPH QIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEET ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYV TEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFN ASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFK EDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEM ARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRD MYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKK MKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILD SRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVV GTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITL ANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESIL PKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIM	8

[0655]

	ERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANL DKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDAT LIHQSITGLYETRIDLSQLGGDGGSPKKKRKV	
dCas9 (D10A H840A) mRNA ORF	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUG GGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUGG GAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUC GACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAAG AUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGCAACG AAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUG GUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGAAACAUCGUCGA CGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGC UGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCA CACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGACCUGAACCCGGA CAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCU GUUCGAAGAAAACCCGAUCAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGA GCGCAAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGCCG GGAGAAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACU GACACCGAACUUCAAGAGCAACUUCGACCUGGCAGAAGACGCAAAGCUGCAGC UGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGA GACCAGUACGCAGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUG CUGAGCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGC AAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGG CACUGGUCAGACAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAG AGCAAGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUU CUACAAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGC UGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAAC GGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAU CCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGCA GAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUC GAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUGAC AAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCU GUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAG AAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAGAAGGCAAUCGUC GACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGA CUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAG ACAGAUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAG GACAAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGU CCUGACACUGACACUGUUCGAAGACAGAGAAAUGAUCGAAGAAAGACUGAAGA CAUACGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGA UACACAGGAUGGGGAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAA GCAGAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCAAACA GAAACUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUC CAGAAGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACACAUCGCAAA CCUGGCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCG UCGACGAACUGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUC GAAAUGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGA AAGAAUGAAGAGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGA AGGAACACCCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUAC UACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAG ACUGAGCGACUACGACGUCGACGCAAUCGUCCCGCAGAGCUUCCUGAAGGACGA CAGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCG ACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGC AGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGACAGC UGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAGCAGA AUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUCAAGGUCAU CACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACA AGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAG UCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUC UACGGAGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACA GGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACU UCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCUG AUCGAAACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUU CGCAACAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAACAUCGUCAAGAAGA CAGAAGUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAAC	9

[0656]

	AGCGACAAGCUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGG AUUCGACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAA AGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUC AUGGAAAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGG AUACAAGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGU UCGAACUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAG AAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGC AAGCCACUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGC UGUUCGUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGC GAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAG CGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCA UCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUCG ACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCA ACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAG CCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGUCUAG	
Cas9 裸编码序列	GACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGAUGGGC AGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUGGGAA ACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGAC AGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAAGAUA CACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGCAACGAAA UGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUC GAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGAAACAUCGUCGACGA AGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGG UCGACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCACACA UGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGACCUGAACCCGGACAAC AGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUC GAAGAAAACCCGAUCAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGAGCGC AAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGCCGGGAG AAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACUGACA CCGAACUUCAAGAGCAACUUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAG CAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCA GUACGCAGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGA GCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGC AUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACU GGUCAGACAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCA AGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUAC AAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGU CAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAA GCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAGG AAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUG ACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGCAGAUU CGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUCGAAG AAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUGACAAAC UUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUAC GAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAGAAGG AAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAGAAGGCAAUCGUCGACC UGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGACUAC UUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAG AUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAGGACA AGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGUCCUG ACACUGACACUGUUCGAAGACAGAGAAAUGAUCGAAGAAAGACUGAAGACAUA CGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGAUACA CAGGAUGGGGAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAAGCAG AGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAA CUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGA AGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUG GCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGA CGAACUGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAA UGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGA AUGAAGAGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGA ACACCCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCU GCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGA GCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGCA UCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAAC GUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAGCUGCU GAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGCAGAGA GAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGACAGCUGGUC	10

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	GAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAGCAGAAUGAA CACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUCAAGGUCAUCACAC UGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACAAGGUC AGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAGUCGUC GGAACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUCUACGG AGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACAGGAAA UCGGAAAGGCAACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUC AAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCUGAUCGA AACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUUCGCAA CAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAACAUCGUCAAGAAGACAGAA GUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGA CAAGCUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCG ACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGA AAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGA AAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACA AGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAA CUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGG AAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCA CUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCG UCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCAUA CAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUCCACC UGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUCGACACA ACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCAACACU GAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGC UGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGUC	
Cas9 切口酶裸编码序列	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGGGC AGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUGGGAA ACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGAC AGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAAGAUA CACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGCAACGAAA UGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUC GAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGAAACAUCGUCGACGA AGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGG UCGACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCACACA UGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGACCUGAACCCGGACAAC AGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUC GAAGAAAACCCGAUCAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGAGCGC AAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGCCGGGAG AAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACUGACA CCGAACUUCAAGAGCAACUUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAG CAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCA GUACGCAGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGA GCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGC AUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACU GGUCAGACAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCA AGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUAC AAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGU CAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAA GCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAGG AAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUG ACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGCAGAUU CGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUCGAAG AAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUGACAAAC UUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUAC GAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAGAAGG AAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAGAAGGCAAUCGUCGACC UGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGACUAC UUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAG AUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAGGACA AGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGUCCUG ACACUGACACUGUUCGAAGACAGAGAAAUGAUCGAAGAAAGACUGAAGACAUA CGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGAUACA CAGGAUGGGGAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAAGCAG AGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAA CUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGA AGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUG	11

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	GCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGA CGAACUGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAA UGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGA AUGAAGAGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGA ACACCCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCU GCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGA GCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGCA UCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAAC GUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAGCUGCU GAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGCAGAGA GAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGACAGCUGGUC GAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAGCAGAAUGAA CACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUCAAGGUCAUCACAC UGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACAAGGUC AGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAGUCGUC GGAACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUCUACGG AGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACAGGAAA UCGGAAAGGCAACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUC AAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCUGAUCGA AACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUUCGCAA CAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAACAUCGUCAAGAAGACAGAA GUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGA CAAGCUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCG ACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGA AAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGA AAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACA AGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAA CUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGG AAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCA CUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCG UCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCAUA CAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUCCACC UGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUCGACACA ACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCAACACU GAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGC UGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGUC	
dCas9裸编码序列	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGGGC AGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUGGGAA ACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGAC AGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAAGAUA CACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGCAACGAAA UGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUC GAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGAAACAUCGUCGACGA AGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGG UCGACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCACACA UGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGACCUGAACCCGGACAAC AGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUC GAAGAAAACCCGAUCAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGAGCGC AAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGCCGGGAG AAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACUGACA CCGAACUUCAAGAGCAACUUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAG CAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCA GUACGCAGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGA GCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGC AUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACU GGUCAGACAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCA AGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUAC AAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGU CAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAA GCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAGG AAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUG ACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGCAGAUU CGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUCGAAG AAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUGACAAAC UUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUAC GAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAGAAGG	12

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	AAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAGAAGGCAAUCGUCGACC UGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGACUAC UUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAG AUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAGGACA AGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGUCCUG ACACUGACACUGUUCGAAGACAGAGAAAUGAUCGAAGAAAGACUGAAGACAUA CGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGAUACA CAGGAUGGGGAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAAGCAG AGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAA CUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGA AGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUG GCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGA CGAACUGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAA UGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGA AUGAAGAGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGA ACACCCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCU GCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGA GCGACUACGACGUCGACGCAAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGCA UCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAAC GUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAGCUGCU GAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGCAGAGA GAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGACAGCUGGUC GAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAGCAGAAUGAA CACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUCAAGGUCAUCACAC UGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACAAGGUC AGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAGUCGUC GGAACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUCUACGG AGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACAGGAAA UCGGAAAGGCAACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUC AAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCUGAUCGA AACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUUCGCAA CAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAACAUCGUCAAGAAGACAGAA GUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGA CAAGCUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCG ACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGA AAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGA AAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACA AGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAA CUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGG AAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCA CUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCG UCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCAUA CAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUCCACC UGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUCGACACA ACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCAACACU GAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGC UGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGUC	
Cas9（无NLS)的氨基酸序列	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGET AEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHP IFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNP DNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKN GLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAA KNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFD QSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPH QIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEET ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYV TEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFN ASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFK EDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEM ARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRD MYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKK MKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILD SRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVV GTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITL ANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESIL	13

[0660]

	PKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIM ERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANL DKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDAT LIHQSITGLYETRIDLSQLGGD	
使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 13的，具有起始和终止密码子的Cas9 mRNA ORF	AUGGACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGAUG GGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUGG GAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUC GACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAAG AUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGCAACG AAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUG GUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGAAACAUCGUCGA CGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGC UGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCA CACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGACCUGAACCCGGA CAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCU GUUCGAAGAAAACCCGAUCAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGA GCGCAAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGCCG GGAGAAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACU GACACCGAACUUCAAGAGCAACUUCGACCUGGCAGAAGACGCAAAGCUGCAGC UGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGA GACCAGUACGCAGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUG CUGAGCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGC AAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGG CACUGGUCAGACAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAG AGCAAGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUU CUACAAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGC UGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAAC GGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAU CCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGCA GAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUC GAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUGAC AAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCU GUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAG AAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAGAAGGCAAUCGUC GACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGA CUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAG ACAGAUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAG GACAAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGU CCUGACACUGACACUGUUCGAAGACAGAGAAAUGAUCGAAGAAAGACUGAAGA CAUACGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGA UACACAGGAUGGGGAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAA GCAGAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCAAACA GAAACUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUC CAGAAGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACACAUCGCAAA CCUGGCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCG UCGACGAACUGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUC GAAAUGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGA AAGAAUGAAGAGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGA AGGAACACCCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUAC UACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAG ACUGAGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGA CAGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCG ACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGC AGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGACAGC UGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAGCAGA AUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUCAAGGUCAU CACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACA AGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAG UCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUC UACGGAGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACA GGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACU UCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCUG AUCGAAACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUU CGCAACAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAACAUCGUCAAGAAGA	14

	CAGAAGUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAAC AGCGACAAGCUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGG AUUCGACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAA AGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUC AUGGAAAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGG AUACAAGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGU UCGAACUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAG AAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGC AAGCCACUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGC UGUUCGUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGC GAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAG CGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCA UCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUCG ACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCA ACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAG CCAGCUGGGAGGAGACUAG	
[0661] 使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 13的Cas9编码序列(无起始或终止密码子;适于包括在融合蛋白质编码序列中)	GACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGAUGGGC AGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUGGGAA ACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGAC AGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAAGAUA CACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGCAACGAAA UGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUC GAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGAAACAUCGUCGACGA AGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGG UCGACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCACACA UGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGACCUGAACCCGGACAAC AGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUC GAAGAAAACCCGAUCAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGAGCGC AAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGCCGGGAG AAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACUGACA CCGAACUUCAAGAGCAACUUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAG CAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCA GUACGCAGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGA GCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGC AUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACU GGUCAGACAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCA AGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUAC AAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGU CAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAA GCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAGG AAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUG ACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGCAGAUU CGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUCGAAG AAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUGACAAAC UUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUAC GAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAGAAGG AAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAGAAGGCAAUCGUCGACC UGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGACUAC UUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAG AUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAGGACA AGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGUCCUG ACACUGACACUGUUCGAAGACAGAGAAAUGAUCGAAGAAAGACUGAAGACAUA CGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGAUACA CAGGAUGGGGAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAAGCAG AGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAA CUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGA AGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUG GCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGA CGAACUGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAA UGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGA AUGAAGAGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGA ACACCCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCU GCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGA GCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGCA UCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAAC GUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAGCUGCU GAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGCAGAGA	15

[0662]

	GAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUCAAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCACUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGCUGGGAGGAGAC	
Cas9 切口酶(无NLS)的氨基酸序列	MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD	16
使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 16的，具有起始和终止密码子的Cas9切口	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGA	17

[0663]

酶 mRNA ORF	GACCAGUACGCAGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUG CUGAGCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGC AAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGG CACUGGUCAGACAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAG AGCAAGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUU CUACAAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGC UGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAAC GGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAU CCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGCA GAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUC GAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUGAC AAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCU GUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAG AAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAGAAGGCAAUCGUC GACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGA CUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAG ACAGAUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAG GACAAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGU CCUGACACUGACACUGUUCGAAGACAGAGAAAUGAUCGAAGAAAGACUGAAGA CAUACGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGA UACACAGGAUGGGGAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAA GCAGAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCAAACA GAAACUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUC CAGAAGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACACAUCGCAAA CCUGGCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCG UCGACGAACUGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUC GAAAUGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGA AAGAAUGAAGAGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGA AGGAACACCCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUAC UACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAG ACUGAGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGA CAGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCG ACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGC AGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGACAGC UGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAGCAGA AUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUCAAGGUCAU CACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACA AGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAG UCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUC UACGGAGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACA GGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACU UCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCUG AUCGAAACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUU CGCAACAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAACAUCGUCAAGAAGA CAGAAGUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAAC AGCGACAAGCUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGG AUUCGACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAA AGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUC AUGGAAAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGG AUACAAGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGU UCGAACUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAG AAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGC AAGCCACUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGC UGUUCGUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGC GAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAG CGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCA UCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUCG ACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCA ACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAG CCAGCUGGGAGGAGACUAG	
使用如表3中所列的最小尿苷	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGGGC AGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUGGGAA ACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGAC AGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAAGAUA CACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGCAACGAAA	18

[0664]

密码子编码SEQ ID NO：16的Cas9切口酶编码序列(无起始或终止密码子;适于包括在融合蛋白质编码序列中)	UGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUC GAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGAAACAUCGUCGACGA AGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGG UCGACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCACACA UGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGACCUGAACCCGGACAAC AGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUC GAAGAAAACCCGAUCAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGAGCGC AAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGCCGGGAG AAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACUGACA CCGAACUUCAAGAGCAACUUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAG CAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCA GUACGCAGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGA GCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGC AUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACU GGUCAGACAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCA AGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUAC AAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGU CAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAA GCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAGG AAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUG ACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGCAGAUU CGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUCGAAG AAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUGACAAAC UUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUAC GAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAGAAGG AAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAGAAGGCAAUCGUCGACC UGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGACUAC UUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAG AUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAGGACA AGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGUCCUG ACACUGACACUGUUCGAAGACAGAGAAAUGAUCGAAGAAAGACUGAAGACAUA CGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGAUACA CAGGAUGGGGAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAAGCAG AGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAA CUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGA AGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUG GCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGA CGAACUGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAA UGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGA AUGAAGAGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGA ACACCCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCU GCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGA GCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGCA UCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAAC GUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAGCUGCU GAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGCAGAGA GAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGACAGCUGGUC GAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAGCAGAAUGAA CACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUCAAGGUCAUCACAC UGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACAAGGUC AGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAGUCGUC GGAACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUCUACGG AGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACAGGAAA UCGGAAAGGCAACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUC AAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCUGAUCGA AACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUUCGCAA CAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAACAUCGUCAAGAAGACAGAA GUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGA CAAGCUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCG ACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGA AAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGA AAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACA AGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAA CUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGG AAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCA CUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCG UCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC	

[0665]

	AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCAUA CAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUCCACC UGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUCGACACA ACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCAACACU GAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGC UGGGAGGAGAC	
dCas9（无NLS)的氨基酸序列	MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGET AEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHP IFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNP DNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKN GLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAA KNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFD QSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPH QIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEET ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYV TEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFN ASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFK EDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEM ARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRD MYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKK MKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILD SRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVV GTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITL ANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESIL PKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIM ERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANL DKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDAT LIHQSITGLYETRIDLSQLGGD	19
使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 19的，具有起始和终止密码子的dCas9 mRNA ORF	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUG GGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUGG GAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUC GACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAAG AUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGCAACG AAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUG GUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGAAACAUCGUCGA CGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGC UGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCA CACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGACCUGAACCCGGA CAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCU GUUCGAAGAAAACCCGAUCAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGA GCGCAAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGCCG GGAGAAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACU GACACCGAACUUCAAGAGCAACUUCGACCUGGCAGAAGACGCAAAGCUGCAGC UGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGA GACCAGUACGCAGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUG CUGAGCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGC AAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGG CACUGGUCAGACAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAG AGCAAGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUU CUACAAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGC UGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAAC GGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAU CCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGCA GAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUC GAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUGAC AAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCU GUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAG AAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAGAAGGCAAUCGUC GACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGA CUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAG ACAGAUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAG GACAAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGU CCUGACACUGACACUGUUCGAAGACAGAGAAAUGAUCGAAGAAAGACUGAAGA CAUACGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGA	20

[0666]

	UACACAGGAUGGGGAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAA GCAGAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCAAACA GAAACUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUC CAGAAGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACACAUCGCAAA CCUGGCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCG UCGACGAACUGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUC GAAAUGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGA AAGAAUGAAGAGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGA AGGAACACCCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUAC UACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAG ACUGAGCGACUACGACGUCGACGCAAUCGUCCCGCAGAGCUUCCUGAAGGACGA CAGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCG ACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGC AGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGACAGC UGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAGCAGA AUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUCAAGGUCAU CACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACA AGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAG UCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUC UACGGAGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACA GGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACU UCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCUG AUCGAAACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUU CGCAACAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAACAUCGUCAAGAAGA CAGAAGUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAAC AGCGACAAGCUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGG AUUCGACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAA AGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUC AUGGAAAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGG AUACAAGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGU UCGAACUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAG AAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGC AAGCCACUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGC UGUUCGUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGC GAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAG CGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCA UCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUCG ACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCA ACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAG CCAGCUGGGAGGAGACUAG	
使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 19的dCas9编码序列(无起始或终止密码子;适于包括在融合蛋白质编码序列中)	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGGGC AGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUGGGAA ACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGAC AGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAAGAUA CACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGCAACGAAA UGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUC GAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGAAACAUCGUCGACGA AGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGG UCGACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCACACA UGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGACCUGAACCCGGACAAC AGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUC GAAGAAAACCCGAUCAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGAGCGC AAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGCCGGGAG AAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACUGACA CCGAACUUCAAGAGCAACUUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAG CAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCA GUACGCAGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGA GCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGC AUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACU GGUCAGACAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCA AGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUAC AAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGU CAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAA GCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAGG AAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUG ACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGCAGAUU	21

[0667]

	CGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUCGAAG AAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUGACAAAC UUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUAC GAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAGAAGG AAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAGAAGGCAAUCGUCGACC UGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGACUAC UUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAG AUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAGGACA AGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGUCCUG ACACUGACACUGUUCGAAGACAGAGAAAUGAUCGAAGAAAGACUGAAGACAUA CGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGAUACA CAGGAUGGGGAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAAGCAG AGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAA CUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGA AGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUG GCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGA CGAACUGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAA UGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGA AUGAAGAGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGA ACACCCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCU GCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGA GCGACUACGACGUCGACGCAAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGCA UCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAAC GUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAGCUGCU GAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGCAGAGA GAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGACAGCUGGUC GAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAGCAGAAUGAA CACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUCAAGGUCAUCACAC UGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACAAGGUC AGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAGUCGUC GGAACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUCUACGG AGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACAGGAAA UCGGAAAGGCAACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUC AAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCUGAUCGA AACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUUCGCAA CAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAACAUCGUCAAGAAGACAGAA GUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGA CAAGCUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCG ACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGA AAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGA AAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACA AGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAA CUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGG AAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCA CUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCG UCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCAUA CAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUCCACC UGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUCGACACA ACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCAACACU GAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGC UGGGAGGAGACGGAGGAGGAAGC	
具有两个核定位信号作为C端氨基酸的Cas9的氨基酸序列	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGET AEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHP IFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNP DNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKN GLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAA KNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFD QSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPH QIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEET ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYV TEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFN ASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFK EDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEM ARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRD MYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKK	22

[0668]

	MKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILD SRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVV GTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITL ANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESIL PKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIM ERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANL DKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDAT LIHQSITGLYETRIDLSQLGGD GSGSPKKKRKVDGSPKKKRKVDSG	
使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 22的，具有起始和终止密码子的Cas9 mRNA ORF	AUGGACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGAUG GGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUGG GAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUC GACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAAG AUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGCAACG AAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUG GUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGAAACAUCGUCGA CGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGC UGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCA CACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGACCUGAACCCGGA CAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCU GUUCGAAGAAAACCCGAUCAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGA GCGCAAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGCCG GGAGAAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACU GACACCGAACUUCAAGAGCAACUUCGACCUGGCAGAAGACGCAAAGCUGCAGC UGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGA GACCAGUACGCAGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUG CUGAGCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGC AAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGG CACUGGUCAGACAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAG AGCAAGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUU CUACAAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGC UGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAAC GGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAU CCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGCA GAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUC GAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUGAC AAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCU GUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAG AAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAGAAGGCAAUCGUC GACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGA CUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAG ACAGAUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAG GACAAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGU CCUGACACUGACACUGUUCGAAGACAGAGAAAUGAUCGAAGAAAGACUGAAGA CAUACGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGA UACACAGGAUGGGGAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAA GCAGAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCAAACA GAAACUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUC CAGAAGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACACAUCGCAAA CCUGGCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCG UCGACGAACUGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUC GAAAUGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGA AAGAAUGAAGAGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGA AGGAACACCCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUAC UACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAG ACUGAGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGA CAGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCG ACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGC AGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGACAGC UGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAGCAGA AUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUCAAGGUCAU CACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACA AGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAG UCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUC UACGGAGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACA	23

[0669]

	GGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACU UCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCUG AUCGAAACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUU CGCAACAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAACAUCGUCAAGAAGA CAGAAGUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAAC AGCGACAAGCUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGG AUUCGACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAA AGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUC AUGGAAAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGG AUACAAGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGU UCGAACUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAG AAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGC AAGCCACUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGC UGUUCGUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGC GAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAG CGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCA UCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUCG ACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCA ACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAG CCAGCUGGGAGGAGACGGAAGCGGAAGCCCGAAGAAGAAGAGAAAGGUCGACG GAAGCCCGAAGAAGAAGAGAAAGGUCGACAGCGGAUAG	
使用如表3中所列的最小尿苷密码子编码SEQ ID NO：23的Cas9编码序列(无起始或终止密码子;适于包括在融合蛋白质编码序列中)	GACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGAUGGGC AGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUGGGAA ACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGAC AGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAAGAUA CACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGCAACGAAA UGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUC GAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGAAACAUCGUCGACGA AGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGG UCGACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCACACA UGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGACCUGAACCCGGACAAC AGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUC GAAGAAAACCCGAUCAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGAGCGC AAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGCCGGGAG AAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACUGACA CCGAACUUCAAGAGCAACUUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAG CAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCA GUACGCAGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGA GCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGC AUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACU GGUCAGACAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCA AGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUAC AAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGU CAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAA GCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAGG AAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUG ACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGCAGAUU CGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUCGAAG AAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUGACAAAC UUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUAC GAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAGAAGG AAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAGAAGGCAAUCGUCGACC UGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGACUAC UUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAG AUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAGGACA AGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGUCCUG ACACUGACACUGUUCGAAGACAGAGAAAUGAUCGAAGAAAGACUGAAGACAUA CGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGAUACA CAGGAUGGGGAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAAGCAG AGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAA CUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGA AGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUG GCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGA CGAACUGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAA UGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGA AUGAAGAGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGA ACACCCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCU	24

	GCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGA GCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGCA UCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAAC GUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAGCUGCU GAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGCAGAGA GAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGACAGCUGGUC GAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAGCAGAAUGAA CACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUCAAGGUCAUCACAC UGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACAAGGUC AGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAGUCGUC GGAACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUCUACGG AGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACAGGAAA UCGGAAAGGCAACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUC AAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCUGAUCGA AACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUUCGCAA CAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAACAUCGUCAAGAAGACAGAA GUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGA CAAGCUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCG ACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGA AAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGA AAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACA AGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAA CUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGG AAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCA CUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCG UCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCAUA CAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUCCACC UGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUCGACACA ACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCAACACU GAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGC UGGGAGGAGACGGAAGCGGAAGCCCGAAGAAGAAGAGAAAGGUCGACGGAAGC CCGAAGAAGAAGAGAAAGGUCGACAGCGGA	
[0670] 具有两个核定位信号作为C端氨基酸的Cas9切口酶的氨基酸序列	MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGET AEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHP IFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNP DNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKN GLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAA KNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFD QSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPH QIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEET ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYV TEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFN ASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFK EDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEM ARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRD MYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKK MKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILD SRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVV GTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITL ANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESIL PKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIM ERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANL DKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDAT LIHQSITGLYETRIDLSQLGGDGSGSPKKKRKVDGSPKKKRKVDSG	25
使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 25的,	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUG GGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUGG GAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUC GACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAAG AUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGCAACG AAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUG GUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGAAACAUCGUCGA CGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGC UGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCA CACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGACCUGAACCCGGA	26

[0671]

具有起始和终止密码子的Cas9切口酶mRNA ORF	CAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCU GUUCGAAGAAAACCCGAUCAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGA GCGCAAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGCCG GGAGAAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACU GACACCGAACUUCAAGAGCAACUUCGACCUGGCAGAAGACGCAAAGCUGCAGC UGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGA GACCAGUACGCAGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUG CUGAGCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGC AAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGG CACUGGUCAGACAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAG AGCAAGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUU CUACAAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGC UGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAAC GGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAU CCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGCA GAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUC GAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUGAC AAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCU GUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAG AAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAGAAGGCAAUCGUC GACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGA CUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAG ACAGAUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAG GACAAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGU CCUGACACUGACACUGUUCGAAGACAGAGAAAUGAUCGAAGAAAGACUGAAGA CAUACGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGA UACACAGGAUGGGGAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAA GCAGAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCAAACA GAAACUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUC CAGAAGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACACAUCGCAAA CCUGGCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCG UCGACGAACUGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUC GAAAUGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGA AAGAAUGAAGAGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGA AGGAACACCCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUAC UACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAG ACUGAGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGA CAGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCG ACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGC AGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGACAGC UGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAGCAGA AUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUCAAGGUCAU CACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACA AGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAG UCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUC UACGGAGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACA GGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACU UCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCUG AUCGAAACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUU CGCAACAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAACAUCGUCAAGAAGA CAGAAGUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAAC AGCGACAAGCUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGG AUUCGACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAA AGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUC AUGGAAAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGG AUACAAGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGU UCGAACUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAG AAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGC AAGCCACUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGC UGUUCGUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGC GAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAG CGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCA UCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUCG ACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCA ACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAG	

[0672]

	CCAGCUGGGAGGAGACGGAAGCGGAAGCCCGAAGAAGAAGAGAAAGGUCGACG GAAGCCCGAAGAAGAAGAGAAAGGUCGACAGCGGAUAG	
使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 25的Cas9切口酶编码序列(无起始或终止密码子;适于包括在融合蛋白质编码序列中)	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGGGC AGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUGGGAA ACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGAC AGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAAGAUA CACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGCAACGAAA UGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUC GAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGAAACAUCGUCGACGA AGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGG UCGACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCACACA UGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGACCUGAACCCGGACAAC AGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUC GAAGAAAACCCGAUCAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGAGCGC AAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGCCGGGAG AAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACUGACA CCGAACUUCAAGAGCAACUUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAG CAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCA GUACGCAGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGA GCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGC AUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACU GGUCAGACAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCA AGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUAC AAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGU CAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAA GCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAGG AAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUG ACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGCAGAUU CGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUCGAAG AAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUGACAAAC UUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUAC GAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAGAAGG AAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAGAAGGCAAUCGUCGACC UGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGACUAC UUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAG AUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAGGACA AGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGUCCUG ACACUGACACUGUUCGAAGACAGAGAAAUGAUCGAAGAAAGACUGAAGACAUA CGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGAUACA CAGGAUGGGGAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAAGCAG AGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAA CUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGA AGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUG GCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGA CGAACUGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAA UGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGA AUGAAGAGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGA ACACCCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCU GCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGA GCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGCA UCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAAC GUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAGCUGCU GAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGCAGAGA GAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGACAGCUGGUC GAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAGCAGAAUGAA CACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUCAAGGUCAUCACAC UGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACAAGGUC AGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAGUCGUC GGAACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUCUACGG AGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACAGGAAA UCGGAAAGGCAACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUC AAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCUGAUCGA AACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUUCGCAA CAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAACAUCGUCAAGAAGACAGAA GUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGA CAAGCUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCG ACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGA	27

[0673]

	AAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGA AAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACA AGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAA CUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGG AAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCA CUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCG UCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCAUA CAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUCCACC UGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUCGACACA ACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCAACACU GAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGC UGGGAGGAGAC GGAAGCGGAAGCCCGAAGAAGAAGAGAAAGGUCGACGGAAGCCCGAAGAAGAA GAGAAAGGUCGACAGCGGA	
具有两个核定位信号作为C端氨基酸的dCas9的氨基酸序列	MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGET AEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHP IFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNP DNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKN GLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAA KNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFD QSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPH QIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEET ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYV TEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFN ASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFK EDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEM ARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRD MYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKK MKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILD SRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVV GTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITL ANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESIL PKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIM ERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANL DKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDAT LIHQSITGLYETRIDLSQLGGDGSGSPKKKRKVDGSPKKKRKVDSG	28
使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 28的，具有起始和终止密码子的dCas9 mRNA ORF	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUG GGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUGG GAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUC GACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAAG AUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGCAACG AAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUG GUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGAAACAUCGUCGA CGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGC UGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCA CACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGACCUGAACCCGGA CAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCU GUUCGAAGAAAACCCGAUCAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGA GCGCAAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGCCG GGAGAAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACU GACACCGAACUUCAAGAGCAACUUCGACCUGGCAGAAGACGCAAAGCUGCAGC UGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGA GACCAGUACGCAGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUG CUGAGCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGC AAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGG CACUGGUCAGACAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAG AGCAAGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUU CUACAAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGC UGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAAC GGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAU CCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGCA GAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUC GAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUGAC	29

[0674]

	AAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCU GUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAG AAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAGAAGGCAAUCGUC GACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGA CUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAG ACAGAUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAG GACAAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGU CCUGACACUGACACUGUUCGAAGACAGAGAAAUGAUCGAAGAAAGACUGAAGA CAUACGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGA UACACAGGAUGGGGAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAA GCAGAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCAAACA GAAACUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUC CAGAAGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACACAUCGCAAA CCUGGCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCG UCGACGAACUGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUC GAAAUGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGA AAGAAUGAAGAGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGA AGGAACACCCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUAC UACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAG ACUGAGCGACUACGACGUCGACGCAAUCGUCCCGCAGAGCUUCCUGAAGGACGA CAGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCG ACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGC AGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGACAGC UGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAGCAGA AUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUCAAGGUCAU CACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACA AGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAG UCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUC UACGGAGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACA GGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACU UCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCUG AUCGAAACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUU CGCAACAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAACAUCGUCAAGAAGA CAGAAGUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAAC AGCGACAAGCUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGG AUUCGACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAA AGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUC AUGGAAAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGG AUACAAGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGU UCGAACUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAG AAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGC AAGCCACUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGC UGUUCGUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGC GAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAG CGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCA UCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUCG ACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCA ACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAG CCAGCUGGGAGGAGAC GGAAGCGGAAGCCCGAAGAAGAAGAGAAAGGUCGACGGAAGCCCGAAGAAGAA GAGAAAGGUCGACAGCGGAUAG	
使用如表3中所列的最小尿苷密码子编码SEQ ID NO：28的dCas9编码序列(无起始或终止密码子；适	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGGGC AGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUGGGAA ACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGAC AGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAAGAUA CACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGCAACGAAA UGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUC GAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGAAACAUCGUCGACGA AGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGG UCGACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCACACA UGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGACCUGAACCCGGACAAC AGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUC GAAGAAAACCCGAUCAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGAGCGC AAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGCCGGGAG AAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACUGACA CCGAACUUCAAGAGCAACUUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAG	30

[0675]

于包括在融合蛋白质编码序列中)	CAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCA GUACGCAGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGA GCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGC AUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACU GGUCAGACAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCA AGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUAC AAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGU CAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAA GCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAGG AAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUG ACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGCAGAUU CGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUCGAAG AAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUGACAAAC UUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUAC GAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAGAAGG AAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAGAAGGCAAUCGUCGACC UGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGACUAC UUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAG AUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAGGACA AGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGUCCUG ACACUGACACUGUUCGAAGACAGAGAAAUGAUCGAAGAAAGACUGAAGACAUA CGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGAUACA CAGGAUGGGGAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAAGCAG AGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAA CUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGA AGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUG GCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGA CGAACUGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAA UGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGA AUGAAGAGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGA ACACCCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCU GCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGA GCGACUACGACGUCGACGCAAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGCA UCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAAC GUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAGCUGCU GAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGCAGAGA GAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGACAGCUGGUC GAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAGCAGAAUGAA CACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUCAAGGUCAUCACAC UGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACAAGGUC AGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAGUCGUC GGAACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUCUACGG AGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACAGGAAA UCGGAAAGGCAACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUC AAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCUGAUCGA AACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUUCGCAA CAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAACAUCGUCAAGAAGACAGAA GUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGA CAAGCUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCG ACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGA AAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGA AAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACA AGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAA CUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGG AAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCA CUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCG UCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCAUA CAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUCCACC UGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUCGACACA ACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCAACACU GAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGC UGGGAGGAGAC GGAAGCGGAAGCCCGAAGAAGAAGAGAAAGGUCGACGGAAGCCCGAAGAAGAA GAGAAAGGUCGACAGCGGA	
T7启动子	TAATACGACTCACTATA	31

[0676]

人类β-血球蛋白5' UTR	ACATTTGCTTCTGACACAACTGTGTTCACTAGCAACCTCAAACAGACACC	32
人类β-血球蛋白3' UTR	GCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGC	33
人类α-血球蛋白5' UTR	CATAAACCCTGGCGCGCTCGCGGCCCGGCACTCTTCTGGTCCCCACAGACTCAGAGAGAACCCACC	34
人类α-血球蛋白3' UTR	GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCGTGGTCTTTGAATAAAGTCTGAGTGGGCGGC	35
有爪蟾蜍β-血球蛋白 5' UTR	AAGCTCAGAATAAACGCTCAACTTTGGCC	36
有爪蟾蜍β-血球蛋白 3' UTR	ACCAGCCTCAAGAACACCCGAATGGAGTCTCTAAGCTACATAATACCAACTTACACTTTACAAAATGTTGTCCCCCAAAATGTAGCCATTCGTATCTGCTCCTAATAAAAAGAAAGTTTCTTCACATTCT	37
牛生长激素 5' UTR	CAGGGTCCTGTGGACAGCTCACCAGCT	38
牛生长激素 3' UTR	TTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCA	39
小家鼠血红素α，成年链 1 (Hba-a1)，3' UTR	GCTGCCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGCACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAG	40
HSD17B4 5' UTR	TCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTTGCAGGCCTTATTC	41
靶向TTR的G282引导RNA	mU*mU*mA*CAGCCACGUCUACAGCAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	42
具有HSD的 5' UTR、对应于SEQ ID NO：4的ORF、Kozak序列及ALB的3' UTR的Cas9转录物	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTTGCAGGCCTTATTCGGATCCGCCACCATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACAT	43

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	CCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAAG AGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGC AGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGC AGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCG ATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAA GACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCC ACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCT GAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTAC GTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGC GAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGC GCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAA AGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTG ACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAG AACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGT CAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAA ATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGC TGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCT GGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAA AGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGA GAAGAAGATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCA GAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGC AAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGAC ATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACACATCGCA AACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCG TCGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGA AATGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAA GAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGG AACACCCGGTCGAAAACACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCT GCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTGAGC GACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCG ACAACAAGGTCCTGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAACGTCC CGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACG CAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAG GACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAA GACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTA CGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAA GCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAATCAAC AACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGA TCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTA CGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACAGC AAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGG CAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAG AAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCAT GCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAA GGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGA CTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTC CTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAG GAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCG ACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCT GCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAG CGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAA CTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAAC GAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCG AACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAA GGTCCTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGA AAACATCATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGT ACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGA CGCAACACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTG AGCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCTA GCTAGCCATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAA TGAAGATCAATAGCTTATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCC TGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAA TAAAAAATGGAAAGAACCTCGAG	
具有HSD的 5'	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTTGCAGG CCTTATTCGGATCCATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAAC AGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCA AGGTCCTGGGAAACACAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACT	44

[0678]

UTR、对应于SEQ ID NO: 4 的 ORF 及 ALB 的 3' UTR 的 Cas9 转录物	GCTGTTCGACAGCGGAGAAACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAG AAGAAGATACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGC AACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCC TGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTCGGAAACATCGTCGA CGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTG GTCGACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACA TGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAG CGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAG AAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACT GAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCCGGGAGAAAAGAA GAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGGACTGACACCGAACTTC AAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCTGCAGCTGAGCAAGGACACA TACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACC TGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAG AGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAAGAGATAC GACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGC CGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATA CATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCGATCCTG GAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTG CTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGG GAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAAGGA CAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGAC CGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAAGAAA CAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACAGA GCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCT GCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAG GTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGA AGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCA GCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGC GGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGA TCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGA CATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTG AAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAA GATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAA GCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGA AACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGA AGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGG CAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGA ACTGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCA AGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAA GAGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCC GGTCGAAAACACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAAC GGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTGAGCGACTACG ACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAA GGTCCTGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGA AGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAAGCT GATCACACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAGGACTGAG CGAACTGGACAAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACAGATC ACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAA AACGACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCA GCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAATCAACAACTACCA CCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAG TACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCA GAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACAGCAAAGTACT TCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGA GAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAGAAATCGTC TGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGCAG GTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGC ATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTGGGAC CCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCG TCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGC TGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCT GGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAA GTACAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGG AGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTG TACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAG AAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGA	

[0679]

	TCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCT GAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACAT CATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCG ACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAA CACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCA GCTGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCTAGCTAGC CATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGA TCAATAGCTTATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTA AAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAATAAAA AATGGAAAGAACCTCGAG	
具有19.36% U含量的替代Cas9 ORF	ATGGATAAGAAGTACTCGATCGGGCTGGATATCGGAACTAATTCCGTGGGTTGGGC AGTGATCACGGATGAATACAAAGTGCCGTCCAAGAAGTTCAAGGTCCTGGGGAAC ACCGATAGACACAGCATCAAGAAGAATCTCATCGGAGCCCTGCTGTTTGACTCCGG CGAAACCGCAGAAGCGACCCGGCTCAAACGTACCGCGAGGCGACGCTACACCCGG CGGAAGAATCGCATCTGCTATCTGCAAGAAATCTTTTCGAACGAAATGGCAAAGGT GGACGACAGCTTCTTCCACCGCCTGGAAGAATCTTTCCTGGTGGAGGAGGACAAG AAGCATGAACGGCATCCTATCTTTGGAAACATCGTGGACGAAGTGGCGTACCACGA AAAGTACCCGACCATCTACCATCTGCGGAAGAAGTTGGTTGACTCAACTGACAAGG CCGACCTCAGATTGATCTACTTGGCCCTCGCCCATATGATCAAATTCCGCGGACACT TCCTGATCGAAGGCGATCTGAACCCTGATAACTCCGACGTGGATAAGCTGTTCATTC AACTGGTGCAGACCTACAACCAACTGTTCGAAGAAAACCCAATCAATGCCAGCGG CGTCGATGCCAAGGCCATCCTGTCCGCCCGGCTGTCGAAGTCGCGGCGCCTCGAAA ACCTGATCGCACAGCTGCCGGGAGAGAAGAAGAACGGACTTTTCGGCAACTTGAT CGCTCTCTCACTGGGACTCACTCCCAATTTCAAGTCCAATTTTGACCTGGCCGAGG ACGCGAAGCTGCAACTCTCAAAGGACACCTACGACGACGACTTGGACAATTTGCT GGCACAAATTGGCGATCAGTACGCGGATCTGTTCCTTGCCGCTAAGAACCTTTCGG ACGCAATCTTGCTGTCCGATATCCTGCGCGTGAACACCGAAATAACCAAAGCGCCG CTTAGCGCCTCGATGATTAAGCGGTACGACGAGCATCACCAGGATCTCACGCTGCT CAAAGCGCTCGTGAGACAGCAACTGCCTGAAAAGTACAAGGAGATTTTCTTCGAC CAGTCCAAGAATGGGTACGCAGGGTACATCGATGGAGGCGCCAGCCAGGAAGAGT TCTATAAGTTCATCAAGCCAATCCTGGAAAAGATGGACGGAACCGAAGAACTGCTG GTCAAGCTGAACAGGGAGGATCTGCTCCGCAAACAGAGAACCTTTGACAACGGAA GCATTCCACACCAGATCCATCTGGGTGAGCTGCACGCCATCTTGCGGCGCCAGGAG GACTTTTACCCATTCCTCAAGGACAACCGGGAAAAGATCGAGAAAATTCTGACGTT CCGCATCCCGTATTACGTGGGCCCACTGGCGCGCGGCAATTCGCGCTTCGCGTGGA TGACTAGAAAATCAGAGGAAACCATCACTCCTTGGAATTTCGAGGAAGTTGTGGAT AAGGGAGCTTCGGCACAATCCTTCATCGAACGAATGACCAACTTCGACAAGAATCT CCCAAACGAGAAGGTGCTTCCTAAGCACAGCCTCCTTTACGAATACTTCACTGTCT ACAACGAACTGACTAAAGTGAAATACGTTACTGAAGGAATGAGGAAGCCGGCCTT TCTGAGCGGAGAACAGAAGAAAGCGATTGTCGATCTGCTGTTCAAGACCAACCGC AAGGTGACCGTCAAGCAGCTTAAAGAGGACTACTTCAAGAAGATCGAGTGTTTCG ACTCAGTGGAAATCAGCGGAGTGGAGGACAGATTCAACGCTTCGCTGGGAACCTA TCATGATCTCCTGAAGATCATCAAGGACAAGGACTTCCTTGACAACGAGGAGAACG AGGACATCCTGGAAGATATCGTCCTGACCTTGACCCTTTTCGAGGATCGCGAGATG ATCGAGGAGAGGCTTAAGACCTACGCTCATCTCTTCGACGATAAGGTCATGAAACA ACTCAAGCGCCGCCGGTACACTGGTTGGGGCCGCCTCTCCCGCAAGCTGATCAAC GGTATTCGCGATAAACAGAGCGGTAAAACTATCCTGGATTTCCTCAAATCGGATGGC TTCGCTAATCGTAACTTCATGCAGTTGATCCACGACGACAGCCTGACCTTTAAGGA GGACATCCAGAAAGCACAAGTGAGCGGACAGGGAGACTCACTCCATGAACACATC GCGAATCTGGCCGGTTCGCCGGCGATTAAGAAGGGAATCCTGCAAACTGTGAAGG TGGTGGACGAGCTGGTGAAGGTCATGGGACGGCACAAACCGGAGAATATCGTGAT TGAAATGGCCCGAGAAAACCAGACTACCCAGAAGGGCCAGAAGAACTCCCGCGA AAGGATGAAGCGGATCGAAGAAGGAATCAAGGAGCTGGGCAGCCAGATCCTGAA AGAGCACCCGGTGGAAAACACGCAGCTGCAGAACGAGAAGCTCTACCTGTACTAT TTGCAAAATGGACGGGACATGTACGTGGACCAAGAGCTGGACATCAATCGGTTGTC TGATTACGACGTGGACCACATCGTTCCACAGTCCTTTCTGAAGGATGACTCCATCGA TAACAAGGTGTTGACTCGCAGCGACAAGAACAGAGGGAAGTCAGATAATGTGCCA TCGGAGGAGGTCGTGAAGAAGATGAAGAATTACTGGCGGCAGCTCCTGAATGCGA AGCTGATTACCCAGAGAAAGTTTGACAATCTCACTAAAGCCGAGCGCGGCGGACT CTCAGAGCTGGATAAGGCTGGATTCATCAAACGGCAGCTGGTCGAGACTCGGCAG ATTACCAAGCACGTGGCGCAGATCCTGGACTCCCGCATGAACACTAAATACGACGA GAACGATAAGCTCATCCGGGAAGTGAAGGTGATTACCCTGAAAAGCAAACTTGTGT CGGACTTTCGGAAGGACTTTCAGTTTTACAAAGTGAGAGAAATCAACAACTACCAT CACGCGCATGACGCATACCTCAACGCTGTGGTCGGCACCGCCCTGATCAAGAAGTA CCCTAAACTTGAATCGGAGTTTGTGTACGGAGACTACAAGGTCTACGACGTGAGGA AGATGATAGCCAAGTCCGAACAGGAAATCGGGAAAGCAACTGCGAAATACTTCTTT TACTCAAACATCATGAACTTCTTCAAGACTGAAATTACGCTGGCCAATGGAGAAAT	45

[0680]

	CAGGAAGAGGCCACTGATCGAAACTAACGGAGAAACGGGCGAAATCGTGTGGGA CAAGGGCAGGGACTTCGCAACTGTTCGCAAAGTGCTCTCTATGCCGCAAGTCAATA TTGTGAAGAAAACCGAAGTGCAAACCGGCGGATTTTCAAAGGAATCGATCCTCCC AAAGAGAAATAGCGACAAGCTCATTGCACGCAAGAAAGACTGGGACCCGAAGAA GTACGGAGGATTCGATTCGCCGACTGTCGCATACTCCGTCCTCGTGGTGGCCAAGG TGGAGAAGGGAAAGAGCAAGAAGCTCAAATCCGTCAAAGAGCTGCTGGGGATTAC CATCATGGAACGATCCTCGTTCGAGAAGAACCCGATTGATTTCCTGGAGGCGAAGG GTTACAAGGAGGTGAAGAAGGATCTGATCATCAAACTGCCCAAGTACTCACTGTTC GAACTGGAAAATGGTCGGAAGCGCATGCTGGCTTCGGCCGGAGAACTCCAGAAAG GAAATGAGCTGGCCTTGCCTAGCAAGTACGTCAACTTCCTCTATCTTGCTTCGCACT ACGAGAAACTCAAAGGGTCACCGGAAGATAACGAACAGAAGCAGCTTTTCGTGGA GCAGCACAAGCATTATCTGGATGAAATCATCGAACAAATCTCCGAGTTTTCAAAGC GCGTGATCCTCGCCGACGCCAACCTCGACAAAGTCCTGTCGGCCTACAATAAGCAT AGAGATAAGCCGATCAGAGAACAGGCCGAGAACATTATCCACTTGTTCACCCTGAC TAACCTGGGAGCTCCAGCCGCCTTCAAGTACTTCGATACTACTATCGACCGCAAAA GATACACGTCCACCAAGGAAGTTCTGGACGCGACCCTGATCCACCAAAGCATCACT GGACTCTACGAAACTAGGATCGATCTGTCGCAGCTGGGTGGCGATGGTGGCGGTGG ATCCTACCCATACGACGTGCCTGACTACGCCTCCGGAGGTGGTGGCCCCAAGAAGA AACGGAAGGTGTGATAG	
具有HSD的5' UTR、对应于SEQ ID NO：45的ORF、Kozak序列及ALB的3' UTR的Cas9转录物	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTTGCAGG CCTTATTCGGATCTGCCACCATGGATAAGAAGTACTCGATCGGGCTGGATATCGGAA CTAATTCCGTGGGTTGGGCAGTGATCACGGATGAATACAAAGTGCCGTCCAAGAAG TTCAAGGTCCTGGGGAACACCGATAGACACAGCATCAAGAAGAATCTCATCGGAG CCCTGCTGTTTGACTCCGGCGAAACCGCAGAAGCGACCCGGCTCAAACGTACCGC GAGGCGACGCTACACCCGGCGGAAGAATCGCATCTGCTATCTGCAAGAAATCTTTT CGAACGAAATGGCAAAGGTGGACGACAGCTTCTTCCACCGCCTGGAAGAATCTTT CCTGGTGGAGGAGGACAAGAAGCATGAACGGCATCCTATCTTTGGAAACATCGTG GACGAAGTGGCGTACCACGAAAAGTACCCGACCATCTACCATCTGCGGAAGAAGT TGGTTGACTCAACTGACAAGGCCGACCTCAGATTGATCTACTTGGCCCTCGCCCATA TGATCAAATTCCGCGGACACTTCCTGATCGAAGGCGATCTGAACCCTGATAACTCC GACGTGGATAAGCTGTTCATTCAACTGGTGCAGACCTACAACCAACTGTTCGAAGA AAACCCAATCAATGCCAGCGGCGTCGATGCCAAGGCCATCCTGTCCGCCCGGCTGT CGAAGTCGCGGCGCCTCGAAAACCTGATCGCACAGCTGCCGGGAGAGAAGAAGA ACGGACTTTTCGGCAACTTGATCGCTCTCTCACTGGGACTCACTCCCAATTTCAAGT CCAATTTTGACCTGGCCGAGGACGCGAAGCTGCAACTCTCAAAGGACACCTACGA CGACGACTTGGACAATTTGCTGGCACAAATTGGCGATCAGTACGCGGATCTGTTCC TTGCCGCTAAGAACCTTTCGGACGCAATCTTGCTGTCCGATATCCTGCGCGTGAACA CCGAAATAACCAAAGCGCCGCTTAGCGCCTCGATGATTAAGCGGTACGACGAGCAT CACCAGGATCTCACGCTGCTCAAAGCGCTCGTGAGACAGCAACTGCCTGAAAAGT ACAAGGAGATTTTCTTCGACCAGTCCAAGAATGGGTACGCAGGGTACATCGATGGA GGCGCCAGCCAGGAAGAGTTCTATAAGTTCATCAAGCCAATCCTGGAAAAGATGGA CGGAACCGAAGAACTGCTGGTCAAGCTGAACAGGGAGGATCTGCTCCGCAAACAG AGAACCTTTGACAACGGAAGCATTCCACACCAGATCCATCTGGGTGAGCTGCACGC CATCTTGCGGCGCCAGGAGGACTTTTACCCATTCCTCAAGGACAACCGGGAAAAGA TCGAGAAAATTCTGACGTTCCGCATCCCGTATTACGTGGGCCCACTGGCGCGCGGC AATTCGCGCTTCGCGTGGATGACTAGAAAATCAGAGGAAACCATCACTCCTTGGAA TTTCGAGGAAGTTGTGGATAAGGGAGCTTCGGCACAATCCTTCATCGAACGAATGA CCAACTTCGACAAGAATCTCCCAAACGAGAAGGTGCTTCCTAAGCACAGCCTCCTT TACGAATACTTCACTGTCTACAACGAACTGACTAAAGTGAAATACGTTACTGAAGG AATGAGGAAGCCGGCCTTTCTGAGCGGAGAACAGAAGAAAGCGATTGTCGATCTG CTGTTCAAGACCAACCGCAAGGTGACCGTCAAGCAGCTTAAAGAGGACTACTTCA AGAAGATCGAGTGTTTCGACTCAGTGGAAATCAGCGGAGTGGAGGACAGATTCAA CGCTTCGCTGGGAACCTATCATGATCTCCTGAAGATCATCAAGGACAAGGACTTCC TTGACAACGAGGAGAACGAGGACATCCTGGAAGATATCGTCCTGACCTTGACCCTT TTCGAGGATCGCGAGATGATCGAGGAGAGGCTTAAGACCTACGCTCATCTCTTCGA CGATAAGGTCATGAAACAACTCAAGCGCCGCCGGTACACTGGTTGGGGCCGCCTCT CCCGCAAGCTGATCAACGGTATTCGCGATAAACAGAGCGGTAAAACTATCCTGGAT TTCCTCAAATCGGATGGCTTCGCTAATCGTAACTTCATGCAGTTGATCCACGACGAC AGCCTGACCTTTAAGGAGGACATCCAGAAAGCACAAGTGAGCGGACAGGGAGAC TCACTCCATGAACACATCGCGAATCTGGCCGGTTCGCCGGCGATTAAGAAGGGAAT CCTGCAAACTGTGAAGGTGGTGGACGAGCTGGTGAAGGTCATGGGACGGCACAAA CCGGAGAATATCGTGATTGAAATGGCCCGAGAAAACCAGACTACCCAGAAGGGCC AGAAGAACTCCCGCGAAAGGATGAAGCGGATCGAAGAAGGAATCAAGGAGCTGG GCAGCCAGATCCTGAAAGAGCACCCGGTGGAAAACACGCAGCTGCAGAACGAGA AGCTCTACCTGTACTATTTGCAAAATGGACGGGACATGTACGTGGACCAAGAGCTG GACATCAATCGGTTGTCTGATTACGACGTGGACCACATCGTTCCACAGTCCTTTCTG AAGGATGACTCCATCGATAACAAGGTGTTGACTCGCAGCGACAAGAACAGAGGGA	46

	AGTCAGATAATGTGCCATCGGAGGAGGTCGTGAAGAAGATGAAGAATTACTGGCG GCAGCTCCTGAATGCGAAGCTGATTACCCAGAGAAAGTTTGACAATCTCACTAAAG CCGAGCGCGGCGGACTCTCAGAGCTGGATAAGGCTGGATTCATCAAACGGCAGCT GGTCGAGACTCGGCAGATTACCAAGCACGTGGCGCAGATCCTGGACTCCCGCATG AACACTAAATACGACGAGAACGATAAGCTCATCCGGGAAGTGAAGGTGATTACCCT GAAAAGCAAACTTGTGTCGGACTTTCGGAAGGACTTTCAGTTTTACAAAGTGAGA GAAATCAACAACTACCATCACGCGCATGACGCATACCTCAACGCTGTGGTCGGCAC CGCCCTGATCAAGAAGTACCCTAAACTTGAATCGGAGTTTGTGTACGGAGACTACA AGGTCTACGACGTGAGGAAGATGATAGCCAAGTCCGAACAGGAAATCGGGAAAGC AACTGCGAAATACTTCTTTTACTCAAACATCATGAACTTCTTCAAGACTGAAATTAC GCTGGCCAATGGAGAAATCAGGAAGAGGCCACTGATCGAAACTAACGGAGAAACG GGCGAAATCGTGTGGGACAAGGGCAGGGACTTCGCAACTGTTCGCAAAGTGCTCT CTATGCCGCAAGTCAATATTGTGAAGAAAACCGAAGTGCAAACCGGCGGATTTTCA AAGGAATCGATCCTCCCAAAGAGAAATAGCGACAAGCTCATTGCACGCAAGAAAG ACTGGGACCCGAAGAAGTACGGAGGATTCGATTCGCCGACTGTCGCATACTCCGTC CTCGTGGTGGCCAAGGTGGAGAAGGGAAAGAGCAAGAAGCTCAAATCCGTCAAA GAGCTGCTGGGGATTACCATCATGGAACGATCCTCGTTCGAGAAGAACCCGATTGA TTTCCTGGAGGCGAAGGGTTACAAGGAGGTGAAGAAGGATCTGATCATCAAACTG CCCAAGTACTCACTGTTCGAACTGGAAAATGGTCGGAAGCGCATGCTGGCTTCGGC CGGAGAACTCCAGAAAGGAAATGAGCTGGCCTTGCCTAGCAAGTACGTCAACTTC CTCTATCTTGCTTCGCACTACGAGAAACTCAAAGGGTCACCGGAAGATAACGAACA GAAGCAGCTTTTCGTGGAGCAGCACAAGCATTATCTGGATGAAATCATCGAACAAA TCTCCGAGTTTTCAAAGCGCGTGATCCTCGCCGACGCCAACCTCGACAAAGTCCTG TCGGCCTACAATAAGCATAGAGATAAGCCGATCAGAGAACAGGCCGAGAACATTAT CCACTTGTTCACCCTGACTAACCTGGGAGCTCCAGCCGCCTTCAAGTACTTCGATA CTACTATCGACCGCAAAAGATACACGTCCACCAAGGAAGTTCTGGACGCGACCCTG ATCCACCAAAGCATCACTGGACTCTACGAAACTAGGATCGATCTGTCGCAGCTGGG TGGCGATGGTGGCGGTGGATCCTACCCATACGACGTGCCTGACTACGCCTCCGGAG GTGGTGGCCCCAAGAAGAAACGGAAGGTGTGATAGCTAGCCATCACATTTAAAAG CATCTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAGCTTATTCAT CTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTC TTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAATAAAAAATGGAAAGAACCTC GAG	
[0681] 具有HSD的5' UTR、对应于SEQ ID NO: 45的ORF及ALB的3' UTR的Cas9转录物	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTTGCAGG CCTTATTCGGATCTATGGATAAGAAGTACTCGATCGGGCTGGATATCGGAACTAATTC CGTGGGTTGGGCAGTGATCACGGATGAATACAAAGTGCCGTCCAAGAAGTTCAAG GTCCTGGGGAACACCGATAGACACAGCATCAAGAAGAATCTCATCGGAGCCCTGCT GTTTGACTCCGGCGAAACCGCAGAAGCGACCCGGCTCAAACGTACCGCGAGGCGA CGCTACACCCGGCGGAAGAATCGCATCTGCTATCTGCAAGAAATCTTTTCGAACGA AATGGCAAAGGTGGACGACAGCTTCTTCCACCGCCTGGAAGAATCTTTCCTGGTGG AGGAGGACAAGAAGCATGAACGGCATCCTATCTTTGGAAACATCGTGGACGAAGT GGCGTACCACGAAAAGTACCCGACCATCTACCATCTGCGGAAGAAGTTGGTTGACT CAACTGACAAGGCCGACCTCAGATTGATCTACTTGGCCCTCGCCCATATGATCAAAT TCCGCGGACACTTCCTGATCGAAGGCGATCTGAACCCTGATAACTCCGACGTGGAT AAGCTGTTCATTCAACTGGTGCAGACCTACAACCAACTGTTCGAAGAAAACCCAAT CAATGCCAGCGGCGTCGATGCCAAGGCCATCCTGTCCGCCCGGCTGTCGAAGTCGC GGCGCCTCGAAAACCTGATCGCACAGCTGCCGGGAGAGAAGAAGAACGGACTTTT CGGCAACTTGATCGCTCTCTCACTGGGACTCACTCCCAATTTCAAGTCCAATTTTGA CCTGGCCGAGGACGCGAAGCTGCAACTCTCAAAGGACACCTACGACGACGACTTG GACAATTTGCTGGCACAAATTGGCGATCAGTACGCGGATCTGTTCCTTGCCGCTAA GAACCTTTCGGACGCAATCTTGCTGTCCGATATCCTGCGCGTGAACACCGAAATAA CCAAAGCGCCGCTTAGCGCCTCGATGATTAAGCGGTACGACGAGCATCACCAGGAT CTCACGCTGCTCAAAGCGCTCGTGAGACAGCAACTGCCTGAAAAGTACAAGGAGA TTTTCTTCGACCAGTCCAAGAATGGGTACGCAGGGTACATCGATGGAGGCGCCAGC CAGGAAGAGTTCTATAAGTTCATCAAGCCAATCCTGGAAAAGATGGACGGAACCG AAGAACTGCTGGTCAAGCTGAACAGGGAGGATCTGCTCCGCAAACAGAGAACCTT TGACAACGGAAGCATTCCACACCAGATCCATCTGGGTGAGCTGCACGCCATCTTGC GGCGCCAGGAGGACTTTTACCCATTCCTCAAGGACAACCGGGAAAAGATCGAGAA AATTCTGACGTTCCGCATCCCGTATTACGTGGGCCCACTGGCGCGCGGCAATTCGCG CTTCGCGTGGATGACTAGAAAATCAGAGGAAACCATCACTCCTTGGAATTTCGAGG AAGTTGTGGATAAGGGAGCTTCGGCACAATCCTTCATCGAACGAATGACCAACTTC GACAAGAATCTCCCAAACGAGAAGGTGCTTCCTAAGCACAGCCTCCTTTACGAATA CTTCACTGTCTACAACGAACTGACTAAAGTGAAATACGTTACTGAAGGAATGAGGA AGCCGGCCTTTCTGAGCGGAGAACAGAAGAAAGCGATTGTCGATCTGCTGTTCAA GACCAACCGCAAGGTGACCGTCAAGCAGCTTAAAGAGGACTACTTCAAGAAGATC GAGTGTTTCGACTCAGTGGAAATCAGCGGAGTGGAGGACAGATTCAACGCTTCGC TGGGAACCTATCATGATCTCCTGAAGATCATCAAGGACAAGGACTTCCTTGACAAC	47

[0682]

	GAGGAGAACGAGGACATCCTGGAAGATATCGTCCTGACCTTGACCCTTTTCGAGGA TCGCGAGATGATCGAGGAGAGGCTTAAGACCTACGCTCATCTCTTCGACGATAAGG TCATGAAACAACTCAAGCGCCGCCGGTACACTGGTTGGGGCCGCCTCTCCCGCAA GCTGATCAACGGTATTCGCGATAAACAGAGCGGTAAAACTATCCTGGATTTCCTCAA ATCGGATGGCTTCGCTAATCGTAACTTCATGCAGTTGATCCACGACGACAGCCTGAC CTTTAAGGAGGACATCCAGAAAGCACAAGTGAGCGGACAGGGAGACTCACTCCAT GAACACATCGCGAATCTGGCCGGTTCGCCGGCGATTAAGAAGGGAATCCTGCAAA CTGTGAAGGTGGTGGACGAGCTGGTGAAGGTCATGGGACGGCACAAACCGGAGA ATATCGTGATTGAAATGGCCCGAGAAAACCAGACTACCCAGAAGGGCCAGAAGAA CTCCCGCGAAAGGATGAAGCGGATCGAAGAAGGAATCAAGGAGCTGGGCAGCCA GATCCTGAAAGAGCACCCGGTGGAAAACACGCAGCTGCAGAACGAGAAGCTCTAC CTGTACTATTTGCAAAATGGACGGGACATGTACGTGGACCAAGAGCTGGACATCAA TCGGTTGTCTGATTACGACGTGGACCACATCGTTCCACAGTCCTTTCTGAAGGATG ACTCCATCGATAACAAGGTGTTGACTCGCAGCGACAAGAACAGAGGGAAGTCAGA TAATGTGCCATCGGAGGAGGTCGTGAAGAAGATGAAGAATTACTGGCGGCAGCTCC TGAATGCGAAGCTGATTACCCAGAGAAAGTTTGACAATCTCACTAAAGCCGAGCGC GGCGGACTCTCAGAGCTGGATAAGGCTGGATTCATCAAACGGCAGCTGGTCGAGA CTCGGCAGATTACCAAGCACGTGGCGCAGATCCTGGACTCCCGCATGAACACTAAA TACGACGAGAACGATAAGCTCATCCGGGAAGTGAAGGTGATTACCCTGAAAAGCA AACTTGTGTCGGACTTTCGGAAGGACTTTCAGTTTTACAAAGTGAGAGAAATCAAC AACTACCATCACGCGCATGACGCATACCTCAACGCTGTGGTCGGCACCGCCCTGAT CAAGAAGTACCCTAAACTTGAATCGGAGTTTGTGTACGGAGACTACAAGGTCTACG ACGTGAGGAAGATGATAGCCAAGTCCGAACAGGAAATCGGGAAAGCAACTGCGA AATACTTCTTTTACTCAAACATCATGAACTTCTTCAAGACTGAAATTACGCTGGCCA ATGGAGAAATCAGGAAGAGGCCACTGATCGAAACTAACGGAGAAACGGGCGAAAT CGTGTGGGACAAGGGCAGGGACTTCGCAACTGTTCGCAAAGTGCTCTCTATGCCG CAAGTCAATATTGTGAAGAAAACCGAAGTGCAAACCGGCGGATTTTCAAAGGAAT CGATCCTCCCAAAGAGAAATAGCGACAAGCTCATTGCACGCAAGAAAGACTGGGA CCCGAAGAAGTACGGAGGATTCGATTCGCCGACTGTCGCATACTCCGTCCTCGTGG TGGCCAAGGTGGAGAAGGGAAAGAGCAAGAAGCTCAAATCCGTCAAAGAGCTGC TGGGGATTACCATCATGGAACGATCCTCGTTCGAGAAGAACCCGATTGATTTCCTGG AGGCGAAGGGTTACAAGGAGGTGAAGAAGGATCTGATCATCAAACTGCCCAAGTA CTCACTGTTCGAACTGGAAAATGGTCGGAAGCGCATGCTGGCTTCGGCCGGAGAA CTCCAGAAAGGAAATGAGCTGGCCTTGCCTAGCAAGTACGTCAACTTCCTCTATCT TGCTTCGCACTACGAGAAACTCAAAGGGTCACCGGAAGATAACGAACAGAAGCAG CTTTTCGTGGAGCAGCACAAGCATTATCTGGATGAAATCATCGAACAAATCTCCGA GTTTTCAAAGCGCGTGATCCTCGCCGACGCCAACCTCGACAAAGTCCTGTCGGCCT ACAATAAGCATAGAGATAAGCCGATCAGAGAACAGGCCGAGAACATTATCCACTTG TTCACCCTGACTAACCTGGGAGCTCCAGCCGCCTTCAAGTACTTCGATACTACTATC GACCGCAAAAGATACACGTCCACCAAGGAAGTTCTGGACGCGACCCTGATCCACC AAAGCATCACTGGACTCTACGAAACTAGGATCGATCTGTCGCAGCTGGGTGGCGAT GGTGGCGGTGGATCCTACCCATACGACGTGCCTGACTACGCCTCCGGAGGTGGTGG CCCCAAGAAGAAACGGAAGGTGTGATAGCTAGCCATCACATTTAAAAGCATCTCAG CCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAGCTTATTCATCTCTTTTT CTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAATCA TTTTGCCTCTTTTCTCTGTGCTTCAATTAATAAAAAATGGAAAGAACCTCGAG	
包含使用通常在人类中高度表达的密码子的Cas9 ORF的Cas9转录物	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTTGCAGG CCTTATTCGGATCCATGCCTAAGAAAAAGCGGAAGGTCGACGGGGATAAGAAGTAC TCAATCGGGCTGGATATCGGAACTAATTCCGTGGGTTGGGCAGTGATCACGGATGA ATACAAAGTGCCGTCCAAGAAGTTCAAGGTCCTGGGGAACACCGATAGACACAGC ATCAAGAAAAATCTCATCGGAGCCCTGCTGTTTGACTCCGGCGAAACCGCAGAAG CGACCCGGCTCAAACGTACCGCGAGGCGACGCTACACCCGGCGGAAGAATCGCAT CTGCTATCTGCAAGAGATCTTTTCGAACGAAATGGCAAAGGTCGACGACAGCTTCT TCCACCGCCTGGAAGAATCTTTCCTGGTGGAGGAGGACAAGAAGCATGAACGGCA TCCTATCTTTGGAAACATCGTCGACGAAGTGGCGTACCACGAAAAGTACCCGACCA TCTACCATCTGCGGAAGAAGTTGGTTGACTCAACTGACAAGGCCGACCTCAGATTG ATCTACTTGGCCCTCGCCCATATGATCAAATTCCGCGGACACTTCCTGATCGAAGGC GATCTGAACCCTGATAACTCCGACGTGGATAAGCTTTTCATTCAACTGGTGCAGACC TACAACCAACTGTTCGAAGAAAACCCAATCAATGCTAGCGGCGTCGATGCCAAGGC CATCCTGTCCGCCCGGCTGTCGAAGTCGCGGCGCCTCGAAAACCTGATCGCACAGC TGCCGGGAGAGAAAAAGAACGGACTTTTCGGCAACTTGATCGCTCTCTCACTGGG ACTCACTCCCAATTTCAAGTCCAATTTTGACCTGGCCGAGGACGCGAAGCTGCAAC TCTCAAAGGACACCTACGACGACGACTTGGACAATTTGCTGGCACAAATTGGCGAT CAGTACGCGGATCTGTTCCTTGCCGCTAAGAACCTTTCGGACGCAATCTTGCTGTCC GATATCCTGCGCGTGAACACCGAAATAACCAAAGCGCCGCTTAGCGCCTCGATGAT TAAGCGGTACGACGAGCATCACCAGGATCTCACGCTGCTCAAAGCGCTCGTGAGA CAGCAACTGCCTGAAAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAATGGGT	48

[0683]

	ACGCAGGGTACATCGATGGAGGCGCTAGCCAGGAAGAGTTCTATAAGTTCATCAAG CCAATCCTGGAAAAGATGGACGGAACCGAAGAACTGCTGGTCAAGCTGAACAGGG AGGATCTGCTCCGGAAACAGAGAACCTTTGACAACGGATCCATTCCCCACCAGATC CATCTGGGTGAGCTGCACGCCATCTTGCGGCGCCAGGAGGACTTTTACCCATTCCT CAAGGACAACCGGGAAAAGATCGAGAAAATTCTGACGTTCCGCATCCCGTATTACG TGGGCCCACTGGCGCGCGGCAATTCGCGCTTCGCGTGGATGACTAGAAAATCAGA GGAAACCATCACTCCTTGGAATTTCGAGGAAGTTGTGGATAAGGGAGCTTCGGCAC AAAGCTTCATCGAACGAATGACCAACTTCGACAAGAATCTCCCAAACGAGAAGGT GCTTCCTAAGCACAGCCTCCTTTACGAATACTTCACTGTCTACAACGAACTGACTAA AGTGAAATACGTTACTGAAGGAATGAGGAAGCCGGCCTTTCTGTCCGGAGAACAG AAGAAAGCAATTGTCGATCTGCTGTTCAAGACCAACCGCAAGGTGACCGTCAAGC AGCTTAAAGAGGACTACTTCAAGAAGATCGAGTGTTTCGACTCAGTGGAAATCAGC GGGGTGGAGGACAGATTCAACGCTTCGCTGGGAACCTATCATGATCTCCTGAAGAT CATCAAGGACAAGGACTTCCTTGACAACGAGGAGAACGAGGACATCCTGGAAGAT ATCGTCCTGACCTTGACCCTTTTCGAGGATCGCGAGATGATCGAGGAGAGGCTTAA GACCTACGCTCATCTCTTCGACGATAAGGTCATGAAACAACTCAAGCGCCGCCGGT ACACTGGTTGGGGCCGCCTCTCCCGCAAGCTGATCAACGGTATTCGCGATAAACAG AGCGGTAAAACTATCCTGGATTTCCTCAAATCGGATGGCTTCGCTAATCGTAACTTC ATGCAATTGATCCACGACGACAGCCTGACCTTTAAGGAGGACATCCAAAAAGCAC AAGTGTCCGGACAGGGAGACTCACTCCATGAACACATCGCGAATCTGGCCGGTTC GCCGGCGATTAAGAAGGGAATTCTGCAAACTGTGAAGGTGGTCGACGAGCTGGTG AAGGTCATGGGACGGCACAAACCGGAGAATATCGTGATTGAAATGGCCCGAGAAA ACCAGACTACCCAGAAGGGCCAGAAAAACTCCCGCGAAAGGATGAAGCGGATCG AAGAAGGAATCAAGGAGCTGGGCAGCCAGATCCTGAAAGAGCACCCGGTGGAAA ACACGCAGCTGCAGAACGAGAAGCTCTACCTGTACTATTTGCAAAATGGACGGGA CATGTACGTGGACCAAGAGCTGGACATCAATCGGTTGTCTGATTACGACGTGGACC ACATCGTTCCACAGTCCTTTCTGAAGGATGACTCGATCGATAACAAGGTGTTGACT CGCAGCGACAAGAACAGAGGGAAGTCAGATAATGTGCCATCGGAGGAGGTCGTGA AGAAGATGAAGAATTACTGGCGGCAGCTCCTGAATGCGAAGCTGATTACCCAGAG AAAGTTTGACAATCTCACTAAAGCCGAGCGCGGCGGACTCTCAGAGCTGGATAAG GCTGGATTCATCAAACGGCAGCTGGTCGAGACTCGGCAGATTACCAAGCACGTGG CGCAGATCTTGGACTCCCGCATGAACACTAAATACGACGAGAACGATAAGCTCATC CGGGAAGTGAAGGTGATTACCCTGAAAAGCAAACTTGTGTCGGACTTTCGGAAGG ACTTTCAGTTTTACAAAGTGAGAGAAATCAACAACTACCATCACGCGCATGACGCA TACCTCAACGCTGTGGTCGGTACCGCCCTGATCAAAAAGTACCCTAAACTTGAATC GGAGTTTGTGTACGGAGACTACAAGGTCTACGACGTGAGGAAGATGATAGCCAAG TCCGAACAGGAAATCGGGAAAGCAACTGCGAAATACTTCTTTTACTCAAACATCAT GAACTTTTTCAAGACTGAAATTACGCTGGCCAATGGAGAAATCAGGAAGAGGCCA CTGATCGAAACTAACGGAGAAACGGGCGAAATCGTGTGGGACAAGGGCAGGGAC TTCGCAACTGTTCGCAAAGTGCTCTCTATGCCGCAAGTCAATATTGTGAAGAAAAC CGAAGTGCAAACCGGCGGATTTTCAAAGGAATCGATCCTCCCAAAGAGAAATAGC GACAAGCTCATTGCACGCAAGAAAGACTGGGACCCGAAGAAGTACGGAGGATTCG ATTCGCCGACTGTCGCATACTCCGTCCTCGTGGTGGCCAAGGTGGAGAAGGGAAA GAGCAAAAAGCTCAAATCCGTCAAAGAGCTGCTGGGGATTACCATCATGGAACGAT CCTCGTTCGAGAAGAACCCGATTGATTTCCTCGAGGCGAAGGGTTACAAGGAGGT GAAGAAGGATCTGATCATCAAACTCCCCAAGTACTCACTGTTCGAACTGGAAAATG GTCGGAAGCGCATGCTGGCTTCGGCCGGAGAACTCCAAAAAGGAAATGAGCTGGC CTTGCCTAGCAAGTACGTCAACTTCCTCTATCTTGCTTCGCACTACGAAAAACTCAA AGGGTCACCGGAAGATAACGAACAGAAGCAGCTTTTCGTGGAGCAGCACAAGCAT TATCTGGATGAAATCATCGAACAAATCTCCGAGTTTTCAAAGCGCGTGATCCTCGCC GACGCCAACCTCGACAAAGTCCTGTCGGCCTACAATAAGCATAGAGATAAGCCGAT CAGAGAACAGGCCGAGAACATTATCCACTTGTTCACCCTGACTAACCTGGGAGCCC CAGCCGCCTTCAAGTACTTCGATACTACTATCGATCGCAAAAGATACACGTCCACCA AGGAAGTTCTGGACGCGACCCTGATCCACCAAAGCATCACTGGACTCTACGAAACT AGGATCGATCTGTCGCAGCTGGGTGGCGATTGATAGTCTAGCCATCACATTTAAAAG CATCTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAGCTTATTCAT CTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTC TTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAATAAAAAATGGAAAGAACCTC GAG	
Cas9 包含Kozak序列以及使用通常在人类中高度	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTTGCAGG CCTTATTCGGATCCGCCACCATGCCTAAGAAAAAGCGGAAGGTCGACGGGGATAAG AAGTACTCAATCGGGCTGGATATCGGAACTAATTCCGTGGGTTGGGCAGTGATCAC GGATGAATACAAAGTGCCGTCCAAGAAGTTCAAGGTCCTGGGGAACACCGATAGA CACAGCATCAAGAAAAATCTCATCGGAGCCCTGCTGTTTGACTCCGGCGAAACCGC AGAAGCGACCCGGCTCAAACGTACCGCGAGGCGACGCTACACCCGGCGGAAGAAT CGCATCTGCTATCTGCAAGAGATCTTTTCGAACGAAATGGCAAAGGTCGACGACAG CTTCTTCCACCGCCTGGAAGAATCTTTCCTGGTGGAGGAGGACAAGAAGCATGAA	49

[0684]

表达的密码子的Cas9 ORF的Cas9转录物	CGGCATCCTATCTTTGGAAACATCGTCGACGAAGTGGCGTACCACGAAAAGTACCC GACCATCTACCATCTGCGGAAGAAGTTGGTTGACTCAACTGACAAGGCCGACCTCA GATTGATCTACTTGGCCCTCGCCCATATGATCAAATTCCGCGGACACTTCCTGATCG AAGGCGATCTGAACCCTGATAACTCCGACGTGGATAAGCTTTTCATTCAACTGGTG CAGACCTACAACCAACTGTTCGAAGAAAACCCAATCAATGCTAGCGGCGTCGATGC CAAGGCCATCCTGTCCGCCCGGCTGTCGAAGTCGCGGCGCCTCGAAAACCTGATCG CACAGCTGCCGGGAGAGAAAAAGAACGGACTTTTCGGCAACTTGATCGCTCTCTC ACTGGGACTCACTCCCAATTTCAAGTCCAATTTTGACCTGGCCGAGGACGCGAAGC TGCAACTCTCAAAGGACACCTACGACGACGACTTGGACAATTTGCTGGCACAAATT GGCGATCAGTACGCGGATCTGTTCCTTGCCGCTAAGAACCTTTCGGACGCAATCTT GCTGTCCGATATCCTGCGCGTGAACACCGAAATAACCAAAGCGCCGCTTAGCGCCT CGATGATTAAGCGGTACGACGAGCATCACCAGGATCTCACGCTGCTCAAAGCGCTC GTGAGACAGCAACTGCCTGAAAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGA ATGGGTACGCAGGGTACATCGATGGAGGCGCTAGCCAGGAAGAGTTCTATAAGTTC ATCAAGCCAATCCTGGAAAAGATGGACGGAACCGAAGAACTGCTGGTCAAGCTGA ACAGGGAGGATCTGCTCCGGAAACAGAGAACCTTTGACAACGGATCCATTCCCCA CCAGATCCATCTGGGTGAGCTGCACGCCATCTTGCGGCGCCAGGAGGACTTTTACC CATTCCTCAAGGACAACCGGGAAAAGATCGAGAAAATTCTGACGTTCCGCATCCCG TATTACGTGGGCCCACTGGCGCGCGGCAATTCGCGCTTCGCGTGGATGACTAGAAA ATCAGAGGAAACCATCACTCCTTGGAATTTCGAGGAAGTTGTGGATAAGGGAGCTT CGGCACAAAGCTTCATCGAACGAATGACCAACTTCGACAAGAATCTCCCAAACGA GAAGGTGCTTCCTAAGCACAGCCTCCTTTACGAATACTTCACTGTCTACAACGAAC TGACTAAAGTGAAATACGTTACTGAAGGAATGAGGAAGCCGGCCTTTCTGTCCGGA GAACAGAAGAAAGCAATTGTCGATCTGCTGTTCAAGACCAACCGCAAGGTGACCG TCAAGCAGCTTAAAGAGGACTACTTCAAGAAGATCGAGTGTTTCGACTCAGTGGA AATCAGCGGGGTGGAGGACAGATTCAACGCTTCGCTGGGAACCTATCATGATCTCC TGAAGATCATCAAGGACAAGGACTTCCTTGACAACGAGGAGAACGAGGACATCCT GGAAGATATCGTCCTGACCTTGACCCTTTTCGAGGATCGCGAGATGATCGAGGAGA GGCTTAAGACCTACGCTCATCTCTTCGACGATAAGGTCATGAAACAACTCAAGCGC CGCCGGTACACTGGTTGGGGCCGCCTCTCCCGCAAGCTGATCAACGGTATTCGCGA TAAACAGAGCGGTAAAACTATCCTGGATTTCCTCAAATCGGATGGCTTCGCTAATCG TAACTTCATGCAATTGATCCACGACGACAGCCTGACCTTTAAGGAGGACATCCAAA AAGCACAAGTGTCCGGACAGGGAGACTCACTCCATGAACACATCGCGAATCTGGC CGGTTCGCCGGCGATTAAGAAGGGAATTCTGCAAACTGTGAAGGTGGTCGACGAG CTGGTGAAGGTCATGGGACGGCACAAACCGGAGAATATCGTGATTGAAATGGCCC GAGAAAACCAGACTACCCAGAAGGGCCAGAAAAACTCCCGCGAAAGGATGAAGC GGATCGAAGAAGGAATCAAGGAGCTGGGCAGCCAGATCCTGAAAGAGCACCCGGT GGAAAACACGCAGCTGCAGAACGAGAAGCTCTACCTGTACTATTTGCAAAATGGA CGGGACATGTACGTGGACCAAGAGCTGGACATCAATCGGTTGTCTGATTACGACGT GGACCACATCGTTCCACAGTCCTTTCTGAAGGATGACTCGATCGATAACAAGGTGT TGACTCGCAGCGACAAGAACAGAGGGAAGTCAGATAATGTGCCATCGGAGGAGGT CGTGAAGAAGATGAAGAATTACTGGCGGCAGCTCCTGAATGCGAAGCTGATTACCC AGAGAAAGTTTGACAATCTCACTAAAGCCGAGCGCGGCGGACTCTCAGAGCTGGA TAAGGCTGGATTCATCAAACGGCAGCTGGTCGAGACTCGGCAGATTACCAAGCACG TGGCGCAGATCTTGGACTCCCGCATGAACACTAAATACGACGAGAACGATAAGCTC ATCCGGGAAGTGAAGGTGATTACCCTGAAAAGCAAACTTGTGTCGGACTTTCGGA AGGACTTTCAGTTTTACAAAGTGAGAGAAATCAACAACTACCATCACGCGCATGAC GCATACCTCAACGCTGTGGTCGGTACCGCCCTGATCAAAAAGTACCCTAAACTTGA ATCGGAGTTTGTGTACGGAGACTACAAGGTCTACGACGTGAGGAAGATGATAGCCA AGTCCGAACAGGAAATCGGGAAAGCAACTGCGAAATACTTCTTTTACTCAAACATC ATGAACTTTTTCAAGACTGAAATTACGCTGGCCAATGGAGAAATCAGGAAGAGGCC ACTGATCGAAACTAACGGAGAAACGGGCGAAATCGTGTGGGACAAGGGCAGGGA CTTCGCAACTGTTCGCAAAGTGCTCTCTATGCCGCAAGTCAATATTGTGAAGAAAA CCGAAGTGCAAACCGGCGGATTTTCAAAGGAATCGATCCTCCCAAAGAGAAATAG CGACAAGCTCATTGCACGCAAGAAAGACTGGGACCCGAAGAAGTACGGAGGATTC GATTCGCCGACTGTCGCATACTCCGTCCTCGTGGTGGCCAAGGTGGAGAAGGGAA AGAGCAAAAAGCTCAAATCCGTCAAAGAGCTGCTGGGGATTACCATCATGGAACG ATCCTCGTTCGAGAAGAACCCGATTGATTTCCTCGAGGCGAAGGGTTACAAGGAGG TGAAGAAGGATCTGATCATCAAACTCCCCAAGTACTCACTGTTCGAACTGGAAAAT GGTCGGAAGCGCATGCTGGCTTCGGCCGGAGAACTCCAAAAAGGAAATGAGCTGG CCTTGCCTAGCAAGTACGTCAACTTCCTCTATCTTGCTTCGCACTACGAAAAACTCA AAGGGTCACCGGAAGATAACGAACAGAAGCAGCTTTTCGTGGAGCAGCACAAGCA TTATCTGGATGAAATCATCGAACAAATCTCCGAGTTTTCAAAGCGCGTGATCCTCGC CGACGCCAACCTCGACAAAGTCCTGTCGGCCTACAATAAGCATAGAGATAAGCCGA TCAGAGAACAGGCCGAGAACATTATCCACTTGTTCACCCTGACTAACCTGGGAGCC CCAGCCGCCTTCAAGTACTTCGATACTACTATCGATCGCAAAAGATACACGTCCACC AAGGAAGTTCTGGACGCGACCCTGATCCACCAAAGCATCACTGGACTCTACGAAA	

[0685]

	CTAGGATCGATCTGTCGCAGCTGGGTGGCGATTGATAGTCTAGCCATCACATTTAAA AGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAGCTTATT CATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAAT TTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAATAAAAAATGGAAAGAAC CTCGAG	
剪接接点经移除;具有 12.75% U 含量的 Cas9 ORF	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGG CAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAA CACAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGC GGAGAAACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACAC AAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCA AAGGTCGACGACAGCTTCTTCCACcggCTGGAAGAAAGCTTCCTGGTCGAAGAAGA CAAGAAGCACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATAC CACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAG ACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAG AGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAG CTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAA CGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAG AAGACTGGAAAACCTGATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTT CGGAAACCTGATCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTC GACCTGGCAGAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGAC CTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACCTGTTCCTGGCAG CAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGA AATCACAAAGGCACCGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCAC CAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACA AGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGG AGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGAAAAGATGGAC GGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAG AGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACG CAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAAGGACAACAGAGAAAA GATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAG GAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTG GAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTTCATCGAAAG AATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGC CTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCAC AGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGT CGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGA CTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGAC AGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACA AGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGAC ACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATACGCA CACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGGAT GGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAA AGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCA GCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTC AGCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCG GCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGG TCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCA GACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAG AAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACA CACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAaAACGGAAGAGACAT GTACGTCGACCAGGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCAC ATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAA GAAGCGACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCA AGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAG AAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAA GGCAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTC GCACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAACGACAAGCTGA TCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAA GGACTTCCAGTTCTACAAGGTCAGAGAAATCAACAACTACCACCACGCACACGAC GCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGG AAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGC AAAGAGCGAACAGGAAATCGGAAAGGCAACAGCAAAGTACTTCTTCTACAGCAAC ATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGA GACCGCTGATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAA GAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTCAA GAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAG AAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGG	50

[0686]

	AGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAAGGTCGAA AAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATC ATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTGGAAGCAAAGGGAT ACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGA ACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGG AAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCCAC TACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTC GAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCA AGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACAACAA GCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACA CTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACA GAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGA GCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGG AGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCTAG	
具有HSD的5' UTR、对应于SEQ ID NO: 50的ORF、Kozak序列及ALB的3' UTR的Cas9转录物	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTTGCAGG CCTTATTCGGATCCGCCACCATGGACAAGAAGTACAGCATCGGACTGGACATCGGA ACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGA AGTTCAAGGTCCTGGGAAACACAGACAGACACAGCATCAAGAAGAACCTGATCGG AGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAACAAGACTGAAGAGAAC AGCAAGAAGAAGATACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATC TTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACcggCTGGAAGAAAG CTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTCGGAAACATC GTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGA AGCTGGTCGACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGC ACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGAC AACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTT CGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGC AAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCCGGGAGA AAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGGACTGACACCG AACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCTGCAGCTGAGCAAG GACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACG CAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACAT CCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAAG AGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGC AGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGC AGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCG ATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAA GACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCC ACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCT GAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTAC GTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGC GAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGC GCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAA AGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTG ACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAG AACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGT CAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAA ATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGC TGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCT GGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAA AGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGA GAAGAAGATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCA GAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGC AAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGAC ATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACACATCGCA AACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCG TCGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGA AATGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAA GAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGG AACACCCGGTCGAAAACACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCT GCAaAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTGAGC GACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCG ACAACAAGGTCCTGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAACGTCC CGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACG CAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAG GACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAA GACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTA	51

		CGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAA GCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAATCAAC AACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGA TCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTA CGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACAGC AAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGG CAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAG AAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCAT GCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAA GGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGA CTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTC CTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAG GAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCG ACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCT GCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAG CGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAA CTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAAC GAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCG AACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAA GGTCCTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGA AAACATCATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGT ACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGA CGCAACACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTG AGCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCTA GCTAGCCATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAA TGAAGATCAATAGCTTATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCC TGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAA TAAAAAATGGAAAGAACCTCGAG	
[0687]	具有一般经常用于人类中的最小尿苷密码子;具有 12.75% U含量的Cas9 ORF	ATGGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACAGCGTGGGCTGGG CCGTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAGTTCAAGGTGCTGGGCAA CACCGACAGACACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGC GGCGAGACCGCCGAGGCCACCAGACTGAAGAGAACCGCCAGAAGAAGATACACC AGAAGAAAGAACAGAATCTGCTACCTGCAGGAGATCTTCAGCAACGAGATGGCCA AGGTGGACGACAGCTTCTTCCACAGACTGGAGGAGAGCTTCCTGGTGGAGGAGGA CAAGAAGCACGAGAGACACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTAC CACGAGAAGTACCCCACCATCTACCACCTGAGAAAGAAGCTGGTGGACAGCACCG ACAAGGCCGACCTGAGACTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCAGA GGCCACTTCCTGATCGAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGC TGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAAC GCCAGCGGCGTGGACGCCAAGGCCATCCTGAGCGCCAGACTGAGCAAGAGCAGA AGACTGGAGAACCTGATCGCCCAGCTGCCCGGCGAGAAGAAGAACGGCCTGTTCG GCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGA CCTGGCCGAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTG GACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTCCTGGCCGCCA AGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGAGAGTGAACACCGAGAT CACCAAGGCCCCCCTGAGCGCCAGCATGATCAAGAGATACGACGAGCACCACCAG GACCTGACCCTGCTGAAGGCCCTGGTGAGACAGCAGCTGCCCGAGAAGTACAAGG AGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGC CAGCCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGC ACCGAGGAGCTGCTGGTGAAGCTGAACAGAGAGGACCTGCTGAGAAAGCAGAGA ACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCAT CCTGAGAAGACAGGAGGACTTCTACCCCTTCCTGAAGGACAACAGAGAGAAGATC GAGAAGATCCTGACCTTCAGAATCCCCTACTACGTGGGCCCCCTGGCCAGAGGCAA CAGCAGATTCGCCTGGATGACCAGAAAGAGCGAGGAGACCATCACCCCCTGGAAC TTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGCTTCATCGAGAGAATGA CCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCT GTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAG GGCATGAGAAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGCCATCGTGGACC TGCTGTTCAAGACCAACAGAAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTT CAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGCGGCGTGGAGGACAGATTC AACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACT TCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGAC CCTGTTCGAGGACAGAGAGATGATCGAGGAGAGACTGAAGACCTACGCCCACCTG TTCGACGACAAGGTGATGAAGCAGCTGAAGAGAAGAAGATACACCGGCTGGGGC AGACTGAGCAGAAAGCTGATCAACGGCATCAGAGACAAGCAGAGCGGCAAGACC ATCCTGGACTTCCTGAAGAGCGACGGCTTCGCCAACAGAAACTTCATGCAGCTGAT CCACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGC	52

[0688]

	CAGGGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCA AGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGG CAGACACAAGCCCGAGAACATCGTGATCGAGATGGCCAGAGAGAACCAGACCACC CAGAAGGGCCAGAAGAACAGCAGAGAGAGAATGAAGAGAATCGAGGAGGGCATC AAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTG CAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCAGAGACATGTACGTGG ACCAGGAGCTGGACATCAACAGACTGAGCGACTACGACGTGGACCACATCGTGCC CCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTGCTGACCAGAAGCGAC AAGAACAGAGGCAAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATG AAGAACTACTGGAGACAGCTGCTGAACGCCAAGCTGATCACCCAGAGAAAGTTCG ACAACCTGACCAAGGCCGAGAGAGGCGGCCTGAGCGAGCTGGACAAGGCCGGCT TCATCAAGAGACAGCTGGTGGAGACCAGACAGATCACCAAGCACGTGGCCCAGAT CCTGGACAGCAGAATGAACACCAAGTACGACGAGAACGACAAGCTGATCAGAGA GGTGAAGGTGATCACCCTGAAGAGCAAGCTGGTGAGCGACTTCAGAAAGGACTTC CAGTTCTACAAGGTGAGAGAGATCAACAACTACCACCACGCCCACGACGCCTACC TGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGGAGAGCGA GTTCGTGTACGGCGACTACAAGGTGTACGACGTGAGAAAGATGATCGCCAAGAGC GAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACAGCAACATCATGAA CTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCAGAAAGAGACCCCTG ATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCAGAGACTTC GCCACCGTGAGAAAGGTGCTGAGCATGCCCCAGGTGAACATCGTGAAGAAGACCG AGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATCCTGCCCAAGAGAAACAGCG ACAAGCTGATCGCCAGAAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCG ACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAA GAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGAGA AGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGG TGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTCGAGCTGGAGAA CGGCAGAAAGAGAATGCTGGCCAGCGCCGGCGAGCTGCAGAAGGGCAACGAGCT GGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCCACTACGAGAAG CTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCAC AAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGAGAGTGA TCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGCACAGAGA CAAGCCCATCAGAGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAAC CTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACAGAAAGAGATA CACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGC CTGTACGAGACCAGAATCGACCTGAGCCAGCTGGGCGGCGACGGCGGCGGCAGCC CCAAGAAGAAGAGAAAGGTGTGA	
具有HSD的 5' UTR、对应于SEQ ID NO：52的ORF 、Kozak序列及ALB的3' UTR的Cas9转录物	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTTGCAGG CCTTATTCGGATCCGCCACCATGGACAAGAAGTACAGCATCGGCCTGGACATCGGC ACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAGA AGTTCAAGGTGCTGGGCAACACCGACAGACACAGCATCAAGAAGAACCTGATCGG CGCCCTGCTGTTCGACAGCGGCGAGACCGCCGAGGCCACCAGACTGAAGAGAACC GCCAGAAGAAGATACACCAGAAGAAAGAACAGAATCTGCTACCTGCAGGAGATCT TCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGACTGGAGGAGAG CTTCCTGGTGGAGGAGGACAAGAAGCACGAGAGACACCCCATCTTCGGCAACATC GTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGAGAAAGA AGCTGGTGGACAGCACCGACAAGGCCGACCTGAGACTGATCTACCTGGCCCTGGC CCACATGATCAAGTTCAGAGGCCACTTCCTGATCGAGGGCGACCTGAACCCCGACA ACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTT CGAGGAGAACCCCATCAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGAGCGCC AGACTGAGCAAGAGCAGAAGACTGGAGAACCTGATCGCCCAGCTGCCCGGCGAG AAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCA ACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCTGCAGCTGAGCAAGGA CACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCC GACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCT GAGAGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCAGCATGATCAAGAGA TACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGAGACAGCAGC TGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGG CTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCC TGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACAGAGAGGACC TGCTGAGAAAGCAGAGAACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCT GGGCGAGCTGCACGCCATCCTGAGAAGACAGGAGGACTTCTACCCCTTCCTGAAG GACAACAGAGAGAAGATCGAGAAGATCCTGACCTTCAGAATCCCCTACTACGTGG GCCCCCTGGCCAGAGGCAACAGCAGATTCGCCTGGATGACCAGAAAGAGCGAGGA GACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAG AGCTTCATCGAGAGAATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGC TGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAA	53

[0689]

	GGTGAAGTACGTGACCGAGGGCATGAGAAAGCCCGCCTTCCTGAGCGGCGAGCAG AAGAAGGCCATCGTGGACCTGCTGTTCAAGACCAACAGAAAGGTGACCGTGAAGC AGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAG CGGCGTGGAGGACAGATTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAG ATCATCAAGGACAAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGG ACATCGTGCTGACCCTGACCCTGTTCGAGGACAGAGAGATGATCGAGGAGAGACT GAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGAGAAGA AGATACACCGGCTGGGGCAGACTGAGCAGAAAGCTGATCAACGGCATCAGAGACA AGCAGAGCGGCAAGACCATCCTGGACTTCCTGAAGAGCGACGGCTTCGCCAACAG AAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTCAAGGAGGACATCCAG AAGGCCCAGGTGAGCGGCCAGGGCGACAGCCTGCACGAGCACATCGCCAACCTG GCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACG AGCTGGTGAAGGTGATGGGCAGACACAAGCCCGAGAACATCGTGATCGAGATGGC CAGAGAGAACCAGACCACCCAGAAGGGCCAGAAGAACAGCAGAGAGAGAATGA AGAGAATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACC CCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAA CGGCAGAGACATGTACGTGGACCAGGAGCTGGACATCAACAGACTGAGCGACTAC GACGTGGACCACATCGTGCCCCAGAGCTTCCTGAAGGACGACAGCATCGACAACA AGGTGCTGACCAGAAGCGACAAGAACAGAGGCAAGAGCGACAACGTGCCCAGCG AGGAGGTGGTGAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCCAAGC TGATCACCCAGAGAAAGTTCGACAACCTGACCAAGGCCGAGAGAGGCGGCCTGA GCGAGCTGGACAAGGCCGGCTTCATCAAGAGACAGCTGGTGGAGACCAGACAGAT CACCAAGCACGTGGCCCAGATCCTGGACAGCAGAATGAACACCAAGTACGACGAG AACGACAAGCTGATCAGAGAGGTGAAGGTGATCACCCTGAAGAGCAAGCTGGTGA GCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTGAGAGAGATCAACAACTACCA CCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAG TACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGA GAAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTT CTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCG AGATCAGAAAGAGACCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTG GGACAAGGGCAGAGACTTCGCCACCGTGAGAAAGGTGCTGAGCATGCCCCAGGTG AACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATCC TGCCCAAGAGAAACAGCGACAAGCTGATCGCCAGAAAGAAGGACTGGGACCCCA AGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGC CAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGG CATCACCATCATGGAGAGAAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAG GCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACA GCCTGTTCGAGCTGGAGAACGGCAGAAAGAGAATGCTGGCCAGCGCCGGCGAGCT GCAGAAGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTG GCCAGCCACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAG CTGTTCGTGGAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCG AGTTCAGCAAGAGAGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGC CTACAACAAGCACAGAGACAAGCCCATCAGAGAGCAGGCCGAGAACATCATCCAC CTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCAC CATCGACAGAAAGAGATACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATC CACCAGAGCATCACCGGCCTGTACGAGACCAGAATCGACCTGAGCCAGCTGGGCG GCGACGGCGGCGGCAGCCCCAAGAAGAAGAGAAAGGTGTGACTAGCCATCACATT TAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAGCT TATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATA AATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAATAAAAAATGGAAAG AACCTCGAG	
具有一般不经常用于人类中的最小尿苷密码子;具有12.75% U含量的Cas9 ORF	ATGGACAAAAAATACAGCATAGGGCTAGACATAGGGACGAACAGCGTAGGGTGGG CGGTAATAACGGACGAATACAAAGTACCGAGCAAAAAATTCAAAGTACTAGGGAA CACGGACCGACACAGCATAAAAAAAAACCTAATAGGGGCGCTACTATTCGACAGC GGGGAAACGGCGGAAGCGACGCGACTAAAACGAACGGCGCGACGACGATACACG CGACGAAAAAACCGAATATGCTACCTACAAGAAATATTCAGCAACGAAATGGCGAA AGTAGACGACAGCTTCTTCCACCGACTAGAAGAAAGCTTCCTAGTAGAAGAAGAC AAAAAACACGAACGACACCCGATATTCGGGAACATAGTAGACGAAGTAGCGTACC ACGAAAAATACCCGACGATATACCACCTACGAAAAAAACTAGTAGACAGCACGGA CAAAGCGGACCTACGACTAATATACCTAGCGCTAGCGCACATGATAAAATTCCGAG GGCACTTCCTAATAGAAGGGGACCTAAACCCGGACAACAGCGACGTAGACAAACT ATTCATACAACTAGTACAAACGTACAACCAACTATTCGAAGAAAACCCGATAAACG CGAGCGGGGTAGACGCGAAAGCGATACTAAGCGCGCGACTAAGCAAAAGCCGAC GACTAGAAAACCTAATAGCGCAACTACCGGGGGAAAAAAAAAACGGGCTATTCGG GAACCTAATAGCGCTAAGCCTAGGGCTAACGCCGAACTTCAAAAGCAACTTCGACC TAGCGGAAGACGCGAAACTACAACTAAGCAAAGACACGTACGACGACGACCTAGA CAACCTACTAGCGCAAATAGGGGACCAATACGCGGACCTATTCCTAGCGGCGAAAA	54

[0690]

	ACCTAAGCGACGCGATACTACTAAGCGACATACTACGAGTAAACACGGAAATAACG AAAGCGCCGCTAAGCGCGAGCATGATAAAACGATACGACGAACACCACCAAGACC TAACGCTACTAAAAGCGCTAGTACGACAACAACTACCGGAAAAATACAAAGAAATA TTCTTCGACCAAAGCAAAAACGGGTACGCGGGGTACATAGACGGGGGGGCGAGCC AAGAAGAATTCTACAAATTCATAAAACCGATACTAGAAAAAATGGACGGGACGGA AGAACTACTAGTAAAACTAAACCGAGAAGACCTACTACGAAAACAACGAACGTTC GACAACGGGAGCATACCGCACCAAATACACCTAGGGGAACTACACGCGATACTACG ACGACAAGAAGACTTCTACCCGTTCCTAAAAGACAACCGAGAAAAAATAGAAAAA ATACTAACGTTCCGAATACCGTACTACGTAGGGCCGCTAGCGCGAGGGAACAGCCG ATTCGCGTGGATGACGCGAAAAAGCGAAGAAACGATAACGCCGTGGAACTTCGAA GAAGTAGTAGACAAAGGGGCGAGCGCGCAAAGCTTCATAGAACGAATGACGAACT TCGACAAAAACCTACCGAACGAAAAAGTACTACCGAAACACAGCCTACTATACGA ATACTTCACGGTATACAACGAACTAACGAAAGTAAAATACGTAACGGAAGGGATGC GAAAACCGGCGTTCCTAAGCGGGGAACAAAAAAAAGCGATAGTAGACCTACTATT CAAAACGAACCGAAAAGTAACGGTAAAACAACTAAAAGAAGACTACTTCAAAAA AATAGAATGCTTCGACAGCGTAGAAATAAGCGGGGTAGAAGACCGATTCAACGCG AGCCTAGGGACGTACCACGACCTACTAAAAATAATAAAAGACAAAGACTTCCTAGA CAACGAAGAAAACGAAGACATACTAGAAGACATAGTACTAACGCTAACGCTATTCG AAGACCGAGAAATGATAGAAGAACGACTAAAAACGTACGCGCACCTATTCGACGA CAAAGTAATGAAACAACTAAAACGACGACGATACACGGGGTGGGGGCGACTAAGC CGAAAACTAATAAACGGGATACGAGACAAACAAAGCGGGAAAACGATACTAGACT TCCTAAAAAGCGACGGGTTCGCGAACCGAAACTTCATGCAACTAATACACGACGA CAGCCTAACGTTCAAAGAAGACATACAAAAAGCGCAAGTAAGCGGGCAAGGGGA CAGCCTACACGAACACATAGCGAACCTAGCGGGGAGCCCGGCGATAAAAAAAGGG ATACTACAAACGGTAAAAGTAGTAGACGAACTAGTAAAAGTAATGGGGCGACACA AACCGGAAAACATAGTAATAGAAATGGCGCGAGAAAACCAAACGACGCAAAAAG GGCAAAAAAACAGCCGAGAACGAATGAAACGAATAGAAGAAGGGATAAAAGAAC TAGGGAGCCAAATACTAAAAGAACACCCGGTAGAAAACACGCAACTACAAAACGA AAAACTATACCTATACTACCTACAAAACGGGCGAGACATGTACGTAGACCAAGAAC TAGACATAAACCGACTAAGCGACTACGACGTAGACCACATAGTACCGCAAAGCTTC CTAAAAGACGACAGCATAGACAACAAAGTACTAACGCGAAGCGACAAAAACCGA GGGAAAAGCGACAACGTACCGAGCGAAGAAGTAGTAAAAAAAATGAAAAACTAC TGGCGACAACTACTAAACGCGAAACTAATAACGCAACGAAAATTCGACAACCTAA CGAAAGCGGAACGAGGGGGGCTAAGCGAACTAGACAAAGCGGGGTTCATAAAAC GACAACTAGTAGAAACGCGACAAATAACGAAACACGTAGCGCAAATACTAGACAG CCGAATGAACACGAAATACGACGAAAACGACAAACTAATACGAGAAGTAAAAGTA ATAACGCTAAAAAGCAAACTAGTAAGCGACTTCCGAAAAGACTTCCAATTCTACAA AGTACGAGAAATAAACAACTACCACCACGCGCACGACGCGTACCTAAACGCGGTA GTAGGGACGGCGCTAATAAAAAAATACCCGAAACTAGAAAGCGAATTCGTATACGG GGACTACAAAGTATACGACGTACGAAAAATGATAGCGAAAAGCGAACAAGAAATA GGGAAAGCGACGGCGAAATACTTCTTCTACAGCAACATAATGAACTTCTTCAAAAC GGAAATAACGCTAGCGAACGGGGAAATACGAAAACGACCGCTAATAGAAACGAAC GGGGAAACGGGGGAAATAGTATGGGACAAAGGGCGAGACTTCGCGACGGTACGA AAAGTACTAAGCATGCCGCAAGTAAACATAGTAAAAAAAACGGAAGTACAAACGG GGGGGTTCAGCAAAGAAAGCATACTACCGAAACGAAACAGCGACAAACTAATAGC GCGAAAAAAAGACTGGGACCCGAAAAAATACGGGGGGTTCGACAGCCCGACGGT AGCGTACAGCGTACTAGTAGTAGCGAAAGTAGAAAAAGGGAAAAGCAAAAAACTA AAAAGCGTAAAAGAACTACTAGGGATAACGATAATGGAACGAAGCAGCTTCGAAA AAAACCCGATAGACTTCCTAGAAGCGAAAGGGTACAAAGAAGTAAAAAAAGACCT AATAATAAAACTACCGAAATACAGCCTATTCGAACTAGAAAACGGGCGAAAACGAA TGCTAGCGAGCGCGGGGGAACTACAAAAAGGGAACGAACTAGCGCTACCGAGCA AATACGTAAACTTCCTATACCTAGCGAGCCACTACGAAAAACTAAAAGGGAGCCCG GAAGACAACGAACAAAAACAACTATTCGTAGAACAACACAAACACTACCTAGACG AAATAATAGAACAAATAAGCGAATTCAGCAAACGAGTAATACTAGCGGACGCGAAC CTAGACAAAGTACTAAGCGCGTACAACAAACACCGAGACAAACCGATACGAGAAC AAGCGGAAAACATAATACACCTATTCACGCTAACGAACCTAGGGGCGCCGGCGGCG TTCAAATACTTCGACACGACGATAGACCGAAAACGATACACGAGCACGAAAGAAG TACTAGACGCGACGCTAATACACCAAAGCATAACGGGGCTATACGAAACGCGAATA GACCTAAGCCAACTAGGGGGGGACGGGGGGGGGAGCCCGAAAAAAAAACGAAAA GTATGA	
具有HSD的5' UTR、对应于SEQ ID	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTTGCAGG CCTTATTCGGATCCGCCACCATGGACAAAAAATACAGCATAGGGCTAGACATAGGG ACGAACAGCGTAGGGTGGGCGGTAATAACGGACGAATACAAAGTACCGAGCAAAA AATTCAAAGTACTAGGGAACACGGACCGACACAGCATAAAAAAAAACCTAATAGG GGCGCTACTATTCGACAGCGGGGAAACGGCGGAAGCGACGCGACTAAAACGAACG GCGCGACGACGATACACGCGACGAAAAAACCGAATATGCTACCTACAAGAAATATT CAGCAACGAAATGGCGAAAGTAGACGACAGCTTCTTCCACCGACTAGAAGAAAGC	55

[0691]

NO: 54 的 ORF 、 Kozak序列及 ALB 的 3' UTR 的 Cas9 转录物	TTCCTAGTAGAAGAAGACAAAAAACACGAACGACACCCGATATTCGGGAACATAGT AGACGAAGTAGCGTACCACGAAAAATACCCGACGATATACCACCTACGAAAAAAA CTAGTAGACAGCACGGACAAAGCGGACCTACGACTAATATACCTAGCGCTAGCGCA CATGATAAAATTCCGAGGGCACTTCCTAATAGAAGGGGACCTAAACCCGGACAACA GCGACGTAGACAAACTATTCATACAACTAGTACAAACGTACAACCAACTATTCGAA GAAAACCCGATAAACGCGAGCGGGGTAGACGCGAAAGCGATACTAAGCGCGCGAC TAAGCAAAAGCCGACGACTAGAAAACCTAATAGCGCAACTACCGGGGGAAAAAAA AAACGGGCTATTCGGGAACCTAATAGCGCTAAGCCTAGGGCTAACGCCGAACTTCA AAAGCAACTTCGACCTAGCGGAAGACGCGAAACTACAACTAAGCAAAGACACGTA CGACGACGACCTAGACAACCTACTAGCGCAAATAGGGGACCAATACGCGGACCTAT TCCTAGCGGCGAAAAACCTAAGCGACGCGATACTACTAAGCGACATACTACGAGTA AACACGGAAATAACGAAAGCGCCGCTAAGCGCGAGCATGATAAAACGATACGACG AACACCACCAAGACCTAACGCTACTAAAAGCGCTAGTACGACAACAACTACCGGA AAAATACAAAGAAATATTCTTCGACCAAAGCAAAAACGGGTACGCGGGGTACATAG ACGGGGGGGCGAGCCAAGAAGAATTCTACAAATTCATAAAACCGATACTAGAAAA AATGGACGGGACGGAAGAACTACTAGTAAAACTAAACCGAGAAGACCTACTACGA AAACAACGAACGTTCGACAACGGGAGCATACCGCACCAAATACACCTAGGGGAAC TACACGCGATACTACGACGACAAGAAGACTTCTACCCGTTCCTAAAAGACAACCGA GAAAAAATAGAAAAAATACTAACGTTCCGAATACCGTACTACGTAGGGCCGCTAGC GCGAGGGAACAGCCGATTCGCGTGGATGACGCGAAAAAGCGAAGAAACGATAAC GCCGTGGAACTTCGAAGAAGTAGTAGACAAAGGGGCGAGCGCGCAAAGCTTCATA GAACGAATGACGAACTTCGACAAAAACCTACCGAACGAAAAAGTACTACCGAAAC ACAGCCTACTATACGAATACTTCACGGTATACAACGAACTAACGAAAGTAAAATACG TAACGGAAGGGATGCGAAAACCGGCGTTCCTAAGCGGGGAACAAAAAAAAGCGAT AGTAGACCTACTATTCAAAACGAACCGAAAAGTAACGGTAAAACAACTAAAAGAA GACTACTTCAAAAAAATAGAATGCTTCGACAGCGTAGAAATAAGCGGGGTAGAAG ACCGATTCAACGCGAGCCTAGGGACGTACCACGACCTACTAAAAATAATAAAAGAC AAAGACTTCCTAGACAACGAAGAAAACGAAGACATACTAGAAGACATAGTACTAA CGCTAACGCTATTCGAAGACCGAGAAATGATAGAAGAACGACTAAAAACGTACGC GCACCTATTCGACGACAAAGTAATGAAACAACTAAAACGACGACGATACACGGGG TGGGGGCGACTAAGCCGAAAACTAATAAACGGGATACGAGACAAACAAAGCGGG AAAACGATACTAGACTTCCTAAAAAGCGACGGGTTCGCGAACCGAAACTTCATGC AACTAATACACGACGACAGCCTAACGTTCAAAGAAGACATACAAAAAGCGCAAGT AAGCGGGCAAGGGGACAGCCTACACGAACACATAGCGAACCTAGCGGGGAGCCC GGCGATAAAAAAAGGGATACTACAAACGGTAAAAGTAGTAGACGAACTAGTAAAA GTAATGGGGCGACACAAACCGGAAAACATAGTAATAGAAATGGCGCGAGAAAACC AAACGACGCAAAAAGGGCAAAAAAACAGCCGAGAACGAATGAAACGAATAGAAG AAGGGATAAAAGAACTAGGGAGCCAAATACTAAAAGAACACCCGGTAGAAAACAC GCAACTACAAAACGAAAAACTATACCTATACTACCTACAAAACGGGCGAGACATGT ACGTAGACCAAGAACTAGACATAAACCGACTAAGCGACTACGACGTAGACCACATA GTACCGCAAAGCTTCCTAAAAGACGACAGCATAGACAACAAAGTACTAACGCGAA GCGACAAAAACCGAGGGAAAAGCGACAACGTACCGAGCGAAGAAGTAGTAAAAA AAATGAAAAACTACTGGCGACAACTACTAAACGCGAAACTAATAACGCAACGAAA ATTCGACAACCTAACGAAAGCGGAACGAGGGGGGCTAAGCGAACTAGACAAAGC GGGGTTCATAAAACGACAACTAGTAGAAACGCGACAAATAACGAAACACGTAGCG CAAATACTAGACAGCCGAATGAACACGAAATACGACGAAAACGACAAACTAATAC GAGAAGTAAAAGTAATAACGCTAAAAAGCAAACTAGTAAGCGACTTCCGAAAAGA CTTCCAATTCTACAAAGTACGAGAAATAAACAACTACCACCACGCGCACGACGCGT ACCTAAACGCGGTAGTAGGGACGGCGCTAATAAAAAAATACCCGAAACTAGAAAG CGAATTCGTATACGGGGACTACAAAGTATACGACGTACGAAAAATGATAGCGAAAA GCGAACAAGAAATAGGGAAAGCGACGGCGAAATACTTCTTCTACAGCAACATAAT GAACTTCTTCAAAACGGAAATAACGCTAGCGAACGGGGAAATACGAAAACGACCG CTAATAGAAACGAACGGGGAAACGGGGGAAATAGTATGGGACAAAGGGCGAGACT TCGCGACGGTACGAAAAGTACTAAGCATGCCGCAAGTAAACATAGTAAAAAAAAC GGAAGTACAAACGGGGGGGTTCAGCAAAGAAAGCATACTACCGAAACGAAACAG CGACAAACTAATAGCGCGAAAAAAAGACTGGGACCCGAAAAAAATACGGGGGGTTC GACAGCCCGACGGTAGCGTACAGCGTACTAGTAGTAGCGAAAGTAGAAAAAGGGA AAAGCAAAAAACTAAAAAGCGTAAAAGAACTACTAGGGATAACGATAATGGAACG AAGCAGCTTCGAAAAAAACCCGATAGACTTCCTAGAAGCGAAAGGGTACAAAGAA GTAAAAAAAGACCTAATAATAAAACTACCGAAATACAGCCTATTCGAACTAGAAAA CGGGCGAAAACGAATGCTAGCGAGCGCGGGGGAACTACAAAAAGGGAACGAACT AGCGCTACCGAGCAAATACGTAAACTTCCTATACCTAGCGAGCCACTACGAAAAAC TAAAAGGGAGCCCGGAAGACAACGAACAAAAACAACTATTCGTAGAACAACACA AACACTACCTAGACGAAATAATAGAACAAATAAGCGAATTCAGCAAACGAGTAATA CTAGCGGACGCGAACCTAGACAAAGTACTAAGCGCGTACAACAAACACCGAGACA AACCGATACGAGAACAAGCGGAAAACATAATACACCTATTCACGCTAACGAACCTA GGGGCGCCGGCGGCGTTCAAATACTTCGACACGACGATAGACCGAAAACGATACA	

[0692]

	CGAGCACGAAAGAAGTACTAGACGCGACGCTAATACACCAAAGCATAACGGGGCT ATACGAAACGCGAATAGACCTAAGCCAACTAGGGGGGGACGGGGGGGGGAGCCC GAAAAAAAAACGAAAAGTATGACTAGCCATCACATTTAAAAGCATCTCAGCCTACC ATGAGAATAAGAGAAAGAAAATGAAGATCAATAGCTTATTCATCTCTTTTTCTTTTT CGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAATCATTTTGC CTCTTTTCTCTGTGCTTCAATTAATAAAAAATGGAAAGAACCTCGAG	
具有AGG作为供与CleanCap™一起使用之前三个核苷酸、HSD的5' UTR、对应于SEQ ID NO: 4的ORF、Kozak序列及ALB的3' UTR的Cas9转录物	AGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTTGCAGG CCTTATTCGGATCCGCCACCATGGACAAGAAGTACAGCATCGGACTGGACATCGGA ACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGA AGTTCAAGGTCCTGGGAAACACAGACAGACACAGCATCAAGAAGAACCTGATCGG AGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAACAAGACTGAAGAGAAC AGCAAGAAGAAGATACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATC TTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAA GCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTCGGAAACAT CGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAG AAGCTGGTCGACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGG CACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGA CAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGT TCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGC AAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCCGGGAGA AAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGGACTGACACCG AACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCTGCAGCTGAGCAAG GACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACG CAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACAT CCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAAG AGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGC AGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGC AGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCG ATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAA GACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCC ACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCT GAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTAC GTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGC GAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGC GCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAA AGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTG ACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAG AACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGT CAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAA ATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGC TGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCT GGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAA AGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGA GAAGAAGATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCA GAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGC AAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGAC ATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACACATCGCA AACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCG TCGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGA AATGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAA GAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGG AACACCCGGTCGAAAACACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCT GCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTGAGC GACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCG ACAACAAGGTCCTGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAACGTCC CGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACG CAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAG GACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAA GACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTA CGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAA GCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAATCAAC AACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGA TCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTA CGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACAGC AAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGG CAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAG AAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCAT	56

		GCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAA GGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGA CTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTC CTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAG GAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCG ACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCT GCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAG CGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAA CTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAAC GAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCG AACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAA GGTCCTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGA AAACATCATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGT ACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGA CGCAACACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTG AGCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCTA GCTAGCCATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAA TGAAGATCAATAGCTTATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCC TGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAA TAAAAAATGGAAAGAACCTCGAG	
[0693]	具有来自CMV的5' UTR、对应于SEQ ID NO: 4的ORF、Kozak序列及ALB的3' UTR的Cas9转录物	GGGCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCG GGACCGATCCAGCCTCCGCGGCCGGGAACGGTGCATTGGAACGCGGATTCCCCGT GCCAAGAGTGACTCACCGTCCTTGACACGGCCACCATGGACAAGAAGTACAGCAT CGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATAC AAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGACACAGCATC AAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCA ACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAAGAACAGAATC TGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCTT CCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACA CCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAA TCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCTGAGACT GATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAG GAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCA GACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCA AAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATC GCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTG AGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAA AGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACA GATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCA ATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGA GCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAA GGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAG AGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCT ACAAGTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGT CAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAG CATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAA GACTTCTACCCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACAT TCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATG GATGACAAGAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGT CGACAAGGGAGCAAGCGCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAA GAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTC ACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGC CGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGAC AAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGA ATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTG GGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACG AAGAAAACGAAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGA CAGAGAAATGATCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAG GTCATGAAGCAGCTGAAGAGAAGAAGATACACAGGATGGGGAAGACTGAGCAGA AAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCC TGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGCTGATCCACGACGACAG CCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAG CCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATC CTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGGGAAGACACAAGC CGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACACAGAAGGGAC AGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGG GAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAGAACGAAA	57

[0694]

	AGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACT GGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTC CTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAGA GGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTAC TGGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGA CAAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGA GACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAG CAGAATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGT CATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTAC AAGGTCAGAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAG TCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTA CGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAA ATCGGAAAGGCAACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAA GACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAAC AAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGT CAGAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAG ACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTG ATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCG ACAGTCGCATACAGCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAG AAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCT TCGAAAAGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAA GGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAAGA AAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTG CCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGG GAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACT ACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGC AGACGCAAACCTGGACAAGGTCCTGAGCGCATACAACAAGCACAGAGACAAGCC GATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGA GCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAA GCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAGCATCACAGGACTGTA CGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGAA GAAGAAGAGAAAGGTCTAGCTAGCCATCACATTTAAAAGCATCTCAGCCTACCATG AGAATAAGAGAAAGAAAATGAAGATCAATAGCTTATTCATCTCTTTTTCTTTTTCGT TGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTC TTTTCTCTGTGCTTCAATTAATAAAAAATGGAAAGAACCTCGAG	
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[0695]

	ATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAA GCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGA CAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTC GAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACG ACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGGATGGGGAAGACTGA GCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGG ACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGCTGATCCACGA CGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGG AGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAA GGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGGGAAGA CACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACACAG AAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAG GAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAG AACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACC AGGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCA GAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAA GAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAA GAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGAC AACCTGACAAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTC ATCAAGAGACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCC TGGACAGCAGAATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAG TCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCA GTTCTACAAGGTCAGAGAAATCAACAACTACCACCACGCACACGACGCATACCTG AACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAAT TCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGA ACAGGAAATCGGAAAGGCAACAGCAAAGTACTTCTTCTACAGCAACATCATGAAC TTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGA TCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCG CAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTCAAGAAGACAGA AGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGA CAAGCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGA CAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGAAAG AGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGA AGCAGCTTCGAAAAGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAG TCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAAAA CGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACT GGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCCACTACGAAAAG CTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCAC AAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCA TCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACAACAAGCACAGAGA CAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAAC CTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGAT ACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAGCATCACAGG ACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAG CCCGAAGAAGAAGAGAAAGGTCTAGctagcgctcgctttcttgctgtccaatttctattaaaggttcctttgttccct aagtccaactactaaactgggggatattatgaagggccttgagcatctggattctgcctaataaaaaacatttattttcattgcctcgag	
具有来自XBG的5' UTR、对应于SEQ ID NO: 4的ORF、Kozak序列及XBG的3' UTR的Cas9转录物	GGGaagctcagaataaacgctcaactttggccggatctgccacCATGGACAAGAAGTACAGCATCGGACT GGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTC CCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGACACAGCATCAAGAAG AACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAACAAGA CTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAAGAACAGAATCTGCTAC CTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACA GACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGA TCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTAC CACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCTGAGACTGATCT ACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGA CCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACA TACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGG CAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACA GCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTG GGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCTGC AGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGG AGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTG CTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAA GCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACT GGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAG AACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGT	59

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	TCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCT GAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCC GCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTC TACCCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAA TCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGAC AAGAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAA GGGAGCAAGCGCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTG CCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTA CAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTC CTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGA AAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCG ACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATA CCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAAC GAAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAA TGATCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAA GCAGCTGAAGAGAAGAAGATACACAGGATGGGGAAGACTGAGCAGAAAGCTGAT CAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAG CGACGGATTCGCAAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACAT TCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACG AACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGAC AGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGAAAA CATCGTCATCGAAATGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAA CAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCA GATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAGAACGAAAAGCTGTAC CTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCA ACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGA CGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAGAGGAAAGAG CGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACA GCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCA GAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAGCTG GTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGA ACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACACT GAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGA GAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTA CAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAA GGCAACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAA TCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAG AAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGG TCCTGAGCATGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGG ATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGA AAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCAT ACAGCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGA GCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAA CCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATC ATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGC TGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGT ACGTCAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGA AGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGACGA AATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAAC CTGGACAAGGTCCTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAAC AGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGC ATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAA GTCCTGGACGCAACACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAA TCGACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGA AAGGTCTAGctagcaccagcctcaagaacacccgaatggagtctctaagctacataataccaacttacactttacaaaatgttgtc ccccaaaatgtagccattcgtatctgctcctaataaaaagaaagtttcttcacattctctcgag	
具有AGG作为供与CleanCap™一起使用之前三个核苷酸、来自XBG的	AGGaagctcagaataaacgctcaactttggccggatctgccacCATGGACAAGAAGTACAGCATCGGACT GGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTC CCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGACACAGCATCAAGAAG AACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAACAAGA CTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAAGAACAGAATCTGCTAC CTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACA GACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGA TCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTAC CACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCTGAGACTGATCT ACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGA	60

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5' UTR、对应于SEQ ID NO: 4的ORF、Kozak序列及XBG的3' UTR的Cas9转录物	CCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACA TACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGG CAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACA GCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTG GGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCTGC AGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGG AGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTG CTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAA GCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACT GGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAG AACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGT TCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCT GAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCC GCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTC TACCCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAA TCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGAC AAGAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAA GGGAGCAAGCGCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTG CCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTA CAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTC CTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGA AAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCG ACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATA CCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAAC GAAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAA TGATCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAA GCAGCTGAAGAGAAGAAGATACACAGGATGGGGAAGACTGAGCAGAAAGCTGAT CAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAG CGACGGATTCGCAAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACAT TCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACG AACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGAC AGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGAAAA CATCGTCATCGAAATGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAA CAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCA GATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAGAACGAAAAGCTGTAC CTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCA ACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGA CGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAGAGGAAAGAG CGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACA GCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCA GAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAGCTG GTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGA ACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACACT GAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGA GAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTA CAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAA GGCAACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAA TCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAG AAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGG TCCTGAGCATGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGG ATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGA AAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCAT ACAGCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGA GCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAA CCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATC ATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGC TGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGT ACGTCAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGA AGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGACGA AATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAAC CTGGACAAGGTCCTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAAC AGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGC ATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAA GTCCTGGACGCAACACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAA TCGACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGA AAGGTCTAGctagcaccagcctcaagaacacccgaatggagtctctaagctacataataccaacttacactttacaaaatgttgtc	

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具有AGG作为供与CleanCap™一起使用之前三个核苷酸、来自HSD的5' UTR、对应于SEQ ID NO: 4的ORF、Kozak序列及ALB的3' UTR的Cas9转录物	AGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTTGCAGG CCTTATTCGGATCCGCCACCATGGACAAGAAGTACAGCATCGGACTGGACATCGGA ACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGA AGTTCAAGGTCCTGGGAAACACAGACAGACACAGCATCAAGAAGAACCTGATCGG AGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAACAAGACTGAAGAGAAC AGCAAGAAGAAGATACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATC TTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAA GCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTCGGAAACAT CGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAG AAGCTGGTCGACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGG CACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGA CAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGT TCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGC AAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCCGGGAGA AAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGGACTGACACCG AACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCTGCAGCTGAGCAAG GACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACG CAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACAT CCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAAG AGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGC AGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGC AGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCG ATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAA GACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCC ACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCT GAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTAC GTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGC GAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGC GCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAA AGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTG ACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAG AACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGT CAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAA ATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGC TGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCT GGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAA AGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGA GAAGAAGATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCA GAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGC AAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGAC ATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACACATCGCA AACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCG TCGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGA AATGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAA GAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGG AACACCCGGTCGAAAACACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCT GCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTGAGC GACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCG ACAACAAGGTCCTGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAACGTCC CGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACG CAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAG GACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAA GACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTA CGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAA GCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAATCAAC AACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGA TCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTA CGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACAGC AAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGG CAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAG AAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCAT GCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAA GGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGA CTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTC CTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAG GAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCG	61

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	ACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCT GCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAG CGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAA CTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAAC GAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCG AACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAA GGTCCTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGA AAACATCATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGT ACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGA CGCAACACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTG AGCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCTA GCTAGCCATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAA TGAAGATCAATAGCTTATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCC TGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAA TAAAAAATGGAAAGAACCTCGAG	
30/30/39 聚-A序列	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGCGAAAAAAAAAAAAAAAAAAA AAAAAAAAAAACCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA A	62
聚-A 100 序列	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	63
G209 引导 RNA	mC*mC*mA*GUCCAGCGAGGCAAAGGGUUUUAGAGCUAGAAAUAGCAAGUUAAA AUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU *mU*U	64
编码脑膜炎双球菌Cas9的ORF	ATGGCAGCATTCAAGCCGAACTCGATCAACTACATCCTGGGACTGGACATCGGAAT CGCATCGGTCGGATGGGCAATGGTCGAAATCGACGAAGAAGAAAACCCGATCAGA CTGATCGACCTGGGAGTCAGAGTCTTCGAAAGAGCAGAAGTCCCGAAGACAGGAG ACTCGCTGGCAATGGCAAGAAGACTGGCAAGATCGGTCAGAAGACTGACAAGAA GAAGAGCACACAGACTGCTGAGAACAAGAAGACTGCTGAAGAGAGAAGGAGTCC TGCAGGCAGCAAACTTCGACGAAAACGGACTGATCAAGTCGCTGCCGAACACACC GTGGCAGCTGAGAGCAGCAGCACTGGACAGAAAGCTGACACCGCTGGAATGGTC GGCAGTCCTGCTGCACCTGATCAAGCACAGAGGATACCTGTCGCAGAGAAAGAAC GAAGGAGAAACAGCAGACAAGGAACTGGGAGCACTGCTGAAGGGAGTCGCAGGA AACGCACACGCACTGCAGACAGGAGACTTCAGAACACCGGCAGAACTGGCACTG AACAAGTTCGAAAAGGAATCGGGACACATCAGAAACCAGAGATCGGACTACTCGC ACACATTCTCGAGAAAGGACCTGCAGGCAGAACTGATCCTGCTGTTCGAAAAGCA GAAGGAATTCGGAAACCCGCACGTCTCGGGAGGACTGAAGGAAGGAATCGAAAC ACTGCTGATGACACAGAGACCGGCACTGTCGGGAGACGCAGTCCAGAAGATGCTG GGACACTGCACATTCGAACCGGCAGAACCGAAGGCAGCAAAGAACACATACACA GCAGAAAGATTCATCTGGCTGACAAAGCTGAACAACCTGAGAATCCTGGAACAGG GATCGGAAAGACCGCTGACAGACACAGAAAGAGCAACACTGATGGACGAACCGT ACAGAAAGTCGAAGCTGACATACGCACAGGCAAGAAAGCTGCTGGGACTGGAAG ACACAGCATTCTTCAAGGGACTGAGATACGGAAAGGACAACGCAGAAGCATCGAC ACTGATGGAAATGAAGGCATACCACGCAATCTCGAGAGCACTGGAAAAGGAAGGA CTGAAGGACAAGAAGTCGCCGCTGAACCTGTCGCCGGAACTGCAGGACGAAATCG GAACAGCATTCTCGCTGTTCAAGACAGACGAAGACATCACAGGAAGACTGAAGGA CAGAATCCAGCCGGAAATCCTGGAAGCACTGCTGAAGCACATCTCGTTCGACAAG TTCGTCCAGATCTCGCTGAAGGCACTGAGAAGAATCGTCCCGCTGATGGAACAGG GAAAGAGATACGACGAAGCATGCGCAGAAATCTACGGAGACCACTACGGAAAGAA GAACACAGAAGAAAAGATCTACCTGCCGCCGATCCCGGCAGACGAAATCAGAAAC CCGGTCGTCCTGAGAGCACTGTCGCAGGCAAGAAAGGTCATCAACGGAGTCGTCA GAAGATACGGATCGCCGGCAAGAATCCACATCGAAACAGCAAGAGAAGTCGGAAA GTCGTTCAAGGACAGAAAGGAAATCGAAAAGAGACAGGAAGAAAACAGAAAGG ACAGAGAAAAGGCAGCAGCAAAGTTCAGAGAATACTTCCCGAACTTCGTCGGAGA ACCGAAGTCGAAGGACATCCTGAAGCTGAGACTGTACGAACAGCAGCACGGAAA GTGCCTGTACTCGGGAAAGGAAATCAACCTGGGAAGACTGAACGAAAAGGGATAC GTCGAAATCGACCACGCACTGCCGTTCTCGAGAACATGGGACGACTCGTTCAACA ACAAGGTCCTGGTCCTGGGATCGGAAAACCAGAACAAGGGAAACCAGACACCGT ACGAATACTTCAACGGAAAGGACAACTCGAGAGAATGGCAGGAATTCAAGGCAAG AGTCGAAACATCGAGATTCCCGAGATCGAAGAAGCAGAGAATCCTGCTGCAGAAG TTCGACGAAGACGGATTCAAGGAAAGAAACCTGAACGACACAAGATACGTCAACA GATTCCTGTGCCAGTTCGTCGCAGACAGAATGAGACTGACAGGAAAGGGAAAGAA GAGAGTCTTCGCATCGAACGGACAGATCACAAACCTGCTGAGAGGATTCTGGGGA CTGAGAAAGGTCAGAGCAGAAAACGACAGACACCACGCACTGGACGCAGTCGTC GTCGCATGCTCGACAGTCGCAATGCAGCAGAAGATCACAAGATTCGTCAGATACAA GGAAATGAACGCATTCGACGGAAAGACAATCGACAAGGAAACAGGAGAAGTCCT GCACCAGAAGACACACTTCCCGCAGCCGTGGGAATTCTTCGCACAGGAAGTCATG	65

[0700]

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编码脑膜炎双球菌Cas9 的ORF (无起始或终止密码子;适于包括在融合蛋白质编码序列中)	GCAGCATTCAAGCCGAACTCGATCAACTACATCCTGGGACTGGACATCGGAATCGC ATCGGTCGGATGGGCAATGGTCGAAATCGACGAAGAAGAAAACCCGATCAGACTG ATCGACCTGGGAGTCAGAGTCTTCGAAAGAGCAGAAGTCCCGAAGACAGGAGACT CGCTGGCAATGGCAAGAAGACTGGCAAGATCGGTCAGAAGACTGACAAGAAGAA GAGCACACAGACTGCTGAGAACAAGAAGACTGCTGAAGAGAGAAGGAGTCCTGC AGGCAGCAAACTTCGACGAAAACGGACTGATCAAGTCGCTGCCGAACACACCGTG GCAGCTGAGAGCAGCAGCACTGGACAGAAAGCTGACACCGCTGGAATGGTCGGC AGTCCTGCTGCACCTGATCAAGCACAGAGGATACCTGTCGCAGAGAAAGAACGAA GGAGAAACAGCAGACAAGGAACTGGGAGCACTGCTGAAGGGAGTCGCAGGAAAC GCACACGCACTGCAGACAGGAGACTTCAGAACACCGGCAGAACTGGCACTGAAC AAGTTCGAAAAGGAATCGGGACACATCAGAAACCAGAGATCGGACTACTCGCACA CATTCTCGAGAAAGGACCTGCAGGCAGAACTGATCCTGCTGTTCGAAAAGCAGAA GGAATTCGGAAACCCGCACGTCTCGGGAGGACTGAAGGAAGGAATCGAAACACTG CTGATGACACAGAGACCGGCACTGTCGGGAGACGCAGTCCAGAAGATGCTGGGAC ACTGCACATTCGAACCGGCAGAACCGAAGGCAGCAAAGAACACATACACAGCAG AAAGATTCATCTGGCTGACAAAGCTGAACAACCTGAGAATCCTGGAACAGGGATC GGAAAGACCGCTGACAGACACAGAAAGAGCAACACTGATGGACGAACCGTACAG AAAGTCGAAGCTGACATACGCACAGGCAAGAAAGCTGCTGGGACTGGAAGACAC AGCATTCTTCAAGGGACTGAGATACGGAAAGGACAACGCAGAAGCATCGACACTG ATGGAAATGAAGGCATACCACGCAATCTCGAGAGCACTGGAAAAGGAAGGACTGA AGGACAAGAAGTCGCCGCTGAACCTGTCGCCGGAACTGCAGGACGAAATCGGAA CAGCATTCTCGCTGTTCAAGACAGACGAAGACATCACAGGAAGACTGAAGGACAG AATCCAGCCGGAAATCCTGGAAGCACTGCTGAAGCACATCTCGTTCGACAAGTTCG TCCAGATCTCGCTGAAGGCACTGAGAAGAATCGTCCCGCTGATGGAACAGGGAAA GAGATACGACGAAGCATGCGCAGAAATCTACGGAGACCACTACGGAAAGAAGAAC ACAGAAGAAAAGATCTACCTGCCGCCGATCCCGGCAGACGAAATCAGAAACCCGG TCGTCCTGAGAGCACTGTCGCAGGCAAGAAAGGTCATCAACGGAGTCGTCAGAAG ATACGGATCGCCGGCAAGAATCCACATCGAAACAGCAAGAGAAGTCGGAAAGTCG TTCAAGGACAGAAAGGAAATCGAAAAGAGACAGGAAGAAAACAGAAAGGACAG AGAAAAGGCAGCAGCAAAGTTCAGAGAATACTTCCCGAACTTCGTCGGAGAACCG AAGTCGAAGGACATCCTGAAGCTGAGACTGTACGAACAGCAGCACGGAAAGTGCC TGTACTCGGGAAAGGAAATCAACCTGGGAAGACTGAACGAAAAGGGATACGTCGA AATCGACCACGCACTGCCGTTCTCGAGAACATGGGACGACTCGTTCAACAACAAG GTCCTGGTCCTGGGATCGGAAAACCAGAACAAGGGAAACCAGACACCGTACGAAT ACTTCAACGGAAAGGACAACTCGAGAGAATGGCAGGAATTCAAGGCAAGAGTCG AAACATCGAGATTCCCGAGATCGAAGAAGCAGAGAATCCTGCTGCAGAAGTTCGA CGAAGACGGATTCAAGGAAAGAAACCTGAACGACACAAGATACGTCAACAGATTC CTGTGCCAGTTCGTCGCAGACAGAATGAGACTGACAGGAAAGGGAAAGAAGAGA GTCTTCGCATCGAACGGACAGATCACAAACCTGCTGAGAGGATTCTGGGGACTGA GAAAGGTCAGAGCAGAAAACGACAGACACCACGCACTGGACGCAGTCGTCGTCG CATGCTCGACAGTCGCAATGCAGCAGAAGATCACAAGATTCGTCAGATACAAGGA AATGAACGCATTCGACGGAAAGACAATCGACAAGGAAACAGGAGAAGTCCTGCAC CAGAAGACACACTTCCCGCAGCCGTGGGAATTCTTCGCACAGGAAGTCATGATCA GAGTCTTCGGAAAGCCGGACGGAAAGCCGGAATTCGAAGAAGCAGACACACTGG AAAAGCTGAGAACACTGCTGGCAGAAAAGCTGTCGTCGAGACCGGAAGCAGTCC ACGAATACGTCACACCGCTGTTCGTCTCGAGAGCACCGAACAGAAAGATGTCGGG ACAGGGACACATGGAAACAGTCAAGTCGGCAAAGAGACTGGACGAAGGAGTCTC GGTCCTGAGAGTCCCGCTGACACAGCTGAAGCTGAAGGACCTGGAAAAGATGGTC AACAGAGAAAGAGAACCGAAGCTGTACGAAGCACTGAAGGCAAGACTGGAAGCA	66

[0701]

	CACAAGGACGACCCGGCAAAGGCATTCGCAGAACCGTTCTACAAGTACGACAAGG CAGGAAACAGAACACAGCAGGTCAAGGCAGTCAGAGTCGAACAGGTCCAGAAGA CAGGAGTCTGGGTCAGAAACCACAACGGAATCGCAGACAACGCAACAATGGTCAG AGTAGACGTCTTCGAAAAGGGAGACAAGTACTACCTGGTCCCGATCTACTCGTGGC AGGTCGCAAAGGGAATCCTGCCGGACAGAGCAGTCGTCCAGGGAAAGGACGAAG AAGACTGGCAGCTGATCGACGACTCGTTCAACTTCAAGTTCTCGCTGCACCCGAAC GACCTGGTCGAAGTCATCACAAAGAAGGCAAGAATGTTCGGATACTTCGCATCGTG CCACAGAGGAACAGGAAACATCAACATCAGAATCCACGACCTGGACCACAAGATC GGAAAGAACGGAATCCTGGAAGGAATCGGAGTCAAGACAGCACTGTCGTTCCAGA AGTACCAGATCGACGAACTGGGAAAGGAAATCAGACCGTGCAGACTGAAGAAGA GACCGCCGGTCAGATCCGGAAAGAGAACAGCAGACGGATCGGAATTCGAATCGCC GAAGAAGAAGAGAAAGGTCGAA	
Tran 包含 SEQ ID NO: 65 (编码脑膜炎双球菌 Cas9)的转录物	GGGAGACCCAAGCTGGCTAGCGTTTAAACTTAAGCTTGGATCCGCCACCATGGCAG CATTCAAGCCGAACTCGATCAACTACATCCTGGGACTGGACATCGGAATCGCATCG GTCGGATGGGCAATGGTCGAAATCGACGAAGAAGAAAACCCGATCAGACTGATCG ACCTGGGAGTCAGAGTCTTCGAAAGAGCAGAAGTCCCGAAGACAGGAGACTCGC TGGCAATGGCAAGAAGACTGGCAAGATCGGTCAGAAGACTGACAAGAAGAAGAG CACACAGACTGCTGAGAACAAGAAGACTGCTGAAGAGAGAAGGAGTCCTGCAGG CAGCAAACTTCGACGAAAACGGACTGATCAAGTCGCTGCCGAACACACCGTGGCA GCTGAGAGCAGCAGCACTGGACAGAAAGCTGACACCGCTGGAATGGTCGGCAGTC CTGCTGCACCTGATCAAGCACAGAGGATACCTGTCGCAGAGAAAGAACGAAGGAG AAACAGCAGACAAGGAACTGGGAGCACTGCTGAAGGGAGTCGCAGGAAACGCAC ACGCACTGCAGACAGGAGACTTCAGAACACCGGCAGAACTGGCACTGAACAAGT TCGAAAAGGAATCGGGACACATCAGAAACCAGAGATCGGACTACTCGCACACATT CTCGAGAAAGGACCTGCAGGCAGAACTGATCCTGCTGTTCGAAAAGCAGAAGGAA TTCGGAAACCCGCACGTCTCGGGAGGACTGAAGGAAGGAATCGAAACACTGCTGA TGACACAGAGACCGGCACTGTCGGGAGACGCAGTCCAGAAGATGCTGGGACACTG CACATTCGAACCGGCAGAACCGAAGGCAGCAAAGAACACATACACAGCAGAAAG ATTCATCTGGCTGACAAAGCTGAACAACCTGAGAATCCTGGAACAGGGATCGGAA AGACCGCTGACAGACACAGAAAGAGCAACACTGATGGACGAACCGTACAGAAAG TCGAAGCTGACATACGCACAGGCAAGAAAGCTGCTGGGACTGGAAGACACAGCAT TCTTCAAGGGACTGAGATACGGAAAGGACAACGCAGAAGCATCGACACTGATGGA AATGAAGGCATACCACGCAATCTCGAGAGCACTGGAAAAGGAAGGACTGAAGGAC AAGAAGTCGCCGCTGAACCTGTCGCCGGAACTGCAGGACGAAATCGGAACAGCAT TCTCGCTGTTCAAGACAGACGAAGACATCACAGGAAGACTGAAGGACAGAATCCA GCCGGAAATCCTGGAAGCACTGCTGAAGCACATCTCGTTCGACAAGTTCGTCCAGA TCTCGCTGAAGGCACTGAGAAGAATCGTCCCGCTGATGGAACAGGGAAAGAGATA CGACGAAGCATGCGCAGAAATCTACGGAGACCACTACGGAAAGAAGAACACAGA AGAAAAGATCTACCTGCCGCCGATCCCGGCAGACGAAATCAGAAACCCGGTCGTC CTGAGAGCACTGTCGCAGGCAAGAAAGGTCATCAACGGAGTCGTCAGAAGATACG GATCGCCGGCAAGAATCCACATCGAAACAGCAAGAGAAGTCGGAAAGTCGTTCAA GGACAGAAAGGAAATCGAAAAGAGACAGGAAGAAAACAGAAAGGACAGAGAAA AGGCAGCAGCAAAGTTCAGAGAATACTTCCCGAACTTCGTCGGAGAACCGAAGTC GAAGGACATCCTGAAGCTGAGACTGTACGAACAGCAGCACGGAAAGTGCCTGTAC TCGGGAAAGGAAATCAACCTGGGAAGACTGAACGAAAAGGGATACGTCGAAATCG ACCACGCACTGCCGTTCTCGAGAACATGGGACGACTCGTTCAACAACAAGGTCCT GGTCCTGGGATCGGAAAACCAGAACAAGGGAAACCAGACACCGTACGAATACTTC AACGGAAAGGACAACTCGAGAGAATGGCAGGAATTCAAGGCAAGAGTCGAAACA TCGAGATTCCCGAGATCGAAGAAGCAGAGAATCCTGCTGCAGAAGTTCGACGAAG ACGGATTCAAGGAAAGAAACCTGAACGACACAAGATACGTCAACAGATTCCTGTG CCAGTTCGTCGCAGACAGAATGAGACTGACAGGAAAGGGAAAGAAGAGAGTCTT CGCATCGAACGGACAGATCACAAACCTGCTGAGAGGATTCTGGGGACTGAGAAAG GTCAGAGCAGAAAACGACAGACACCACGCACTGGACGCAGTCGTCGTCGCATGCT CGACAGTCGCAATGCAGCAGAAGATCACAAGATTCGTCAGATACAAGGAAATGAA CGCATTCGACGGAAAGACAATCGACAAGGAAACAGGAGAAGTCCTGCACCAGAA GACACACTTCCCGCAGCCGTGGGAATTCTTCGCACAGGAAGTCATGATCAGAGTCT TCGGAAAGCCGGACGGAAAGCCGGAATTCGAAGAAGCAGACACACTGGAAAAGC TGAGAACACTGCTGGCAGAAAAGCTGTCGTCGAGACCGGAAGCAGTCCACGAATA CGTCACACCGCTGTTCGTCTCGAGAGCACCGAACAGAAAGATGTCGGGACAGGGA CACATGGAAACAGTCAAGTCGGCAAAGAGACTGGACGAAGGAGTCTCGGTCCTGA GAGTCCCGCTGACACAGCTGAAGCTGAAGGACCTGGAAAAGATGGTCAACAGAG AAAGAGAACCGAAGCTGTACGAAGCACTGAAGGCAAGACTGGAAGCACACAAGG ACGACCCGGCAAAGGCATTCGCAGAACCGTTCTACAAGTACGACAAGGCAGGAAA CAGAACACAGCAGGTCAAGGCAGTCAGAGTCGAACAGGTCCAGAAGACAGGAGT CTGGGTCAGAAACCACAACGGAATCGCAGACAACGCAACAATGGTCAGAGTAGAC GTCTTCGAAAAGGGAGACAAGTACTACCTGGTCCCGATCTACTCGTGGCAGGTCGC AAAGGGAATCCTGCCGGACAGAGCAGTCGTCCAGGGAAAGGACGAAGAAGACTG	67

[0702]

	GCAGCTGATCGACGACTCGTTCAACTTCAAGTTCTCGCTGCACCCGAACGACCTGGTCGAAGTCATCACAAAGAAGGCAAGAATGTTCGGATACTTCGCATCGTGCCACAGAGGAACAGGAAACATCAACATCAGAATCCACGACCTGGACCACAAGATCGGAAAGAACGGAATCCTGGAAGGAATCGGAGTCAAGACAGCACTGTCGTTCCAGAAGTACCAGATCGACGAACTGGGAAAGGAAATCAGACCGTGCAGACTGAAGAAGAGACCGCCGGTCAGATCCGGAAAGAGAACAGCAGACGGATCGGAATTCGAATCGCCGAAGAAGAAGAGAAAGGTCGAATGATAGCTAGCTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGG	
脑膜炎双球菌Cas9的氨基酸序列	MAAFKPNSINYILGLDIGIASVGWAMVEIDEEENPIRLIDLGVRVFERAEVPKTGDSLAMARRLARSVRRLTRRRAHRLLRTRRLLKREGVLQAANFDENGLIKSLPNTPWQLRAAALDRKLTPLEWSAVLLHLIKHRGYLSQRKNEGETADKELGALLKGVAGNAHALQTGDFRTPAELALNKFEKESGHIRNQRSDYSHTFSRKDLQAELILLFEKQKEFGNPHVSGGLKEGIETLLMTQRPALSGDAVQKMLGHCTFEPAEPKAAKNTYTAERFIWLTKLNNLRILEQGSERPLTDTERATLMDEPYRKSKLTYAQARKLLGLEDTAFFKGLRYGKDNAEASTLMEMKAYHAISRALEKEGLKDKKSPLNLSPELQDEIGTAFSLFKTDEDITGRLKDRIQPEILEALLKHISFDKFVQISLKALRRIVPLMEQGKRYDEACAEIYGDHYGKKNTEEKIYLPPIPADEIRNPVVLRALSQARKVINGVVRRYGSPARIHIETAREVGKSFKDRKEIEKRQEENRKDREKAAAKFREYFPNFVGEPKSKDILKLRLYEQQHGKCLYSGKEINLGRLNEKGYVEIDHALPFSRTWDDSFNNKVLVLGSENQNKGNQTPYEYFNGKDNSREWQEFKARVETSRFPRSKKQRILLQKFDEDGFKERNLNDTRYVNRFLCQFVADRMRLTGKGKKRVFASNGQITNLLRGFWGLRKVRAENDRHHALDAVVVACSTVAMQQKITRFVRYKEMNAFDGKTIDKETGEVLHQKTHFPQPWEFFAQEVMIRVFGKPDGKPEFEEADTLEKLRTLLAEKLSSRPEAVHEYVTPLFVSRAPNRKMSGQGHMETVKSAKRLDEGVSVLRVPLTQLKLKDLEKMVNREREPKLYEALKARLEAHKDDPAKAFAEPFYKYDKAGNRTQQVKAVRVEQVQKTGVWVRNHNGIADNATMVRVDVFEKGDKYYLVPIYSWQVAKGILPDRAVVQGKDEEDWQLIDDSFNFKFSLHPNDLVEVITKKARMFGYFASCHRGTGNINIRIHDLDHKIGKNGILEGIGVKTALSFQKYQIDELGKEIRPCRLKKRPPVRSGKRTADGSEFESPKKKRKVE	68
G390引导RNA	mG*mC*mC*GAGUCUGGAGAGCUGCAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	69
G502引导RNA	mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	70
G509引导RNA	mA*mA*mA*GUUCUAGAUGCCGUCCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	71
G534引导RNA	mA*mC*mG*CAAAUAUCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	72
eGFP的DNA编码序列	TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTCTAATACGACTCACTATAGGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTTGCAGGCCTTATTCGGATCCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAATAGGAATTATGCAG	73

[0703]

	TCTAGCCATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAA TGAAGATCAATAGCTTATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCC TGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAA TAAAAAATGGAAAGAACCTCGAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAATCTAGACTTAAGCTTGATGAGCTCTAGCTTGGCGTAAT CATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACAT ACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTC ACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCA GCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCT CTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCG GTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGC AGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGC CGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATC GACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTT TCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATA CCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAG GTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCC CCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGG TAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGC GAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACA CTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAA AGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTT TGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGA TCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTG GTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGT TTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAA TCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACT CCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTG CAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAG CCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCA GTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCG CAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGC TTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTG CAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCG CAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATC CGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTG TATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCAC ATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTC TCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAA CTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAA GGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCAT ACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGAT ACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCC GAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAA ATAGGCGTATCACGAGGCCCTTTCGTCG	
经修饰的sgRNA模式，其中N为编码引导序列的核苷酸	mN*mN*mN*NNNNNNNNNNNNNNNNNGUUUUAG AmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAA AUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmC mGmGmUmGmCmU*mU*mU*mU	74
CMV-1 5' UTR	CAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCAT	75
CMV-2 5' UTR	AGAAGACACCGGGACCGATCCAGCCTCCGCGGCCGGGAACGG	76
CMV-3 5' UTR	TGCATTGGAACGCGGATTCCCCGTGCCAAGAGTGACTCACCG	77
SV40 NLS	PKKKRKV	78
示例性NLS 1	LAAKRSRTT	79
示例性NLS 2	QAAKRSRTT	80

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示例性NLS 3	PAPAKRERTT	81
示例性NLS 4	QAAKRPRTT	82
示例性NLS 5	RAAKRPRTT	83
示例性NLS 6	AAAKRSWSMAA	84
示例性NLS 7	AAAKRVWSMAF	85
示例性NLS 8	AAAKRSWSMAF	86
示例性NLS 9	AAAKRKYFAA	87
示例性NLS 10	RAAKRKAFAA	88
示例性NLS 11	RAAKRKYFAV	89
替代SV40 NLS	PKKKRRV	90
核质蛋白NLS	KRPAATKKAGQAKKKK	91
SV40 NLS的示例性编码序列	CCGAAGAAGAAGAGAAAGGTC	92
NLS1的示例性编码序列	CTGGCAGCAAAGAGAAGCAGAACAACA	93
NLS2的示例性编码序列	CAGGCAGCAAAGAGAAGCAGAACAACA	94
NLS3的示例性编码序列	CCGGCACCGGCAAAGAGAGAAAGAACAACA	95
NLS4的示例性编码序列	CAGGCAGCAAAGAGACCGAGAACAACA	96
NLS5的示例性编码序列	AGAGCAGCAAAGAGACCGAGAACAACA	97
NLS6的示例性编码序列	GCAGCAGCAAAGAGAAGCTGGAGCATGGCAGCA	98
NLS7的示例性编码序列	GCAGCAGCAAAGAGAGTCTGGAGCATGGCATTC	99
NLS8的示例性编码序列	GCAGCAGCAAAGAGAAGCTGGAGCATGGCATTC	100
NLS9的示例性编码序列	GCAGCAGCAAAGAGAAAGTACTTCGCAGCA	101
NLS10的示例性编码序列	AGAGCAGCAAAGAGAAAGGCATTCGCAGCA	102
NLS11的示例性编码序列	AGAGCAGCAAAGAGAAAGTACTTCGCAGTC	103
替代SV40 NLS的示例	CCGAAGAAGAAGAGAAGAGTC	104

[0705]

性编码序列		
示例性Kozak序列	gccgccRccAUGG	105
	未使用	106
使用表4的长半衰期密码子，具有起始及终止密码子的Cas9 ORF	ATGGACAAGAAGTACTCTATCGGTTTGGACATCGGTACCAACTCTGTCGGTTGGGCCG TCATCACCGACGAATACAAGGTCCCATCTAAGAAGTTCAAGGTCTTGGGTAACACCG ACAGACACTCTATCAAGAAGAACTTGATCGGTGCCTTGTTGTTCGACTCTGGTGAAAC CGCCGAAGCCACCAGATTGAAGAGAACCGCCAGAAGAAGATACACCAGAAGAAAGA ACAGAATCTGCTACTTGCAAGAAATCTTCTCTAACGAAATGGCCAAGGTCGACGACT CTTTCTTCCACAGATTGGAAGAATCTTTCTTGGTCGAAGAAGACAAGAAGCACGAAA GACACCCAATCTTCGGTAACATCGTCGACGAAGTCGCCTACCACGAAAAGTACCCAA CCATCTACCACTTGAGAAAGAAGTTGGTCGACTCTACCGACAAGGCCGACTTGAGAT TGATCTACTTGGCCTTGGCCCACATGATCAAGTTCAGAGGTCACTTCTTGATCGAAGG TGACTTGAACCCAGACAACTCTGACGTCGACAAGTTGTTCATCCAATTGGTCCAAAC CTACAACCAATTGTTCGAAGAAAACCCAATCAACGCCTCTGGTGTCGACGCCAAGGC CATCTTGTCTGCCAGATTGTCTAAGAGCAGAAGATTGGAAAACTTGATCGCCCAATTG CCAGGTGAAAAGAAGAACGGTTTGTTCGGTAACTTGATCGCCTTGTCTTTGGGTTTGA CCCCAAACTTCAAGTCTAACTTCGACTTGGCCGAAGACGCCAAGTTGCAATTGTCTA AGGACACCTACGACGACGACTTGGACAACTTGTTGGCCCAAATCGGTGACCAATACG CCGACTTGTTCTTGGCCGCCAAGAACTTGTCTGACGCCATCTTGTTGTCTGACATCTT GAGAGTCAACACCGAAATCACCAAGGCCCCATTGTCTGCCTCTATGATCAAGAGATAC GACGAACACCACCAAGACTTGACCTTGTTGAAGGCCTTGGTCAGACAACAATTGCCA GAAAAGTACAAGGAAATCTTCTTCGACCAATCTAAGAACGGTTACGCCGGTTACATCG ACGGTGGTGCCTCTCAAGAAGAATTCTACAAGTTCATCAAGCCAATCTTGGAAAAGA TGGACGGTACCGAAGAATTGTTGGTCAAGTTGAACAGAGAAGACTTGTTGAGAAAG CAAAGAACCTTCGACAACGGTTCTATCCCACACCAAATCCACTTGGGTGAATTGCAC GCCATCTTGAGAAGACAAGAAGACTTCTACCCATTCTTGAAGGACAACAGAGAAAAG ATCGAAAAGATCTTGACCTTCAGAATCCCATACTACGTCGGTCCATTGGCCAGAGGTA ACAGCAGATTCGCCTGGATGACCAGAAAGTCTGAAGAAACCATCACCCCATGGAACT TCGAAGAAGTCGTCGACAAGGGTGCCTCTGCCCAATCTTTCATCGAAAGAATGACCA ACTTCGACAAGAACTTGCCAAACGAAAAGGTCTTGCCAAAGCACTCTTTGTTGTACG AATACTTCACCGTCTACAACGAATTGACCAAGGTCAAGTACGTCACCGAAGGTATGA GAAAGCCAGCCTTCTTGTCTGGTGAACAAAAGAAGGCCATCGTCGACTTGTTGTTCA AGACCAACAGAAAGGTCACCGTCAAGCAATTGAAGGAAGACTACTTCAAGAAGATC GAATGCTTCGACTCTGTCGAAATCTCTGGTGTCGAAGACAGATTCAACGCCTCTTTGG GTACCTACCACGACTTGTTGAAGATCATCAAGGACAAGGACTTCTTGGACAACGAAG AAAACGAAGACATCTTGGAAGACATCGTCTTGACCTTGACCTTGTTCGAAGACAGAG AAATGATCGAAGAAAGATTGAAGACCTACGCCCACTTGTTCGACGACAAGGTCATGA AGCAATTGAAGAGAAGAAGATACACCGGTTGGGGTAGATTGAGCAGAAAGTTGATCA ACGGTATCAGAGACAAGCAATCTGGTAAGACCATCTTGGACTTCTTGAAGTCTGACG GTTTCGCCAACAGAAACTTCATGCAATTGATCCACGACGACTCTTTGACCTTCAAGGA AGACATCCAAAAGGCCCAAGTCTCTGGTCAAGGTGACTCTTTGCACGAACACATCGC CAACTTGGCCGGTTCTCCAGCCATCAAGAAGGGTATCTTGCAAACCGTCAAGGTCGT CGACGAATTGGTCAAGGTCATGGGTAGACACAAGCCAGAAAACATCGTCATCGAAAT GGCCAGAGAAAACCAAACCACCCAAAAGGGTCAAAAGAACAGCAGAGAAAGAATG AAGAGAATCGAAGAAGGTATCAAGGAATTGGGTTCTCAAATCTTGAAGGAACACCCA GTCGAAAACACCCAATTGCAAAACGAAAAGTTGTACTTGTACTACTTGCAAAACGGT AGAGACATGTACGTCGACCAAGAATTGGACATCAACAGATTGTCTGACTACGACGTC GACCACATCGTCCCACAATCTTTCTTGAAGGACGACTCTATCGACAACAAGGTCTTGA CCAGATCTGACAAGAACAGAGGTAAGTCTGACAACGTCCCATCTGAAGAAGTCGTCA AGAAGATGAAGAACTACTGGAGACAATTGTTGAACGCCAAGTTGATCACCCAAAGAA AGTTCGACAACTTGACCAAGGCCGAAAGAGGTGGTTTGTCTGAATTGGACAAGGCC GGTTTCATCAAGAGACAATTGGTCGAAACCAGACAAATCACCAAGCACGTCGCCCAA ATCTTGGACAGCAGAATGAACACCAAGTACGACGAAAACGACAAGTTGATCAGAGA AGTCAAGGTCATCACCTTGAAGTCTAAGTTGGTCTCTGACTTCAGAAAGGACTTCCAA TTCTACAAGGTCAGAGAAATCAACAACTACCACCACGCCCACGACGCCTACTTGAAC GCCGTCGTCGGTACCGCCTTGATCAAGAAGTACCCAAAGTTGGAATCTGAATTCGTCT ACGGTGACTACAAGGTCTACGACGTCAGAAAGATGATCGCCAAGTCTGAACAAGAAA TCGGTAAGGCCACCGCCAAGTACTTCTTCTACTCTAACATCATGAACTTCTTCAAGAC CGAAATCACCTTGGCCAACGGTGAAATCAGAAAGAGACCATTGATCGAAACCAACGG TGAAACCGGTGAAATCGTCTGGGACAAGGGTAGAGACTTCGCCACCGTCAGAAAGG TCTTGTCTATGCCACAAGTCAACATCGTCAAGAAGACCGAAGTCCAAACCGGTGGTT TCTCTAAGGAATCTATCTTGCCAAAGAGAAACTCTGACAAGTTGATCGCCAGAAAGA AGGACTGGGACCCAAAGAAGTACGGTGGTTTCGACTCTCCAACCGTCGCCTACTCTG	107

[0706]

	TCTTGGTCGTCGCCAAGGTCGAAAAGGGTAAGTCTAAGAAGTTGAAGTCTGTCAAGG AATTGTTGGGTATCACCATCATGGAAAGATCTTCTTTCGAAAAGAACCCAATCGACTT CTTGGAAGCCAAGGGTTACAAGGAAGTCAAGAAGGACTTGATCATCAAGTTGCCAAA GTACTCTTTGTTCGAATTGGAAAACGGTAGAAAGAGAATGTTGGCCTCTGCCGGTGA ATTGCAAAAGGGTAACGAATTGGCCTTGCCATCTAAGTACGTCAACTTCTTGTACTTG GCCTCTCACTACGAAAAGTTGAAGGGTTCTCCAGAAGACAACGAACAAAAGCAATT GTTCGTCGAACAACACAAGCACTACTTGGACGAAATCATCGAACAAATCTCTGAATTC TCTAAGAGAGTCATCTTGGCCGACGCCAACTTGGACAAGGTCTTGTCTGCCTACAAC AAGCACAGAGACAAGCCAATCAGAGAACAAGCCGAAAACATCATCCACTTGTTCAC CTTGACCAACTTGGGTGCCCCAGCCGCCTTCAAGTACTTCGACACCACCATCGACAG AAAGAGATACACCTCTACCAAGGAAGTCTTGGACGCCACCTTGATCCACCAATCTATC ACCGGTTTGTACGAAACCAGAATCGACTTGTCTCAATTGGGTGGTGACGGTGGTGGT TCTCCAAAGAAGAAGAGAAAGGTCTAA	
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[0707]

	ATAAAGGTCGTGATTTTGCTACTGTTCGTAAAGTTTTATCTATGCCTCAAGTTAATATTG TTAAAAAAACTGAAGTTCAAACTGGTGGTTTTTCTAAAGAATCTATTTTACCTAAACG TAATTCTGATAAATTAATTGCTCGTAAAAAAGATTGGGATCCTAAAAAATATGGTGGTT TTGATTCTCCTACTGTTGCTTATTCTGTTTTAGTTGTTGCTAAAGTTGAAAAAGGTAAA TCTAAAAAATTAAAATCTGTTAAAGAATTATTAGGTATTACTATTATGGAACGTTCTTCT TTTGAAAAAAATCCTATTGATTTTTTAGAAGCTAAAGGTTATAAAGAAGTTAAAAAAG ATTTAATTATTAAATTACCTAAATATTCTTTATTTGAATTAGAAAATGGTCGTAAACGTAT GTTAGCTTCTGCTGGTGAATTACAAAAAGGTAATGAATTAGCTTTACCTTCTAAATATG TTAATTTTTTATATTTAGCTTCTCATTATGAAAAATTAAAAGGTTCTCCTGAAGATAATG AACAAAAACAATTATTTGTTGAACAACATAAACATTATTTAGATGAAATTATTGAACAA ATTTCTGAATTTTCTAAACGTGTTATTTTAGCTGATGCTAATTTAGATAAAGTTTTATCT GCTTATAATAAACATCGTGATAAACCTATTCGTGAACAAGCTGAAAATATTATTCATTTA TTTACTTTAACTAATTTAGGTGCTCCTGCTGCTTTTAAATATTTTGATACTACTATTGATC GTAAACGTTATACTTCTACTAAAGAAGTTTTAGATGCTACTTTAATTCATCAATCTATTA CTGGTTTATATGAAACTCGTATTGATTTATCTCAATTAGGTGGTGATGGTGGTGGTTCTC CTAAAAAAAAACGTAAAGTTTGA	
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	GAAAAGACTTCCAATTCTACAAAGTCAGAGAAATCAACAACTACCACCACGCCCACG ACGCCTACCTCAACGCCGTCGTCGGCACCGCCCTCATCAAAAAATACCCCAAACTCG AATCCGAATTCGTCTACGGCGACTACAAAGTCTACGACGTCAGAAAAATGATCGCCA AATCCGAACAAGAAATCGGCAAAGCCACCGCCAAATACTTCTTCTACTCCAACATCAT GAACTTCTTCAAAACCGAAATCACCCTCGCCAACGGCGAAATCAGAAAAAGACCCCT CATCGAAACCAACGGCGAAACCGGCGAAATCGTCTGGGACAAAGGCAGAGACTTCG CCACCGTCAGAAAAGTCCTCTCCATGCCCCAAGTCAACATCGTCAAAAAAACCGAAG TCCAAACCGGCGGCTTCTCCAAAGAATCCATCCTCCCCAAAAGAAACTCCGACAAAC TCATCGCCAGAAAAAAAGACTGGGACCCCAAAAAATACGGCGGCTTCGACTCCCCCA CCGTCGCCTACTCCGTCCTCGTCGTCGCCAAAGTCGAAAAAGGCAAATCCAAAAAAC TCAAATCCGTCAAAGAACTCCTCGGCATCACCATCATGGAAAGATCCTCCTTCGAAAA AAACCCCATCGACTTCCTCGAAGCCAAAGGCTACAAAGAAGTCAAAAAAGACCTCAT CATCAAACTCCCCAAATACTCCCTCTTCGAACTCGAAAACGGCAGAAAAAGAATGCT CGCCTCCGCCGGCGAACTCCAAAAAGGCAACGAACTCGCCCTCCCCTCCAAATACGT CAACTTCCTCTACCTCGCCTCCCACTACGAAAAACTCAAAGGCTCCCCCGAAGACAA CGAACAAAAACAACTCTTCGTCGAACAACACAAACACTACCTCGACGAAATCATCGA ACAAATCTCCGAATTCTCCAAAAGAGTCATCCTCGCCGACGCCAACCTCGACAAAGT CCTCTCCGCCTACAACAAACACAGAGACAAACCCATCAGAGAACAAGCCGAAAACA TCATCCACCTCTTCACCCTCACCAACCTCGGCGCCCCCGCCGCCTTCAAATACTTCGA CACCACCATCGACAGAAAAAGATACACCTCCACCAAAGAAGTCCTCGACGCCACCCT CATCCACCAATCCATCACCGGCCTCTACGAAACCAGAATCGACCTCTCCCAACTCGGC GGCGACGGCGGCGGCTCCCCCAAAAAAAAAAGAAAAGTCTGA	
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[0709]

	GGTGGTGAAGAAGATGAAGAATTATTGGAGACAGTTGTTGAATGCTAAGTTGATTACA CAGAGAAAGTTTGATAATTTGACAAAGGCTGAGAGAGGAGGATTGAGTGAGTTGGAT AAGGCTGGATTTATTAAGAGACAGTTGGTGGAGACAAGACAGATTACAAAGCATGTG GCTCAGATTTTGGATAGTAGAATGAATACAAAGTATGATGAGAATGATAAGTTGATTAG AGAGGTGAAGGTGATTACATTGAAGAGTAAGTTGGTGAGTGATTTTAGAAAGGATTTT CAGTTTTATAAGGTGAGAGAGATTAATAATTATCATCATGCTCATGATGCTTATTTGAAT GCTGTGGTGGGAACAGCTTTGATTAAGAAGTATCCTAAGTTGGAGAGTGAGTTTGTGT ATGGAGATTATAAGGTGTATGATGTGAGAAAGATGATTGCTAAGAGTGAGCAGGAGAT TGGAAAGGCTACAGCTAAGTATTTTTTTTATAGTAATATTATGAATTTTTTTAAGACAGA GATTACATTGGCTAATGGAGAGATTAGAAAGAGACCTTTGATTGAGACAAATGGAGA GACAGGAGAGATTGTGTGGGATAAGGGAAGAGATTTTGCTACAGTGAGAAAGGTGTT GAGTATGCCTCAGGTGAATATTGTGAAGAAGACAGAGGTGCAGACAGGAGGATTTAG TAAGGAGAGTATTTTGCCTAAGAGAAATAGTGATAAGTTGATTGCTAGAAAGAAGGAT TGGGATCCTAAGAAGTATGGAGGATTTGATAGTCCTACAGTGGCTTATAGTGTGTTGGT GGTGGCTAAGGTGGAGAAGGGAAAGAGTAAGAAGTTGAAGAGTGTGAAGGAGTTGT TGGGAATTACAATTATGGAGAGAAGTAGTTTTGAGAAGAATCCTATTGATTTTTTGGA GGCTAAGGGATATAAGGAGGTGAAGAAGGATTTGATTATTAAGTTGCCTAAGTATAGT TTGTTTGAGTTGGAGAATGGAAGAAAGAGAATGTTGGCTAGTGCTGGAGAGTTGCAG AAGGGAAATGAGTTGGCTTTGCCTAGTAAGTATGTGAATTTTTTGTATTTGGCTAGTCA TTATGAGAAGTTGAAGGGAAGTCCTGAGGATAATGAGCAGAAGCAGTTGTTTGTGGA GCAGCATAAGCATTATTTGGATGAGATTATTGAGCAGATTAGTGAGTTTAGTAAGAGA GTGATTTTGGCTGATGCTAATTTGGATAAGGTGTTGAGTGCTTATAATAAGCATAGAGA TAAGCCTATTAGAGAGCAGGCTGAGAATATTATTCATTTGTTTACATTGACAAATTTGG GAGCTCCTGCTGCTTTTAAGTATTTTGATACAACAATTGATAGAAAGAGATATACAAGT ACAAAGGAGGTGTTGGATGCTACATTGATTCATCAGAGTATTACAGGATTGTATGAGA CAAGAATTGATTTGAGTCAGTTGGGAGGAGATGGAGGAGGAAGTCCTAAGAAGAAG AGAAAGGTGTGA	
使用表4的低A密码子，具有起始及终止密码子的Cas9 ORF	ATGGACAAGAAGTACTCCATCGGCCTGGACATCGGCACCAACTCCGTGGGCTGGGCC GTGATCACCGACGAGTACAAGGTGCCCTCCAAGAAGTTCAAGGTGCTGGGCAACACC GACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAG ACCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAA GAACCGGATCTGCTACCTGCAGGAGATCTTCTCCAACGAGATGGCCAAGGTGGACGA CTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGA GCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCC CACCATCTACCACCTGCGGAAGAAGCTGGTGGACTCCACCGACAAGGCCGACCTGCG GCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAG GGCGACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAG ACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCTCCGGCGTGGACGCCAAG GCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAG CTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGC CTGACCCCCAACTTCAAGTCCAACTTCGACCTGGCCGAGGACGCCAAGCTGCAGCTG TCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCA GTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGA CATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGTCCGCCTCCATGATCAA GCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGC AGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCG GCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCCT GGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGC TGCGGAAGCAGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCG AGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGACAACC GGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGG CCCGGGGCAACTCCCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCC CCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCCAGTCCTTCATCGAGC GGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCC CTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACC GAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAAGGCCATCGTGGA CCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACT TCAAGAAGATCGAGTGCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCA ACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCC TGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTG TTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGAC GACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGTC CCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTT CCTGAAGTCCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATCCACGACGACTC CCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGACTCCCT GCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCA GACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGA	111

	ACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAAC TCCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGAT CCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGT ACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC TGTCCGACTACGACGTGGACCACATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCAT CGACAACAAGGTGCTGACCCGGTCCGACAAGAACCGGGGCAAGTCCGACAACGTGC CCTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCC AAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCT GTCCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGA TCACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGA ACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCG ACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACCACG CCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCA AGCTGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGA TCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACTCCA ACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGC GGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGG GACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAG ACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCC GACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGA CTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTC CAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTC CTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGA AGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGA AGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCC TCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCC CCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGA CGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAA CCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGC AGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCT TCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGC TGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACC TGTCCCAGCTGGGCGGCGACGGCGGCGGCTCCCCCAAGAAGAAGCGGAAGGTGTGA	

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使用表4的低A/U密码子，具有起始及终止密码子的Cas9 ORF	ATGGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACAGCGTGGGCTGGGCC GTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAGTTCAAGGTGCTGGGCAACAC CGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCG AGACCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGG AAGAACCGGATCTGCTACCTGCAGGAGATCTTCAGCAACGAGATGGCCAAGGTGGAC GACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCA CGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTA CCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACAGCACCGACAAGGCCGACCT GCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATC GAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGT GCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGCGGCGTGGACG CCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATC GCCCAGCTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAG CCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCT GCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCG GCGACCAGTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGC TGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCAGCA TGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGC GGCAGCAGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGC TACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAG CCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGA GGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCA CCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAA GGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGG CCCCCTGGCCCGGGGCAACAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGA CCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGC TTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCC AAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAG TACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGC CATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGG AGGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGCGGCGTGGAG GACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGAC CCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCA	112

[0711]

	CCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGG GCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACC ATCCTGGACTTCCTGAAGAGCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATC CACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCA GGGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGA AGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGG CACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAA GGGCCAGAAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAG CTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGA GAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCT GGACATCAACCGGCTGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCT GAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGACAAGAACCGGGGC AAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCG GCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGG CCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTG GTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAA CACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGA AGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAG ATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCC CTGATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTG TACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGC CAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCC AACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGAT CGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGC ATCCTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCC CAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGC CAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGC ATCACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCC AAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCT GTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGA AGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCC ACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTG GAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAA GCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGC ACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGA CCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGC GGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCG GCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACGGCGGCGGCAGC CCCAAGAAGAAGCGGAAGGTGTGA	
使用表4的低A密码子，具有两个C端NLS序列及起始及终止密码子的Cas9 ORF	ATGGACAAGAAGTACTCCATCGGCCTGGACATCGGCACCAACTCCGTGGGCTGGGCC GTGATCACCGACGAGTACAAGGTGCCCTCCAAGAAGTTCAAGGTGCTGGGCAACACC GACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAG ACCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAA GAACCGGATCTGCTACCTGCAGGAGATCTTCTCCAACGAGATGGCCAAGGTGGACGA CTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGA GCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCC CACCATCTACCACCTGCGGAAGAAGCTGGTGGACTCCACCGACAAGGCCGACCTGCG GCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAG GGCGACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAG ACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCTCCGGCGTGGACGCCAAG GCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAG CTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGC CTGACCCCCAACTTCAAGTCCAACTTCGACCTGGCCGAGGACGCCAAGCTGCAGCTG TCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCA GTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGA CATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGTCCGCCTCCATGATCAA GCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGC AGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCG GCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCCT GGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGC TGCGGAAGCAGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCG AGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGACAACC GGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGG CCCGGGGCAACTCCCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCC CCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCCAGTCCTTCATCGAGC GGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCC	113

[0712]

	CTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACC GAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAAGGCCATCGTGGA CCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACT TCAAGAAGATCGAGTGCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCA ACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCC TGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTG TTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGAC GACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGTC CCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTT CCTGAAGTCCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATCCACGACGACTC CCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGACTCCCT GCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCA GACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGA ACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAAC TCCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGAT CCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGT ACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC TGTCCGACTACGACGTGGACCACATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCAT CGACAACAAGGTGCTGACCCGGTCCGACAAGAACCGGGGCAAGTCCGACAACGTGC CCTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCC AAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCT GTCCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGA TCACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGA ACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCG ACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACCACG CCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCA AGCTGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGA TCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACTCCA ACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGC GGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGG GACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAG ACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCC GACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGA CTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTC CAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTC CTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGA AGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGA AGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCC TCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCC CCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGA CGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAA CCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGC AGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCT TCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGC TGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACC TGTCCCAGCTGGGCGGCGACGGCTCCGGCTCCCCCAAGAAGAAGCGGAAGGTGGAC GGCTCCCCCAAGAAGAAGCGGAAGGTGGACTCCGGCTGA	
使用表4的低A密码子，具有起始及终止密码子的Cas9切口酶ORF	ATGGACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCC GTGATCACCGACGAGTACAAGGTGCCCTCCAAGAAGTTCAAGGTGCTGGGCAACACC GACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAG ACCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAA GAACCGGATCTGCTACCTGCAGGAGATCTTCTCCAACGAGATGGCCAAGGTGGACGA CTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGA GCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCC CACCATCTACCACCTGCGGAAGAAGCTGGTGGACTCCACCGACAAGGCCGACCTGCG GCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAG GGCGACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAG ACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCTCCGGCGTGGACGCCAAG GCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAG CTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGC CTGACCCCCAACTTCAAGTCCAACTTCGACCTGGCCGAGGACGCCAAGCTGCAGCTG TCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCA GTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGA CATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGTCCGCCTCCATGATCAA GCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGC AGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCG GCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCCT	114

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	GGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGC TGCGGAAGCAGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCG AGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGACAACC GGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGG CCCGGGGCAACTCCCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCC CCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCCAGTCCTTCATCGAGC GGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCC CTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACC GAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAAGGCCATCGTGGA CCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACT TCAAGAAGATCGAGTGCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCA ACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCC TGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTG TTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGAC GACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGTC CCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTT CCTGAAGTCCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATCCACGACGACTC CCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGACTCCCT GCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCA GACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGA ACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAAC TCCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGAT CCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGT ACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC TGTCCGACTACGACGTGGACCACATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCAT CGACAACAAGGTGCTGACCCGGTCCGACAAGAACCGGGGCAAGTCCGACAACGTGC CCTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCC AAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCT GTCCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGA TCACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGA ACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCG ACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACCACG CCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCA AGCTGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGA TCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACTCCA ACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGC GGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGG GACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAG ACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCC GACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGA CTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTC CAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTC CTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGA AGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGA AGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCC TCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCC CCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGA CGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAA CCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGC AGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCT TCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGC TGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACC TGTCCCAGCTGGGCGGCGACGGCGGCGGCTCCCCCAAGAAGAAGCGGAAGGTGTGA	
使用表4的低A密码子，具有起始及终止密码子且无 NLS 的Cas9切口酶ORF	ATGGACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCC GTGATCACCGACGAGTACAAGGTGCCCTCCAAGAAGTTCAAGGTGCTGGGCAACACC GACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAG ACCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAA GAACCGGATCTGCTACCTGCAGGAGATCTTCTCCAACGAGATGGCCAAGGTGGACGA CTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGA GCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCC CACCATCTACCACCTGCGGAAGAAGCTGGTGGACTCCACCGACAAGGCCGACCTGCG GCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAG GGCGACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAG ACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCTCCGGCGTGGACGCCAAG GCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAG CTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGC CTGACCCCCAACTTCAAGTCCAACTTCGACCTGGCCGAGGACGCCAAGCTGCAGCTG	115

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	TCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCA GTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGA CATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGTCCGCCTCCATGATCAA GCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGC AGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCG GCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCCT GGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGC TGCGGAAGCAGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCG AGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGACAACC GGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGG CCCGGGGCAACTCCCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCC CCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCCAGTCCTTCATCGAGC GGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCC CTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACC GAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAAGGCCATCGTGGA CCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACT TCAAGAAGATCGAGTGCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCA ACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCC TGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTG TTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGAC GACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGTC CCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTT CCTGAAGTCCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATCCACGACGACTC CCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGACTCCCT GCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCA GACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGA ACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAAC TCCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGAT CCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGT ACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC TGTCCGACTACGACGTGGACCACATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCAT CGACAACAAGGTGCTGACCCGGTCCGACAAGAACCGGGGCAAGTCCGACAACGTGC CCTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCC AAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCT GTCCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGA TCACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGA ACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCG ACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACCACG CCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCA AGCTGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGA TCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACTCCA ACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGC GGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGG GACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAG ACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCC GACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGA CTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTC CAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTC CTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGA AGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGA AGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCC TCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCC CCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGA CGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAA CCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGC AGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCT TCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGC TGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACC TGTCCCAGCTGGGCGGCGACTGA	
使用表4的低A密码子，具有两个C端NLS序列及起始及终止	ATGGACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCC GTGATCACCGACGAGTACAAGGTGCCCTCCAAGAAGTTCAAGGTGCTGGGCAACACC GACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAG ACCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAA GAACCGGATCTGCTACCTGCAGGAGATCTTCTCCAACGAGATGGCCAAGGTGGACGA CTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGA GCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCC CACCATCTACCACCTGCGGAAGAAGCTGGTGGACTCCACCGACAAGGCCGACCTGCG	116

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密码子的Cas9切口酶ORF	GCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAG GGCGACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAG ACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCTCCGGCGTGGACGCCAAG GCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAG CTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGC CTGACCCCCAACTTCAAGTCCAACTTCGACCTGGCCGAGGACGCCAAGCTGCAGCTG TCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCA GTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGA CATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGTCCGCCTCCATGATCAA GCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGC AGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCG GCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCCT GGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGC TGCGGAAGCAGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCG AGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGACAACC GGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGG CCCGGGGCAACTCCCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCC CCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCCAGTCCTTCATCGAGC GGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCC CTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACC GAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAAGGCCATCGTGGA CCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACT TCAAGAAGATCGAGTGCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCA ACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCC TGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTG TTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGAC GACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGTC CCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTT CCTGAAGTCCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATCCACGACGACTC CCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGACTCCCT GCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCA GACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGA ACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAAC TCCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGAT CCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGT ACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC TGTCCGACTACGACGTGGACCACATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCAT CGACAACAAGGTGCTGACCCGGTCCGACAAGAACCGGGGCAAGTCCGACAACGTGC CCTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCC AAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCT GTCCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGA TCACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGA ACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCG ACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACCACG CCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCA AGCTGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGA TCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACTCCA ACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGC GGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGG GACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAG ACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCC GACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGA CTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTC CAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTC CTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGA AGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGA AGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCC TCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCC CCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGA CGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAA CCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGC AGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCT TCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGC TGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACC TGTCCCAGCTGGGCGGCGACGGCTCCGGCTCCCCCAAGAAGAAGCGGAAGGTGGAC GGCTCCCCCAAGAAGAAGCGGAAGGTGGACTCCGGCTGA	

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使用表4的低A密码子，具有起始及终止密码子的dCas9 ORF	ATGGACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCC GTGATCACCGACGAGTACAAGGTGCCCTCCAAGAAGTTCAAGGTGCTGGGCAACACC GACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAG ACCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAA GAACCGGATCTGCTACCTGCAGGAGATCTTCTCCAACGAGATGGCCAAGGTGGACGA CTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGA GCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCC CACCATCTACCACCTGCGGAAGAAGCTGGTGGACTCCACCGACAAGGCCGACCTGCG GCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAG GGCGACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAG ACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCTCCGGCGTGGACGCCAAG GCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAG CTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGC CTGACCCCCAACTTCAAGTCCAACTTCGACCTGGCCGAGGACGCCAAGCTGCAGCTG TCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCA GTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGA CATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGTCCGCCTCCATGATCAA GCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGC AGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCG GCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCCT GGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGC TGCGGAAGCAGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCG AGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGACAACC GGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGG CCCGGGGCAACTCCCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCC CCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCCAGTCCTTCATCGAGC GGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCC CTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACC GAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAAGGCCATCGTGGA CCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACT TCAAGAAGATCGAGTGCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCA ACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCC TGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTG TTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGAC GACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGTC CCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTT CCTGAAGTCCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATCCACGACGACTC CCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGACTCCCT GCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCA GACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGA ACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAAC TCCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGAT CCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGT ACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC TGTCCGACTACGACGTGGACGCCATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCAT CGACAACAAGGTGCTGACCCGGTCCGACAAGAACCGGGGCAAGTCCGACAACGTGC CCTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCC AAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCT GTCCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGA TCACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGA ACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCG ACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACCACG CCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCA AGCTGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGA TCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACTCCA ACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGC GGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGG GACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAG ACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCC GACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGA CTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTC CAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTC CTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGA AGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGA AGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCC TCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCC CCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGA	117

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	CGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAA CCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGC AGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCT TCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGC TGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACC TGTCCCAGCTGGGCGGCGACGGCGGCGGCTCCCCCAAGAAGAAGCGGAAGGTGTGA	
使用表4的低A密码子，具有起始及终止密码子且无 NLS 的dCas9 ORF	ATGGACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCC GTGATCACCGACGAGTACAAGGTGCCCTCCAAGAAGTTCAAGGTGCTGGGCAACACC GACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAG ACCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAA GAACCGGATCTGCTACCTGCAGGAGATCTTCTCCAACGAGATGGCCAAGGTGGACGA CTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGA GCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCC CACCATCTACCACCTGCGGAAGAAGCTGGTGGACTCCACCGACAAGGCCGACCTGCG GCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAG GGCGACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAG ACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCTCCGGCGTGGACGCCAAG GCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAG CTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGC CTGACCCCCAACTTCAAGTCCAACTTCGACCTGGCCGAGGACGCCAAGCTGCAGCTG TCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCA GTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGA CATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGTCCGCCTCCATGATCAA GCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGC AGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCG GCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCCT GGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGC TGCGGAAGCAGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCG AGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGACAACC GGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGG CCCGGGGCAACTCCCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCC CCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCCAGTCCTTCATCGAGC GGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCC CTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACC GAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAAGGCCATCGTGGA CCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACT TCAAGAAGATCGAGTGCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCA ACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCC TGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTG TTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGAC GACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGTC CCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTT CCTGAAGTCCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATCCACGACGACTC CCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGACTCCCT GCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCA GACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGA ACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAAC TCCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGAT CCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGT ACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC TGTCCGACTACGACGTGGACGCCATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCAT CGACAACAAGGTGCTGACCCGGTCCGACAAGAACCGGGGCAAGTCCGACAACGTGC CCTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCC AAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCT GTCCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGA TCACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGA ACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCG ACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACCACG CCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCA AGCTGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGA TCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACTCCA ACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGC GGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGG GACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAG ACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCC GACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGA CTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTC	118

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	CAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTC CTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGA AGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGA AGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCC TCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCC CCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGA CGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAA CCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGC AGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCT TCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGC TGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACC TGTCCCAGCTGGGCGGCGACTGA	
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[0719]

	ACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGC GGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGG GACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAG ACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCC GACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGA CTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTC CAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTC CTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGA AGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGA AGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCC TCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCC CCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGA CGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAA CCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGC AGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCT TCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGC TGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACC TGTCCCAGCTGGGCGGCGACGGCTCCGGCTCCCCCAAGAAGAAGCGGAAGGTGGAC GGCTCCCCCAAGAAGAAGCGGAAGGTGGACTCCGGCTGA	
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	CCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTG GTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAA CACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGA AGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAG ATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCC CTGATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTG TACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGC CAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCC AACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGAT CGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGC ATCCTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCC CAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGC CAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGC ATCACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCC AAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCT GTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGA AGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCC ACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTG GAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAA GCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGC ACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGA CCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGC GGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCG GCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACGGCAGCGGCAGC CCCAAGAAGAAGCGGAAGGTGGACGGCAGCCCCAAGAAGAAGCGGAAGGTGGACA GCGGCTGA	
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[0720]

	CACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAA GGGCCAGAAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAG CTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGA GAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCT GGACATCAACCGGCTGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCT GAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGACAAGAACCGGGGC AAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCG GCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGG CCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTG GTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAA CACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGA AGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAG ATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCC CTGATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTG TACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGC CAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCC AACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGAT CGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGC ATCCTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCC CAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGC CAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGC ATCACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCC AAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCT GTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGA AGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCC ACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTG GAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAA GCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGC ACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGA CCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGC GGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCG GCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACTGA	
[0721] 使用表4的低A/U密码子，具有起始及终止密码子的Cas9切口酶ORF	ATGGACAAGAAGTACAGCATCGGCCTGGCCATCGGCACCAACAGCGTGGGCTGGGCC GTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAGTTCAAGGTGCTGGGCAACAC CGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCG AGACCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGG AAGAACCGGATCTGCTACCTGCAGGAGATCTTCAGCAACGAGATGGCCAAGGTGGAC GACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCA CGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTA CCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACAGCACCGACAAGGCCGACCT GCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATC GAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGT GCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGCGGCGTGGACG CCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATC GCCCAGCTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAG CCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCT GCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCG GCGACCAGTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGC TGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCAGCA TGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGC GGCAGCAGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGC TACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAG CCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGA GGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCA CCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAA GGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGG CCCCCTGGCCCGGGGCAACAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGA CCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGC TTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCC AAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAG TACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGC CATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGG AGGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGCGGCGTGGAG GACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGAC CCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCA	122

[0722]

	CCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGG GCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACC ATCCTGGACTTCCTGAAGAGCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATC CACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCA GGGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGA AGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGG CACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAA GGGCCAGAAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAG CTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGA GAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCT GGACATCAACCGGCTGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCT GAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGACAAGAACCGGGGC AAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCG GCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGG CCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTG GTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAA CACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGA AGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAG ATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCC CTGATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTG TACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGC CAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCC AACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGAT CGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGC ATCCTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCC CAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGC CAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGC ATCACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCC AAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCT GTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGA AGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCC ACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTG GAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAA GCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGC ACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGA CCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGC GGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCG GCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACGGCGGCGGCAGC CCCAAGAAGAAGCGGAAGGTGTGA	
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[0723]

	AAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAG TACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGC CATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGG AGGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGCGGCGTGGAG GACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGAC CCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCA CCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGG GCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACC ATCCTGGACTTCCTGAAGAGCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATC CACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCA GGGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGA AGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGG CACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAA GGGCCAGAAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAG CTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGA GAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCT GGACATCAACCGGCTGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCT GAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGACAAGAACCGGGGC AAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCG GCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGG CCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTG GTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAA CACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGA AGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAG ATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCC CTGATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTG TACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGC CAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCC AACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGAT CGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGC ATCCTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCC CAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGC CAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGC ATCACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCC AAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCT GTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGA AGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCC ACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTG GAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAA GCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGC ACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGA CCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGC GGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCG GCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACGGCAGCGGCAGC CCCAAGAAGAAGCGGAAGGTGGACGGCAGCCCCAAGAAGAAGCGGAAGGTGGACA GCGGCTGA	
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[0724]

	TACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAG CCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGA GGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCA CCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAA GGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGG CCCCCTGGCCCGGGGCAACAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGA CCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGC TTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCC AAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAG TACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGC CATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGG AGGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGCGGCGTGGAG GACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGAC CCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCA CCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGG GCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACC ATCCTGGACTTCCTGAAGAGCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATC CACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCA GGGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGA AGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGG CACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAA GGGCCAGAAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAG CTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGA GAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCT GGACATCAACCGGCTGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCT GAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGACAAGAACCGGGGC AAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCG GCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGG CCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTG GTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAA CACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGA AGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAG ATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCC CTGATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTG TACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGC CAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCC AACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGAT CGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGC ATCCTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCC CAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGC CAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGC ATCACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCC AAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCT GTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGA AGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCC ACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTG GAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAA GCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGC ACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGA CCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGC GGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCG GCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACTGA	
使用表4的低A/U密码子，具有起始及终止密码子的 dCas9 ORF	ATGGACAAGAAGTACAGCATCGGCCTGGcCATCGGCACCAACAGCGTGGGCTGGGCC GTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAGTTCAAGGTGCTGGGCAACAC CGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCG AGACCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGG AAGAACCGGATCTGCTACCTGCAGGAGATCTTCAGCAACGAGATGGCCAAGGTGGAC GACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCA CGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTA CCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACAGCACCGACAAGGCCGACCT GCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATC GAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGT GCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGCGGCGTGGACG CCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATC GCCCAGCTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAG	125

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	CCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCT GCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCG GCGACCAGTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGC TGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCAGCA TGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGC GGCAGCAGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGC TACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAG CCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGA GGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCA CCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAA GGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGG CCCCCTGGCCCGGGGCAACAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGA CCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGC TTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCC AAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAG TACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGC CATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGG AGGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGCGGCGTGGAG GACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGAC CCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCA CCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGG GCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACC ATCCTGGACTTCCTGAAGAGCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATC CACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCA GGGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGA AGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGG CACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAA GGGCCAGAAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAG CTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGA GAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCT GGACATCAACCGGCTGAGCGACTACGACGTGGACgcCATCGTGCCCCAGAGCTTCCTG AAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGACAAGAACCGGGGCA AGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGG CAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGC CGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGG TGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAAC ACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAA GAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGAT CAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCT GATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTA CGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGCCA AGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAA CGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCG TGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCCAG GTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCAT CCTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCA AGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGCCA AGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGCAT CACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAA GGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTT CGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGAAGG GCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCCACT ACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGA GCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGCG GGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGCACC GGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCA ACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGT ACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGC CTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACGGCGGCGGCAGCCC CAAGAAGAAGCGGAAGGTGTGA	
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起始及终止密码子的dCas9 ORF	CGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTA CCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACAGCACCGACAAGGCCGACCT GCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATC GAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGT GCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGCGGCGTGGACG CCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATC GCCCAGCTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAG CCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCT GCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCG GCGACCAGTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGC TGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCAGCA TGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGC GGCAGCAGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGC TACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAG CCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGA GGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCA CCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAA GGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGG CCCCCTGGCCCGGGGCAACAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGA CCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGC TTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCC AAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAG TACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGC CATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGG AGGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGCGGCGTGGAG GACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGAC CCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCA CCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGG GCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACC ATCCTGGACTTCCTGAAGAGCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATC CACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCA GGGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGA AGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGG CACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAA GGGCCAGAAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAG CTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGA GAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCT GGACATCAACCGGCTGAGCGACTACGACGTGGACGCCATCGTGCCCCAGAGCTTCCT GAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGACAAGAACCGGGGC AAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCG GCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGG CCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTG GTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAA CACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGA AGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAG ATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCC CTGATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTG TACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGC CAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCC AACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGAT CGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGC ATCCTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCC CAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGC CAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGC ATCACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCC AAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCT GTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGA AGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCC ACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTG GAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAA GCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGC ACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGA CCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGC GGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCG GCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACGGCAGCGGCAGC	

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	CCCAAGAAGAAGCGGAAGGTGGACGGCAGCCCCAAGAAGAAGCGGAAGGTGGACA GCGGCTGA	
使用表4的低A/U密码子，具有起始及终止密码子且无NLS的dCas9 ORF	ATGGACAAGAAGTACAGCATCGGCCTGGcCATCGGCACCAACAGCGTGGGCTGGGCC GTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAGTTCAAGGTGCTGGGCAACAC CGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCG AGACCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGG AAGAACCGGATCTGCTACCTGCAGGAGATCTTCAGCAACGAGATGGCCAAGGTGGAC GACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCA CGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTA CCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACAGCACCGACAAGGCCGACCT GCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATC GAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGT GCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGCGGCGTGGACG CCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATC GCCCAGCTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAG CCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCT GCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCG GCGACCAGTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGC TGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCAGCA TGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGC GGCAGCAGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGC TACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAG CCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGA GGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCA CCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAA GGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGG CCCCCTGGCCCGGGGCAACAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGA CCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGC TTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCC AAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAG TACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGC CATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGG AGGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGCGGCGTGGAG GACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGAC CCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCA CCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGG GCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACC ATCCTGGACTTCCTGAAGAGCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATC CACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCA GGGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGA AGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGG CACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAA GGGCCAGAAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAG CTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGA GAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCT GGACATCAACCGGCTGAGCGACTACGACGTGGACgcCATCGTGCCCCAGAGCTTCCTG AAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGACAAGAACCGGGGCA AGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGG CAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGC CGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGG TGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAAC ACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAA GAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGAT CAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCT GATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTA CGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGCCA AGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAA CGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCG TGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCCAG GTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCAT CCTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCA AGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGCCA AGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGCAT CACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAA GGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTT CGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGAAGG	127

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	GCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCCACT ACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGA GCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGCG GGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGCACC GGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCA ACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGT ACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGC CTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACTGA	
使用表4的低A密码子，具有起始及终止密码子的Nme Cas9 ORF	ATGGCCGCCTTCAAGCCCAACTCCATCAACTACATCCTGGGCCTGGACATCGGCATCG CCTCCGTGGGCTGGGCCATGGTGGAGATCGACGAGGAGGAGAACCCCATCCGGCTGA TCGACCTGGGCGTGCGGGTGTTCGAGCGGGCCGAGGTGCCCAAGACCGGCGACTCC CTGGCCATGGCCCGGCGGCTGGCCCGGTCCGTGCGGCGGCTGACCCGGCGGCGGGC CCACCGGCTGCTGCGGACCCGGCGGCTGCTGAAGCGGGAGGGCGTGCTGCAGGCCG CCAACTTCGACGAGAACGGCCTGATCAAGTCCCTGCCCAACACCCCCTGGCAGCTGC GGGCCGCCGCCCTGGACCGGAAGCTGACCCCCCTGGAGTGGTCCGCCGTGCTGCTGC ACCTGATCAAGCACCGGGGCTACCTGTCCCAGCGGAAGAACGAGGGCGAGACCGCC GACAAGGAGCTGGGCGCCCTGCTGAAGGGCGTGGCCGGCAACGCCCACGCCCTGCA GACCGGCGACTTCCGGACCCCCGCCGAGCTGGCCCTGAACAAGTTCGAGAAGGAGT CCGGCCACATCCGGAACCAGCGGTCCGACTACTCCCACACCTTCTCCCGGAAGGACC TGCAGGCCGAGCTGATCCTGCTGTTCGAGAAGCAGAAGGAGTTCGGCAACCCCCAC GTGTCCGGCGGCCTGAAGGAGGGCATCGAGACCCTGCTGATGACCCAGCGGCCCGCC CTGTCCGGCGACGCCGTGCAGAAGATGCTGGGCCACTGCACCTTCGAGCCCGCCGAG CCCAAGGCCGCCAAGAACACCTACACCGCCGAGCGGTTCATCTGGCTGACCAAGCTG AACAACCTGCGGATCCTGGAGCAGGGCTCCGAGCGGCCCCTGACCGACACCGAGCG GGCCACCCTGATGGACGAGCCCTACCGGAAGTCCAAGCTGACCTACGCCCAGGCCCG GAAGCTGCTGGGCCTGGAGGACACCGCCTTCTTCAAGGGCCTGCGGTACGGCAAGG ACAACGCCGAGGCCTCCACCCTGATGGAGATGAAGGCCTACCACGCCATCTCCCGGG CCCTGGAGAAGGAGGGCCTGAAGGACAAGAAGTCCCCCCTGAACCTGTCCCCCGAG CTGCAGGACGAGATCGGCACCGCCTTCTCCCTGTTCAAGACCGACGAGGACATCACC GGCCGGCTGAAGGACCGGATCCAGCCCGAGATCCTGGAGGCCCTGCTGAAGCACATC TCCTTCGACAAGTTCGTGCAGATCTCCCTGAAGGCCCTGCGGCGGATCGTGCCCCTGA TGGAGCAGGGCAAGCGGTACGACGAGGCCTGCGCCGAGATCTACGGCGACCACTAC GGCAAGAAGAACACCGAGGAGAAGATCTACCTGCCCCCCATCCCCGCCGACGAGATC CGGAACCCCGTGGTGCTGCGGGCCCTGTCCCAGGCCCGGAAGGTGATCAACGGCGT GGTGCGGCGGTACGGCTCCCCCGCCCGGATCCACATCGAGACCGCCCGGGAGGTGGG CAAGTCCTTCAAGGACCGGAAGGAGATCGAGAAGCGGCAGGAGGAGAACCGGAAG GACCGGGAGAAGGCCGCCGCCAAGTTCCGGGAGTACTTCCCCAACTTCGTGGGCGA GCCCAAGTCCAAGGACATCCTGAAGCTGCGGCTGTACGAGCAGCAGCACGGCAAGT GCCTGTACTCCGGCAAGGAGATCAACCTGGGCCGGCTGAACGAGAAGGGCTACGTG GAGATCGACCACGCCCTGCCCTTCTCCCGGACCTGGGACGACTCCTTCAACAACAAG GTGCTGGTGCTGGGCTCCGAGAACCAGAACAAGGGCAACCAGACCCCCTACGAGTA CTTCAACGGCAAGGACAACTCCCGGGAGTGGCAGGAGTTCAAGGCCCGGGTGGAGA CCTCCCGGTTCCCCCGGTCCAAGAAGCAGCGGATCCTGCTGCAGAAGTTCGACGAGG ACGGCTTCAAGGAGCGGAACCTGAACGACACCCGGTACGTGAACCGGTTCCTGTGCC AGTTCGTGGCCGACCGGATGCGGCTGACCGGCAAGGGCAAGAAGCGGGTGTTCGCC TCCAACGGCCAGATCACCAACCTGCTGCGGGGCTTCTGGGGCCTGCGGAAGGTGCGG GCCGAGAACGACCGGCACCACGCCCTGGACGCCGTGGTGGTGGCCTGCTCCACCGT GGCCATGCAGCAGAAGATCACCCGGTTCGTGCGGTACAAGGAGATGAACGCCTTCGA CGGCAAGACCATCGACAAGGAGACCGGCGAGGTGCTGCACCAGAAGACCCACTTCC CCCAGCCCTGGGAGTTCTTCGCCCAGGAGGTGATGATCCGGGTGTTCGGCAAGCCCG ACGGCAAGCCCGAGTTCGAGGAGGCCGACACCCTGGAGAAGCTGCGGACCCTGCTG GCCGAGAAGCTGTCCTCCCGGCCCGAGGCCGTGCACGAGTACGTGACCCCCCTGTTC GTGTCCCGGGCCCCCAACCGGAAGATGTCCGGCCAGGGCCACATGGAGACCGTGAA GTCCGCCAAGCGGCTGGACGAGGGCGTGTCCGTGCTGCGGGTGCCCCTGACCCAGCT GAAGCTGAAGGACCTGGAGAAGATGGTGAACCGGGAGCGGGAGCCCAAGCTGTACG AGGCCCTGAAGGCCCGGCTGGAGGCCCACAAGGACGACCCCGCCAAGGCCTTCGCC GAGCCCTTCTACAAGTACGACAAGGCCGGCAACCGGACCCAGCAGGTGAAGGCCGT GCGGGTGGAGCAGGTGCAGAAGACCGGCGTGTGGGTGCGGAACCACAACGGCATCG CCGACAACGCCACCATGGTGCGGGTGGACGTGTTCGAGAAGGGCGACAAGTACTAC CTGGTGCCCATCTACTCCTGGCAGGTGGCCAAGGGCATCCTGCCCGACCGGGCCGTG GTGCAGGGCAAGGACGAGGAGGACTGGCAGCTGATCGACGACTCCTTCAACTTCAA GTTCTCCCTGCACCCCAACGACCTGGTGGAGGTGATCACCAAGAAGGCCCGGATGTT CGGCTACTTCGCCTCCTGCCACCGGGGCACCGGCAACATCAACATCCGGATCCACGA CCTGGACCACAAGATCGGCAAGAACGGCATCCTGGAGGGCATCGGCGTGAAGACCG CCCTGTCCTTCCAGAAGTACCAGATCGACGAGCTGGGCAAGGAGATCCGGCCCTGCC GGCTGAAGAAGCGGCCCCCCGTGCGGTCCGGCAAGCGGACCGCCGACGGCTCCGAG TTCGAGTCCCCCAAGAAGAAGCGGAAGGTGGAGTGA	128

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使用表4的低A/U密码子，具有起始及终止密码子的Nme Cas9 ORF	ATGGCCGCCTTCAAGCCCAACAGCATCAACTACATCCTGGGCCTGGACATCGGCATCG CCAGCGTGGGCTGGGCCATGGTGGAGATCGACGAGGAGGAGAACCCCATCCGGCTG ATCGACCTGGGCGTGCGGGTGTTCGAGCGGGCCGAGGTGCCCAAGACCGGCGACAG CCTGGCCATGGCCCGGCGGCTGGCCCGGAGCGTGCGGCGGCTGACCCGGCGGCGG CCCACCGGCTGCTGCGGACCCGGCGGCTGCTGAAGCGGGAGGGCGTGCTGCAGGCC GCCAACTTCGACGAGAACGGCCTGATCAAGAGCCTGCCCAACACCCCCTGGCAGCTG CGGGCCGCCGCCCTGGACCGGAAGCTGACCCCCCTGGAGTGGAGCGCCGTGCTGCT GCACCTGATCAAGCACCGGGGCTACCTGAGCCAGCGGAAGAACGAGGGCGAGACCG CCGACAAGGAGCTGGGCGCCCTGCTGAAGGGCGTGGCCGGCAACGCCCACGCCCTG CAGACCGGCGACTTCCGGACCCCCGCCGAGCTGGCCCTGAACAAGTTCGAGAAGGA GAGCGGCCACATCCGGAACCAGCGGAGCGACTACAGCCACACCTTCAGCCGGAAGG ACCTGCAGGCCGAGCTGATCCTGCTGTTCGAGAAGCAGAAGGAGTTCGGCAACCCCC ACGTGAGCGGCGGCCTGAAGGAGGGCATCGAGACCCTGCTGATGACCCAGCGGCCC GCCCTGAGCGGCGACGCCGTGCAGAAGATGCTGGGCCACTGCACCTTCGAGCCCGCC GAGCCCAAGGCCGCCAAGAACACCTACACCGCCGAGCGGTTCATCTGGCTGACCAA GCTGAACAACCTGCGGATCCTGGAGCAGGGCAGCGAGCGGCCCCTGACCGACACCG AGCGGGCCACCCTGATGGACGAGCCCTACCGGAAGAGCAAGCTGACCTACGCCCAG GCCCGGAAGCTGCTGGGCCTGGAGGACACCGCCTTCTTCAAGGGCCTGCGGTACGGC AAGGACAACGCCGAGGCCAGCACCCTGATGGAGATGAAGGCCTACCACGCCATCAG CCGGGCCCTGGAGAAGGAGGGCCTGAAGGACAAGAAGAGCCCCCTGAACCTGAGCC CCGAGCTGCAGGACGAGATCGGCACCGCCTTCAGCCTGTTCAAGACCGACGAGGAC ATCACCGGCCGGCTGAAGGACCGGATCCAGCCCGAGATCCTGGAGGCCCTGCTGAAG CACATCAGCTTCGACAAGTTCGTGCAGATCAGCCTGAAGGCCCTGCGGCGGATCGTG CCCCTGATGGAGCAGGGCAAGCGGTACGACGAGGCCTGCGCCGAGATCTACGGCGA CCACTACGGCAAGAAGAACACCGAGGAGAAGATCTACCTGCCCCCCATCCCCGCCGA CGAGATCCGGAACCCCGTGGTGCTGCGGGCCCTGAGCCAGGCCCGGAAGGTGATCA ACGGCGTGGTGCGGCGGTACGGCAGCCCCGCCCGGATCCACATCGAGACCGCCCGGG AGGTGGGCAAGAGCTTCAAGGACCGGAAGGAGATCGAGAAGCGGCAGGAGGAGAA CCGGAAGGACCGGGAGAAGGCCGCCGCCAAGTTCCGGGAGTACTTCCCCAACTTCG TGGGCGAGCCCAAGAGCAAGGACATCCTGAAGCTGCGGCTGTACGAGCAGCAGCAC GGCAAGTGCCTGTACAGCGGCAAGGAGATCAACCTGGGCCGGCTGAACGAGAAGGG CTACGTGGAGATCGACCACGCCCTGCCCTTCAGCCGGACCTGGGACGACAGCTTCAA CAACAAGGTGCTGGTGCTGGGCAGCGAGAACCAGAACAAGGGCAACCAGACCCCCT ACGAGTACTTCAACGGCAAGGACAACAGCCGGGAGTGGCAGGAGTTCAAGGCCCGG GTGGAGACCAGCCGGTTCCCCCGGAGCAAGAAGCAGCGGATCCTGCTGCAGAAGTT CGACGAGGACGGCTTCAAGGAGCGGAACCTGAACGACACCCGGTACGTGAACCGGT TCCTGTGCCAGTTCGTGGCCGACCGGATGCGGCTGACCGGCAAGGGCAAGAAGCGG GTGTTCGCCAGCAACGGCCAGATCACCAACCTGCTGCGGGGCTTCTGGGGCCTGCGG AAGGTGCGGGCCGAGAACGACCGGCACCACGCCCTGGACGCCGTGGTGGTGGCCTG CAGCACCGTGGCCATGCAGCAGAAGATCACCCGGTTCGTGCGGTACAAGGAGATGAA CGCCTTCGACGGCAAGACCATCGACAAGGAGACCGGCGAGGTGCTGCACCAGAAGA CCCACTTCCCCCAGCCCTGGGAGTTCTTCGCCCAGGAGGTGATGATCCGGGTGTTCG GCAAGCCCGACGGCAAGCCCGAGTTCGAGGAGGCCGACACCCTGGAGAAGCTGCGG ACCCTGCTGGCCGAGAAGCTGAGCAGCCGGCCCGAGGCCGTGCACGAGTACGTGAC CCCCCTGTTCGTGAGCCGGGCCCCCAACCGGAAGATGAGCGGCCAGGGCCACATGG AGACCGTGAAGAGCGCCAAGCGGCTGGACGAGGGCGTGAGCGTGCTGCGGGTGCCC CTGACCCAGCTGAAGCTGAAGGACCTGGAGAAGATGGTGAACCGGGAGCGGGAGCC CAAGCTGTACGAGGCCCTGAAGGCCCGGCTGGAGGCCCACAAGGACGACCCCGCCA AGGCCTTCGCCGAGCCCTTCTACAAGTACGACAAGGCCGGCAACCGGACCCAGCAG GTGAAGGCCGTGCGGGTGGAGCAGGTGCAGAAGACCGGCGTGTGGGTGCGGAACCA CAACGGCATCGCCGACAACGCCACCATGGTGCGGGTGGACGTGTTCGAGAAGGGCG ACAAGTACTACCTGGTGCCCATCTACAGCTGGCAGGTGGCCAAGGGCATCCTGCCCG ACCGGGCCGTGGTGCAGGGCAAGGACGAGGAGGACTGGCAGCTGATCGACGACAGC TTCAACTTCAAGTTCAGCCTGCACCCCAACGACCTGGTGGAGGTGATCACCAAGAAG GCCCGGATGTTCGGCTACTTCGCCAGCTGCCACCGGGGCACCGGCAACATCAACATC CGGATCCACGACCTGGACCACAAGATCGGCAAGAACGGCATCCTGGAGGGCATCGGC GTGAAGACCGCCCTGAGCTTCCAGAAGTACCAGATCGACGAGCTGGGCAAGGAGAT CCGGCCCTGCCGGCTGAAGAAGCGGCCCCCCGTGCGGAGCGGCAAGCGGACCGCCG ACGGCAGCGAGTTCGAGAGCCCCAAGAAGAAGCGGAAGGTGGAGTGA	129
具有NLS1的Cas9的开放阅读框架，其具有起始及终止密码子	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGC AGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACA CAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGA GAAACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAA GAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCG ACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAG CACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAG TACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGA	130

[0730]

	CCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTG ATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCT GGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCG ACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTG ATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACT GAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAA AGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAG ATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATC CTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGC AAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACT GGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGA ACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCA TCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAAC AGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCA GATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTT CCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTA CGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCG AAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCA CAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGT CCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAA GGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGA AGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAG CTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGA GTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATC AAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGT CCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACAT ACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACA GGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGG AAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCA GCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCA GCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCA ATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATG GGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAAC ACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATC AAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCA GAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCA GGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAG CTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACA GAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTAC TGGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGAC AAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGA CAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAG AATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCA CACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCCTGGCAGCAAAGAGAAGCAGAACAACATAG	

[0731]

具有NLS2的Cas9的开放阅读框架，其具有起始及终止密码子	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGC AGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACA CAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGA GAAACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAA GAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCG ACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAG CACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAG TACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGA CCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTG ATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCT GGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCG ACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTG ATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACT GAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAA AGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAG ATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATC CTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGC AAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACT GGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGA ACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCA TCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAAC AGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCA GATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTT CCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTA CGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCG AAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCA CAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGT CCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAA GGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGA AGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAG CTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGA GTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATC AAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGT CCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACAT ACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACA GGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGG AAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCA GCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCA GCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCA ATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATG GGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAAC ACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATC AAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCA GAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCA GGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAG CTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACA GAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTAC TGGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGAC AAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGA CAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAG AATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCA CACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT	131

[0732]

	GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCCAGGCAGCAAAGAGAAGCAGAACAACATAG	
具有NLS3的Cas9的开放阅读框架，其具有起始及终止密码子	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGC AGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACA CAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGA GAAACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAA GAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCG ACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAG CACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAG TACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGA CCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTG ATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCT GGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCG ACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTG ATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACT GAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAA AGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAG ATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATC CTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGC AAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACT GGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGA ACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCA TCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAAC AGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCA GATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTT CCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTA CGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCG AAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCA CAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGT CCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAA GGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGA AGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAG CTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGA GTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATC AAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGT CCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACAT ACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACA GGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGG AAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCA GCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCA GCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCA ATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATG GGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAAC ACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATC AAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCA GAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCA GGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAG CTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACA GAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTAC TGGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGAC AAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGA CAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAG AATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCA CACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG	132

[0733]

	GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCCCGGCACCGGCAAAGAGAGAAAGAACAACATAG	
具有NLS4的Cas9的开放阅读框架，其具有起始及终止密码子	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGC AGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACA CAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGA GAAACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAA GAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCG ACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAG CACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAG TACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGA CCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTG ATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCT GGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCG ACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTG ATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACT GAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAA AGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAG ATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATC CTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGC AAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACT GGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGA ACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCA TCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAAC AGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCA GATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTT CCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTA CGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCG AAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCA CAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGT CCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAA GGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGA AGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAG CTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGA GTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATC AAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGT CCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACAT ACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACA GGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGG AAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCA GCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCA GCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCA ATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATG GGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAAC ACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATC AAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCA GAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCA GGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAG CTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACA GAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTAC TGGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGAC AAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGA CAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAG AATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCA CACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA	133

[0734]

	CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCCAGGCAGCAAAGAGACCGAGAACAACATAG	
具有NLS5的Cas9的开放阅读框架，其具有起始及终止密码子	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGC AGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACA CAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGA GAAACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAA GAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCG ACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAG CACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAG TACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGA CCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTG ATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCT GGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCG ACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTG ATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACT GAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAA AGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAG ATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATC CTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGC AAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACT GGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGA ACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCA TCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAAC AGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCA GATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTT CCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTA CGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCG AAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCA CAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGT CCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAA GGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGA AGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAG CTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGA GTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATC AAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGT CCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACAT ACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACA GGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGG AAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCA GCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCA GCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCA ATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATG GGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAAC ACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATC AAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCA GAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCA GGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAG CTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACA	134

[0735]

	GAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTAC TGGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGAC AAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGA CAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAG AATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCA CACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCAGAGCAGCAAAGAGACCGAGAACAACATAG	
具有NLS6的Cas9的开放阅读框架，其具有起始及终止密码子	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGC AGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACA CAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGA GAAACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAA GAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCG ACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAG CACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAG TACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGA CCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTG ATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCT GGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCG ACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTG ATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACT GAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAA AGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAG ATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATC CTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGC AAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACT GGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGA ACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCA TCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAAC AGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCA GATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTT CCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTA CGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCG AAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCA CAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGT CCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAA GGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGA AGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAG CTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGA GTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATC AAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGT CCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACAT ACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACA GGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGG AAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCA GCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCA GCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCA	135

[0736]

	ATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATG GGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAAC ACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATC AAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCA GAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCA GGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAG CTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACA GAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTAC TGGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGAC AAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGA CAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAG AATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCA CACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCGCAGCAGCAAAGAGAAGCTGGAGCATGGCAGCATAG	
具有NLS7的Cas9的开放阅读框架，其具有起始及终止密码子	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGC AGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACA CAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGA GAAACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAA GAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCG ACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAG CACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAG TACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGA CCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTG ATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCT GGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCG ACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTG ATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACT GAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAA AGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAG ATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATC CTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGC AAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACT GGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGA ACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCA TCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAAC AGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCA GATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTT CCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTA CGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCG AAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCA CAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGT CCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAA GGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGA AGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAG CTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGA GTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATC	136

[0737]

	AAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGT CCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACAT ACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACA GGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGG AAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCA GCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCA GCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCA ATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATG GGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAAC ACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATC AAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCA GAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCA GGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAG CTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACA GAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTAC TGGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGAC AAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGA CAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAG AATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCA CACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCGCAGCAGCAAAGAGAGTCTGGAGCATGGCATTCTAG	
具有NLS8的Cas9的开放阅读框架，其具有起始及终止密码子	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGC AGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACA CAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGA GAAACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAA GAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCG ACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAG CACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAG TACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGA CCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTG ATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCT GGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCG ACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTG ATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACT GAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAA AGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAG ATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATC CTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGC AAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACT GGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGA ACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCA TCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAAC AGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCA GATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTT CCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTA CGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCG	137

[0738]

	AAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCA CAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGT CCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAA GGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGA AGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAG CTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGA GTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATC AAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGT CCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACAT ACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACA GGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGG AAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCA GCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCA GCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCA ATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATG GGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAAC ACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATC AAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCA GAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCA GGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAG CTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACA GAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTAC TGGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGAC AAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGA CAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAG AATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCA CACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCGCAGCAGCAAAGAGAAGCTGGAGCATGGCATTCTAG	
具有NLS9的Cas9的开放阅读框架，其具有起始及终止密码子	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGC AGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACA CAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGA GAAACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAA GAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCG ACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAG CACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAG TACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGA CCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTG ATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCT GGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCG ACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTG ATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACT GAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAA AGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAG ATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATC CTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGC AAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACT	138

[0739]

	GGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGA ACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCA TCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAAC AGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCA GATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTT CCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTA CGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCG AAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCA CAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGT CCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAA GGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGA AGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAG CTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGA GTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATC AAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGT CCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACAT ACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACA GGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGG AAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCA GCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCA GCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCA ATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATG GGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAAC ACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATC AAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCA GAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCA GGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAG CTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACA GAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTAC TGGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGAC AAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGA CAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAG AATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCA CACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCGCAGCAGCAAAGAGAAAGTACTTCGCAGCATAG	
具有NLS10的Cas9的开放阅读框架，其具有起始及终止密码子	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGC AGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACA CAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGA GAAACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAA GAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCG ACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAG CACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAG TACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGA CCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTG ATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCT GGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCG	139

[0740]

	ACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTG ATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACT GAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAA AGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAG ATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATC CTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGC AAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACT GGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGA ACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCA TCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAAC AGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCA GATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTT CCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTA CGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCG AAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCA CAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGT CCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAA GGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGA AGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAG CTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGA GTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATC AAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGT CCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACAT ACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACA GGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGG AAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCA GCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCA GCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCA ATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATG GGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAAC ACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATC AAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCA GAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCA GGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAG CTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACA GAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTAC TGGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGAC AAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGA CAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAG AATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCA CACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCAGAGCAGCAAAGAGAAAGGCATTCGCAGCATAG	
具有NLS11的Cas9的开	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGC AGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACA CAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGA GAAACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAA	140

[0741]

放阅读框架，其具有起始及终止密码子	GAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCG ACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAG CACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAG TACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGA CCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTG ATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCT GGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCG ACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTG ATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACT GAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAA AGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAG ATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATC CTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGC AAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACT GGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGA ACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCA TCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAAC AGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCA GATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTT CCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTA CGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCG AAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCA CAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGT CCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAA GGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGA AGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAG CTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGA GTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATC AAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGT CCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACAT ACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACA GGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGG AAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCA GCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCA GCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCA ATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATG GGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAAC ACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATC AAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCA GAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCA GGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAG CTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACA GAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTAC TGGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGAC AAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGA CAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAG AATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCA CACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC	

[0742]

	AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCAGAGCAGCAAAGAGAAAGTACTTCGCAGTCTAG	
使用通常在人类中高度表达的密码子的 Cas9 ORF (无起始或终止密码子;适于包括在融合蛋白质编码序列中)	CCTAAGAAAAAGCGGAAGGTCGACGGGGATAAGAAGTACTCAATCGGGCTGGATATC GGAACTAATTCCGTGGGTTGGGCAGTGATCACGGATGAATACAAAGTGCCGTCCAAG AAGTTCAAGGTCCTGGGGAACACCGATAGACACAGCATCAAGAAAAATCTCATCGGA GCCCTGCTGTTTGACTCCGGCGAAACCGCAGAAGCGACCCGGCTCAAACGTACCGCG AGGCGACGCTACACCCGGCGGAAGAATCGCATCTGCTATCTGCAAGAGATCTTTTCGA ACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACCGCCTGGAAGAATCTTTCCTGG TGGAGGAGGACAAGAAGCATGAACGGCATCCTATCTTTGGAAACATCGTCGACGAAG TGGCGTACCACGAAAAGTACCCGACCATCTACCATCTGCGGAAGAAGTTGGTTGACT CAACTGACAAGGCCGACCTCAGATTGATCTACTTGGCCCTCGCCCATATGATCAAATT CCGCGGACACTTCCTGATCGAAGGCGATCTGAACCCTGATAACTCCGACGTGGATAA GCTTTTCATTCAACTGGTGCAGACCTACAACCAACTGTTCGAAGAAAACCCAATCAAT GCTAGCGGCGTCGATGCCAAGGCCATCCTGTCCGCCCGGCTGTCGAAGTCGCGGCGC CTCGAAAACCTGATCGCACAGCTGCCGGGAGAGAAAAAGAACGGACTTTTCGGCAA CTTGATCGCTCTCTCACTGGGACTCACTCCCAATTTCAAGTCCAATTTTGACCTGGCC GAGGACGCGAAGCTGCAACTCTCAAAGGACACCTACGACGACGACTTGGACAATTT GCTGGCACAAATTGGCGATCAGTACGCGGATCTGTTCCTTGCCGCTAAGAACCTTTCG GACGCAATCTTGCTGTCCGATATCCTGCGCGTGAACACCGAAATAACCAAAGCGCCG CTTAGCGCCTCGATGATTAAGCGGTACGACGAGCATCACCAGGATCTCACGCTGCTCA AAGCGCTCGTGAGACAGCAACTGCCTGAAAAGTACAAGGAGATCTTCTTCGACCAGT CCAAGAATGGGTACGCAGGGTACATCGATGGAGGCGCTAGCCAGGAAGAGTTCTATA AGTTCATCAAGCCAATCCTGGAAAAGATGGACGGAACCGAAGAACTGCTGGTCAAG CTGAACAGGGAGGATCTGCTCCGGAAACAGAGAACCTTTGACAACGGATCCATTCCC CACCAGATCCATCTGGGTGAGCTGCACGCCATCTTGCGGCGCCAGGAGGACTTTTACC CATTCCTCAAGGACAACCGGGAAAAGATCGAGAAAATTCTGACGTTCCGCATCCCGT ATTACGTGGGCCCACTGGCGCGCGGCAATTCGCGCTTCGCGTGGATGACTAGAAAATC AGAGGAAACCATCACTCCTTGGAATTTCGAGGAAGTTGTGGATAAGGGAGCTTCGGC ACAAAGCTTCATCGAACGAATGACCAACTTCGACAAGAATCTCCCAAACGAGAAGGT GCTTCCTAAGCACAGCCTCCTTTACGAATACTTCACTGTCTACAACGAACTGACTAAA GTGAAATACGTTACTGAAGGAATGAGGAAGCCGGCCTTTCTGTCCGGAGAACAGAAG AAAGCAATTGTCGATCTGCTGTTCAAGACCAACCGCAAGGTGACCGTCAAGCAGCTT AAAGAGGACTACTTCAAGAAGATCGAGTGTTTCGACTCAGTGGAAATCAGCGGGGTG GAGGACAGATTCAACGCTTCGCTGGGAACCTATCATGATCTCCTGAAGATCATCAAGG ACAAGGACTTCCTTGACAACGAGGAGAACGAGGACATCCTGGAAGATATCGTCCTGA CCTTGACCCTTTTCGAGGATCGCGAGATGATCGAGGAGAGGCTTAAGACCTACGCTCA TCTCTTCGACGATAAGGTCATGAAACAACTCAAGCGCCGCCGGTACACTGGTTGGGG CCGCCTCTCCCGCAAGCTGATCAACGGTATTCGCGATAAACAGAGCGGTAAAACTATC CTGGATTTCCTCAAATCGGATGGCTTCGCTAATCGTAACTTCATGCAATTGATCCACGA CGACAGCCTGACCTTTAAGGAGGACATCCAAAAAGCACAAGTGTCCGGACAGGGAG ACTCACTCCATGAACACATCGCGAATCTGGCCGGTTCGCCGGCGATTAAGAAGGGAA TTCTGCAAACTGTGAAGGTGGTCGACGAGCTGGTGAAGGTCATGGGACGGCACAAA CCGGAGAATATCGTGATTGAAATGGCCCGAGAAAACCAGACTACCCAGAAGGGCCAG AAAAACTCCCGCGAAAGGATGAAGCGGATCGAAGAAGGAATCAAGGAGCTGGGCAG CCAGATCCTGAAAGAGCACCCGGTGGAAAACACGCAGCTGCAGAACGAGAAGCTCT ACCTGTACTATTTGCAAAATGGACGGGACATGTACGTGGACCAAGAGCTGGACATCAA TCGGTTGTCTGATTACGACGTGGACCACATCGTTCCACAGTCCTTTCTGAAGGATGAC TCGATCGATAACAAGGTGTTGACTCGCAGCGACAAGAACAGAGGGAAGTCAGATAAT GTGCCATCGGAGGAGGTCGTGAAGAAGATGAAGAATTACTGGCGGCAGCTCCTGAAT GCGAAGCTGATTACCCAGAGAAAGTTTGACAATCTCACTAAAGCCGAGCGCGGCGGA CTCTCAGAGCTGGATAAGGCTGGATTCATCAAACGGCAGCTGGTCGAGACTCGGCAG ATTACCAAGCACGTGGCGCAGATCTTGGACTCCCGCATGAACACTAAATACGACGAG AACGATAAGCTCATCCGGGAAGTGAAGGTGATTACCCTGAAAAGCAAACTTGTGTCG GACTTTCGGAAGGACTTTCAGTTTTACAAAGTGAGAGAAATCAACAACTACCATCAC GCGCATGACGCATACCTCAACGCTGTGGTCGGTACCGCCCTGATCAAAAAGTACCCTA AACTTGAATCGGAGTTTGTGTACGGAGACTACAAGGTCTACGACGTGAGGAAGATGA TAGCCAAGTCCGAACAGGAAATCGGGAAAGCAACTGCGAAATACTTCTTTTACTCAA ACATCATGAACTTTTTCAAGACTGAAATTACGCTGGCCAATGGAGAAATCAGGAAGA GGCCACTGATCGAAACTAACGGAGAAACGGGCGAAATCGTGTGGGACAAGGGCAGG GACTTCGCAACTGTTCGCAAAGTGCTCTCTATGCCGCAAGTCAATATTGTGAAGAAAA CCGAAGTGCAAACCGGCGGATTTTCAAAGGAATCGATCCTCCCAAAGAGAAATAGCG ACAAGCTCATTGCACGCAAGAAAGACTGGGACCCGAAGAAGTACGGAGGATTCGATT CGCCGACTGTCGCATACTCCGTCCTCGTGGTGGCCAAGGTGGAGAAGGGAAAGAGC AAAAAGCTCAAATCCGTCAAAGAGCTGCTGGGGATTACCATCATGGAACGATCCTCG TTCGAGAAGAACCCGATTGATTTCCTCGAGGCGAAGGGTTACAAGGAGGTGAAGAA GGATCTGATCATCAAACTCCCCAAGTACTCACTGTTCGAACTGGAAAATGGTCGGAA	141

[0743]

	GCGCATGCTGGCTTCGGCCGGAGAACTCCAAAAAGGAAATGAGCTGGCCTTGCCTAG CAAGTACGTCAACTTCCTCTATCTTGCTTCGCACTACGAAAAACTCAAAGGGTCACCG GAAGATAACGAACAGAAGCAGCTTTTCGTGGAGCAGCACAAGCATTATCTGGATGAA ATCATCGAACAAATCTCCGAGTTTTCAAAGCGCGTGATCCTCGCCGACGCCAACCTCG ACAAAGTCCTGTCGGCCTACAATAAGCATAGAGATAAGCCGATCAGAGAACAGGCCG AGAACATTATCCACTTGTTCACCCTGACTAACCTGGGAGCCCCAGCCGCCTTCAAGTA CTTCGATACTACTATCGATCGCAAAAGATACACGTCCACCAAGGAAGTTCTGGACGCG ACCCTGATCCACCAAAGCATCACTGGACTCTACGAAACTAGGATCGATCTGTCGCAGC TGGGTGGCGAT	
使用表4的长半衰期密码子的Cas9 ORF (无起始或终止密码子;适于包括在融合蛋白质编码序列中)	GACAAGAAGTACTCTATCGGTTTGGACATCGGTACCAACTCTGTCGGTTGGGCCGTCA TCACCGACGAATACAAGGTCCCATCTAAGAAGTTCAAGGTCTTGGGTAACACCGACA GACACTCTATCAAGAAGAACTTGATCGGTGCCTTGTTGTTCGACTCTGGTGAAACCGC CGAAGCCACCAGATTGAAGAGAACCGCCAGAAGAAGATACACCAGAAGAAAGAACA GAATCTGCTACTTGCAAGAAATCTTCTCTAACGAAATGGCCAAGGTCGACGACTCTTT CTTCCACAGATTGGAAGAATCTTTCTTGGTCGAAGAAGACAAGAAGCACGAAAGAC ACCCAATCTTCGGTAACATCGTCGACGAAGTCGCCTACCACGAAAAGTACCCAACCAT CTACCACTTGAGAAAGAAGTTGGTCGACTCTACCGACAAGGCCGACTTGAGATTGAT CTACTTGGCCTTGGCCCACATGATCAAGTTCAGAGGTCACTTCTTGATCGAAGGTGAC TTGAACCCAGACAACTCTGACGTCGACAAGTTGTTCATCCAATTGGTCCAAACCTACA ACCAATTGTTCGAAGAAAACCCAATCAACGCCTCTGGTGTCGACGCCAAGGCCATCT TGTCTGCCAGATTGTCTAAGAGCAGAAGATTGGAAAACTTGATCGCCCAATTGCCAG GTGAAAAGAAGAACGGTTTGTTCGGTAACTTGATCGCCTTGTCTTTGGGTTTGACCCC AAACTTCAAGTCTAACTTCGACTTGGCCGAAGACGCCAAGTTGCAATTGTCTAAGGA CACCTACGACGACGACTTGGACAACTTGTTGGCCCAAATCGGTGACCAATACGCCGA CTTGTTCTTGGCCGCCAAGAACTTGTCTGACGCCATCTTGTTGTCTGACATCTTGAGA GTCAACACCGAAATCACCAAGGCCCCATTGTCTGCCTCTATGATCAAGAGATACGACG AACACCACCAAGACTTGACCTTGTTGAAGGCCTTGGTCAGACAACAATTGCCAGAAA AGTACAAGGAAATCTTCTTCGACCAATCTAAGAACGGTTACGCCGGTTACATCGACGG TGGTGCCTCTCAAGAAGAATTCTACAAGTTCATCAAGCCAATCTTGGAAAAGATGGA CGGTACCGAAGAATTGTTGGTCAAGTTGAACAGAGAAGACTTGTTGAGAAAGCAAA GAACCTTCGACAACGGTTCTATCCCACACCAAATCCACTTGGGTGAATTGCACGCCAT CTTGAGAAGACAAGAAGACTTCTACCCATTCTTGAAGGACAACAGAGAAAAGATCG AAAAGATCTTGACCTTCAGAATCCCATACTACGTCGGTCCATTGGCCAGAGGTAACAG CAGATTCGCCTGGATGACCAGAAAGTCTGAAGAAACCATCACCCCATGGAACTTCGA AGAAGTCGTCGACAAGGGTGCCTCTGCCCAATCTTTCATCGAAAGAATGACCAACTT CGACAAGAACTTGCCAAACGAAAAGGTCTTGCCAAAGCACTCTTTGTTGTACGAATA CTTCACCGTCTACAACGAATTGACCAAGGTCAAGTACGTCACCGAAGGTATGAGAAA GCCAGCCTTCTTGTCTGGTGAACAAAAGAAGGCCATCGTCGACTTGTTGTTCAAGAC CAACAGAAAGGTCACCGTCAAGCAATTGAAGGAAGACTACTTCAAGAAGATCGAAT GCTTCGACTCTGTCGAAATCTCTGGTGTCGAAGACAGATTCAACGCCTCTTTGGGTAC CTACCACGACTTGTTGAAGATCATCAAGGACAAGGACTTCTTGGACAACGAAGAAAA CGAAGACATCTTGGAAGACATCGTCTTGACCTTGACCTTGTTCGAAGACAGAGAAAT GATCGAAGAAAGATTGAAGACCTACGCCCACTTGTTCGACGACAAGGTCATGAAGCA ATTGAAGAGAAGAAGATACACCGGTTGGGGTAGATTGAGCAGAAAGTTGATCAACGG TATCAGAGACAAGCAATCTGGTAAGACCATCTTGGACTTCTTGAAGTCTGACGGTTTC GCCAACAGAAACTTCATGCAATTGATCCACGACGACTCTTTGACCTTCAAGGAAGAC ATCCAAAAGGCCCAAGTCTCTGGTCAAGGTGACTCTTTGCACGAACACATCGCCAAC TTGGCCGGTTCTCCAGCCATCAAGAAGGGTATCTTGCAAACCGTCAAGGTCGTCGAC GAATTGGTCAAGGTCATGGGTAGACACAAGCCAGAAAACATCGTCATCGAAATGGCC AGAGAAAACCAAACCACCCAAAAGGGTCAAAAGAACAGCAGAGAAAGAATGAAGA GAATCGAAGAAGGTATCAAGGAATTGGGTTCTCAAATCTTGAAGGAACACCCAGTCG AAAACACCCAATTGCAAAACGAAAAGTTGTACTTGTACTACTTGCAAAACGGTAGAG ACATGTACGTCGACCAAGAATTGGACATCAACAGATTGTCTGACTACGACGTCGACC ACATCGTCCCACAATCTTTCTTGAAGGACGACTCTATCGACAACAAGGTCTTGACCAG ATCTGACAAGAACAGAGGTAAGTCTGACAACGTCCCATCTGAAGAAGTCGTCAAGAA GATGAAGAACTACTGGAGACAATTGTTGAACGCCAAGTTGATCACCCAAAGAAAGTT CGACAACTTGACCAAGGCCGAAAGAGGTGGTTTGTCTGAATTGGACAAGGCCGGTTT CATCAAGAGACAATTGGTCGAAACCAGACAAATCACCAAGCACGTCGCCCAAATCTT GGACAGCAGAATGAACACCAAGTACGACGAAAACGACAAGTTGATCAGAGAAGTCA AGGTCATCACCTTGAAGTCTAAGTTGGTCTCTGACTTCAGAAAGGACTTCCAATTCTA CAAGGTCAGAGAAATCAACAACTACCACCACGCCCACGACGCCTACTTGAACGCCGT CGTCGGTACCGCCTTGATCAAGAAGTACCCAAAGTTGGAATCTGAATTCGTCTACGGT GACTACAAGGTCTACGACGTCAGAAAGATGATCGCCAAGTCTGAACAAGAAATCGGT AAGGCCACCGCCAAGTACTTCTTCTACTCTAACATCATGAACTTCTTCAAGACCGAAA TCACCTTGGCCAACGGTGAAATCAGAAAGAGACCATTGATCGAAACCAACGGTGAA ACCGGTGAAATCGTCTGGGACAAGGGTAGAGACTTCGCCACCGTCAGAAAGGTCTTG TCTATGCCACAAGTCAACATCGTCAAGAAGACCGAAGTCCAAACCGGTGGTTTCTCT	142

[0744]

	AAGGAATCTATCTTGCCAAAGAGAAACTCTGACAAGTTGATCGCCAGAAAGAAGGAC TGGGACCCAAAGAAGTACGGTGGTTTCGACTCTCCAACCGTCGCCTACTCTGTCTTG GTCGTCGCCAAGGTCGAAAAGGGTAAGTCTAAGAAGTTGAAGTCTGTCAAGGAATTG TTGGGTATCACCATCATGGAAAGATCTTCTTTCGAAAAGAACCCAATCGACTTCTTGG AAGCCAAGGGTTACAAGGAAGTCAAGAAGGACTTGATCATCAAGTTGCCAAAGTACT CTTTGTTCGAATTGGAAAACGGTAGAAAGAGAATGTTGGCCTCTGCCGGTGAATTGC AAAAGGGTAACGAATTGGCCTTGCCATCTAAGTACGTCAACTTCTTGTACTTGGCCTC TCACTACGAAAAGTTGAAGGGTTCTCCAGAAGACAACGAACAAAAGCAATTGTTCGT CGAACAACACAAGCACTACTTGGACGAAATCATCGAACAAATCTCTGAATTCTCTAA GAGAGTCATCTTGGCCGACGCCAACTTGGACAAGGTCTTGTCTGCCTACAACAAGCA CAGAGACAAGCCAATCAGAGAACAAGCCGAAAACATCATCCACTTGTTCACCTTGAC CAACTTGGGTGCCCCAGCCGCCTTCAAGTACTTCGACACCACCATCGACAGAAAGAG ATACACCTCTACCAAGGAAGTCTTGGACGCCACCTTGATCCACCAATCTATCACCGGT TTGTACGAAACCAGAATCGACTTGTCTCAATTGGGTGGTGACGGTGGTGGTTCTCCAA AGAAGAAGAGAAAGGTC	
使用表4的富含U的密码子的Cas9 ORF (无起始或终止密码子;适于包括在融合蛋白质编码序列中)	GATAAAAAATATTCTATTGGTTTAGATATTGGTACTAATTCTGTTGGTTGGGCTGTTATT ACTGATGAATATAAAGTTCCTTCTAAAAAATTTAAAGTTTTAGGTAATACTGATCGTCA TTCTATTAAAAAAAATTTAATTGGTGCTTTATTATTTGATTCTGGTGAAACTGCTGAAG CTACTCGTTTAAAACGTACTGCTCGTCGTCGTTATACTCGTCGTAAAAATCGTATTTGTT ATTTACAAGAAATTTTTTCTAATGAAATGGCTAAAGTTGATGATTCTTTTTTTCATCGTT TAGAAGAATCTTTTTTAGTTGAAGAAGATAAAAAACATGAACGTCATCCTATTTTTGGT AATATTGTTGATGAAGTTGCTTATCATGAAAAATATCCTACTATTTATCATTTACGTAAA AAATTAGTTGATTCTACTGATAAAGCTGATTTACGTTTAATTTATTTAGCTTTAGCTCATA TGATTAAATTTCGTGGTCATTTTTTAATTGAAGGTGATTTAAATCCTGATAATTCTGATG TTGATAAATTATTTATTCAATTAGTTCAAACTTATAATCAATTATTTGAAGAAAATCCTAT TAATGCTTCTGGTGTTGATGCTAAAGCTATTTTATCTGCTCGTTTATCTAAATCTCGTCG TTTAGAAAATTTAATTGCTCAATTACCTGGTGAAAAAAAAAAATGGTTTATTTGGTAATT TAATTGCTTTATCTTTAGGTTTAACTCCTAATTTTAAATCTAATTTTGATTTAGCTGAAGA TGCTAAATTACAATTATCTAAAGATACTTATGATGATGATTTAGATAATTTATTAGCTCAA ATTGGTGATCAATATGCTGATTTATTTTTAGCTGCTAAAAATTTATCTGATGCTATTTTAT TATCTGATATTTTACGTGTTAATACTGAAATTACTAAAGCTCCTTTATCTGCTTCTATGAT TAAACGTTATGATGAACATCATCAAGATTTAACTTTATTAAAAGCTTTAGTTCGTCAAC AATTACCTGAAAAATATAAAGAAATTTTTTTTGATCAATCTAAAAATGGTTATGCTGGT TATATTGATGGTGGTGCTTCTCAAGAAGAATTTTATAAATTTATTAAACCTATTTTAGAA AAAATGGATGGTACTGAAGAATTATTAGTTAAATTAAATCGTGAAGATTTATTACGTAA ACAACGTACTTTTGATAATGGTTCTATTCCTCATCAAATTCATTTAGGTGAATTACATGC TATTTTACGTCGTCAAGAAGATTTTTATCCTTTTTTAAAAGATAATCGTGAAAAAATTG AAAAAATTTTAACTTTTCGTATTCCTTATTATGTTGGTCCTTTAGCTCGTGGTAATTCTC GTTTTGCTTGGATGACTCGTAAATCTGAAGAAACTATTACTCCTTGGAATTTTGAAGA AGTTGTTGATAAAGGTGCTTCTGCTCAATCTTTTATTGAACGTATGACTAATTTTGATAA AAATTTACCTAATGAAAAAGTTTTACCTAAACATTCTTTATTATATGAATATTTTACTGTT TATAATGAATTAACTAAAGTTAAATATGTTACTGAAGGTATGCGTAAACCTGCTTTTTTA TCTGGTGAACAAAAAAAAAGCTATTGTTGATTTATTATTTAAAACTAATCGTAAAGTTAC TGTTAAACAATTAAAAGAAGATTATTTTAAAAAAATTGAATGTTTTGATTCTGTTGAAA TTTCTGGTGTTGAAGATCGTTTTAATGCTTCTTTAGGTACTTATCATGATTTATTAAAAA TTATTAAAGATAAAGATTTTTTAGATAATGAAGAAAATGAAGATATTTTAGAAGATATT GTTTTAACTTTAACTTTATTTGAAGATCGTGAAATGATTGAAGAACGTTTAAAAACTTA TGCTCATTTATTTGATGATAAAGTTATGAAACAATTAAAACGTCGTCGTTATACTGGTT GGGGTCGTTTATCTCGTAAATTAATTAATGGTATTCGTGATAAACAATCTGGTAAAACTA TTTTAGATTTTTTAAAATCTGATGGTTTTGCTAATCGTAATTTTATGCAATTAATTCATGA TGATTCTTTAACTTTTAAAGAAGATATTCAAAAAGCTCAAGTTTCTGGTCAAGGTGATT CTTTACATGAACATATTGCTAATTTAGCTGGTTCTCCTGCTATTAAAAAAGGTATTTTAC AAACTGTTAAAGTTGTTGATGAATTAGTTAAAGTTATGGGTCGTCATAAACCTGAAAAT ATTGTTATTGAAATGGCTCGTGAAAATCAAACTACTCAAAAAGGTCAAAAAAATTCTC GTGAACGTATGAAACGTATTGAAGAAGGTATTAAAGAATTAGGTTCTCAAATTTTAAA AGAACATCCTGTTGAAAATACTCAATTACAAAATGAAAAATTATATTTATATTATTTACA AAATGGTCGTGATATGTATGTTGATCAAGAATTAGATATTAATCGTTTATCTGATTATGAT GTTGATCATATTGTTCCTCAATCTTTTTTAAAAGATGATTCTATTGATAATAAAGTTTTA ACTCGTTCTGATAAAAATCGTGGTAAATCTGATAATGTTCCTTCTGAAGAAGTTGTTAA AAAAATGAAAAATTATTGGCGTCAATTATTAAATGCTAAATTAATTACTCAACGTAAAT TTGATAATTTAACTAAAGCTGAACGTGGTGGTTTATCTGAATTAGATAAAGCTGGTTTT ATTAAACGTCAATTAGTTGAAACTCGTCAAATTACTAAACATGTTGCTCAAATTTTAGA TTCTCGTATGAATACTAAATATGATGAAAATGATAAATTAATTCGTGAAGTTAAAGTTAT TACTTTAAAATCTAAATTAGTTTCTGATTTTCGTAAAGATTTTCAATTTTATAAAGTTCG TGAAATTAATAATTATCATCATGCTCATGATGCTTATTTAAATGCTGTTGTTGGTACTGC TTTAATTAAAAAATATCCTAAATTAGAATCTGAATTTGTTTATGGTGATTATAAAGTTTAT GATGTTCGTAAAATGATTGCTAAATCTGAACAAGAAATTGGTAAAGCTACTGCTAAATA	143

[0745]

	TTTTTTTTATTCTAATATTATGAATTTTTTTAAAACTGAAATTACTTTAGCTAATGGTGAA ATTCGTAAACGTCCTTTAATTGAAACTAATGGTGAAACTGGTGAAATTGTTTGGGATA AAGGTCGTGATTTTGCTACTGTTCGTAAAGTTTTATCTATGCCTCAAGTTAATATTGTTA AAAAAACTGAAGTTCAAACTGGTGGTTTTTCTAAAGAATCTATTTTACCTAAACGTAA TTCTGATAAATTAATTGCTCGTAAAAAAGATTGGGATCCTAAAAAATATGGTGGTTTTG ATTCTCCTACTGTTGCTTATTCTGTTTTAGTTGTTGCTAAAGTTGAAAAAGGTAAATCT AAAAAATTAAAATCTGTTAAAGAATTATTAGGTATTACTATTATGGAACGTTCTTCTTTT GAAAAAAATCCTATTGATTTTTTAGAAGCTAAAGGTTATAAAGAAGTTAAAAAAGATT TAATTATTAAATTACCTAAATATTCTTTATTTGAATTAGAAAATGGTCGTAAACGTATGTT AGCTTCTGCTGGTGAATTACAAAAAGGTAATGAATTAGCTTTACCTTCTAAATATGTTA ATTTTTTATATTTAGCTTCTCATTATGAAAAATTAAAAGGTTCTCCTGAAGATAATGAAC AAAAACAATTATTTGTTGAACAACATAAACATTATTTAGATGAAATTATTGAACAAATT TCTGAATTTTCTAAACGTGTTATTTTAGCTGATGCTAATTTAGATAAAGTTTTATCTGCT TATAATAAACATCGTGATAAACCTATTCGTGAACAAGCTGAAAATATTATTCATTTATTT ACTTTAACTAATTTAGGTGCTCCTGCTGCTTTTAAATATTTTGATACTACTATTGATCGT AAACGTTATACTTCTACTAAAGAAGTTTTAGATGCTACTTTAATTCATCAATCTATTACT GGTTTATATGAAACTCGTATTGATTTATCTCAATTAGGTGGTGATGGTGGTGGTTCTCCT AAAAAAAAACGTAAAGTT	
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[0746]

	CGTCGCCCAAATCCTCGACTCCAGAATGAACACCAAATACGACGAAAACGACAAACT CATCAGAGAAGTCAAAGTCATCACCCTCAAATCCAAACTCGTCTCCGACTTCAGAAA AGACTTCCAATTCTACAAAGTCAGAGAAATCAACAACTACCACCACGCCCACGACGC CTACCTCAACGCCGTCGTCGGCACCGCCCTCATCAAAAAATACCCCAAACTCGAATCC GAATTCGTCTACGGCGACTACAAAGTCTACGACGTCAGAAAAATGATCGCCAAATCC GAACAAGAAATCGGCAAAGCCACCGCCAAATACTTCTTCTACTCCAACATCATGAACT TCTTCAAAACCGAAATCACCCTCGCCAACGGCGAAATCAGAAAAAGACCCCTCATCG AAACCAACGGCGAAACCGGCGAAATCGTCTGGGACAAAGGCAGAGACTTCGCCACC GTCAGAAAAGTCCTCTCCATGCCCCAAGTCAACATCGTCAAAAAAACCGAAGTCCAA ACCGGCGGCTTCTCCAAAGAATCCATCCTCCCCAAAAGAAACTCCGACAAACTCATC GCCAGAAAAAAAGACTGGGACCCCAAAAAATACGGCGGCTTCGACTCCCCCACCGT CGCCTACTCCGTCCTCGTCGTCGCCAAAGTCGAAAAAGGCAAATCCAAAAAACTCAA ATCCGTCAAAGAACTCCTCGGCATCACCATCATGGAAAGATCCTCCTTCGAAAAAAA CCCCATCGACTTCCTCGAAGCCAAAGGCTACAAAGAAGTCAAAAAAGACCTCATCAT CAAACTCCCCAAATACTCCCTCTTCGAACTCGAAAACGGCAGAAAAAGAATGCTCGC CTCCGCCGGCGAACTCCAAAAAGGCAACGAACTCGCCCTCCCCTCCAAATACGTCAA CTTCCTCTACCTCGCCTCCCACTACGAAAAACTCAAAGGCTCCCCCGAAGACAACGA ACAAAAACAACTCTTCGTCGAACAACACAAACACTACCTCGACGAAATCATCGAACA AATCTCCGAATTCTCCAAAAGAGTCATCCTCGCCGACGCCAACCTCGACAAAGTCCT CTCCGCCTACAACAAACACAGAGACAAACCCATCAGAGAACAAGCCGAAAACATCA TCCACCTCTTCACCCTCACCAACCTCGGCGCCCCCGCCGCCTTCAAATACTTCGACAC CACCATCGACAGAAAAAGATACACCTCCACCAAAGAAGTCCTCGACGCCACCCTCAT CCACCAATCCATCACCGGCCTCTACGAAACCAGAATCGACCTCTCCCAACTCGGCGG CGACGGCGGCGGCTCCCCCAAAAAAAAAAAGAAAAGTC	
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[0747]

	TATGATGTGGATCATATTGTGCCTCAGAGTTTTTTGAAGGATGATAGTATTGATAATAAG GTGTTGACAAGAAGTGATAAGAATAGAGGAAAGAGTGATAATGTGCCTAGTGAGGAG GTGGTGAAGAAGATGAAGAATTATTGGAGACAGTTGTTGAATGCTAAGTTGATTACAC AGAGAAAGTTTGATAATTTGACAAAGGCTGAGAGAGGAGGATTGAGTGAGTTGGATA AGGCTGGATTTATTAAGAGACAGTTGGTGGAGACAAGACAGATTACAAAGCATGTGG CTCAGATTTTGGATAGTAGAATGAATACAAAGTATGATGAGAATGATAAGTTGATTAGA GAGGTGAAGGTGATTACATTGAAGAGTAAGTTGGTGAGTGATTTTAGAAAGGATTTTC AGTTTTATAAGGTGAGAGAGATTAATAATTATCATCATGCTCATGATGCTTATTTGAATG CTGTGGTGGGAACAGCTTTGATTAAGAAGTATCCTAAGTTGGAGAGTGAGTTTGTGTA TGGAGATTATAAGGTGTATGATGTGAGAAAGATGATTGCTAAGAGTGAGCAGGAGATT GGAAAGGCTACAGCTAAGTATTTTTTTTATAGTAATATTATGAATTTTTTTAAGACAGAG ATTACATTGGCTAATGGAGAGATTAGAAAGAGACCTTTGATTGAGACAAATGGAGAG ACAGGAGAGATTGTGTGGGATAAGGGAAGAGATTTTGCTACAGTGAGAAAGGTGTTG AGTATGCCTCAGGTGAATATTGTGAAGAAGACAGAGGTGCAGACAGGAGGATTTAGT AAGGAGAGTATTTTGCCTAAGAGAAATAGTGATAAGTTGATTGCTAGAAAGAAGGATT GGGATCCTAAGAAGTATGGAGGATTTGATAGTCCTACAGTGGCTTATAGTGTGTTGGT GGTGGCTAAGGTGGAGAAGGGAAAGAGTAAGAAGTTGAAGAGTGTGAAGGAGTTGT TGGGAATTACAATTATGGAGAGAAGTAGTTTTGAGAAGAATCCTATTGATTTTTTGGA GGCTAAGGGATATAAGGAGGTGAAGAAGGATTTGATTATTAAGTTGCCTAAGTATAGT TTGTTTGAGTTGGAGAATGGAAGAAAGAGAATGTTGGCTAGTGCTGGAGAGTTGCAG AAGGGAAATGAGTTGGCTTTGCCTAGTAAGTATGTGAATTTTTTGTATTTGGCTAGTCA TTATGAGAAGTTGAAGGGAAGTCCTGAGGATAATGAGCAGAAGCAGTTGTTTGTGGA GCAGCATAAGCATTATTTGGATGAGATTATTGAGCAGATTAGTGAGTTTAGTAAGAGA GTGATTTTGGCTGATGCTAATTTGGATAAGGTGTTGAGTGCTTATAATAAGCATAGAGA TAAGCCTATTAGAGAGCAGGCTGAGAATATTATTCATTTGTTTACATTGACAAATTTGG GAGCTCCTGCTGCTTTTAAGTATTTTGATACAACAATTGATAGAAAGAGATATACAAGT ACAAAGGAGGTGTTGGATGCTACATTGATTCATCAGAGTATTACAGGATTGTATGAGA CAAGAATTGATTTGAGTCAGTTGGGAGGAGATGGAGGAGGAAGTCCTAAGAAGAAG AGAAAGGTG	
使用表4的低A密码子的Cas9 ORF(无起始或终止密码子;适于包括在融合蛋白质编码序列中)	GACAAGAAGTACTCCATCGGCCTGGACATCGGCACCAACTCCGTGGGCTGGGCCGTG ATCACCGACGAGTACAAGGTGCCCTCCAAGAAGTTCAAGGTGCTGGGCAACACCGA CCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGAC CGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAGA ACCGGATCTGCTACCTGCAGGAGATCTTCTCCAACGAGATGGCCAAGGTGGACGACT CCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAG CGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCC ACCATCTACCACCTGCGGAAGAAGCTGGTGGACTCCACCGACAAGGCCGACCTGCGG CTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGG GCGACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGA CCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCTCCGGCGTGGACGCCAAGG CCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGC TGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCC TGACCCCCAACTTCAAGTCCAACTTCGACCTGGCCGAGGACGCCAAGCTGCAGCTGT CCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGT ACGCCGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACA TCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGTCCGCCTCCATGATCAAGC GGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAG CTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGC TACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCCTG GAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCT GCGGAAGCAGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGA GCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGACAACCG GGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGC CCGGGGCAACTCCCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCCC CTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCCAGTCCTTCATCGAGCG GATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCT GCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGA GGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAAGGCCATCGTGGACCT GCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCA AGAAGATCGAGTGCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACG CCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGG ACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCG AGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGAC AAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGTCCCG GAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCT GAAGTCCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATCCACGACGACTCCCT GACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGACTCCCTGC	146

[0748]

	ACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGA CCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAAC ATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACTC CCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCC TGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTAC TACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGCTG TCCGACTACGACGTGGACCACATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCATC GACAACAAGGTGCTGACCCGGTCCGACAAGAACCGGGGCAAGTCCGACAACGTGCC CTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCA AGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTG TCCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGAT CACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGA ACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCG ACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACCACG CCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCA AGCTGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGA TCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACTCCA ACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGC GGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGG GACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAG ACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCC GACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGA CTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTC CAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTC CTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGA AGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGA AGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCC TCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCC CCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGA CGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAA CCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGC AGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCT TCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGC TGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACC TGTCCCAGCTGGGCGGCGACGGCGGCGGCTCCCCCAAGAAGAAGCGGAAGGTG	
使用表4的低A/U密码子的Cas9 ORF(无起始或终止密码子;适于包括在融合蛋白质编码序列中)	GACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACAGCGTGGGCTGGGCCGT GATCACCGACGAGTACAAGGTGCCCAGCAAGAAGTTCAAGGTGCTGGGCAACACCG ACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAG ACCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAA GAACCGGATCTGCTACCTGCAGGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGA CAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACG AGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACC CCACCATCTACCACCTGCGGAAGAAGCTGGTGGACAGCACCGACAAGGCCGACCTG CGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCG AGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTG CAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGCGGCGTGGACGC CAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCG CCCAGCTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGC CTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCT GCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCG GCGACCAGTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGC TGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCAGCA TGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGC GGCAGCAGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGC TACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAG CCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGA GGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCA CCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAA GGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGG CCCCCTGGCCCGGGGCAACAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGA CCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGC TTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCC AAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAG TACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGC CATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGG AGGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGCGGCGTGGAG GACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC	147

[0749]

	AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGAC CCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCA CCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGG GCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACC ATCCTGGACTTCCTGAAGAGCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATC CACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCA GGGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGA AGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGG CACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAA GGGCCAGAAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAG CTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGA GAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCT GGACATCAACCGGCTGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCT GAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGACAAGAACCGGGGC AAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCG GCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGG CCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTG GTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAA CACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGA AGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAG ATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCC CTGATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTG TACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGC CAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCC AACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGAT CGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGC ATCCTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCC CAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGC CAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGC ATCACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCC AAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCT GTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGA AGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCC ACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTG GAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAA GCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGC ACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGA CCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGC GGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCG GCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACGGCGGCGGCAGC CCCAAGAAGAAGCGGAAGGTG	
使用表4的低A密码子，具有两个C端NLS序列的Cas9 ORF(无起始或终止密码子；适于包括在融合蛋白质编码序列中)	GACAAGAAGTACTCCATCGGCCTGGACATCGGCACCAACTCCGTGGGCTGGGCCGTG ATCACCGACGAGTACAAGGTGCCCTCCAAGAAGTTCAAGGTGCTGGGCAACACCGA CCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGAC CGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAGA ACCGGATCTGCTACCTGCAGGAGATCTTCTCCAACGAGATGGCCAAGGTGGACGACT CCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAG CGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCC ACCATCTACCACCTGCGGAAGAAGCTGGTGGACTCCACCGACAAGGCCGACCTGCGG CTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGG GCGACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGA CCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCTCCGGCGTGGACGCCAAGG CCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGC TGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCC TGACCCCCAACTTCAAGTCCAACTTCGACCTGGCCGAGGACGCCAAGCTGCAGCTGT CCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGT ACGCCGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACA TCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGTCCGCCTCCATGATCAAGC GGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAG CTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGC TACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCCTG GAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCT GCGGAAGCAGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGA GCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGACAACCG GGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGC CCGGGGCAACTCCCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCCC	148

[0750]

	CTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCCAGTCCTTCATCGAGCG GATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCT GCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGA GGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAAGGCCATCGTGGACCT GCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCA AGAAGATCGAGTGCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACG CCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGG ACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCG AGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGAC AAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGTCCCG GAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCT GAAGTCCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATCCACGACGACTCCCT GACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGACTCCCTGC ACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGA CCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAAC ATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACTC CCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCC TGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTAC TACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGCTG TCCGACTACGACGTGGACCACATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCATC GACAACAAGGTGCTGACCCGGTCCGACAAGAACCGGGGCAAGTCCGACAACGTGCC CTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCA AGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTG TCCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGAT CACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGA ACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCG ACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACCACG CCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCA AGCTGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGA TCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACTCCA ACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGC GGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGG GACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAG ACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCC GACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGA CTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTC CAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTC CTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGA AGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGA AGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCC TCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCC CCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGA CGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAA CCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGC AGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCT TCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGC TGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACC TGTCCCAGCTGGGCGGCGACGGCTCCGGCTCCCCCAAGAAGAAGCGGAAGGTGGAC GGCTCCCCCAAGAAGAAGCGGAAGGTGGACTCCGGC	
使用表4的低A密码子的Cas9切口酶ORF (无起始或终止密码子;适于包括在融合蛋白质编码序列中)	GACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCCGTG ATCACCGACGAGTACAAGGTGCCCTCCAAGAAGTTCAAGGTGCTGGGCAACACCGA CCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGAC CGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAGA ACCGGATCTGCTACCTGCAGGAGATCTTCTCCAACGAGATGGCCAAGGTGGACGACT CCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAG CGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCC ACCATCTACCACCTGCGGAAGAAGCTGGTGGACTCCACCGACAAGGCCGACCTGCGG CTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGG GCGACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGA CCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCTCCGGCGTGGACGCCAAGG CCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGC TGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCC TGACCCCCAACTTCAAGTCCAACTTCGACCTGGCCGAGGACGCCAAGCTGCAGCTGT CCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGT ACGCCGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACA TCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGTCCGCCTCCATGATCAAGC GGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAG	149

[0751]

	CTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGC TACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCCTG GAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCT GCGGAAGCAGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGA GCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGACAACCG GGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGC CCGGGGCAACTCCCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCCC CTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCCAGTCCTTCATCGAGCG GATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCT GCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGA GGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAAGGCCATCGTGGACCT GCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCA AGAAGATCGAGTGCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACG CCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGG ACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCG AGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGAC AAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGTCCCG GAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCT GAAGTCCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATCCACGACGACTCCCT GACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGACTCCCTGC ACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGA CCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAAC ATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACTC CCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCC TGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTAC TACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGCTG TCCGACTACGACGTGGACCACATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCATC GACAACAAGGTGCTGACCCGGTCCGACAAGAACCGGGGCAAGTCCGACAACGTGCC CTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCA AGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTG TCCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGAT CACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGA ACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCG ACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACCACG CCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCA AGCTGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGA TCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACTCCA ACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGC GGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGG GACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAG ACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCC GACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGA CTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTC CAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTC CTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGA AGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGA AGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCC TCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCC CCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGA CGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAA CCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGC AGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCT TCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGC TGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACC TGTCCCAGCTGGGCGGCGACGGCGGCGGCTCCCCCAAGAAGAAGCGGAAGGTG	
使用表4的低A密码子的Cas9切口酶ORF (无NLS且无起始或终止密码子；适于包括在融合蛋白质	GACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCCGTG ATCACCGACGAGTACAAGGTGCCCTCCAAGAAGTTCAAGGTGCTGGGCAACACCGA CCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGAC CGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAGA ACCGGATCTGCTACCTGCAGGAGATCTTCTCCAACGAGATGGCCAAGGTGGACGACT CCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAG CGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCC ACCATCTACCACCTGCGGAAGAAGCTGGTGGACTCCACCGACAAGGCCGACCTGCGG CTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGG GCGACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGA CCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCTCCGGCGTGGACGCCAAGG CCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGC	150

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编码序列中)	TGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCC TGACCCCCAACTTCAAGTCCAACTTCGACCTGGCCGAGGACGCCAAGCTGCAGCTGT CCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGT ACGCCGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACA TCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGTCCGCCTCCATGATCAAGC GGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAG CTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGC TACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCCTG GAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCT GCGGAAGCAGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGA GCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGACAACCG GGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGC CCGGGGCAACTCCCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCCC CTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCCAGTCCTTCATCGAGCG GATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCT GCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGA GGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAAGGCCATCGTGGACCT GCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCA AGAAGATCGAGTGCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACG CCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGG ACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCG AGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGAC AAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGTCCCG GAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCT GAAGTCCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATCCACGACGACTCCCT GACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGACTCCCTGC ACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGA CCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAAC ATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACTC CCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCC TGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTAC TACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGCTG TCCGACTACGACGTGGACCACATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCATC GACAACAAGGTGCTGACCCGGTCCGACAAGAACCGGGGCAAGTCCGACAACGTGCC CTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCA AGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTG TCCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGAT CACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGA ACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCG ACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACCACG CCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCA AGCTGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGA TCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACTCCA ACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGC GGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGG GACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAG ACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCC GACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGA CTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTC CAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTC CTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGA AGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGA AGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCC TCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCC CCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGA CGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAA CCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGC AGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCT TCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGC TGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACC TGTCCCAGCTGGGCGGCGAC	
使用表4的低A密码子，具有两个C端NLS序列的	GACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCCGTG ATCACCGACGAGTACAAGGTGCCCTCCAAGAAGTTCAAGGTGCTGGGCAACACCGA CCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGAC CGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAGA ACCGGATCTGCTACCTGCAGGAGATCTTCTCCAACGAGATGGCCAAGGTGGACGACT CCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAG	151

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Cas9切口酶ORF (无起始或终止密码子; 适于包括在融合蛋白质编码序列中)	CGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCC ACCATCTACCACCTGCGGAAGAAGCTGGTGGACTCCACCGACAAGGCCGACCTGCGG CTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGG GCGACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGA CCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCTCCGGCGTGGACGCCAAGG CCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGC TGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCC TGACCCCCAACTTCAAGTCCAACTTCGACCTGGCCGAGGACGCCAAGCTGCAGCTGT CCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGT ACGCCGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACA TCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGTCCGCCTCCATGATCAAGC GGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAG CTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGC TACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCCTG GAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCT GCGGAAGCAGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGA GCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGACAACCG GGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGC CCGGGGCAACTCCCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCCC CTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCCAGTCCTTCATCGAGCG GATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCT GCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGA GGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAAGGCCATCGTGGACCT GCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCA AGAAGATCGAGTGCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACG CCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGG ACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCG AGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGAC AAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGTCCCG GAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCT GAAGTCCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATCCACGACGACTCCCT GACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGACTCCCTGC ACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGA CCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAAC ATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACTC CCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCC TGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTAC TACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGCTG TCCGACTACGACGTGGACCACATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCATC GACAACAAGGTGCTGACCCGGTCCGACAAGAACCGGGGCAAGTCCGACAACGTGCC CTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCA AGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTG TCCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGAT CACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGA ACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCG ACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACCACG CCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCA AGCTGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGA TCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACTCCA ACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGC GGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGG GACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAG ACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCC GACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGA CTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTC CAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTC CTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGA AGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGA AGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCC TCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCC CCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGA CGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAA CCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGC AGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCT TCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGC TGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACC TGTCCCAGCTGGGCGGCGACGGCTCCGGCTCCCCCAAGAAGAAGCGGAAGGTGGAC	

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	GGCTCCCCCAAGAAGAAGCGGAAGGTGGACTCCGGC	
使用表4的低A密码子的dCas9 ORF(无起始或终止密码子;适于包括在融合蛋白质编码序列中)	GACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCCGTG ATCACCGACGAGTACAAGGTGCCCTCCAAGAAGTTCAAGGTGCTGGGCAACACCGA CCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGAC CGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAGA ACCGGATCTGCTACCTGCAGGAGATCTTCTCCAACGAGATGGCCAAGGTGGACGACT CCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAG CGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCC ACCATCTACCACCTGCGGAAGAAGCTGGTGGACTCCACCGACAAGGCCGACCTGCGG CTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGG GCGACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGA CCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCTCCGGCGTGGACGCCAAGG CCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGC TGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCC TGACCCCCAACTTCAAGTCCAACTTCGACCTGGCCGAGGACGCCAAGCTGCAGCTGT CCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGT ACGCCGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACA TCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGTCCGCCTCCATGATCAAGC GGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAG CTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGC TACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCCTG GAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCT GCGGAAGCAGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGA GCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGACAACCG GGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGC CCGGGGCAACTCCCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCCC CTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCCAGTCCTTCATCGAGCG GATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCT GCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGA GGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAAGGCCATCGTGGACCT GCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCA AGAAGATCGAGTGCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACG CCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGG ACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCG AGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGAC AAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGTCCCG GAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCT GAAGTCCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATCCACGACGACTCCCT GACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGACTCCCTGC ACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGA CCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAAC ATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACTC CCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCC TGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTAC TACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGCTG TCCGACTACGACGTGGACGCCATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCATC GACAACAAGGTGCTGACCCGGTCCGACAAGAACCGGGGCAAGTCCGACAACGTGCC CTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCA AGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTG TCCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGAT CACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGA ACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCG ACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACCACG CCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCA AGCTGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGA TCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACTCCA ACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGC GGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGG GACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAG ACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCC GACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGA CTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTC CAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTC CTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGA AGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGA AGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCC TCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCC	152

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	CCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGA CGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAA CCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGC AGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCT TCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGC TGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACC TGTCCCAGCTGGGCGGCGACGGCGGCGGCTCCCCCAAGAAGAAGCGGAAGGTG	
使用表4的低A密码子的dCas9 ORF(无NLS且无起始或终止密码子;适于包括在融合蛋白质编码序列中)	GACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCCGTG ATCACCGACGAGTACAAGGTGCCCTCCAAGAAGTTCAAGGTGCTGGGCAACACCGA CCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGAC CGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAGA ACCGGATCTGCTACCTGCAGGAGATCTTCTCCAACGAGATGGCCAAGGTGGACGACT CCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAG CGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCC ACCATCTACCACCTGCGGAAGAAGCTGGTGGACTCCACCGACAAGGCCGACCTGCGG CTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGG GCGACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGA CCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCTCCGGCGTGGACGCCAAGG CCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGC TGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCC TGACCCCCAACTTCAAGTCCAACTTCGACCTGGCCGAGGACGCCAAGCTGCAGCTGT CCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGT ACGCCGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACA TCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGTCCGCCTCCATGATCAAGC GGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAG CTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGC TACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCCTG GAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCT GCGGAAGCAGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGA GCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGACAACCG GGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGC CCGGGGCAACTCCCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCCC CTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCCAGTCCTTCATCGAGCG GATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCT GCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGA GGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAAGGCCATCGTGGACCT GCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCA AGAAGATCGAGTGCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACG CCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGG ACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCG AGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGAC AAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGTCCCG GAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCT GAAGTCCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATCCACGACGACTCCCT GACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGACTCCCTGC ACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGA CCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAAC ATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACTC CCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCC TGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTAC TACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGCTG TCCGACTACGACGTGGACGCCATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCATC GACAACAAGGTGCTGACCCGGTCCGACAAGAACCGGGGCAAGTCCGACAACGTGCC CTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCA AGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTG TCCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGAT CACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGA ACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCG ACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACCACG CCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCA AGCTGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGA TCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACTCCA ACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGC GGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGG GACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAG ACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCC GACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGA	153

	CTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTC CAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTC CTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGA AGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGA AGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCC TCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCC CCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGA CGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAA CCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGC AGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCT TCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGC TGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACC TGTCCCAGCTGGGCGGCGAC	
使用表4的低A密码子，具有两个C端NLS序列的dCas9 ORF(无起始或终止密码子；适于包括在融合蛋白质编码序列中)	GACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCCGTG ATCACCGACGAGTACAAGGTGCCCTCCAAGAAGTTCAAGGTGCTGGGCAACACCGA CCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGAC CGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAGA ACCGGATCTGCTACCTGCAGGAGATCTTCTCCAACGAGATGGCCAAGGTGGACGACT CCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAG CGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCC ACCATCTACCACCTGCGGAAGAAGCTGGTGGACTCCACCGACAAGGCCGACCTGCGG CTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGG GCGACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGA CCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCTCCGGCGTGGACGCCAAGG CCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGC TGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCC TGACCCCCAACTTCAAGTCCAACTTCGACCTGGCCGAGGACGCCAAGCTGCAGCTGT CCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGT ACGCCGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACA TCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGTCCGCCTCCATGATCAAGC GGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAG CTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGC TACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCCTG GAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCT GCGGAAGCAGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGA GCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGACAACCG GGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGC CCGGGGCAACTCCCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCCC CTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCCAGTCCTTCATCGAGCG GATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCT GCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGA GGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAAGGCCATCGTGGACCT GCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCA AGAAGATCGAGTGCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACG CCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGG ACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCG AGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGAC AAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGTCCCG GAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCT GAAGTCCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATCCACGACGACTCCCT GACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGACTCCCTGC ACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGA CCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAAC ATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACTC CCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCC TGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTAC TACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGCTG TCCGACTACGACGTGGACGCCATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCATC GACAACAAGGTGCTGACCCGGTCCGACAAGAACCGGGGCAAGTCCGACAACGTGCC CTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCA AGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTG TCCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGAT CACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGA ACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCG ACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACCACG CCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCA AGCTGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGA	154

[0756]

[0757]

	TCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACTCCA ACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGC GGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGG GACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAG ACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCC GACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGA CTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTC CAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTC CTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGA AGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGA AGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCC TCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCC CCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGA CGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAA CCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGC AGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCT TCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGC TGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACC TGTCCCAGCTGGGCGGCGACGGCTCCGGCTCCCCCAAGAAGAAGCGGAAGGTGGAC GGCTCCCCCAAGAAGAAGCGGAAGGTGGACTCCGGC	
使用表4的低A/U密码子，具有两个C端NLS序列的Cas9 ORF(无起始或终止密码子；适于包括在融合蛋白质编码序列中)	GACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACAGCGTGGGCTGGGCCGT GATCACCGACGAGTACAAGGTGCCCAGCAAGAAGTTCAAGGTGCTGGGCAACACCG ACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAG ACCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAA GAACCGGATCTGCTACCTGCAGGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGA CAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACG AGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACC CCACCATCTACCACCTGCGGAAGAAGCTGGTGGACAGCACCGACAAGGCCGACCTG CGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCG AGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTG CAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGCGGCGTGGACGC CAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCG CCCAGCTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGC CTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCT GCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCG GCGACCAGTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGC TGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCAGCA TGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGC GGCAGCAGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGC TACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAG CCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGA GGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCA CCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAA GGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGG CCCCCTGGCCCGGGGCAACAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGA CCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGC TTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCC AAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAG TACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGC CATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGG AGGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGCGGCGTGGAG GACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGAC CCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCA CCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGG GCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACC ATCCTGGACTTCCTGAAGAGCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATC CACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCA GGGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGA AGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGG CACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAA GGGCCAGAAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAG CTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGA GAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCT GGACATCAACCGGCTGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCT GAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGACAAGAACCGGGGC AAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCG	155

[0758]

	GCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGG CCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTG GTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAA CACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGA AGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAG ATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCC CTGATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTG TACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGC CAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCC AACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGAT CGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGC ATCCTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCC CAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGC CAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGC ATCACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCC AAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCT GTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGA AGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCC ACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTG GAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAA GCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGC ACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGA CCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGC GGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCG GCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACGGCAGCGGCAGC CCCAAGAAGAAGCGGAAGGTGGACGGCAGCCCCAAGAAGAAGCGGAAGGTGGACA GCGGC	
使用表4的低A/U密码子的Cas9 ORF(无NLS且无起始或终止密码子；适于包括在融合蛋白质编码序列中)	GACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACAGCGTGGGCTGGGCCGT GATCACCGACGAGTACAAGGTGCCCAGCAAGAAGTTCAAGGTGCTGGGCAACACCG ACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAG ACCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAA GAACCGGATCTGCTACCTGCAGGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGA CAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACG AGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACC CCACCATCTACCACCTGCGGAAGAAGCTGGTGGACAGCACCGACAAGGCCGACCTG CGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCG AGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTG CAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGCGGCGTGGACGC CAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCG CCCAGCTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGC CTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCT GCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCG GCGACCAGTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGC TGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCAGCA TGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGC GGCAGCAGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGC TACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAG CCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGA GGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCA CCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAA GGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGG CCCCCTGGCCCGGGGCAACAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGA CCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGC TTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCC AAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAG TACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGC CATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGG AGGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGCGGCGTGGAG GACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGAC CCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCA CCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGG GCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACC ATCCTGGACTTCCTGAAGAGCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATC CACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCA GGGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGA	156

[0759]

	AGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGG CACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAA GGGCCAGAAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAG CTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGA GAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCT GGACATCAACCGGCTGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCT GAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGACAAGAACCGGGGC AAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCG GCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGG CCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTG GTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAA CACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGA AGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAG ATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCC CTGATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTG TACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGC CAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCC AACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGAT CGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGC ATCCTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCC CAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGC CAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGC ATCACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCC AAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCT GTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGA AGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCC ACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTG GAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAA GCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGC ACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGA CCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGC GGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCG GCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGAC	
使用表4的低A/U 密码子的 Cas9 切口酶ORF (无起始或终止密码子；适于包括在融合蛋白质编码序列中)	GACAAGAAGTACAGCATCGGCCTGGCCATCGGCACCAACAGCGTGGGCTGGGCCGT GATCACCGACGAGTACAAGGTGCCCAGCAAGAAGTTCAAGGTGCTGGGCAACACCG ACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAG ACCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAA GAACCGGATCTGCTACCTGCAGGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGA CAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACG AGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACC CCACCATCTACCACCTGCGGAAGAAGCTGGTGGACAGCACCGACAAGGCCGACCTG CGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCG AGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTG CAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGCGGCGTGGACGC CAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCG CCCAGCTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGC CTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCT GCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCG GCGACCAGTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGC TGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCAGCA TGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGC GGCAGCAGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGC TACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAG CCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGA GGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCA CCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAA GGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGG CCCCCTGGCCCGGGGCAACAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGA CCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGC TTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCC AAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAG TACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGC CATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGG AGGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGCGGCGTGGAG GACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGAC	157

[0760]

	CCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCA CCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGG GCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACC ATCCTGGACTTCCTGAAGAGCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATC CACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCA GGGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGA AGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGG CACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAA GGGCCAGAAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAG CTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGA GAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCT GGACATCAACCGGCTGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCT GAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGACAAGAACCGGGGC AAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCG GCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGG CCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTG GTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAA CACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGA AGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAG ATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCC CTGATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTG TACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGC CAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCC AACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGAT CGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGC ATCCTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCC CAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGC CAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGC ATCACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCC AAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCT GTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGA AGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCC ACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTG GAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAA GCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGC ACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGA CCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGC GGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCG GCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACGGCGGCGGCAGC CCCAAGAAGAAGCGGAAGGTG	
使用表4的低A/U密码子，具有两个C端NLS序列的Cas9切口酶ORF (无起始或终止密码子；适于包括在融合蛋白质编码序列中)	GACAAGAAGTACAGCATCGGCCTGGCCATCGGCACCAACAGCGTGGGCTGGGCCGT GATCACCGACGAGTACAAGGTGCCCAGCAAGAAGTTCAAGGTGCTGGGCAACACCG ACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAG ACCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAA GAACCGGATCTGCTACCTGCAGGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGA CAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACG AGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACC CCACCATCTACCACCTGCGGAAGAAGCTGGTGGACAGCACCGACAAGGCCGACCTG CGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCG AGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTG CAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGCGGCGTGGACGC CAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCG CCCAGCTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGC CTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCT GCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCG GCGACCAGTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGC TGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCAGCA TGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGC GGCAGCAGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGC TACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAG CCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGA GGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCA CCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAA GGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGG CCCCCTGGCCCGGGGCAACAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGA CCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGC	158

[0761]

	TTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCC AAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAG TACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGC CATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGG AGGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGCGGCGTGGAG GACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGAC CCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCA CCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGG GCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACC ATCCTGGACTTCCTGAAGAGCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATC CACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCA GGGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGA AGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGG CACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAA GGGCCAGAAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAG CTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGA GAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCT GGACATCAACCGGCTGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCT GAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGACAAGAACCGGGGC AAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCG GCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGG CCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTG GTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAA CACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGA AGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAG ATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCC CTGATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTG TACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGC CAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCC AACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGAT CGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGC ATCCTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCC CAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGC CAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGC ATCACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCC AAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCT GTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGA AGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCC ACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTG GAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAA GCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGC ACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGA CCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGC GGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCG GCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACGGCAGCGGCAGC CCCAAGAAGAAGCGGAAGGTGGACGGCAGCCCCAAGAAGAAGCGGAAGGTGGACA GCGGC	
使用表4的低A/U密码子的Cas9切口酶ORF（无NLS且无起始或终止密码子;适于包括在融合蛋白质编码序列中)	GACAAGAAGTACAGCATCGGCCTGGcCATCGGCACCAACAGCGTGGGCTGGGCCGTG ATCACCGACGAGTACAAGGTGCCCAGCAAGAAGTTCAAGGTGCTGGGCAACACCGA CCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGA CCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAG AACCGGATCTGCTACCTGCAGGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGAC AGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGA GCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCC CACCATCTACCACCTGCGGAAGAAGCTGGTGGACAGCACCGACAAGGCCGACCTGC GGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGA GGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGC AGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGCGGCGTGGACGCC AAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCGC CCAGCTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCT GGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCTGC AGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGC GACCAGTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTG AGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCAGCATG ATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCG	159

[0762]

	GCAGCAGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCT ACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAGC CCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAG GACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCAC CTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAG GACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGC CCCCTGGCCCGGGGCAACAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGAC CATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGCT TCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCA AGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGT ACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGCC ATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGA GGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGCGGCGTGGAGG ACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACA AGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACC CTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCAC CTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGG CCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACCA TCCTGGACTTCCTGAAGAGCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATCC ACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAG GGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAA GGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGC ACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAG GGCCAGAAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCT GGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGA AGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGG ACATCAACCGGCTGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCTGA AGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGACAAGAACCGGGGCAA GAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGC AGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCC GAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGT GGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAACA CCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAG AGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATC AACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTG ATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTAC GACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGCCAA GTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAAC GGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGT GTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCCAGG TGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATC CTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAA GAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGCCAA GGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGCATC ACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAG GGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTC GAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGAAGG GCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCCACT ACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGA GCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGCG GGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGCACC GGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCA ACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGT ACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGC CTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGAC	
使用表4的低A/U密码子的 dCas9 ORF (无起始或终止密码子;适于包括在融合蛋白质编码序列	GACAAGAAGTACAGCATCGGCCTGGcCATCGGCACCAACAGCGTGGGCTGGGCCGTG ATCACCGACGAGTACAAGGTGCCCAGCAAGAAGTTCAAGGTGCTGGGCAACACCGA CCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGA CCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAG AACCGGATCTGCTACCTGCAGGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGAC AGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGA GCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCC CACCATCTACCACCTGCGGAAGAAGCTGGTGGACAGCACCGACAAGGCCGACCTGC GGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGA GGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGC AGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGCGGCGTGGACGCC AAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCGC	160

[0763]

中)	CCAGCTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCT GGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCTGC AGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGC GACCAGTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTG AGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCAGCATG ATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCG GCAGCAGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCT ACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAGC CCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAG GACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCAC CTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAG GACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGC CCCCTGGCCCGGGGCAACAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGAC CATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGCT TCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCA AGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGT ACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGCC ATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGA GGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGCGGCGTGGAGG ACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACA AGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACC CTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCAC CTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGG CCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACCA TCCTGGACTTCCTGAAGAGCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATCC ACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAG GGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAA GGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGC ACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAG GGCCAGAAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCT GGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGA AGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGG ACATCAACCGGCTGAGCGACTACGACGTGGACgcCATCGTGCCCCAGAGCTTCCTGAA GGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGACAAGAACCGGGGCAAG AGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCA GCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCG AGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTG GAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAACAC CAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGA GCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCA ACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGA TCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACG ACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGCCAAG TACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACG GCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTG TGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCCAGGT GAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATCC TGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAG AAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGCCAAG GTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGCATCA CCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGG GCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTCG AGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGAAGGGC AACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCCACTAC GAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGC AGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGCGG GTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGCACCG GGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAA CCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTA CACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGCCT GTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACGGCGGCGGCAGCCCCA AGAAGAAGCGGAAGGTG	
使用表4的低A/U密码子，具有两个C端	GACAAGAAGTACAGCATCGGCCTGGCCATCGGCACCAACAGCGTGGGCTGGGCCGT GATCACCGACGAGTACAAGGTGCCCAGCAAGAAGTTCAAGGTGCTGGGCAACACCG ACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAG ACCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAA GAACCGGATCTGCTACCTGCAGGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGA	161

[0764]

NLS序列的dCas9 ORF(无起始或终止密码子;适于包括在融合蛋白质编码序列中)	CAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACG AGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACC CCACCATCTACCACCTGCGGAAGAAGCTGGTGGACAGCACCGACAAGGCCGACCTG CGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCG AGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTG CAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGCGGCGTGGACGC CAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCG CCCAGCTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGC CTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCT GCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCG GCGACCAGTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGC TGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCAGCA TGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGC GGCAGCAGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGC TACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAG CCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGA GGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCA CCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAA GGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGG CCCCCTGGCCCGGGGCAACAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGA CCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGC TTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCC AAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAG TACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGC CATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGG AGGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGCGGCGTGGAG GACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGAC CCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCA CCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGG GCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACC ATCCTGGACTTCCTGAAGAGCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATC CACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCA GGGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGA AGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGG CACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAA GGGCCAGAAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAG CTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGA GAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCT GGACATCAACCGGCTGAGCGACTACGACGTGGACGCCATCGTGCCCCAGAGCTTCCT GAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGACAAGAACCGGGGC AAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCG GCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGG CCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTG GTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAA CACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGA AGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAG ATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCC CTGATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTG TACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGC CAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCC AACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGAT CGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGC ATCCTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCC CAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGC CAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGC ATCACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCC AAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCT GTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGA AGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCC ACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTG GAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAA GCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGC ACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGA CCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGC GGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCG	

[0765]

	GCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACGGCAGCGGCAGC CCCAAGAAGAAGCGGAAGGTGGACGGCAGCCCCAAGAAGAAGCGGAAGGTGGACA GCGGC	
使用表4的低A/U密码子的 dCas9 ORF (无NLS且无起始或终止密码子；适于包括在融合蛋白质编码序列中)	GACAAGAAGTACAGCATCGGCCTGGcCATCGGCACCAACAGCGTGGGCTGGGCCGTG ATCACCGACGAGTACAAGGTGCCCAGCAAGAAGTTCAAGGTGCTGGGCAACACCGA CCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGA CCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAG AACCGGATCTGCTACCTGCAGGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGAC AGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGA GCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCC CACCATCTACCACCTGCGGAAGAAGCTGGTGGACAGCACCGACAAGGCCGACCTGC GGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGA GGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGC AGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGCGGCGTGGACGCC AAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCGC CCAGCTGCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCT GGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCTGC AGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGC GACCAGTACGCCGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTG AGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCAGCATG ATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCG GCAGCAGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCT ACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAGC CCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAG GACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCAC CTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAG GACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGC CCCCTGGCCCGGGGCAACAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGAC CATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGCT TCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCA AGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGT ACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGCC ATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGA GGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGCGGCGTGGAGG ACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACA AGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACC CTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTACGCCCAC CTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGG CCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACCA TCCTGGACTTCCTGAAGAGCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATCC ACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAG GGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAA GGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGC ACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAG GGCCAGAAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCT GGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGA AGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGG ACATCAACCGGCTGAGCGACTACGACGTGGACgcCATCGTGCCCCAGAGCTTCCTGAA GGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGACAAGAACCGGGGCAAG AGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCA GCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCG AGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTG GAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAACAC CAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGA GCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCA ACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGA TCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACG ACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGCCAAG TACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACG GCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTG TGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCCAGGT GAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATCC TGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAG AAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGCCAAG GTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGCATCA CCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGG GCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTCG	162

[0766]

	AGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGAAGGGC AACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCCACTAC GAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGC AGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGCGG GTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGCACCG GGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAA CCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTA CACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGCCT GTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGAC	
使用表4的低A密码子的Nme Cas9 ORF (无起始或终止密码子;适于包括在融合蛋白质编码序列中)	GCCGCCTTCAAGCCCAACTCCATCAACTACATCCTGGGCCTGGACATCGGCATCGCCT CCGTGGGCTGGGCCATGGTGGAGATCGACGAGGAGGAGAACCCCATCCGGCTGATCG ACCTGGGCGTGCGGGTGTTCGAGCGGGCCGAGGTGCCCAAGACCGGCGACTCCCTG GCCATGGCCCGGCGGCTGGCCCGGTCCGTGCGGCGGCTGACCCGGCGGCGGGCCCA CCGGCTGCTGCGGACCCGGCGGCTGCTGAAGCGGGAGGGCGTGCTGCAGGCCGCCA ACTTCGACGAGAACGGCCTGATCAAGTCCCTGCCCAACACCCCCTGGCAGCTGCGGG CCGCCGCCCTGGACCGGAAGCTGACCCCCCTGGAGTGGTCCGCCGTGCTGCTGCACC TGATCAAGCACCGGGGCTACCTGTCCCAGCGGAAGAACGAGGGCGAGACCGCCGAC AAGGAGCTGGGCGCCCTGCTGAAGGGCGTGGCCGGCAACGCCCACGCCCTGCAGAC CGGCGACTTCCGGACCCCCGCCGAGCTGGCCCTGAACAAGTTCGAGAAGGAGTCCG GCCACATCCGGAACCAGCGGTCCGACTACTCCCACACCTTCTCCCGGAAGGACCTGC AGGCCGAGCTGATCCTGCTGTTCGAGAAGCAGAAGGAGTTCGGCAACCCCCACGTGT CCGGCGGCCTGAAGGAGGGCATCGAGACCCTGCTGATGACCCAGCGGCCCGCCCTGT CCGGCGACGCCGTGCAGAAGATGCTGGGCCACTGCACCTTCGAGCCCGCCGAGCCC AAGGCCGCCAAGAACACCTACACCGCCGAGCGGTTCATCTGGCTGACCAAGCTGAAC AACCTGCGGATCCTGGAGCAGGGCTCCGAGCGGCCCCTGACCGACACCGAGCGGGC CACCCTGATGGACGAGCCCTACCGGAAGTCCAAGCTGACCTACGCCCAGGCCCGGAA GCTGCTGGGCCTGGAGGACACCGCCTTCTTCAAGGGCCTGCGGTACGGCAAGGACA ACGCCGAGGCCTCCACCCTGATGGAGATGAAGGCCTACCACGCCATCTCCCGGGCCC TGGAGAAGGAGGGCCTGAAGGACAAGAAGTCCCCCCTGAACCTGTCCCCCGAGCTG CAGGACGAGATCGGCACCGCCTTCTCCCTGTTCAAGACCGACGAGGACATCACCGGC CGGCTGAAGGACCGGATCCAGCCCGAGATCCTGGAGGCCCTGCTGAAGCACATCTCC TTCGACAAGTTCGTGCAGATCTCCCTGAAGGCCCTGCGGCGGATCGTGCCCCTGATG GAGCAGGGCAAGCGGTACGACGAGGCCTGCGCCGAGATCTACGGCGACCACTACGG CAAGAAGAACACCGAGGAGAAGATCTACCTGCCCCCCATCCCCGCCGACGAGATCCG GAACCCCGTGGTGCTGCGGGCCCTGTCCCAGGCCCGGAAGGTGATCAACGGCGTGGT GCGGCGGTACGGCTCCCCCGCCCGGATCCACATCGAGACCGCCCGGGAGGTGGGCAA GTCCTTCAAGGACCGGAAGGAGATCGAGAAGCGGCAGGAGGAGAACCGGAAGGAC CGGGAGAAGGCCGCCGCCAAGTTCCGGGAGTACTTCCCCAACTTCGTGGGCGAGCCC AAGTCCAAGGACATCCTGAAGCTGCGGCTGTACGAGCAGCAGCACGGCAAGTGCCT GTACTCCGGCAAGGAGATCAACCTGGGCCGGCTGAACGAGAAGGGCTACGTGGAGA TCGACCACGCCCTGCCCTTCTCCCGGACCTGGGACGACTCCTTCAACAACAAGGTGC TGGTGCTGGGCTCCGAGAACCAGAACAAGGGCAACCAGACCCCCTACGAGTACTTC AACGGCAAGGACAACTCCCGGGAGTGGCAGGAGTTCAAGGCCCGGGTGGAGACCTC CCGGTTCCCCCGGTCCAAGAAGCAGCGGATCCTGCTGCAGAAGTTCGACGAGGACG GCTTCAAGGAGCGGAACCTGAACGACACCCGGTACGTGAACCGGTTCCTGTGCCAGT TCGTGGCCGACCGGATGCGGCTGACCGGCAAGGGCAAGAAGCGGGTGTTCGCCTCC AACGGCCAGATCACCAACCTGCTGCGGGGCTTCTGGGGCCTGCGGAAGGTGCGGGC CGAGAACGACCGGCACCACGCCCTGGACGCCGTGGTGGTGGCCTGCTCCACCGTGG CCATGCAGCAGAAGATCACCCGGTTCGTGCGGTACAAGGAGATGAACGCCTTCGACG GCAAGACCATCGACAAGGAGACCGGCGAGGTGCTGCACCAGAAGACCCACTTCCCC CAGCCCTGGGAGTTCTTCGCCCAGGAGGTGATGATCCGGGTGTTCGGCAAGCCCGAC GGCAAGCCCGAGTTCGAGGAGGCCGACACCCTGGAGAAGCTGCGGACCCTGCTGGC CGAGAAGCTGTCCTCCCGGCCCGAGGCCGTGCACGAGTACGTGACCCCCCTGTTCGT GTCCCGGGCCCCCAACCGGAAGATGTCCGGCCAGGGCCACATGGAGACCGTGAAGT CCGCCAAGCGGCTGGACGAGGGCGTGTCCGTGCTGCGGGTGCCCCTGACCCAGCTG AAGCTGAAGGACCTGGAGAAGATGGTGAACCGGGAGCGGGAGCCCAAGCTGTACGA GGCCCTGAAGGCCCGGCTGGAGGCCCACAAGGACGACCCCGCCAAGGCCTTCGCCG AGCCCTTCTACAAGTACGACAAGGCCGGCAACCGGACCCAGCAGGTGAAGGCCGTG CGGGTGGAGCAGGTGCAGAAGACCGGCGTGTGGGTGCGGAACCACAACGGCATCGC CGACAACGCCACCATGGTGCGGGTGGACGTGTTCGAGAAGGGCGACAAGTACTACCT GGTGCCCATCTACTCCTGGCAGGTGGCCAAGGGCATCCTGCCCGACCGGGCCGTGGT GCAGGGCAAGGACGAGGAGGACTGGCAGCTGATCGACGACTCCTTCAACTTCAAGT TCTCCCTGCACCCCAACGACCTGGTGGAGGTGATCACCAAGAAGGCCCGGATGTTCG GCTACTTCGCCTCCTGCCACCGGGGCACCGGCAACATCAACATCCGGATCCACGACCT GGACCACAAGATCGGCAAGAACGGCATCCTGGAGGGCATCGGCGTGAAGACCGCCC TGTCCTTCCAGAAGTACCAGATCGACGAGCTGGGCAAGGAGATCCGGCCCTGCCGGC TGAAGAAGCGGCCCCCCGTGCGGTCCGGCAAGCGGACCGCCGACGGCTCCGAGTTC	163

[0767]

	GAGTCCCCCAAGAAGAAGCGGAAGGTGGAG	
使用表4的低A/U密码子的Nme Cas9 ORF (无起始或终止密码子；适于包括在融合蛋白质编码序列中)	GCCGCCTTCAAGCCCAACAGCATCAACTACATCCTGGGCCTGGACATCGGCATCGCCA GCGTGGGCTGGGCCATGGTGGAGATCGACGAGGAGGAGAACCCCATCCGGCTGATCG ACCTGGGCGTGCGGGTGTTCGAGCGGGCCGAGGTGCCCAAGACCGGCGACAGCCTG GCCATGGCCCGGCGGCTGGCCCGGAGCGTGCGGCGGCTGACCCGGCGGCGGGCCCA CCGGCTGCTGCGGACCCGGCGGCTGCTGAAGCGGGAGGGCGTGCTGCAGGCCGCCA ACTTCGACGAGAACGGCCTGATCAAGAGCCTGCCCAACACCCCCTGGCAGCTGCGG GCCGCCGCCCTGGACCGGAAGCTGACCCCCCTGGAGTGGAGCGCCGTGCTGCTGCA CCTGATCAAGCACCGGGGCTACCTGAGCCAGCGGAAGAACGAGGGCGAGACCGCCG ACAAGGAGCTGGGCGCCCTGCTGAAGGGCGTGGCCGGCAACGCCCACGCCCTGCAG ACCGGCGACTTCCGGACCCCCGCCGAGCTGGCCCTGAACAAGTTCGAGAAGGAGAG CGGCCACATCCGGAACCAGCGGAGCGACTACAGCCACACCTTCAGCCGGAAGGACC TGCAGGCCGAGCTGATCCTGCTGTTCGAGAAGCAGAAGGAGTTCGGCAACCCCCAC GTGAGCGGCGGCCTGAAGGAGGGCATCGAGACCCTGCTGATGACCCAGCGGCCCGC CCTGAGCGGCGACGCCGTGCAGAAGATGCTGGGCCACTGCACCTTCGAGCCCGCCG AGCCCAAGGCCGCCAAGAACACCTACACCGCCGAGCGGTTCATCTGGCTGACCAAGC TGAACAACCTGCGGATCCTGGAGCAGGGCAGCGAGCGGCCCCTGACCGACACCGAG CGGGCCACCCTGATGGACGAGCCCTACCGGAAGAGCAAGCTGACCTACGCCCAGGC CCGGAAGCTGCTGGGCCTGGAGGACACCGCCTTCTTCAAGGGCCTGCGGTACGGCAA GGACAACGCCGAGGCCAGCACCCTGATGGAGATGAAGGCCTACCACGCCATCAGCCG GGCCCTGGAGAAGGAGGGCCTGAAGGACAAGAAGAGCCCCCTGAACCTGAGCCCCG AGCTGCAGGACGAGATCGGCACCGCCTTCAGCCTGTTCAAGACCGACGAGGACATCA CCGGCCGGCTGAAGGACCGGATCCAGCCCGAGATCCTGGAGGCCCTGCTGAAGCACA TCAGCTTCGACAAGTTCGTGCAGATCAGCCTGAAGGCCCTGCGGCGGATCGTGCCCC TGATGGAGCAGGGCAAGCGGTACGACGAGGCCTGCGCCGAGATCTACGGCGACCACT ACGGCAAGAAGAACACCGAGGAGAAGATCTACCTGCCCCCCATCCCCGCCGACGAG ATCCGGAACCCCGTGGTGCTGCGGGCCCTGAGCCAGGCCCGGAAGGTGATCAACGG CGTGGTGCGGCGGTACGGCAGCCCCGCCCGGATCCACATCGAGACCGCCCGGGAGGT GGGCAAGAGCTTCAAGGACCGGAAGGAGATCGAGAAGCGGCAGGAGGAGAACCGG AAGGACCGGGAGAAGGCCGCCGCCAAGTTCCGGGAGTACTTCCCCAACTTCGTGGG CGAGCCCAAGAGCAAGGACATCCTGAAGCTGCGGCTGTACGAGCAGCAGCACGGCA AGTGCCTGTACAGCGGCAAGGAGATCAACCTGGGCCGGCTGAACGAGAAGGGCTAC GTGGAGATCGACCACGCCCTGCCCTTCAGCCGGACCTGGGACGACAGCTTCAACAAC AAGGTGCTGGTGCTGGGCAGCGAGAACCAGAACAAGGGCAACCAGACCCCCTACGA GTACTTCAACGGCAAGGACAACAGCCGGGAGTGGCAGGAGTTCAAGGCCCGGGTGG AGACCAGCCGGTTCCCCCGGAGCAAGAAGCAGCGGATCCTGCTGCAGAAGTTCGAC GAGGACGGCTTCAAGGAGCGGAACCTGAACGACACCCGGTACGTGAACCGGTTCCT GTGCCAGTTCGTGGCCGACCGGATGCGGCTGACCGGCAAGGGCAAGAAGCGGGTGT TCGCCAGCAACGGCCAGATCACCAACCTGCTGCGGGGCTTCTGGGGCCTGCGGAAG GTGCGGGCCGAGAACGACCGGCACCACGCCCTGGACGCCGTGGTGGTGGCCTGCAG CACCGTGGCCATGCAGCAGAAGATCACCCGGTTCGTGCGGTACAAGGAGATGAACGC CTTCGACGGCAAGACCATCGACAAGGAGACCGGCGAGGTGCTGCACCAGAAGACCC ACTTCCCCCAGCCCTGGGAGTTCTTCGCCCAGGAGGTGATGATCCGGGTGTTCGGCA AGCCCGACGGCAAGCCCGAGTTCGAGGAGGCCGACACCCTGGAGAAGCTGCGGACC CTGCTGGCCGAGAAGCTGAGCAGCCGGCCCGAGGCCGTGCACGAGTACGTGACCCC CCTGTTCGTGAGCCGGGCCCCCAACCGGAAGATGAGCGGCCAGGGCCACATGGAGA CCGTGAAGAGCGCCAAGCGGCTGGACGAGGGCGTGAGCGTGCTGCGGGTGCCCCTG ACCCAGCTGAAGCTGAAGGACCTGGAGAAGATGGTGAACCGGGAGCGGGAGCCCAA GCTGTACGAGGCCCTGAAGGCCCGGCTGGAGGCCCACAAGGACGACCCCGCCAAGG CCTTCGCCGAGCCCTTCTACAAGTACGACAAGGCCGGCAACCGGACCCAGCAGGTGA AGGCCGTGCGGGTGGAGCAGGTGCAGAAGACCGGCGTGTGGGTGCGGAACCACAAC GGCATCGCCGACAACGCCACCATGGTGCGGGTGGACGTGTTCGAGAAGGGCGACAA GTACTACCTGGTGCCCATCTACAGCTGGCAGGTGGCCAAGGGCATCCTGCCCGACCG GGCCGTGGTGCAGGGCAAGGACGAGGAGGACTGGCAGCTGATCGACGACAGCTTCA ACTTCAAGTTCAGCCTGCACCCCAACGACCTGGTGGAGGTGATCACCAAGAAGGCCC GGATGTTCGGCTACTTCGCCAGCTGCCACCGGGGCACCGGCAACATCAACATCCGGA TCCACGACCTGGACCACAAGATCGGCAAGAACGGCATCCTGGAGGGCATCGGCGTG AAGACCGCCCTGAGCTTCCAGAAGTACCAGATCGACGAGCTGGGCAAGGAGATCCG GCCCTGCCGGCTGAAGAAGCGGCCCCCCGTGCGGAGCGGCAAGCGGACCGCCGACG GCAGCGAGTTCGAGAGCCCCAAGAAGAAGCGGAAGGTGGAG	164
具有NLS1的Cas9的开放阅读框架(无起始或终止	GACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGT CATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAG ACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAA ACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAA AGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACG ACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCAC	165

[0768]

密码子；适于包括在融合蛋白质编码序列中)	GAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTAC CCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCT GAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATC GAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGT CCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACG CAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATC GCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAG CCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGC TGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATC GGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCT GCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAA GCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGG TCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAAC GGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATC AAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAG AGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGA TCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCC TGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACG TCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAA GAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACA GAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCC TGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGG TCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAG AAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCT GAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGT CGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAA GGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCC TGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATAC GCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGG ATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAA AGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGC TGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGC GGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAAT CAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGG GAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACA CAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCA AGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAG AACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAG GAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGC TTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAG AGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACT GGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACA AAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGAC AGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGA ATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCAC ACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG	

[0769]

	GAGGAAGCCTGGCAGCAAAGAGAAGCAGAACAACA	
具有NLS2的Cas9的开放阅读框架(无起始或终止密码子;适于包括在融合蛋白质编码序列中)	GACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGT CATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAG ACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAA ACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAA AGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACG ACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCAC GAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTAC CCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCT GAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATC GAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGT CCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACG CAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATC GCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAG CCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGC TGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATC GGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCT GCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAA GCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGG TCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAAC GGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATC AAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAG AGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGA TCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCC TGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACG TCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAA GAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACA GAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCC TGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGG TCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAG AAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCT GAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGT CGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAA GGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCC TGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATAC GCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGG ATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAA AGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGC TGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGC GGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAAT CAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGG GAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACA CAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCA AGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAG AACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAG GAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGC TTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAG AGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACT GGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACA AAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGAC AGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGA ATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCAC ACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG	166

[0770]

	CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCCAGGCAGCAAAGAGAAGCAGAACAACA	
具有NLS3的Cas9的开放阅读框架(无起始或终止密码子;适于包括在融合蛋白质编码序列中)	GACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGT CATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAG ACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAA ACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAA AGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACG ACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCAC GAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTAC CCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCT GAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATC GAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGT CCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACG CAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATC GCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAG CCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGC TGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATC GGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCT GCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAA GCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGG TCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAAC GGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATC AAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAG AGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGA TCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCC TGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACG TCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAA GAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACA GAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCC TGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGG TCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAG AAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCT GAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGT CGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAA GGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCC TGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATAC GCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGG ATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAA AGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGC TGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGC GGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAAT CAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGG GAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACA CAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCA AGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAG AACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAG GAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGC TTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAG AGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACT GGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACA AAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGAC AGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGA ATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCAC ACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG	167

[0771]

	GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCCCGGCACCGGCAAAGAGAGAAAGAACAACA	
具有NLS4的Cas9的开放阅读框架(无起始或终止密码子;适于包括在融合蛋白质编码序列中)	GACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGT CATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAG ACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAA ACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAA AGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACG ACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCAC GAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTAC CCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCT GAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATC GAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGT CCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACG CAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATC GCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAG CCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGC TGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATC GGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCT GCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAA GCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGG TCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAAC GGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATC AAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAG AGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGA TCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCC TGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACG TCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAA GAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACA GAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCC TGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGG TCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAG AAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCT GAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGT CGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAA GGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCC TGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATAC GCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGG ATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAA AGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGC TGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGC GGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAAT CAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGG GAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACA CAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCA AGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAG AACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAG GAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGC TTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAG AGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACT GGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACA AAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGAC AGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGA ATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCAC ACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA	168

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	GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCCAGGCAGCAAAGAGACCGAGAACAACA	
具有NLS5的Cas9的开放阅读框架(无起始或终止密码子;适于包括在融合蛋白质编码序列中)	GACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGT CATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAG ACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAA ACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAA AGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACG ACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCAC GAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTAC CCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCT GAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATC GAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGT CCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACG CAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATC GCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAG CCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGC TGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATC GGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCT GCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAA GCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGG TCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAAC GGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATC AAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAG AGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGA TCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCC TGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACG TCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAA GAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACA GAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCC TGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGG TCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAG AAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCT GAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGT CGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAA GGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCC TGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATAC GCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGG ATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAA AGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGC TGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGC GGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAAT CAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGG GAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACA CAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCA AGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAG AACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAG GAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGC	169

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	TTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAG AGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACT GGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACA AAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGAC AGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGA ATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCAC ACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCAGAGCAGCAAAGAGACCGAGAACAACA	
具有NLS6的Cas9的开放阅读框架(无起始或终止密码子;适于包括在融合蛋白质编码序列中)	GACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGT CATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAG ACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAA ACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAA AGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACG ACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCAC GAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTAC CCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCT GAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATC GAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGT CCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACG CAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATC GCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAG CCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGC TGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATC GGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCT GCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAA GCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGG TCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAAC GGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATC AAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAG AGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGA TCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCC TGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACG TCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAA GAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACA GAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCC TGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGG TCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAG AAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCT GAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGT CGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAA GGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCC TGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATAC GCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGG ATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAA AGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGC TGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGC	170

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	GGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAAT CAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGG GAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACA CAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCA AGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAG AACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAG GAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGC TTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAG AGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACT GGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACA AAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGAC AGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGA ATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCAC ACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCGCAGCAGCAAAGAGAAGCTGGAGCATGGCAGCA	
具有NLS7的Cas9的开放阅读框架(无起始或终止密码子;适于包括在融合蛋白质编码序列中)	GACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGT CATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAG ACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAA ACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAA AGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACG ACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCAC GAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTAC CCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCT GAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATC GAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGT CCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACG CAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATC GCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAG CCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGC TGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATC GGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCT GCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAA GCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGG TCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAAC GGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATC AAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAG AGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGA TCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCC TGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACG TCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAA GAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACA GAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCC TGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGG TCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAG AAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCT GAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGT	171

[0775]

	CGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAA GGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCC TGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATAC GCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGG ATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAA AGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGC TGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGC GGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAAT CAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGG GAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACA CAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCA AGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAG AACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAG GAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGC TTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAG AGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACT GGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACA AAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGAC AGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGA ATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCAC ACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCGCAGCAGCAAAGAGAGTCTGGAGCATGGCATTC	
具有NLS8的Cas9的开放阅读框架(无起始或终止密码子；适于包括在融合蛋白质编码序列中)	GACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGT CATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAG ACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAA ACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAA AGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACG ACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCAC GAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTAC CCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCT GAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATC GAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGT CCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACG CAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATC GCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAG CCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGC TGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATC GGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCT GCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAA GCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGG TCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAAC GGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATC AAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAG AGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGA TCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCC TGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACG	172

[0776]

	TCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAA GAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACA GAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCC TGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGG TCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAG AAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCT GAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGT CGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAA GGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCC TGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATAC GCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGG ATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAA AGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGC TGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGC GGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAAT CAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGG GAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACA CAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCA AGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAG AACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAG GAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGC TTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAG AGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACT GGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACA AAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGAC AGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGA ATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCAC ACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCGCAGCAGCAAAGAGAAGCTGGAGCATGGCATTC	
具有NLS9的Cas9的开放阅读框架(无起始或终止密码子；适于包括在融合蛋白质编码序列中)	GACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGT CATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAG ACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAA ACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAA AGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACG ACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCAC GAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTAC CCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCT GAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATC GAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGT CCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACG CAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATC GCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAG CCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGC TGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATC GGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCT GCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAA	173

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	GCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGG TCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAAC GGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATC AAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAG AGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGA TCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCC TGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACG TCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAA GAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACA GAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCC TGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGG TCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAG AAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCT GAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGT CGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAA GGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCC TGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATAC GCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGG ATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAA AGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGC TGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGC GGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAAT CAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGG GAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACA CAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCA AGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAG AACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAG GAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGC TTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAG AGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACT GGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACA AAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGAC AGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGA ATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCAC ACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCGCAGCAGCAAAGAGAAAGTACTTCGCAGCA	
具有NLS10的Cas9的开放阅读框架(无起始或终止密码子;适于包括在融	GACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGT CATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAG ACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAA ACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAA AGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACG ACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCAC GAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTAC CCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCT GAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATC GAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGT	174

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合蛋白质编码序列中)	CCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACG CAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATC GCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAG CCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGC TGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATC GGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCT GCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAA GCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGG TCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAAC GGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATC AAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAG AGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGA TCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCC TGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACG TCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAA GAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACA GAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCC TGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGG TCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAG AAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCT GAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGT CGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAA GGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCC TGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATAC GCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGG ATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAA AGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGC TGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGC GGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAAT CAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGG GAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACA CAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCA AGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAG AACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAG GAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGC TTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAG AGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACT GGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACA AAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGAC AGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGA ATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCAC ACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCAGAGCAGCAAAGAGAAAGGCATTCGCAGCA	
具有NLS11的Cas9的开	GACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGT CATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAG ACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAA	175

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放阅读框架(无起始或终止密码子;适于包括在融合蛋白质编码序列中)	ACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAA AGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACG ACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCAC GAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTAC CCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCT GAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATC GAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGT CCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACG CAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATC GCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAG CCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGC TGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATC GGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCT GCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAA GCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGG TCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAAC GGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATC AAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAG AGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGA TCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCC TGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACG TCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAA GAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACA GAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCC TGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGG TCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAG AAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCT GAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGT CGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAA GGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCC TGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATAC GCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGG ATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAA AGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGC TGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGC GGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAAT CAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGG GAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACA CAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCA AGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAG AACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAG GAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGC TTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAG AGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACT GGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACA AAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGAC AGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGA ATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCAC ACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCA GAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAA CAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACA AGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACAC TGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGG AGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCA TGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAG GAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTG GGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGT CGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTG CTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTG GAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTA CAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC	

[0780]

	ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCAGAGCAGCAAAGAGAAAGTACTTCGCAGTC	
具有XBG UTR及含有表4的低U 1密码子的Cas9 ORF的mRNA转录物	GGGAAGCUCAGAAUAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGGACAAGAA GUACAGCAUCGGCCUGGACAUCGGCACCAACAGCGUGGGCUGGGCCGUGAUCACC GACGAGUACAAGGUGCCCAGCAAGAAGUUCAAGGUGCUGGGCAACACCGACAGAC ACAGCAUCAAGAAGAACCUGAUCGGCGCCCUGCUGUUCGACAGCGGCGAGACCGC CGAGGCCACCAGACUGAAGAGAACCGCCAGAAGAAGAUACACCAGAAGAAAGAAC AGAAUCUGCUACCUGCAGGAGAUCUUCAGCAACGAGAUGGCCAAGGUGGACGACA GCUUCUUCCACAGACUGGAGGAGAGCUUCCUGGUGGAGGAGGACAAGAAGCACGA GAGACACCCCAUCUUCGGCAACAUCGUGGACGAGGUGGCCUACCACGAGAAGUAC CCCACCAUCUACCACCUGAGAAAGAAGCUGGUGGACAGCACCGACAAGGCCGACC UGAGACUGAUCUACCUGGCCCUGGCCCACAUGAUCAAGUUCAGAGGCCACUUCCU GAUCGAGGGCGACCUGAACCCCGACAACAGCGACGUGGACAAGCUGUUCAUCCAG CUGGUGCAGACCUACAACCAGCUGUUCGAGGAGAACCCCAUCAACGCCAGCGGCG UGGACGCCAAGGCCAUCCUGAGCGCCAGACUGAGCAAGAGCAGAAGACUGGAGAA CCUGAUCGCCCAGCUGCCCGGCGAGAAGAAGAACGGCCUGUUCGGCAACCUGAUC GCCCUGAGCCUGGGCCUGACCCCCAACUUCAAGAGCAACUUCGACCUGGCCGAGG ACGCCAAGCUGCAGCUGAGCAAGGACACCUACGACGACGACCUGGACAACCUGCU GGCCCAGAUCGGCGACCAGUACGCCGACCUGUUCCUGGCCGCCAAGAACCUGAGC GACGCCAUCCUGCUGAGCGACAUCCUGAGAGUGAACACCGAGAUCACCAAGGCCC CCCUGAGCGCCAGCAUGAUCAAGAGAUACGACGAGCACCACCAGGACCUGACCCU GCUGAAGGCCCUGGUGAGACAGCAGCUGCCCGAGAAGUACAAGGAGAUCUUCUUC GACCAGAGCAAGAACGGCUACGCCGGCUACAUCGACGGCGGCGCCAGCCAGGAGG AGUUCUACAAGUUCAUCAAGCCCAUCCUGGAGAAGAUGGACGGCACCGAGGAGCU GCUGGUGAAGCUGAACAGAGAGGACCUGCUGAGAAAGCAGAGAACCUUCGACAAC GGCAGCAUCCCCCACCAGAUCCACCUGGGCGAGCUGCACGCCAUCCUGAGAAGAC AGGAGGACUUCUACCCCUUCCUGAAGGACAACAGAGAGAAGAUCGAGAAGAUCCU GACCUUCAGAAUCCCCUACUACGUGGGCCCCCUGGCCAGAGGCAACAGCAGAUUC GCCUGGAUGACCAGAAAGAGCGAGGAGACCAUCACCCCCUGGAACUUCGAGGAGG UGGUGGACAAGGGCGCCAGCGCCCAGAGCUUCAUCGAGAGAAUGACCAACUUCGA CAAGAACCUGCCCAACGAGAAGGUGCUGCCCAAGCACAGCCUGCUGUACGAGUAC UUCACCGUGUACAACGAGCUGACCAAGGUGAAGUACGUGACCGAGGGCAUGAGAA AGCCCGCCUUCCUGAGCGGCGAGCAGAAGAAGGCCAUCGUGGACCUGCUGUUCAA GACCAACAGAAAGGUGACCGUGAAGCAGCUGAAGGAGGACUACUUCAAGAAGAU CGAGUGCUUCGACAGCGUGGAGAUCAGCGGCGUGGAGGACAGAUUCAACGCCAGC CUGGGCACCUACCACGACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACA ACGAGGAGAACGAGGACAUCCUGGAGGACAUCGUGCUGACCCUGACCCUGUUCGA GGACAGAGAGAUGAUCGAGGAGAGACUGAAGACCUACGCCCACCUGUUCGACGAC AAGGUGAUGAAGCAGCUGAAGAGAAGAAGAUACACCGGCUGGGGCAGACUGAGC AGAAAGCUGAUCAACGGCAUCAGAGACAAGCAGAGCGGCAAGACCAUCCUGGACU UCCUGAAGAGCGACGGCUUCGCCAACAGAAACUUCAUGCAGCUGAUCCACGACGA CAGCCUGACCUUCAAGGAGGACAUCCAGAAGGCCCAGGUGAGCGGCCAGGGCGAC AGCCUGCACGAGCACAUCGCCAACCUGGCCGGCAGCCCCGCCAUCAAGAAGGGCA UCCUGCAGACCGUGAAGGUGGUGGACGAGCUGGUGAAGGUGAUGGGCAGACACA AGCCCGAGAACAUCGUGAUCGAGAUGGCCAGAGAGAACCAGACCACCCAGAAGGG CCAGAAGAACAGCAGAGAGAGAAUGAAGAGAAUCGAGGAGGGCAUCAAGGAGCU GGGCAGCCAGAUCCUGAAGGAGCACCCCGUGGAGAACACCCAGCUGCAGAACGAG AAGCUGUACCUGUACUACCUGCAGAACGGCAGAGACAUGUACGUGGACCAGGAGC UGGACAUCAACAGACUGAGCGACUACGACGUGGACCACAUCGUGCCCCAGAGCUU CCUGAAGGACGACAGCAUCGACAACAAGGUGCUGACCAGAAGCGACAAGAACAGA GGCAAGAGCGACAACGUGCCCAGCGAGGAGGUGGUGAAGAAGAUGAAGAACUAC UGGAGACAGCUGCUGAACGCCAAGCUGAUCACCCAGAGAAAGUUCGACAACCUGA CCAAGGCCGAGAGAGGCGGCCUGAGCGAGCUGGACAAGGCCGGCUUCAUCAAGAG ACAGCUGGUGGAGACCAGACAGAUCACCAAGCACGUGGCCCAGAUCCUGGACAGC AGAAUGAACACCAAGUACGACGAGAACGACAAGCUGAUCAGAGAGGUGAAGGUG AUCACCCUGAAGAGCAAGCUGGUGAGCGACUUCAGAAAGGACUUCCAGUUCUACA AGGUGAGAGAGAUCAACAACUACCACCACGCCCACGACGCCUACCUGAACGCCGU GGUGGGCACCGCCCUGAUCAAGAAGUACCCCAAGCUGGAGAGCGAGUUCGUGUAC GGCGACUACAAGGUGUACGACGUGAGAAAGAUGAUCGCCAAGAGCGAGCAGGAG AUCGGCAAGGCCACCGCCAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUCA AGACCGAGAUCACCCUGGCCAACGGCGAGAUCAGAAAGAGACCCCUGAUCGAGAC CAACGGCGAGACCGGCGAGAUCGUGUGGGACAAGGGCAGAGACUUCGCCACCGUG AGAAAGGUGCUGAGCAUGCCCCAGGUGAACAUCGUGAAGAAGACCGAGGUGCAG ACCGGCGGCUUCAGCAAGGAGAGCAUCCUGCCCAAGAGAAACAGCGACAAGCUGA UCGCCAGAAAGAAGGACUGGGACCCCAAGAAGUACGGCGGCUUCGACAGCCCCAC	176

[0781]

	CGUGGCCUACAGCGUGCUGGUGGUGGCCAAGGUGGAGAAGGGCAAGAGCAAGAAGCUGAAGAGCGUGAAGGAGCUGCUGGGCAUCACCAUCAUGGAGAGAAGCAGCUUCGAGAAGAACCCCAUCGACUUCCUGGAGGCCAAGGGCUACAAGGAGGUGAAGAAGGACCUGAUCAUCAAGCUGCCCAAGUACAGCCUGUUCGAGCUGGAGAACGGCAGAAAGAGAAUGCUGGCCAGCGCCGGCGAGCUGCAGAAGGGCAACGAGCUGGCCCUGCCCAGCAAGUACGUGAACUUCCUGUACCUGGCCAGCCACUACGAGAAGCUGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCUGUUCGUGGAGCAGCACAAGCACUACCUGGACGAGAUCAUCGAGCAGAUCAGCGAGUUCAGCAAGAGAGUGAUCCUGGCCGACGCCAACCUGGACAAGGUGCUGAGCGCCUACAACAAGCACAGAGACAAGCCCAUCAGAGAGCAGGCCGAGAACAUCAUCCACCUGUUCACCCUGACCAACCUGGGCGCCCCCGCCGCCUUCAAGUACUUCGACACCACCAUCGACAGAAAGAGAUACACCAGCACCAAGGAGGUGCUGGACGCCACCCUGAUCCACCAGAGCAUCACCGGCCUGUACGAGACCAGAAUCGACCUGAGCCAGCUGGGCGGCGACGGCGGCGGCAGCCCCAAGAAGAAGAGAAAGGUGUGACUAGCACCAGCCUCAAGAACACCCGAAUGGAGUCUCUAAGCUACAUAAUACCAACUUACACUUUACAAAAUGUUGUCCCCCAAAAUGUAGCCAUUCGUAUCUGCUCCUAAUAAAAAGAAAGUUUCUUCACAUUCUCUCGAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAUCUAG	
具有XBG UTR及含有表4的低A密码子的Cas9 ORF的mRNA转录物	GGGAAGCUCAGAAUAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGGACAAGAAGUACUCCAUCGGCCUGGACAUCGGCACCAACUCCGUGGGCUGGGCCGUGAUCACCGACGAGUACAAGGUGCCCUCCAAGAAGUUCAAGGUGCUGGGCAACACCGACCGGCACUCCAUCAAGAAGAACCUGAUCGGCGCCCUGCUGUUCGACUCCGGCGAGACCGCCGAGGCCACCCGGCUGAAGCGGACCGCCCGGCGGCGGUACACCCGGCGGAAGAACCGGAUCUGCUACCUGCAGGAGAUCUUCUCCAACGAGAUGGCCAAGGUGGACGACUCCUUCUUCCACCGGCUGGAGGAGUCCUUCCUGGUGGAGGAGGACAAGAAGCACGAGCGGCACCCCAUCUUCGGCAACAUCGUGGACGAGGUGGCCUACCACGAGAAGUACCCCACCAUCUACCACCUGCGGAAGAAGCUGGUGGACUCCACCGACAAGGCCGACCUGCGGCUGAUCUACCUGGCCCUGGCCCACAUGAUCAAGUUCCGGGGCCACUUCCUGAUCGAGGGCGACCUGAACCCCGACAACUCCGACGUGGACAAGCUGUUCAUCCAGCUGGUGCAGACCUACAACCAGCUGUUCGAGGAGAACCCCAUCAACGCCUCCGGCGUGGACGCCAAGGCCAUCCUGUCCGCCCGGCUGUCCAAGUCCCGGCGGCUGGAGAACCUGAUCGCCCAGCUGCCCGGCGAGAAGAAGAACGGCCUGUUCGGCAACCUGAUCGCCCUGUCCCUGGGCCUGACCCCCAACUUCAAGUCCAACUUCGACCUGGCCGAGGACGCCAAGCUGCAGCUGUCCAAGGACACCUACGACGACGACCUGGACAACCUGCUGGCCCAGAUCGGCGACCAGUACGCCGACCUGUUCCUGGCCGCCAAGAACCUGUCCGACGCCAUCCUGCUGUCCGACAUCCUGCGGGUGAACACCGAGAUCACCAAGGCCCCCCUGUCCGCCUCCAUGAUCAAGCGGUACGACGAGCACCACCAGGACCUGACCCUGCUGAAGGCCCUGGUGCGGCAGCAGCUGCCCGAGAAGUACAAGGAGAUCUUCUUCGACCAGUCCAAGAACGGCUACGCCGGCUACAUCGACGGCGGCGCCUCCCAGGAGGAGUUCUACAAGUUCAUCAAGCCCAUCCUGGAGAAGAUGGACGGCACCGAGGAGCUGCUGGUGAAGCUGAACCGGGAGGACCUGCUGCGGAAGCAGCGGACCUUCGACAACGGCUCCAUCCCCCACCAGAUCCACCUGGGCGAGCUGCACGCCAUCCUGCGGCGGCAGGAGGACUUCUACCCCUUCCUGAAGGACAACCGGGAGAAGAUCGAGAAGAUCCUGACCUUCCGGAUCCCCUACUACGUGGGCCCCCUGGCCCGGGGCAACUCCCGGUUCGCCUGGAUGACCCGGAAGUCCGAGGAGACCAUCACCCCCUGGAACUUCGAGGAGGUGGUGGACAAGGGCGCCUCCGCCCAGUCCUUCAUCGAGCGGAUGACCAACUUCGACAAGAACCUGCCCAACGAGAAGGUGCUGCCCAAGCACUCCCUGCUGUACGAGUACUUCACCGUGUACAACGAGCUGACCAAGGUGAAGUACGUGACCGAGGGCAUGCGGAAGCCCGCCUUCCUGUCCGGCGAGCAGAAGAAGGCCAUCGUGGACCUGCUGUUCAAGACCAACCGGAAGGUGACCGUGAAGCAGCUGAAGGAGGACUACUUCAAGAAGAUCGAGUGCUUCGACUCCGUGGAGAUCUCCGGCGUGGAGGACCGGUUCAACGCCUCCCUGGGCACCUACCACGACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAGGAGAACGAGGACAUCCUGGAGGACAUCGUGCUGACCCUGACCCUGUUCGAGGACCGGGAGAUGAUCGAGGAGCGGCUGAAGACCUACGCCCACCUGUUCGACGACAAGGUGAUGAAGCAGCUGAAGCGGCGGCGGUACACCGGCUGGGGCCGGCUGUCCCGGAAGCUGAUCAACGGCAUCCGGGACAAGCAGUCCGGCAAGACCAUCCUGGACUUCCUGAAGUCCGACGGCUUCGCCAACCGGAACUUCAUGCAGCUGAUCCACGACGACUCCCUGACCUUCAAGGAGGACAUCCAGAAGGCCCAGGUGUCCGGCCAGGGCGACUCCCUGCACGAGCACAUCGCCAACCUGGCCGGCUCCCCCGCCAUCAAGAAGGGCAUCCUGCAGACCGUGAAGGUGGUGGACGAGCUGGUGAAGGUGAUGGGCCGGCACAAGCCCGAGAACAUCGUGAUCGAGAUGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACUCCCGGGAGCGGAUGAAGCGGAUCGAGGAGGGCAUCAAGGAGCUGGGCUCCCAGAUCCUGAAGGAGCACCCCGUGGAGAACACCCAGCUGCAGAACGAGAAGCUGUACCUGUACUACCUGCAGAACGGCCGGGACAUGUACGUGGACCAGGAGCUGGACAUCAACCGGCUGUCCGACUACGACGUGGACCACAUCGUGCCCCAGUCCUUCCUGAAGGACGACUCCAUCGACAACAAGGUGCUGACCCGGUCCGACAAGAACCGGGGCAAGUCCGACAACGUGCCCUCCGAGGAGGUGGUGAAGAAGAUGAAGAACUACUGGCGGC	177

		AGCUGCUGAACGCCAAGCUGAUCACCCAGCGGAAGUUCGACAACCUGACCAAGGC CGAGCGGGGCGGCCUGUCCGAGCUGGACAAGGCCGGCUUCAUCAAGCGGCAGCUG GUGGAGACCCGGCAGAUCACCAAGCACGUGGCCCAGAUCCUGGACUCCCGGAUGA ACACCAAGUACGACGAGAACGACAAGCUGAUCCGGGAGGUGAAGGUGAUCACCCU GAAGUCCAAGCUGGUGUCCGACUUCCGGAAGGACUUCCAGUUCUACAAGGUGCGG GAGAUCAACAACUACCACCACGCCCACGACGCCUACCUGAACGCCGUGGUGGGCA CCGCCCUGAUCAAGAAGUACCCCAAGCUGGAGUCCGAGUUCGUGUACGGCGACUA CAAGGUGUACGACGUGCGGAAGAUGAUCGCCAAGUCCGAGCAGGAGAUCGGCAAG GCCACCGCCAAGUACUUCUUCUACUCCAACAUCAUGAACUUCUUCAAGACCGAGA UCACCCUGGCCAACGGCGAGAUCCGGAAGCGGCCCCUGAUCGAGACCAACGGCGA GACCGGCGAGAUCGUGUGGGACAAGGGCCGGGACUUCGCCACCGUGCGGAAGGUG CUGUCCAUGCCCCAGGUGAACAUCGUGAAGAAGACCGAGGUGCAGACCGGCGGCU UCUCCAAGGAGUCCAUCCUGCCCAAGCGGAACUCCGACAAGCUGAUCGCCCGGAA GAAGGACUGGGACCCCAAGAAGUACGGCGGCUUCGACUCCCCCACCGUGGCCUAC UCCGUGCUGGUGGUGGCCAAGGUGGAGAAGGGCAAGUCCAAGAAGCUGAAGUCC GUGAAGGAGCUGCUGGGCAUCACCAUCAUGGAGCGGUCCUCCUUCGAGAAGAACC CCAUCGACUUCCUGGAGGCCAAGGGCUACAAGGAGGUGAAGAAGGACCUGAUCAU CAAGCUGCCCAAGUACUCCCUGUUCGAGCUGGAGAACGGCCGGAAGCGGAUGCUG GCCUCCGCCGGCGAGCUGCAGAAGGGCAACGAGCUGGCCCUGCCCUCCAAGUACG UGAACUUCCUGUACCUGGCCUCCCACUACGAGAAGCUGAAGGGCUCCCCCGAGGA CAACGAGCAGAAGCAGCUGUUCGUGGAGCAGCACAAGCACUACCUGGACGAGAUC AUCGAGCAGAUCUCCGAGUUCUCCAAGCGGGUGAUCCUGGCCGACGCCAACCUGG ACAAGGUGCUGUCCGCCUACAACAAGCACCGGGACAAGCCCAUCCGGGAGCAGGC CGAGAACAUCAUCCACCUGUUCACCCUGACCAACCUGGGCGCCCCCGCCGCCUUCA AGUACUUCGACACCACCAUCGACCGGAAGCGGUACACCUCCACCAAGGAGGUGCU GGACGCCACCCUGAUCCACCAGUCCAUCACCGGCCUGUACGAGACCCGGAUCGAC CUGUCCCAGCUGGGCGGCGACGGCGGCGGCUCCCCCAAGAAGAAGCGGAAGGUGU GACUAGCACCAGCCUCAAGAACACCCGAAUGGAGUCUCUAAGCUACAUAAUACCA ACUUACACUUUACAAAAUGUUGUCCCCCAAAAUGUAGCCAUUCGUAUCUGCUCCU AAUAAAAAGAAAGUUUCUUCACAUUCUCUCGAGAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAUCUAG	
[0782]	具有XBG UTR及含有表4的低U/A密码子的Cas9 ORF的mRNA转录物	GGGAAGCUCAGAAUAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGGACAAGAA GUACAGCAUCGGCCUGGACAUCGGCACCAACAGCGUGGGCUGGGCCGUGAUCACC GACGAGUACAAGGUGCCCAGCAAGAAGUUCAAGGUGCUGGGCAACACCGACCGGC ACAGCAUCAAGAAGAACCUGAUCGGCGCCCUGCUGUUCGACAGCGGCGAGACCGC CGAGGCCACCCGGCUGAAGCGGACCGCCCGGCGGCGGUACACCCGGCGGAAGAAC CGGAUCUGCUACCUGCAGGAGAUCUUCAGCAACGAGAUGGCCAAGGUGGACGACA GCUUCUUCCACCGGCUGGAGGAGAGCUUCCUGGUGGAGGAGGACAAGAAGCACGA GCGGCACCCCAUCUUCGGCAACAUCGUGGACGAGGUGGCCUACCACGAGAAGUAC CCCACCAUCUACCACCUGCGGAAGAAGCUGGUGGACAGCACCGACAAGGCCGACC UGCGGCUGAUCUACCUGGCCCUGGCCCACAUGAUCAAGUUCCGGGGCCACUUCCU GAUCGAGGGCGACCUGAACCCCGACAACAGCGACGUGGACAAGCUGUUCAUCCAG CUGGUGCAGACCUACAACCAGCUGUUCGAGGAGAACCCCAUCAACGCCAGCGGCG UGGACGCCAAGGCCAUCCUGAGCGCCCGGCUGAGCAAGAGCCGGCGGCUGGAGAA CCUGAUCGCCCAGCUGCCCGGCGAGAAGAAGAACGGCCUGUUCGGCAACCUGAUC GCCCUGAGCCUGGGCCUGACCCCCAACUUCAAGAGCAACUUCGACCUGGCCGAGG ACGCCAAGCUGCAGCUGAGCAAGGACACCUACGACGACGACCUGGACAACCUGCU GGCCCAGAUCGGCGACCAGUACGCCGACCUGUUCCUGGCCGCCAAGAACCUGAGC GACGCCAUCCUGCUGAGCGACAUCCUGCGGGUGAACACCGAGAUCACCAAGGCCC CCCUGAGCGCCAGCAUGAUCAAGCGGUACGACGAGCACCACCAGGACCUGACCCU GCUGAAGGCCCUGGUGCGGCAGCAGCUGCCCGAGAAGUACAAGGAGAUCUUCUUC GACCAGAGCAAGAACGGCUACGCCGGCUACAUCGACGGCGGCGCCAGCCAGGAGG AGUUCUACAAGUUCAUCAAGCCCAUCCUGGAGAAGAUGGACGGCACCGAGGAGCU GCUGGUGAAGCUGAACCGGGAGGACCUGCUGCGGAAGCAGCGGACCUUCGACAAC GGCAGCAUCCCCCACCAGAUCCACCUGGGCGAGCUGCACGCCAUCCUGCGGCGGC AGGAGGACUUCUACCCCUUCCUGAAGGACAACCGGGAGAAGAUCGAGAAGAUCCU GACCUUCCGGAUCCCCUACUACGUGGGCCCCCUGGCCCGGGGCAACAGCCGGUUC GCCUGGAUGACCCGGAAGAGCGAGGAGACCAUCACCCCCUGGAACUUCGAGGAGG UGGUGGACAAGGGCGCCAGCGCCCAGAGCUUCAUCGAGCGGAUGACCAACUUCGA CAAGAACCUGCCCAACGAGAAGGUGCUGCCCAAGCACAGCCUGCUGUACGAGUAC UUCACCGUGUACAACGAGCUGACCAAGGUGAAGUACGUGACCGAGGGCAUGCGGA AGCCCGCCUUCCUGAGCGGCGAGCAGAAGAAGGCCAUCGUGGACCUGCUGUUCAA GACCAACCGGAAGGUGACCGUGAAGCAGCUGAAGGAGGACUACUUCAAGAAGAUC GAGUGCUUCGACAGCGUGGAGAUCAGCGGCGUGGAGGACCGGUUCAACGCCAGCC UGGGCACCUACCACGACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAA CGAGGAGAACGAGGACAUCCUGGAGGACAUCGUGCUGACCCUGACCCUGUUCGAG	178

[0783]

	GACCGGGAGAUGAUCGAGGAGCGGCUGAAGACCUACGCCCACCUGUUCGACGACA AGGUGAUGAAGCAGCUGAAGCGGCGGCGGUACACCGGCUGGGGCCGGCUGAGCCG GAAGCUGAUCAACGGCAUCCGGGACAAGCAGAGCGGCAAGACCAUCCUGGACUUC CUGAAGAGCGACGGCUUCGCCAACCGGAACUUCAUGCAGCUGAUCCACGACGACA GCCUGACCUUCAAGGAGGACAUCCAGAAGGCCCAGGUGAGCGGCCAGGGCGACAG CCUGCACGAGCACAUCGCCAACCUGGCCGGCAGCCCCGCCAUCAAGAAGGGCAUC CUGCAGACCGUGAAGGUGGUGGACGAGCUGGUGAAGGUGAUGGGCCGGCACAAG CCCGAGAACAUCGUGAUCGAGAUGGCCCGGGAGAACCAGACCACCCAGAAGGGCC AGAAGAACAGCCGGGAGCGGAUGAAGCGGAUCGAGGAGGGCAUCAAGGAGCUGG GCAGCCAGAUCCUGAAGGAGCACCCCGUGGAGAACACCCAGCUGCAGAACGAGAA GCUGUACCUGUACUACCUGCAGAACGGCCGGGACAUGUACGUGGACCAGGAGCUG GACAUCAACCGGCUGAGCGACUACGACGUGGACCACAUCGUGCCCCAGAGCUUCC UGAAGGACGACAGCAUCGACAACAAGGUGCUGACCCGGAGCGACAAGAACCGGGG CAAGAGCGACAACGUGCCCAGCGAGGAGGUGGUGAAGAAGAUGAAGAACUACUG GCGGCAGCUGCUGAACGCCAAGCUGAUCACCCAGCGGAAGUUCGACAACCUGACC AAGGCCGAGCGGGGCGGCCUGAGCGAGCUGGACAAGGCCGGCUUCAUCAAGCGGC AGCUGGUGGAGACCCGGCAGAUCACCAAGCACGUGGCCCAGAUCCUGGACAGCCG GAUGAACACCAAGUACGACGAGAACGACAAGCUGAUCCGGGAGGUGAAGGUGAU CACCCUGAAGAGCAAGCUGGUGAGCGACUUCCGGAAGGACUUCCAGUUCUACAAG GUGCGGGAGAUCAACAACUACCACCACGCCCACGACGCCUACCUGAACGCCGUGG UGGGCACCGCCCUGAUCAAGAAGUACCCCAAGCUGGAGAGCGAGUUCGUGUACGG CGACUACAAGGUGUACGACGUGCGGAAGAUGAUCGCCAAGAGCGAGCAGGAGAUC GGCAAGGCCACCGCCAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUCAAGA CCGAGAUCACCCUGGCCAACGGCGAGAUCCGGAAGCGGCCCCUGAUCGAGACCAA CGGCGAGACCGGCGAGAUCGUGUGGGACAAGGGCCGGGACUUCGCCACCGUGCGG AAGGUGCUGAGCAUGCCCCAGGUGAACAUCGUGAAGAAGACCGAGGUGCAGACCG GCGGCUUCAGCAAGGAGAGCAUCCUGCCCAAGCGGAACAGCGACAAGCUGAUCGC CCGGAAGAAGGACUGGGACCCCAAGAAGUACGGCGGCUUCGACAGCCCCACCGUG GCCUACAGCGUGCUGGUGGUGGCCAAGGUGGAGAAGGGCAAGAGCAAGAAGCUG AAGAGCGUGAAGGAGCUGCUGGGCAUCACCAUCAUGGAGCGGAGCAGCUUCGAGA AGAACCCCAUCGACUUCCUGGAGGCCAAGGGCUACAAGGAGGUGAAGAAGGACCU GAUCAUCAAGCUGCCCAAGUACAGCCUGUUCGAGCUGGAGAACGGCCGGAAGCGG AUGCUGGCCAGCGCCGGCGAGCUGCAGAAGGGCAACGAGCUGGCCCUGCCCAGCA AGUACGUGAACUUCCUGUACCUGGCCAGCCACUACGAGAAGCUGAAGGGCAGCCC CGAGGACAACGAGCAGAAGCAGCUGUUCGUGGAGCAGCACAAGCACUACCUGGAC GAGAUCAUCGAGCAGAUCAGCGAGUUCAGCAAGCGGGUGAUCCUGGCCGACGCCA ACCUGGACAAGGUGCUGAGCGCCUACAACAAGCACCGGGACAAGCCCAUCCGGGA GCAGGCCGAGAACAUCAUCCACCUGUUCACCCUGACCAACCUGGGCGCCCCCGCCG CCUUCAAGUACUUCGACACCACCAUCGACCGGAAGCGGUACACCAGCACCAAGGA GGUGCUGGACGCCACCCUGAUCCACCAGAGCAUCACCGGCCUGUACGAGACCCGG AUCGACCUGAGCCAGCUGGGCGGCGACGGCGGCGGCAGCCCCAAGAAGAAGCGGA AGGUGUGACUAGCACCAGCCUCAAGAACACCCGAAUGGAGUCUCUAAGCUACAUA AUACCAACUUACACUUUACAAAAUGUUGUCCCCCAAAAUGUAGCCAUUCGUAUCU GCUCCUAAUAAAAAGAAAGUUUCUUCACAUUCUCUCGAGAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAUCUAG	
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[0784]

	GGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGA ACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACA ACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGA CAGGAAGACTTCTACCCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCT GACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGC ATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCG TCGACAAGGGAGCAAGCGCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAG AACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACA GTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGC ATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACA GAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTC GACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATA CCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACG AAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGA TCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAG CTGAAGAGAAGAAGATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGG AATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGAT TCGCAAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAG ACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACACATCGCA AACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGT CGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAAA TGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAT GAAGAGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACC CGGTCGAAAACACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAAC GGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTGAGCGACTACGA CGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGT CCTGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAA GTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATCAC ACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAGGACTGAGCGAACTG GACAAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACAGATCACAAAGCA CGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAACGACAAGC TGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGA AAGGACTTCCAGTTCTACAAGGTCAGAGAAATCAACAACTACCACCACGCACACGAC GCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGGAA AGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAG AGCGAACAGGAAATCGGAAAGGCAACAGCAAAGTACTTCTTCTACAGCAACATCATG AACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCT GATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCG CAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTCAAGAAGACAGAA GTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAA GCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCC CGACAGTCGCATACAGCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAG AAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTT CGAAAAGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGG ACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAAGAAAG AGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGA GCAAGTACGTCAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCC CGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGAC GAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAAC CTGGACAAGGTCCTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACA GGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGCATT CAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCC TGGACGCAACACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACC TGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCAG CGAAAGCGCAACACCGGAAAGCGTCAGCGGATGGAGACTGTTCAAGAAGATCAGCT AGCTAGCCATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAAT GAAGATCAATAGCTTATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTG TCTAAAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAATAA AAAATGGAAAGAACCTCGAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAATCTAG	
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CMV-1 5'UTR及人类ALB 3'UTR的mRNA转录物	CTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAA GACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGA CAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCTGAGA CTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAG GAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAG ACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAA GGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCAC AGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTG GGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCTGCA GCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAG ACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGA GCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATG ATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAG ACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGAT ACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGC CGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAA GACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCA CCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAA GGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGG ACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAAGAAA CAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGC TTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCC GAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGGTCAA GTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGG CAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAG GAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAA GACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGAC ACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATACGCAC ACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGGATGG GGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGA CAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGCTGA TCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGA CAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAA GAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGGGAA GACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACACAG AAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGG AACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAGAAC GAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAA CTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTC CTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAGAG GAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGG AGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAA GGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAG CTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAAT GAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACAC TGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGA GAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACA GCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACAAG GTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAAC AGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTG GCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAG AAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATG CCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGA AAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTGGG ACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCG TCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCT GGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTGGA AGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACA GCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTG CAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGC AAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTG TTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTC AGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACAA CAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCA CACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACA GAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAGC	

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	ATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGG AGGAAGCCCGAAGAAGAAGAGAAAGGTCAGCGAAAGCGCAACACCGGAAAGCGTC AGCGGATGGAGACTGTTCAAGAAGATCAGCTAGCTAGCCATCACATTTAAAAGCATCT CAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAGCTTATTCATCTCTTT TTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAATCA TTTTGCCTCTTTTCTCTGTGCTTCAATTAATAAAAAATGGAAAGAACCTCGAGAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	
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	GCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGG AAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTGG GACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTC GTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGC TGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTGG AAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTAC AGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACT GCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGG CAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCT GTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATT CAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTC ACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGAC AGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAG GAGGAAGCCCGAAGAAGAAGAGAAAGGTCAGCGAAAGCGCAACACCGGAAAGCGT CAGCGGATGGAGACTGTTCAAGAAGATCAGCTAGCTAGCCATCACATTTAAAAGCATC TCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAGCTTATTCATCTCTT TTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAATC ATTTTGCCTCTTTTCTCTGTGCTTCAATTAATAAAAAATGGAAAGAACCTCGAGAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	
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	CTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTC CTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAGAG GAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGG AGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAA GGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAG CTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAAT GAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACAC TGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGA GAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACA GCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACAAG GTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAAC AGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTG GCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAG AAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATG CCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGA AAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTGGG ACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCG TCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCT GGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTGGA AGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACA GCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTG CAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGC AAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTG TTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTC AGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACAA CAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCA CACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACA GAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAGC ATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGG AGGAAGCCCGAAGAAGAAGAGAAAGGTCAGCGAAAGCGCAACACCGGAAAGCGTC AGCGGATGGAGACTGTTCAAGAAGATCAGCTAGCTAGCCATCACATTTAAAAGCATCT CAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAGCTTATTCATCTCTTT TTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAATCA TTTTGCCTCTTTTCTCTGTGCTTCAATTAATAAAAAATGGAAAGAACCTCGAGAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	
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	GAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTC GACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATA CCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACG AAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGA TCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAG CTGAAGAGAAGAAGATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGG AATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGAT TCGCAAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAG ACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACACATCGCA AACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGT CGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAAA TGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAT GAAGAGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACC CGGTCGAAAACACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAAC GGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTGAGCGACTACGA CGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGT CCTGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAA GTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATCAC ACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAGGACTGAGCGAACTG GACAAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACAGATCACAAAGCA CGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAACGACAAGC TGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGA AAGGACTTCCAGTTCTACAAGGTCAGAGAAATCAACAACTACCACCACGCACACGAC GCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGGAA AGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAG AGCGAACAGGAAATCGGAAAGGCAACAGCAAAGTACTTCTTCTACAGCAACATCATG AACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCT GATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCG CAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTCAAGAAGACAGAA GTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAA GCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCC CGACAGTCGCATACAGCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAG AAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTT CGAAAAGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGG ACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAAGAAAG AGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGA GCAAGTACGTCAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCC CGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGAC GAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAAC CTGGACAAGGTCCTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACA GGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGCATT CAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCC TGGACGCAACACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACC TGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCAG CGAAAGCGCAACACCGGAAAGCGTCAGCGGATGGAGACTGTTCAAGAAGATCAGCT AGCTAGCCATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAAT GAAGATCAATAGCTTATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTG TCTAAAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAATAA AAAATGGAAAGAACCTCGAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA	
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	TGGCACAGATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGC GACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACC GCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCT GAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACC AGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTC TACAAGTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTC AAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCAT CCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACT TCTACCCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAA TCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAA GAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGA GCAAGCGCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAA CGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGA ACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCG GAGAACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACA GTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAA ATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTG AAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGA AGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACT GAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAA GATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAG CAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAA CTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGC ACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAA GCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTC AAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAA CCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAA GAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACAC ACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTA CGTCGACCAGGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGT CCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCG ACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGAT GAAGAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCG ACAACCTGACAAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATT CATCAAGAGACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCC TGGACAGCAGAATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTC AAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTC TACAAGGTCAGAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCA GTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTAC GGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAAT CGGAAAGGCAACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGAC AGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACG GAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAA GGTCCTGAGCATGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAG GATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGA AAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATA CAGCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGC GTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCC GATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAA GCTGCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAA GCGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAAC TTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGA ACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACA GATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCT GAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCA TCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACA CAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTG ATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGA GGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCAGCGAAAGCGCAACAC CGGAAAGCGTCAGCGGATGGAGACTGTTCAAGAAGATCAGCTAGCTAGCCATCACAT TTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAGCTT ATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAA TTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAATAAAAAATGGAAAGAAC CTCGAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	
具有编码带	GGGAAGCTCAGAATAAACGCTCAACTTTGGCCGGATCTCGCCACCATGGACAAGAAG TACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGAC	185

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有HiBiT标记标签的Cas9的ORF、XBG 5' UTR及人类ALB 3' UTR的mRNA转录物	GAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGACACAG CATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAG CAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAAGAACAGAAT CTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCTT CCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACAC CCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCT ACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCTGAGACTGATC TACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGAC CTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATAC AACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAAT CCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGC CGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGGACTG ACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCTGCAGCTGAG CAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGT ACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACA TCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAAG AGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCA GCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAG GATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCGATCC TGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTG CTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGG AGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAAGGACA ACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGC TGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATC ACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTTCAT CGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGC ACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGGTCAAGTACG TCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATC GTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGA CTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAG ATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGA CTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGACACTGAC ACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATACGCACACCTGTT CGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGGATGGGGAAGAC TGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTG GACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGCTGATCCACGAC GACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGA CAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAA TCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGGGAAGACACAAG CCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACACAGAAGGGACA GAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGGGA AGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAGAACGAAAAGCT GTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACAT CAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGA CGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAGAGGAAAGAGC GACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCT GCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGA GAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAGCTGGTCGAA ACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAA GTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCA AGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAATCAACA ACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCA AGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACG TCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACAGCAAAGTAC TTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGA GAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAGAAATCGTCTG GGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCA ACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTG CCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGA AGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAAGG TCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCAC AATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTGGAAGCAAAGG GATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCG AACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGG AAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCCACTA CGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAA CAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGA	

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	GTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACAACAAGCACAG AGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAA ACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGAT ACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAGCATCACAGGA CTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCCC GAAGAAGAAGAGAAAGGTCAGCGAAAGCGCAACACCGGAAAGCGTCAGCGGATGG AGACTGTTCAAGAAGATCAGCTAGCTAGCCATCACATTTAAAAGCATCTCAGCCTACC ATGAGAATAAGAGAAAGAAAATGAAGATCAATAGCTTATTCATCTCTTTTTCTTTTTCG TTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTCT TTTCTCTGTGCTTCAATTAATAAAAAATGGAAAGAACCTCGAGAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	
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具有NLS2的Cas9的氨基酸序列	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAE ATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGN IVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDV DKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLI ALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAIL LSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGY IDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILR RQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDK GASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGE QKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKD KDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHI ANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMK RIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLT KAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSK LVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVR KMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDF ATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFV EQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAP AAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSQAAKRSRTT	187
具有NLS3的Cas9的氨基酸序列	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAE ATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGN IVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDV DKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLI ALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAIL LSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGY IDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILR RQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDK	188

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	GASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGE QKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKD KDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHI ANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMK RIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLT KAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSK LVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVR KMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDF ATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFV EQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAP AAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSPAPAKRERTT	
具有NLS4的Cas9的氨基酸序列	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAE ATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGN IVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDV DKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLI ALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAIL LSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGY IDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILR RQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDK GASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGE QKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKD KDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHI ANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMK RIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLT KAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSK LVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVR KMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDF ATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFV EQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAP AAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSQAAKRPRTT	189
具有NLS5的Cas9的氨基酸序列	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAE ATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGN IVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDV DKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLI ALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAIL LSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGY IDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILR RQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDK GASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGE QKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKD KDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHI ANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMK RIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLT KAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSK LVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVR KMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDF ATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFV EQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAP AAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSRAAKRPRTT	190
具有NLS6的Cas9的氨基酸序列	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAE ATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGN IVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDV DKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLI ALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAIL	191

[0794]

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具有NLS7的Cas9的氨基酸序列	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAE ATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGN IVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDV DKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLI ALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAIL LSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGY IDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILR RQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDK GASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGE QKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKD KDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHI ANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMK RIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLT KAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSK LVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVR KMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDF ATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFV EQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAP AAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSAAAKRVWSM AF	192
具有NLS8的Cas9的氨基酸序列	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAE ATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGN IVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDV DKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLI ALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAIL LSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGY IDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILR RQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDK GASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGE QKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKD KDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHI ANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMK RIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLT KAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSK LVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVR KMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDF ATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFV EQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAP AAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSAAAKRSWSM	193

[0795]

	AF	
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[0796]

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[0797] *＝PS键;'m'＝2'-O-Me核苷酸。

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<120> 用于基因组编辑的多核苷酸、组合物及方法

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<151> 2017-09-29

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tga 4143

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<223> 合成的：Cas9 氨基酸序列

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35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
325 330 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
340 345 350

[0006] Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
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Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
370 375 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385 390 395 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
405 410 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
420 425 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
435 440 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
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Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465 470 475 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
485 490 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
500 505 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
515 520 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
530 535 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545 550 555 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
565 570 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
580 585 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
595 600 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
610 615 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625 630 635 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655

[0007] Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
660 665 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
675 680 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
690 695 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705 710 715 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
725 730 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
740 745 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
755 760 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
770 775 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785 790 795 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
805 810 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
820 825 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
835 840 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865 870 875 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
885 890 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
900 905 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
915 920 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
930 935 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945 950 955 960

[0008] Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
965 970 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
995 1000 1005

Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala
1010 1015 1020

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
1025 1030 1035

Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala
1040 1045 1050

Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu
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Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val
1070 1075 1080

Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr
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Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys

1100 1105 1110

Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1115 1120 1125

Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
1130 1135 1140

Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys
1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
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Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
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Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
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Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

[0009]

Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
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Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
1250 1255 1260

His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys
1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
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Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
1295 1300 1305

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
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uga 4143

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<212> PRT
<213> 人工序列

<220>
<223> 合成的：Cas9 切口酶 (D10A) 氨基酸序列

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Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

[0014] His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
325 330 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
340 345 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
355 360 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
370 375 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385 390 395 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
405 410 415

[0015] Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
420 425 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
435 440 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
450 455 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465 470 475 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
485 490 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
500 505 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
515 520 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
530 535 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545 550 555 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
565 570 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
580 585 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
595 600 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
610 615 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625 630 635 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
660 665 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
675 680 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
690 695 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705 710 715 720

[0016] His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
725 730 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
740 745 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
755 760 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
770 775 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785 790 795 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
805 810 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
820 825 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
835 840 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys

865 870 875 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
885 890 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
900 905 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
915 920 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
930 935 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945 950 955 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
965 970 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
995 1000 1005

[0017] Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala
1010 1015 1020

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
1025 1030 1035

Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala
1040 1045 1050

Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu
1055 1060 1065

Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val
1070 1075 1080

Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr
1085 1090 1095

Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys
1100 1105 1110

Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1115 1120 1125

Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
1130 1135 1140

Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys
1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
1175 1180 1185

Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
1190 1195 1200

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
1205 1210 1215

Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1235 1240 1245

Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
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His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys
1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
1280 1285 1290

[0018] Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
1295 1300 1305

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
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Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
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Gly Gly Gly Ser Pro Lys Lys Lys Arg Lys Val
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aacaucauga acuucuucaa gacagaaauc acacuggcaa acggagaaau cagaaagaga 3180

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<220>
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Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

[0021] Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
325 330 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
340 345 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
355 360 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
370 375 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385 390 395 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
405 410 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
420 425 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
435 440 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
450 455 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465 470 475 480

[0022] Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
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Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
500 505 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
515 520 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
530 535 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545 550 555 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
565 570 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
580 585 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
595 600 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
610 615 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala

625 630 635 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
660 665 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
675 680 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
690 695 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705 710 715 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
725 730 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
740 745 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
755 760 765

[0023] Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
770 775 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785 790 795 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
805 810 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
820 825 830

Leu Ser Asp Tyr Asp Val Asp Ala Ile Val Pro Gln Ser Phe Leu Lys
835 840 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865 870 875 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
885 890 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
900 905 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
915 920 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
930 935 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945 950 955 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
965 970 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
995 1000 1005

Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala
1010 1015 1020

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
1025 1030 1035

Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala
1040 1045 1050

Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu
1055 1060 1065

[0024] Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val
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Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr
1085 1090 1095

Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys
1100 1105 1110

Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1115 1120 1125

Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
1130 1135 1140

Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys
1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
1175 1180 1185

Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
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Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
1205 1210 1215

Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1235 1240 1245

Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
1250 1255 1260

His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys
1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
1280 1285 1290

Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
1295 1300 1305

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
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[0025] Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
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cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa 180

gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc 240

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cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga 360

aacaucgucg acgaagucgc auaccacgaa aaguacccga caaucuacca ccugagaaag 420

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cuggcagacg caaaccugga caagguccug agcgcauaca acaagcacag agacaagccg 3900

aucagagaac aggcagaaaa caucauccac cuguucacac ugacaaaccu gggagcaccg 3960

gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

guccuggacg caacacugau ccaccagagc aucacaggac uguacgaaac aagaaucgac 4080

[0029] cugagccagc ugggaggaga cggaggagga agcccgaaga agaagagaaa gguc 4134

<210> 11
<211> 4134
<212> RNA
<213> 人工序列

<220>
<223> 合成的：Cas9 切口酶裸编码序列

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agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180

acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360

aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420

cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540

gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600

aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660

cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720

aucgcacuga gccugggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780

gcaaagcugc agcugagcaa ggacacauac gacgacgacc uggacaaccu gcuggcacag 840
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cugagcgaca uccugagagu caacacagaa aucacaaagg caccgcugag cgcaagcaug 960
aucaagagau acgacgaaca ccaccaggac cugacacugc ugaaggcacu ggucagacag 1020
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ggagucgaag acagauucaa cgcaagccug ggaacauacc acgaccugcu gaagaucauc 1800
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cuggucagcg acuucagaaa ggacuuccag uucuacaagg ucagagaaau caacaacuac 2940
caccacgcac acgacgcaua ccugaacgca gucgucggaa cagcacugau caagaaguac 3000

ccgaagcugg aaagcgaauu cgucuacgga gacuacaagg ucuacgacgu cagaaagaug 3060
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gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020
guccuggacg caacacugau ccaccagagc aucacaggac uguacgaaac aagaaucgac 4080
cugagccagc ugggaggaga cggaggagga agcccgaaga agaagagaaa gguc 4134

[0031]

<210> 12
<211> 4134
<212> RNA
<213> 人工序列

<220>
<223> 合成的：dCas9 裸编码序列

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agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180
acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240
cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300
gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360
aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420
cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480
aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540
gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600
aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660
cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720
aucgcacuga gccugggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780
gcaaagcugc agcugagcaa ggacacauac gacgacgacc uggacaaccu gcuggcacag 840

aucggagacc aguacgcaga ccuguuccug gcagcaaaga accugagcga cgcaauccug 900
cugagcgaca uccugagagu caacacagaa aucacaaagg caccgcugag cgcaagcaug 960
aucaagagau acgacgaaca ccaccaggac cugacacugc ugaaggcacu ggucagacag 1020
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uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140
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aagggaaacg aacuggcacu gccgagcaag uacgucaacu uccuguaccu ggcaagccac 3720
uacgaaaagc ugaagggaag cccggaagac aacgaacaga agcagcuguu cgucgaacag 3780
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cugagccagc ugggaggaga cggaggagga agcccgaaga agaagagaaa gguc 4134

[0033]

<210> 13
<211> 1368
<212> PRT
<213> 人工序列

<220>
<223> 合成的: Cas9(无NLS)的氨基酸序列

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Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
            20   25   30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
        35   40   45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
    50   55   60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65   70   75   80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85   90   95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100   105   110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

[0034] Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
325 330 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
340 345 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
355 360 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
370 375 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385 390 395 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
405 410 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
420 425 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
435 440 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
450 455 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465 470 475 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
485 490 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
500 505 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
515 520 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
530 535 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545 550 555 560

[0035] Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
565 570 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
580 585 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
595 600 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
610 615 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625 630 635 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
660 665 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
675 680 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
690 695 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705 710 715 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
725 730 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
740 745 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
755 760 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
770 775 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785 790 795 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
805 810 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
820 825 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
835 840 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860

[0036] Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865 870 875 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
885 890 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
900 905 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
915 920 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
930 935 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945 950 955 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
965 970 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
995 1000 1005

Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala

1010 1015 1020

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
1025 1030 1035

Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala
1040 1045 1050

Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu
1055 1060 1065

Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val
1070 1075 1080

Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr
1085 1090 1095

Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys
1100 1105 1110

Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1115 1120 1125

Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
1130 1135 1140

[0037] Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys
1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
1175 1180 1185

Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
1190 1195 1200

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
1205 1210 1215

Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1235 1240 1245

Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
1250 1255 1260

His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys
1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
1280 1285 1290

Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
1295 1300 1305

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
1340 1345 1350

Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

<210> 14
<211> 4107
<212> RNA
<213> 人工序列

<220>
<223> 合成的:使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 13的，具有起始和终止密码子的Cas9 mRNA ORF

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cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa 180
gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc 240
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cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga 360
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aagcuggucg acagcacaga caaggcagac cugagacuga ucuaccuggc acuggcacac 480
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<210> 15
<211> 4101
<212> RNA
<213> 人工序列

<220>
<223> 合成的：使用如表3中所列的最小尿苷密码子编码SEQ ID NO：13的Cas9编码序列(无起始或终止密码子；适于包括在融合蛋白质编码序列中)

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agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180

acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

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cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540

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cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720

aucgcacuga gccugggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780

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uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140

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aucagagaac aggcagaaaa caucauccac cuguucacac ugacaaaccu gggagcaccg 3960

gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

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cugagccagc ugggaggaga c 4101

<210> 16
<211> 1368
<212> PRT
<213> 人工序列

<220>
<223> 合成的: Cas9切口酶(无NLS)的氨基酸序列

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Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

[0042] Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
325 330 335

[0043] Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
340 345 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
355 360 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
370 375 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385 390 395 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
405 410 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
420 425 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
435 440 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
450 455 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465 470 475 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
485 490 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
500 505 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
515 520 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
530 535 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545 550 555 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
565 570 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
580 585 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
595 600 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
610 615 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625 630 635 640

[0044] His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
660 665 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
675 680 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
690 695 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705 710 715 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
725 730 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
740 745 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
755 760 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
770 775 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785 790 795 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
805 810 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
820 825 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
835 840 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865 870 875 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
885 890 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
900 905 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
915 920 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
930 935 940

[0045] Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945 950 955 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
965 970 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
995 1000 1005

Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala
1010 1015 1020

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
1025 1030 1035

Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala
1040 1045 1050

Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu
1055 1060 1065

Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val
1070 1075 1080

Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr

1085 1090 1095

Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys
1100 1105 1110

Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1115 1120 1125

Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
1130 1135 1140

Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys
1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
1175 1180 1185

Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
1190 1195 1200

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
1205 1210 1215

[0046] Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1235 1240 1245

Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
1250 1255 1260

His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys
1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
1280 1285 1290

Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
1295 1300 1305

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
1340 1345 1350

Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

<210> 17
<211> 4107
<212> RNA
<213> 人工序列

<220>
<223> 合成的:使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 16的，具有起始和终止密码子的Cas9切口酶mRNA ORF

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aucaaggaca aggacuuccu ggacaacgaa gaaaacgaag acauccugga agacaucguc 1860
cugacacuga cacuguucga agacagagaa augaucgaag aaagacugaa gacauacgca 1920
caccuguucg acgacaaggu caugaagcag cugaagagaa gaagauacac aggaugggga 1980

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<210> 18
<211> 4101

<212> RNA
<213> 人工序列

<220>
<223> 合成的：使用如表3中所列的最小尿苷密码子编码SEQ ID NO：16的Cas9切口酶编码序列(无起始或终止密码子；适于包括在融合蛋白质编码序列中)

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agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180
acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240
cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300
gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360
aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420
cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480
aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540
gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600
aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660
cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720
aucgcacuga gccugggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780
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cugagcagaa agcugaucaa cggaaucaga gacaagcaga gcggaaagac aauccuggac 2040

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<210> 19
<211> 1368
<212> PRT

<213> 人工序列

<220>
<223> 合成的: dCas9 (无NLS)的氨基酸序列

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Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

[0051] His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
325 330 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
340 345 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
355 360 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
370 375 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385 390 395 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
405 410 415

[0052] Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
420 425 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
435 440 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
450 455 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465 470 475 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
485 490 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
500 505 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
515 520 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
530 535 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545 550 555 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
565 570 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
580 585 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
595 600 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
610 615 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625 630 635 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
660 665 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
675 680 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
690 695 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705 710 715 720

[0053] His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
725 730 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
740 745 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
755 760 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
770 775 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785 790 795 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
805 810 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
820 825 830

Leu Ser Asp Tyr Asp Val Asp Ala Ile Val Pro Gln Ser Phe Leu Lys
835 840 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865 870 875 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
885 890 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
900 905 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
915 920 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
930 935 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945 950 955 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
965 970 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
995 1000 1005

Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala
1010 1015 1020

[0054] Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
1025 1030 1035

Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala
1040 1045 1050

Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu
1055 1060 1065

Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val
1070 1075 1080

Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr
1085 1090 1095

Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys
1100 1105 1110

Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1115 1120 1125

Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
1130 1135 1140

Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys
1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser

1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
1175 1180 1185

Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
1190 1195 1200

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
1205 1210 1215

Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1235 1240 1245

Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
1250 1255 1260

His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys
1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
1280 1285 1290

Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
1295 1300 1305

[0055]

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
1340 1345 1350

Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

<210> 20
<211> 4107
<212> RNA
<213> 人工序列

<220>
<223> 合成的:使用如表3中所列的最小尿苷密码子编码SEQ ID NO: 19的，具有起始和终止密码子的dCas9 mRNA ORF

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cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa 180

gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc 240

uaccugcagg aaaucuucag caacgaaaug gcaaaggucg acgacagcuu cuuccacaga 300

cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga 360 aacaucgucg acgaagucgc auaccacgaa aaguacccga caaucuacca ccugagaaag 420

aagcuggucg acagcacaga caaggcagac cugagacuga ucuaccuggc acuggcacac 480

augaucaagu ucagaggaca cuuccugauc gaaggagacc ugaacccgga caacagcgac 540

gucgacaagc uguucaucca gcugguccag acauacaacc agcuguucga agaaaacccg 600

aucaacgcaa gcggagucga cgcaaaggca auccugagcg caagacugag caagagcaga 660

agacuggaaa accugaucgc acagcugccg ggagaaaaga agaacggacu guucggaaac 720

cugaucgcac ugagccuggg acugacaccg aacuucaaga gcaacuucga ccuggcagaa 780

gacgcaaagc ugcagcugag caaggacaca uacgacgacg accuggacaa ccugcuggca 840

cagaucggag accaguacgc agaccuguuc cuggcagcaa agaaccugag cgacgcaauc 900

cugcugagcg acauccugag agucaacaca gaaaucacaa aggcaccgcu gagcgcaagc 960

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cagcagcugc cggaaaagua caaggaaauc uucuucgacc agagcaagaa cggauacgca 1080

ggauacaucg acggaggagc aagccaggaa gaauucuaca aguucaucaa gccgauccug 1140

gaaaagaugg acggaacaga agaacugcug gucaagcuga acagagaaga ccugcugaga 1200

aagcagagaa cauucgacaa cggaagcauc ccgcaccaga uccaccuggg agaacugcac 1260

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aucaaggaca aggacuuccu ggacaacgaa gaaaacgaag acauccugga agacaucguc 1860

cugacacuga cacuguucga agacagagaa augaucgaag aaagacugaa gacauacgca 1920

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<212> RNA
<213> 人工序列

<220>
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cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480
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<211> 1392
<212> PRT
<213> 人工序列

<220>
<223> 合成的：具有两个核定位信号作为C端氨基酸的Cas9的氨基酸序列

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Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu

50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

[0060] Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
325 330 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
340 345 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
355 360 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
370 375 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385 390 395 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
405 410 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
420 425 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
435 440 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
450 455 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465 470 475 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
485 490 495

[0061] Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
500 505 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
515 520 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
530 535 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545 550 555 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
565 570 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
580 585 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
595 600 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
610 615 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625 630 635 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655

```
Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800
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[0062]

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Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960
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Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
965 970 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
995 1000 1005

Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala
1010 1015 1020

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
1025 1030 1035

Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala
1040 1045 1050

Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu
1055 1060 1065

Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val
1070 1075 1080

Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr
1085 1090 1095

[0063] Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys
1100 1105 1110

Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1115 1120 1125

Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
1130 1135 1140

Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys
1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
1175 1180 1185

Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
1190 1195 1200

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
1205 1210 1215

Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser

1235 1240 1245

Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
1250 1255 1260

His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys
1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
1280 1285 1290

Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
1295 1300 1305

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
1340 1345 1350

Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

Gly Ser Gly Ser Pro Lys Lys Lys Arg Lys Val Asp Gly Ser Pro
1370 1375 1380

[0064]

Lys Lys Lys Arg Lys Val Asp Ser Gly
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<210> 23
<211> 4179
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<220>
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gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc 240
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agacuggaaa accugaucgc acagcugccg ggagaaaaga agaacggacu guucggaaac 720

cugaucgcac ugagccuggg acugacaccg aacuucaaga gcaacuucga ccuggcagaa 780
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ccgaucagag aacaggcaga aaacaucauc caccuguuca cacugacaaa ccugggagca 3960

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<210> 24
<211> 4173
<212> RNA
<213> 人工序列

<220>
<223> 合成的：使用如表3中所列的最小尿苷密码子编码SEQ ID NO：23的Cas9编码序列(无起始或终止密码子；适于包括在融合蛋白质编码序列中)

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agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180

acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360

aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420

cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540

gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600

aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660

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aucgcacuga gccugggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780
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agcccgaaga agaagagaaa ggucgacagc gga 4173

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<211> 1392
<212> PRT
<213> 人工序列

<220>
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Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser

85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

[0069] Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
325 330 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
340 345 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
355 360 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
370 375 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385 390 395 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
405 410 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
420 425 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
435 440 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
450 455 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465 470 475 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
485 490 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
500 505 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
515 520 525

[0070] Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
530 535 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545 550 555 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
565 570 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
580 585 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
595 600 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
610 615 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625 630 635 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
660 665 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
675 680 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
690 695 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705 710 715 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
725 730 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
740 745 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
755 760 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
770 775 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785 790 795 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
805 810 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
820 825 830

[0071] Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
835 840 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865 870 875 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
885 890 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
900 905 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
915 920 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
930 935 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945 950 955 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
965 970 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
995 1000 1005

Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala
1010 1015 1020

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
1025 1030 1035

Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala
1040 1045 1050

Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu
1055 1060 1065

Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val
1070 1075 1080

Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr
1085 1090 1095

Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys
1100 1105 1110

Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1115 1120 1125

[0072] Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
1130 1135 1140

Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys
1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
1175 1180 1185

Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
1190 1195 1200

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
1205 1210 1215

Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1235 1240 1245

Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
1250 1255 1260

His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys

1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
1280 1285 1290

Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
1295 1300 1305

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
1340 1345 1350

Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

Gly Ser Gly Ser Pro Lys Lys Lys Arg Lys Val Asp Gly Ser Pro
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Lys Lys Lys Arg Lys Val Asp Ser Gly
1385 1390

[0073]
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<211> 4179
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cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa 180
gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc 240
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cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga 360
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gucgacaagc uguucaucca gcugguccag acauacaacc agcuguucga agaaaacccg 600
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gcaacaguca gaaagguccu gagcaugccg caggucaaca ucgucaagaa gacagaaguc 3300
cagacaggag gauucagcaa ggaaagcauc cugccgaaga gaaacagcga caagcugauc 3360
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cagcacaagc acuaccugga cgaaaucauc gaacagauca gcgaauucag caagagaguc 3840
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ccgaucagag aacaggcaga aaacaucauc caccuguuca cacugacaaa ccugggagca 3960
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[0075] <210> 27
<211> 4173
<212> RNA
<213> 人工序列

<220>
<223> 合成的：使用如表3中所列的最小尿苷密码子编码SEQ ID NO：25的Cas9切口酶编码序列(无起始或终止密码子；适于包括在融合蛋白质编码序列中)

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agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180
acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240
cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300
gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360
aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420
cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480
aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540
gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600
aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660
cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720
aucgcacuga gccugggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780
gcaaagcugc agcugagcaa ggacacauac gacgacgacc uggacaaccu gcuggcacag 840
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cugagcgaca uccugagagu caacacagaa aucacaaagg caccgcugag cgcaagcaug 960
aucaagagau acgacgaaca ccaccaggac cugacacugc ugaaggcacu ggucagacag 1020
cagcugccgg aaaaguacaa ggaaaucuuc uucgaccaga gcaagaacgg auacgcagga 1080
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gaacugcugg gaaucacaau cauggaaaga agcagcuucg aaaagaaccc gaucgacuuc 3540

cuggaagcaa agggauacaa ggaagucaag aaggaccuga ucaucaagcu gccgaaguac 3600

agccuguucg aacuggaaaa cggaagaaag agaaugcugg caagcgcagg agaacugcag 3660

aagggaaacg aacuggcacu gccgagcaag uacgucaacu uccuguaccu ggcaagccac 3720

uacgaaaagc ugaagggaag cccggaagac aacgaacaga agcagcuguu cgucgaacag 3780

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cuggcagacg caaaccugga caagguccug agcgcauaca acaagcacag agacaagccg 3900

aucagagaac aggcagaaaa caucauccac cuguucacac ugacaaaccu gggagcaccg 3960

gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

guccuggacg caacacugau ccaccagagc aucacaggac uguacgaaac aagaaucgac 4080

cugagccagc ugggaggaga cggaagcgga agcccgaaga agaagagaaa ggucgacgga 4140

agcccgaaga agaagagaaa ggucgacagc gga 4173

[0077]

<210> 28
<211> 1392
<212> PRT
<213> 人工序列

<220>
<223> 合成的：具有两个核定位信号作为C端氨基酸的dCas9的氨基酸序列

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Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
            20   25   30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
        35   40   45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
    50   55   60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65   70   75   80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85   90   95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100   105   110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

[0078] Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
325 330 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
340 345 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
355 360 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
370 375 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385 390 395 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
405 410 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
420 425 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
435 440 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
450 455 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465 470 475 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
485 490 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
500 505 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
515 520 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
530 535 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545 550 555 560

[0079] Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
565 570 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
580 585 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
595 600 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
610 615 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625 630 635 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
660 665 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
675 680 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
690 695 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705 710 715 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
725 730 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
740 745 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
755 760 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
770 775 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785 790 795 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
805 810 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
820 825 830

Leu Ser Asp Tyr Asp Val Asp Ala Ile Val Pro Gln Ser Phe Leu Lys
835 840 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860

[0080] Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865 870 875 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
885 890 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
900 905 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
915 920 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
930 935 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945 950 955 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
965 970 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
995 1000 1005

Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala

1010 1015 1020

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
1025 1030 1035

Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala
1040 1045 1050

Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu
1055 1060 1065

Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val
1070 1075 1080

Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr
1085 1090 1095

Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys
1100 1105 1110

Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1115 1120 1125

Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
1130 1135 1140

[0081] Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys
1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
1175 1180 1185

Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
1190 1195 1200

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
1205 1210 1215

Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1235 1240 1245

Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
1250 1255 1260

His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys
1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
1280 1285 1290

Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
1295 1300 1305

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
1340 1345 1350

Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

Gly Ser Gly Ser Pro Lys Lys Lys Arg Lys Val Asp Gly Ser Pro
1370 1375 1380

Lys Lys Lys Arg Lys Val Asp Ser Gly
1385 1390

<210> 29
<211> 4179
<212> RNA
<213> 人工序列

<220>
<223> 合成的：使用如表3中所列的最小尿苷密码子编码SEQ ID NO：28的，具有起始和终止密码子的dCas9 mRNA ORF

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cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa 180
gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc 240
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cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga 360
aacaucgucg acgaagucgc auaccacgaa aaguacccga caaucuacca ccugagaaag 420
aagcuggucg acagcacaga caaggcagac cugagacuga ucuaccuggc acuggcacac 480
augaucaagu ucagaggaca cuuccugauc gaaggagacc ugaacccgga caacagcgac 540
gucgacaagc uguucaucca gcugguccag acauacaacc agcuguucga agaaaacccg 600
aucaacgcaa gcggagucga cgcaaaggca auccugagcg caagacugag caagagcaga 660
agacuggaaa accugaucgc acagcugccg ggagaaaaga agaacggacu guucggaaac 720
cugaucgcac ugagccuggg acugacaccg aacuucaaga gcaacuucga ccuggcagaa 780
gacgcaaagc ugcagcugag caaggacaca uacgacgacg accuggacaa ccugcuggca 840
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uuccuggaag caaagggaua caaggaaguc aagaaggacc ugaucaucaa gcugccgaag 3600

uacagccugu ucgaacugga aaacggaaga aagagaaugc uggcaagcgc aggagaacug 3660

cagaagggaa acgaacuggc acugccgagc aaguacguca acuuccugua ccuggcaagc 3720

cacuacgaaa agcugaaggg aagcccggaa gacaacgaac agaagcagcu guucgucgaa 3780

cagcacaagc acuaccugga cgaaaucauc gaacagauca gcgaauucag caagagaguc 3840

auccuggcag acgcaaaccu ggacaagguc cugagcgcau acaacaagca cagagacaag 3900

ccgaucagag aacaggcaga aaacaucauc caccuguuca cacugacaaa ccugggagca 3960

ccggcagcau ucaaguacuu cgacacaaca aucgacagaa agagauacac aagcacaaag 4020

gaaguccugg acgcaacacu gauccaccag agcaucacag gacuguacga aacaagaauc 4080

gaccugagcc agcugggagg agacggaagc ggaagcccga agaagaagag aaaggucgac 4140

ggaagcccga agaagaagag aaaggucgac agcggauag 4179

<210> 30
<211> 4173
<212> RNA
<213> 人工序列

<220>
<223> 合成的：使用如表3中所列的最小尿苷密码子编码SEQ ID NO：28的dCas9编码序列(无起始或终止密码子；适于包括在融合蛋白质编码序列中)

[0084] <400> 30
gacaagaagu acagcaucgg acuggcaauc ggaacaaaca gcgucggaug ggcagucauc 60

acagacgaau acaaggucccc gagcaagaag uucaaggucc ugggaaacac agacagacac 120

agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180

acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360

aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420

cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540

gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600

aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660

cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720

aucgcacuga gccugggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780

gcaaagcugc agcugagcaa ggacacauac gacgacgacc uggacaaccu gcuggcacag 840

aucggagacc aguacgcaga ccuguuccug gcagcaaaga accugagcga cgcaauccug 900

cugagcgaca uccugagagu caacacagaa aucacaaagg caccgcugag cgcaagcaug 960

aucaagagau acgacgaaca ccaccaggac cugacacugc ugaaggcacu ggucagacag 1020

cagcugccgg aaaaguacaa ggaaaucuuc uucgaccaga gcaagaacgg auacgcagga 1080

uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140

aagauggacg gaacagaaga acugcugguc aagcugaaca gagaagaccu gcugagaaag 1200
cagagaacau ucgacaacgg aagcaucccg caccagaucc accugggaga acugcacgca 1260
auccugagaa gacaggaaga cuucuacccg uuccugaagg acaacagaga aaagaucgaa 1320
aagauccuga cauucagaau cccguacuac gucggaccgc uggcaagagg aaacagcaga 1380
uucgcaugga ugacaagaaa gagcgaagaa acaaucacac cguggaacuu cgaagaaguc 1440
gucgacaagg gagcaagcgc acagagcuuc aucgaaagaa ugacaaacuu cgacaagaac 1500
cugccgaacg aaaagguccu gccgaagcac agccugcugu acgaauacuu cacagucuac 1560
aacgaacuga caaaggucaa guacgucaca gaaggaauga gaaagccggc auuccugagc 1620
ggagaacaga agaaggcaau cgucgaccug cuguucaaga caaacagaaa ggucacaguc 1680
aagcagcuga aggaagacua cuucaagaag aucgaaugcu ucgacagcgu cgaaaucagc 1740
ggagucgaag acagauucaa cgcaagccug ggaacauacc acgaccugcu gaagaucauc 1800
aaggacaagg acuuccugga caacgaagaa aacgaagaca uccuggaaga caucguccug 1860
acacugacac uguucgaaga cagagaaaug aucgaagaaa gacugaagac auacgcacac 1920
cuguucgacg acaaggucau gaagcagcug aagagaagaa gauacacagg augggggaaga 1980
cugagcagaa agcugaucaa cggaaucaga gacaagcaga gcggaaagac aauccuggac 2040
uuccugaaga gcgacggauu cgcaaacaga aacuucaugc agcugaucca cgacgacagc 2100
cugacauuca aggaagacau ccagaaggca caggucagcg gacagggaga cagccugcac 2160
gaacacaucg caaaccuggc aggaagcccg gcaaucaaga agggaauccu gcagacaguc 2220
[0085] aaggucgucg acgaacuggu caaggucaug ggaagacaca agccggaaaa caucgucauc 2280
gaaauggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaaug 2340
aagagaaucg aagaaggaau caaggaacug ggaagccaga uccugaagga acacccgguc 2400
gaaaacacac agcugcagaa cgaaaagcug uaccuguacu accugcagaa cggaagagac 2460
auguacgucg accaggaacu ggacaucaac agacugagcg acuacgacgu cgacgcaauc 2520
gucccgcaga gcuuccugaa ggacgacagc aucgacaaca agguccugac aagaagcgac 2580
aagaacagag gaaagagcga caacgucccg agcgaagaag ucgucaagaa gaugaagaac 2640
uacuggagac agcugcugaa cgcaaagcug aucacacaga gaaaguucga caaccugaca 2700
aaggcagaga gaggaggacu gagcgaacug gacaaggcag gauucaucaa gagacagcug 2760
gucgaaacaa gacagaucac aaagcacguc gcacagaucc uggacagcag aaugaacaca 2820
aaguacgacg aaaacgacaa gcugaucaga gaagucaagg ucaucacacu gaagagcaag 2880
cuggucagcg acuucagaaa ggacuuccag uucuacaagg ucagagaaau caacaacuac 2940
caccacgcac acgacgcaua ccugaacgca gucgucggaa cagcacugau caagaaguac 3000
ccgaagcugg aaagcgaauu cgucuacgga gacuacaagg ucuacgacgu cagaaagaug 3060
aucgcaaaga gcgaacagga aaucggaaag gcaacagcaa aguacuucuu cuacagcaac 3120
aucaugaacu ucuucaagac agaaaucaca cuggcaaacg gagaaaucag aaagagaccg 3180
cugaucgaaa caaacggaga aacaggagaa aucgucuggg acaagggaag agacuucgca 3240
acagucagaa agguccugag caugccgcag gucaacaucg ucaagaagac agaaguccag 3300
acaggaggau ucagcaagga aagcauccug ccgaagagaa acagcgacaa gcugaucgca 3360

agaaagaagg acugggaccc gaagaaguac ggaggauucg acagcccgac agucgcauac 3420

agcguccugg ucgucgcaaa ggucgaaaag ggaaagagca agaagcugaa gagcgucaag 3480

gaacugcugg gaaucacaau cauggaaaga agcagcuucg aaaagaaccc gaucgacuuc 3540

cuggaagcaa agggauacaa ggaagucaag aaggaccuga ucaucaagcu gccgaaguac 3600

agccuguucg aacuggaaaa cggaagaaag agaaugcugg caagcgcagg agaacugcag 3660

aagggaaacg aacuggcacu gccgagcaag uacgucaacu uccuguaccu ggcaagccac 3720

uacgaaaagc ugaagggaag cccggaagac aacgaacaga agcagcuguu cgucgaacag 3780

cacaagcacu accuggacga aaucaucgaa cagaucagcg aauucagcaa gagagucauc 3840

cuggcagacg caaaccugga caagguccug agcgcauaca acaagcacag agacaagccg 3900

aucagagaac aggcagaaaa caucauccac cuguucacac ugacaaaccu gggagcaccg 3960

gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

guccuggacg caacacugau ccaccagagc aucacaggac uguacgaaac aagaaucgac 4080

cugagccagc ugggaggaga cggaagcgga agcccgaaga agaagagaaa ggucgacgga 4140

agcccgaaga agaagagaaa ggucgacagc gga 4173

<210> 31
<211> 17
<212> DNA
<213> 人工序列

<220>
<223> 合成的: T7 启动子

[0086]

<400> 31
taatacgact cactata 17

<210> 32
<211> 50
<212> DNA
<213> 智人

<220>
<221> misc_feature
<222> (1)..(50)
<223> 人类β-血球蛋白 5 UTR

<400> 32
acatttgctt ctgacacaac tgtgttcact agcaacctca aacagacacc 50

<210> 33
<211> 132
<212> DNA
<213> 智人

<220>
<221> misc_feature
<222> (1)..(132)
<223> 人类β-血球蛋白 3 UTR

<400> 33
gctcgctttc ttgctgtcca atttctatta aaggttcctt tgttccctaa gtccaactac 60

taaactgggg gatattatga agggccttga gcatctggat tctgcctaat aaaaaacatt 120

tattttcatt gc 132

<210> 34
<211> 66
<212> DNA
<213> 智人

<220>
<221> misc_feature
<222> (1)..(66)
<223> 人类α-血球蛋白 5 UTR

<400> 34
cataaaccct ggcgcgctcg cggcccggca ctcttctggt ccccacagac tcagagagaa 60

cccacc 66

<210> 35
<211> 110
<212> DNA
<213> 智人

<220>
<221> misc_feature
<222> (1)..(110)
<223> 人类α-血球蛋白 3 UTR

<400> 35
gctggagcct cggtggccat gcttcttgcc ccttgggcct ccccccagcc cctcctcccc 60

ttcctgcacc cgtacccccg tggtctttga ataaagtctg agtgggcggc 110

<210> 36
<211> 29
<212> DNA
[0087] <213> 有爪蟾蜍

<220>
<221> misc_feature
<222> (1)..(29)
<223> 有爪蟾蜍β-血球蛋白 5 UTR

<400> 36
aagctcagaa taaacgctca actttggcc 29

<210> 37
<211> 130
<212> DNA
<213> 有爪蟾蜍

<220>
<221> misc_feature
<222> (1)..(130)
<223> 有爪蟾蜍β-血球蛋白 3 UTR

<400> 37
accagcctca agaacacccg aatggagtct ctaagctaca taataccaac ttacacttta 60

caaaatgttg tcccccaaaa tgtagccatt cgtatctgct cctaataaaa agaaagtttc 120

ttcacattct 130

<210> 38
<211> 27
<212> DNA
<213> 普通牛

<220>

<221> misc_feature
<222> (1)..(27)
<223> 牛生长激素 5 UTR

<400> 38
cagggtcctg tggacagctc accagct 27

<210> 39
<211> 102
<212> DNA
<213> 普通牛

<220>
<221> misc_feature
<222> (1)..(102)
<223> 牛生长激素 3 UTR

<400> 39
ttgccagcca tctgttgttt gcccctcccc cgtgccttcc ttgaccctgg aaggtgccac 60

tcccactgtc ctttcctaat aaaatgagga aattgcatcg ca 102

<210> 40
<211> 93
<212> DNA
<213> 小家鼠

<220>
<221> misc_feature
<222> (1)..(93)
<223> 小家鼠血红素α， 成年链1 (Hba-a1), 3UTR

[0088] <400> 40
gctgccttct gcggggcttg ccttctggcc atgcccttct tctctccctt gcacctgtac 60

ctcttggtct ttgaataaag cctgagtagg aag 93

<210> 41
<211> 61
<212> DNA
<213> 人工序列

<220>
<223> 合成的: HSD17B4 5 UTR

<400> 41
tcccgcagtc ggcgtccagc ggctctgctt gttcgtgtgt gtgtcgttgc aggccttatt 60

c 61

<210> 42
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的: 靶向TTR的G282引导RNA

<220>
<221> 修饰的_碱基
<222> (1)..(3)
<223> PS键, 2'-O-Me 核苷酸

<220>
<221> 修饰的_碱基
<222> (29)..(40)
<223> 2'-O-Me 核苷酸

<220>
<221> 修饰的_碱基
<222> (69)..(96)
<223> 2'-O-Me 核苷酸

<220>
<221> 修饰的_碱基
<222> (97)..(100)
<223> PS键, 2'-O-Me 核苷酸

<400> 42
uuacagccac gucuacagca guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 43
<211> 4411
<212> DNA
<213> 人工序列

<220>
<223> 合成的: 具有HSD的5' UTR、对应于SEQ ID NO: 4的ORF、Kozak序列及ALB的3' UTR的Cas9转录物

<400> 43
gggtcccgca gtcggcgtcc agcggctctg cttgttcgtg tgtgtgtcgt tgcaggcctt 60

attcggatcc gccaccatgg acaagaagta cagcatcgga ctggacatcg gaacaaacag 120

cgtcggatgg gcagtcatca cagacgaata caaggtcccg agcaagaagt tcaaggtcct 180

gggaaacaca gacagacaca gcatcaagaa gaacctgatc ggagcactgc tgttcgacag 240

cggagaaaca gcagaagcaa caagactgaa gagaacagca agaagaagat acacaagaag 300

aaagaacaga atctgctacc tgcaggaaat cttcagcaac gaaatggcaa aggtcgacga 360

[0089] cagcttcttc cacagactgg aagaaagctt cctggtcgaa gaagacaaga agcacgaaag 420

acacccgatc ttcggaaaca tcgtcgacga agtcgcatac cacgaaaagt acccgacaat 480

ctaccacctg agaaagaagc tggtcgacag cacagacaag gcagacctga gactgatcta 540

cctggcactg gcacacatga tcaagttcag aggacacttc ctgatcgaag gagacctgaa 600

cccggacaac agcgacgtcg acaagctgtt catccagctg gtccagacat acaaccagct 660

gttcgaagaa aacccgatca acgcaagcgg agtcgacgca aaggcaatcc tgagcgcaag 720

actgagcaag agcagaagac tggaaaacct gatcgcacag ctgccgggag aaaagaagaa 780

cggactgttc ggaaacctga tcgcactgag cctgggactg acaccgaact tcaagagcaa 840

cttcgacctg gcagaagacg caaagctgca gctgagcaag gacacatacg acgacgacct 900

ggacaacctg ctggcacaga tcggagacca gtacgcagac ctgttcctgg cagcaaagaa 960

cctgagcgac gcaatcctgc tgagcgacat cctgagagtc aacacagaaa tcacaaaggc 1020

accgctgagc gcaagcatga tcaagagata cgacgaacac caccaggacc tgacactgct 1080

gaaggcactg gtcagacagc agctgccgga aaagtacaag gaaatcttct tcgaccagag 1140

caagaacgga tacgcaggat acatcgacgg aggagcaagc caggaagaat tctacaagtt 1200

catcaagccg atcctggaaa agatggacgg aacagaagaa ctgctggtca agctgaacag 1260

agaagacctg ctgagaaagc agagaacatt cgacaacgga agcatcccgc accagatcca 1320

cctgggagaa ctgcacgcaa tcctgagaag acaggaagac ttctacccgt tcctgaagga 1380

caacagagaa aagatcgaaa agatcctgac attcagaatc ccgtactacg tcggaccgct 1440

ggcaagagga aacagcagat tcgcatggat gacaagaaag agcgaagaaa caatcacacc 1500

gtggaacttc gaagaagtcg tcgacaaggg agcaagcgca cagagcttca tcgaaagaat 1560
gacaaacttc gacaagaacc tgccgaacga aaaggtcctg ccgaagcaca gcctgctgta 1620
cgaatacttc acagtctaca acgaactgac aaaggtcaag tacgtcacag aaggaatgag 1680
aaagccggca ttcctgagcg gagaacagaa gaaggcaatc gtcgacctgc tgttcaagac 1740
aaacagaaag gtcacagtca agcagctgaa ggaagactac ttcaagaaga tcgaatgctt 1800
cgacagcgtc gaaatcagcg gagtcgaaga cagattcaac gcaagcctgg gaacatacca 1860
cgacctgctg aagatcatca aggacaagga cttcctggac aacgaagaaa acgaagacat 1920
cctggaagac atcgtcctga cactgacact gttcgaagac agagaaatga tcgaagaaag 1980
actgaagaca tacgcacacc tgttcgacga caaggtcatg aagcagctga agagaagaag 2040
atacacagga tggggaagac tgagcagaaa gctgatcaac ggaatcagag acaagcagag 2100
cggaaagaca atcctggact tcctgaagag cgacggattc gcaaacagaa acttcatgca 2160
gctgatccac gacgacagcc tgacattcaa ggaagacatc cagaaggcac aggtcagcgg 2220
acagggagac agcctgcacg aacacatcgc aaacctggca ggaagcccgg caatcaagaa 2280
gggaatcctg cagacagtca aggtcgtcga cgaactggtc aaggtcatgg gaagacacaa 2340
gccggaaaac atcgtcatcg aaatggcaag agaaaaccag acaacacaga agggacagaa 2400
gaacagcaga gaaagaatga agagaatcga agaaggaatc aaggaactgg gaagccagat 2460
cctgaaggaa cacccggtcg aaaacacaca gctgcagaac gaaaagctgt acctgtacta 2520
cctgcagaac ggaagagaca tgtacgtcga ccaggaactg gacatcaaca gactgagcga 2580
ctacgacgtc gaccacatcg tcccgcagag cttcctgaag gacgacagca tcgacaacaa 2640
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ggtcctgaca agaagcgaca agaacagagg aaagagcgac aacgtcccga gcgaagaagt 2700
cgtcaagaag atgaagaact actggagaca gctgctgaac gcaaagctga tcacacagag 2760
aaagttcgac aacctgacaa aggcagagag aggaggactg agcgaactgg acaaggcagg 2820
attcatcaag agacagctgg tcgaaacaag acagatcaca aagcacgtcg cacagatcct 2880
ggacagcaga atgaacacaa agtacgacga aaacgacaag ctgatcagag aagtcaaggt 2940
catcacactg aagagcaagc tggtcagcga cttcagaaag gacttccagt tctacaaggt 3000
cagagaaatc aacaactacc accacgcaca cgacgcatac ctgaacgcag tcgtcggaac 3060
agcactgatc aagaagtacc cgaagctgga aagcgaattc gtctacggag actacaaggt 3120
ctacgacgtc agaaagatga tcgcaaagag cgaacaggaa atcggaaagg caacagcaaa 3180
gtacttcttc tacagcaaca tcatgaactt cttcaagaca gaaatcacac tggcaaacgg 3240
agaaatcaga aagagaccgc tgatcgaaac aaacggagaa acaggagaaa tcgtctggga 3300
caagggaaga gacttcgcaa cagtcagaaa ggtcctgagc atgccgcagg tcaacatcgt 3360
caagaagaca gaagtccaga caggaggatt cagcaaggaa agcatcctgc cgaagagaaa 3420
cagcgacaag ctgatcgcaa gaaagaagga ctgggacccg aagaagtacg gaggattcga 3480
cagcccgaca gtcgcataca gcgtcctggt cgtcgcaaag gtcgaaaagg gaaagagcaa 3540
gaagctgaag agcgtcaagg aactgctggg aatcacaatc atggaaagaa gcagcttcga 3600
aaagaacccg atcgacttcc tggaagcaaa gggatacaag gaagtcaaga aggacctgat 3660
catcaagctg ccgaagtaca gcctgttcga actggaaaac ggaagaaaga gaatgctggc 3720
aagcgcagga gaactgcaga agggaaacga actggcactg ccgagcaagt acgtcaactt 3780

cctgtacctg gcaagccact acgaaaagct gaagggaagc ccggaagaca acgaacagaa 3840

gcagctgttc gtcgaacagc acaagcacta cctggacgaa atcatcgaac agatcagcga 3900

attcagcaag agagtcatcc tggcagacgc aaacctggac aaggtcctga gcgcatacaa 3960

caagcacaga gacaagccga tcagagaaca ggcagaaaac atcatccacc tgttcacact 4020

gacaaacctg ggagcaccgg cagcattcaa gtacttcgac acaacaatcg acagaaagag 4080

atacacaagc acaaaggaag tcctggacgc aacactgatc caccagagca tcacaggact 4140

gtacgaaaca agaatcgacc tgagccagct gggaggagac ggaggaggaa gcccgaagaa 4200

gaagagaaag gtctagctag ccatcacatt taaaagcatc tcagcctacc atgagaataa 4260

gagaaagaaa atgaagatca atagcttatt catctctttt tctttttcgt tggtgtaaag 4320

ccaacaccct gtctaaaaaa cataaatttc tttaatcatt ttgcctcttt tctctgtgct 4380

tcaattaata aaaaatggaa agaacctcga g 4411

<210> 44
<211> 4405
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有HSD的5' UTR、对应于SEQ ID NO：4的ORF及ALB的3' UTR的Cas9转录物

<400> 44
gggtcccgca gtcggcgtcc agcggctctg cttgttcgtg tgtgtgtcgt tgcaggcctt 60

attcggatcc atggacaaga agtacagcat cggactggac atcggaacaa acagcgtcgg 120

[0091] atgggcagtc atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa 180

cacagacaga cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga 240

aacagcagaa gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa 300

cagaatctgc tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt 360

cttccacaga ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc 420

gatcttcgga aacatcgtcg acgaagtcgc ataccacgaa aagtacccga caatctacca 480

cctgagaaag aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc 540

actggcacac atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga 600

caacagcgac gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga 660

agaaaacccg atcaacgcaa gcggagtcga cgcaaaggca atcctgagcg caagactgag 720

caagagcaga agactggaaa acctgatcgc acagctgccg ggagaaaaga agaacggact 780

gttcggaaac ctgatcgcac tgagcctggg actgacaccg aacttcaaga gcaacttcga 840

cctggcagaa gacgcaaagc tgcagctgag caaggacaca tacgacgacg acctggacaa 900

cctgctggca cagatcggag accagtacgc agacctgttc ctggcagcaa agaacctgag 960

cgacgcaatc ctgctgagcg acatcctgag agtcaacaca gaaatcacaa aggcaccgct 1020

gagcgcaagc atgatcaaga gatacgacga acaccaccag gacctgacac tgctgaaggc 1080

actggtcaga cagcagctgc cggaaaagta caaggaaatc ttcttcgacc agagcaagaa 1140

cggatacgca ggatacatcg acggaggagc aagccaggaa gaattctaca agttcatcaa 1200

gccgatcctg gaaaagatgg acggaacaga agaactgctg gtcaagctga acagagaaga 1260

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<210> 45
<211> 4188
<212> DNA
<213> 人工序列

[0093] <220>
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<210> 46
<211> 4459
<212> DNA
<213> 人工序列

[0095] <220>
<223> 合成的：具有HSD的5' UTR、对应于SEQ ID NO：45的ORF、Kozak序列及ALB的3' UTR的Cas9转录物

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aaagaacctc gag 4453

<210> 48
<211> 4403
<212> DNA
<213> 人工序列

<220>
<223> 合成的：包含使用通常在人类中高度表达的密码子的Cas9 ORF的Cas9转录物

<400> 48
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gctggatatc ggaactaatt ccgtgggttg ggcagtgatc acggatgaat acaaagtgcc 180

gtccaagaag ttcaaggtcc tggggaacac cgatagacac agcatcaaga aaaatctcat 240

cggagccctg ctgtttgact ccggcgaaac cgcagaagcg acccggctca aacgtaccgc 300

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cgaaatggca aaggtcgacg acagcttctt ccaccgcctg gaagaatctt tcctggtgga 420

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<210> 49
<211> 4409
<212> DNA
<213> 人工序列

<220>
<223> 合成的：Cas9 包含Kozak序列以及使用通常在人类中高度表达的密码子的Cas9 ORF的Cas9转录物

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<210> 50
<211> 4140
<212> DNA
<213> 人工序列

<220>
<223> 合成的：剪接接点经移除；具有12.75% U含量的Cas9 ORF

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<210> 51
<211> 4411
<212> DNA
<213> 人工序列

<220>
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<400> 51

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<210> 52

<211> 4140
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有一般经常用于人类中的最小尿苷密码子；具有12.75% U含量的Cas9 ORF

<400> 52

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<210> 53
<211> 4411
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<213>　人工序列

<220>
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<400>　53

[0110]

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<210> 54
<211> 4140
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有一般不经常用于人类中的最小尿苷密码子；具有12.75% U含量的Cas9 ORF

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cacagcataa aaaaaaacct aataggggcg ctactattcg acagcgggga aacggcggaa 180

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<210> 55
<211> 4411
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有HSD的5' UTR、对应于SEQ ID NO：54的ORF、Kozak序列及ALB的3' UTR的Cas9转录物

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<210> 56
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<220>
<223> 合成的：具有AGG作为供与CleanCapTM一起使用之前三个核苷酸、HSD的5' UTR、对应于SEQ ID NO：4的ORF、Kozak序列及ALB的3' UTR的Cas9转录物

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<210> 57
<211> 4481
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有来自CMV的5’ UTR、对应于SEQ ID NO：4的ORF、Kozak序列及ALB的3’ UTR的Cas9转录物

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<210> 58
<211> 4348
<212> DNA
<213> 人工序列

<220>
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[0122]
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<213> 人工序列

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<213> 人工序列

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<212> DNA

<213> 人工序列

<220>
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<220>
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<210> 66
<211> 3306
<212> DNA
<213> 人工序列

<220>
<223> 合成的：编码脑膜炎双球菌Cas9的ORF（无起始或终止密码子；适于包括在融合蛋白质编码序列中）

<400> 66
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ggagtcagag tcttcgaaag agcagaagtc ccgaagacag gagactcgct ggcaatggca 180
agaagactgg caagatcggt cagaagactg acaagaagaa gagcacacag actgctgaga 240
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gtcgaa 3306

<210> 67
<211> 3636
<212> DNA
<213> 人工序列

<220>
<223> 合成的: Tran包含SEQ ID NO: 65 (编码脑膜炎双球菌Cas9)的转录物

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agtcttcgaa agagcagaag tcccgaagac aggagactcg ctggcaatgg caagaagact 240
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<210> 68
<211> 1103
<212> PRT
<213> 人工序列

<220>
<223> 合成的：脑膜炎双球菌Cas9的氨基酸序列

<400> 68

Met Ala Ala Phe Lys Pro Asn Ser Ile Asn Tyr Ile Leu Gly Leu Asp
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Ile Gly Ile Ala Ser Val Gly Trp Ala Met Val Glu Ile Asp Glu Glu
20 25 30

Glu Asn Pro Ile Arg Leu Ile Asp Leu Gly Val Arg Val Phe Glu Arg
35 40 45

Ala Glu Val Pro Lys Thr Gly Asp Ser Leu Ala Met Ala Arg Arg Leu
50 55 60

Ala Arg Ser Val Arg Arg Leu Thr Arg Arg Arg Ala His Arg Leu Leu
65 70 75 80

Arg Thr Arg Arg Leu Leu Lys Arg Glu Gly Val Leu Gln Ala Ala Asn
85 90 95

Phe Asp Glu Asn Gly Leu Ile Lys Ser Leu Pro Asn Thr Pro Trp Gln
100 105 110

Leu Arg Ala Ala Ala Leu Asp Arg Lys Leu Thr Pro Leu Glu Trp Ser
115 120 125

Ala Val Leu Leu His Leu Ile Lys His Arg Gly Tyr Leu Ser Gln Arg
130 135 140

Lys Asn Glu Gly Glu Thr Ala Asp Lys Glu Leu Gly Ala Leu Leu Lys
145 150 155 160

Gly Val Ala Gly Asn Ala His Ala Leu Gln Thr Gly Asp Phe Arg Thr
165 170 175

Pro Ala Glu Leu Ala Leu Asn Lys Phe Glu Lys Glu Ser Gly His Ile
180 185 190

Arg Asn Gln Arg Ser Asp Tyr Ser His Thr Phe Ser Arg Lys Asp Leu
195 200 205

Gln Ala Glu Leu Ile Leu Leu Phe Glu Lys Gln Lys Glu Phe Gly Asn
210 215 220

[0135] Pro His Val Ser Gly Gly Leu Lys Glu Gly Ile Glu Thr Leu Leu Met
225 230 235 240

Thr Gln Arg Pro Ala Leu Ser Gly Asp Ala Val Gln Lys Met Leu Gly
245 250 255

His Cys Thr Phe Glu Pro Ala Glu Pro Lys Ala Ala Lys Asn Thr Tyr
260 265 270

Thr Ala Glu Arg Phe Ile Trp Leu Thr Lys Leu Asn Asn Leu Arg Ile
275 280 285

Leu Glu Gln Gly Ser Glu Arg Pro Leu Thr Asp Thr Glu Arg Ala Thr
290 295 300

Leu Met Asp Glu Pro Tyr Arg Lys Ser Lys Leu Thr Tyr Ala Gln Ala
305 310 315 320

Arg Lys Leu Leu Gly Leu Glu Asp Thr Ala Phe Phe Lys Gly Leu Arg
325 330 335

Tyr Gly Lys Asp Asn Ala Glu Ala Ser Thr Leu Met Glu Met Lys Ala
340 345 350

Tyr His Ala Ile Ser Arg Ala Leu Glu Lys Glu Gly Leu Lys Asp Lys
355 360 365

Lys Ser Pro Leu Asn Leu Ser Pro Glu Leu Gln Asp Glu Ile Gly Thr

370 375 380

Ala Phe Ser Leu Phe Lys Thr Asp Glu Asp Ile Thr Gly Arg Leu Lys
385 390 395 400

Asp Arg Ile Gln Pro Glu Ile Leu Glu Ala Leu Leu Lys His Ile Ser
405 410 415

Phe Asp Lys Phe Val Gln Ile Ser Leu Lys Ala Leu Arg Arg Ile Val
420 425 430

Pro Leu Met Glu Gln Gly Lys Arg Tyr Asp Glu Ala Cys Ala Glu Ile
435 440 445

Tyr Gly Asp His Tyr Gly Lys Lys Asn Thr Glu Glu Lys Ile Tyr Leu
450 455 460

Pro Pro Ile Pro Ala Asp Glu Ile Arg Asn Pro Val Val Leu Arg Ala
465 470 475 480

Leu Ser Gln Ala Arg Lys Val Ile Asn Gly Val Val Arg Arg Tyr Gly
485 490 495

Ser Pro Ala Arg Ile His Ile Glu Thr Ala Arg Glu Val Gly Lys Ser
500 505 510

[0136] Phe Lys Asp Arg Lys Glu Ile Glu Lys Arg Gln Glu Glu Asn Arg Lys
515 520 525

Asp Arg Glu Lys Ala Ala Ala Lys Phe Arg Glu Tyr Phe Pro Asn Phe
530 535 540

Val Gly Glu Pro Lys Ser Lys Asp Ile Leu Lys Leu Arg Leu Tyr Glu
545 550 555 560

Gln Gln His Gly Lys Cys Leu Tyr Ser Gly Lys Glu Ile Asn Leu Gly
565 570 575

Arg Leu Asn Glu Lys Gly Tyr Val Glu Ile Asp His Ala Leu Pro Phe
580 585 590

Ser Arg Thr Trp Asp Asp Ser Phe Asn Asn Lys Val Leu Val Leu Gly
595 600 605

Ser Glu Asn Gln Asn Lys Gly Asn Gln Thr Pro Tyr Glu Tyr Phe Asn
610 615 620

Gly Lys Asp Asn Ser Arg Glu Trp Gln Glu Phe Lys Ala Arg Val Glu
625 630 635 640

Thr Ser Arg Phe Pro Arg Ser Lys Lys Gln Arg Ile Leu Leu Gln Lys
645 650 655

Phe Asp Glu Asp Gly Phe Lys Glu Arg Asn Leu Asn Asp Thr Arg Tyr
660 665 670

Val Asn Arg Phe Leu Cys Gln Phe Val Ala Asp Arg Met Arg Leu Thr
675 680 685

Gly Lys Gly Lys Lys Arg Val Phe Ala Ser Asn Gly Gln Ile Thr Asn
690 695 700

Leu Leu Arg Gly Phe Trp Gly Leu Arg Lys Val Arg Ala Glu Asn Asp
705 710 715 720

Arg His His Ala Leu Asp Ala Val Val Val Ala Cys Ser Thr Val Ala
725 730 735

Met Gln Gln Lys Ile Thr Arg Phe Val Arg Tyr Lys Glu Met Asn Ala
740 745 750

Phe Asp Gly Lys Thr Ile Asp Lys Glu Thr Gly Glu Val Leu His Gln
755 760 765

Lys Thr His Phe Pro Gln Pro Trp Glu Phe Phe Ala Gln Glu Val Met
770 775 780

Ile Arg Val Phe Gly Lys Pro Asp Gly Lys Pro Glu Phe Glu Glu Ala
785 790 795 800

Asp Thr Leu Glu Lys Leu Arg Thr Leu Leu Ala Glu Lys Leu Ser Ser
805 810 815

[0137] Arg Pro Glu Ala Val His Glu Tyr Val Thr Pro Leu Phe Val Ser Arg
820 825 830

Ala Pro Asn Arg Lys Met Ser Gly Gln Gly His Met Glu Thr Val Lys
835 840 845

Ser Ala Lys Arg Leu Asp Glu Gly Val Ser Val Leu Arg Val Pro Leu
850 855 860

Thr Gln Leu Lys Leu Lys Asp Leu Glu Lys Met Val Asn Arg Glu Arg
865 870 875 880

Glu Pro Lys Leu Tyr Glu Ala Leu Lys Ala Arg Leu Glu Ala His Lys
885 890 895

Asp Asp Pro Ala Lys Ala Phe Ala Glu Pro Phe Tyr Lys Tyr Asp Lys
900 905 910

Ala Gly Asn Arg Thr Gln Gln Val Lys Ala Val Arg Val Glu Gln Val
915 920 925

Gln Lys Thr Gly Val Trp Val Arg Asn His Asn Gly Ile Ala Asp Asn
930 935 940

Ala Thr Met Val Arg Val Asp Val Phe Glu Lys Gly Asp Lys Tyr Tyr
945 950 955 960

Leu Val Pro Ile Tyr Ser Trp Gln Val Ala Lys Gly Ile Leu Pro Asp
965 970 975

Arg Ala Val Val Gln Gly Lys Asp Glu Glu Asp Trp Gln Leu Ile Asp
980 985 990

Asp Ser Phe Asn Phe Lys Phe Ser Leu His Pro Asn Asp Leu Val Glu
995 1000 1005

Val Ile Thr Lys Lys Ala Arg Met Phe Gly Tyr Phe Ala Ser Cys
1010 1015 1020

His Arg Gly Thr Gly Asn Ile Asn Ile Arg Ile His Asp Leu Asp
1025 1030 1035

His Lys Ile Gly Lys Asn Gly Ile Leu Glu Gly Ile Gly Val Lys
1040 1045 1050

Thr Ala Leu Ser Phe Gln Lys Tyr Gln Ile Asp Glu Leu Gly Lys
1055 1060 1065

Glu Ile Arg Pro Cys Arg Leu Lys Lys Arg Pro Pro Val Arg Ser
1070 1075 1080

Gly Lys Arg Thr Ala Asp Gly Ser Glu Phe Glu Ser Pro Lys Lys
1085 1090 1095

Lys Arg Lys Val Glu
1100

[0138] <210> 69
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的: G390 引导 RNA

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<222> (1)..(3)
<223> PS键, 2'-O-Me 核苷酸

<220>
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<222> (29)..(40)
<223> 2'-O-Me 核苷酸

<220>
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<222> (69)..(96)
<223> 2'-O-Me 核苷酸

<220>
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<222> (97)..(100)
<223> PS键, 2'-O-Me 核苷酸

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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 70
<211> 100
<212> RNA

<213> 人工序列

<220>
<223> 合成的: G502 引导 RNA

<220>
<221> 修饰的_碱基
<222> (1)..(3)
<223> PS键, 2'-O-Me 核苷酸

<220>
<221> 修饰的_碱基
<222> (29)..(40)
<223> 2'-O-Me 核苷酸

<220>
<221> 修饰的_碱基
<222> (69)..(96)
<223> 2'-O-Me 核苷酸

<220>
<221> 修饰的_碱基
<222> (97)..(100)
<223> PS键, 2'-O-Me 核苷酸

<400> 70
acacaaauac caguccagcg guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 71
<211> 100
<212> RNA
<213> 人工序列

[0139]

<220>
<223> 合成的: G509 引导 RNA

<220>
<221> 修饰的_碱基
<222> (1)..(3)
<223> PS键, 2'-O-Me 核苷酸

<220>
<221> 修饰的_碱基
<222> (29)..(40)
<223> 2'-O-Me 核苷酸

<220>
<221> 修饰的_碱基
<222> (69)..(96)
<223> 2'-O-Me 核苷酸

<220>
<221> 修饰的_碱基
<222> (97)..(100)
<223> PS键, 2'-O-Me 核苷酸

<400> 71
aaaguucuag augccguccg guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 72
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的: G534 引导 RNA

<220>
<221> 修饰的_碱基
<222> (1)..(3)
<223> PS键, 2'-O-Me 核苷酸

<220>
<221> 修饰的_碱基
<222> (29)..(40)
<223> 2'-O-Me 核苷酸

<220>
<221> 修饰的_碱基
<222> (69)..(96)
<223> 2'-O-Me 核苷酸

<220>
<221> 修饰的_碱基
<222> (97)..(100)
<223> PS键, 2'-O-Me 核苷酸

<400> 72
acgcaaauau caguccagcg guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 73
<211> 3783
<212> DNA
<213> 人工序列

<220>
<223> 合成的: eGFP的DNA编码序列

<400> 73
tcgcgcgttt cggtgatgac ggtgaaaacc tctgacacat gcagctcccg gagacggtca 60

[0140] cagcttgtct gtaagcggat gccgggagca gacaagcccg tcagggcgcg tcagcgggtg 120

ttggcgggtg tcggggctgg cttaactatg cggcatcaga gcagattgta ctgagagtgc 180

accatatgcg gtgtgaaata ccgcacagat gcgtaaggag aaaataccgc atcaggcgcc 240

attcgccatt caggctgcgc aactgttggg aagggcgatc ggtgcgggcc tcttcgctat 300

tacgccagct ggcgaaaggg ggatgtgctg caaggcgatt aagttgggta acgccagggt 360

tttcccagtc acgacgttgt aaaacgacgg ccagtgaatt ctaatacgac tcactatagg 420

gtcccgcagt cggcgtccag cggctctgct tgttcgtgtg tgtgtcgttg caggccttat 480

tcggatccat ggtgagcaag ggcgaggagc tgttcaccgg ggtggtgccc atcctggtcg 540

agctggacgg cgacgtaaac ggccacaagt tcagcgtgtc cggcgagggc gagggcgatg 600

ccacctacgg caagctgacc ctgaagttca tctgcaccac cggcaagctg cccgtgccct 660

ggcccaccct cgtgaccacc ctgacctacg gcgtgcagtg cttcagccgc taccccgacc 720

acatgaagca gcacgacttc ttcaagtccg ccatgcccga aggctacgtc caggagcgca 780

ccatcttctt caaggacgac ggcaactaca agacccgcgc cgaggtgaag ttcgagggcg 840

acaccctggt gaaccgcatc gagctgaagg gcatcgactt caaggaggac ggcaacatcc 900

tggggcacaa gctggagtac aactacaaca gccacaacgt ctatatcatg gccgacaagc 960

agaagaacgg catcaaggtg aacttcaaga tccgccacaa catcgaggac ggcagcgtgc 1020

agctcgccga ccactaccag cagaacaccc ccatcggcga cggccccgtg ctgctgcccg 1080

acaaccacta cctgagcacc cagtccgccc tgagcaaaga ccccaacgag aagcgcgatc 1140

acatggtcct gctggagttc gtgaccgccg ccgggatcac tctcggcatg gacgagctgt 1200

acaagtaata ggaattatgc agtctagcca tcacatttaa aagcatctca gcctaccatg 1260
agaataagag aaagaaaatg aagatcaata gcttattcat ctctttttct ttttcgttgg 1320
tgtaaagcca acaccctgtc taaaaaacat aaatttcttt aatcattttg cctcttttct 1380
ctgtgcttca attaataaaa aatggaaaga acctcgagaa aaaaaaaaaa aaaaaaaaaa 1440
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1500
aaaaaaaaaa aaaaaaaatc tagacttaag cttgatgagc tctagcttgg cgtaatcatg 1560
gtcatagctg tttcctgtgt gaaattgtta tccgctcaca attccacaca acatacgagc 1620
cggaagcata aagtgtaaag cctggggtgc ctaatgagtg agctaactca cattaattgc 1680
gttgcgctca ctgcccgctt tccagtcggg aaacctgtcg tgccagctgc attaatgaat 1740
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tgactcgctg cgctcggtcg ttcggctgcg gcgagcggta tcagctcact caaaggcggt 1860
aatacggtta tccacagaat caggggataa cgcaggaaag aacatgtgag caaaaggcca 1920
gcaaaaggcc aggaaccgta aaaaggccgc gttgctggcg tttttccata ggctccgccc 1980
ccctgacgag catcacaaaa atcgacgctc aagtcagagg tggcgaaacc cgacaggact 2040
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gccgcttacc ggatacctgt ccgcctttct cccttcggga agcgtggcgc tttctcatag 2160
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cgaacccccc gttcagcccg accgctgcgc cttatccggt aactatcgtc ttgagtccaa 2280
[0141] cccggtaaga cacgacttat cgccactggc agcagccact ggtaacagga ttagcagagc 2340
gaggtatgta ggcggtgcta cagagttctt gaagtggtgg cctaactacg gctacactag 2400
aagaacagta tttggtatct gcgctctgct gaagccagtt accttcggaa aaagagttgg 2460
tagctcttga tccggcaaac aaaccaccgc tggtagcggt ggtttttttg tttgcaagca 2520
gcagattacg cgcagaaaaa aaggatctca agaagatcct ttgatctttt ctacggggtc 2580
tgacgctcag tggaacgaaa actcacgtta agggattttg gtcatgagat tatcaaaaag 2640
gatcttcacc tagatccttt taaattaaaa atgaagtttt aaatcaatct aaagtatata 2700
tgagtaaact tggtctgaca gttaccaatg cttaatcagt gaggcaccta tctcagcgat 2760
ctgtctattt cgttcatcca tagttgcctg actccccgtc gtgtagataa ctacgatacg 2820
ggagggctta ccatctggcc ccagtgctgc aatgataccg cgagacccac gctcaccggc 2880
tccagattta tcagcaataa accagccagc cggaagggcc gagcgcagaa gtggtcctgc 2940
aactttatcc gcctccatcc agtctattaa ttgttgccgg gaagctagag taagtagttc 3000
gccagttaat agtttgcgca acgttgttgc cattgctaca ggcatcgtgg tgtcacgctc 3060
gtcgtttggt atggcttcat tcagctccgg ttcccaacga tcaaggcgag ttacatgatc 3120
ccccatgttg tgcaaaaaag cggttagctc cttcggtcct ccgatcgttg tcagaagtaa 3180
gttggccgca gtgttatcac tcatggttat ggcagcactg cataattctc ttactgtcat 3240
gccatccgta agatgctttt ctgtgactgg tgagtactca accaagtcat tctgagaata 3300
gtgtatgcgg cgaccgagtt gctcttgccc ggcgtcaata cgggataata ccgcgccaca 3360
tagcagaact ttaaaagtgc tcatcattgg aaaacgttct tcggggcgaa aactctcaag 3420

gatcttaccg ctgttgagat ccagttcgat gtaacccact cgtgcaccca actgatcttc 3480

agcatctttt actttcacca gcgtttctgg gtgagcaaaa acaggaaggc aaaatgccgc 3540

aaaaaaggga ataagggcga cacggaaatg ttgaatactc atactcttcc tttttcaata 3600

ttattgaagc atttatcagg gttattgtct catgagcgga tacatatttg aatgtattta 3660

gaaaaataaa caaatagggg ttccgcgcac atttccccga aaagtgccac ctgacgtcta 3720

agaaaccatt attatcatga cattaaccta taaaaatagg cgtatcacga ggccctttcg 3780

tcg 3783

<210> 74
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的：经修饰的sgRNA模式，其中N为编码引导序列的核苷酸

<220>
<221> misc_feature
<222> (1)..(20)
<223> n 是 a, c, g, 或u

<220>
<221> 修饰的_碱基
<222> (1)..(3)
<223> PS键, 2'-O-Me 核苷酸

[0142]

<220>
<221> 修饰的_碱基
<222> (29)..(40)
<223> 2'-O-Me 核苷酸

<220>
<221> 修饰的_碱基
<222> (69)..(96)
<223> 2'-O-Me 核苷酸

<220>
<221> 修饰的_碱基
<222> (97)..(100)
<223> PS键, 2'-O-Me 核苷酸

<400> 74
nnnnnnnnnn nnnnnnnnnn guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 75
<211> 42
<212> DNA
<213> 人工序列

<220>
<223> 合成的：CMV-1 5 UTR

<400> 75
cagatcgcct ggagacgcca tccacgctgt tttgacctcc at 42

<210> 76
<211> 42
<212> DNA
<213> 人工序列

<220>
<223> 合成的：CMV-2 5 UTR

<400> 76
agaagacacc gggaccgatc cagcctccgc ggccgggaac gg 42

<210> 77
<211> 42
<212> DNA
<213> 人工序列

<220>
<223> 合成的: CMV-3 5 UTR

<400> 77
tgcattggaa cgcggattcc ccgtgccaag agtgactcac cg 42

<210> 78
<211> 7
<212> PRT
<213> 人工序列

<220>
<223> 合成的: SV40 NLS

<400> 78

Pro Lys Lys Lys Arg Lys Val
1 5

<210> 79
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成的: 示例性 NLS 1

[0143]

<400> 79

Leu Ala Ala Lys Arg Ser Arg Thr Thr
1 5

<210> 80
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成的: 示例性 NLS 2

<400> 80

Gln Ala Ala Lys Arg Ser Arg Thr Thr
1 5

<210> 81
<211> 10
<212> PRT
<213> 人工序列

<220>
<223> 合成的: 示例性 NLS 3

<400> 81

Pro Ala Pro Ala Lys Arg Glu Arg Thr Thr
1 5 10

<210> 82
<211> 9
<212> PRT

<213> 人工序列

<220>
<223> 合成的: 示例性 NLS 4

<400> 82

Gln Ala Ala Lys Arg Pro Arg Thr Thr
1   5

<210> 83
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成的: 示例性 NLS 5

<400> 83

Arg Ala Ala Lys Arg Pro Arg Thr Thr
1   5

<210> 84
<211> 11
<212> PRT
<213> 人工序列

<220>
<223> 合成的: 示例性 NLS 6

<400> 84

Ala Ala Ala Lys Arg Ser Trp Ser Met Ala Ala
[0144] 1   5   10

<210> 85
<211> 11
<212> PRT
<213> 人工序列

<220>
<223> 合成的: 示例性 NLS 7

<400> 85

Ala Ala Ala Lys Arg Val Trp Ser Met Ala Phe
1   5   10

<210> 86
<211> 11
<212> PRT
<213> 人工序列

<220>
<223> 合成的: 示例性 NLS 8

<400> 86

Ala Ala Ala Lys Arg Ser Trp Ser Met Ala Phe
1   5   10

<210> 87
<211> 10
<212> PRT
<213> 人工序列

<220>
<223> 合成的: 示例性 NLS 9

<400> 87

Ala Ala Ala Lys Arg Lys Tyr Phe Ala Ala
1 5 10

<210> 88
<211> 10
<212> PRT
<213> 人工序列

<220>
<223> 合成的：示例性 NLS 10

<400> 88

Arg Ala Ala Lys Arg Lys Ala Phe Ala Ala
1 5 10

<210> 89
<211> 10
<212> PRT
<213> 人工序列

<220>
<223> 合成的：示例性 NLS 11

<400> 89

Arg Ala Ala Lys Arg Lys Tyr Phe Ala Val
1 5 10

<210> 90
<211> 7
<212> PRT
[0145] <213> 人工序列

<220>
<223> 合成的：替代SV40 NLS

<400> 90

Pro Lys Lys Lys Arg Arg Val
1 5

<210> 91
<211> 16
<212> PRT
<213> 人工序列

<220>
<223> 合成的：核质蛋白NLS

<400> 91

Lys Arg Pro Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys Lys Lys
1 5 10 15

<210> 92
<211> 21
<212> DNA
<213> 人工序列

<220>
<223> 合成的：SV40 NLS的示例性编码序列

<400> 92
ccgaagaaga agagaaaggt c 21

<210> 93

<211> 27
<212> DNA
<213> 人工序列

<220>
<223> 合成的：NLS1的示例性编码序列

<400> 93
ctggcagcaa agagaagcag aacaaca 27

<210> 94
<211> 27
<212> DNA
<213> 人工序列

<220>
<223> 合成的：NLS2的示例性编码序列

<400> 94
caggcagcaa agagaagcag aacaaca 27

<210> 95
<211> 30
<212> DNA
<213> 人工序列

<220>
<223> 合成的：NLS3的示例性编码序列

<400> 95
ccggcaccgg caaagagaga aagaacaaca 30

[0146] <210> 96
<211> 27
<212> DNA
<213> 人工序列

<220>
<223> 合成的：NLS4的示例性编码序列

<400> 96
caggcagcaa agagaccgag aacaaca 27

<210> 97
<211> 27
<212> DNA
<213> 人工序列

<220>
<223> 合成的：NLS5的示例性编码序列

<400> 97
agagcagcaa agagaccgag aacaaca 27

<210> 98
<211> 33
<212> DNA
<213> 人工序列

<220>
<223> 合成的：NLS6的示例性编码序列

<400> 98
gcagcagcaa agagaagctg gagcatggca gca 33

<210> 99
<211> 33
<212> DNA
<213> 人工序列

<220>
<223> 合成的：NLS7的示例性编码序列

<400> 99
gcagcagcaa agagagtctg gagcatggca ttc 33

<210> 100
<211> 33
<212> DNA
<213> 人工序列

<220>
<223> 合成的：NLS8的示例性编码序列

<400> 100
gcagcagcaa agagaagctg gagcatggca ttc 33

<210> 101
<211> 30
<212> DNA
<213> 人工序列

<220>
<223> 合成的：NLS9的示例性编码序列

<400> 101
gcagcagcaa agagaaagta cttcgcagca 30

<210> 102
<211> 30
<212> DNA
<213> 人工序列

[0147] <220>
<223> 合成的：NLS10的示例性编码序列

<400> 102
agagcagcaa agagaaaggc attcgcagca 30

<210> 103
<211> 30
<212> DNA
<213> 人工序列

<220>
<223> 合成的：NLS11的示例性编码序列

<400> 103
agagcagcaa agagaaagta cttcgcagtc 30

<210> 104
<211> 21
<212> DNA
<213> 人工序列

<220>
<223> 合成的：替代SV40 NLS的示例性编码序列

<400> 104
ccgaagaaga agagaagagt c 21

<210> 105
<211> 13
<212> RNA
<213> 人工序列

<220>
<223> 合成的：示例性 Kozak 序列

<400> 105
gccgccrcca ugg 13

<210> 106
<211> 0
<212> DNA
<213> N/A

<400> 106
000

<210> 107
<211> 4140
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的长半衰期密码子，具有起始及终止密码子的Cas9 ORF

<400> 107
atggacaaga agtactctat cggtttggac atcggtacca actctgtcgg ttgggccgtc 60
atcaccgacg aatacaaggt cccatctaag aagttcaagg tcttgggtaa caccgacaga 120
cactctatca agaagaactt gatcggtgcc ttgttgttcg actctggtga aaccgccgaa 180
gccaccagat tgaagagaac cgccagaaga agatacacca gaagaaagaa cagaatctgc 240
tacttgcaag aaatcttctc taacgaaatg gccaaggtcg acgactcttt cttccacaga 300
ttggaagaat ctttcttggt cgaagaagac aagaagcacg aaagacaccc aatcttcggt 360
aacatcgtcg acgaagtcgc ctaccacgaa aagtacccaa ccatctacca cttgagaaag 420
aagttggtcg actctaccga caaggccgac ttgagattga tctacttggc cttggcccac 480
[0148] atgatcaagt tcagaggtca cttcttgatc gaaggtgact tgaacccaga caactctgac 540
gtcgacaagt tgttcatcca attggtccaa acctacaacc aattgttcga agaaaaccca 600
atcaacgcct ctggtgtcga cgccaaggcc atcttgtctg ccagattgtc taagagcaga 660
agattggaaa acttgatcgc ccaattgcca ggtgaaaaga agaacggttt gttcggtaac 720
ttgatcgcct tgtctttggg tttgacccca aacttcaagt ctaacttcga cttggccgaa 780
gacgccaagt tgcaattgtc taaggacacc tacgacgacg acttggacaa cttgttggcc 840
caaatcggtg accaatacgc cgacttgttc ttggccgcca agaacttgtc tgacgccatc 900
ttgttgtctg acatcttgag agtcaacacc gaaatcacca aggccccatt gtctgcctct 960
atgatcaaga gatacgacga acaccaccaa gacttgacct tgttgaaggc cttggtcaga 1020
caacaattgc cagaaaagta caaggaaatc ttcttcgacc aatctaagaa cggttacgcc 1080
ggttacatcg acggtggtgc ctctcaagaa gaattctaca agttcatcaa gccaatcttg 1140
gaaaagatgg acggtaccga agaattgttg gtcaagttga acagagaaga cttgttgaga 1200
aagcaaagaa ccttcgacaa cggttctatc ccacaccaaa tccacttggg tgaattgcac 1260
gccatcttga gaagacaaga agacttctac ccattcttga aggacaacag agaaaagatc 1320
gaaaagatct tgaccttcag aatcccatac tacgtcggtc cattggccag aggtaacagc 1380
agattcgcct ggatgaccag aaagtctgaa gaaaccatca ccccatggaa cttcgaagaa 1440
gtcgtcgaca agggtgcctc tgcccaatct ttcatcgaaa gaatgaccaa cttcgacaag 1500
aacttgccaa acgaaaaggt cttgccaaag cactctttgt tgtacgaata cttcaccgtc 1560
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tctggtgaac aaaagaaggc catcgtcgac ttgttgttca agaccaacag aaaggtcacc 1680
gtcaagcaat tgaaggaaga ctacttcaag aagatcgaat gcttcgactc tgtcgaaatc 1740
tctggtgtcg aagacagatt caacgcctct ttgggtacct accacgactt gttgaagatc 1800
atcaaggaca aggacttctt ggacaacgaa gaaaacgaag acatcttgga agacatcgtc 1860
ttgaccttga ccttgttcga agacagagaa atgatcgaag aaagattgaa gacctacgcc 1920
cacttgttcg acgacaaggt catgaagcaa ttgaagagaa gaagatacac cggttggggt 1980
agattgagca gaaagttgat caacggtatc agagacaagc aatctggtaa gaccatcttg 2040
gacttcttga agtctgacgg tttcgccaac agaaacttca tgcaattgat ccacgacgac 2100
tctttgacct tcaaggaaga catccaaaag gcccaagtct ctggtcaagg tgactctttg 2160
cacgaacaca tcgccaactt ggccggttct ccagccatca agaagggtat cttgcaaacc 2220
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atcgaaatgg ccagagaaaa ccaaaccacc caaaagggtc aaaagaacag cagagaaaga 2340
atgaagagaa tcgaagaagg tatcaaggaa ttgggttctc aaatcttgaa ggaacaccca 2400
gtcgaaaaca cccaattgca aaacgaaaag ttgtacttgt actacttgca aaacggtaga 2460
gacatgtacg tcgaccaaga attggacatc aacagattgt ctgactacga cgtcgaccac 2520
atcgtcccac aatctttctt gaaggacgac tctatcgaca acaaggtctt gaccagatct 2580
gacaagaaca gaggtaagtc tgacaacgtc ccatctgaag aagtcgtcaa gaagatgaag 2640
aactactgga gacaattgtt gaacgccaag ttgatcaccc aaagaaagtt cgacaacttg 2700
[0149] accaaggccg aaagaggtgg tttgtctgaa ttggacaagg ccggtttcat caagagacaa 2760
ttggtcgaaa ccagacaaat caccaagcac gtcgcccaaa tcttggacag cagaatgaac 2820
accaagtacg acgaaaacga caagttgatc agagaagtca aggtcatcac cttgaagtct 2880
aagttggtct ctgacttcag aaaggacttc caattctaca aggtcagaga aatcaacaac 2940
taccaccacg cccacgacgc ctacttgaac gccgtcgtcg gtaccgcctt gatcaagaag 3000
tacccaaagt tggaatctga attcgtctac ggtgactaca aggtctacga cgtcagaaag 3060
atgatcgcca agtctgaaca agaaatcggt aaggccaccg ccaagtactt cttctactct 3120
aacatcatga acttcttcaa gaccgaaatc accttggcca acggtgaaat cagaaagaga 3180
ccattgatcg aaaccaacgg tgaaaccggt gaaatcgtct gggacaaggg tagagacttc 3240
gccaccgtca gaaaggtctt gtctatgcca caagtcaaca tcgtcaagaa gaccgaagtc 3300
caaaccggtg gtttctctaa ggaatctatc ttgccaaaga gaaactctga caagttgatc 3360
gccagaaaga aggactggga cccaaagaag tacggtggtt tcgactctcc aaccgtcgcc 3420
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caacacaagc actacttgga cgaaatcatc gaacaaatct ctgaattctc taagagagtc 3840

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ccaatcagag aacaagccga aaacatcatc cacttgttca ccttgaccaa cttgggtgcc 3960
ccagccgcct tcaagtactt cgacaccacc atcgacagaa agagatacac ctctaccaag 4020
gaagtcttgg acgccacctt gatccaccaa tctatcaccg gtttgtacga aaccagaatc 4080
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<210> 108
<211> 4140
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的富含U的密码子，具有起始及终止密码子的Cas9 ORF

<400> 108
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cattctatta aaaaaaattt aattggtgct ttattatttg attctggtga aactgctgaa 180
gctactcgtt taaaacgtac tgctcgtcgt cgttatactc gtcgtaaaaa tcgtatttgt 240
tatttacaag aaatttttc taatgaaatg gctaaagttg atgattcttt ttttcatcgt 300
ttagaagaat ctttttagt tgaagaagat aaaaaacatg aacgtcatcc tatttttggt 360
aatattgttg atgaagttgc ttatcatgaa aaatatccta ctatttatca tttacgtaaa 420
aaattagttg attctactga taaagctgat ttacgtttaa tttatttagc tttagctcat 480
atgattaaat ttcgtggtca tttttttaatt gaaggtgatt taaatcctga taattctgat 540
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attaatgctt ctggtgttga tgctaaagct attttatctg ctcgtttatc taaatctcgt 660
cgtttagaaa atttaattgc tcaattacct ggtgaaaaaa aaaatggttt atttggtaat 720
ttaattgctt tatctttagg tttaactcct aattttaaat ctaattttga tttagctgaa 780
gatgctaaat tacaattatc taaagatact tatgatgatg atttagataa tttattagct 840
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gaagttttag atgctacttt aattcatcaa tctattactg gtttatatga aactcgtatt 4080

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<210> 109
<211> 4140
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低G密码子，具有起始及终止密码子的Cas9 ORF

<400> 109
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atcaccgacg aatacaaagt cccctccaaa aaattcaaag tcctcggcaa caccgacaga 120

cactccatca aaaaaaacct catcggcgcc ctcctcttcg actccggcga aaccgccgaa 180

gccaccagac tcaaaagaac cgccagaaga agatacacca gaagaaaaaa cagaatctgc 240

tacctccaag aaatcttctc caacgaaatg gccaaagtcg acgactcctt cttccacaga 300

ctcgaagaat ccttcctcgt cgaagaagac aaaaaacacg aaagacaccc catcttcggc 360

aacatcgtcg acgaagtcgc ctaccacgaa aaataccccа ccatctacca cctcagaaaa 420

aaactcgtcg actccaccga caaagccgac ctcagactca tctacctcgc cctcgcccac 480

atgatcaaat tcagaggcca cttcctcatc gaaggcgacc tcaaccccga caactccgac 540

[0152] gtcgacaaac tcttcatcca actcgtccaa acctacaacc aactcttcga agaaaacccc 600

atcaacgcct ccggcgtcga cgccaaagcc atcctctccg ccagactctc caaatccaga 660

agactcgaaa acctcatcgc ccaactcccc ggcgaaaaaa aaaacggcct cttcggcaac 720

ctcatcgccc tctccctcgg cctcaccccc aacttcaaat ccaacttcga cctcgccgaa 780

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caaatcggcg accaatacgc cgacctcttc ctcgccgcca aaaacctctc cgacgccatc 900

ctcctctccg acatcctcag agtcaacacc gaaatcacca aagccccccт ctccgcctcc 960

atgatcaaaa gatacgacga acaccaccaa gacctcaccc tcctcaaagc cctcgtcaga 1020

caacaactcc ccgaaaaata caaagaaatc ttcttcgacc aatccaaaaa cggctacgcc 1080

ggctacatcg acggcggcgc ctcccaagaa gaattctaca aattcatcaa acccatcctc 1140

gaaaaaatgg acggcaccga agaactcctc gtcaaactca acagagaaga cctcctcaga 1200

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agattcgcct ggatgaccag aaaatccgaa gaaaccatca cccccctggaa cttcgaagaa 1440

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<210> 110
<211> 4140
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低C密码子，具有起始及终止密码子的Cas9 ORF

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attacagatg agtataaggt gcctagtaag aagtttaagg tgttgggaaa tacagataga 120
catagtatta agaagaattt gattggagct ttgttgtttg atagtggaga gacagctgag 180
gctacaagat tgaagagaac agctagaaga agatatacaa gaagaaagaa tagaatttgt 240
tatttgcagg agatttttag taatgagatg gctaaggtgg atgatagttt ttttcataga 300
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gaggtgttgg atgctacatt gattcatcag agtattacag gattgtatga gacaagaatt 4080

gatttgagtc agttgggagg agatggagga ggaagtccta agaagaagag aaaggtgtga 4140

<210> 111
<211> 4140
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A密码子，具有起始及终止密码子的Cas9 ORF

<400> 111
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atcaccgacg agtacaaggt gccctccaag aagttcaagg tgctgggcaa caccgaccgg 120

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<210> 112
<211> 4140
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A/U密码子，具有起始及终止密码子的Cas9 ORF

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<211> 4179
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A密码子，具有两个C端NLS序列及起始及终止密码子的Cas9 ORF

<400> 113

[0160]

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<220>
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[0162]

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<211> 4107
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A密码子，具有起始及终止密码子且无NLS的Cas9切口酶ORF

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<213> 人工序列

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<223> 合成的：使用表4的低A密码子，具有两个C端NLS序列及起始及终止密码子的Cas9切口酶ORF

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<210> 118
<211> 4107
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A密码子，具有起始及终止密码子且无NLS的dCas9 ORF

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<211> 4179
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A密码子，具有两个C端NLS序列及起始及终止密码子的dCas9 ORF

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<210> 120
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<223> 合成的：使用表4的低A/U密码子，具有两个C端NLS序列及起始及终止密码子的Cas9 ORF

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<210> 124
<211> 4107
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A/U密码子，具有起始及终止密码子且无NLS的Cas9切口酶ORF

<400> 124
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<211> 4140
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A/U密码子，具有起始及终止密码子的dCas9 ORF

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<210> 126
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<220>
<223> 合成的：使用表4的低A/U密码子，具有两个C端NLS序列及起始及终止密码子的dCas9 ORF

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<210> 127
<211> 4107
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A/U密码子，具有起始及终止密码子且无NLS的dCas9 ORF

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atcaccgacg agtacaaggt gcccagcaag aagttcaagg tgctgggcaa caccgaccgg 120
cacagcatca agaagaacct gatcggcgcc ctgctgttcg acagcggcga gaccgccgag 180

gccacccggc tgaagcggac cgcccggcgg cggtacaccc ggcggaagaa ccggatctgc 240
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aacatcgtgg acgaggtggc ctaccacgag aagtacccca ccatctacca cctgcggaag 420
aagctggtgg acagcaccga caaggccgac ctgcggctga tctacctggc cctggcccac 480
atgatcaagt tccggggcca cttcctgatc gagggcgacc tgaaccccga caacagcgac 540
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<210> 128
<211> 3312
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A密码子，具有起始及终止密码子的Nme Cas9 ORF

<400> 128
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tccgtgggct gggccatggt ggagatcgac gaggaggaga accccatccg gctgatcgac 120
ctgggcgtgc gggtgttcga gcgggccgag gtgcccaaga ccggcgactc cctggccatg 180
gcccggcggc tggcccggtc cgtgcggcgg ctgacccggc ggcgggccca ccggctgctg 240

cggacccggc ggctgctgaa gcgggagggc gtgctgcagg ccgccaactt cgacgagaac 300
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taccagatcg acgagctggg caaggagatc cggccctgcc ggctgaagaa gcggcccccc 3240
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aaggtggagt ga 3312

[0191]
<210> 129
<211> 3312
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A/U密码子，具有起始及终止密码子的Nme Cas9 ORF

<400> 129
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agcgtgggct gggccatggt ggagatcgac gaggaggaga accccatccg gctgatcgac 120
ctgggcgtgc gggtgttcga gcgggccgag gtgcccaaga ccggcgacag cctggccatg 180
gcccggcggc tggcccggag cgtgcggcgg ctgacccggc ggcgggccca ccggctgctg 240
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ctgagccagc ggaagaacga gggcgagacc gccgacaagg agctgggcgc cctgctgaag 480
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taccagatcg acgagctggg caaggagatc cggccctgcc ggctgaagaa gcggcccccc 3240
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aaggtggagt ga 3312

<210> 130
<211> 4146
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有NLS1的Cas9的开放阅读框架，其具有起始及终止密码子

<400> 130
atggacaaga agtacagcat cggactggac atcggaacaa acagcgtcgg atgggcagtc 60

atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa cacagacaga 120

cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180

gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240

tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccacaga 300

ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc gatcttcgga 360

aacatcgtcg acgaagtcgc ataccacgaa aagtacccga caatctacca cctgagaaag 420

aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc actggcacac 480

atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga caacagcgac 540

gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga agaaaacccg 600

atcaacgcaa gcggagtcga cgcaaaggca atcctgagcg caagactgag caagagcaga 660

agactggaaa acctgatcgc acagctgccg ggagaaaaga agaacggact gttcggaaac 720

ctgatcgcac tgagcctggg actgacaccg aacttcaaga gcaacttcga cctggcagaa 780

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<210> 131
<211> 4146
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有NLS2的Cas9的开放阅读框架，其具有起始及终止密码子

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<210> 132
<211> 4149
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有NLS3的Cas9的开放阅读框架，其具有起始及终止密码子

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<210> 133
<211> 4146
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有NLS4的Cas9的开放阅读框架，其具有起始及终止密码子

<400> 133

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acatag 4146

<210> 134
<211> 4146
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有NLS5的Cas9的开放阅读框架，其具有起始及终止密码子

<400> 134
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gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240
tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccacaga 300
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acatag 4146

<210> 135
<211> 4152
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有NLS6的Cas9的开放阅读框架，其具有起始及终止密码子

<400> 135
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atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa cacagacaga 120
cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180
gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240
tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccacaga 300
ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc gatcttcgga 360
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aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc actggcacac 480
atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga caacagcgac 540
gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga agaaaacccg 600
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atggcagcat ag 4152

<210> 136
<211> 4152
<212> DNA
<213> 人工序列

<220>
<223> 合成的: 具有NLS7的Cas9的开放阅读框架，其具有起始及终止密码子

<400> 136
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atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa cacagacaga 120
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atggcattct ag 4152

<210> 137
<211> 4152
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有NLS8的Cas9的开放阅读框架，其具有起始及终止密码子

<400> 137
atggacaaga agtacagcat cggactggac atcggaacaa acagcgtcgg atgggcagtc 60
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cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180
gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240
tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccacaga 300
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atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga caacagcgac 540
gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga agaaaacccg 600
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ccgctgatcg aaacaaacgg agaaacagga gaaatcgtct gggacaaggg aagagacttc 3240
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atggcattct ag 4152

<210> 138
<211> 4149
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有NLS9的Cas9的开放阅读框架，其具有起始及终止密码子

<400> 138
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atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa cacagacaga 120
cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180
gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240
tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccacaga 300
ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc gatcttcgga 360
aacatcgtcg acgaagtcgc ataccacgaa aagtacccga caatctacca cctgagaaag 420
aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc actggcacac 480
atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga caacagcgac 540
gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga agaaaacccg 600
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agactggaaa acctgatcgc acagctgccg ggagaaaaga agaacggact gttcggaaac 720
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agattcgcat ggatgacaag aaagagcgaa gaaacaatca caccgtggaa cttcgaagaa 1440
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gcagcatag 4149

<210> 139
<211> 4149
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有NLS10的Cas9的开放阅读框架，其具有起始及终止密码子

<400> 139
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atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa cacagacaga 120
cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180
gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240
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atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga caacagcgac 540
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aagctggtca gcgacttcag aaaggacttc cagttctaca aggtcagaga aatcaacaac 2940
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ccgatcagag aacaggcaga aaacatcatc cacctgttca cactgacaaa cctgggagca 3960
ccggcagcat tcaagtactt cgacacaaca atcgacagaa agagatacac aagcacaaag 4020
gaagtcctgg acgcaacact gatccaccag agcatcacag gactgtacga aacaagaatc 4080
gacctgagcc agctgggagg agacggagga ggaagcagag cagcaaagag aaaggcattc 4140
gcagcatag 4149

<210> 140
<211> 4149
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有NLS11的Cas9的开放阅读框架，其具有起始及终止密码子

<400> 140
atggacaaga agtacagcat cggactggac atcggaacaa acagcgtcgg atgggcagtc 60
atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa cacagacaga 120
cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180
gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240
tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccacaga 300
ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc gatcttcgga 360
aacatcgtcg acgaagtcgc ataccacgaa aagtacccga caatctacca cctgagaaag 420
aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc actggcacac 480
atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga caacagcgac 540
gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga agaaaacccg 600
atcaacgcaa gcggagtcga cgcaaaggca atcctgagcg caagactgag caagagcaga 660
agactggaaa acctgatcgc acagctgccg ggagaaaaga agaacggact gttcggaaac 720
ctgatcgcac tgagcctggg actgacaccg aacttcaaga gcaacttcga cctggcagaa 780
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gaaaagatcc tgacattcag aatcccgtac tacgtcggac cgctggcaag aggaaacagc 1380
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agcggagtcg aagacagatt caacgcaagc ctgggaacat accacgacct gctgaagatc 1800
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gtcgaaaaca cacagctgca gaacgaaaag ctgtacctgt actacctgca gaacggaaga 2460
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gacaagaaca gaggaaagag cgacaacgtc ccgagcgaag aagtcgtcaa gaagatgaag 2640
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tacagcctgt tcgaactgga aaacggaaga aagagaatgc tggcaagcgc aggagaactg 3660
cagaagggaa acgaactggc actgccgagc aagtacgtca acttcctgta cctggcaagc 3720
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ccgatcagag aacaggcaga aaacatcatc cacctgttca cactgacaaa cctgggagca 3960
ccggcagcat tcaagtactt cgacacaaca atcgacagaa agagatacac aagcacaaag 4020
gaagtcctgg acgcaacact gatccaccag agcatcacag gactgtacga aacaagaatc 4080
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gcagtctag 4149

<210> 141
<211> 4128
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用通常在人类中高度表达的密码子的Cas9 ORF（无起始或终止密码子；适于包括在融合蛋白质编码序列中）

<400> 141
cctaagaaaa agcggaaggt cgacggggat aagaagtact caatcgggct ggatatcgga 60
actaattccg tgggttgggc agtgatcacg gatgaataca aagtgccgtc caagaagttc 120
aaggtcctgg ggaacaccga tagacacagc atcaagaaaa atctcatcgg agccctgctg 180
tttgactccg gcgaaaccgc agaagcgacc cggctcaaac gtaccgcgag gcgacgctac 240
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ccgaccatct accatctgcg gaagaagttg gttgactcaa ctgacaaggc cgacctcaga 480
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acgctgctca aagcgctcgt gagacagcaa ctgcctgaaa agtacaagga gatcttcttc 1080
gaccagtcca agaatgggta cgcagggtac atcgatggag gcgctagcca ggaagagttc 1140
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gaacgaatga ccaacttcga caagaatctc ccaaacgaga aggtgcttcc taagcacagc 1560
ctcctttacg aatacttcac tgtctacaac gaactgacta aagtgaaata cgttactgaa 1620
ggaatgagga agccggcctt tctgtccgga gaacagaaga aagcaattgt cgatctgctg 1680
ttcaagacca accgcaaggt gaccgtcaag cagcttaaag aggactactt caagaagatc 1740
gagtgtttcg actcagtgga aatcagcggg gtggaggaca gattcaacgc ttcgctggga 1800
acctatcatg atctcctgaa gatcatcaag gacaaggact tccttgacaa cgaggagaac 1860
gaggacatcc tggaagatat cgtcctgacc ttgacccttt tcgaggatcg cgagatgatc 1920

gaggagaggc ttaagaccta cgctcatctc ttcgacgata aggtcatgaa acaactcaag 1980
cgccgccggt acactggttg gggccgcctc tcccgcaagc tgatcaacgg tattcgcgat 2040
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cggcacaaac cggagaatat cgtgattgaa atggcccgag aaaaccagac tacccagaag 2340
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ttgtctgatt acgacgtgga ccacatcgtt ccacagtcct ttctgaagga tgactcgatc 2580
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gaggaggtcg tgaagaagat gaagaattac tggcggcagc tcctgaatgc gaagctgatt 2700
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cagatcttgg actcccgcat gaacactaaa tacgacgaga acgataagct catccgggaa 2880
gtgaaggtga ttaccctgaa aagcaaactt gtgtcggact ttcggaagga ctttcagttt 2940
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ggattcgatt cgccgactgt cgcatactcc gtcctcgtgg tggccaaggt ggagaaggga 3480
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ttcaccctga ctaacctggg agccccagcc gccttcaagt acttcgatac tactatcgat 4020
cgcaaaagat acacgtccac caaggaagtt ctggacgcga ccctgatcca ccaaagcatc 4080
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<210> 142
<211> 4134
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的长半衰期密码子的Cas9 ORF（无起始或终止密码子；适于包括在融合蛋白质编码序列中）

<400> 142

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gacaagaagt actctatcgg tttggacatc ggtaccaact ctgtcggttg ggccgtcatc      60
accgacgaat acaaggtccc atctaagaag ttcaaggtct tgggtaacac cgacagacac     120
tctatcaaga agaacttgat cggtgccttg ttgttcgact ctggtgaaac cgccgaagcc     180
accagattga agagaaccgc cagaagaaga tacaccagaa gaaagaacag aatctgctac     240
ttgcaagaaa tcttctctaa cgaaatggcc aaggtcgacg actctttctt ccacagattg     300
gaagaatctt tcttggtcga agaagacaag aagcacgaaa gacacccaat cttcggtaac     360
atcgtcgacg aagtcgccta ccacgaaaag tacccaacca tctaccactt gagaaagaag     420
ttggtcgact ctaccgacaa ggccgacttg agattgatct acttggcctt ggcccacatg     480
atcaagttca gaggtcactt cttgatcgaa ggtgacttga acccagacaa ctctgacgtc     540
gacaagttgt tcatccaatt ggtccaaacc tacaaccaat tgttcgaaga aaacccaatc     600
aacgcctctg gtgtcgacgc caaggccatc ttgtctgcca gattgtctaa gagcagaaga     660
ttggaaaact tgatcgccca attgccaggt gaaaagaaga acggtttgtt cggtaacttg     720
atcgccttgt ctttgggttt gaccccaaac ttcaagtcta acttcgactt ggccgaagac     780
gccaagttgc aattgtctaa ggacacctac gacgacgact tggacaactt gttggcccaa     840
atcggtgacc aatacgccga cttgttcttg gccgccaaga acttgtctga cgccatcttg     900
ttgtctgaca tcttgagagt caacaccgaa atcaccaagg ccccattgtc tgcctctatg     960
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tacatcgacg gtggtgcctc tcaagaagaa ttctacaagt tcatcaagcc aatcttggaa    1140
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caaagaacct tcgacaacgg ttctatccca caccaaatcc acttgggtga attgcacgcc    1260
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aacgaattga ccaaggtcaa gtacgtcacc gaaggtatga gaaagccagc cttcttgtct    1620
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accttgacct tgttcgaaga cagagaaatg atcgaagaaa gattgaagac ctacgcccac    1920
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[0217]

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gaacacatcg ccaacttggc cggttctcca gccatcaaga agggtatctt gcaaaccgtc 2220
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gaaaacaccc aattgcaaaa cgaaaagttg tacttgtact acttgcaaaa cggtagagac 2460
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<210> 143

〈211〉 4134
〈212〉 DNA
〈213〉 人工序列

〈220〉
〈223〉 合成的：使用表4的富含U的密码子的Cas9 ORF（无起始或终止密码子；适于包括在融合蛋白质编码序列中）

〈400〉 143

[0219]

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actcgtttaa aacgtactgc tcgtcgtcgt tatactcgtc gtaaaaatcg tatttgttat     240
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<210> 144
<211> 4134
<212> DNA

<213> 人工序列

<220>
<223> 合成的：使用表4的低G密码子的Cas9 ORF（无起始或终止密码子；适于包括在融合蛋白质编码序列中）

<400> 144

[0221]

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<210> 145
<211> 4134
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低C密码子的Cas9 ORF（无起始或终止密码子；适于包括在融合蛋白质编码序列中）

<400> 145

[0223]

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<210> 146
<211> 4134
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A密码子的Cas9 ORF（无起始或终止密码子；适于包括在融合蛋白质编码序列中）

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<212> DNA
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<223> 合成的：使用表4的低A/U密码子的Cas9 ORF（无起始或终止密码子；适于包括在融合蛋白质编码序列中）

<400> 147

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<210> 148
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<212> DNA
<213> 人工序列

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<223> 合成的：使用表4的低A密码子，具有两个C端NLS序列的Cas9 ORF（无起始或终止密码子；适于包括在融合蛋白质编码序列中）

<400> 148
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<210> 150
<211> 4101
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A密码子的Cas9切口酶ORF（无NLS且无起始或终止密码子；适于包括在融合蛋白质编码序列中）

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<210> 151
<211> 4173
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A密码子，具有两个C端NLS序列的Cas9切口酶ORF（无起始或终止密码子；适于包括在融合蛋白质编码序列中）

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<210> 152
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<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A密码子的dCas9 ORF（无起始或终止密码子；适于包括在融合蛋白质编码序列中）

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<211> 4101
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A密码子的dCas9 ORF（无NLS且无起始或终止密码子；适于包括在融合蛋白质编码序列中）

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<210> 154
<211> 4173
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A密码子，具有两个C端NLS序列的dCas9 ORF（无起始或终止密码子；适于包括在融合蛋白质编码序列中）

<400> 154
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<211> 4173
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<223> 合成的：使用表4的低A/U密码子，具有两个C端NLS序列的Cas9 ORF（无起始或终止密码子；适于包括在融合蛋白质编码序列中）

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<211> 4101
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A/U密码子的Cas9 ORF（无NLS且无起始或终止密码子；适于包括在融合蛋白质编码序列中）

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<210> 157
<211> 4134
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A/U密码子的Cas9切口酶ORF（无起始或终止密码子；适于包括在融合蛋白质编码序列中）

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<210> 158
<211> 4173
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A/U密码子，具有两个C端NLS序列的Cas9切口酶ORF（无起始或终止密码子；适于包括在融合蛋白质编码序列中）

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<210> 159
<211> 4101
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A/U密码子的Cas9切口酶ORF（无NLS且无起始或终止密码子；适于包括在融合蛋白质编码序列中）

<400> 159
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<210> 160
<211> 4134
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A/U密码子的dCas9 ORF（无起始或终止密码子；适于包括在融合蛋白质编码序列中）

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<210> 161
<211> 4173
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A/U密码子，具有两个C端NLS序列的dCas9 ORF（无起始或终止密码子；适于包括在融合蛋白质编码序列中）

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gtgctggacg ccaccctgat ccaccagagc atcaccggcc tgtacgagac ccggatcgac 4080
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<210> 162
<211> 4101
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A/U密码子的dCas9 ORF（无NLS且无起始或终止密码子；适于包括在融合蛋白质编码序列中）

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agcatcaaga agaacctgat cggcgccctg ctgttcgaca gcggcgagac cgccgaggcc 180
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gaggagagct tcctggtgga ggaggacaag aagcacgagc ggcaccccat cttcggcaac 360
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<210> 163
<211> 3306
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A密码子的Nme Cas9 ORF（无起始或终止密码子；适于包括在融合蛋白质编码序列中）

<400> 163
gccgccttca agcccaactc catcaactac atcctgggcc tggacatcgg catcgcctcc 60
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cggcggctgg cccggtccgt gcggcggctg acccggcggc gggcccaccg gctgctgcgg 240
acccggcggc tgctgaagcg ggagggcgtg ctgcaggccg ccaacttcga cgagaacggc 300
ctgatcaagt ccctgcccaa cacccccctgg cagctgcggg ccgccgccct ggaccggaag 360

ctgaccccc tggagtggtc cgccgtgctg ctgcacctga tcaagcaccg gggctacctg 420
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cggtccggca agcggaccgc cgacggctcc gagttcgagt cccccaagaa gaagcggaag 3300

gtggag 3306

<210> 164
<211> 3306
<212> DNA
<213> 人工序列

<220>
<223> 合成的：使用表4的低A/U密码子的Nme Cas9 ORF（无起始或终止密码子；适于包括在融合蛋白质编码序列中）

<400> 164
gccgccttca agcccaacag catcaactac atcctgggcc tggacatcgg catcgccagc 60

[0260] gtgggctggg ccatggtgga gatcgacgag gaggagaacc ccatccggct gatcgacctg 120

ggcgtgcggg tgttcgagcg ggccgaggtg cccaagaccg gcgacagcct ggccatggcc 180

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accttcagcc ggaaggacct gcaggccgag ctgatcctgc tgttcgagaa gcagaaggag 660

ttcggcaacc cccacgtgag cggcggcctg aaggagggca tcgagaccct gctgatgacc 720

cagcggcccg ccctgagcgg cgacgccgtg cagaagatgc tgggccactg caccttcgag 780

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gtggag 3306

<210> 165
<211> 4140

<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有NLS1的Cas9的开放阅读框架(无起始或终止密码子；适于包括在融合蛋白质编码序列中)

<400> 165

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<212> DNA
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<211> 4140
<212> DNA
<213> 人工序列

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<223> 合成的：具有NLS5的Cas9的开放阅读框架(无起始或终止密码子；适于包括在融合蛋白质编码序列中)5

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agcatcaaga agaacctgat cggagcactg ctgttcgaca gcggagaaac agcagaagca 180
acaagactga agagaacagc aagaagaaga tacacaagaa gaaagaacag aatctgctac 240
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gacaagctgt tcatccagct ggtccagaca tacaaccagc tgttcgaaga aaacccgatc 600
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<212> DNA
<213> 人工序列

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agcatcaaga agaacctgat cggagcactg ctgttcgaca gcggagaaac agcagaagca 180

acaagactga agagaacagc aagaagaaga tacacaagaa gaaagaacag aatctgctac 240

ctgcaggaaa tcttcagcaa cgaaatggca aaggtcgacg acagcttctt ccacagactg 300

gaagaaagct tcctggtcga agaagacaag aagcacgaaa gacacccgat cttcggaaac 360

atcgtcgacg aagtcgcata ccacgaaaag tacccgacaa tctaccacct gagaaagaag 420

ctggtcgaca gcacagacaa ggcagacctg agactgatct acctggcact ggcacacatg 480

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tacatcgacg gaggagcaag ccaggaagaa ttctacaagt tcatcaagcc gatcctggaa 1140

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aacgaactga caaaggtcaa gtacgtcaca gaaggaatga gaaagccggc attcctgagc 1620

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<223> 合成的：具有NLS7的Cas9的开放阅读框架(无起始或终止密码子；适于包括在融合蛋白质编码序列中)

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ctggtcgaca gcacagacaa ggcagacctg agactgatct acctggcact ggcacacatg 480

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aagatcctga cattcagaat cccgtactac gtcggaccgc tggcaagagg aaacagcaga 1380

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aaggacaagg acttcctgga caacgaagaa aacgaagaca tcctggaaga catcgtcctg 1860

acactgacac tgttcgaaga cagagaaatg atcgaagaaa gactgaagac atacgcacac 1920

ctgttcgacg acaaggtcat gaagcagctg aagagaagaa gatacacagg atggggaaga 1980

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gcattc 4146

<210> 172
<211> 4146
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有NLS8的Cas9的开放阅读框架(无起始或终止密码子；适于包括在融合蛋白质编码序列中)

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agcatcaaga agaacctgat cggagcactg ctgttcgaca gcggagaaac agcagaagca 180
acaagactga agagaacagc aagaagaaga tacacaagaa gaaagaacag aatctgctac 240
ctgcaggaaa tcttcagcaa cgaaatggca aaggtcgacg acagcttctt ccacagactg 300
gaagaaagct tcctggtcga agaagacaag aagcacgaaa gacacccgat cttcggaaac 360
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aacgcaagcg gagtcgacgc aaaggcaatc ctgagcgcaa gactgagcaa gagcagaaga 660
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gcattc 4146

<210> 173
<211> 4143
<212> DNA
<213> 人工序列

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<223> 合成的：具有NLS9的Cas9的开放阅读框架(无起始或终止密码子；适于包括在融合蛋白质编码序列中)

<400> 173
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acagacgaat acaaggtccc gagcaagaag ttcaaggtcc tgggaaacac agacagacac 120
agcatcaaga agaacctgat cggagcactg ctgttcgaca gcggagaaac agcagaagca 180
acaagactga agagaacagc aagaagaaga tacacaagaa gaaagaacag aatctgctac 240
ctgcaggaaa tcttcagcaa cgaaatggca aaggtcgacg acagcttctt ccacagactg 300
gaagaaagct tcctggtcga agaagacaag aagcacgaaa gacacccgat cttcggaaac 360
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ctggtcgaca gcacagacaa ggcagacctg agactgatct acctggcact ggcacacatg 480
atcaagttca gaggacactt cctgatcgaa ggagacctga acccggacaa cagcgacgtc 540
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gca 4143

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<211> 4143
<212> DNA
<213> 人工序列

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<223> 合成的：具有NLS10的Cas9的开放阅读框架(无起始或终止密码子；适于包括在融合蛋白质编码序列中)

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atcaagttca gaggacactt cctgatcgaa ggagacctga acccggacaa cagcgacgtc 540
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gca 4143

<210> 175
<211> 4143
<212> DNA
<213> 人工序列

<220>
<223> 合成的：具有NLS11的Cas9的开放阅读框架(无起始或终止密码子；适于包括在融合蛋白质编码序列中)

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<210> 176
<211> 4423
<212> RNA
<213> 人工序列

<220>
<223> 合成的：具有XBG UTR及含有表4的低U 1密码子的Cas9 ORF的mRNA转录物

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<210> 177
<211> 4423

<212> RNA
<213> 人工序列

<220>
<223> 合成的：具有XBG UTR及含有表4的低A密码子的Cas9 ORF的mRNA转录物

<400> 177

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35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
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Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
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Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
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[0306] Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
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Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
355 360 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
370 375 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385 390 395 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
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Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
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Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp

450 455 460

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Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
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Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
500 505 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
515 520 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
530 535 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545 550 555 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
565 570 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
580 585 590

[0307] Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
595 600 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
610 615 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625 630 635 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
660 665 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
675 680 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
690 695 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705 710 715 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
725 730 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
740 745 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
755 760 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
770 775 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785 790 795 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
805 810 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
820 825 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
835 840 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865 870 875 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
885 890 895

[0308] Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
900 905 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
915 920 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
930 935 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945 950 955 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
965 970 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
995 1000 1005

Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala
1010 1015 1020

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
1025 1030 1035

Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala
1040 1045 1050

Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu
1055 1060 1065

Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val
1070 1075 1080

Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr
1085 1090 1095

Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys
1100 1105 1110

Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1115 1120 1125

Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
1130 1135 1140

Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys
1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
1175 1180 1185

[0309] Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
1190 1195 1200

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
1205 1210 1215

Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1235 1240 1245

Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
1250 1255 1260

His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys
1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
1280 1285 1290

Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
1295 1300 1305

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
1340 1345 1350

Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

Gly Gly Gly Ser Leu Ala Ala Lys Arg Ser Arg Thr Thr
1370 1375 1380

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1 5 10 15

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

[0310] Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
325 330 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
340 345 350

[0311] Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
355 360 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
370 375 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385 390 395 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
405 410 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
420 425 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
435 440 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
450 455 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465 470 475 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
485 490 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
500 505 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
515 520 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
530 535 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545 550 555 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
565 570 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
580 585 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
595 600 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
610 615 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625 630 635 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655

[0312] Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
660 665 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
675 680 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
690 695 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705 710 715 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
725 730 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
740 745 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
755 760 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
770 775 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785 790 795 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu

805 810 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
820 825 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
835 840 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865 870 875 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
885 890 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
900 905 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
915 920 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
930 935 940

[0313] Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945 950 955 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
965 970 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
995 1000 1005

Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala
1010 1015 1020

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
1025 1030 1035

Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala
1040 1045 1050

Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu
1055 1060 1065

Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val
1070 1075 1080

Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr
1085 1090 1095

Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys
1100 1105 1110

Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1115 1120 1125

Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
1130 1135 1140

Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys
1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
1175 1180 1185

Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
1190 1195 1200

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
1205 1210 1215

Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

[0314] Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1235 1240 1245

Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
1250 1255 1260

His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys
1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
1280 1285 1290

Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
1295 1300 1305

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
1340 1345 1350

Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

Gly Gly Gly Ser Gln Ala Ala Lys Arg Ser Arg Thr Thr
1370 1375 1380

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Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
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Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

[0315] Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
325 330 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
340 345 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
355 360 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
370 375 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385 390 395 400

[0316] Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
405 410 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
420 425 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
435 440 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
450 455 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465 470 475 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
485 490 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
500 505 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
515 520 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
530 535 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545 550 555 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
565 570 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
580 585 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
595 600 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
610 615 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625 630 635 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
660 665 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
675 680 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
690 695 700

[0317] Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705 710 715 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
725 730 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
740 745 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
755 760 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
770 775 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785 790 795 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
805 810 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
820 825 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
835 840 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865 870 875 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
885 890 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
900 905 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
915 920 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
930 935 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945 950 955 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
965 970 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
995 1000 1005

[0318] Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala
1010 1015 1020

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
1025 1030 1035

Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala
1040 1045 1050

Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu
1055 1060 1065

Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val
1070 1075 1080

Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr
1085 1090 1095

Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys
1100 1105 1110

Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1115 1120 1125

Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
1130 1135 1140

Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys

1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
1175 1180 1185

Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
1190 1195 1200

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
1205 1210 1215

Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1235 1240 1245

Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
1250 1255 1260

His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys
1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
1280 1285 1290

[0319]

Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
1295 1300 1305

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
1340 1345 1350

Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

Gly Gly Gly Ser Pro Ala Pro Ala Lys Arg Glu Arg Thr Thr
1370 1375 1380

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<220>
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<400> 189

Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
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Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

[0320] Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser

305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
325 330 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
340 345 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
355 360 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
370 375 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385 390 395 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
405 410 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
420 425 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
435 440 445

[0321] Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
450 455 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465 470 475 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
485 490 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
500 505 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
515 520 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
530 535 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545 550 555 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
565 570 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
580 585 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
595 600 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
610 615 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625 630 635 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
660 665 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
675 680 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
690 695 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705 710 715 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
725 730 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
740 745 750

[0322] Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
755 760 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
770 775 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785 790 795 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
805 810 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
820 825 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
835 840 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865 870 875 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
885 890 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
900 905 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
915 920 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
930 935 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945 950 955 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
965 970 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
995 1000 1005

Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala
1010 1015 1020

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
1025 1030 1035

Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala
1040 1045 1050

[0323] Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu
1055 1060 1065

Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val
1070 1075 1080

Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr
1085 1090 1095

Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys
1100 1105 1110

Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1115 1120 1125

Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
1130 1135 1140

Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys
1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
1175 1180 1185

Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
1190 1195 1200

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
1205 1210 1215

Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1235 1240 1245

Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
1250 1255 1260

His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys
1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
1280 1285 1290

Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
1295 1300 1305

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

[0324] Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
1340 1345 1350

Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

Gly Gly Gly Ser Gln Ala Ala Lys Arg Pro Arg Thr Thr
1370 1375 1380

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<212> PRT
<213> 人工序列

<220>
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<400> 190

Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1 5 10 15

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

[0325] Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
325 330 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
340 345 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
355 360 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
370 375 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385 390 395 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
405 410 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
420 425 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
435 440 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
450 455 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465 470 475 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
485 490 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
500 505 510

[0326] Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
515 520 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
530 535 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545 550 555 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
565 570 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
580 585 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
595 600 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
610 615 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625 630 635 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp

660 665 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
675 680 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
690 695 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705 710 715 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
725 730 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
740 745 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
755 760 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
770 775 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785 790 795 800

[0327] Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
805 810 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
820 825 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
835 840 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865 870 875 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
885 890 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
900 905 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
915 920 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
930 935 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945 950 955 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
965 970 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
995 1000 1005

Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala
1010 1015 1020

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
1025 1030 1035

Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala
1040 1045 1050

Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu
1055 1060 1065

Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val
1070 1075 1080

Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr
1085 1090 1095

[0328] Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys
1100 1105 1110

Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1115 1120 1125

Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
1130 1135 1140

Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys
1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
1175 1180 1185

Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
1190 1195 1200

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
1205 1210 1215

Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1235 1240 1245

Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
1250 1255 1260

His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys
1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
1280 1285 1290

Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
1295 1300 1305

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
1340 1345 1350

Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

Gly Gly Gly Ser Arg Ala Ala Lys Arg Pro Arg Thr Thr
1370 1375 1380

[0329]

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<220>
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<400> 191

Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
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Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

[0330] Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
325 330 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
340 345 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
355 360 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
370 375 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385 390 395 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
405 410 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
420 425 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
435 440 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
450 455 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465 470 475 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
485 490 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
500 505 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
515 520 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
530 535 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545 550 555 560

[0331] Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
565 570 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
580 585 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
595 600 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
610 615 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625 630 635 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
660 665 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
675 680 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
690 695 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705 710 715 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
725 730 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
740 745 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
755 760 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
770 775 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785 790 795 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
805 810 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
820 825 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
835 840 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860

[0332] Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865 870 875 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
885 890 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
900 905 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
915 920 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
930 935 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945 950 955 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
965 970 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
995 1000 1005

Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala

1010 1015 1020

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
1025 1030 1035

Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala
1040 1045 1050

Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu
1055 1060 1065

Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val
1070 1075 1080

Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr
1085 1090 1095

Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys
1100 1105 1110

Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1115 1120 1125

Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
1130 1135 1140

[0333] Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys
1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
1175 1180 1185

Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
1190 1195 1200

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
1205 1210 1215

Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1235 1240 1245

Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
1250 1255 1260

His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys
1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
1280 1285 1290

Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
1295 1300 1305

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
1340 1345 1350

Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

Gly Gly Gly Ser Ala Ala Ala Lys Arg Ser Trp Ser Met Ala Ala
1370 1375 1380

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<213> 人工序列

<220>
<223> 合成的：具有NLS7的Cas9的氨基酸序列

<400> 192

Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1 5 10 15

[0334]

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro

165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

[0335] Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
325 330 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
340 345 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
355 360 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
370 375 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385 390 395 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
405 410 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
420 425 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
435 440 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
450 455 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465 470 475 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
485 490 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
500 505 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
515 520 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
530 535 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545 550 555 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
565 570 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
580 585 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
595 600 605

[0336] Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
610 615 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625 630 635 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
660 665 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
675 680 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
690 695 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705 710 715 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
725 730 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
740 745 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
755 760 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
770 775 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785 790 795 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
805 810 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
820 825 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
835 840 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865 870 875 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
885 890 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
900 905 910

[0337] Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
915 920 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
930 935 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945 950 955 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
965 970 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
995 1000 1005

Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala
1010 1015 1020

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
1025 1030 1035

Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala
1040 1045 1050

Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu
1055 1060 1065

Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val
1070 1075 1080

Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr
1085 1090 1095

Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys
1100 1105 1110

Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1115 1120 1125

Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
1130 1135 1140

Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys
1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
1175 1180 1185

Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
1190 1195 1200

[0338] Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
1205 1210 1215

Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1235 1240 1245

Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
1250 1255 1260

His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys
1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
1280 1285 1290

Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
1295 1300 1305

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr

1340 1345 1350

Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

Gly Gly Gly Ser Ala Ala Ala Lys Arg Val Trp Ser Met Ala Phe
1370 1375 1380

<210> 193
<211> 1383
<212> PRT
<213> 人工序列

<220>
<223> 合成的：具有NLS8的Cas9的氨基酸序列

<400> 193

Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1 5 10 15

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

[0339] Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
325 330 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
340 345 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
355 360 365

[0340] Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
370 375 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385 390 395 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
405 410 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
420 425 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
435 440 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
450 455 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465 470 475 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
485 490 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
500 505 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys

515 520 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
530 535 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545 550 555 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
565 570 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
580 585 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
595 600 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
610 615 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625 630 635 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655

[0341]

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
660 665 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
675 680 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
690 695 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705 710 715 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
725 730 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
740 745 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
755 760 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
770 775 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785 790 795 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
805 810 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
820 825 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
835 840 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865 870 875 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
885 890 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
900 905 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
915 920 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
930 935 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945 950 955 960

[0342] Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
965 970 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
995 1000 1005

Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala
1010 1015 1020

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
1025 1030 1035

Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala
1040 1045 1050

Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu
1055 1060 1065

Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val
1070 1075 1080

Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr
1085 1090 1095

Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys
1100 1105 1110

Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1115 1120 1125

Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
1130 1135 1140

Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys
1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
1175 1180 1185

Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
1190 1195 1200

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
1205 1210 1215

Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1235 1240 1245

[0343] Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
1250 1255 1260

His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys
1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
1280 1285 1290

Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
1295 1300 1305

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
1340 1345 1350

Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

Gly Gly Gly Ser Ala Ala Ala Lys Arg Ser Trp Ser Met Ala Phe
1370 1375 1380

<210> 194
<211> 1382

<212> PRT
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<220>
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<400> 194

[0344]

Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1 5 10 15

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
325 330 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
340 345 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
355 360 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
370 375 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385 390 395 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
405 410 415

[0345] Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
420 425 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
435 440 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
450 455 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465 470 475 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
485 490 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
500 505 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
515 520 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
530 535 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545 550 555 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
565 570 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
580 585 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
595 600 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
610 615 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625 630 635 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
660 665 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
675 680 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
690 695 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705 710 715 720

[0346] His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
725 730 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
740 745 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
755 760 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
770 775 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785 790 795 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
805 810 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
820 825 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
835 840 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys

865 870 875 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
885 890 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
900 905 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
915 920 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
930 935 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945 950 955 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
965 970 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
995 1000 1005

[0347] Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala
1010 1015 1020

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
1025 1030 1035

Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala
1040 1045 1050

Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu
1055 1060 1065

Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val
1070 1075 1080

Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr
1085 1090 1095

Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys
1100 1105 1110

Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1115 1120 1125

Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
1130 1135 1140

Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys
1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
1175 1180 1185

Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
1190 1195 1200

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
1205 1210 1215

Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1235 1240 1245

Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
1250 1255 1260

His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys
1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
1280 1285 1290

Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
[0348] 1295 1300 1305

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
1340 1345 1350

Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

Gly Gly Gly Ser Ala Ala Ala Lys Arg Lys Tyr Phe Ala Ala
1370 1375 1380

<210> 195
<211> 1382
<212> PRT
<213> 人工序列

<220>
<223> 合成的：具有NLS10的Cas9的氨基酸序列

<400> 195

Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1 5 10 15

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe

20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

[0349] Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
325 330 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
340 345 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
355 360 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
370 375 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385 390 395 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
405 410 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
420 425 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
435 440 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
450 455 460

[0350] Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465 470 475 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
485 490 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
500 505 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
515 520 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
530 535 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545 550 555 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
565 570 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
580 585 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
595 600 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
610 615 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625 630 635 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
660 665 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
675 680 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
690 695 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705 710 715 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
725 730 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
740 745 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
755 760 765

[0351] Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
770 775 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785 790 795 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
805 810 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
820 825 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
835 840 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865 870 875 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
885 890 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
900 905 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
915 920 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
930 935 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945 950 955 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
965 970 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
995 1000 1005

Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala
1010 1015 1020

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
1025 1030 1035

Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala
1040 1045 1050

Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu
1055 1060 1065

[0352] Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val
1070 1075 1080

Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr
1085 1090 1095

Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys
1100 1105 1110

Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1115 1120 1125

Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
1130 1135 1140

Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys
1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
1175 1180 1185

Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
1190 1195 1200

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly

1205 1210 1215

Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1235 1240 1245

Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
1250 1255 1260

His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys
1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
1280 1285 1290

Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
1295 1300 1305

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
1340 1345 1350

[0353]

Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

Gly Gly Gly Ser Arg Ala Ala Lys Arg Lys Ala Phe Ala Ala
1370 1375 1380

<210> 196
<211> 1382
<212> PRT
<213> 人工序列

<220>
<223> 合成的：具有NLS11的Cas9的氨基酸序列

<400> 196

Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1 5 10 15

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

[0354] Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
325 330 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
340 345 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
355 360 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp

370 375 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385 390 395 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
405 410 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
420 425 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
435 440 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
450 455 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465 470 475 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
485 490 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
500 505 510

[0355] Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
515 520 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
530 535 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545 550 555 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
565 570 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
580 585 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
595 600 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
610 615 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625 630 635 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
660 665 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
675 680 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
690 695 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705 710 715 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
725 730 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
740 745 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
755 760 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
770 775 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785 790 795 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
805 810 815

[0356] Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
820 825 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
835 840 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865 870 875 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
885 890 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
900 905 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
915 920 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
930 935 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945 950 955 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
965 970 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
995 1000 1005

Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala
1010 1015 1020

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
1025 1030 1035

Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala
1040 1045 1050

Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu
1055 1060 1065

Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val
1070 1075 1080

Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr
1085 1090 1095

Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys
1100 1105 1110

[0357] Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1115 1120 1125

Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
1130 1135 1140

Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys
1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
1175 1180 1185

Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
1190 1195 1200

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
1205 1210 1215

Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1235 1240 1245

Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
1250 1255 1260

His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys
1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
1280 1285 1290

Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
1295 1300 1305

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
1340 1345 1350

Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

Gly Gly Gly Ser Arg Ala Ala Lys Arg Lys Tyr Phe Ala Val
1370 1375 1380

[0358]

<210> 197
<211> 100
<212> RNA
<213> 人工序列

<220>
<223> 合成的: G506 引导 RNA

<220>
<221> 修饰的_碱基
<222> (1)..(3)
<223> PS键, 2'-O-Me 核苷酸

<220>
<221> 修饰的_碱基
<222> (29)..(40)
<223> 2'-O-Me 核苷酸

<220>
<221> 修饰的_碱基
<222> (69)..(97)
<223> 2'-O-Me 核苷酸

<220>
<221> 修饰的_碱基
<222> (97)..(100)
<223> PS键, 2'-O-Me 核苷酸

<400> 197
auaccagucc agcgaggcag guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 198
<211> 100
<212> RNA
<213> 人工序列

<220>

<223> 合成的：G510 引导 RNA

<220>
<221> 修饰的_碱基
<222> (1)..(3)
<223> PS键，2'-O-Me 核苷酸

<220>
<221> 修饰的_碱基
<222> (29)..(40)
<223> 2'-O-Me 核苷酸

[0359] <220>
<221> 修饰的_碱基
<222> (69)..(96)
<223> 2'-O-Me 核苷酸

<220>
<221> 修饰的_碱基
<222> (97)..(100)
<223> PS键，2'-O-Me 核苷酸

<400> 198
acuugucuuc ucuauaccca guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

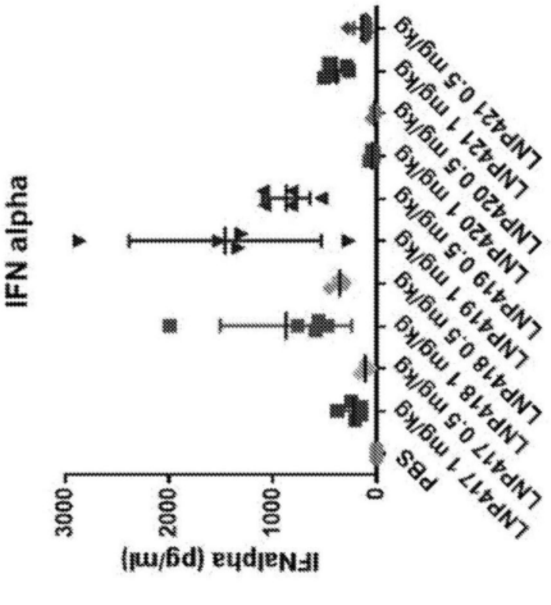

IFN alpha
IFNalpha (pg/ml)
3000
2000
1000
0
PBS
LNP417 1 mg/kg
LNP417 0.5 mg/kg
LNP418 1 mg/kg
LNP418 0.5 mg/kg
LNP419 1 mg/kg
LNP419 0.5 mg/kg
LNP420 1 mg/kg
LNP420 0.5 mg/kg
LNP421 1 mg/kg
LNP421 0.5 mg/kg

图1A

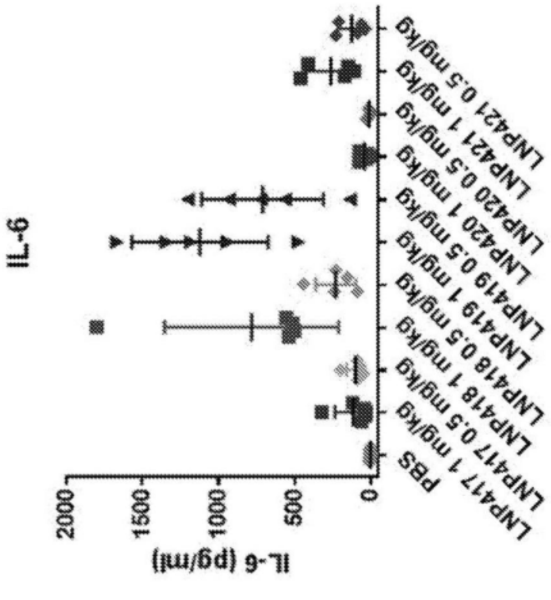

IL-6
IL-6 (pg/ml)
2000
1500
1000
500
0
PBS
LNP417 1 mg/kg
LNP417 0.5 mg/kg
LNP418 1 mg/kg
LNP418 0.5 mg/kg
LNP419 1 mg/kg
LNP419 0.5 mg/kg
LNP420 1 mg/kg
LNP420 0.5 mg/kg
LNP421 1 mg/kg
LNP421 0.5 mg/kg

图1B

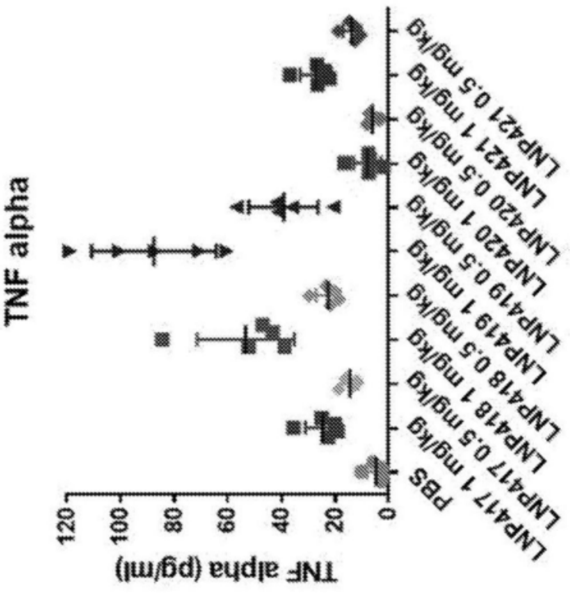

TNF alpha
TNF alpha (pg/ml)
120
100
80
60
40
20
0
PBS
LNP417 1 mg/kg
LNP417 0.5 mg/kg
LNP418 1 mg/kg
LNP418 0.5 mg/kg
LNP419 1 mg/kg
LNP419 0.5 mg/kg
LNP420 1 mg/kg
LNP420 0.5 mg/kg
LNP421 1 mg/kg
LNP421 0.5 mg/kg

图1C

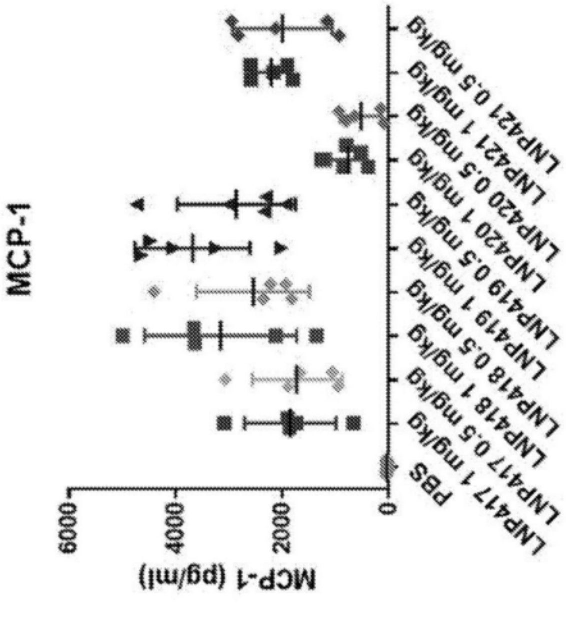
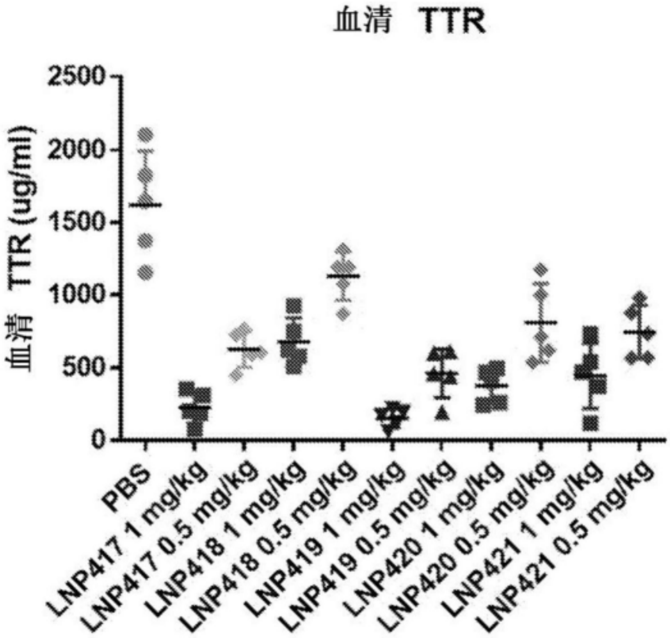
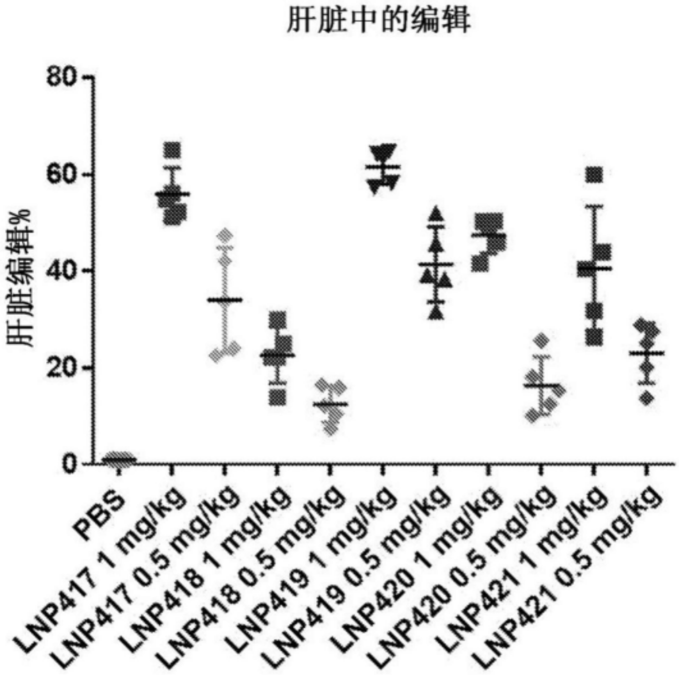

MCP-1
MCP-1 (pg/ml)
6000
4000
2000
0
PBS
LNP417 1 mg/kg
LNP417 0.5 mg/kg
LNP418 1 mg/kg
LNP418 0.5 mg/kg
LNP419 1 mg/kg
LNP419 0.5 mg/kg
LNP420 1 mg/kg
LNP420 0.5 mg/kg
LNP421 1 mg/kg
LNP421 0.5 mg/kg

图1D



血清 TTR
血清 TTR (ug/ml)
2500
2000
1500
1000
500
0
PBS
LNP417 1 mg/kg
LNP417 0.5 mg/kg
LNP418 1 mg/kg
LNP418 0.5 mg/kg
LNP419 1 mg/kg
LNP419 0.5 mg/kg
LNP420 1 mg/kg
LNP420 0.5 mg/kg
LNP421 1 mg/kg
LNP421 0.5 mg/kg

图2A



肝脏中的编辑
肝脏编辑%
80
60
40
20
0
PBS
LNP417 1 mg/kg
LNP417 0.5 mg/kg
LNP418 1 mg/kg
LNP418 0.5 mg/kg
LNP419 1 mg/kg
LNP419 0.5 mg/kg
LNP420 1 mg/kg
LNP420 0.5 mg/kg
LNP421 1 mg/kg
LNP421 0.5 mg/kg

图2B

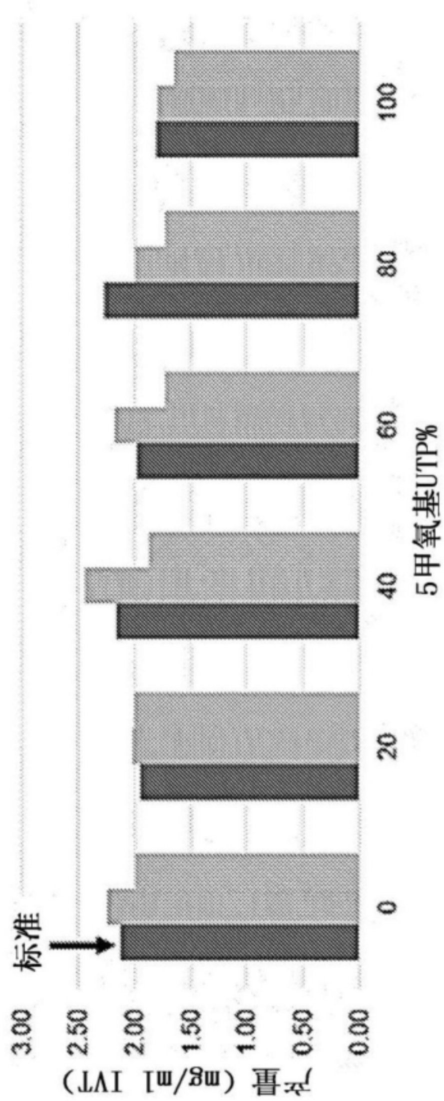

产量 (mg/ml IVT)
3.00
2.50
2.00
1.50
1.00
0.50
0.00
标准
0
20
40
60
80
100
5甲氧基UTP%

图3

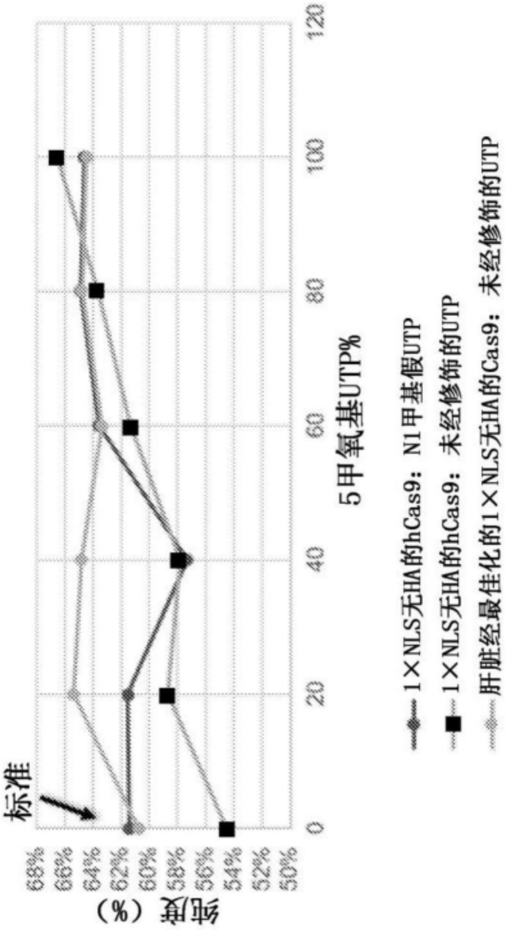

标准
纯度（%）
68%
66%
64%
62%
60%
58%
56%
54%
52%
50%
0
20
40
60
80
100
120
5甲氧基UTP%
1×NLS无HA的hCas9：N1甲基假UTP
1×NLS无HA的hCas9：未经修饰的UTP
肝脏经最佳化的1×NLS无HA的Cas9：未经修饰的UTP

图4

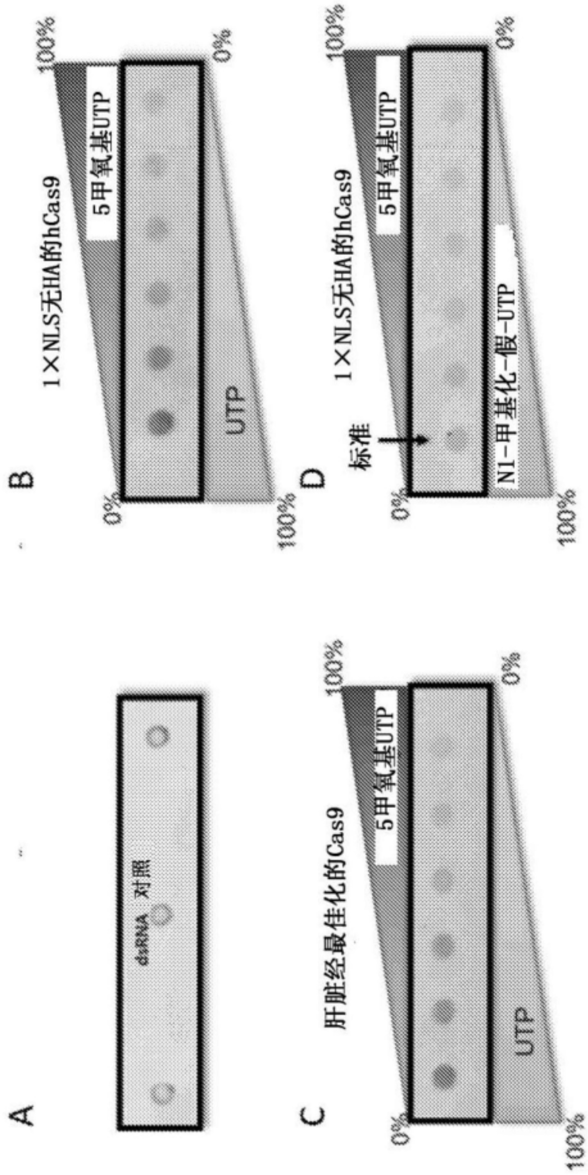

A
dsRNA 对照
B
1×NLS无HA的hCas9
5甲氧基UTP
UTP
100%
0%
0%
100%
C
肝脏经最佳化的Cas9
5甲氧基UTP
UTP
100%
0%
0%
100%
D
1×NLS无HA的hCas9
5甲氧基UTP
N1-甲基化-假-UTP
标准
100%
0%
0%
100%

图5

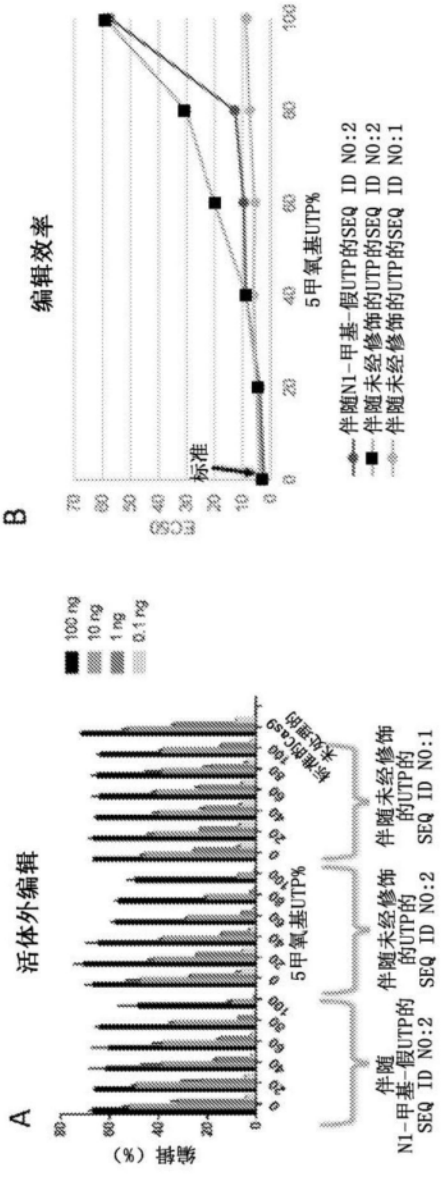

A
活体外编辑
编辑（%）
5甲氧基UTP%
100 ng
10 ng
1 ng
0.1 ng
伴随N1-甲基-假UTP的SEQ ID NO:2
伴随未经修饰的UTP的SEQ ID NO:2
伴随未经修饰的UTP的SEQ ID NO:1
B
编辑效率
EC50
标准
5甲氧基UTP%
伴随N1-甲基-假UTP的SEQ ID NO:2
伴随未经修饰的UTP的SEQ ID NO:2
伴随未经修饰的UTP的SEQ ID NO:1

图6

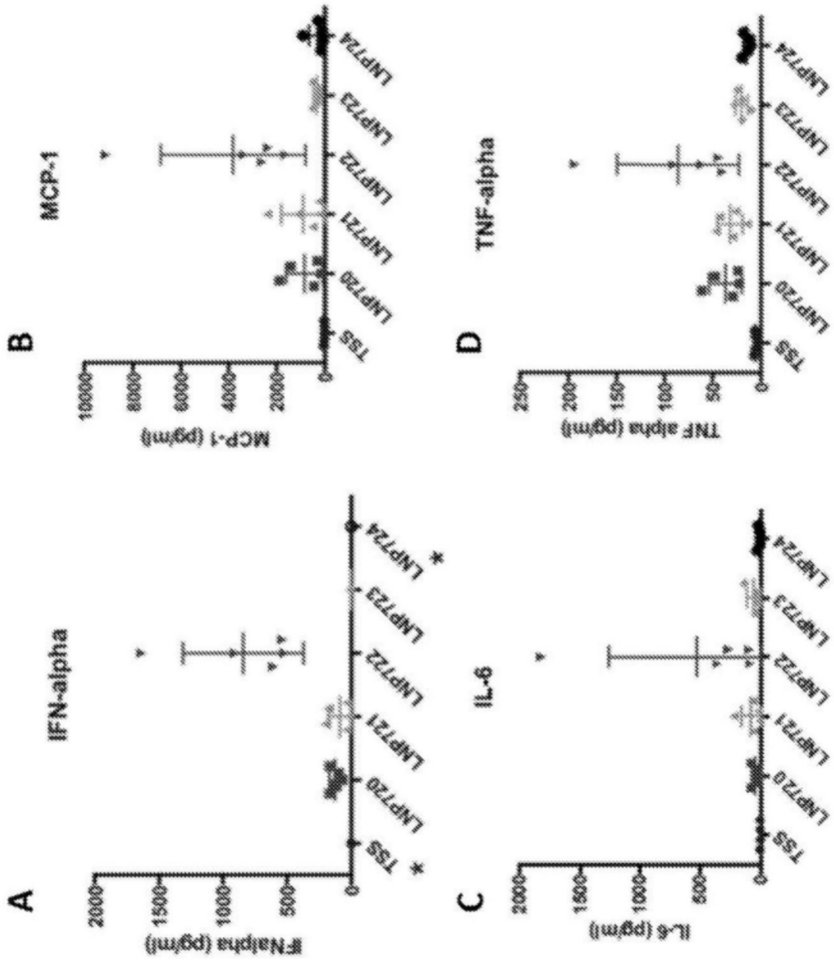

A
IFN-alpha
IFNalpha (pg/ml)
B
MCP-1
MCP-1 (pg/ml)
C
IL-6
IL-6 (pg/ml)
D
TNF-alpha
TNF alpha (pg/ml)
TSS
LNP720
LNP721
LNP722
LNP723
LNP724

图7

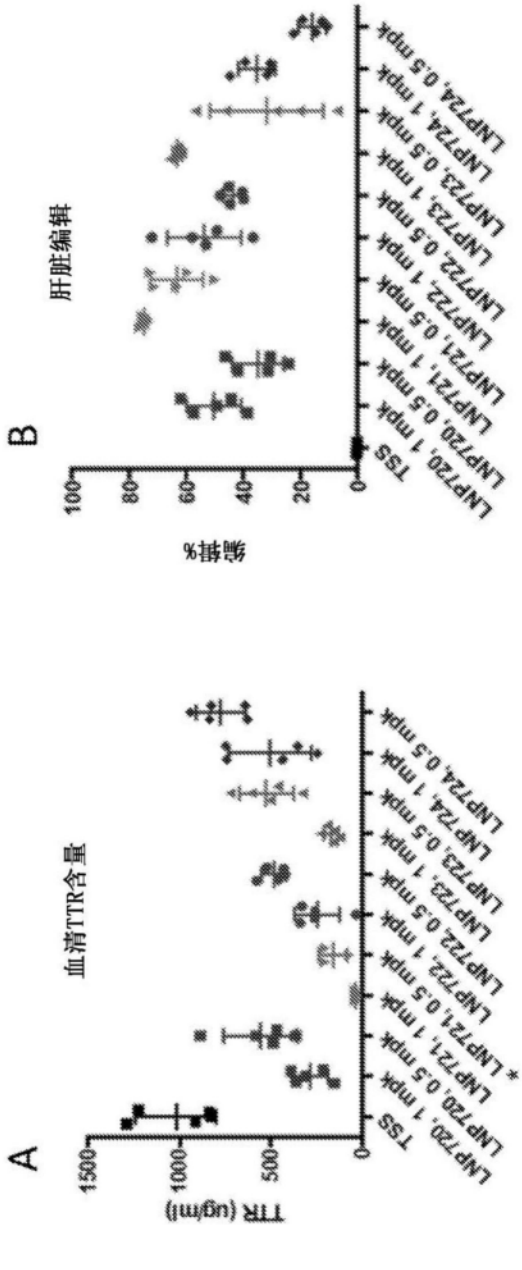

B
肝脏编辑
编辑%
100
80
60
40
20
0
TSS
LNP720, 1 mpk
LNP720, 0.5 mpk
LNP721, 1 mpk
LNP721, 0.5 mpk
LNP722, 1 mpk
LNP722, 0.5 mpk
LNP723, 1 mpk
LNP723, 0.5 mpk
LNP724, 1 mpk
LNP724, 0.5 mpk
A
血清TTR含量
TTR (ug/ml)
1500
1000
500
0
TSS
LNP720, 1 mpk
LNP720, 0.5 mpk
LNP721, 1 mpk
LNP721, 0.5 mpk
LNP722, 1 mpk
LNP722, 0.5 mpk
LNP723, 1 mpk
LNP723, 0.5 mpk
LNP724, 1 mpk
LNP724, 0.5 mpk

图8

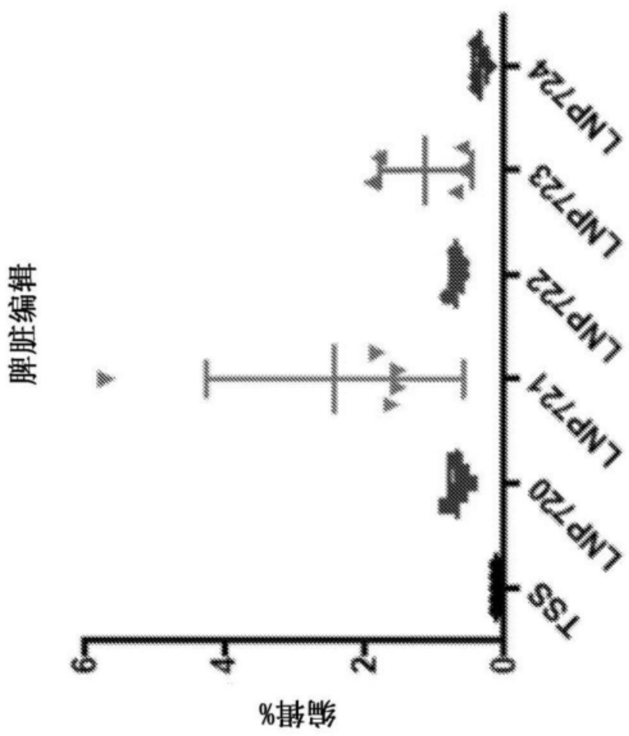


图9

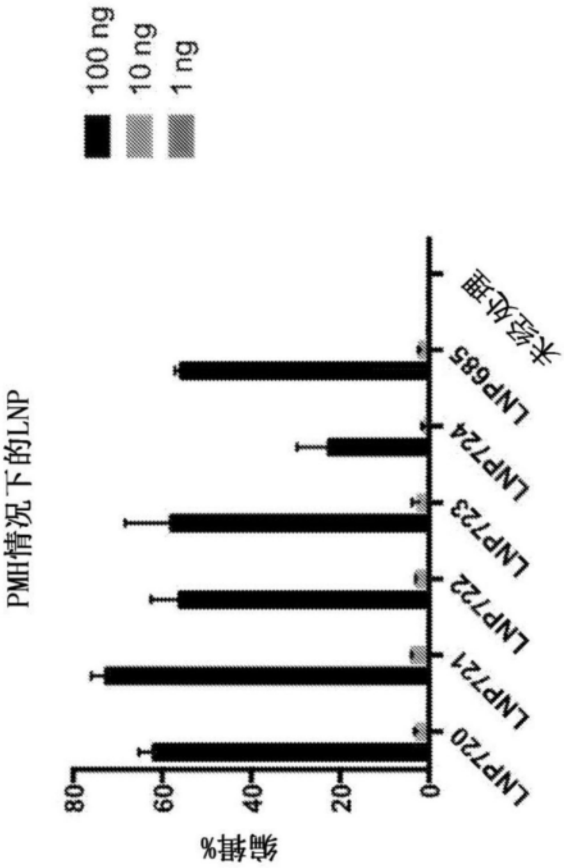

PMH情况下的LNP
编辑%
100 ng
10 ng
1 ng
LNP720
LNP721
LNP722
LNP723
LNP724
LNP685
未经处理
0
20
40
60
80

图10

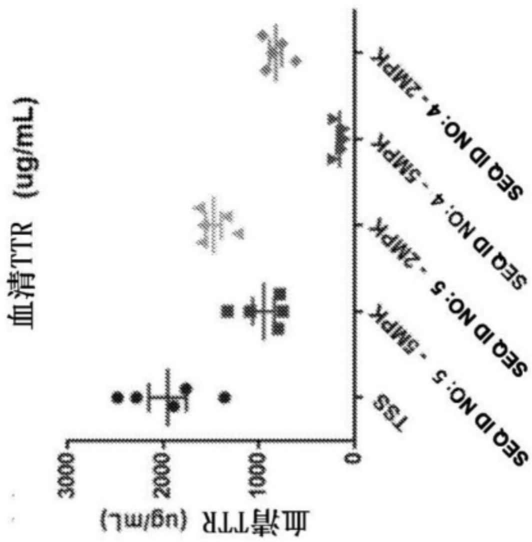

血清TTR (ug/mL)
TSS
SEQ ID NO: 5 - 5MPK
SEQ ID NO: 5 - 2MPK
SEQ ID NO: 4 - 5MPK
SEQ ID NO: 4 - 2MPK
0
1000
2000
3000

图11A

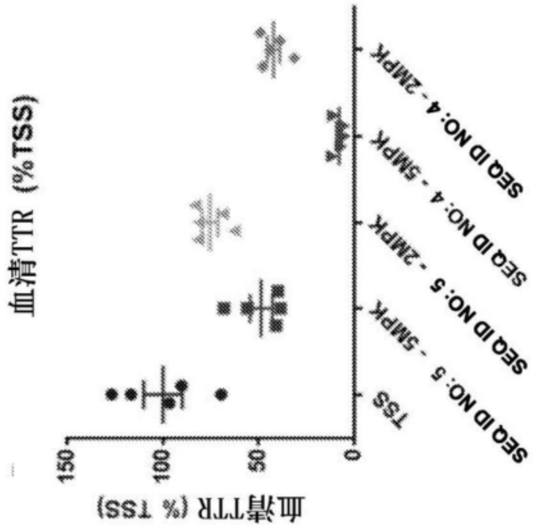

血清TTR (%TSS)
血清TTR (% TSS)
150
100
50
0
TSS
SEQ ID NO: 5 - 5MPK
SEQ ID NO: 5 - 2MPK
SEQ ID NO: 4 - 5MPK
SEQ ID NO: 4 - 2MPK

图11B

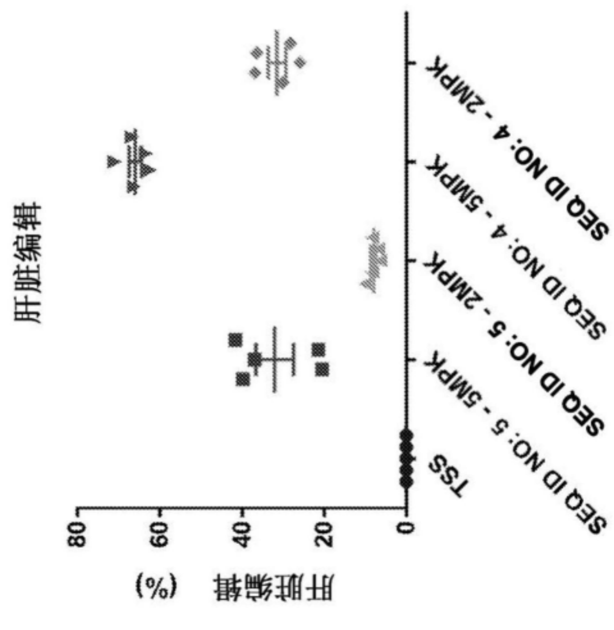

肝脏编辑
肝脏编辑 (%)
80
60
40
20
0
TSS
SEQ ID NO: 5 - 5MPK
SEQ ID NO: 5 - 2MPK
SEQ ID NO: 4 - 5MPK
SEQ ID NO: 4 - 2MPK

图12

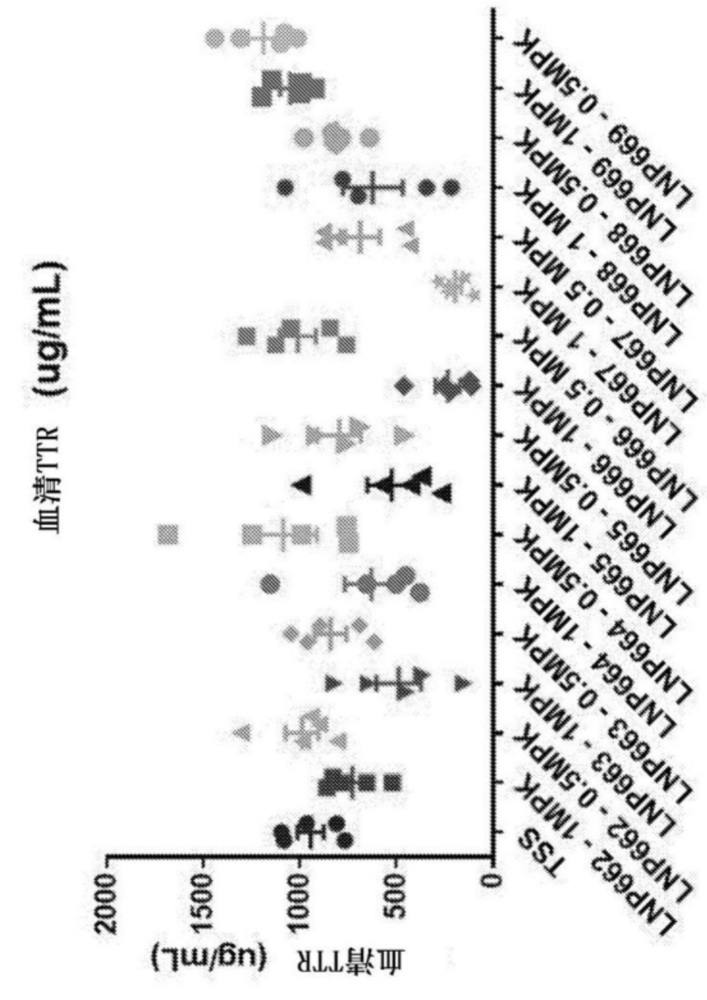

血清TTR (ug/mL)
血清TTR (ug/mL)
2000
1500
1000
500
0
TSS
LNP662 - 1MPK
LNP662 - 0.5MPK
LNP663 - 1MPK
LNP663 - 0.5MPK
LNP664 - 1MPK
LNP664 - 0.5MPK
LNP665 - 1MPK
LNP665 - 0.5MPK
LNP666 - 1MPK
LNP666 - 0.5 MPK
LNP667 - 1 MPK
LNP667 - 0.5 MPK
LNP668 - 1 MPK
LNP668 - 0.5MPK
LNP669 - 1MPK
LNP669 - 0.5MPK

图13A

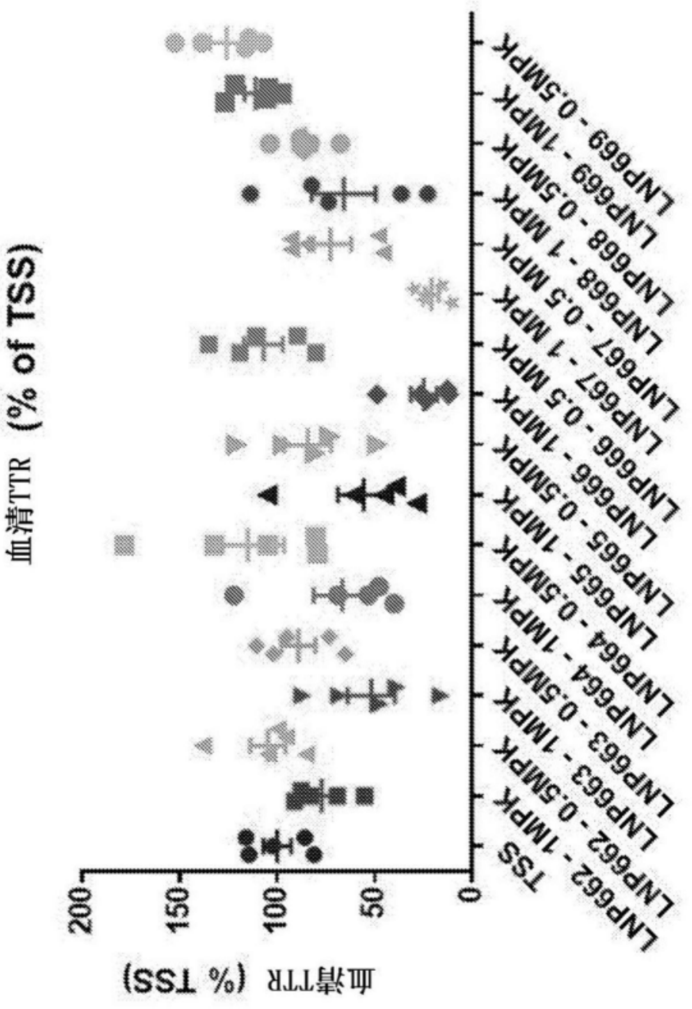

血清TTR (% of TSS)
血清TTR (% TSS)
200
150
100
50
0
TSS
LNP662 - 1MPK
LNP662 - 0.5MPK
LNP663 - 1MPK
LNP663 - 0.5MPK
LNP664 - 1MPK
LNP664 - 0.5MPK
LNP665 - 1MPK
LNP665 - 0.5MPK
LNP666 - 1MPK
LNP666 - 0.5 MPK
LNP667 - 1 MPK
LNP667 - 0.5 MPK
LNP668 - 1 MPK
LNP668 - 0.5MPK
LNP669 - 1MPK
LNP669 - 0.5MPK

图13B

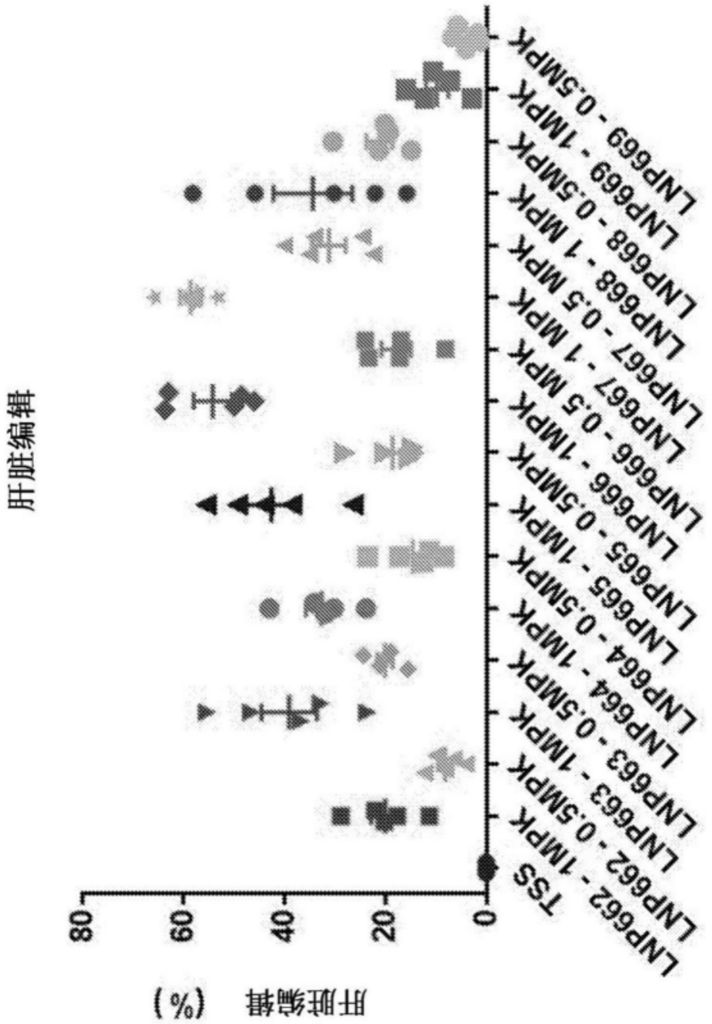

肝脏编辑
肝脏编辑 (%)
0
20
40
60
80
TSS
LNP662 - 1MPK
LNP662 - 0.5MPK
LNP663 - 1MPK
LNP663 - 0.5MPK
LNP664 - 1MPK
LNP664 - 0.5MPK
LNP665 - 1MPK
LNP665 - 0.5MPK
LNP666 - 1MPK
LNP666 - 0.5MPK
LNP667 - 1 MPK
LNP667 - 0.5 MPK
LNP668 - 1 MPK
LNP668 - 0.5MPK
LNP669 - 1MPK
LNP669 - 0.5MPK

图13C

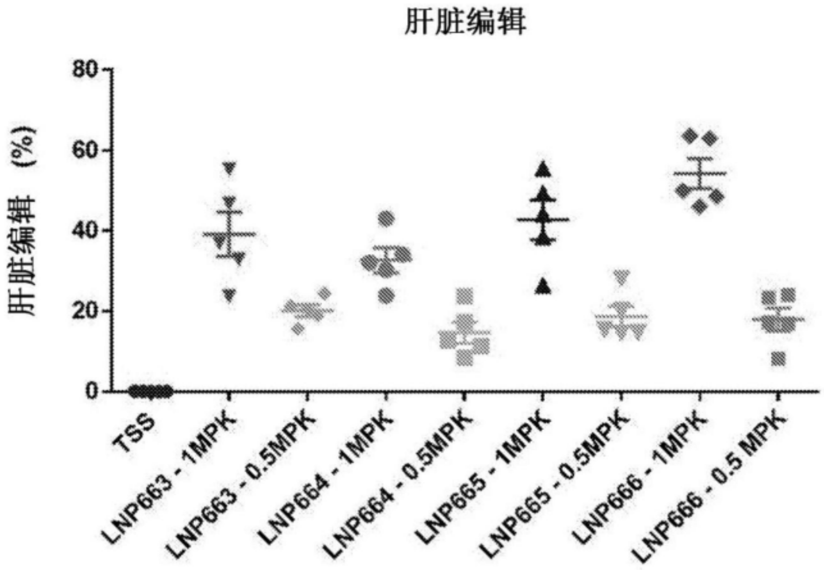

肝脏编辑
肝脏编辑 (%)
80
60
40
20
0
TSS
LNP663 - 1MPK
LNP663 - 0.5MPK
LNP664 - 1MPK
LNP664 - 0.5MPK
LNP665 - 1MPK
LNP665 - 0.5MPK
LNP666 - 1MPK
LNP666 - 0.5 MPK

图13D

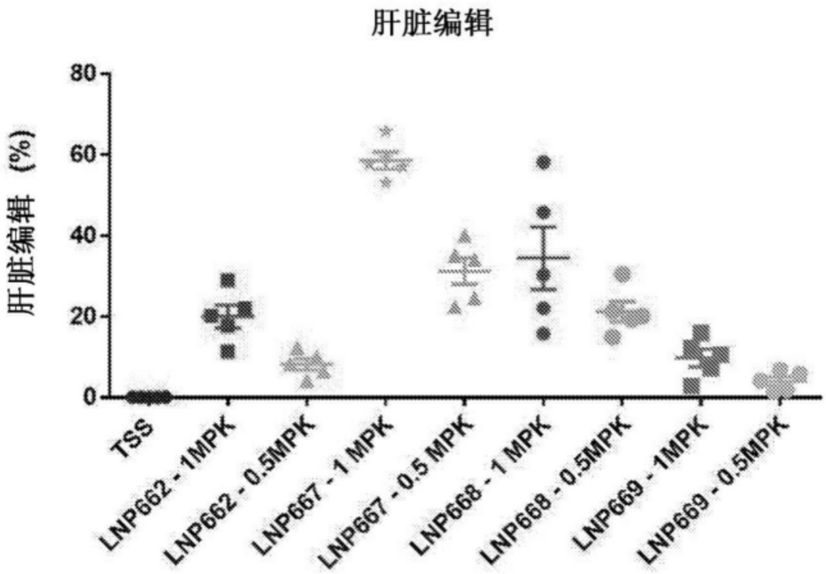

肝脏编辑
肝脏编辑 (%)
80
60
40
20
0
TSS
LNP662 - 1MPK
LNP662 - 0.5MPK
LNP667 - 1 MPK
LNP667 - 0.5 MPK
LNP668 - 1 MPK
LNP668 - 0.5MPK
LNP669 - 1MPK
LNP669 - 0.5MPK

图13E

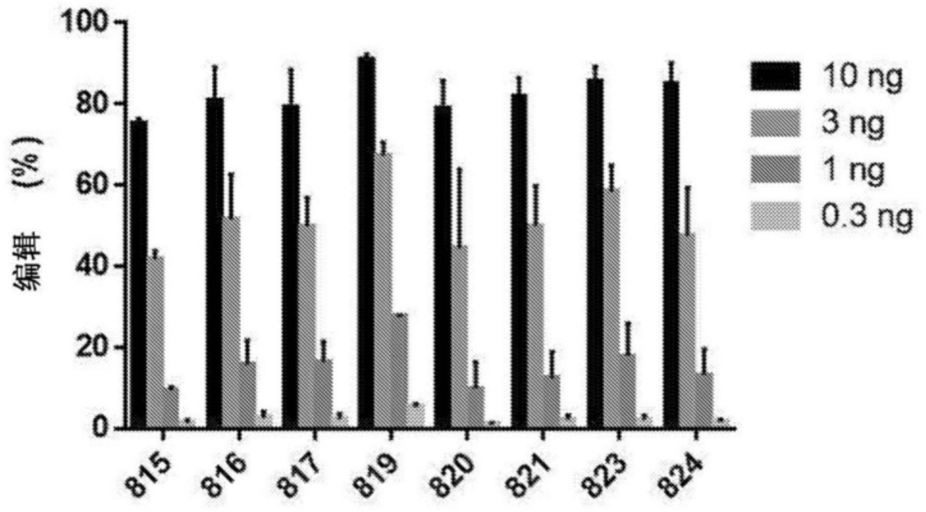


图14

血清TTR含量- SEQ ID NO:5

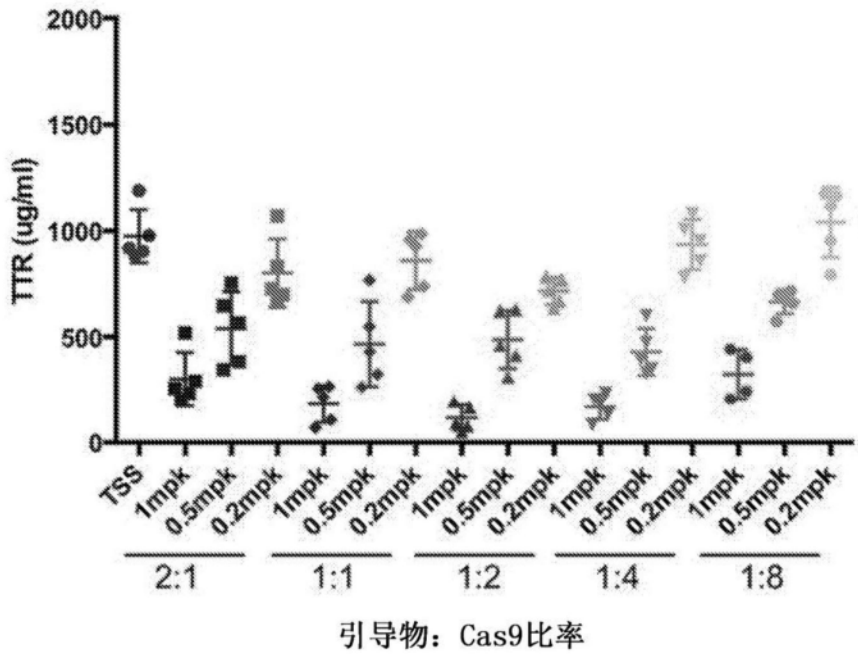


图15A

血清TTR含量- SEQ ID NO:4

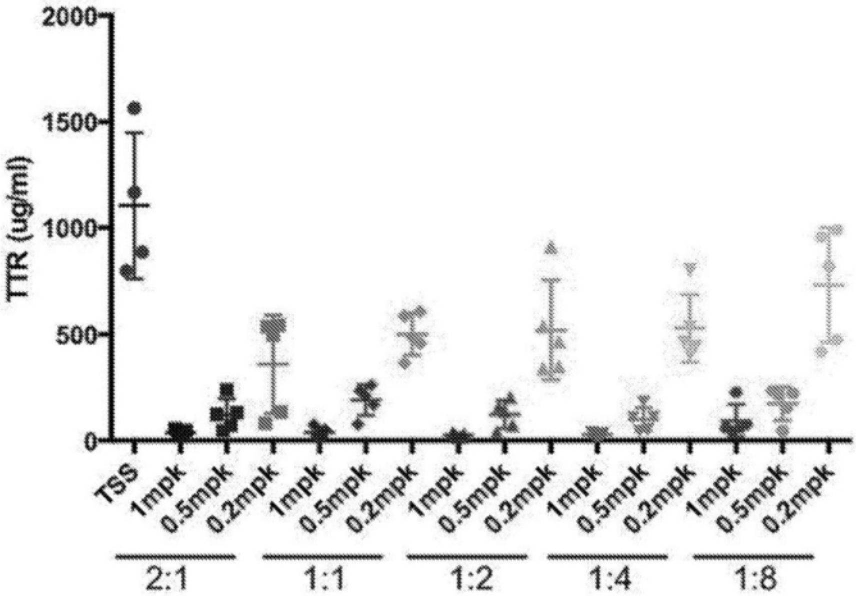


图15B

肝脏编辑- SEQ ID NO:5

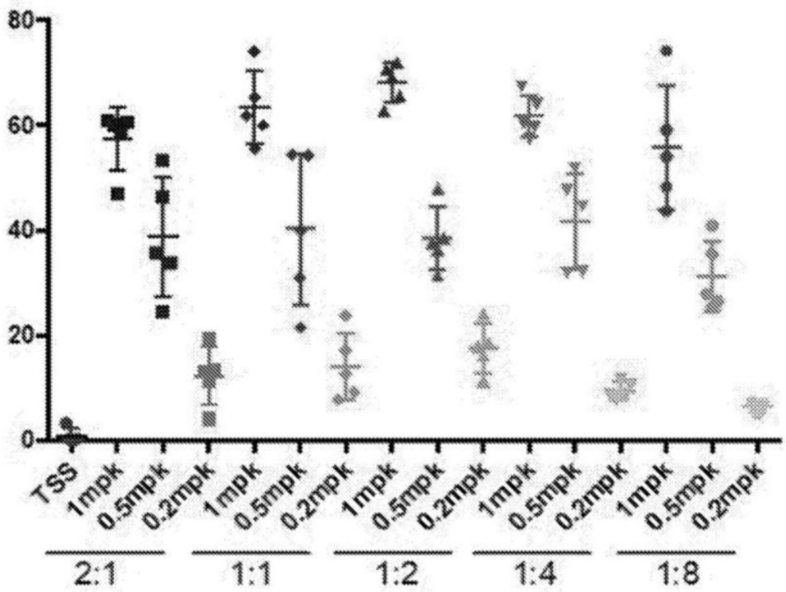


图16A

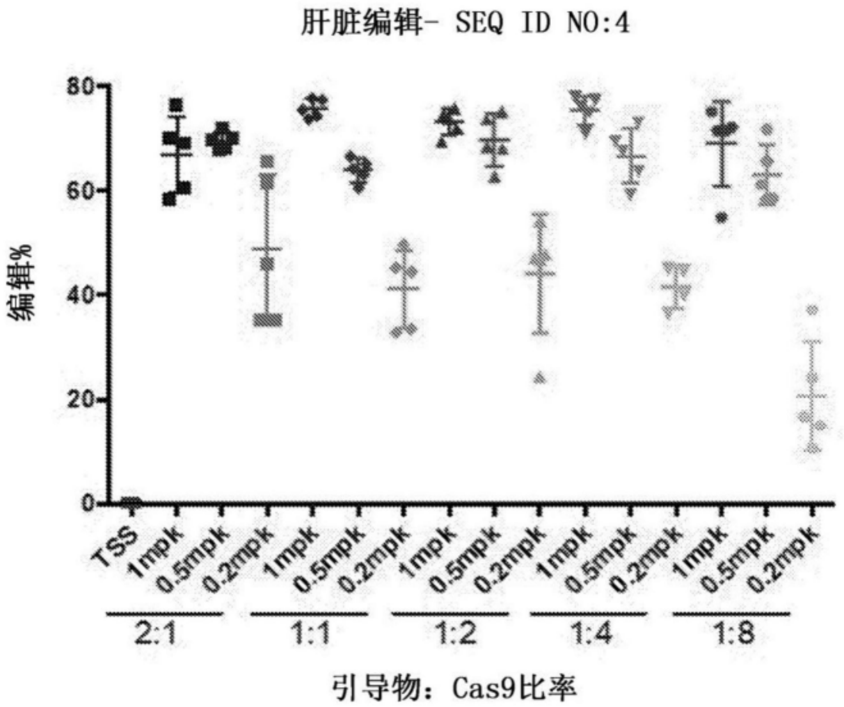

肝脏编辑- SEQ ID NO:4
编辑%
80
60
40
20
0
TSS
1mpk
0.5mpk
0.2mpk
2:1
1:1
1:2
1:4
1:8
引导物：Cas9比率

图16B

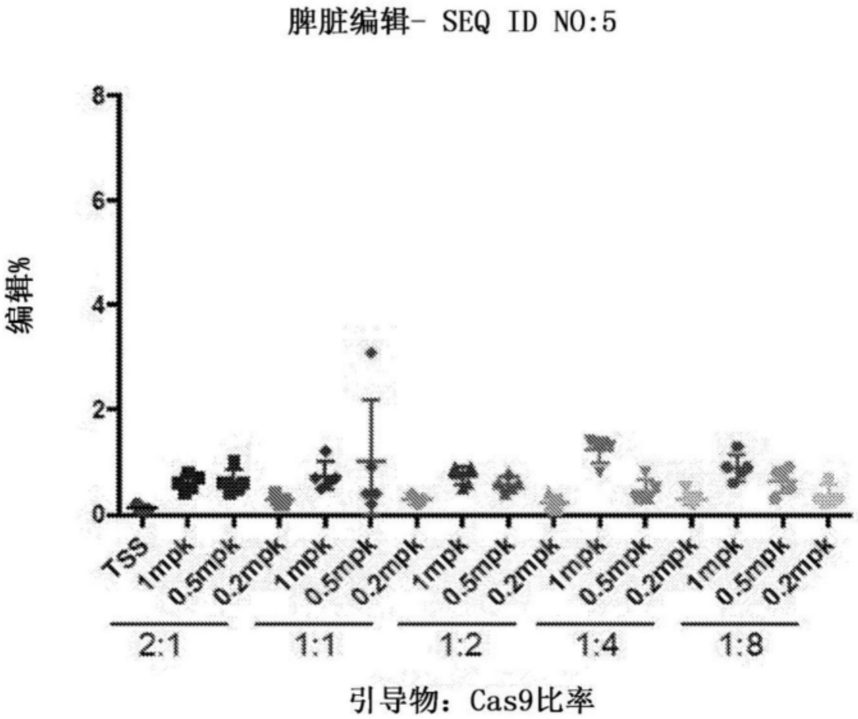

脾脏编辑- SEQ ID NO:5
编辑%
8
6
4
2
0
TSS
1mpk
0.5mpk
0.2mpk
2:1
1:1
1:2
1:4
1:8
引导物：Cas9比率

图17A

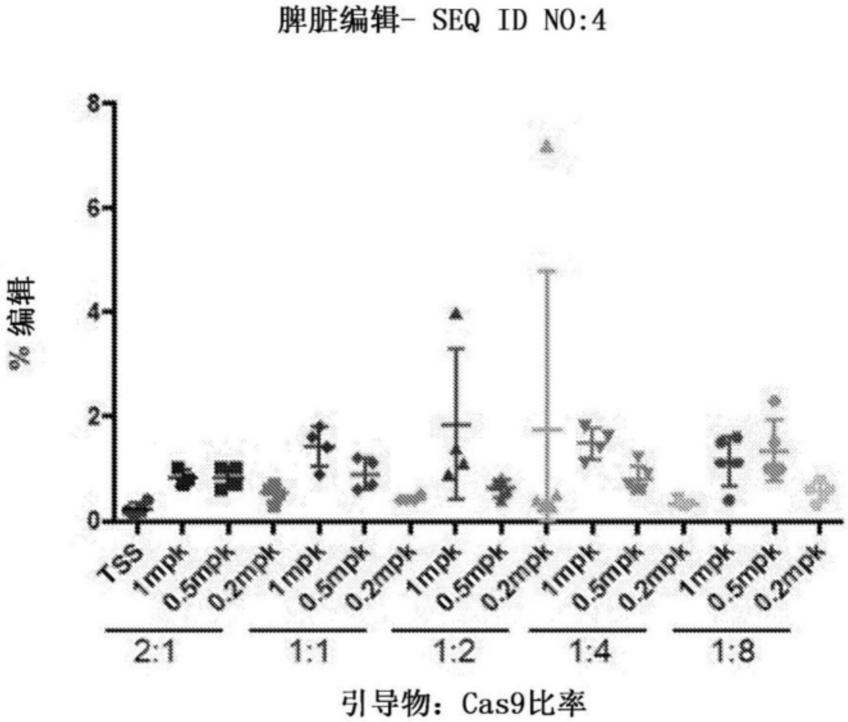

脾脏编辑- SEQ ID NO:4
8
6
4
2
0
% 编辑
TSS
1mpk
0.5mpk
0.2mpk
1mpk
0.5mpk
0.2mpk
1mpk
0.5mpk
0.2mpk
1mpk
0.5mpk
0.2mpk
1mpk
0.5mpk
0.2mpk
2:1
1:1
1:2
1:4
1:8
引导物：Cas9比率

图17B

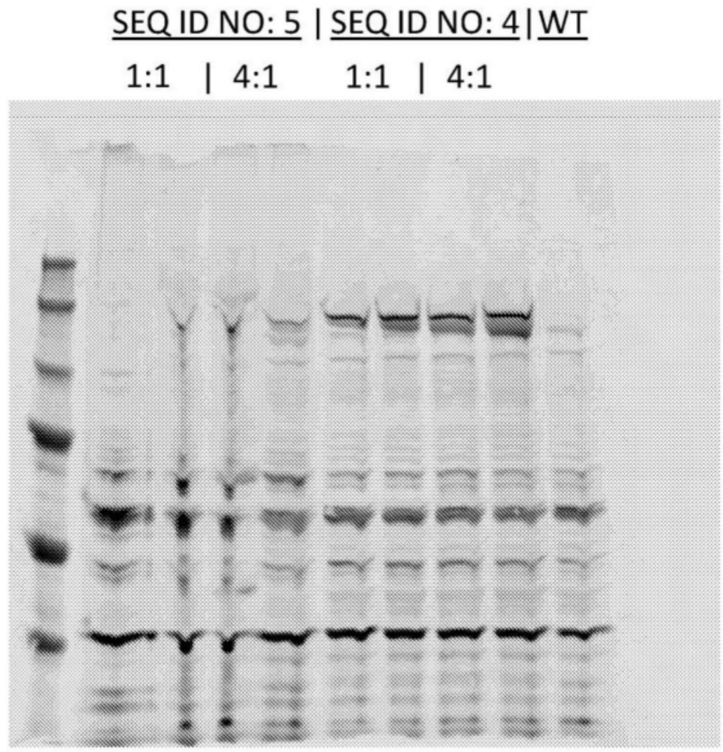

SEQ ID NO: 5 | SEQ ID NO: 4 | WT
1:1 | 4:1 1:1 | 4:1

图18

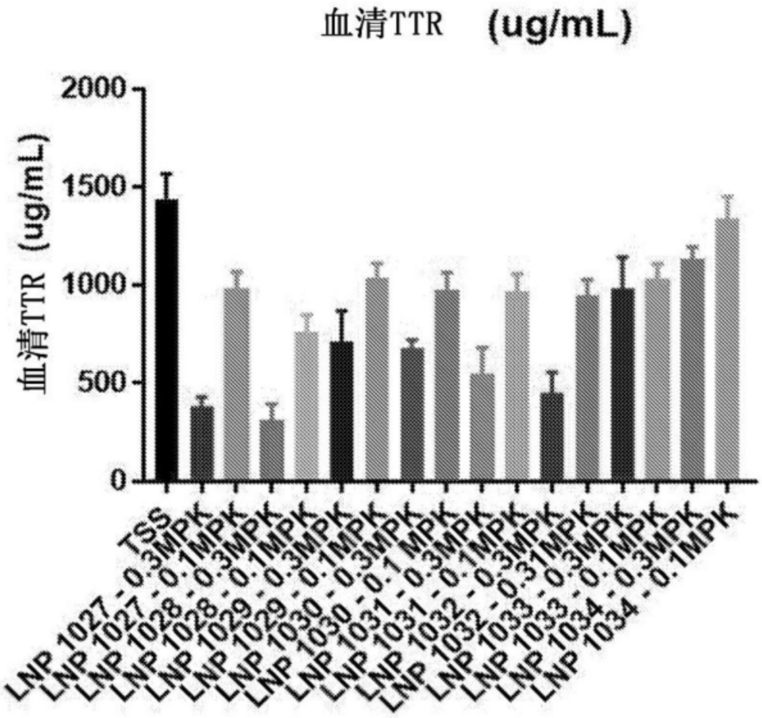

血清TTR (ug/mL)
2000
1500
1000
500
0
血清TTR (ug/mL)
TSS
LNP 1027 - 0.3MPK
LNP 1027 - 0.1MPK
LNP 1028 - 0.3MPK
LNP 1028 - 0.1MPK
LNP 1029 - 0.3MPK
LNP 1029 - 0.1MPK
LNP 1030 - 0.3MPK
LNP 1030 - 0.1 MPK
LNP 1031 - 0.3MPK
LNP 1031 - 0.1MPK
LNP 1032 - 0.3MPK
LNP 1032 - 0.31MPK
LNP 1033 - 0.3MPK
LNP 1033 - 0.1MPK
LNP 1034 - 0.3MPK
LNP 1034 - 0.1MPK

图19A

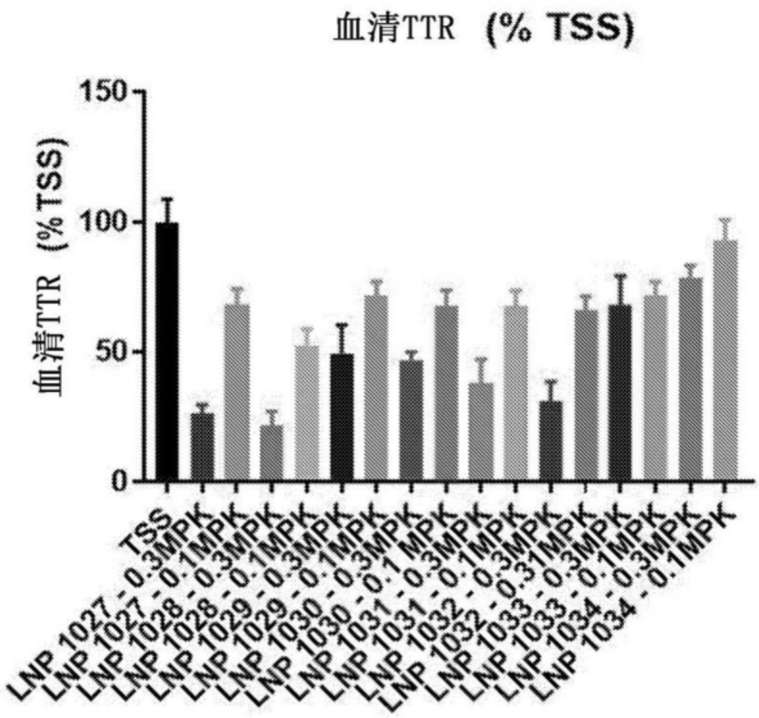

血清TTR (% TSS)
150
100
50
0
血清TTR (% TSS)
TSS
LNP 1027 - 0.3MPK
LNP 1027 - 0.1MPK
LNP 1028 - 0.3MPK
LNP 1028 - 0.1MPK
LNP 1029 - 0.3MPK
LNP 1029 - 0.1MPK
LNP 1030 - 0.3MPK
LNP 1030 - 0.1 MPK
LNP 1031 - 0.3MPK
LNP 1031 - 0.1MPK
LNP 1032 - 0.3MPK
LNP 1032 - 0.31MPK
LNP 1033 - 0.3MPK
LNP 1033 - 0.1MPK
LNP 1034 - 0.3MPK
LNP 1034 - 0.1MPK

图19B

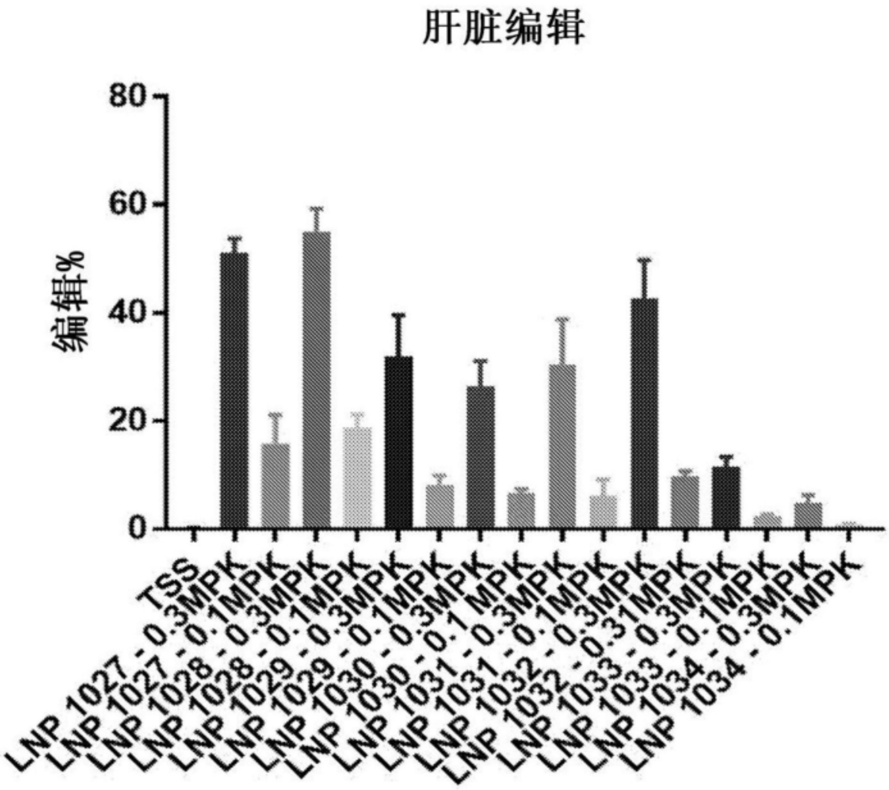

肝脏编辑
80
60
40
20
0
编辑%
TSS
LNP 1027 - 0.3MPK
LNP 1027 - 0.1MPK
LNP 1028 - 0.3MPK
LNP 1028 - 0.1MPK
LNP 1029 - 0.3MPK
LNP 1029 - 0.1MPK
LNP 1030 - 0.3MPK
LNP 1030 - 0.1 MPK
LNP 1031 - 0.3MPK
LNP 1031 - 0.1MPK
LNP 1032 - 0.3MPK
LNP 1032 - 0.31MPK
LNP 1033 - 0.3MPK
LNP 1033 - 0.1MPK
LNP 1034 - 0.3MPK
LNP 1034 - 0.1MPK

图20

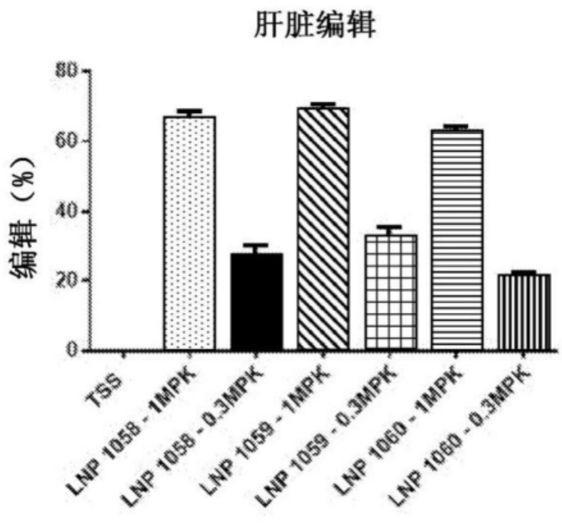

肝脏编辑
80
60
40
20
0
编辑（%）
TSS
LNP 1058 - 1MPK
LNP 1058 - 0.3MPK
LNP 1059 - 1MPK
LNP 1059 - 0.3MPK
LNP 1060 - 1MPK
LNP 1060 - 0.3MPK

图21A

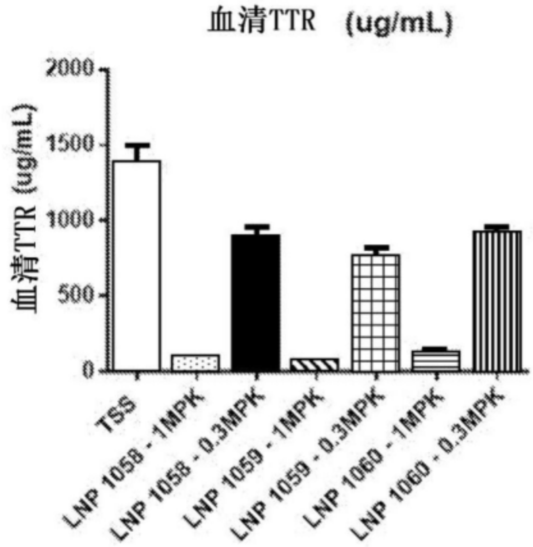

血清TTR (ug/mL)
血清TTR (ug/mL)
2000
1500
1000
500
0
TSS
LNP 1058 - 1MPK
LNP 1058 - 0.3MPK
LNP 1059 - 1MPK
LNP 1059 - 0.3MPK
LNP 1060 - 1MPK
LNP 1060 - 0.3MPK

图21B

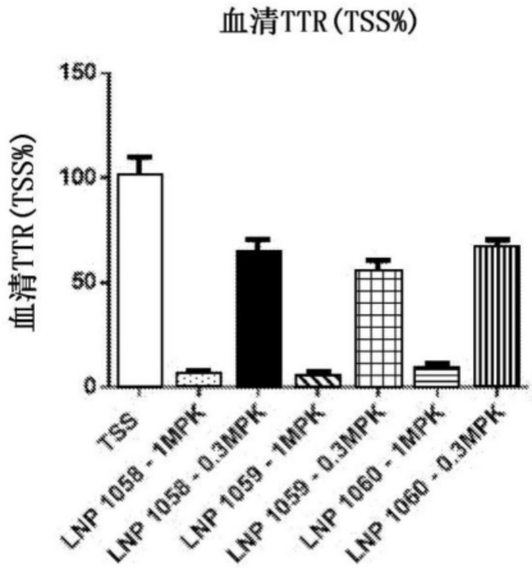

血清TTR(TSS%)
血清TTR(TSS%)
150
100
50
0
TSS
LNP 1058 - 1MPK
LNP 1058 - 0.3MPK
LNP 1059 - 1MPK
LNP 1059 - 0.3MPK
LNP 1060 - 1MPK
LNP 1060 - 0.3MPK

图21C

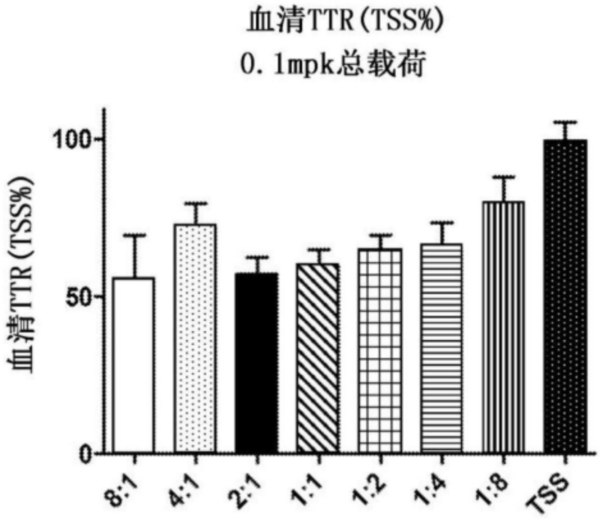

血清TTR(TSS%)
0.1mpk总载荷
血清TTR(TSS%)
100
50
0
8:1
4:1
2:1
1:1
1:2
1:4
1:8
TSS

图22A

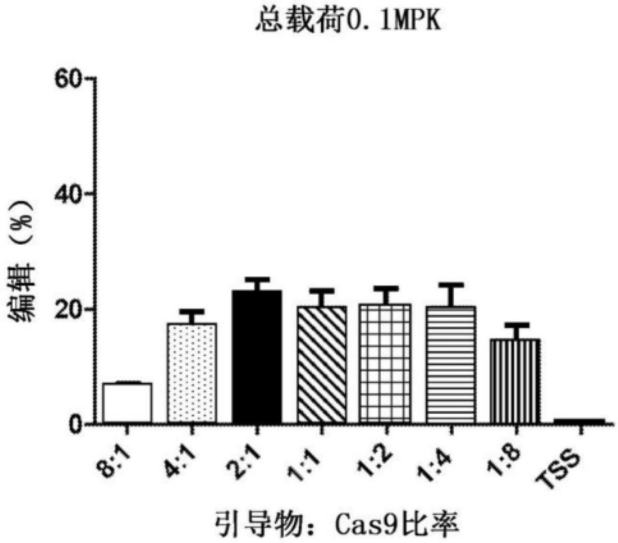

总载荷0.1MPK
60
40
20
0
编辑（%）
8:1
4:1
2:1
1:1
1:2
1:4
1:8
TSS
引导物：Cas9比率

图22B

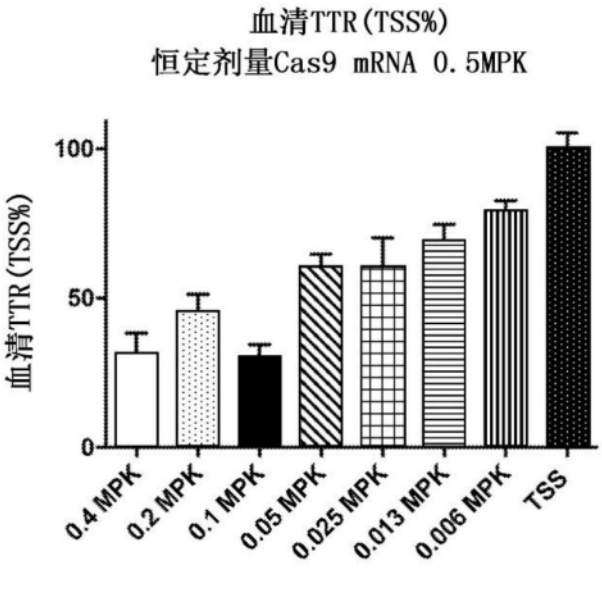

血清TTR(TSS%)
恒定剂量Cas9 mRNA 0.5MPK
血清TTR(TSS%)
100
50
0
0.4 MPK
0.2 MPK
0.1 MPK
0.05 MPK
0.025 MPK
0.013 MPK
0.006 MPK
TSS

图22C

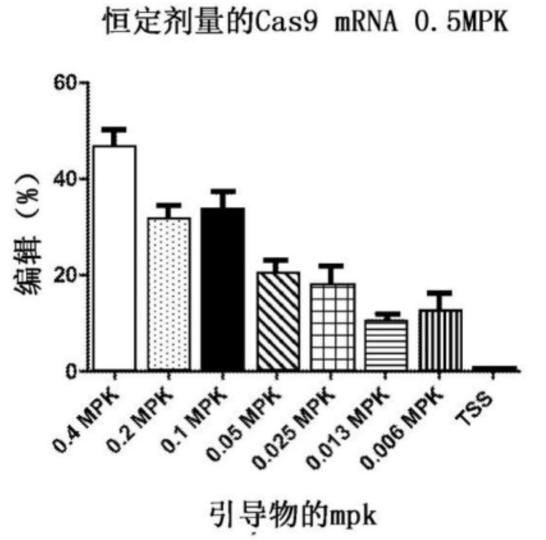

恒定剂量的Cas9 mRNA 0.5MPK
编辑（%）
60
40
20
0
0.4 MPK
0.2 MPK
0.1 MPK
0.05 MPK
0.025 MPK
0.013 MPK
0.006 MPK
TSS
引导物的mpk

图22D

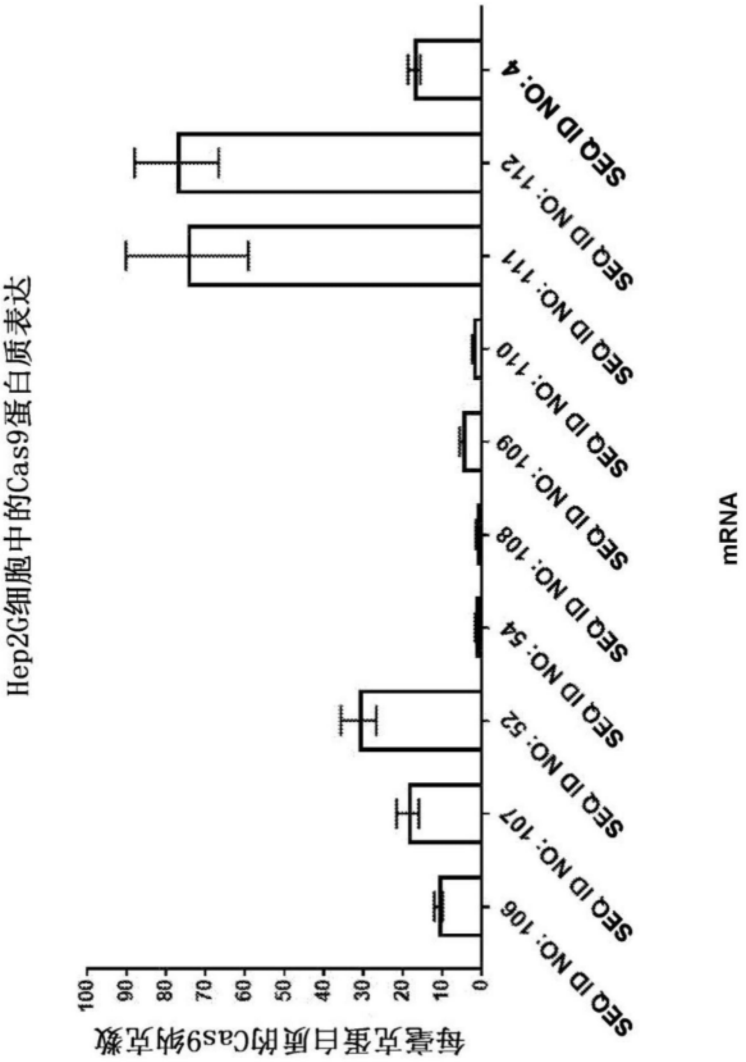

Hep2G细胞中的Cas9蛋白质表达
每毫克蛋白质的Cas9纳克数
100
90
80
70
60
50
40
30
20
10
0
SEQ ID NO: 106
SEQ ID NO: 107
SEQ ID NO: 52
SEQ ID NO: 54
SEQ ID NO: 108
SEQ ID NO: 109
SEQ ID NO: 110
SEQ ID NO: 111
SEQ ID NO: 112
SEQ ID NO: 4
mRNA

图23

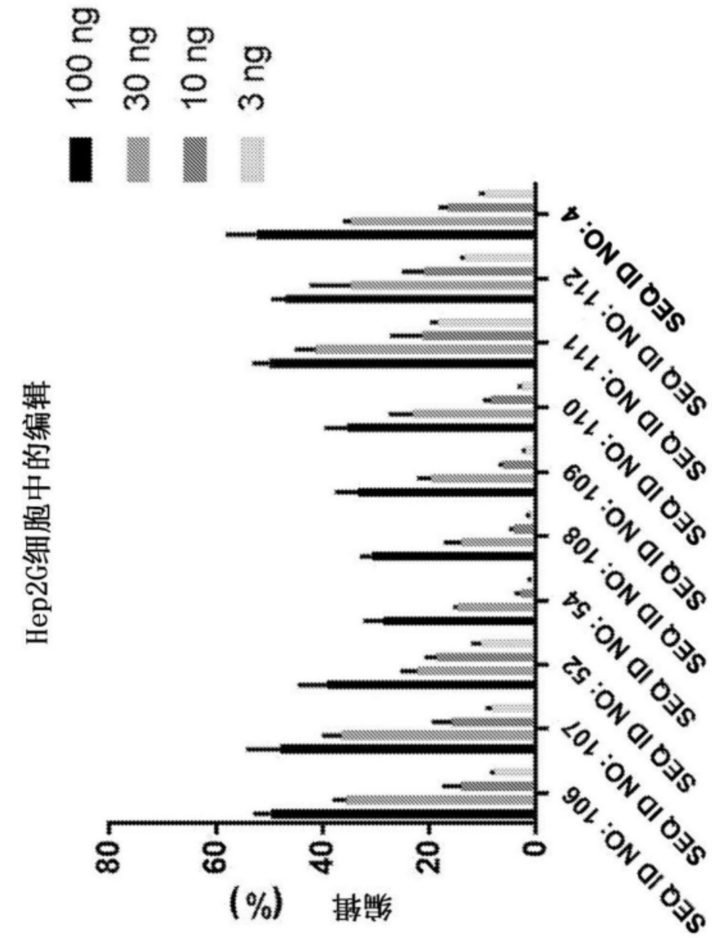

Hep2G细胞中的编辑
100 ng
30 ng
10 ng
3 ng
编辑 (%)
0
20
40
60
80
SEQ ID NO: 106
SEQ ID NO: 107
SEQ ID NO: 52
SEQ ID NO: 54
SEQ ID NO: 108
SEQ ID NO: 109
SEQ ID NO: 110
SEQ ID NO: 111
SEQ ID NO: 112
SEQ ID NO: 4

图24

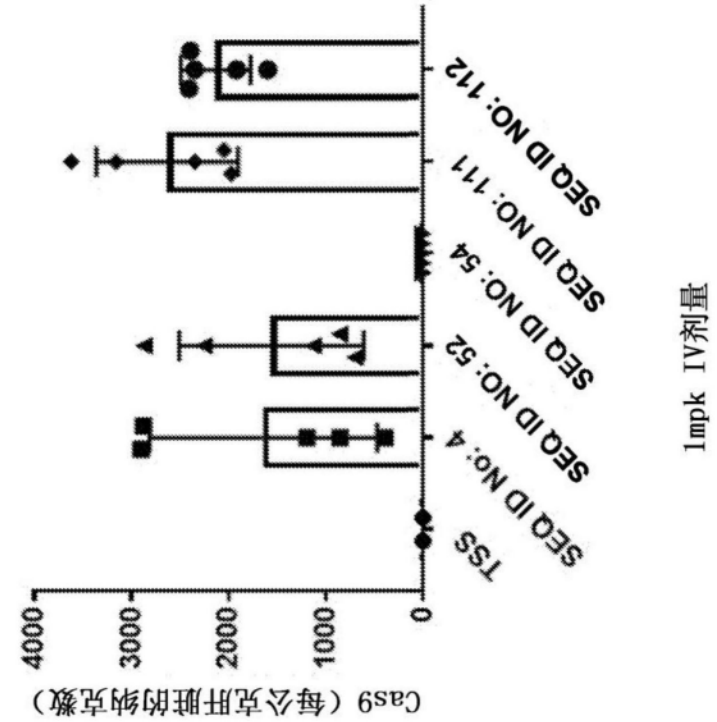

Cas9（每公克肝脏的纳克数）
4000
3000
2000
1000
0
TSS
SEQ ID No: 4
SEQ ID NO: 52
SEQ ID NO: 54
SEQ ID NO: 111
SEQ ID NO: 112
1mpk IV剂量

图25

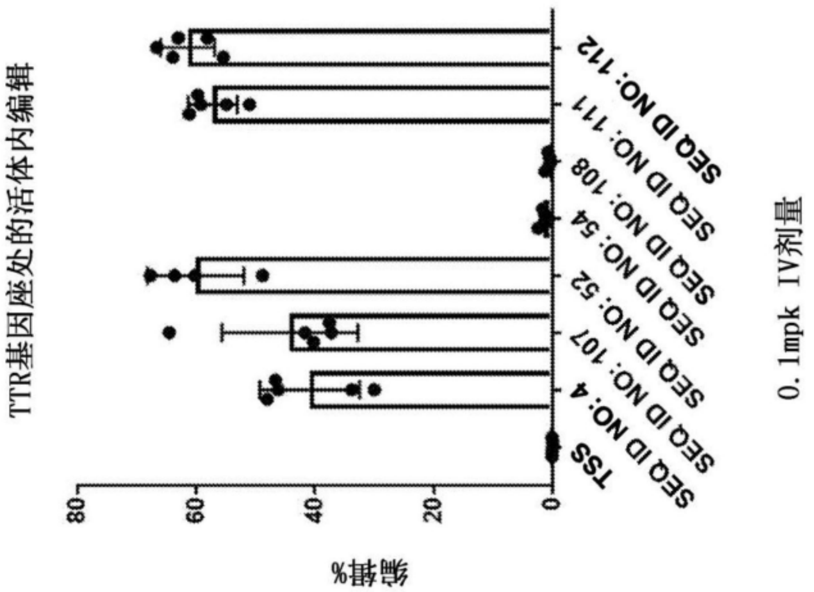

TTR基因座处的活体内编辑
编辑%
80
60
40
20
0
TSS
SEQ ID NO: 4
SEQ ID NO: 107
SEQ ID NO: 52
SEQ ID NO: 54
SEQ ID NO: 108
SEQ ID NO: 111
SEQ ID NO: 112
0.1mpk IV剂量

图26

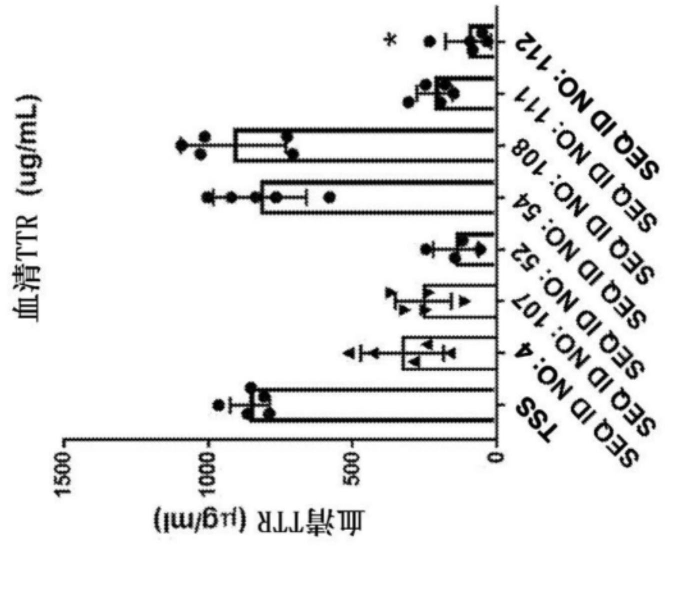

血清TTR (ug/mL)
血清TTR (μg/ml)
1500
1000
500
0
TSS
SEQ ID NO: 4
SEQ ID NO: 107
SEQ ID NO: 52
SEQ ID NO: 54
SEQ ID NO: 108
SEQ ID NO: 111
SEQ ID NO: 112
*

图27A

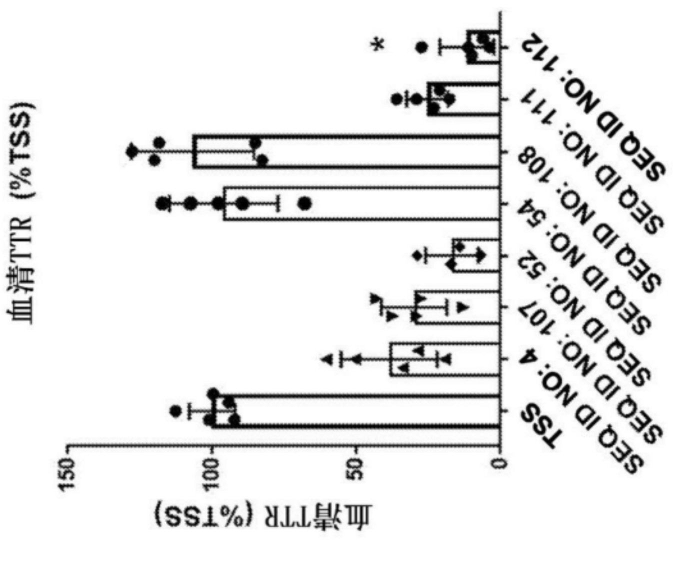

血清TTR (%TSS)
血清TTR (%TSS)
150
100
50
0
TSS
SEQ ID NO: 4
SEQ ID NO: 107
SEQ ID NO: 52
SEQ ID NO: 54
SEQ ID NO: 108
SEQ ID NO: 111
SEQ ID NO: 112
*

图27B

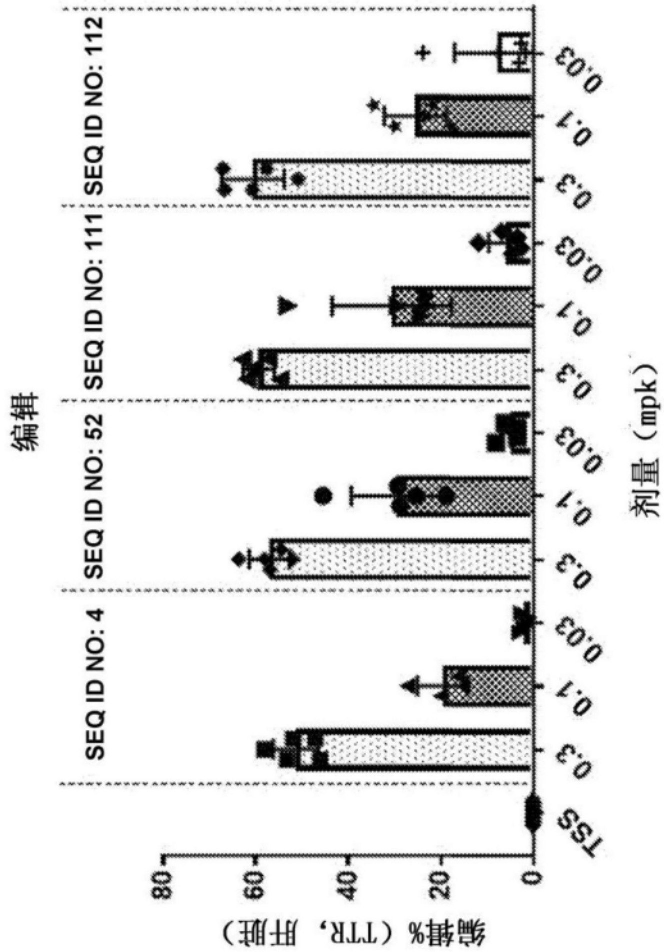

编辑
SEQ ID NO: 4
SEQ ID NO: 52
SEQ ID NO: 111
SEQ ID NO: 112
编辑%（TTR，肝脏）
80
60
40
20
0
TSS
0.3
0.1
0.03
剂量（mpk）

图28

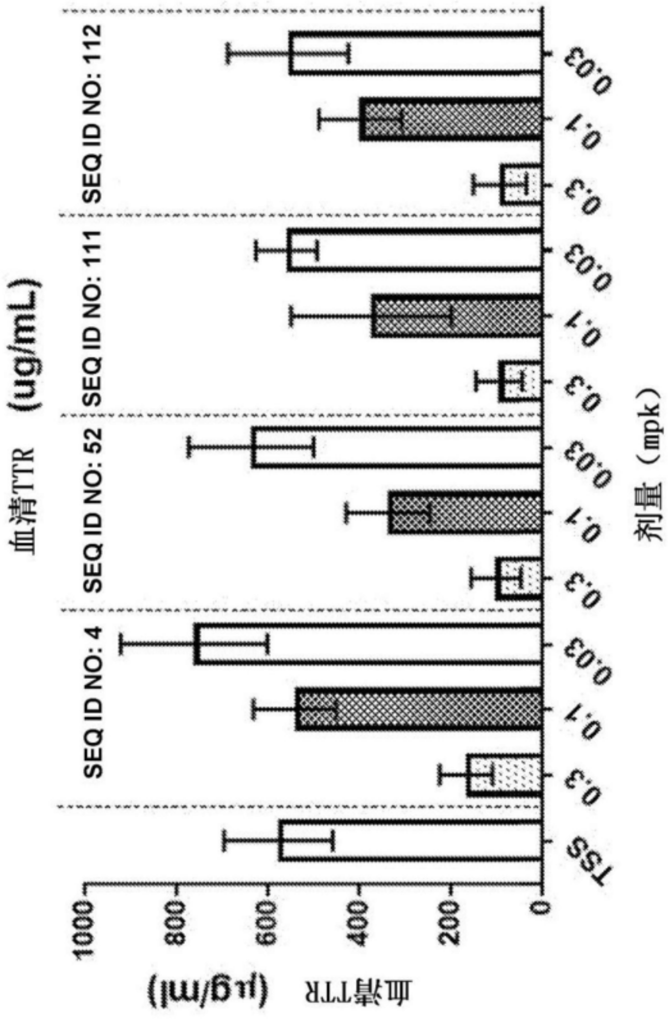

血清TTR (ug/mL)
SEQ ID NO: 4
SEQ ID NO: 52
SEQ ID NO: 111
SEQ ID NO: 112
TSS
0.3
0.1
0.03
剂量（mpk）
血清TTR (μg/ml)
0
200
400
600
800
1000

图29A

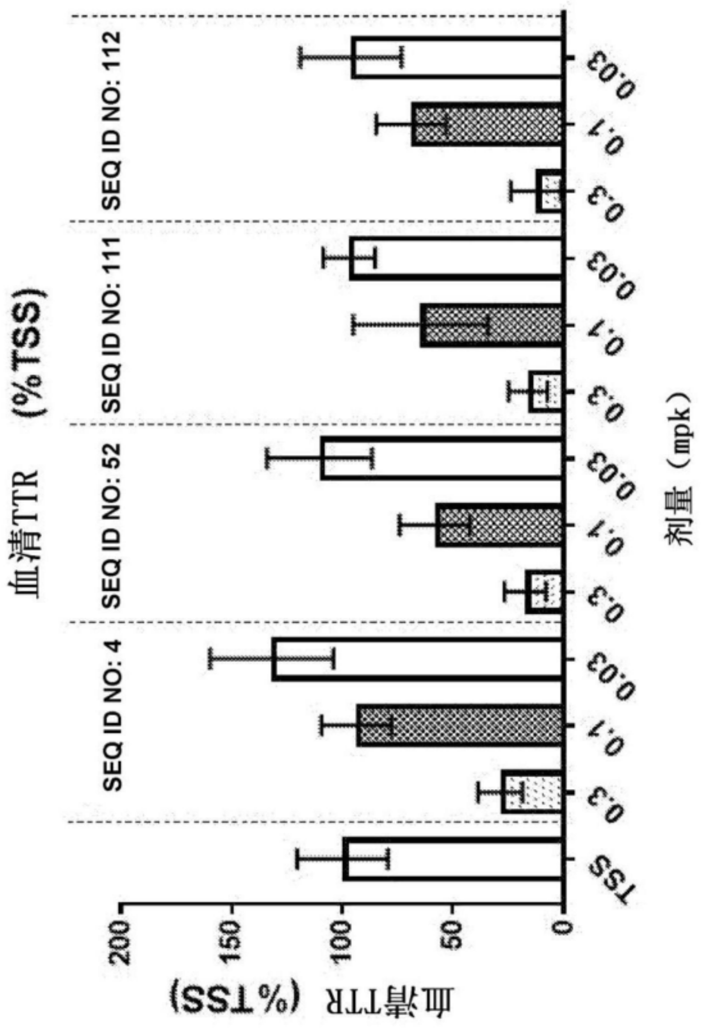

血清TTR　(%TSS)
SEQ ID NO: 4
SEQ ID NO: 52
SEQ ID NO: 111
SEQ ID NO: 112
200
150
100
50
0
血清TTR　(%TSS)
TSS
0.3
0.1
0.03
剂量（mpk）

图29B

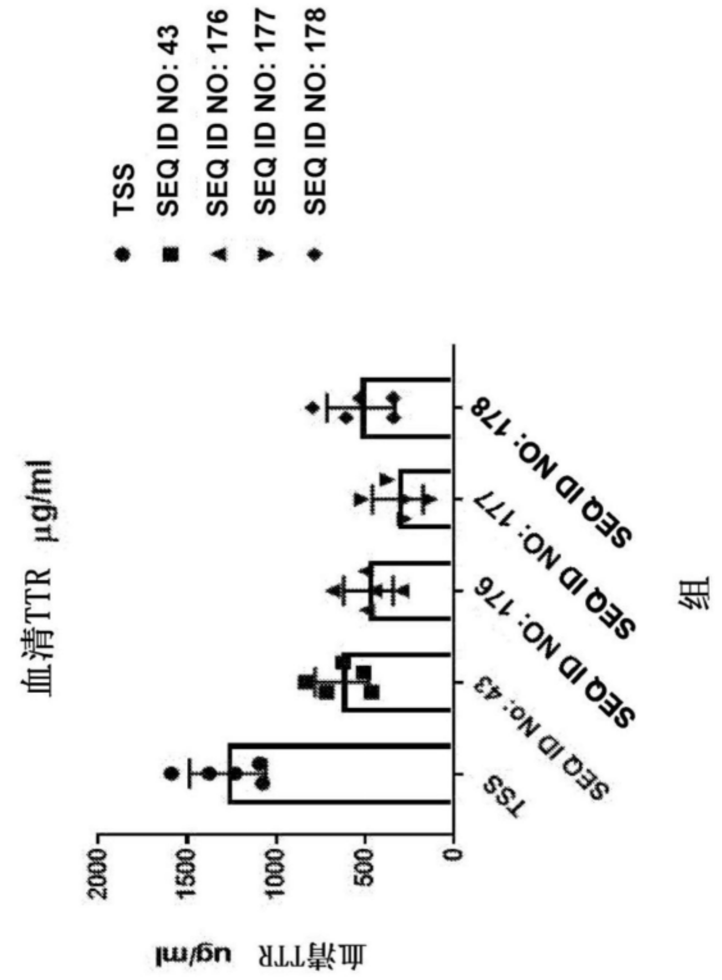

血清TTR μg/ml
血清TTR ug/ml
2000
1500
1000
500
0
TSS
SEQ ID NO: 43
SEQ ID NO: 176
SEQ ID NO: 177
SEQ ID NO: 178
组

图30A

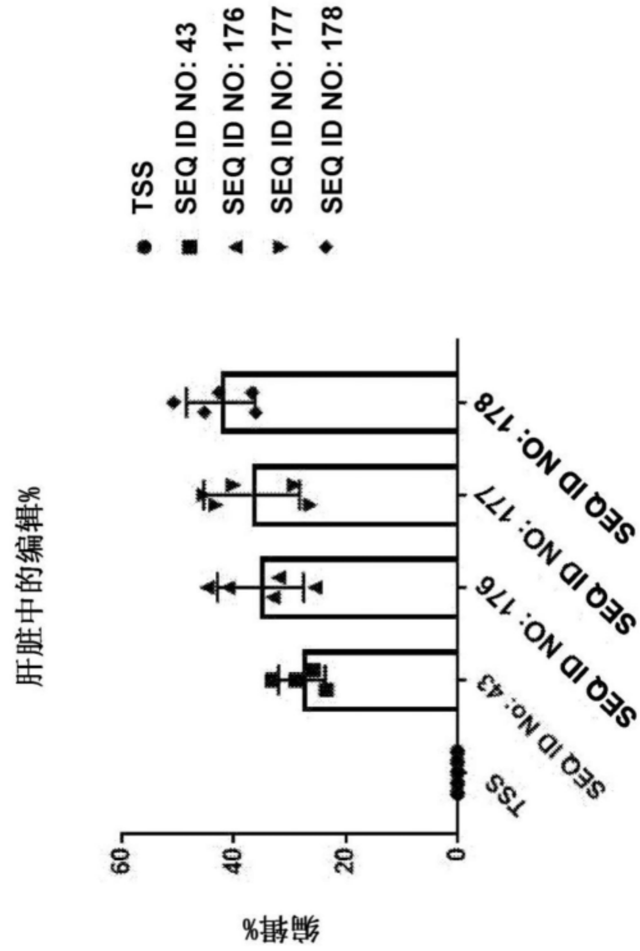

肝脏中的编辑%
编辑%
60
40
20
0
TSS
SEQ ID NO: 43
SEQ ID NO: 176
SEQ ID NO: 177
SEQ ID NO: 178

图30B

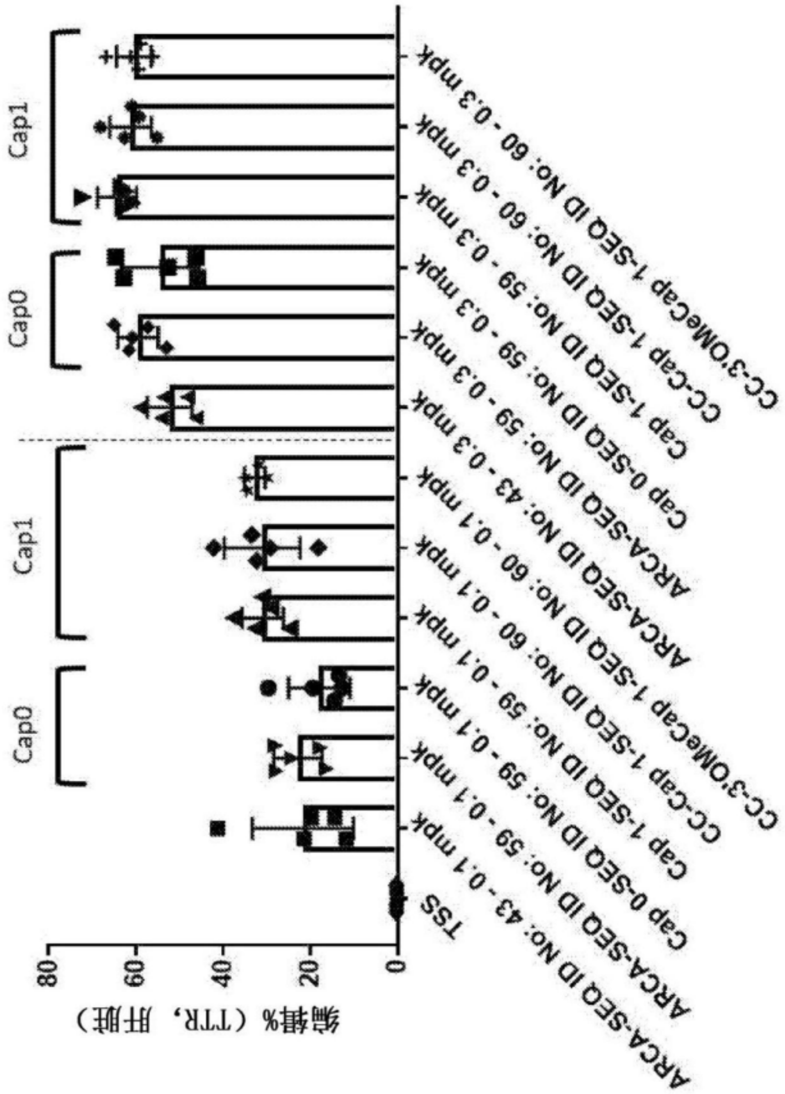

编辑%（TTR，肝脏）
0
20
40
60
80
Cap0
Cap1
Cap0
Cap1
TSS
ARCA-SEQ ID No: 43 - 0.1 mpk
Cap 0-SEQ ID No: 59 - 0.1 mpk
Cap 1-SEQ ID No: 59 - 0.1 mpk
CC-Cap 1-SEQ ID No: 60 - 0.1 mpk
CC-3'OMeCap 1-SEQ ID No: 60 - 0.1 mpk
ARCA-SEQ ID No: 60 - 0.1 mpk
ARCA-SEQ ID No: 43 - 0.3 mpk
Cap 0-SEQ ID No: 59 - 0.3 mpk
Cap 1-SEQ ID No: 59 - 0.3 mpk
CC-Cap 1-SEQ ID No: 60 - 0.3 mpk
CC-3'OMeCap 1-SEQ ID No: 60 - 0.3 mpk

图31

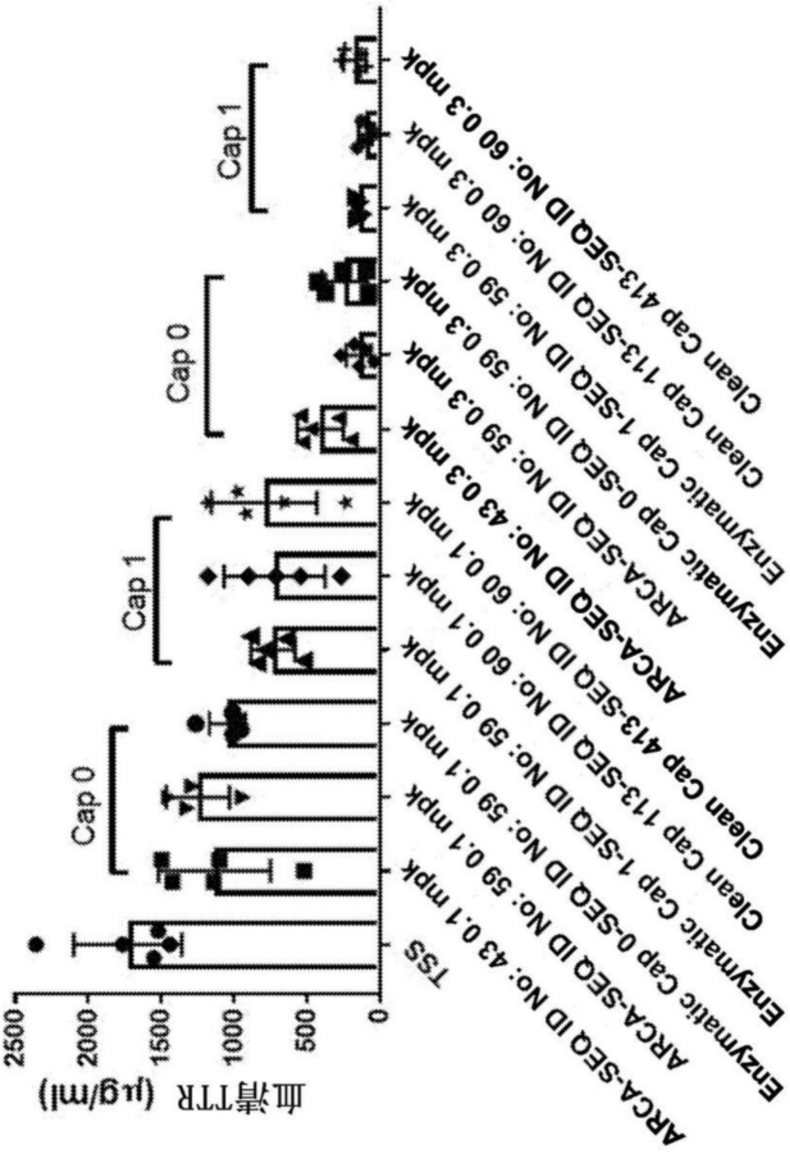

血清TTR (μg/ml)
2500
2000
1500
1000
500
0
TSS
ARCA-SEQ ID No: 43 0.1 mpk
Enzymatic Cap 0-SEQ ID No: 59 0.1 mpk
Enzymatic Cap 1-SEQ ID No: 59 0.1 mpk
Clean Cap 113-SEQ ID No: 60 0.1 mpk
Clean Cap 413-SEQ ID No: 60 0.1 mpk
ARCA-SEQ ID No: 43 0.3 mpk
Enzymatic Cap 0-SEQ ID No: 59 0.3 mpk
Enzymatic Cap 1-SEQ ID No: 59 0.3 mpk
Clean Cap 113-SEQ ID No: 60 0.3 mpk
Clean Cap 413-SEQ ID No: 60 0.3 mpk
Cap 0
Cap 1
Cap 0
Cap 1

图32

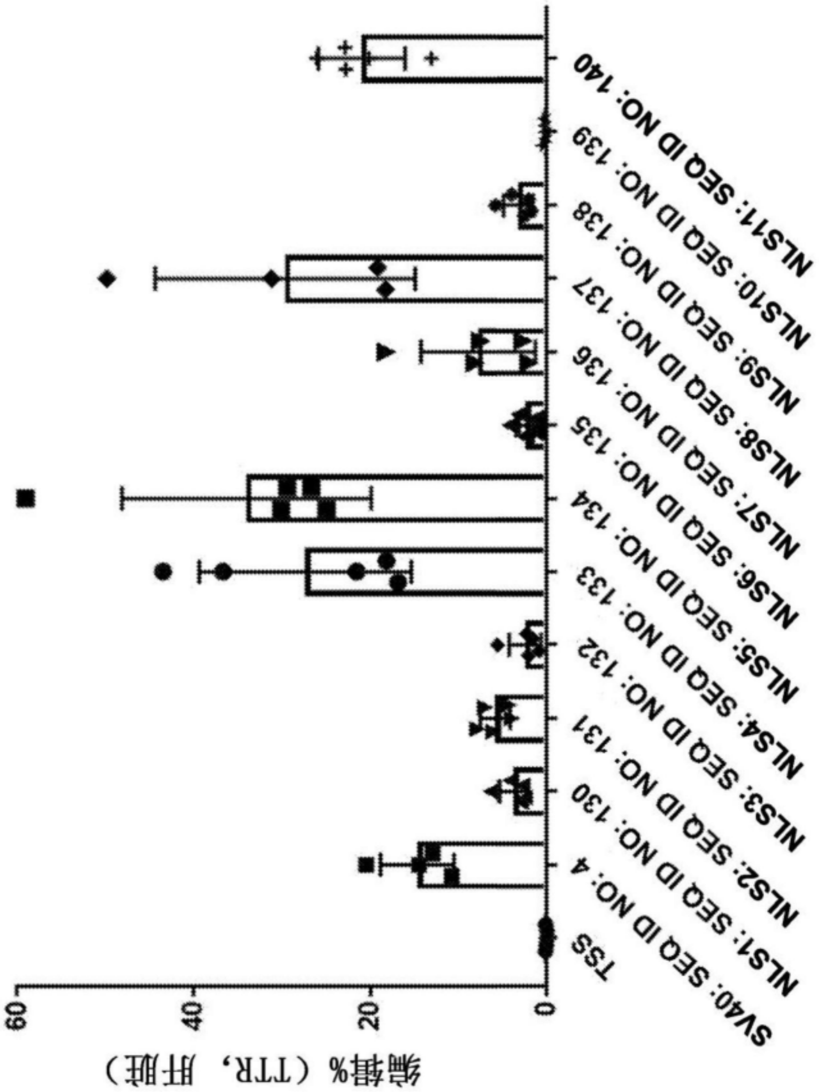

编辑% (TTR, 肝脏)
0
20
40
60
TSS
SV40: SEQ ID NO: 4
NLS1: SEQ ID NO: 130
NLS2: SEQ ID NO: 131
NLS3: SEQ ID NO: 132
NLS4: SEQ ID NO: 133
NLS5: SEQ ID NO: 134
NLS6: SEQ ID NO: 135
NLS7: SEQ ID NO: 136
NLS8: SEQ ID NO: 137
NLS9: SEQ ID NO: 138
NLS10: SEQ ID NO: 139
NLS11: SEQ ID NO: 140

图33

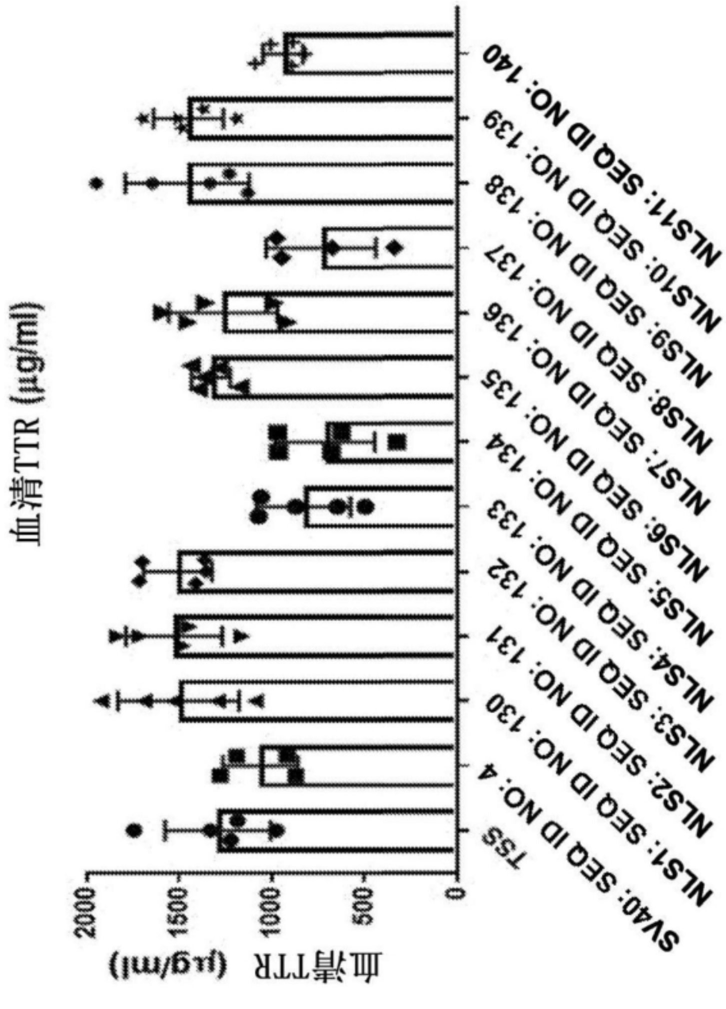

血清TTR (μg/ml)
2000
1500
1000
500
0
TSS
SV40: SEQ ID NO: 4
NLS1: SEQ ID NO: 130
NLS2: SEQ ID NO: 131
NLS3: SEQ ID NO: 132
NLS4: SEQ ID NO: 133
NLS5: SEQ ID NO: 134
NLS6: SEQ ID NO: 135
NLS7: SEQ ID NO: 136
NLS8: SEQ ID NO: 137
NLS9: SEQ ID NO: 138
NLS10: SEQ ID NO: 139
NLS11: SEQ ID NO: 140

图34A

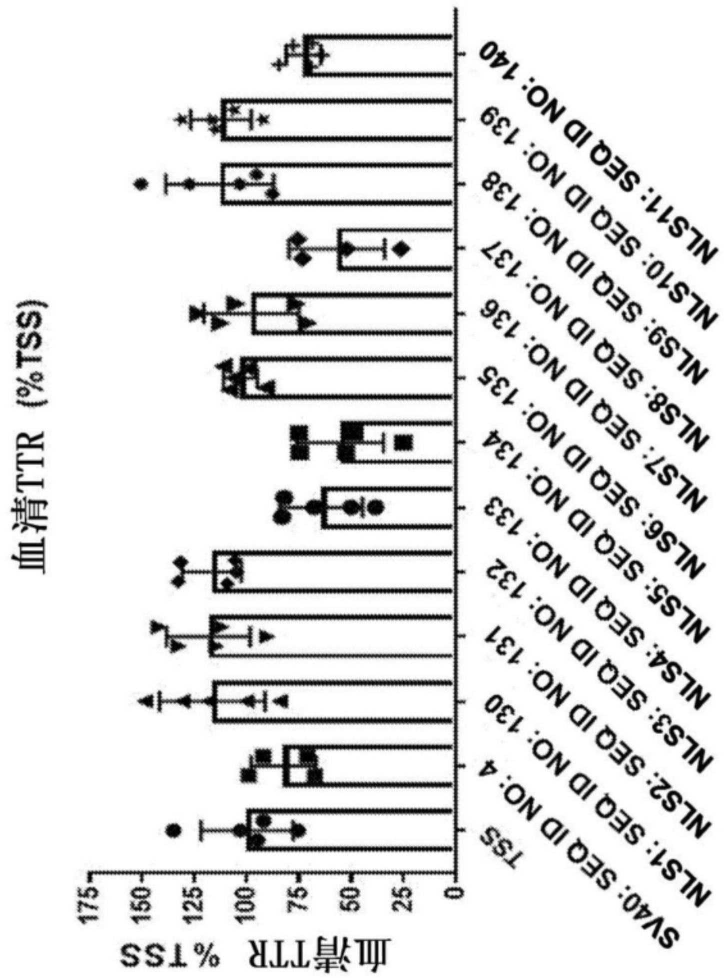

血清TTR (%TSS)
血清TTR %TSS
0
25
50
75
100
125
150
175
TSS
SV40: SEQ ID NO: 4
NLS1: SEQ ID NO: 130
NLS2: SEQ ID NO: 131
NLS3: SEQ ID NO: 132
NLS4: SEQ ID NO: 133
NLS5: SEQ ID NO: 134
NLS6: SEQ ID NO: 135
NLS7: SEQ ID NO: 136
NLS8: SEQ ID NO: 137
NLS9: SEQ ID NO: 138
NLS10: SEQ ID NO: 139
NLS11: SEQ ID NO: 140

图34B

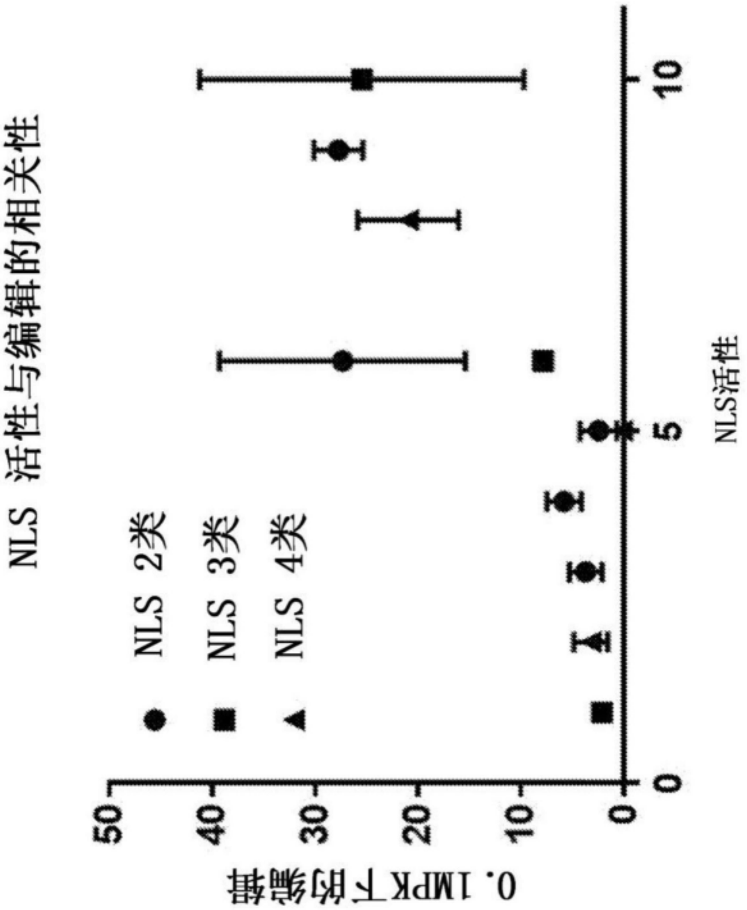

NLS 活性与编辑的相关性
NLS 2类
NLS 3类
NLS 4类
0.1MPK下的编辑
50
40
30
20
10
0
0
5
10
NLS活性

图35

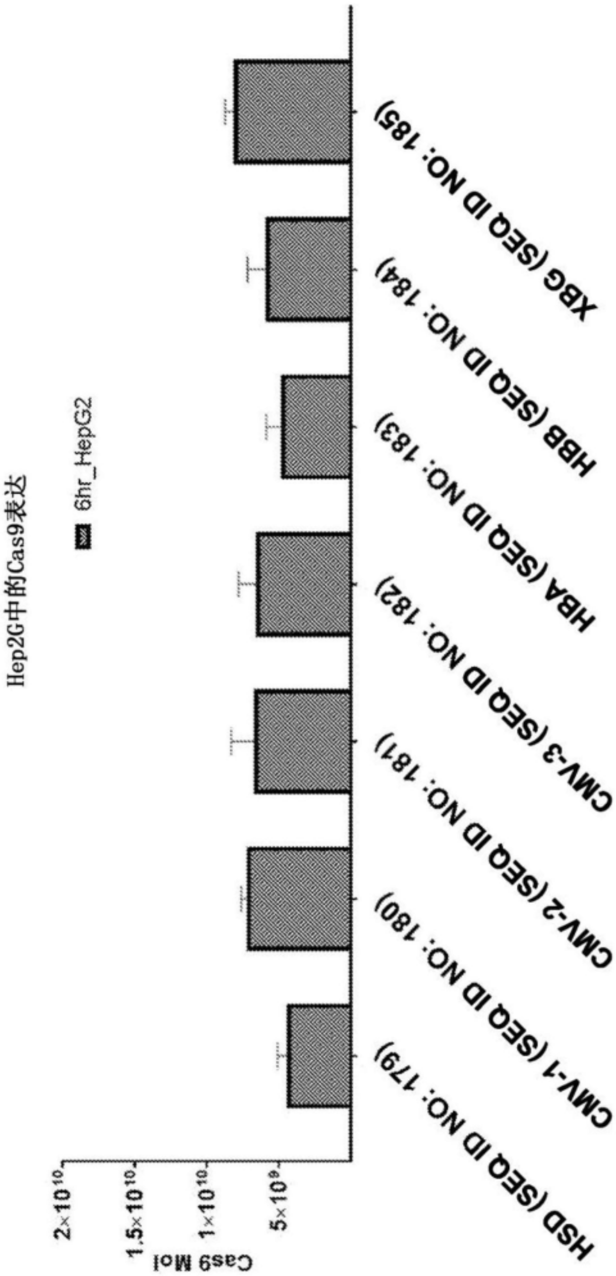

Hep2G中的Cas9表达
6hr_HepG2
Cas9 Mol
2×10¹⁰
1.5×10¹⁰
1×10¹⁰
5×10⁹
HSD (SEQ ID NO: 179)
CMV-1 (SEQ ID NO: 180)
CMV-2 (SEQ ID NO: 181)
CMV-3 (SEQ ID NO: 182)
HBA (SEQ ID NO: 183)
HBB (SEQ ID NO: 184)
XBG (SEQ ID NO: 185)

图36